OMPHALINA

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Newsletter of FORAY NEWFOUNDLAND AND LABRADOR

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Feb. 2, 2015

FORAY NEWFOUNDLAND AND LABRADOR

is an amateur, volunteer-run, community, not-for-profit organization with a mission to organize enjoyable and informative amateur mushroom forays in Newfoundland and Labrador and disseminate the knowledge gained.

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OMPHALINA, *newsletter of Foray Newfoundland & Labrador, has no fixed schedule of publication, and no promise to appear again. Its primary purpose is to serve as a conduit of information to registrants of the upcoming foray and secondarily as a communications tool with members.*

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seened AT *gmail* DOT *com*,

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COVER

Sarcosoma globosum photographed by Vello Liiv in Estonia. No, this red-listed fungus does not grow in Newfoundland and Labrador. Neither does the beautiful *Hepatica* bloomimg around it. However, as the contents will demonstrate, search for this unusual mushroom may lead to other mushrooms, also not known to fruit here, some of which may. Maybe.

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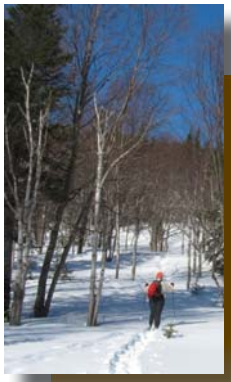
CONTENT



Message from the Editor

Q: *How do you get material for an issue? Where do ideas for a theme come from?*

A: All you need to do is sit and wait, and the ideas seek you out. Really. For example, consider this SOIL FUNGI issue. The ideas as well as the contents just landed in our lap back in 2011 through this series of three (possibly fictitious) e-mails.

June 2011
Dear L:

The rare *Sarcosoma globosum* is known from nearby Nova Scotia and Maine, but not Newfoundland and Labrador. Reputedly it prefers old growth spruce forests, and we have a foray planned into our oldest spruce forest, one never "harvested" by man. If I were to collect some soil samples, would you be willing to look for traces of its DNA? a

June 2011
Dear A:

The presence or absence of *Sarcosoma globosum* in some remote corner of the world is of such marginal interest that it hardly warrants the expense of soil sequencing. L

November 2011
Dear A:

We have received funding for an ambitious project to study global fungal diversity. Could you help by collecting soil samples from Newfoundland and Labrador? As a side benefit you'd find out whether *Sarcosoma globosum* grows in your area. L

How could we resist this backpedaling volte face? Better make the most of it, because this may be our only opportunity to participate in a study like this. Living on an island, obviously we cannot go on forever sending away part of our land mass.

The global study is now published in Science. Our lead article provides the broad brush strokes of that study. Even summarized and simplified, it may be pretty heavy going. Mostly, it gives an idea of the current direction of research in mycology. Phylogeny studies, all the rage a while back, are yesterday's news. The stage belongs to the powerful tool of statistical analysis combined with the ability to identify environmental DNA.

We asked the Principal Investigator to give us a summary of the findings for NL. He sent the entire world database, suggesting we plumb it ourselves. For an idea of the amount of data: the original MicroSoft Excel database file occupied 87,716,118 bytes. Once pruned to show only the Newfoundland findings, the file shrank to 327,478 bytes.

