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Our major research findings may be divided into two categories: (1) comparative information on cellular development and structure in land plants and (2) contributions to clarifying evolutionary trends and resolving phylogenetic relationships among basal embryophytes. Our studies have provided detailed descriptions of sperm cell architecture and cellular development in a wide range of pteridophytes and in selected bryophytes, green algae and seed plants. Male gametes were poorly described or entirely unknown for most of these plants (16 of 24). Three-dimensional drawings were constructed for mature spermatozooids of most taxa investigated. Structural and developmental complexity in plant sperm cells are unsurpassed in any other group of organisms, and prior to our work remained unknown. The role of the cytoskeleton in this process is complicated; our studies have systematically identified the involvement of these cellular components during spermatogenesis in representatives of the major plant groups. Our most significant findings on the cytoskeleton relate to the distribution and function of centrin and actin. Centrin is a primary constituent of diverse structures in plant sperm cells, namely the lamellar strip, amorphous zone, the stellate pattern of the basal body and fibrous connective structures. Actin is also abundant in spermatogenous cells and it is essential for normal sperm cell differentiation. Differences in these proteins among plant groups provide informative morphological characters for phylogenetic analyses.

Our research program has generated new data, assembled published data and analysed the most comprehensive data bases of both morphological and molecular data (either alone or together) associated with the phylogeny of land plants. These analyses show that diverse data sets could converge on a common answer. Our cladograms based exclusively on sperm gametogenesis resolve four or five primary lineages within terrestrial plants: (1) seed plants, (2) a large fern assemblage including *Equisetum*, *Psilotum* and *Botrychium*, (3) lycopsids, (4) mosses and liverworts, with clade 4 either including hornworts or placing this assemblage (5) basal on the land plant tree. The ability of the male gametogenesis data alone to resolve these natural assemblages suggests that processes of spermatogenesis are highly conserved within land plants. Several of our primary conclusions were supported by subsequent molecular analyses. Most notable are the conclusions that *Equisetum* and *Psilotum* are members of the fern clade and that mosses plus liverworts form a monophyletic group (1).

Since 1995, Dr. Renzaglia's lab team has generated 26 published manuscripts (2-27 in the References Cited), one submitted articles (28), 27 published abstracts and 12 additional abstracts for local or regional meetings. Publications include co-authorships with two M.S. students, one doctoral student and four undergraduates (stared and bold in References Cited). A recent invited review, published as a separate issue of Critical Reviews in Plant Sciences (29), synthesizes the results from this research program and incorporates virtually all of the available literature on male gametes in plants.

Support from DEB- 9527735 has had a tremendous impact on education at both the undergraduate and graduate levels. Nine undergraduates at SIUC have participated in the research enterprise under Dr. Renzaglia's supervision and the REU program supported all of these students. Undergraduates have presented at local, regional, national and international meetings, for a total of 16 published abstracts and 10 additional abstracts. Undergraduates are co-authors on six manuscripts and they have received numerous small grants and awards for research and travel. One doctoral student (Angel Maden), and three master's students (Lawrence Mainwaring, Gayleen Cochran, Marlies Weber) worked on plant spermatogenesis since 1995. Dr. Renzaglia has also conducted workshops and presented papers on the use of bryophytes and pteridophytes in teaching plant biology and plant development. The inquiry-based exercises she has developed as a result of her studies of plant spermatogenesis have been adopted repeatedly for use in plant biology courses. A web site called Land Plants Online (<http://www.science.siu.edu/landplants/index.html>) was developed with Dan Nickrent at SIUC to disseminate a broad range of information on the biology of land plants, including extensive information on plant spermatogenesis.

PROJECT DESCRIPTION

I. INTRODUCTION AND OBJECTIVES

As the oldest living lineages of land-dwelling organisms, the bryophytes (mosses, liverworts and hornworts) represent pivotal groups for evaluating early patterns of terrestrial biodiversification. While mosses and liverworts have experienced a recent resurgence of interest in regards to biodiversity and taxonomy (30,31, and refs. cited therein), one group of bryophytes, the **hornworts**, has remained relatively overlooked. Hypothesized to be the basal most land plants (8,13,14,29,32-39) the hornworts (anthocerotes) are a small group (5-12 genera with 100-150 species worldwide) of morphologically distinct, seemingly simple land plants. Many structural features of these plants represent adaptations to life on land that are the products of deep divergences and thus are unparalleled in other groups of plants (7). Even with these unique innovations, the hornworts possess a bewildering array of characters that are algal-like, liverwort-like, moss-like, and yet others that are similar to vascular plants (e.g., continued sporophyte growth and embedded archegonia). Some of these features, especially chloroplast ultrastructure and sperm cell architecture, are viewed as plesiomorphic (29) while others are considered advanced characters among bryophytes (e.g., complicated sporophyte and presence of stomata in some genera) (39). It is because of the unique structural innovations and the curious shared characteristics with other plant and algal lineages that this ancient group of land plants is particularly intriguing from an evolutionary perspective.

While hornworts clearly represent a monophyletic group, taxonomic boundaries and interrelationships among hornwort species and genera remain confused (7). Existing classification schemes for the group are incongruent and conflicting generic concepts recognize from five to nine, totalling 12, genera (40-43). Although diagnostic morphological features exist in some cases, other critical characters for delineation of taxa show considerable variation. Additional complications leading to these taxonomic incongruities include the inconsistent use of characters to delineate taxa, and the paucity of fundamental developmental, ultrastructural and biochemical data. Confounding this is the complete absence of any molecular phylogenetic analysis that focuses on the group. Indeed, the anthocerotes are the **only** major group of land plants for which a molecular study has not yet been undertaken at **any** taxonomic level. Due to the absence of molecular phylogenetic studies and the lack of morphological data, it is virtually impossible to evaluate hypothesis of adaptive evolution within the hornworts and to place these innovations into the broader context of land plant evolution. Clearly, new data and new insight into analyzing existing data are required to clarify the systematics and evolution of this important, ancient plant lineage.

We propose a **global examination of hornworts** that will develop a portrait of the biodiversity, phylogeny and biogeography of this historically significant plant assemblage. Our collaborative research will provide the necessary data on genetic/morphological diversity and geographic distributions of hornworts to achieve the following goals:

1) Establish a robust estimate of hornwort relationships. To achieve this goal we will conduct analyses of multiple molecular data sets and comprehensive data sets representing morphological, ultrastructural, functional and developmental characters. Gene sequences will be generated from all three genomes, and morphological data will be collected on features that are emphasized in taxonomic treatments. This goal is essential to assess character evolution and to accomplish the remaining goals.

2) Produce a revised classification of the hornworts. Rather than simply providing phylogenetic relationships to the systematics community, we feel strongly that a modern reclassification of the hornworts should result from our efforts. We will approach the classification from a cladistic perspective applying clades derived from phylogenetic analyses to the organisms included in our study. This approach will clarify the presently poorly defined familial, generic, and species boundaries.

