

NORTHEASTERN CAVE CONSERVANCY, INC.

Minutes

September 9, 2012 10:00 Melrose, NY

Meeting called to order at 10:20

1. Introduction and greetings – President Bob Addis
2. Attendance:
Officers: B. Addis, B. Folsom, C. Hay
Trustees: M Chu, K. Dumont, T. Engel, A. Traino, P. Woodell, P. Youngbaer, A. Hicks, M. Warner
3. Absent with Proxy: John Dunham for Morgan Ingalls
4. Absent without Proxy: Vince Kappler (Vince contacted secretary to state the board can assign proxy; board decided at meeting proxy was not needed as there was no other members present)
5. President's Report – Bob Addis- Attachment A
6. Vice President's Report –Vince Kappler – Attachment B
7. Treasurer's Report –Bill Folsom - Attachment C
8. Secretary's Report – Christa Hay
 - Status quo
 - August EC meeting minutes – Attachment D
9. Science Committee – Larry Davis – Attachment E
 - I have received and reviewed the attached research proposal. After consulting with the cave manager, I am strongly recommending that the board accept the proposal as written. This is important work; the proposal is thorough and detailed. The cave and its visitors will not be harmed or inconvenienced.

If the board approves, I would add the usual requirements for providing reports, access to data, contacting the manager before research visits, etc., etc.
10. Acquisitions Committee Report –Chuck Porter
 - This will be my last report, as I am today resigning as Acquisitions Chair, in protest of the Board's attitude against conservation easements.

Also, I've been Acquisitions Chair for well over 10 years and it might be good to have some new blood in the position.

I will continue to support the NCC in other ways, such as being co-steward of Clarksville Cave. When we begin to get conservation easements on our existing preserves I would then be willing to consider resuming as Acquisitions Chair.
11. Bylaws Committee Report – Joe Levinson
 - I have been asked to look and make some comments to the following bylaw's discussions. My comments are in red (italicized).

ARTICLE III: MEMBERSHIP

2. Categories of membership shall be:

- a. Regular: have all privileges of membership, including receiving all membership mailings and publications, receiving notices of meetings of the Board of Directors, having the right to vote, and other privileges as established by the Board of Directors.
- b. Family: have all privileges of Regular membership except Family membership shall be nonvoting and only one mailing per family shall be sent out.
- c. Student: have all privileges of Regular membership except students shall be less than 18 years of age or have valid student identification, and shall be nonvoting.
- d. Benefactor: have all privileges of Regular membership except Benefactors have paid an addition amount as determined by the Board of Directors above the established Regular dues.
- e. Institutional: have all privileges of Regular membership except Institutional members are organizations and not individuals.
- f. Life: have all privileges of Regular membership but will receive publications digitally when available. Life members pay a onetime membership fee determined by the Board.

Discussion: Institutional Members and voting. The way the bylaw is written IMs can vote but some board members

opined (1) that it should be clearly stated that the IM has only one vote, and (2) is it customary for organizations to allow IMs to vote at all.

This is good, but should also indicate the IM should not be permitted to serve on as a trustee, officer, or committee chair.

ARTICLE IV: BOARD OF DIRECTORS

1. The Board of Directors shall consist of the Trustees and of the Officers.
2. Each Officer and each Trustee shall have one vote at meetings of the Board of Directors.
3. Between regularly scheduled meetings of the NCC Board, urgent business may be conducted by a majority vote of the Board by responding to either an electronic or a US Postal Service offering. There shall be no less than two (2) calendar weeks between the initial posting and the deadline for ballots to be received. There shall be an attempt to foster discussion by listing this on the NCC's electronic discussion group at the time of the initial posting. Results of the electronic or postal voting will be made available to the Board as soon as practical after the voting.

Discussion: E-voting. The board feels that it needs more guidance on how to conduct an e-vote. Should the bylaw simply state that e-voting is permissible and the board then adopt policies on when and how to conduct the vote or should the process be spelled out in the bylaws. An advantage of using policy is that policy can be amended faster than a bylaw to accommodate unusual situations and/or glitches that surface when actually conducting an e-vote.

Who decides if business is urgent, what types of business can and cannot be considered urgent, a set time frame for discussion, followed by a set time for voting, are all important points. The board feels these items need to be defined someplace.

The current process of board members discussing/voting simultaneously is definitely counterproductive.

Urgent business would be defined by the board, it should not be in a policy, and a board member always has the potential to table a motion they do not feel is urgent until the next meeting. By defining urgent in a policy circumvents the membership from having a direct say in the bylaws. When I served on the board, our urgent business was typically land purchases that the board was cognizant of.

ARTICLE IX: MEETINGS

4. Quorum
 - a. Executive Committee: Three voting members of the Executive Committee shall constitute a quorum.
 - b. Board of Directors: Seven members on the Board of Directors shall constitute a quorum.
 - c. Trustees. Two thirds of the Trustees shall constitute a quorum.

Discussion: A Board of Directors meeting includes the nine trustees and four officers. A quorum of the BoD is seven thus a meeting could be held without any officers present or a meeting could be held with four officers and three trustees. However unlikely, some board members feel that any meeting conducted under either situation is not representative enough and thus unacceptable.

Is two officers and five trustees a satisfactory compromise as a quorum for a meeting?

If you want to have a larger quorum for a meeting, make it three officers, and 6 trustees, which provide a quorum for both groups. Also for discussion, is there any desire to have members present?

12. Publications/Publicity Committee Report - Christa Hay
 - Brochure – want to market to non cavers as well – hiking on the preserves...yes still working on this- after NRO
13. Fundraising Committee Report – Open
14. Office Committee Report – Emily Davis / Mike Warner
 - Problems: None
Plans: With the closing of daily service for the Loaves and Fishes Cafe (after 25,000 meals served) we will be back on track for monthly office reports.
Progress: Status Quo
 - Memo from Emily delivered at meeting.
Dear NCC Officers and Board
I would like to thank all of you for your support of Schoharie Recovery. First in the efforts of many of you to help with the early cleanup next with the generous donation that was made last year.

Last, I would like to thank you for up with the inconsistent and sporadic office reports while I will managing the Loaves and Fishes Café. We have closed for daily service after one year and over 25, 0000 meals served. We will only be serving on occasional days we have large numbers of volunteers.

The office committee is committed to restoring our month or more reports now.

15. Knox Cave Preserve Report –Emily Davis

- Problems: None
Plans: To continue to work with the groups that are doing research in Knox. (Could someone add it a report from the opening of the new Knox room?)
Progress: The first report arrived from the research group setting up Data Loggers.
- Knox Cave room – (Knox Museum) – Roger Dinsmore was a major explorer in the early days of Knox. Good Community interaction. Mike Nardacci is the liaison.

16. Onesquethaw Cave Preserve Report – Thom Engel

- There has been only 8 permits plus the 8 group permits issued as of 8/22 for the period of the last board meeting to this board meeting. A work day will be scheduled for later in the fall. Alan Traino, Bill Folsom, and others did a sinkhole clean-up.

17. Sellecks Karst Preserve Report – Alan Traino

- nothing to report

18. Clarksville Preserve Report – Mike Chu, Thom Engel, Chuck Porter

- A storm cell brought down some trees on the property. This cell dropped about six inches of rain. This has further eroded the eastern access to the upper parking lot. Since we don't own this upper lot, there doesn't appear to be much we can do here.
There are also reports of graffiti in the cave. This and on-going needs to remove debris from the Gregory parcel still will require a clean-up day. The culvert on the Gregory parcel has been removed.
We have noticed an increase in the size of some informal groups. This seems to be related to "meet-up" groups using the internet to bring people together. Often these groups can nearly fill the parking area. (One trip of 13 had come in 9 vehicles.) While I do not recommend requiring such groups to get a permit, asking them to tell us they are planning to come would seem to be reasonable and allow better planning with respect to formal groups. I think we need to discuss this and WNS issues regarding such groups.
There were two problem groups. In both cases the groups were permitted to bring one group and showed up with two groups. In both cases they were told they had to limit their group to a total of 15 and they'd have to decide who wasn't going into the cave.
- Looking to set up cleanup - possible the weekend of the Barn Dance

19. Bentleys Preserve Report - Jonah Spivak

20. Ella Armstrong Preserve – Alan Traino

- Property being maintained

21. Merlins Cave Preserve – Morgan Ingalls

- Leader Training: John and I ran a leader training on the 4th of August. We had six cavers come to the training and ideally we'd like to have at least one Merlins leader for each grotto in the NRO in the near future. Currently we have leaders from VCA, HHG, NNJG, CCG, SOC, and BG (I think). Two trips have been led since the leader training, and there are at least three more that are planned for before October 1st. I'm currently trying to put together a calendar for all trip leaders so that trips can be coordinated. Finally, I feel like having some small recreational trips to Merlins is important as I've been hearing some complaints about individuals and grottos donating to the purchase of the cave, and then not being allowed it. I also hope that by having a few more individuals see the save, there will be more understanding about a.) how delicate the cave is and b.) how challenging the cave can be.