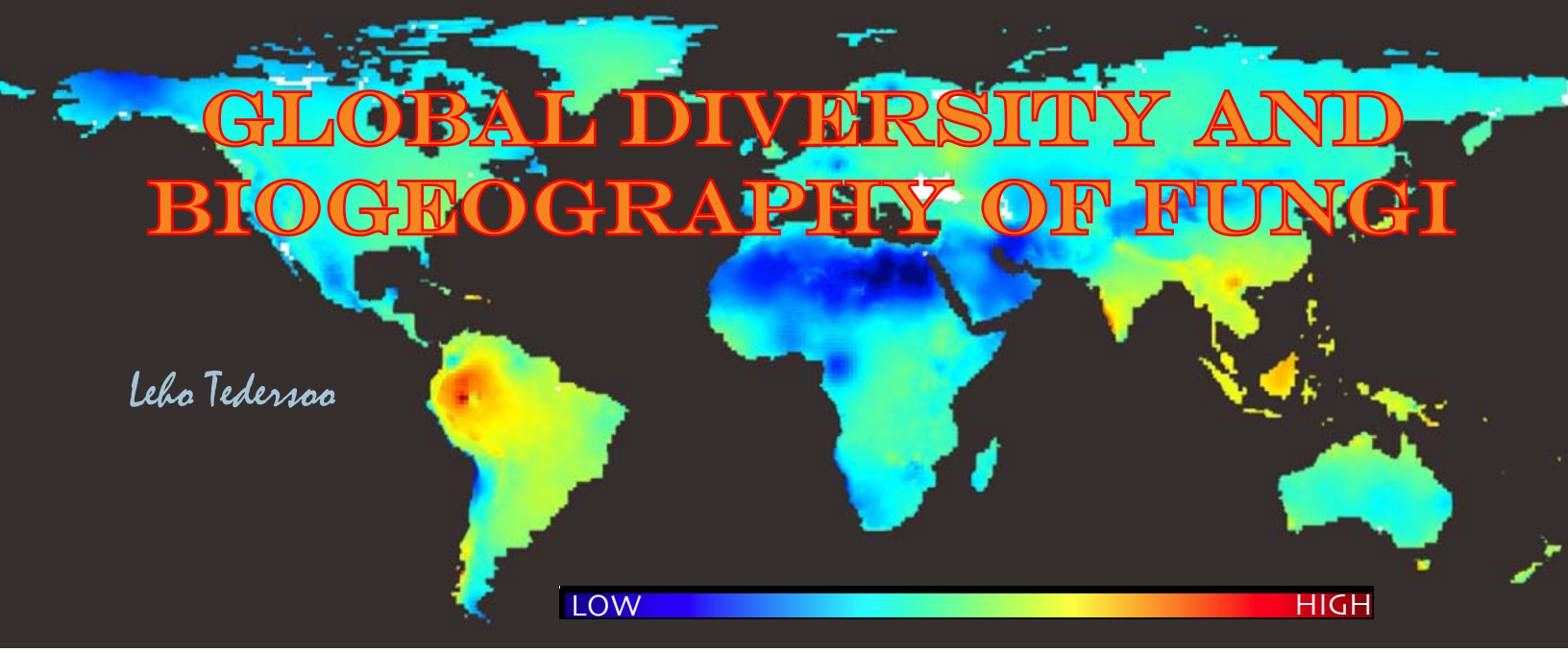
We have tried to sift through these 327,478 bytes for matters that might interest our readers about our own mycota, selecting interesting finds from our soil that have never been seen here. Even if the two soil articles are a bit of a slog, look at these mushrooms, unknown here to date, whose DNA seemed relatively common in our soil. It will be interesting to see if anybody finds them fruiting here.

OK, now you see how it is done. Sit by your computer and wait for the e-mails to come in. They will write the issue for you. All you have to do is mail it out and take the credit. Guaranteed.

Happy 2015!

andrus

GLOBAL DIVERSITY AND BIOGEOGRAPHY OF FUNGI



Fungal diversity is estimated at 1.5-5 million species, the largest group of eukaryotes after insects. Despite their enormous diversity and pivotal role in ecosystem function, little is known about the distribution of diversity and biogeography of functional fungal groups on a global scale.

This investigation aims to 1) define the relative roles of climate, soil, plant diversity, latitude and biome variables on global fungal diversity, and 2) examine biome and continental relationships based on major fungal functional and taxonomic groups.

Methods

40 soil samples from each of 365 sites across the world (see next page) were analyzed for fungal DNA. Single species sequences (44.6%) were excluded from the analysis.