3) Identify patterns of hornwort diversification. To gain a greater appreciation for the spatial and temporal patterns of hornwort diversification we propose to examine world-wide biogeographic patterns. The antiquity of hornworts presents unique opportunities to test biogeographic hypotheses of vicariance versus recent colonization, typically generated from studies of seed plants and other bryophytes

(44). We propose to (a) identify general global biogeographic patterns, (b) examine specific biogeographic hypotheses involving the Australasian landmasses, and (c) explore infra-specific variability (45) with intense evaluation of the controversial cosmopolitan *Phaeoceros laevis* L. (= *P. laevis* Prosk. + *P. carolinianus* Prosk. + *P. mohrii* Aust. + *P. oreganus* Aust. + >10 additional named segregates worldwide).

To address these goals we request three years of support to conduct two highly integrated and collaborative projects. The first (J. Duff) employs molecular phylogenetic methodologies whereas the second (K. Renzaglia) involves comparative morphogenetic and ultrastructural studies. Integral to the project are further collaborations with Chris Cargill from the Australian National Herbarium at the Center for Plant Biodiversity Research (CANB) and Jeff Duckett from the University of London (see attached letters). As experts on hornwort taxonomy and morphology, C. Cargill and J. Duckett will collect intensively in the Australasian landmasses, southeast Asia and Africa and will play integral roles in morphological data collection and analyses. Additionally, we have called on collaborators from around the world, including Jiro Hasegawa (Japan), Noris Salazar (Panama), Gabriella Hassel de Menendez (Argentina), Benito Tan (Singapore), Roberto Ligrone (Italy), A. K. Asthana (India), S. C. Srivastava (India), Kevin Vaughn (Mississippi-USA) and Paul Davison (southeastern USA), to assist in specimen collection and to collaborate on our studies (see attached letters).

II. HOW DOES THIS PROPOSAL DIFFER FROM THAT OF PAST SUBMISSIONS?

This proposal has some similarities in content to previous submissions, most recently in 1999 (DEB-9974135). The current proposal takes on a very different focus in part due to suggestions and criticisms from reviewers and in part to recently acquired preliminary data. Below we describe how this proposal differs in both spirit and substance from its predecessors.

Research focus. A consistent comment across previous submissions was that the question of hornwort phylogeny is lost in the more controversial, and potentially intractable question of how they fit into the context of basal embryophyte phylogeny. A significant portion of previous submissions was devoted to addressing the nuances of this debate and anticipated objections. For the present submission we wish to put the larger question of the position of the hornworts among land plants aside in favor of focusing on questions important within the group itself. With a robust phylogeny and comprehensive taxonomic revision, it will be possible to evaluate global patterns of distribution of anthocerotales, an exercise that is long overdue but untenable without clear concepts of generic/species limits. Thus, a significant addition to the current proposal is to examine the biogeography of hornworts.

Principles investigators. This proposal includes a change in principle investigator. Former submissions involved two PIs, D. Nickrent and K. Renzaglia. The present submission involves collaborative research between J. Duff and K. Renzaglia. As D. Nickrent's postdoctoral fellow from 1995-1999 and since attaining his present faculty position, J. Duff has been intensively involved in systematics studies of hornworts (see preliminary data). Indeed, these investigations are the primary focus of his research laboratory.

Molecular and morphological data. Prior submissions emphasized molecular and morphological characters that were critical for solving problems in establishing basal land plant phylogeny. Our focus within the hornwort clade necessitates a new examination of key characters and phylogenetic tools. As such, we will examine key morphological, ultrastructural, function and developmental features, in addition to gene sequences appropriate to questions of within hornwort phylogeny. Such multiplicity of data sets will allow extensive congruency tests to be undertaken that were not possible in past manifestations of this proposal (eg. gametophytic vs. sporophytic morphological characters).

Taxon Sampling. In accordance with the focus on hornwort systematics we are proposing to increase significantly taxon sampling in hornworts. With the assistance of colleagues from around the world, we will collect and investigate at least five species from each of the five most often recognized genera (*Anthoceros*, *Dendroceros*, *Megaceros*, *Notothylas*, and *Phaeoceros*) and a minimum of two species from each of the more recently proposed genera and subgenera (see details in Research Plan and

letters of support). Especially extensive collections from around the world will be acquired for the cosmopolitan, but poorly characterized genus *Phaeoceros* and for taxa in the Australasian region.

III. BACKGROUND

A. Unique Hornwort Characters: The peculiar structure and development of the sporophyte separates hornworts from all other land plants. This generation consists of a **long cylindrical sporangium** that **perpetually elongates** from a basal meristem and produces spores in a **non-synchronized** fashion during the entire growing season. Histogenic regions from outside to inside include an epidermis with or without **stomata**, an assimilative tissue, sporogenous tissue and a **central columella**. Although similar in appearance to those of mosses and tracheophytes, hornwort stomata apparently **do not open and close**, i.e., once open, they remain that way. The sexual phase or gametophyte generation, is a simple flattened thallus or ribbon that appears undifferentiated. However, internal differentiation is evident in the chambers and cavities that house sex organs and **colonies of *Nostoc***, a cyanobacterial symbiont. Peculiar to this group is the abundant production of **mucilage** by virtually any cell. Indeed, reproductive organs and vulnerable tissues are consistently enclosed in **internal, mucilage-filled chambers** that provide protection against desiccation, ionizing radiation and mechanical damage (8). At the cellular level, hornworts are unique among land plants in the possession of **solitary chloroplasts with pyrenoids**, features that are shared with green algae (46). **Morphological variability among genera** is primarily seen in reproductive features such as the architecture of the sporangium, spores, pseudoelaters, antheridia and antheridial chambers, but generic differences are also seen in the thallus structure, number and microanatomy of chloroplasts, and the biochemistry of these plants.

B. Questionable Hornwort Affinities: Hornworts are in the center of a heated debate over land plant evolution (8,32,37,47-49). As noted above, recent molecular and morphological evidence suggests the hornworts are the oldest living lineage of land plants (8,13,14,29,32-39). An equally viable hypothesis suggests that liverworts may be the basal lineage of extant land plant with either hornworts or mosses sister to the vascular plants (47-53). Fossil evidence does little in the way to resolve this issue as the first unambiguous hornwort fossils date to the Cretaceous (54). However, ornamentation of Paleozoic spores from the Silurian (>410 Mya) have been compared to that of *Anthoceros* thus raising the **possibility** that hornworts were the first bryophytes to diversify (55). Although resolution of this fundamental question in evolutionary biology is not our focus, further characterization of hornwort phylogeny and character evolution is essential in providing a complete picture for interpretation of the evolutionary events that accompanied land colonization. For example, if the directionality of evolution in structures such as stomata and chloroplasts is clarified within hornworts, then the homology with similar structures in other plant and algal groups can be ascertained.