Survey: John Dunham as been leading survey trips weekly (more or less) throughout the summer. Roughly 1450 feet have been mapped, with roughly 550 feet left to survey, and surveys will continue until the cave closes in October. Hoping to have done by October 1 and work on the map over the winter.

Bolting: As most of you know, the sketchy traverse to the high room climb was bolted and a traverse line was rigged at the spring NRO, which will be removed on the last trip in this fall (the last Sunday of September). This now allows cavers to clip in to the safety when doing both the traverse and the climb. Personally, I think this is a huge improvement and greatly reduces the risk of injury in this particular passage.

Hawthorne Valley Farmscape Ecology Program: Chuck and I have been in touch with a few members of the Hawthorne Valley Farmscape Ecology Program who went and visited the preserve in early August. They inventoried

vascular plants, spiders, ants, ground beetles, and mosses. While still waiting for the results from the spiders, ants, ground beetles, and mosses, they did find some county-rare vascular plants including American Ginseng. Sadly, they also found some invasive, especially in the parking lot area. I hope to be able to go down and meet with the botanists doing the work and see if we can come up with a plan for controlling the invasive and protecting rare species.

Finally, the cave will be closing for the hibernation season starting October 1st.

- Bob spoke with Tom Metzgar (Mid Atlantic Karst Conservancy) about National Wood Lot Association – they might provide liability insurance. John will pass information along to Morgan.

22. Education Committee Report – Thom Engel

- Nothing to report. I did look into exhibiting at STEM, but their standard exhibitor fee is \$450.

23. Membership Committee – Peter Youngbaer

- The Membership Committee is pleased to report two new Life Members during this quarter: Mitchell Berger #19, Michael Tennant # 20, Bru Randell #21, Christa Hay#22 and Joe Light #23. We sent new renewal notices to everyone whose dues were up in August, September, and October, and sent reminder notices to those folks who hadn't yet renewed for the period ending July 31.

Several folks sent renewals prior to the Old Timers' Reunion, including three Institutional Members - CCG, HHG, and a new one - Berlin High School - which had been a group user. Given the timing of the OTR and this meeting, I expect I will have updates this week from the Office Committee and Bob and Christa and can give updated membership numbers at the meeting.

The database is current with the information I had through August 26, and appropriate thank yous have been sent.

24. Website Subcommittee Report – Mike Chu

25. Legal Committee Report – Open

26. Special and Group Use Coordinators –Thom Engel/Emily Davis interim advertize

- As of today permits have been issued as follows:

Clarksville 105 groups – In addition 14 reservations were received for which permits were not issued.

Knox 17 groups

Onesquethaw 8 groups

Bentleys, Sellecks, Ella Armstrong, and Merlins had zero (0) group permits issued.

Total group permits issued for NCC caves = 130.

Total group permits issued for NSS caves = 17.

Total permits issued for NCC & NSS = 147.

Several situations arose that suggested that permittees were not reading the conditions on the 2nd page of the permit. Thus, we have made a slight change to the permit and recipients now need to initial the front to “activate” the permit. Their initials show they have read and understand the conditions on the 2nd page.

There were situations where groups either failed to ask for all the dates they wanted or asked for the wrong dates.

Consequently, next year, I plan go back to the requestor with a summary of their requested dates and ask them to verify that the dates submitted are what they want.

I have made updates to the general waiver. These primarily remove the MHLC from the form, but also clarify some of the requested information. How do I get it on the website?

27. Financial and Investments Committee – Joe Levinson

- Nothing to report

28. Volunteer Value Committee – Vince Kappler

- Progress: Year to date totals are: 792 hours of volunteer work have been recorded on NCC projects and members drove 5455 miles for a total value of \$25,438.
- Plans: I will send periodic VV reminders to the membership and monitor data collection.
- Problems: None at this time

29. Nominating Committee – Bob Simmons

30. Ad hoc Committee – Surprise Cave – Bob Simmons

31. Ad hoc Committee- Tory's Cave – Bob Simmons

32. Ad hoc Risk Management Committee - Bob Addis, Peter Youngbaer, Vince Kappler
 - Vince- I have not heard back from the charity lawyer about our questions on violating provisions of the sportsmen law and loosing immunity. I'll send him another note after the holiday asking to restart the conversation.
 - Bob and Vince to meet with pro bono lawyer and then talk with Peter Y about the interaction between the two lawyers the NCC is meeting with.

33. Mohawk River Basin Karst Ad hoc committee- Chuck Porter and Art Palmer

34. Transmission pipeline karst Impact ad hoc committee – Thom Engel
 - Constitution Pipeline (ad hoc) – Nothing to report in this period. The pipeline is encountering considerable opposition along its route. This line will be seeking FERC (Federal Energy Regulatory Commission) approval which comes with the right of eminent domain. However, the company can be kept off property even though they may need to analyze the impacts on it as part of their FERC filing.

35. Action Items (from previous meeting)
 - Christa to find a display FLAG for and table skirt - Christa has been checking prices
 - Bob to contact Jean Devries to formalize the conservancy survey for the Risk Management Committee – will contact – will talk to Jean at convention
 - Need to advertize the position for Special and Group Use Coordinator – person has to be able to answer email daily, Legal, Newsletter, Fundraising,
 - **Year excluding hibernation time. Amend all on management plans Vince to up date all –**
 - Bob spoke with Tom Metzgar (Mid Atlantic Karst Conservancy) about National Wood Lot Association – they might provide liability insurance. John will pass information along to Morgan.
 - Bob and Vince to meet with pro bono lawyer and then talk with Peter Y about the interaction between the two lawyers the NCC is meeting with.
 - Thom has updated the waivers. Removed the MHLC from it since we now own the Onesquthaw property. He will send to Mike Chu for the website

36. Addis moved to open the Committee of the Whole. The Treasurer will preside. Items can be entered as new business.

2nd by Hay
For – Rest Opposed - Traino

 - John Boyd Thacher State Park Master Plan – Thom Engel - Nothing formal has been received as a result of our comments for the Thacher Master Plan. I had a conversation with the park manager, Chris Fallon. He says the consensus of those working on the master plan is to allow access to the caves. Exactly, what this means is not clear. On a related matter, I have been asked to serve on a special committee exploring the possibility of a visitor's center for the park. (This would be apart from the existing nature center.)
 - Davis/Warner - We will be asking for the NCC to co sponsor the North east Bat Working Group Meeting. This will cost the group no money but will allow NEBWG to avoid paying taxes on the hotel rental. It is related to our purpose and is similar to what we did for the National Cave Management meeting. The event is January 9-11 in Albany at the Airport Best Western.
 - Barn Dance - working on getting donations and promoting the dance. Bob will talk with Mike Nardarcii about opening the Knox Cave room one hour before the dance begins.
 - Science Projects – discussion on science policy procedure..needs tweaking. If there is a negative impact to caves, karts etc we should not allow but if we do not agree with a science project that should not automatically vote it down. We will send some concerns to Science Chair for his input and work on reworking the procedure.
 - Thacher Park Fall Festival / Club day EMS – Saturday October 6- would like help covering the two events. Thom will represent NCC at Thacher Park.
 - Open Positions- need newsletter at the very least. Advertize all open positions. Mike Chu will take back the newsletter! Have an article for the membership in each newsletter. Peter sends out thank yous to members etc and does state we have positions that need help etc.
 - Conservation easement/ liability – Merlins – we had several questions concerning the easement and we are working on it. The board **did** vote to approve the conservation easement for Merlins so we are not against conservation easements as suggested. We wanted to talk to attorney about language in easement and also checking the timing for the easement as stated in our EC meeting in February 2012. Bob will talk with Vince and move forward. CLC knows we passed the motion to have a conservation easement.
 - Thom has updated the waivers. Removed the MHLC from it since we now own the Onesquthaw property. He will send to Mike Chu for the website

37. The Vice President moved to close the Committee of the Whole.
2nd by Dumont
Passed unanimously

38. Hay moved to accept the minutes of the June 10, 2012 meeting.
2nd by Youngbaer
For- Rest Abstained- Hay
Passed

The following motion postponed from previous meeting

39. Addis moves \$10,000.00 be directed to the Rensselaer Land Trust (RLT) for a conservation easement to be held by RLT on the Bentley property. This will be paid in five equal annual installments of \$2,000.00 each, the first payment being due upon the acceptance of the conservation easement agreement by RLT. If requested, the NCC shall forward a copy of the survey and the walking right-of-way into the property.
2nd by Traino
Postponed from September 2011 meeting

Engel moved to postpone until Merlins conservation easement is completed and the recommendation of the ad hoc risk committee are given
2nd by Youngbaer
For- Rest Abstained Porter

We have not received the Ad hoc committee recommendation so is still postponed.