Results

Overall

Over 80,000 "species" of fungi were identified; the study group was reduced to 44,563 after removing singletons. All major fungal phyla and classes, except Microsporidia were represented. The commonest soil phyla were ascomycetes (48.7%) and basidiomycetes (41.8%). Nearly 6% could not be assigned to any known class of fungi. Distribution by lifestyle was saprotrophs, 43.8%, ectomycorrhizal (ECM) mutualists, 23.3%, plant pathogens, 4.0%, other <1%), and uncertain 27.7%. Only 9.8% matched fully identified specimens with a scientific binomial.

Climate

Mean annual precipitation was a powerful predictor of fungal diversity, correlating strongly with the diversity of saprotrophs and animal parasites.

Soil

Calcium concentration or pH was the most important soil predictor of fungal diversity. ECM fungal diversity correlated best with soil pH (greatest in slightly acidic to neutral soils), and the diversity of basidiomycetes, especially agarics, correlated best with increased soil calcium concentration. Saprotrophs, especially white rot decomposers, were more diverse in moderately to strongly acidic soils. Diversity of plant pathogens declined with the soil Carbon to Nitrogen ratio. Phosporus concentration or its ratio to Carbon was also a predictor for some groups, e.g. geoglossums and mucromycetes.

Plant life

Plant and fungal diversity were positively correlated, but the plant to fungus diversity ratio decreased exponentially toward the poles (Figure 1). Major functional and taxonomic groups showed dramatic differences in latitudinal diversity distribution. ECM fungal diversity was directly proportional to the number and diversity of ECM plants.








Photos of other teams and sites around the world. Clockwise from upper left: Guyana, photo courtesy Terry Henkel; Panama, photo courtesy Meike Piepenbring; Benin, West Africa, photo courtesy of Nourou Yorou; same as previous; New Zealand, photo courtesy Gwen Grelet; Panama, photo courtesy Meike Piepenbring; Malaysia, photo courtesy Su See Lee; middle insert principal investigator Leho Tedersoo in Benin, photo courtesy Nourou Yorou. For Newfoundland and Labrador team in action, see next article.

Latitude

Fungal diversity:

- Increased/peaked toward the equator:
  - Ascomycota, in particular Archaeorhizomycetes, Dothideomycetes, Eurotiomycetes, Orbiliomycetes, and Sordariomycetes
  - Saprobes, soil fungi, plant pathogens, animal parasites
- Increased/peaked toward the poles:
  - Lecanoromycetes, Leotiomycetes, Microbotryomycetes, Mortierellomycotina, and Mucoromycotina
- Peaked in the middle temperate zones:
  - Agaricomycetes, Pezizomycetes, and Tremellomycetes
  - ECM fungi
- Greater in the Northern Hemisphere:
  - Agaricomycetes
- Greater in the Southern Hemisphere:
  - Microbotryomycetes, Tremellomycetes, Wallemiomycetes

Biomes

- Taxonomic distribution
  - The Ascomycota:Basidiomycota ratio was highest in grasslands (1.86), shrublands (1.86), and tropical dry forests (1.64), and lowest in temperate deciduous forests (0.88).
  - The diversity of moulds peaked in the tundra and was lowest in tropical dry forests.
- Functional distribution
  - ECM fungi made up 34.1% of all taxa in northern temperate deciduous forests, but only 11.9% in grasslands and shrublands.
  - The distribution of ECM fungi was similar for southern temperate forests, tropical montane forests, grasslands, shrublands, lowlands and Mediterranean biomes.
  - The distribution of plant pathogens and yeasts was similar in tropical montane forests and tropical lowland biomes.
  - Plant pathogens were more diverse in tropical forests.
- Biome distribution
  - Boreal and temperate forests shared the most fungal groups and species.