C. Problematic Hornwort Taxonomy and Classification: Cladistic analyses involving morphological features of a limited number of samples have been conducted on hornworts (41,50), and some of the resultant phylogenetic hypotheses have been used to develop classifications schemes (40-43) (Figure 1). Of the twelve proposed genera of hornworts, only five have gained wide recognition, namely *Anthoceros*, *Phaeoceros*, *Notothylas*, *Megaceros* and *Dendroceros*. As evidenced by the lack of agreement on classification schemes, the hierarchical organization of hornwort taxa remains equivocal and taxonomic boundaries are poorly resolved. Mishler and Churchill (50) noted the problematic relationships within and among hornwort genera and stated that almost all of the apparent homoplasy they detected in their morphological analysis of bryophytes involved the hornworts.

Hyvönen and Piippo, 1993 (42)	Hasegawa, 1994 (43)
Anthocerotales Limpricht in Cohn	Anthocerotales Limpricht in Cohn
Anthocerotaceae Dum.	Notothyladaceae (Milde) Müll.
<i>Anthoceros</i> (Mich.) L.	<i>Notothylas</i> Sull.
<i>Folioceros</i> Bharadwaj	Anthocerotaceae Dum.
<i>Leiosporoceros</i> Hässel de Men.	Subfam. Anthocerotoideae
<i>Mesoceros</i> Piippo	<i>Anthoceros</i> (Mich.) L.
<i>Phaeoceros</i> Prosk.	Subgen. <i>Anthoceros</i>
<i>Spaerosporoceros</i> Hässel de Men.	Subgen. <i>Folioceros</i>
Dendrocerotaceae (Milde) Hässel de Men.	<i>Leiosporoceros</i> Hässel de Men.
<i>Dendroceros</i> Nees	<i>Hattorioceros</i> Hasegawa
<i>Megaceros</i> Campbell	<i>Phaeoceros</i> Prosk.
Nothothylales	Subfam. Dendrocerotoideae
Notothyladaceae (Milde) Müll.	<i>Dendroceros</i> Nees
<i>Notothylas</i> Sull.	Subgen. <i>Dendroceros</i>
	Subgen. <i>Apoceros</i> Schuster
	<i>Megaceros</i> Campbell
	<i>Notoceros</i> (Schuster) Hasegawa

Figure 1. Two recent classification schemes proposed for the hornworts.

An evaluation of the divergent interpretations of the evolutionary origins of *Notothylas* and the conflicting views on the placement of this genus among hornworts highlights the critical need for a modern taxonomic revision of the hornworts. With a relatively simple, unelaborated sporophyte, *Notothylas* may represent 1) an apomorphic form that has undergone extensive evolutionary reduction or 2) a plesiomorphic, unspecialized condition. Schuster (39) stated that, "The consensus (among bryologists) has been that a reduced genus, derived from *Anthoceros* s. str. is at hand." Alternatively, he cited evidence, including the absence of stomata, faintly spiraled pseudoelaters and spore wall ornamentation, that *Notothylas* may be related by reduction to *Dendroceros*, *Megaceros* or *Phaeoceros*. He noted that the generic characters are spurious or minor and the circumscription of the genus problematic. Corroborating the views of Schuster (39), Proskauer (56) stated that *Notothylas* may be a possible polyphyletic, reduced derivative from *Anthoceros*-like forms. In contrast, a basal position of *Notothylas* was proposed by Mishler and Churchill (50) and is supported by characters interpreted as retained plesiomorphies, many of which are shared with liverwort taxa, e.g., indehiscent sporophyte, determinate sporophyte growth, poorly developed pseudoelaters, and poorly-developed or absent columella (42,43,57). Because of the prevailing view that *Notothylas* represents an early divergent hornwort, this genus is often utilized as the sole anthocerote examined in studies aimed at elucidating evolutionary modifications of subcellular features such as plasmodesmata, placental structure and cell wall organization (58,59). Resolution of the phylogenetic position of *Notothylas* within the anthocerotes is essential for future molecular, morphological and physiological studies in which a single exemplar taxon is selected to represent a basal hornwort.

In contrast, there appears to be some agreement on evolutionary affinities between *Anthoceros* (= Schuster's *Aspiromitus*) and *Phaeoceros* (= Schuster's *Anthoceros*), and between *Dendroceros* and *Megaceros*, albeit all four of these taxa have been segregated into multiple genera and subgenera (Figure 1). The putative sister group relationship between *Phaeoceros* and *Anthoceros* is particularly intriguing in light of our recent studies. An emerging consensus from our preliminary molecular analyses is that *Anthoceros* is monophyletic and basal to all other hornworts (Figure 2 and discussion below). Sister relationships between *Dendroceros* and *Megaceros* and between *Phaeoceros* and *Notothylas* are supported, while the monophyly of *Phaeoceros* is called into question. When morphological data are evaluated in light of this hypothesis, a new somewhat surprising interpretation of character evolution emerges (see discussion in section V.C). Clearly, a comprehensive re-evaluation of hornwort systematics and evolution is warranted.

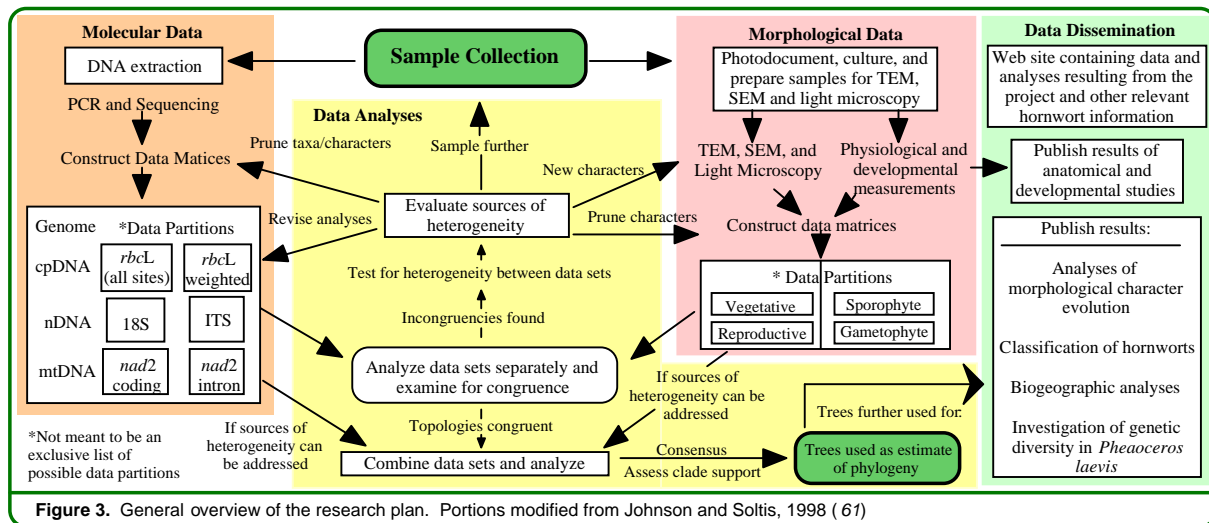
D. Why have hornwort biodiversity, phylogeny and biogeography not been reexamined?:

Evolutionary biologists and bryologists face a number of difficulties in studying hornworts. First, the acquisition of specimens and identification of taxa are two major obstacles. To overcome the former, we have networked with many of the world experts on hornworts and they are eager to participate in this study

observations may be made from these analyses that are relevant to derivation of the stated goals of the proposed research: **1)** Genetic divergence among putative genera is substantial, validating the suggestion that there are deep divergence points among extant taxa; **2)** The taxa fall into well-supported clades that show general agreement with current concepts of generic limits, but the hypothesized interrelationship of these taxa (eg. basal position of *Anthoceros*) is novel; **3)** Rate heterogeneity in the *rbcL* sequences observed in liverworts (48), a group also characterized by deep divergences, is not apparent, suggesting that differential rates of RNA editing in this gene will not pose a problem for subsequent analyses. Preliminary analysis of the 18S sequences also suggests a lack of significant rate heterogeneity; **4)** The position of *Phaeoceros hirticalyx* and *P. coriaceus* in the *Megaceros* clade and *Anthoceros lamuniferus* within *A. punctatus* challenges generic and species limits and calls into question the monophyly of taxa. Partial sequences of 18S and *nad2* (mitochondrial DNA) of *P. hirticalyx* and *P. coriaceus* also substantiate a close tie to *Megaceros* rather than *Phaeoceros*; **5)** Both *Phaeoceros laevis* s.lat. and *Anthoceros punctatus* exhibit genetic divergence among samples that suggests the possible presence of cryptic species. Clearly, many more taxa are necessary to characterize and test global and regional biogeographical patterns, define familial, generic, and subgeneric limits and examine cryptic speciation. It is with these preliminary observations in mind that we have formulated the following research plan.

V. RESEARCH PLAN

Figure 3 and the descriptions below provide an overview of our proposed research program that involves the integration of multiple research approaches, techniques and methods of analyses.



A. Sample acquisition, proposed collection and preparation: We will obtain samples (see attached letters and Budget Justification of KSR) from around the world that represent the entire spectrum of hornwort diversity. These will include species of all the named generic segregates and subgenera (Figures 1 and 4). In order to obtain the complete suite of morphological data, including ultrastructural information, living specimens are preferred. In the event that living specimens cannot be obtained and to insure that we have sampled as much of the genetic diversity in this group as possible, we will seek to obtain DNA from herbarium specimens. Of the 100-150 named species worldwide we will gather approximately 70 species representing those with expansive and restrictive ranges. Especially intensive sampling will be conducted from the Australasian regions and India where endemism and species diversity are particularly great. Specimens will be cultured at Southern Illinois University and the University of Akron and voucher specimens will be deposited at CANB with duplicates donated to the herbarium at the

Missouri Botanical Garden (MBOT). Collection data, including sample photos will be documented on a web site constructed for his project (<http://www.uakron.edu/biology/hornworts/hornworts.html>).

Figure 4. Hornwort species targeted for this study. The following subgenera (a-e) were recognized as genera by: (a) Hassel de Menendez, 1988 (41), (b) Bharadwaj, 1971 (63), (c) Piipo, 1993 (64), (d) Hassel de Menendez 1986 (65), and (e,f) Haegawa (1994 (43). Locations: W=Worldwide E=Europe A=Africa SEA=Southeast Asia CA=Central America SA=South America P=Paletropica NZ=New Zealand I=India PB=Pamboreal Aust=Australia NA=North America J=Japan WI=West Indies. Bold are species we have samples prepared for morphological and molecular analyses. Asterisks denote species for which we have more than one sample.

Anthoceros L.	Location	Notothyllas Sull.	Location
<i>Anthoceros</i>		<i>Notothyllas</i>	
A. punctatus L.*	W	N. orbicularis (Schwein.) Sull*	PB
A. agrestis Paton *	E	<i>N. breutelii</i> (Gott.) Gott.	WI,NA
<i>A. expansus</i> Steph.	A	<i>N. javanicus</i> Sande Lac.) Gott.	SEA
<i>A. fusiformis</i> Aust.	NA,J	<i>N. temperata</i> Haseg.	EA
<i>A. pinnatus</i> Steph.	A	<i>N. indica</i> Kash.	I
<i>A. cavernosus</i> Steph.	WI	<i>N. galapagensis</i> Howe	CA
<i>A. erectus</i> Kash.	I	<i>Depressisporae</i>	
<i>A. glandulosus</i> L. & L.	I	<i>N. depressisporae</i> Haseg.	J
<i>A. angustus</i> Steph.	SEA	<i>N. dissecta</i> Steph.	I,CA
A. lamuniferus Steph.	NZ		
<i>A. myriandroecium</i> Steph.	A		
<i>A. tuberculatas</i> Lehm.	CA		
<i>A. hispidus</i> Steph.	SA		
<i>A. simulans</i> Howe	CA		
<i>Sphaerosporoceros</i>		Phaeoceros Prosk.	
<i>A. adscendens</i> L. & L.	NA	<i>Phaeoceros</i>	
<i>A. macounii</i> Howe	NA	P. laevis (L.) Prosk*	W
<i>A. capricornii</i> Cargill & Scott	Aust	P. carolinianus (Michaux) Prosk.*	W
<i>Folioceros</i>		P. oregonus (Aust.) Hassel	W
<i>F. fuciformis</i> (Mont.) Bharadw.	Aust.	P. morhii (Aust.) Hassel	NA
<i>F. appendiculatas</i> (Steph.) Haseg.	SEA	<i>P. pearsonii</i> (Howe) Prosk.	NA
<i>F. amboinensis</i> (Schiffn.) Piippo	SEA	<i>P. parvulus</i> (Schiffn.) Haseg.	SEA
<i>F. dilatatus</i> (Steph.) comb. nov.	A	<i>P. gemmifer</i> (Horik.) Haseg.	J
<i>F. apiahynus</i> (Steph.) Hassel	SA	<i>P. hallii</i> (Aust.) Prosk.	NA
<i>F. indicus</i> Bharad.	I	P. coriaceus (Steph.) comb. nov.	NZ
		P. hirticalyx (Steph.) Haseg.	NZ
		<i>P. fimbriatus</i> (Gott.) Gradst.	SA
		<i>P. foveatus</i> Haseg.	SEA
		<i>P. bulbiculosus</i> (Brotero) Prosk.	E,A
		<i>Mesoceros</i>	
		<i>M. mesophoras</i> Piippo	SEA
		<i>M. porcatus</i> Piippo	SEA
		<i>Leiosporoceros</i>	
		<i>P. dussii</i> (Steph.) Hassel	WI
		<i>Hattorioceros</i>	
		<i>P. striatisporus</i> (Haseg.) Haseg.	I
Dendroceros Nees		Megaceros Camp.	
<i>Dendroceros</i>		<i>Megaceros</i>	
D. crispus (Sw.) Nees *	Aust	M. denticulatas (Lehm.) Steph.*	NZ
D. japonicus Steph.	J	<i>M. leptohymeius</i>	
<i>D. acutilobus</i> Steph.	SEA	(Hook. f. & Taylor.) Steph	NZ
<i>D. javanicus</i> (Nees) Nees	SEA	<i>M. arachnoideus</i> (Steph.) Steph.	NZ
D. tubercularis Hatt.	J	M. pellucidus	
<i>D. borbonicus</i> Steph.	SEA	(Colenso) E.A.Hodgs.	NZ
D. ganulatus Mitt.	SEA	M. cyanus Nom. Herb.*	Aust
D. canaliculatus Pagan*	CA	M. vincentianus (L.&L.) Steph.*	CA
<i>D. vallidus</i> Steph.	NZ	M. aenigmaticus Schust.*	NA
<i>Apoceros</i>		M. flagellaris (Mitt.) Steph.*	P
<i>D. difficilis</i> Steph.	SEA	<i>M. fusiformis</i>	J
<i>D. pedunculatus</i> Steph.	SEA	<i>Nothoceros</i>	
<i>D. cavernosus</i> Haseg.	SEA	M. endiviaefolius Mont.	SA
		M. giganteus (L.&L.) Haseg.	NZ