40. Addis moved the approval of the proposal titled *Fungal Biocontrol Agents for Alleviation or Remediation of Geomyces destructans* as described in the report of the science committee in the September 9, 2012 minutes with conditions that the science coordinator communicates to the researchers that there is nothing implicit in this approval that Knox Cave would necessary be approved for the application of biological controls and that the researchers be informed of the presence of the other on going science project that was approved at the June 10, 2012 Board meeting in Knox Cave. Further that we inform cave visitors of the presence of scientific research going on in the cave.
2nd by Dunham
For- Rest. Abstain – Engle, Warner, Hicks
Passed

41. Engel moved all management plans will be modified to contain a new section to be titled “Research Rules.” This section shall be placed in the plans prior to the “Explorations Rules” and should contain the following minimum “boilerplate language”

“All research carried out on the NCC preserve must meet the following criteria:

- 1) Researchers must initially contact the NCC science coordinator.
- 2) The goals and objectives of the research must be clearly defined.
- 3) There must be a clear beginning and end to each project, with the exception of long-term monitoring studies.
- 4) The work must not cause permanent damage to any caves, natural features, native biota, or historical resources nor interfere with natural hydrologic or chemical processes.
- 5) The research plan must assure the maximum safety of all concerned.
- 6) The work must not interfere with the “experience” of other property visitors.
- 7) Unless specifically authorized by the NCC Board, researchers must operate within the confines of the established management plans for each property.

2nd by Hicks

Folsom moves to table this discussion.

2nd by Traino

For – Hicks, Traino, Folsom Against- Youngbaer, Engel, Warner, Woodell, Chu Abstained – Dumont, Dunham, Hay
Failed

Original motion vote

Passed unanimously

42. Warner moved that the NCC co-sponsor the Northeastern Bat Working Group Meeting in January 2012.
2nd by Traino
Passed unanimously

43. Informational Point: Executive committee meeting will be November 5th at 7pm. NCC Conference Call: 605-475-6111, Access Code: 814008

44. Addis moved that the next NCC Board meeting will be December 9th at 10:00 am at Speleobooks.
2nd by Engel
Passed unanimously
45. Addis moves to thanks Mike Chu for hosting the meeting.
2nd by Hay
Passed unanimously
46. Addis moves to adjourn.
2nd by Dunham
Passed unanimously

Meeting adjourned at 1:09

Attachment A
President's Report

Business for the NCC continues and here are a few highlights.

1. NCC Legal Advice. We have a pro bono lawyer in Albany through an application process for nonprofit organizations, BJ Costello, and one item discussed is that Section 9-103 of the NY State General Obligations Law is valid and has not been overturned in legal cases to date. It is the so-called Sportsman's Law that affords some landowner liability protection to the NCC but obviously is not a cure all. Vince Kappler and I will be discussing this and other issues with Mr. Costello this fall.

We are exploring possibilities through Peter Youngbaer of another specialist attorney offering opinions on various issues affecting nonprofits. Details to follow.

2. During the NSS Convention in the last week of June, NCC Charter Member Aaron Jarvis of Parkersburg, WV suggested that the NCC do a fund raiser for the new NSS Office. He thought that since it was the 40th anniversary of the establishment of the world's record for stalagmite sitting in the Lost World Caverns near Lewisburg by yours truly, we should offer photo taking of cavers and regular tourists with the World's Champion. Peter Jones of Camden, ME and a noted cave photographer donated his time and equipment and \$205 was raised in one evening.

This addresses more the aspect of having fun with your friends underground than raising large sums of money. The evening was highlighted with the arrival of Bob Liebman, the 1971 assistant to the event and of Cliff Forman, the owner of Lost World Caverns, Inc. at the time. Photos taken, history made. The NCC booth was on exhibit during the week.

3. Roger Dinsmore, early explorer of Knox Cave. Over the past four years a few NCC members have kept up an email conversation with the daughter of Roger Dinsmore, one of the three 1950's explorers credited with major discoveries beyond the Gunbarrel in Knox Cave. They were Dinsmore for the Pit Room, Mitchnier for the Alabaster Room, and Negley for the alleged Football Room. None of us current NCC "old timers" knew this early crowd who represented a previous generation of cavers so it was a great pleasure to finally meet Roger, view his amazing caving scrapbook and pick his brain.

Unfortunately Roger passed away in early August at the age of 92. NCC members attending his wake and funeral and were warmly greeted as "family" for that special part of Roger's life – caving. A greater tribute came on August 19 at the Re-Dedication of the Knox Cave Room at the Knox Museum when several of Roger's relatives were present to witness his presence becoming part of the Knox Cave Room. Later several relatives joined NCC members for their first tour of Knox Cave. They were all deeply appreciative for the cave trip and gained further insight into why Roger always spoke so passionately about caving. The NCC gained two new members and from the follow-up emails we will be seeing more of them.

If you will recall that the NCC was incorporated in 1978 to accept Knox Cave as its first acquisition, you will see the historical importance of this link with the Dinsmore Family. As well, it indicates more community involvement, a good thing.

4. An OTR Activity. Besides manning the NCC Booth on Vendor's Row this year, once again our Ideas Man, Aaron Jarvis suggested that we take a break and visit Stratosphere Balloon Cave on the show caves ground of Seneca Caverns. Aaron suggested that I call ahead for a group discount and as I was talking to Russell Andrews, the General Manager he wanted us as a cave conservancy to come over and analyze his new cave guides' training program. Offering lunch and a free tour of Stratosphere, we accepted. Russell ran a power point during lunch and frankly we were all quite impressed with the guides' training program and told him so. It had been prepared by a consultant and it contained adequate amounts of science specific to the two caves, conservation messages including WNS updates, first aid responses, and handling a group in an emergency, to name a few topics.

Russell and two guides then took us into Stratosphere Balloon Cave, so-named because of a large formation resembling a balloon. This cave was commercialized in the 1930's but closed for decades due to the dangerous nature of rotting wooden staircases and platforms. More recently Seneca Caverns has been removing the old stairs and substituting rope handrails for the continual steep descent/ascent. It never had electric lighting and today it's a semi-wild tour using their helmets, lights and gloves. For more money they offer a wilder tour featuring crawling and climbing ladders. We had a good time on the semi-wild tour and made some good friends there.

5. An NCC Retreat. No, we are not pulling out of the cave business! Hopefully in 2013 the Board, Committee Chairs and guests will get away somewhere to have a structured look at the NCC, how it operates today, and what we can do in the future to make it better. On a personal note, I will be attending a retreat for my work on September 26. Since this is the first retreat for me in many years, I will view it as a learning experience and one that I can share with the NCC.
6. Reminder of Fall Events: Sept. 14-16 – NRO Weekend. Oct. – Knox Barn Dance Fund Raiser

Attachment B
Vice President's Report

Liability Insurance

I received the binder for our liability insurance on June 26 and advised the EC and Risk Committee that the insurance policy was in effect. The binder was forwarded to the Office Committee after convention for filing and safekeeping. In early July, I received a document from the insurance company asking me (the NCC) to verify that we had made a diligent effort to identify an insurance company in New York State that could provide the coverage we needed but were unsuccessful. I wrote a critique of my efforts to identify a suitable carrier and the rationale used in selecting a broker from Florida. I forwarded the critique along with the document to the president for his signature and return to the insurance company.

EC Meeting

I participated in the EC phone meeting held on August 1. As a result of this meeting, I was tasked with (1) soliciting a review of several bylaws from the Bylaw Committee, and (2) continuing the conversation with our Charity Corps Attorney, Mr. Barry Costello.

Bylaws: I asked the Bylaws Committee for comments and suggestions on how best to address the following bylaws questions;

- Institutional Members and voting: The way the bylaw is written IMs can vote but some board members opined (1) that it should be clearly stated that the IM has only one vote, and (2) is it customary for organizations to allow IMs to vote at all.
- E-vote: The board feels that it needs more guidance on how to conduct an e-vote. Should the bylaw simply state that e-voting is permissible and the board then adopt policies on when and how to conduct the vote or should the process be spelled out in the bylaws. Who decides if business is urgent, what types of business can and cannot be considered urgent, a set time frame for discussion, followed by a set time for voting, are all important points that need clarification.
- Quorum: A quorum of the BoD is seven thus a meeting could be held without any officers present or a meeting could be held with four officers and three trustees. However unlikely, some board members feel that any meeting conducted under either situation is not representative enough and thus unacceptable. Should quorum requirements be re-written to ensure a more representative mix of officers and trustees is required to conduct NCC business?

Charity Corps Attorney: Mr. Costello felt that the "Sportsmen Law" provided a good level of protection providing we don't violate any of the provisions of the law. He recommended that we look into insurance for "yourselves" which I take to mean a director/officer liability policy. He also offered to continue providing advice and guidance on specific insurance topics as well as conservation easements. Mr. Costello was out of his office for a month and in mid-August I emailed a request to continue our conversation and asked for some examples of how the NCC might unknowingly violate provisions of the sportsmen law and thus lose any immunity the law provided.