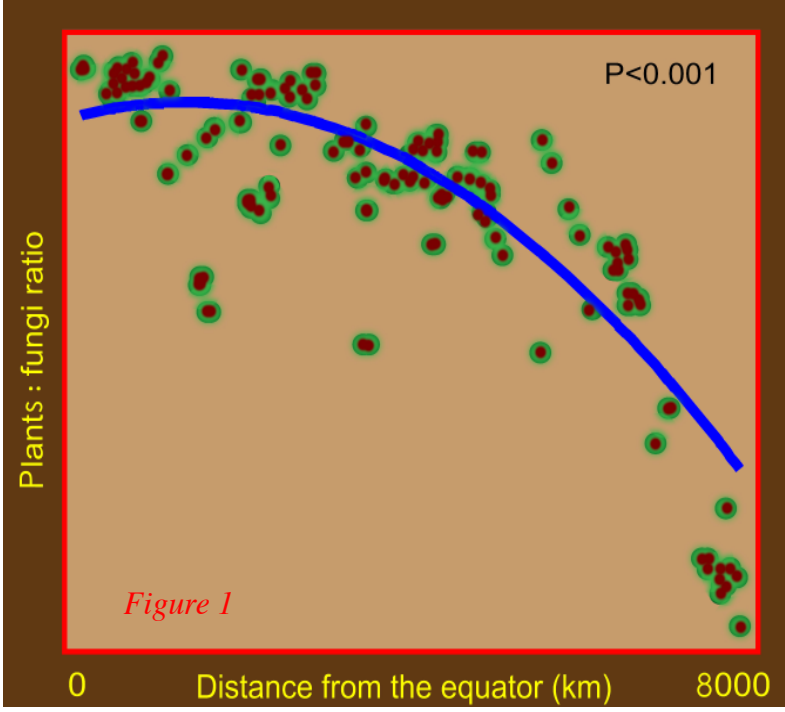


Figure 1

Multivariate analysis

Combined climate, soil and plant variables were the strongest predictors for all fungal, ECM, and plant pathogen community compositions, but had little effect on saprobe distribution, which was mostly related to latitude. Of specific variables, potential evapotranspiration (PET) and soil pH exerted a significant effect on total fungal community composition. Specifically, PET contributed significantly to the distribution of saprotrophs, plant pathogens, and yeasts. Distance from the equator and soil pH were the strongest predictors of ECM fungal community composition. Mean annual temperature and distance from the equator correlated best with variations in animal parasite and mycoparasite communities.

Cross-biome and cross-continental relationships of functional and taxonomic groups

The mean latitudinal range of fungi increased strongly toward the poles, linearly for ECM fungi and plant pathogens, and unimodally with a temperate peak for saprotrophic fungi. Diversity of saprotrophic and ECM fungi decreased with increasing latitude. Major taxonomic and functional groups of fungi differed markedly in the frequency of occurrence across sites. Saprotrophs and plant pathogens had a broader distribution range than ECM and AM root symbionts. Moulds and parasites were most widely distributed, with the greatest range for animal parasites.

Figure 2 shows the clustering of geographic zones, and biomes, as determined by shared fungal groups. Europe, West Asia, East Asia and North America clustered tightly, forming a sister group to Southern