B. Molecular phylogenetic methods: Given the potential antiquity of the hornworts (possibly 400 -700 my, 61), it is likely that the degree of genetic divergence between species will vary greatly. Hence, no single gene sequence will provide an appropriate number of informative sites to resolve hornwort relationships at multiple hierarchical levels. To address this problem, we will employ a strategy of collecting multiple molecular data sets. These data will be partitioned so that individual analyses may be completed while allowing extensive tests of congruence between a variety of independent data partitions. The molecular phylogenetic methodologies we will employ are described below.

1. DNA extraction. Extraction of total DNAs will be done on fresh material when available. The DNeasy Plant kit (Qiagen) has proven to give high quality DNAs from hornworts where other methods

have failed. Furthermore, this kit has been used successfully in J. Duff's lab for DNA extraction and subsequent PCR from herbarium specimens of hornworts up to 25 years old.

2. Selection of gene sequences and data partitions. Several candidate genes that could contribute to resolving relationships within hornworts have been examined. These include the small and large subunit nuclear ribosomal DNA genes (18S and 26S), nuclear internal transcribed spacer (ITS), *rbcL* (chloroplast), and *nad5*, *nad2* and small subunit rDNA (19S) genes from the mitochondrial genome. Of these gene regions, we have chosen sequences based on their usefulness for assessing phylogenetic relationships at different taxonomic levels. To allow for multiple outgroup options and for comparison of relative rates of sequence evolution, we selected genes with sufficient available sequences from related groups. The lack of introns in the *nad5* gene (mtDNA) in hornworts (36, Duff unpublished data) precluded its use on different taxonomic levels but recent examination of *nad2* has suggested that this gene has phylogenetic utility among bryophytes (66) and does retain two large introns in the hornworts. Our preliminary analysis suggests the first intron is present in all genera. We examined ca. 2100 bp of 5' end of this gene including a 1400 bp intron from six hornworts. Intron sequences from three samples of *Phaeoceros laevis* s. lat. can be readily aligned while exhibiting ca. 2% sequence divergence (comparable to the ITS). Alignments of the intron sequences among genera resulted in large portions that could be aligned although sequence divergence may preclude their use in analyses of relationships above the generic level. Hornwort *rbcL* sequences have been shown to exhibit high levels of RNA editing (67) and *nad2* sequences are also subject to RNA editing (66). Although it has been suggested (68), and our preliminary analyses confirm, that editing will have little effect on phylogeny reconstruction, we intend to examine the effects of those edited sites by way of elimination of known or postulated edited sites. Figure 5 shows the target sequences and their anticipated resolution based on preliminary data. This should be considered a minimal set of data partitions as a variety of weighting schemes will be employed.

Figure 5. Data partitions to be analyzed. * denotes combinations of data partitions that will be examined if criteria outlined in the combined conditional approach are met. (a) The *rbcL* data partition will involve several sub-partitions including alternative weighting of third base positions and the removal of known or postulated RNA editing sites. Additional analyses would include the use of different outgroup taxa. Relevant literature and preliminary data suggest the following relationships among the sequences with respect to substitution rates in the target sequences: **ITS>*nad2* intron>*rbcL*>18S>*nad2* exon**

Data Partition	Location	Aligned sequence	Resolution
18S SSU rDNA	Nucleus	1720 bp	Generic and above
ITS (Internal Transcribed Spacer)	Nucleus	950 bp including 5.8S	Generic and below
<i>rbcL</i> ^a	Chloroplast	1400 bp	Generic and below
<i>nad2</i> exon	Mitochondrion	700 bp	Generic and below
<i>nad2</i> intron	Mitochondrion	1400 bp	Generic and below
*18S+ <i>rbcL</i> + <i>nad2</i> exon	N,CP,MT	3820 bp	Generic and above
* <i>rbcL</i> +ITS+ <i>nad2</i> intron	N,CP,MT	3750 bp	Generic and below

3. PCR and sequencing. Amplification of gene sequences from hornworts has been problematic in the past. For the present study, we have developed and tested new hornwort specific primers for amplifying each of the target sequences. These primers have proven effective at selecting the hornwort sequence from total DNA extractions from field collections or herbarium specimens that may contain cyanobacteria or algal contaminants. In addition, we have developed sets of primers that allow for complete sequencing of each gene in both directions. Other aspects of gene amplification, cloning of products if necessary, product purification, and DNA sequencing reactions will follow standard protocols. Sequencing is accomplished using an ABI 310 automated sequencer available in the Department of Biology at the University of Akron. In total, we anticipate generating at least 70 new 18S rDNA + ITS [1720 + 950 bp] *rbcL* [1400 bp] and partial *nad2* [2100 bp] sequences for a total of 432 kb. Additional ITS and *nad2* intron sequences will be obtained for multiple samples of *Phaeoceros laevis* s. lat.

4. Outgroup selection. Given the conflicting hypotheses on the position of hornworts among land plants (see Background), we will analyze a number of charophycean green algae (69), liverworts and mosses as outgroup members. Heretofore, analysis of the *rbcL* and 18S sequences show that any combination of outgroup taxa has no effect on the topology of the hornwort clade.

5. Sequence alignment. Alignment of all sequences will be done visually employing computer programs SeqApp (70) and GenDoc (71). Dr. Duff has extensive experience aligning these genes in the context of basal land plants (32,72). Preliminary results show that ITS and *nad2* intron sequences are not likely to be aligned among all genera along their entire length due primarily to multiplicity of indels, but may be readily aligned among species in the same genus. If homology cannot be determined for portions of any sequence, these portions will be excluded from subsequent analyses. Gap-coding as described by Simmons (73,74) will be explored to gain greater utility from these intervening sequences. In the case of the *nad2* intron and ITS, limited portions of such sequences may be used for generic and familial analyses while larger portions may be utilized for analyses within genera and species.

Presently there are only 10 relevant hornwort sequences deposited in Genbank. For the **majority** of these sequences **we suspect error** in either the sequence itself (eg. the *Notothylas* 18S sequence appears to contain multiple errors, primarily indels, based on secondary structure reconstructions) or species identifications. Hence, we propose to use only hornwort sequences generated as part of the proposed research for our molecular phylogenetic analyses. We will deposit all sequences into Genbank as well as provide alignments of all data sets on our project web site hosted at the University of Akron: (<http://www.uakron.edu/biology/hornworts/hornworts.html>).