Attachment C
Treasurer's Report
September 9, 2012

Balance Sheet

As of September 1, 2012

	<u>Sep 1, 12</u>
Current Assets	
Checking/Savings	
Checking	4,069.28
Money Market	10,036.65
NSF Account	43,302.86
PayPal Checking	<u>689.61</u>
Total Checking/Savings	<u>58,098.40</u>
 Total Current Assets	 <u>58,098.40</u>

The 2nd and final IRS Form 8868 was filed, giving us an additional 3-month extension to complete the 2011 filing, so the tax forms are due now on 11/15/12. The tax preparation work was completed last week, with forms ready to be printed and signed.

Profit & Loss Budget vs. Actual

January 1 through September 1, 2012

09/02/2012

	Jan 1 - Sep 1, 12	Budget
Ordinary Income/Expense		
Income		
Donations		
Auction Donations	434.00	1,000.00
Donations - Other	982.04	4,000.00
NRO Donations	0.00	0.00
Total Donations	<u>1,416.04</u>	<u>5,000.00</u>
Donations Restricted		
NCC White Nose Fund	50.00	0.00
Total Donations Restricted	<u>50.00</u>	<u>0.00</u>
Interest Earned	3.38	50.00
Membership Income	<u>2,820.00</u>	<u>5,200.00</u>
Total Income	4,289.42	10,250.00
Expense		
Acquisitions	0.00	500.00
Bank Charges	20.42	20.00
Development	0.00	1,445.00
Donations-outgoing	205.76	200.00
Easements	0.00	1,500.00
Education	0.00	100.00
Executive		
President	0.00	150.00
Secretary	0.00	75.00
Treasurer	0.00	275.00

VP	0.00	75.00
Total Executive	0.00	575.00
Insurance	971.10	0.00
Legal Fees	0.00	2,000.00
Licenses & Permits	0.00	300.00
Meeting Expense	0.00	100.00
Membership Expenses	0.00	100.00
Miscellaneous	0.00	100.00
Office Expense	0.00	100.00
Postage	55.00	150.00
Preserves-Maintenance		
Bentleys	0.00	100.00
Clarksville	0.00	300.00
Ella Armstrong	0.00	100.00
Knox	0.00	100.00
Merlins	0.00	100.00
Onesquethaw	0.00	100.00
Sellecks	0.00	200.00
Total Preserves-Maintenance	0.00	1,000.00
Printing	0.00	200.00
Promotion/Fundraising	30.00	800.00
Publishing		
Mailings	0.00	200.00
Website	0.00	60.00
Total Publishing	0.00	260.00
Sponsorships	0.00	500.00
Taxes on Properties	13.25	300.00
Total Expense	1,295.53	10,250.00
Net Ordinary Income	2,993.89	0.00
Other Income/Expense		
Other Income		
In Kind donations	0.00	0.00
Volunteer Value	25,438.00	0.00
Total Other Income	25,438.00	0.00
Other Expense		
In Kind Out	0.00	0.00
Volunteer Value Exp	25,438.00	0.00
Total Other Expense	25,438.00	0.00
Net Other Income	0.00	0.00
Net Income	2,993.89	0.00
Net Gain or Loss from NSF Fund	1,888.78	
NET Income after NSF Fund Gain/Loss	4,882.67	

Attachment D
EC Minutes

EC Meeting
08/01/2012
Conference Call

Conference call: Bob, Bill, Vince, Christa

Barn Dance – October 13, **Bob will check with Emily about the door prizes etc. Question is can we charge the full price if we do not have fun stuff to give away.**

Pro-bono Legal work – Vince contacted the lawyer. General obligation law is ok. Lawyer sent email but Vince still has questions. Lawyer is out until August 15, **Vince and maybe Bob will talk to him to clarify about general obligation and internal insurance questions.**

Upcoming Activities:

- Clarksville Heritage Day – August 4 – Thom Engel will represent
- Knox Museum Knox Cave Room rededication ceremony
- OTR – NCC will be there.
- Fall NRO – NCC will be there
- Barn Dance

Bylaw – issue with clarifying Quorum, way its written could be no EC members at a meeting. Institutional voting – can they vote? E-vote issues. **Vince will need to contact Joe Levinson have him prepare something by next meeting.**

Release Form –updating our website, need to change remove Hudson Mohawk Land Conservancy since we now own the property. Maybe we could have the pro-bono lawyer check on the release forms as well. *Vince will add this to his questions to the lawyer.*

Property taxes – for Merlins property in Town of Cannon – these have been paid.

Bob will send a form he has been working on to the Board/Preserve Managers....Status of Preserves – etc..do we have them posted, survey...

Bob will check with Peter Youngbaer on his contacts with lawyer that deals with conservation easements.

Fungal Biocontrol Agents for Alleviation or Remediation of *Geomyces destructans*

Outline of proposed sampling locations and sampling protocol
Prepared 8-17-2012

By
Alan Hicks

We are sampling at least four hibernacula (Table 1) and will collect samples on three occasions from each. One sample will be collected during August 2012, another during the winter of 2012-2013 that will be concurrent to winter hibernacula surveys while bats are present, and the last during the spring of 2013 after bats have exited. We will select sites that represent a range of success by their respective bat colonies.

At each hibernaculum we will collect eight samples. These will include sampling two sites that meet each of the following requirements:

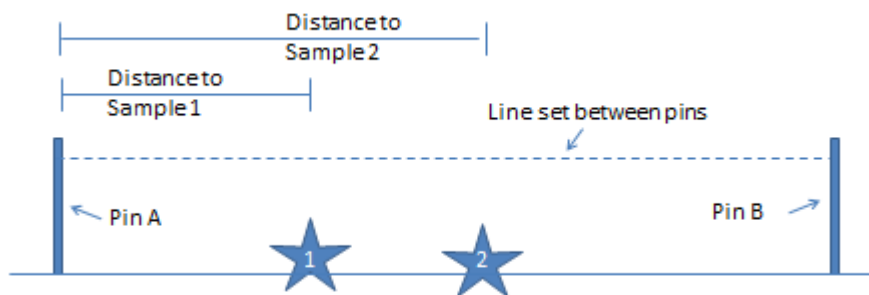
- floors that are clearly under roosts
- floors clearly not under roosts
- walls, drill holes/ or cracks that are clearly roosts
- walls drill holes/ or cracks that are clearly not roosts

When possible, samples will be collected from locations within the hibernacula that have the least amount of human traffic to minimize the chance of human contamination.

Sample locations will be fixed for at least the duration of the project by setting pins on opposite sides of the intended sample locations (stars in figure 1), leaving a margin of at least 0.5 meters between the drilled holes and the intended sampling locations. Holes for the pins will be drilled into the substrate using the smallest diameter masonry drill bits that are available. Pins will be aluminum rods that will extend above the surface by 5-7 cm. A line will be tied between the pins a couple of centimeters above the surface and samples will be collected from directly below the line. Distances to the intended sample sites will be measured from pin A, marked on the line, and the distance will be recorded. The location will also be photographed with the marked string in place. In sampling locations where sediment is available a sample of several cubic centimeters will be collected with sterile scoops and placed into a sterile plastic bag. In sample sites where there is no sediment (walls / crevices, bore holes), two sterile cotton swabs will be rubbed over an area of 2-3 cm² and placed in a separate sterile containers that are labeled with date, hibernacula name, sample site and sample number. There will be at least two samples collected from each sample location. When sampling is completed the line will be removed but the pins will stay in place. In the event that drilling is prohibited we can use any two easily identified and permanent structures to identify the beginning and end point of the sample transect. At the completion of the study, pins will be removed and holes will be filled with on- site materials.

All equipment will be decontaminated between visits, Tyvek suits, and surgical gloves disinfected rubber boots will be used on all collection trips. Samples will be kept in coolers approximating hibernacula temperatures until delivered to the NYSDOH laboratory.

Figure 1. Site sampling diagram



By the time the third sample is collected we will know the fungal community identified forms the first two samples. We will be prepared to select a subset of sampling locations from which a small volume (approximately 1 liter) of material consisting of sediment and/or loose rocks from the floor will be extracted to create a laboratory model of a the hibernacula . In these “lab hibernacula” we will test candidate fungal strains as *Geomyces Destructans* control agents.

Table 1. Candidate sites for fungal sampling. At least four sites will be selected that represent a range of apparent success by their respective colonies of hibernating bats. Status refers to the status of the wintering colony of bats. An (+) indicated a site with better than average success, (0) average success (-) less than average success (- -) nearly, or completely, devoid of hibernating bats. Bridgewater and Greely have contained no bats since March of 2010 and are screened to exclude them.