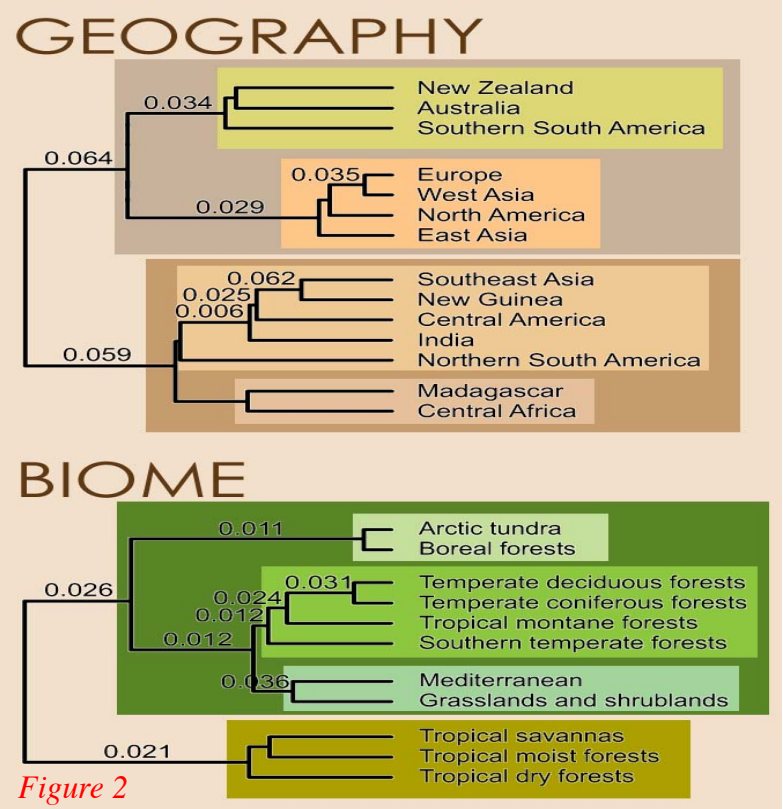


Figure 2

Hemisphere regions. Despite the large geographical distance and lack of terrestrial contact, tropical biogeographic regions clustered together.

Biogeographic clustering of regions deviated markedly for certain functional groups of fungi. Based on ECM fungi, the Southern hemisphere and tropical regions clustered together. Based on yeasts, New Zealand and Southern South America clustered with the Northern Hemisphere regions, and Australia clustered with Central Africa and Madagascar.

Discussion

This is the first study of the distribution of fungal taxonomic and functional groups on a global scale. Analysis of over 1,200 soil samples distributed globally is without precedent, revealing an unprecedented fungal diversity and several striking biogeographic and macroecological patterns. Our results are strongly at odds with current fungal diversity estimates, based on a constant plant to fungus ratio. The unprecedented size of our dataset notwithstanding, it cannot be used for reliable richness estimates. These rely on the frequency of the rarest species, recovered but a single time, which make up almost one-half of species lists, but which were rejected in our study to exclude sequencing errors.

Fungal taxonomic groups originated >150 million years ago during the existence of the Pangaea supercontinent, when they had sufficient time to disperse over all the land. Current differences in distribution patterns suggest modification by more recent factors. Favorable climatic or soil conditions may have stimulated radiation (adaptive speciation) of these taxonomic groups in some biogeographic regions. Most fungal classes responded strongly to environmental predictors; even more striking effects can be expected at the order to genus levels, due to greater niche conservatism (retention of ancestral traits) in younger groups.

These biogeographic analyses complement community-level analyses suggesting that both climate and biogeographic history shape fungal macroecological patterns. Co-migration with hosts over Pleistocene land bridges (Beringia, Wallacea, Panama, etc.) and long-distance dispersal by spores play important roles in shaping fungal distribution. Climate and associations with host plants probably contribute to differences in distribution range and biogeographic relationships among fungal functional groups.

Our analyses revealed a strong influence of host density on ECM fungal diversity, suggesting that the type of root symbiosis drives the functional composition of soil fungi. ECM associations have evolved relatively recently in multiple plant and fungal taxonomic groups. The peak of their taxonomic and phylogenetic richness in northern temperate biomes coincides with the geographical distribution and dominance of the Pinaceae, the oldest extant ECM host. AM fungi exhibit even more promiscuous associations with tree and understory species and, therefore, their diversity is expected to be causally unrelated to plant diversity. Finally, the paucity of matches to available database sequences indicates that a vast majority of soil-inhabiting fungi have remained undetected.

Conclusions

Except for ectomycorrhizal symbionts, richness of fungal functional groups is unrelated to plant diversity; plants respond similarly to climate resulting in a similar diversity pattern. The plant:fungus ratio declines exponentially towards the poles, questioning the validity of current fungal diversity estimates. Climatic factors, followed by soil and latitude, constitute the best predictors of global fungal diversity. ECM fungi differ from other groups by exhibiting a reversed latitudinal diversity gradient and strong dependence on the density of their host plants.

Dmitry Sveshnikov, Henry Mann, Leho Tedersoo, Andrus Voitk

DOES *SARCOSOMA GLOBOSUM* GROW IN NL?

This report makes a brief foray into soil samples from western Newfoundland in a global study,[1] to learn what they may divulge of local mycota.

