DEAN'S ADVISORY COMMITTEE
STUDENT VENTURE GRANT APPLICATION

Please read all instructions and regulations on the reverse side of this sheet prior to the completion of this form. **The original plus 6 copies of your proposal are due in the Dean's Office on the 2nd Friday of the Block by 1:00 p.m.**

DATE SUBMITTED September 20th, 2004

NAME [redacted] **CLASS** Snr. **WORNER BOX** [redacted] **EXT.** ____

ID # [redacted] **HOMETOWN (Not Address)** Ashland, OR

NAME ____ **CLASS** ____ **WORNER BOX** ____ **EXT.** ____

ID # ____ **HOMETOWN (Not Address)** ____

NAME OF ORGANIZATION ____

BRIEF DESCRIPTION OF INTENDED USE OF FUNDS
Genetic Analysis of Variance among Scaphosepalum suertifolium specimens → Funds requested for orchid plants, PCR materials, and sequencing costs.

PROPOSED DATE/BLOCK OF USE Continuous during 2004-2005 academic year

NAME OF FACULTY SPONSOR Dr. Mark Wilson

HAVE YOU BEEN THE RECIPIENT OF A PREVIOUS VENTURE GRANT Yes (No)

IF SO, WHAT AMOUNT? ____ **WHEN?** ____ **REPORT SUBMITTED?** ____

TOTAL AMOUNT OF VENTURE FUNDS NOW REQUESTED ____

ARE YOU SEEKING OTHER FUNDING FOR THIS PROPOSAL? Yes (No)

IF YES, WHAT IS THE SOURCE? ____

If this proposal is approved, I understand that it is my responsibility to notify the Dean's Office immediately if I do not pursue my project as proposed to the Dean's Advisory Committee. I further understand that all funds are to be used according to the proposal as submitted and approved by the Dean's Advisory Committee. Any changes to an approved project must be submitted to the Chair of the Committee for approval. **Please note: the IRS requires that we report Venture Funds as taxable income.**

SIGNATURE [redacted] **DATE** 9/20/04

**********DO NOT WRITE BELOW THIS LINE**********

DATE ____ **ACTION TAKEN** ____ **DATE** ____

BLOCKS TO BE USED ____ **REPORT SUBMITTED** ____
COMMENTS AND SUGGESTIONS:

[redacted]
9/20/2004

Venture Grant Proposal: Analysis of the Genetic Variability of the Ecuadorian Orchid *Scaphosepalum swertifolium*

The orchid family is the largest plant family and incredibly diverse in areas of the South American Neotropics such as Ecuador. Unfortunately nations like Ecuador are experiencing increasing population and development pressures which imperil large numbers of their endemic plant species, many them orchids. It is estimated that there are 5,000 species of orchids in Ecuador alone. The exact number of species is unclear for a variety of reasons. Part of the reasons for this gap in scientific understanding is due to the lack of competent botanists exploring Ecuador's remaining forest. The other part of the information gap is due to a lack of genetic and systematics resources. Modern techniques with genetics allow plant scientists to investigate the inherited differences between visually or geographically differentiated plants in order to define the evolutionary difference between them. This type of research allows botanists to more clearly define species or subspecies, which is key to understanding the evolution of orchids in this part of the world. If conservationists do not know that an orchid species is a species, rather than a variety, it will not be protected.

Genetic techniques available at Colorado College make it possible to detect the differences in species through a method known as PCR (polymorphism chain reaction). With PCR it is possible to amplify the genetic region of interest with DNA extracted in a separate process. Finally, the resulting sequence for a species or variety can be sent away to be analyzed at a separate lab. The steps that can be accomplished at Colorado College are time consuming as it is not necessarily obvious what region of the genome will show differences in species. Past research in orchids has been successful using ribosomal DNA internal transcribed spacers (ITS) regions, which widely vary between species because they do not code for ribosome genes, and therefore may vary without harming the organism's survival. However, the differences found in previous studies were not necessarily at the fine scale (subspecies) that might exist in varieties of *Scaphosepalum swertifolium*. We might then have to work with DNA from other parts of the plant cell, such as plastid or mitochondrial DNA, or even with an entirely different technique than PCR. When researching a group of species that are relatively unknown from the genetic perspective, initial difficulties are expected.