Site	Site status	Number of sample locations by category			
		Floor under a roost	Floor not under a roost	Walls, crevices, or bore holes that are roosts	Walls, crevices, or bore holes that are not roosts
Williams Hotel Mine	+	2	2	2	2
Williams Preserve Mine	0	2	2	2	2
Williams Lake Mine	-	2	2	2	2
Bridgewater Mine		2	2	2	2
Greely Mine		2	2	2	2
Hailes Cave	+	2	2	2	2
Knox Cave	+	2	2	2	2
Howe Cave/Caverns	0	2	2	2	2
Lincoln Pond Mine	- -	2	2	2	2
Rock Pond Mine	- -	2	2	2	2
Cranberry Mine	- -	2	2	2	2

1) Cover Page

Project Title:

Fungal Biocontrol Agents for Alleviation or Remediation of *Geomyces destructans*

Principle investigator(s)

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Consultants

Alan C. Hicks, Formerly Mammalian Biologist, New York State Department of Environmental Conservation, Currently working with Mike Cooper for Vesper Environmental LLC, Hurley, NY 12443 achicks@nycap.rr.com Tel. 518-860-8805

Hazel A. Barton, Associate Professor of Biology /Associate Professor of Geology and Environmental Science, Department of Biology, University of Akron/ B216 Auburn Science and Engineering Center, Akron, OH 44325-3908 bartonh@uakron.edu Tel: 330-972-2518

Key Collaborators

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Joseph Okoniewski, Biologist, New York State Department of Environmental Conservation, Wildlife Resources Center, Delmar, NY 12156 Jokoniewski@gw.dec.state.ny.us Tel. 518-402-8854

Project Duration

One-year (05/01/2012 – 04/30/2013)

Total budget costs

\$96,074 (\$24,018/ \$24,018/ \$24,018/ \$24,018)

PIs and Key Collaborators are aware of the project and agree to the terms of the proposal.

2) Executive Summary

“Fungal Biocontrol Agents for Alleviation or Remediation of *Geomyces destructans*”

On September 2, 2010, US Fish and Wildlife Service issued administrative guidelines for the management of bat White Nose Syndrome (WNS) in the National Wildlife Refuge System. The recommended measures included closures of mines and caves. This guidance was conceived before conclusive evidence was available on the role of *Geomyces destructans* in bat mortality and disease spread. However, recent scientific publications have provided strong support for this action namely that a single clone of the fungus has spread in New York and that WNS is a communicable disease. Thus, *G. destructans* fits the characteristics of a newly emerging pathogen that spreads rapidly in the susceptible populations. An effective remedial measure in such instances is to break the disease cycle by reducing or eliminating the environmental sources of the pathogen, which lowers the rates of new infections. Fortunately, the first clues about the natural habitat of *G. destructans* are now emerging on account of its recovery from affected sites along with other fungi. Fungal biocontrol agents refer to fungi that destroy plant pathogenic fungi, nematodes, insects and invasive plant species. These agents are highly desirable because, they are effective, cheap, and environment friendly and most importantly, they do not infect native plants and animals. The central hypothesis of the proposed study is that the biocontrol fungi are a viable tool to alleviate or remediate *G. destructans* in natural sites. Our preliminary observations support this hypothesis as biocontrol fungus *Trichoderma atroviride* impedes the growth of *G. destructans* in the laboratory. Therefore, the main objective of this study is to identify most effective fungal biocontrol agent (s) against *G. destructans*. A number of experimental approaches are proposed for challenging *G. destructans* with fungi obtained from systematic surveys in New York and Vermont and with well-known biocontrol fungi *T. atroviride*, *T. harzianum* and insect and mite pathogens *Beuveria bassiana*, and *Metarhizium anisoplae*. Both dual cultures and experimental seeding of soil and rock samples from the affected sites will be investigated. Additionally, the culture-free products of target fungi will be tested for bioremediation. A commercial product containing *T. harzianum* will also be tested in these experiments. The fate of *G. destructans* and biocontrol agent will be monitored by culture, microscopy, and molecular tests. The results will be used to develop a ‘cave or mine in a lab’ model for the study of biocontrol fungi. This model will be made available to FWS for testing the efficacy of other promising biocontrol agents. Finally, the most promising agent from our laboratory studies will be identified for future field trials at a select location (s) with close monitoring of the fate of *G. destructans*, biocontrol agent and other microbes. The study will deploy the strengths of an inter-disciplinary team of mycologists, bat experts and cave microbiologist. All necessary tools including biocontrol fungi and specialized tests for *G. destructans* are available in the investigators’ laboratory and team has ready access to affected sites. The proposed study will leverage a FWS-funded systematic survey of *G. destructans* currently underway in New York and Vermont. The proposal has high potential to become a showcase for the integration of natural survey with remediation measures for controlling the spread of *G. destructans*. This one-year project has a total budget outlay of \$96,074.

3) Body of the proposal

3) A. INTRODUCTION

3A.i. Background and problem statement

Geomyces destructans (*Gd*) is a cold-loving ('psychrophile') fungus that causes white nose syndrome (WNS) in hibernating bats [1]. The disease leads to mass mortality of affected bats in the US Northeast, Midwest, South and some provinces in Canada [1,2]. A reference set of criteria have been proposed for the field and laboratory confirmation of WNS and *G. dermatitidis* [3,4]. An identical fungus has been reported from bats in Europe without the associated mortality [5,6,7]. The cause of mortality in affected animals is fungal infection itself or a myriad of other factors including severe physiological changes in the wing membranes [8,9].

Our laboratory has worked on WNS since its first discovery near Albany, NY. We have characterized morphological and molecular features of *G. destructans* [10]. A proposal was published for naming of the disease as geomycosis [11]. Another study described a rapid real time PCR test for *G. destructans* identification from fungal culture, bat tissues and environmental substrates [12]. We have also carried out laboratory studies to identify optimal antifungals and disinfectants for this pathogen [13].

Overall, the focus of our laboratory is to determine if this emerging infectious disease is caused by a novel or an endemic pathogen, because this information is important for devising conservation measures [14]. Therefore, we analyzed 16 isolates of *G. destructans* by multilocus sequence typing (MLST) and found a single genotype in different parts of New York [15]. These findings raised the possibility of clonal spread of a new pathogen. We extended this genotyping study to *G. destructans* isolates from Midwestern and Southern United States in collaboration with Dr. Kevin Keele. The data indicated the spread of the same clone of *G. destructans* in bats captured from Pennsylvania, West Virginia, Ohio and North Carolina {Ren et. al. *Emerging Infectious Diseases*, 2011, submitted}. This genetic evidence of a single fungal clone raised the possibility that a rapid spread of *G. destructans* is taking place along summer and winter migration routes of bats. These migrations might present ample opportunities for inter-mixing among healthy and infected animals and further spread of the disease. A mechanism for disease transmission by contact between healthy and infected animals was recently proposed by Blehert and colleagues [9]. At present, we do not have direct evidence about the role of anthropomorphic activities (occupational or recreational) in this disease spread [16]. Similarly, the environmental factors that led to the introduction or re-emergence of *G. destructans* in mines and caves remain unknown and their contribution in spread of the fungus via air, water and soil is yet to be determined [17].

On September 2, 2010, US Fish and Wildlife Service issued administrative guidelines for the management of bat White Nose Syndrome (WNS) in National Wildlife Refuge System. The recommended measures included closures of mines and caves. This guidance was conceived in order to control a communicable disease, which is easily transmitted from infected to uninfected animals. Recent published studies have provided strong support for these measures as *G. destructans* appears to be a newly introduced or emerging pathogen that spreads rapidly in the susceptible populations [9,15]. An effective remedial measure in such instances is to break the disease cycle by reducing or eliminating the environmental sources of the pathogen to reduce the rates of new infections. Fortuitously, the first clues about the natural habitat of *G. destructans* are now emerging on account

of positive DNA evidence obtained from soil of affected sites [18]. However, it is not yet known if *G. destructans* is exclusively adapted to bats similar to the characteristics of obligate zoophilic pathogenic fungi [19]. It is also possible that *G. destructans* thrives in soil, rocks and animal detritus found in contaminated sites in the vicinity of the infected animals. A third possibility is that *G. destructans* conidia, after being shed from the infected bats, survive in the natural substrates without any visible fungal growth. Additional studies are needed to understand the ecology of *G. dermatitidis*. However, any of the three scenarios outlined here would provide a readymade reservoir of the pathogen in the affected sites, which could pose risk of infections to healthy animals. As fungal conidia could persist for long periods in the environment, the contaminated sites could continue to pose risk. Therefore, it is important to carry out environmental alleviation or remediation at such sites to break the disease cycle especially by reducing the fungal burden available for new infections.