C. Morphological/ ultrastructure studies: The second major component of this project involves the accumulation and interpretation of morphological data. Critical to all future morphological inquiry is an accurate detailing of developmental and ultrastructural features. Our studies are designed to provide reliable contemporary morphological data that will correct errors, clarify ambiguity and augment information available in the literature, hence original data will be collected for all taxa investigated. These data will consist of 1) gross anatomical and morphological features that can be derived from light microscope observation of living and dried material and 2) ultrastructural, developmental and physiological data that require tissue preparation and observation in the TEM, SEM, fluorescence or light microscope. In order to focus our morphological studies, we have identified characters that are specific to hornworts and that are designed to approach questions relating to generic boundaries and relationships among anthocerototes. A list of 67 characters (available on the project web site) was compiled from previous cladistic analyses (41-43) and more recent data generated by K. Renzaglia and collaborators (7,13,75). These characters will serve as a **baseline** for data collection and will be modified substantially as characters are evaluated and character states defined. It is anticipated that the acquisition of crucial ultrastructural and morphogenetic characters will significantly enhance resolution of morphological data.

In addition to accumulating general information on plant morphology, we will conduct intensive studies of the key structural features and processes described below.

1. Chloroplasts. The ultrastructure of plastids (especially chloroplasts) is a key feature in circumscription of hornwort genera (46,76). In all but *Megaceros*, the chloroplast is typically solitary (one per cell) and contains a central pyrenoid and channel thylakoids. However, multiple chloroplasts that lack pyrenoids are also seen in species of *Notothylas* (77) and *Phaeoceros* (see below) and thus are not restricted to *Megaceros*. Pyrenoid substructure and location of plastoglobuli provide further taxonomic characters that define genera and species. It is generally agreed that pyrenoids were lost and plastid numbers increased in *Megaceros* (78).

2. Stomata. Although stomata are considered to be a critical morphological adaptation to life on land and the existence of these structures is viewed as a primary link between hornworts, mosses and tracheophytes (49-51), no modern studies have been published on hornwort stomata. We propose to examine the occurrence, development and structure of stomata in all taxa by examination in the SEM, TEM and fluorescence microscope. Physiological studies in progress in our lab (28), indicate that

hornwort stomata do not open and close and thus do not function as do those of tracheophytes. In addition to continuing studies of stomatal function, we propose to detail variations in structure with the goal of evaluating stomatal evolution within hornworts. The structure and ontogeny of sporophytic stomata will be compared with those of the stoma-like clefts found in the gametophyte of hornworts. Although we hypothesize that these structures are not homologous, others have referred to the mucilage clefts in the gametophyte as true stomata and this issue is of particular importance when comparisons are made with early fossil land plants that possess gametophytic stomata (49,56,79).

3. Sporogenesis, spores and pseudoelaters. Spore shape and wall ornamentation, tetrad organization and pseudoelater characters are widely used in classification of hornwort genera and species (41-43,77). To ascertain variations in spore and pseudoelater differentiation, we will conduct cytomorphogenetic investigations of sporogenesis in all taxa. Non-synchronized spore development in hornworts is viewed as a synapomorphy (51). *Notothylas* is the exception among anthocerotales in that sporogenesis appears to be synchronized (40-43). Our studies of sporogenesis in *Notothylas* suggest that the duration of meristem activity is dramatically shortened and spore development is not truly synchronized but rather abbreviated in duration. In regards to spore wall ornamentation, little is known of ultrastructural details (80). To test the axiom that wall ornamentation is an informative diagnostic feature of hornwort taxa, we will observe surface spore wall features with the SEM and correlate these with details of internal organization and development as gleaned from the TEM. Elongated single-celled pseudoelaters of *Dendroceros* and *Megaceros* have spiraled wall thickenings while variations in pseudoelater shape and wall thickenings are diagnostic of species (41). Likewise, fluorescence patterns of pseudoelaters (81) are considered taxonomically informative and these will be recorded for all species.

4. Placenta. The zone of contact between sporophyte and gametophyte provides a number of characters of potential phylogenetic value. Hornworts are among the few archegoniates with transfer cells restricted to the gametophyte side, a feature shared with *Coleochaete* (82-84). More precise ultrastructural traits such as the occurrence of protein crystals between generations and the structure of chloroplasts specific to each generation have been shown to be diagnostic of hornwort taxa (84).

Newly acquired developmental and ultrastructural data when evaluated based on results of phylogenetic analyses will provide a more thorough understanding of biocomplexity within the hornworts and will enable elucidation of historical modifications at the cell, tissue and organ levels. As an example, below we highlight two **new interpretations** of relative timing and directionality of structural evolution within hornworts **based on our preliminary analysis of *rbcL* sequences**. As our working hypothesis, this tree presents novel relationships that are highly intuitive when morphological characters are reevaluated, thus providing interesting new insights into character evolution. **1) Stomatal evolution:** Analysis of *rbcL* sequences (Figure 2) suggests that stomata are plesiomorphic (present in *Anthoceros* and *Phaeoceros*) and that they have been lost independently at least three times during hornwort evolution (*Notothylas*, *Megaceros* and *Dendroceros*). Corroborating this interpretation of stomata loss is the existence of malformed and incomplete stomata in *Phaeoceros coriaceus* (Renzaglia, unpublished), a potentially basal taxon in the *Megaceros* clade. If with increased taxon sampling and phylogenetic analyses of additional data sets, the affinity between *P. coriaceus* and the *Megaceros* clade is strengthened, ultrastructural examination of these stomata will elucidate structural changes that have accompanied stomatal loss in the hornwort lineage. **2) Chloroplast evolution:** Although the sporophyte in *P. hirticalyx* and *P. coriaceus* shows more similarity with *Phaeoceros* (including 16-celled columella, non-spiralled pseudoelaters, stomata), features of the chloroplast in these two species are diagnostic of *Megaceros*. As in *Megaceros*, these chloroplasts lack a pyrenoid and often number more than one per cell. This supports the interpretation that within *Megaceros*, pyrenoid loss and increase in chloroplast number occurred prior to evolutionary changes in the sporophyte. Moreover, these data suggest that contrary to current opinion, features of the chloroplasts are potentially more informative in defining genera than sporophytic characteristics.