Methods

Two habitats were surveyed with 40 samples from each: a deciduous woods (primarily *Betula*—Figure 1) leading to Barry's Lookout behind Humber Village, and two coniferous woods, one near Pasadena (primarily *Picea*) and the other near the campus of Sir Wilfred Grenfell College in Corner Brook (primarily *Abies*). Soil was collected and habitat observations made according to a standardized protocol and DNA and related analyses done as reported in the global study.[1]

Terminology

For ease of interpretation, this report makes two assumptions, which may not be true, using terms or concepts more loosely than strict scientific observation would support. 1. "Species" is used to refer to a genetically distinct entity. In the study, because all distinct DNA units were not identified to species, and those that did match an identified species may have been questionable, the original study referred to the genetically distinct entities as Operational Taxonomic Units (OTUs). 2. The relative number of recordings of ribosomal DNA is used as an indicator of frequency or commonness. Strictly, this number represents the relative amount of an organism's rDNA in the soil sample when compared to that of others, which may not be the same as the relative frequency of the organism in the soil.

Results

NL samples yielded 665 genetically distinct fungal "species," from which 129 sequences matched species named in publicly available genetic databanks. The full list is available on request to the editor.

Table 1 lists the phyla from kingdom Fungi in order of diversity. Basidiomycetes and ascomycetes accounted for 84% of the species found. Table 2 shows their distribution in the two woods, divided into saprobes and mycorrhizals. The total number of species from each woods was almost the same, but the communities were different, with only 7% of the total species found in both sites.

Table 3 lists the sequences recovered 10 or more times that matched named species.

Phylum	Nr spp.
Basidiomycota	357
Ascomycota	193
Zygomycota	85
Chytridiomycota	12
Roziellida	10

Table 1. Fungal phyla from NL soil samples in order of diversity. The ascomycete:basidiomycete ratio is low. Note that Roziellida is here considered in kingdom Fungi.

Discussion

The original study was designed to examine global diversity and distribution of fungi. Applying these data to the study of regional fungi at the species level may not be reliable. For example, blasts (techniques of matching DNA to that available on record) depend on various algorithms, each with its own strengths and weaknesses in any given situation. An algorithm suited for the purpose of the original study may select the most "convenient" sequence segment for matching, not always the closest. To be in the same clade as Species X is different from being Species X. Even if the DNA really does match fully, whether it is the species identified depends on how accurately the deposited material was identified. This is a problem that we have encountered in many of our other studies, and much more a problem when dealing with DNA from soil samples, where there are no fruiting bodies to fall back on. Less than 20% of unique DNA samples from NL matched material in public genetic depositories identified to species with a binomial name. Therefore, even if the matching is impeccably reliable, this species list sampling but 20% of recovered mycota may not be representative of the mycota of our region.

	Ascomycetes				Basidiomycetes			
	ECM	Sap-robe	Undeter-mined	Total asco	ECM	Sap-robe	Undeter-mined	Total basidio
Deciduous woods	16	63	26	106	114	51	13	178
Conifer-ous woods	16	61	30	107	113	78	15	206

Table 2. Ascomycetes and basidiomycetes divided into ectomycorrhizals (ECM), saprobes and undetermined, for both deciduous and coniferous woods. One lichenized ascomycete was classified as a mycorrhizal, and parasites were classified as saprobes (a photobiont is a photobiont, regardless of size, and organic matter is organic matter, regardless whether living or dead).

This is not a provincial study: the samples were taken from two forests (coniferous and deciduous) in a small area of Western Newfoundland, in one of the nine ecoregions of the Island. Even with 80 samples from this small region, not all regional fungal taxa are recovered. People familiar with the territory will recognize several species that have not been recovered, some of them relatively common (e.g. the total absence of *Cortinarius armillatus*, the commonest observed macrofungus in the sampled birchwoods). Given these caveats, the broader findings described under Results, may be expected to be more reliable than specific ones, as the former are more in line with the aims of the original study.