3.A.ii. Objectives and hypothesis tested and anticipated products

The central hypothesis of this study is that the biocontrol fungi are a viable tool to alleviate or remediate *G. destructans* in natural sites. Fungal biocontrol agents refer to fungi that destroy plant pathogenic fungi, nematodes, insects and invasive plant species [20,21,22]. There are possibly hundreds of fungi with biocontrol activities with well-known species among them now being commercially developed for widespread use in agriculture and horticulture[23,24]. Similarly, some bacteria also have biocontrol properties, but they will not be the subject of our investigations due to lack of expertise in that area [25,26]. Biocontrol agents are highly desirable because, they are effective, cheap, and environment friendly and most importantly, they do not infect native plants and animals. Interestingly, a zooplankton *Daphnia magna* was recently suggested as a promising biocontrol agent for *Batrachochytrium dendrobatidis*, which causes deadly chytridiomycosis in amphibians[27].

US FWS funded our laboratory in 2011 to carry out a systematic survey of *G. destructans*, which has allowed us to start cataloging fungi found in caves and mines along with *G. dermatitidis*. To our surprise, we isolated *Trichoderma* species from negative samples in few instances. This is significant as *Trichoderma* species are one of the most valuable biocontrol agents [28,29,30,31]. We followed up this observation with a preliminary experiment and we found that *Trichoderma atroviride* impeded the growth of *G. destructans* in dual cultures (details in next section). Therefore, there are strong indications that biocontrol fungi could be one of the options for alleviation or remediation of *G. destructans*. The main objective of our investigation is to identify most effective fungal biocontrol agent (s) against *G. destructans*. We anticipate that the proposed study would also yield a ‘cave or mine in a lab’ model for systematic testing of efficacy of biocontrol fungi and their commercial products against *G. destructans*. The results will set the stage for future field trial of the fungal biocontrol agents identified in this study.

3. A.iii. How the project addresses the identified priorities

The proposed study directly addresses FWS priority areas of investigation. Specifically, the study focus will be exclusively on item 3, “ Identification of non-chemical control options to reduce the severity of WNS among wild bats: - Is there a biological means to disrupt transmission, disrupt/kill Gd, or otherwise decrease infection rates and/or bat mortality?”

3) B. METHODS

3.B.i. Background for testing biocontrol agents

There is a vast volume of literature on biocontrol fungi and several highly effective agents are now approved for commercial use in USA and other parts of the world [20,25,31]. Biocontrol fungi could be used alone or in combinations to combine their effectiveness against the target organism. A number of standardized methods are available for the screening of local strains of fungi to narrow the search for an effective agent against a novel pathogen [24,32,33]. All biocontrol agents employ one or more of the following mechanisms of actions against the target organism: secretion of antibiotics to inhibit growth, competition for space and nutrients, suppression of spore formation and mycoparasitism. A good understanding of these mechanisms is immensely useful for the design of appropriate field trials. It is also important to standardize methods for large-scale growth of the biocontrol agent and formulate them for safe and sustained environmental release.

3.B.ii. Preliminary studies

Three ongoing projects in our laboratory have enhanced our capabilities to undertake the proposed investigations of biocontrol fungi against *G. destructans*:

Natural survey of *G. destructans* – We have employed fungal culture and DNA methods for ongoing surveys of the *G. destructans* in NY and VT. The preliminary results are summarized in the accompanying table:

Site of Sample Collection	Survey Date	<i>G. destructans</i> No. Positive/Total Samples		Other Fungi Identified
		Culture & ITS-Sequencing	Real Time PCR	
Aeolus Cave, E. Dorset, VT	8/4/2010	0/15	13/15	<i>Penicillium</i> sp., <i>Mucor</i> sp., <i>Geomyces</i> sp., <i>Trichosporon</i> sp., <i>Geomyces pannorum</i> , <i>Mortierella</i> sp., <i>Oidiodendron</i> sp., <i>Fusarium</i> sp., <i>Polypaecilum</i> sp.
Hitchcock Mine, Paradox, NY	1/17/2010	0/2	1/2	<i>Mucor</i> sp., <i>Geomyces pannorum</i> , <i>Mortierella</i> sp., <i>Polypaecilum</i> sp., <i>Chaetomium</i> sp.
Barton Hill, Mineville, NY	2/11/2010	0/2	1/2	<i>Geomyces pannorum</i> , <i>Helicostylum elegans</i>
Williams Mine Complex, Kingston, NY	11/8/2010	9/26	Not done	<i>Penicillium</i> sp., <i>Mucor</i> sp., <i>Geomyces</i> sp., <i>Trichosporon</i> sp., <i>Geomyces pannorum</i> , <i>Mortierella</i> sp., <i>Oidiodendron</i> sp., <i>Arthroderma</i> sp., <i>Polypaecilum</i> sp., <i>Candida</i> sp., <u><i>Trichoderma</i> sp.</u> , <i>Dothiorella</i> sp., <i>Cladosporium</i> sp., <i>Helicostylum elegans</i>
Graphite Mine, Hague, NY	1/25/2011	13/19	6/12 tested	Pending
Barton Mine, Moriah, NY	2/8/2011	12/18	5/8 tested	Pending
Hailes Cave, Guilderland, NY	2/18/2011	9/12	3/8 tested	Pending

The data indicates that a variety of fungi are found in the affected sites including other *Geomyces* species and *Trichoderma* species. This work will provide us with a number of test fungi for screening against *G. destructans* as explained later in this section.

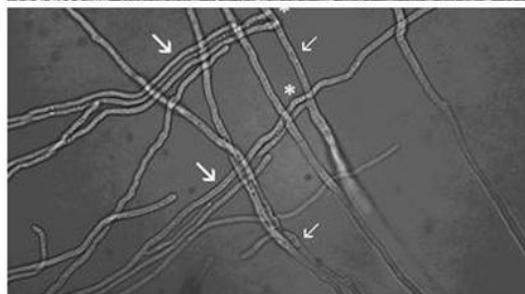
Self-nonsel self recognition in *G. destructans* – Fungi have exquisite ability to distinguish self hyphae from non-self hyphae in the environment to maintain genetic homogeneity. Any



Dual cultures of *G. destructans*



SEM - Dual cultures of *G. destructans*

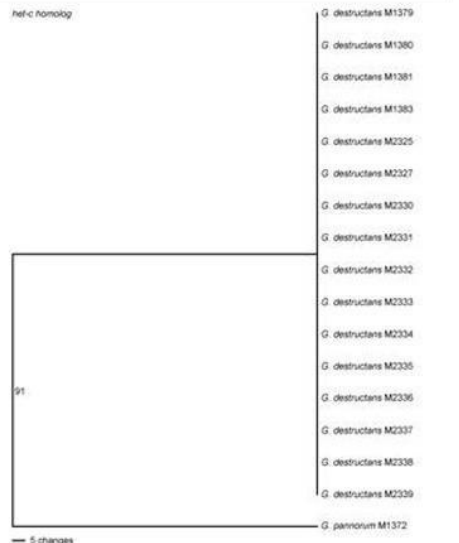


Microscopy - *G. destructans* dual cultures

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Gd-HCH  FVYTRRLGQYFPRHIDRQVADNRDQKPRRGGQK-HHIDIPN*HMKNYIAN 174
Gd-HCL  FVYTRRLGQYFPRHIDRQVADNRDQKPRRGGQK-HHIDIPN*HMKNYIAN 174
Ef-HCH  FVYTRRLGQYFPRHIDRQVADNRDQKPRRGGQK-HHIDIPN*HMKNYIAN 176
No-HET-C  FVYTRRLGQYFPRHIDRQVADNRDQKPRRGGQK-HHIDIPN*HMKNYIAN 176
No-HET-CA  FVYTRRLGQYFPRHIDRQVADNRDQKPRRGGQK-HHIDIPN*HMKNYIAN 176
Gd-HCH  G---GGGMAH...H...TZYV...H...V...EN...G...S...D...A...H...L...C...G...H... 229
Gd-HCL  HIG---...T...G...V...L...F...D...C...G...H...---R...K...A...H...A...H...H... 227
Ef-HCH  ---S...G...L...H...V...F...F...A...H...H...H...G...B...H...H...H...A...L... 230
No-HET-C  RLANR...S...M...H...V...F...F...A...H...H...H...G...B...H...H...H...A...L... 235
No-HET-CA  RLAY...Q...Q...M...H...V...F...F...A...H...H...H...G...B...H...H...H...A...L... 235
Gd-HCH  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 267
Gd-HCL  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 267
Ef-HCH  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 268
No-HET-C  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 280
No-HET-CA  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 284
Gd-HCH  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 294
Gd-HCL  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 318
Ef-HCH  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 305
No-HET-C  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 323
No-HET-CA  G...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H...H... 44
  
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Genes for MCG in *G. destructans*