D. Phylogenetic Inference. The assembly of a data set that includes multiple DNA loci and morphological characters designed to examine questions at different taxonomic levels necessitates a multifaceted approach to data analyses. In general we will be following the conditional combination approach as outlined in Figure 3 (61,85,86). Both morphological and molecular data sets will be analyzed separately. Tree constructing algorithms including distance, maximum parsimony and maximum likelihood will be employed as implemented in the software package PAUP* 4.0 (60). Prior to combining data sets, congruence among the data sets will be assessed using the partition-homogeneity test (ILD of 87) and the Templeton's Significantly Less Parsimonious Test (SLP_T; 61,88). In the unlikely event that no incongruencies are noted between the resultant trees, the data sets will be combined and a second round of analyses conducted. If significant incongruencies are found, data sets will be reexamined in light of possible effects of transition/transversion ratios, base composition bias, variations in evolutionary rates among lineages and among sites to identify sources of heterogeneity. If significant heterogeneity still exists and cannot be addressed by differential weighting schemes, deletion of problematic taxa or other methods, then the data sets in question will not be combined. Combined data sets will be further analyzed with methods used for individual data partitions. Confidence levels for various clades will be determined using bootstrap (89) and decay analyses (90) implemented with AUTODECAY (91). PAUP* will also be used to constrain trees to other topologies (eg., topologies resulting from data partitions not included in the combined analyses due to significant heterogeneity between data sets) thus allowing determination of the costs (in steps) relative to the most-parsimonious solution. Morphological character evolution will be examined by employing the program MacClade (92).

Approach to Analysis of Morphological Data: The fact that morphological characters have produced highly conflicting phylogenetic hypotheses in the past indicates that some characters utilized in taxonomic studies of hornworts are not appropriate for phylogenetic reconstruction. At this time we cannot say whether these incongruencies are the result of real convergent and parallel evolution of particular features, the result of our ignorance of character homology, or both. Hence, we will examine key ultrastructural, functional and developmental features, which will enable us to better assess the homology of morphological characters. We will initially develop four combinations of the data (see Figure 3) that will allow comparisons of characters from different life stages (gametophyte vs. sporophyte) and tissue types (vegetative vs. reproductive). With our revised morphological data sets, phylogenetic analyses will be conducted and the resultant trees compared to the results obtained from molecular data. The advantage of the conditional combination approach we are using is that the close examination of sources of incongruence among data sets will serve as a feedback mechanism suggesting problematic characters that we will investigate further.

E. Biogeography and biodiversity: hornworts in spatial and temporal perspective.

Our phylogenetic analyses will provide a framework wherein we can investigate biogeographic patterns in the hornworts. Similar to the mosses and liverworts, hornwort species exhibit unusual distribution patterns. Many species are described as cosmopolitan while others show extremely limited ranges (39). Our taxon sampling includes species that represent both of these extremes: we will conduct intensive sampling in New Zealand and India, areas that exhibit high levels of endemism (76), as well as acquire multiple samples from widely dispersed geographic localities of cosmopolitan species. Because of the presence of both pockets of high endemism and widespread taxa, hornworts present opportunities to investigate many fundamental issues relating to biogeography and speciation. Presently, we seek to identify general worldwide biogeographic patterns, examine specific biogeographic hypotheses involving the Australasian landmasses, and explore species limits in the controversial cosmopolitan species *Phaeoceros laevis*. This approach of examining biogeographic patterns at multiple levels will allow us to address questions such as: Are regions of high endemism as determined by morphology also characterized by higher levels of genetic diversity? Do species that exhibit cosmopolitan distributions show evidence of widespread gene flow thus reflecting extensive long-range dispersal or might there be cryptic species that have gone undiscovered? Is the high diversity of New Zealand hornworts ancient or recent? Do

biogeographic patterns exhibited by hornworts reflect patterns observed in other plants?

1. Worldwide patterns of distribution: To gain a greater appreciation for diversification, we propose to conduct dispersal-vicariance analyses (93) of hornworts collected from all continents except Antarctica. Owing to the antiquity of this group, one might presume that patterns of biogeographical distribution among hornworts would reflect vicariance due to the break-up of supercontinent Pangaea and later Gondwanaland and Laurasia. The sequence divergence observed in *rbcL* and 18S sequences suggests that many of the modern genera diversified at or before the breakup of the supercontinent Pangaea (>250 mya). By comparison, estimations of divergence times in the Lycopodiaceae using *rbcL* sequences suggest that the extant genera are over 250 mya (94). Assuming similar rates of sequence evolution in hornworts, the observed sequence divergence between genera would suggest they are of similar age. Unfortunately, direct evaluation of divergence times among hornwort genera is not possible because of the difficulty in establishing minimum and maximum divergence times from paleontological evidence as is possible for the lycopods. Nevertheless, the data collected thus far is not inconsistent with deep divergences among extant genera. By examining species found on all landmasses, we will be able to test (see methods below) whether patterns of diversity reflect vicariant events. This hypothesis will be tested with the data gathered for phylogenetic analyses.

2. Australasian patterns of distribution: On a more limited geographic scale we can provide a more rigorous test of biogeographic and biodiversity patterns among hornworts. We have already obtained material from a large number of taxa from the Australasian landmasses. The Australasian landmasses consist of Australia (including Lord Howe Island, Norfolk Island and Macquarie Island), New Zealand (including Auckland Islands, Campbell Island, Chatham Island, Kermadec Islands and Bounty Islands), New Caledonia (including Loyalty Islands), Vanuatu (formerly New Hebrides), Solomon Islands (including Santa Cruz Islands), Bougainville, New Britain, and Papua New Guinea (95). These locations, most of which have hornworts, have been of particular interest to biogeographers because of the generally accepted patterns of continental evolution of the region (96,97), although alternative vicariance models have been obtained from molecular phylogenetic studies (98,99). A variety of studies (98,100-105) focusing on this region have implicated both vicariance events and long-distance dispersal in explaining the current biogeographic patterns of the family. But, despite the presence of a generally accepted geological vicariance model for the austral region (NZ(SA(AUST)), recent studies applying molecular phylogenetic data to biogeographic questions in taxa distributed over this region have recognized dispersal as being the causal agent of the observed patterns of biotic distributions (106-109). In addition to Australia, only Tasmania has yielded definitive evidence of an ancient Gondwana flora reflecting a vicariant biogeographic pattern (110). Some uncertainties remain with respect to the geological histories of several locations included in most biogeographic studies of the area. These include whether New Caledonia, New Zealand, New Guinea, and Chatham Islands have remained emergent (and thus available for colonization) since their initial isolation from their continental relatives (111). If they have, then vicariant events may have played a role in the current distribution of the flora and fauna of these places.

The presence of multiple species from several genera on each of the Australasian landmasses presents an opportunity to examine and compare biogeographical patterns of hornworts with those of other paleoaustral plant taxa for which molecular data have been obtained. This will be accomplished by examining members of *Megaceros* which are particularly well represented on all of the Australasian landmasses of interest and thus our analyses will focus on deciphering the patterns of diversification of this genus. *Dendroceros* and, to a lesser extent, *Phaeoceros* will also be investigated. For each of these groups of taxa various biogeographical analyses will be applied to the phylogenetic trees. In particular we will conduct dispersal-vicariance analyses using the computer package DIVA as described by Ronquist (93,112). A second method, weighted ancestral areas analysis (113) will be used and the results of these two analyses compared for congruence using the model of *Nothofagus* (99,10)

3. Cryptic speciation and hornwort biogeography: As noted previously, in contrast to many groups of organisms, the bryophytes generally exhibit broad geographical distributions that span multiple continents.