The marked difference in the funga of broadleaved and coniferous forests is interesting. We seem more ready to accept specificity in mycorrhizal relationships, but clearly the preferences of decomposers are every bit as specific, even when they are not decomposing the primary trees involved. For example, some balsam fir and spruce can always be found in our birchwoods, yet the majority of organisms from coniferous woods were not recovered from soil in the birchwoods. This bespeaks of systems with complex interdependence of their parts. The obverse is that extinction of a

Species	T	D	C
Cortinarius obtusus	402	0	402
Melanophyllum haematospermum	117	117	0
Humidicutis cf. *marginata*	70	70	0
Trichosporon porosum	43	18	25
Cortinarius duracinus var. *raphanicus*	25	0	25
Tomentella subclavigera	24	24	0
Cryptococcus podzolicus	24	24	0
Cortinarius boulderensis	22	0	22
Russula griseascens	22	0	22
Cryptococcus terricola	21	20	1
Byssoporia terrestris	20	0	20
Cortinarius acutus	19	0	19
Clavaria acuta	19	19	0
Cortinarius mucifluus	18	0	18
Lecythophora cf. *mutabilis*	12	12	0
Hygrocybe laeta	12	0	12
Piloderma sphaerosporum	11	11	0
Lactarius tabidus	11	11	0
Lactarius aff. *lignyotus*	11	0	11
Cenococcum geophilum	10	1	9

Table 3. Species matched to deposited sample with identified binomial, recorded 10 or more times (T= total, D=deciduous woods, C=coniferous woods). Specific examples of interest will be discussed in the text.

Sampling of Newfoundland birchwoods. Primarily Betula payrifera, *with 20%* B. alleghanensis, *and 10%* Acer, Abies, Picea, *plus a deciduous arbuscular understory.*

Here we see 75% of the authors (L to R: HM, AV, DS), 67% of them doing 99% of the sampling associated work.

species from its econiche may have far wider repercussions than the loss of a single species.

While the original study was not designed to identify or study individual species, several such interesting observations were seen in the data for our region. For example, the three commonest birchwoods macrofungal species were *Humidicutis* cf. *marginata*, *Clavaria acuta* and *Lactarius tabidus*. The first and last have been documented at our forays, but there is no record of *Clavaria acuta*. AV's personal collections had but one example of this "common" species.

In the coniferous forest, *Cortinarius obtusus* was by far the commonest recovered species: identified 402 times in a database where the second-commonest species (*Cortinarius duracinus* var. *raphanicus*) was identified only 25 times, and most species only once. While *C. obtusus* is seen occasionally in these woods, the fruiting body is not encountered nearly as frequently.

Clearly, there can be quite a wide dichotomy between the proportion of fruiting bodies and environmental DNA samples. Of the many other interesting finds in the data, three deserve special mention. All were reasonably commonly encountered macrofungi, in soil samples and should, therefore, be familiar, yet all bear species names we have not encountered hitherto.

Russula griseascens (99% match with 2 collections determined by Jukka Vauras) was encountered 22 times. This is a member of the *Russula emetica* complex, and since *R. emetica* was not recovered, it is conceivable that in our coniferous woods *R. griseascens* may be the commonest species of the complex.

Cortinarius boulderensis was identified 22 times (third-commonest macrofungus) in the soil samples from the coniferous forest, yet the species has been unknown here. Hitherto it has been thought to be a North American species limited to the Pacific Northwest. However, the high number of occurrences in our soil is difficult to ignore. How reliable are the matches? Well, on blast they make a 99% match with DNA from a specimen from British Columbia,[2] and the DNA of that specimen matches that for the holotype.[3] Thus, despite the potential inaccuracies of the methodology for identifying individual species, the likelihood is quite high that our soil Isolate is indeed *Cortinarius boulderensis*.