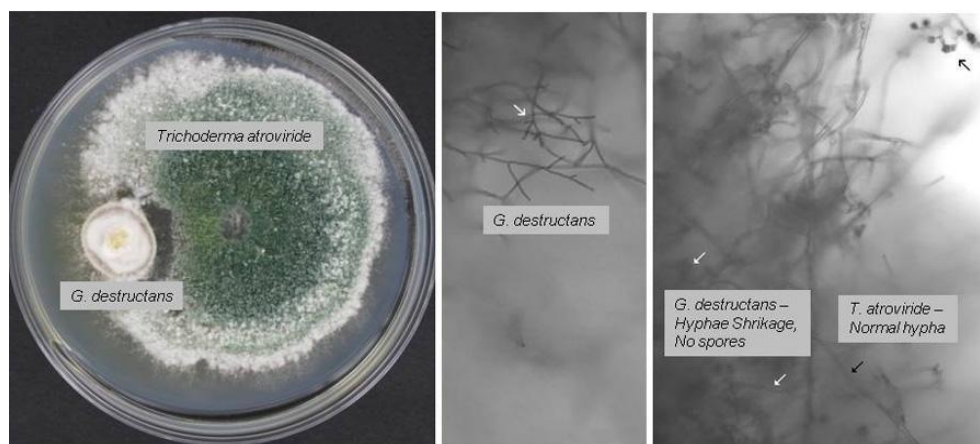


Identical MCG Genes in *G. destructans*

encounters with nonself fungal members could lead to growth arrest and lysis. This property has been used to devise mycelial compatibility groupings (MCG) as a fungal classification scheme. For *G. destructans*, we found that two different strains grow well with each other without any deleterious activities and their hyphae remained healthy (left panel). Furthermore, all *G. destructans* strains in our collection have genetic machinery (*Het-C* locus) to distinguish self from non-self; as these genes are identical, they belonged to one MCG (right panel. These findings have been submitted for publication (Rajkumar and Chaturvedi, 2011, *Applied and Environmental Microbiology*, under review). This study has important bearing on the proposed biocontrol investigations as we have acquired experience with standardized methods for dual cultures of fungi.

G. destructans – *T. atroviride* dual culture - A dual test challenge was performed by inoculating *G. destructans* on PDA plates and followed two-weeks later with *T. atroviride* to ensure that both fungi attain good growth (left panel). After one-week, the biocontrol fungus completely engulfed the plate surface including *G. destructans* and suppressed its growth. The zone of interaction between two fungi were carefully excised and observed under the microscope. It was observed that hyphal damages occurred in both fungi, but at dramatically

different degrees. *G. destructans* suffered noticeable shrinkage of hyphae and lost the ability to produce spores (middle panel). In case of *T. atroviride*, the hyphae remained intact while some loss of spore formation was observed (right panel). These observations are being further examined to define the biocontrol potential of *T. atroviride* and other *Trichoderma* species.



3.B.iii. Screening for new biocontrol agents

The results from natural surveys in NY and VT will provide us with a number of fungi from the sites with and without *G. destructans*. We will analyze respective sites to find out if there is any correlation between the absence of *G. destructans* and the occurrence of a particular soil fungus ('Fungus A'). Such a correlation might be due to an inhibitory activity especially if *G. destructans* can be isolated in adjoining sites in the absence of 'Fungus A'. This approach would allow us to build an inventory of fungi from various sites that might have novel biocontrol activities against *G. destructans*[24]. They will be tested as described below for *Trichoderma* species.

3.B.iv. Testing with *Trichoderma*

Trichoderma species are the most widely evaluated biocontrol fungi due to their activities against a wide variety of other fungi under a variety of geoclimatic conditions; they are also most common ingredients of commercial biocontrol formulations [28,29,30,31]. We will test two important species: *T. atroviride* and *T. harzianum* procured from ARS Fungal Collection at the Cornell University. *T. harzianum*T22 is also available commercially (RootShield, BioWorks, Inc.) and the product will be included in our evaluation. Other *Trichoderma* spp will be considered as needed.

Dual culture will be set-up by first measuring the relative growth rates of *G. destructans* and *Trichoderma* spp. individually on potato dextrose agar (PDA) plates. The cultures will be grown at 6°C and 15°C as previously described [10]. A growth rate for each fungus will be determined by linear regression of colony diameter (mm) against days of growth from three independent experiments [34]. Dual cultures will be set-up on PDA plates containing sterile dialysis membrane with 12-kDa cutoff. The membranes will be first inoculated with 10-mm disk of *G. dermatitidis* obtained from a two-week old growth. After one-week, a 10-mm disk of *Trichoderma* species will be placed on the membrane 45-mm apart from *G. dermatitidis* growth. The growth rates in dual cultures will be measured and macroscopic changes will be photographed over 2-8 week observation period

with a Photosimile image creator[®] Macro-photography system (Ortery Technologies). The type of fungal interaction and Index of Dominance (I_D) will be recorded as follows: mutual intermingling{1}; mutual antagonism on contact {2}; mutual antagonism at a distance{3}; dominance on contact{4 for dominant species, 0 for the inhibited species}, and dominance at distance{5 for dominant species, 0 for the inhibited species}[34,35]. The anticipated results for biocontrol will be I_{D4} and I_{D5} .

Once a promising *Trichoderma* spp. is identified, the growth conditions in dual cultures will be further evaluated for variables such as low carbon and nitrogen contents and water activities using appropriate formulations [32,34]. The results from biocontrol experiments will identify most promising *Trichoderma* spp. and other effective fungal species from local sites.

Light and scanning electron microscopy (SEM) will be performed to examine changes in the morphological appearance and spore formation. This is important to correlate macroscopic observation from dual culture experiments. Light microscopy will require setting up dual micro-cultures on glass slides placed in sterile plates with 50-mm long, thin blocks of PDA. The cultures will be inoculated as 5-mm disks of two fungi placed 10- mm apart and covered with a cover slip. The fungal growth will be examined by light microscopy to record changes in morphology of hyphae and presence/absence of spores. SEM will be done by processing dialysis membrane squares with fungal growth obtained at various intervals from dual cultures, fixed in glutaraldehyde, dehydrated in ethanol and coated with gold [36,37]. They will be examined using a Zeiss Scanning Electron Microscope per standard methods in our laboratory [10]. The microscopic results will be used to match I_{D4} and I_{D5} reactions of effective biocontrol agents identified by dual cultures.

Diffusible inhibitory metabolites of *Trichoderma* spp will be tested by growing *T. atroviridae* and *T. harzianum* on PDA agar plates containing sterile dialysis membrane with 12-kDa cutoff. After one-week growth, the membrane blocks with *Trichoderma* spp will be removed. Fresh sterile membranes will be placed on these PDA plates and inoculated with *G. destructans*. These cultures plates along with control PDA plates will be incubated for 2-4 weeks to compare growth inhibition in the presence and absence of *Trichoderma* spp diffuse metabolites.

Antibiosis effects of *Trichoderma* spp. and other promising fungi will be measured by growing them in complete synthetic medium containing either 1% dextrose or 1% homogenate of *G. destructans* mycelia. The liquid cultures will be filtered (0.45 μ m) after 7-days and filtrate treated at 90°C for 10 min to denature hydrolyzing enzymes. The treated filtrate will be mixed with sterile agar at 50°C and poured into plates. Filtrate agar plates will be tested in parallel with PDA agar for suppression of growth of *G. dermatitidis*; any inhibition of growth in the former would indicate secretory antibiotics from *Trichoderma* spp. [38,39]. The sterile filtrate will also be added at 0.1:1, 0.5:1, 1:1 ratio to PDA broth. These mixed media will be tested for their ability to suppress *G. destructans* growth. Any growth inhibition in comparison to PDA broth control would indicate heat-sensitive antibiosis effects from *Trichoderma* spp. and other fungi. We anticipate final results from antibiosis experiments to either correlate with I_{D4} and I_{D5} values obtained in dual cultures or to show no correlation with them, thereby indicating an additional mode of action of biocontrol agents.

3.B.v. Testing with entomopathogenic fungi

All bats including Little Brown Bat (*Myotis lucifugus*) are infected by ectoparasites such as mite, bed bug, tick and flea [40,41]. These ectoparasites impose energetic grooming costs, which might indirectly affect the survival of hibernating bats [42]. Although a direct connection between

bat ectoparasites and *G. dermatitidis* has not yet been shown, it is worthwhile to consider ectoparasites as vectors of the fungus at roosting sites [43,44]. Therefore, we propose to investigate the biocontrol potential of entomopathogenic fungi for possible application at affected sites to directly and indirectly control *G. dermatitidis* via control of bat ectoparasites. Entomopathogenic fungi attack mites, insects and nematodes [22,45]. Beside control of agriculture pests, they have shown great promise for the control of insect borne pathogens such as malaria [46,47]. The most effective entomopathogenic fungi are *Beauveria bassiana* and *Metarhizium anisopliae* [48]. We have procured standard cultures of *B. bassiana* and *M. anisopliae* from ARS Fungal Culture Collection, Cornell University. These fungi will be tested per methods detailed for *Trichoderma* and other fungi in previous sections to identify most promising agents against *G. dermatitidis*. The objective is to find out if this psychrophile is inhibited by entomopathogenic fungi directly or their application will only have indirect effect via reduction in the number of ectoparasites. Please note that we are not proposing any direct study of ectoparasites - *G. dermatitidis* in this proposal.