The lack of morphological variation across wide ranges historically has not been attributed to widespread gene flow but rather to the view that bryophytes are ‘unmoving, unchanging sphinxes of the past’ (114). More recent molecular work has begun to probe the extent of genetic diversity among bryophytes. These studies of mosses and liverworts with widespread occurrence over several continents have demonstrated surprising genetic complexity within species that exhibit negligible morphological differentiation (45,115-117). The patterns of this genetic diversity frequently do not follow geographical patterns and many cryptic species of mosses are strewn across several continents. Indeed, populations on a single continent may show greater genetic variability among themselves than between populations of a different continent (118). The *Phaeoceros laevis* species complex with its many named segregates (see Introduction) is an ideal taxon for studies of speciation and genetic diversity in hornworts. Indeed our preliminary data, using *rbcL* and *nad2* sequences, reveal considerable genetic heterogeneity both within and between continents. Using samples from different continents we will attempt to answer the following questions: Is the present worldwide genetic landscape of *P. laevis* the result of recurrent gene flow consequent on persistent long distance dispersal or are populations on different land masses relicts of ancient dispersal patterns? Is *P. laevis* less diverse genetically in areas of the northern hemisphere that were recolonized since the last ice age and particularly after the spread of agriculture and the accompanying deforestation of lands? Is *P. carolinianus* derived from *P. laevis* or vice versa and thus can the origin (or multiple origins) of monoecism and dioecism be ascertained in these taxa? Are the overall patterns of genetic diversity in *P. laevis* the same as those in mosses and hepatics.

VI. Timetable and Future Goals

The large number of collections, morphological observations and DNA sequences makes the collaboration of the two PIs in this project vital to the success of the program. In general, molecular sequence data will be generated in the lab of J. Duff (U. Akron) while morphological investigations will take place in K. Renzaglia’s lab (SIU). Morphological investigations will be ongoing and the 18S and *rbcL* genes will be a first priority in line with our initial goal to define broad relationships among the hornworts. An emphasis in Year 1 will be to acquire most of the specimens from around the world and process them for analyses. A large portion of this work will involve collection from the Australasian region by Chris Cargill and Jeff Duckett with additional support from K. Renzaglia who will travel to Australia to assist in collection and preparation of materials. Year 2 will see a continuation of sample collection and processing and the initiation of sequencing of ITS and *nad2* genes. At this time, data sets for the 18S, *rbcL* and morphological data for a large number of taxa will be complete, allowing preliminary analyses to be conducted, which will inform decisions on additional sampling and/or morphological character examinations. In Year 3 we expect to complete sequencing of the ITS and *nad2* sequences from those samples for which infrageneric and infraspecific relationships are of interest. We expect that early publications will result from *rbcL* sequences and morphological investigations. Subsequent papers will follow in areas outlined in Figure 3.

The described research will enable us to achieve our goals while providing a qualitative step toward establishing a **long term research program** aimed at 1) continued evaluation of hypotheses of adaptive evolution within the group and placing morphological innovations diagnostic of hornworts into the broader context of land plant evolution, 2) providing an exhaustive taxonomic treatment of the entire group worldwide, 3) elucidating the placement of hornworts within the global phylogeny of plants, 4) placing biogeographical patterns in specific regions of the world observed in hornworts in context with other bryophyte and vascular plants, and 5) exploring bryophyte morphological and genetic complexity, including the existence of cryptic speciation, through the examination of additional cosmopolitan species.

VII. Intellectual Merit and Educational Impact

Our collaborative project involves input from scientists around the world. Distribution patterns and information on biodiversity will be accumulated for the first time for hornworts collected from multiple geographic regions by an international assemblage of experts on these plants. Our three focus

areas (molecular, morphological and biogeographical) are centered around questions pertinent to both hornwort systematics and more global issues relating to biological diversification and biocomplexity. The hornworts are the only major group of land plants for which a detailed molecular phylogeny is lacking. Existing classification schemes based on morphology are in great conflict; there is no agreement on species, generic or familial limits, and the interrelationships among taxa remain obscure. The lack of a consensus taxonomic treatment is indicative of the shortage of biological information on these plants and the shortcoming of classical approaches in adequately resolving affinities within the group. Consequently, it is not possible to identify a single basal exemplar taxon. Our proposed research will remedy these inadequacies. Morphological studies, coupled with molecular analyses, will provide the necessary data to define taxonomic boundaries and enable accurate estimates of taxonomic diversity. The construction of a worldwide phylogeny of the hornworts will serve as the foundation for a new classification system for the group, and when evaluated in conjunction with distribution areas of the hornwort species will enable a first reconstruction of the biogeographic history of the group.

Rare among the extant groups of eukaryotes, the hornworts represent a living historical record of as many as 500 (-700) million years of evolutionary adaptation. Our proposed developmental and morphological studies on a wide range of anthocerotales will provide valuable data on biocomplexity at the cellular, tissue and organismal levels. The available studies, e.g., on the cell cycle and sperm structure, have identified cellular complexity that is unique to these plants. Similarly, this relatively obscure group of small plants exhibits general architectural features of the gametophyte and sporophyte, as well as strategies for survival on dry land that are unparalleled in other eukaryotes. The comparative morphological investigations of hornworts that we have outlined will provide critical data in evaluating hypotheses of adaptive evolution in land plants.

The results of previous NSF grants regenerated 26 published manuscripts since 1995. We expect the currently proposed work to result in numerous manuscripts in many international journals as reflecting the interests of our large group of collaborators. To further augment the dissemination and exchange of data among other researchers and raise awareness of hornwort biology among the public we have established a website dedicated to the project (<http://www.uakron.edu/biology/hornworts/hornworts.html>).

The proposed research will have an impact far beyond the value of the data collected. Both PIs work in research rich environments where significant importance is placed on one-on-one interactions with both undergraduate and graduate students. Our approach to training students is to provide a supportive, stimulating environment that stresses teamwork and provides a strong mentoring network (119-121). K. Renzaglia has a successful record of providing supportive research and professional opportunities for students. Her long-standing commitment to enhancing undergraduate education by providing meaningful, individualized research experience enabled her to attain her present position as director of the Undergraduate Research Program at SIUC. In this capacity and as an active member of the Center for Systematic Biology, K. Renzaglia is in an ideal position to provide a broad base of experiences for training students in plant systematics. She will encourage her students to attend the numerous research workshops, seminars, discussion and training sessions available at SIUC and she will facilitate their professional development by providing a supportive mentoring network and a wide variety of professional opportunities. As a new researcher (since Fall, 1999) at a master's granting institution in Ohio, J. Duff has already trained seven undergraduates and one graduate student in his lab. Because he is the only molecular systematist at the University of Akron and among only four in northern Ohio, his laboratory and course offerings attract students interested in a wide range of organisms and provide the foundation for students to explore biosystematics and conservation biology. In 2001, J. Duff received the Outstanding Teaching Award from the Honors College in recognition of his commitment to excellence in teaching. He is also a co-PI/senior personnel on three educational grant proposals aimed at developing inquiry-based laboratories and providing effective mentoring networks among undergraduates, graduates, and high school students.