This species is proposed as a research subject because it is an Ecuadorian orchid in the group of interest, the subtribe Pleurothallidinae, it appears to be highly variable in appearance (indicating possible genetic differences), and many varieties are available in the United States. It was initially suggested as a research subject by Dr. Andrew Hirtz, one of the foremost authorities on orchids of Ecuador, based on his personal experience with the large geographic range and considerable morphological differences in plants identified as *Scaphosepalum swertifolium*. Defining the genetic variation of this species is expected to help in resolving some of the questions regarding morphologically varying species "complexes" in other Pleurothallidinae genera, many of which are rare or endangered.

I expect that this project will be valuable not only personally, but also for the college community, and possibly even other scientists working with Pleurothallidinae genetics and systematics. Ever since hearing of Andy Shephard's phenomenal work with the *Teagueia* genus around Baños, Ecuador I have been intrigued by the possibilities of orchid speciation in the Neotropical cloud forest environments. I then went on to work with Lou Jost, Andy Shephard's advisor, when I studied abroad in Ecuador. I ended up collecting more than a 100 species of orchids, most of them Pleurothallidinae species, in a square kilometer area close to Baños. Now I find myself fascinated by the puzzles presented by this group and frustrated by the lack of information on many species. Why are some species geographically restricted, like the *Teagueias*, whereas others appear to exist in fragmented populations from Colombia to Peru? What makes a species a species? This research project offers the chance to use genetics to address these questions. It will also allow me to connect an amazing study abroad field botany experience with cutting edge plants genetics research. Ultimately, this experience with plant genetic methods may prove invaluable in my pursuit of graduate school and research opportunities.

Orchid genetics research at Colorado College offers a variety of benefits for the college community. Though this research will probably not lead to a thesis, the results may be presented at Biology Day, a spring biology department event, for the enrichment of the campus community. The techniques used, and their relative success, will be documented so that others may replicate the process or improve on it. This project may eventually lead to a great number of potential Colorado College student research projects involving Ecuadorian orchids or even other Ecuadorian plant families. In the last few years some CC students have investigated aspects of orchid genetics or Ecuadorian orchid diversity. Notably, Andy Shephard discovered dozens of species in the Ecuadorian Andes during his Venture Grant funded thesis project. Others have sequenced orchid species in the college greenhouse. This avenue of research will probably continue in some capacity after my departure. Dr. Mark Wilson is developing further research possibilities with Ecuadorian Orchidaceae, and this research project will form the building blocks for countless other projects by exploring methods for analyzing genetic differences in the Pleurothallidinae group. Hopefully the unique relationship developing between Ecuadorian botanists and Colorado College will lead to further findings, the sharing of resources and knowledge, and most importantly, the information necessary to help save the incredibly diverse flora of Ecuador.

Proposed Budget

Plant material:

- plants of *Scaphosepalum swertifolium* from each source, the most important of which is Ecuagenera (Gualaceo, Ecuador)

(i) Ecuagenera:

S. swertifolium IC	*$15
S. swertifolium 1	$15
S. swertifolium 2	$15
S. swertifolium 3	$15
S. swertifolium big	$15

**cheaper because imported plants must be bare root*

(ii)	Santa Barbara Orchid Estate		
		S. swertifolium (inc. S&H)	$25
(iii)	SLO Gardens:	SLO1225	$20
		SLO1226	$20
		SLO1227	$20
(iv)	Orchids in Our Tropics		
		S. swertifolium 'Robert Engle'	$40
		S. swertifolium 'Ruby Wings'	$40
(v)	Tropical Orchid Farm		
		S. swertifolium var. roseum	$30
(vi)	Petite Plaisance	S. swertifolium red form	$40
		S. swertifolium yellow form	$40
(vii)	Hoosier Orchids	S. swertifolium	$26

(2) PCR amplification:
- Ready-to-Go PCR Beads (AP Biotech) $170

(3) Clean-up of PCR products:
- Qiaquick gel extraction kits [50 rxns] (Qiagen) $79

(4) UV transparent cuvettes to estimate concentration of PCR product:
- UVettes (Eppendorf; carried by Fisher) $82

(5) Sequencing:
- it is necessary to sequence the ITS in two directions, therefore, the minimum number of sequencing reactions (ie. excluding repeats of failed reactions) will be: cost per reaction x number of plants x 2 directions (ie. $6 x 15 plants x 2 directions = $180)

$180

Total **$887**

This total is the bare minimum. Ideally we would like to have two of each type of plant in order to grow them out and observe morphological differences as well as to have plenty of plant material for analysis. That would be 376$ in orchid material, for a total of 1263$. Further sources for funding have not yet been explored for this project.