3.B.vii. Testing biocontrol in soil, rock and other substrate

We are collecting soils, sediments, rocks and other substrates from a number of affected sites in NY and VT as recorded in preliminary observations. Our analyses have already provided us with an estimate of presence of *G. destructans* and other fungi in these materials. They would serve as starting materials for testing of promising biocontrol fungi as a simulation of field conditions. The substrates will be divided into two parts: one will remain untreated and the other sterilized by autoclaving. They will be housed in glass enclosures with high and low humidity at 6°C and 15°C. A graded amount of *G. destructans* spores (10^7 , 10^5 and 10^3 /g substrate) will be seeded. After a week, spores of *Trichoderma* spp or other biocontrol fungi in similar graded dose will be added with thorough mixing. The mixed cultures will be followed over 4 -12 weeks with regular sampling. A dilution plating technique will be used for the culture recovery of *G. dermatitidis*, *Trichoderma* spp and other fungi. We have developed a real time PCR for estimation of *G. dermatitidis* in soil and a similar method is available for quantification of *Trichoderma* spp .[12,49]

The fate of microbes commonly found in subterranean systems -bacterial, archaeal and fungal, will be examined following application of biocontrol agent to non-sterile substrates from affected sites. This work will be carried out in collaboration with Dr. Hazel Barton using a combination of RNA and density gradient gel electrophoresis (DGGE) analyses [50,51,52].

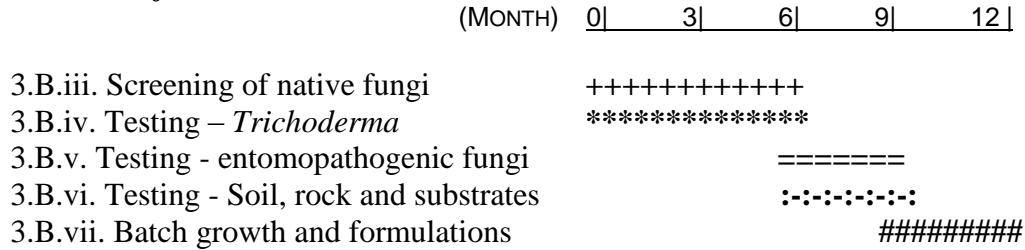
3.B.vii. Batch growth and formulations

After the identification of a promising biocontrol fungus, batch culture conditions will be standardized. These will include a defined synthetic or semi-synthetic medium, and solid or liquid culture conditions that enhance spore formation [53,54,55]. Since spore formulations also affect effectiveness in field conditions, we will attempt to process final spores with microencapsulation or pellet formation for testing with natural substrates [55,56].

3.B.viii. Statistical methods

The analysis of variance (ANOVA) will be used to determine dual culture growth rates and inhibitions with significance set at $p < 0.01$. Each experimental mean in other treatments will be compared with the control mean by Dunnett's test ($p < 0.01$). The statistical tests will be carried out using Microsoft Excel and SigmaStat software.

3. B.ix. Timeline of activities



3) C. DISCUSSION

3.C.i. Application of anticipated results and implications for disease management

This proof of concept study includes laboratory and field simulations. There is good prospect of biocontrol fungal agent (s) being validated in the laboratory for control of *G. destructans*. At the end of one-year, we will have an expert team, fungal production and tools for environmental monitoring. This should enable us to quickly conduct a field trial at site (s) in NY and VT positive for *G. destructans* if the necessary clearances are given by FWS and other agencies. We will also have ‘caver or mine in a lab’ set-up for testing of other biocontrol agents identified by FWS.

3.C.ii. Feasibility of biocontrol approaches and potential side effects

There are legitimate concerns about the unintended side effects that could result from introduction of biological agents in non-native habitats [23,57,58]. This concern is not applicable for *Trichoderma* spp as they have passed through rigorous regulatory approval process in USA and have a long track record of efficacy without side effects for soil and foliar applications [23,59]. Like all other fungi that can grow at 37°C, some *Trichoderma* spp other than the ones being tested in this study rarely cause infection in humans with server immune system disorders [60,61]. This concern is unlikely to be a major hindrance as potential application sites are all far removed from human habitation and many fungi being considered in the present study are native to soil and substrates.

3. C.iii. The relation, if any, of the project to existing research efforts

Both principal investigators have pledged substantial efforts for the investigation of WNS using many unique resources available at the Mycology Laboratory, New York State Department of Health. They have an active team in place and ongoing collaborations with the experts from other New York State Agencies. A recently funded FWS Study on systematic survey of *G. destructans* has further enhanced the research capabilities of this team. Thus, there is great synergy among existing and proposed projects on WNS - *G. destructans*.

3. C.iv. Implications for WNS investigations if this project is not funded at this time

We believe that this is an opportune time to explore the feasibility of biocontrol measures to break cycle of transmission of *G. destructans* and lower the incidence of new geomycosis cases. Our preliminary observations support detailed examination of this approach. If this project is not funded then our capabilities to expand upon these promising findings will be severely curtailed.

3) D. BUDGET

3.D.i. *The amount and source of any matching funds (matching funds are not required)*

Personnel	\$ 37,368	Supplies	\$ 10,614	Contractual	\$ 10,000
Fringe	\$ 7,922	Other	\$ 3,313		
Total (Direct)	\$ 69,217				
Total (Indirect)	\$ 26,857				
Grand total	\$ 96,074				

Details	1 st Quarter	2 nd Quarter	3 rd Quarter	4 th Quarter
Year 1	\$ 24,018	\$ 24,018	\$ 24,018	\$ 24,018

Budget Justification – The budget projection is based upon 100% efforts of one post-doctoral fellow who will assist both principal investigators in carrying out majority of laboratory experiments. The contractual arrangements have been made to collect relevant samples for this study from NY and VT including sites closed off to public. There is also a consulting arrangement in place to model impact of biocontrol agent release on subterranean microbes. The other item includes institutional mandated partial recovery of PIs' salaries. The supply budget will cover disposable laboratory supplies, chemicals and reagents for microbiology and molecular biology tests and publication charges. The indirect reflects 59.3% Federal approved rate.

Matching Funds - None.

3.D.ii. *Partner Contributions.*

Although no formal partner contributions are being projected for this effort, our colleagues from the New York State Department of Environmental Conservation are readily available for collaborations including ongoing efforts on a USFWS funded project. Similar arrangements are in effect with the Vermont Fish & Wildlife Department.

4) PI Qualifications

Principal Investigators

Vishnu Chaturvedi: Dr. Chaturvedi is a medical mycologist with more than 15-year experience as the director of Mycology Laboratory, Wadsworth Center, New York State Department of Health (NYSDOH). He worked on the association of bats with pathogenic fungi for his Ph.D. dissertation. He has published extensively on the epidemiology and ecology of pathogenic fungi, antifungals, pathogenic mechanisms and clinical diagnostics. He and his collaborators have been involved with WNS investigations since the very beginning and are currently funded by FWS to carry out systematic survey of *G. destructans* in NY and VT.

Sudha Chaturvedi: Dr. S. Chaturvedi is a medical mycologist with more than 20-year experience. Her area of expertise includes fungal genes and proteins, host-pathogen interactions, and HIV and *Toxoplasma*. Her special expertise includes the development of rapid and more accurate molecular tests for the identification of pathogenic fungi. She has developed a rapid and specific real time PCR

for specific identification of *G. destructans* in culture, tissues and soil samples. She is the co-PI of FWS Grant to survey *G. destrutans* in NY and VT.

Consultants

Alan Hicks: Mr. Hicks was formerly the bat specialist for the endangered Species unit of the NYS Department of Environmental Conservation for over three decades. His program discovered WNS, led the initial investigations into the disease, and co-hosted and organized the first national meeting after the discovery of the disease. He has partnered with Mr. Michael Cooper to form Vesper Environmental LLC for providing consulting services related to WNS, bat monitoring and conservation.

Hazel Barton: Dr. Barton is a pre-eminent cave microbiologist. She pioneered the initial decontamination protocols for WNS. She has vast experience in enumerating microbial species in subterranean environment.

Key Collaborators

Carl Herzog: Mr. Herzog is a wildlife biologist with 8 years of experience working with bats for NYSDEC. He has been closely involved with WNS investigations since the disease was first discovered and currently coordinates all NYSDEC activities related to the disease.

Scott Darling: Mr. Darling is a supervisory biologist and bat specialist for State of Vermont with extensive experience in dealing with bat research and management. He has been involved in the investigations of WNS since its first diagnosis in eastern New York.

Joseph Okoniewski: Mr. Okoniewski is a biologist with Wildlife Disease Unit of NYSDEC with extensive experience in field sampling and pathology of wildlife diseases seen in New York and adjoining States. He has played a key role in the publication describing first report of WNS in bats.

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