A somewhat similar situation occurs with *Lactarius auriolla*, found five times. It was described in 1984 by Kytövouri, who described a series of similar, hairy-capped, slimy, yellow *Lactarius* species.[4] The DNA of our soil fungus matches 100% with DNA found in the soil in Alaska, which, in turn, is a 99% match for DNA extracted from a specimen near the type locality, its identification confirmed by its author.[5] This makes the likelihood very high that this European

Sampling of Newfoundland softwoods. Primarily a pure Picea glauca *stand, although a small* Abies balsamea *can be seen in the foreground, on the left. One co-author examines the soil for gold nuggets (to be removed before sending for DNA), and the other fine-tunes the digger's carburetor.*

species also exists in Newfoundland and Labrador (as well as Alaska), and has been overlooked to date. These results suggest that we need to work up the large genera, *Cortinarius*, *Lactarius*, and *Russula*, in our province.

Among several other interesting observations was the finding of two real truffles, unnamed species of *Tuber*, and our cosmopolitan morel, Mel-19, in the process of being formally reported. Because the species mentioned here may represent undetected but relatively common species in our woods, or are otherwise of interest, they are described in more detail elsewhere in this issue.

This mere glimpse should give a hint at the sort of information such studies can provide. Because of potential problems with reliability due to methodology, matches need to be examined and referred to type or other reliable material, as illustrated here, before conclusions can be formed.

The power of environmental sampling and DNA analysis is readily evident: it took FNL six forays of 45-65 participants before we had accumulated 665 species. This approach, combined to the power of statistical analysis can unleash massive amounts of information; the global study from which this report is culled has barely scratched the surface of the potential of this technology.

Oh, and do we have *Sarcosoma globosum* in NL? According to this study, no. Is that certain? No. After all, soil samples were only studied from a very limited area of the province, and not from areas most likely to harbour the species. However, the entire global database contained very few samples identified as *Sarcosoma*, and none were identified to species, so possibly the technique was not ideal to answer this question in any case. These considerations aside, it is our best guess that *Sarcosoma globosum* does not exist in this province. But soil for sampling does.

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Cortinarius boulderensis Smith

Joe Ammirati

Cortinarius boulderensis Smith was described from an old growth conifer forest along Boulder Creek, Olympic Hot Springs, in Olympic National Park. Alex Smith made the type collection of this very distinctive *Telamonia* in 1941, and since then it has been documented in the Pacific Northwest from several locations scattered along the west side of the Cascades and Olympic Mountains from Washington south into western Oregon and northwestern California, and north into western British Columbia. *Cortinarius boulderensis* prefers moist, well established conifer forests of *Abies*, *Tsuga* and/or *Pseudotsuga*, sometimes also including *Picea*.

The coloration of fresh specimens in prime condition is remarkable. This medium sized *Telamonia* features a slightly violaceous brown to rich brown, moist, silky pileus with slight striations and a pale to whitish edge. The lamellae are grayish lilac at first, later becoming dull cinnamon, and the silky stipe is violaceous above becoming brownish below, and ending in a slightly bulbous base. The most striking feature is the universal veil that leaves a distinct testaceous band on the upper stipe and similarly colored fibrils or a sheath-like covering below. The medium sized spores are not particularly distinctive, but are helpful in separating it from unrelated species with similarly colored veils.

Alex Smith considered *Cortinarius boulderensis* to be related to *C. armillatus* and other telamonias with more or less reddish colored universal veils. Phylogenetic studies by Kare Liimatainen and Tuula Niskanen and have shown that telamonias with reddish universal veils represent different evolutionary lineages and that *C. boulderensis* and *C. rubrovioleipes* are sister taxa that are morphologically the same and genetically similar. Both of these taxa are related to a western subalpine species, *C. pseudobovinus*, which feature a greyish brown to fuscous universal veil.

Tuula Niskanen, Kare Liimatainen and Ilkka Kytövuori published on *Cortinarius boulderensis* in Karstenia in 2006. This prompted me to contact them about this species which resulted in a very enjoyable and rich collaboration on *Cortinarius* taxonmy and evolution, for which I am thankful.

Lactarius auriolla Kytövouri

Kuulo Kalamees

Cap: yolk yellow, deeply funnel shaped, edge inrolled for a long time, from which hang distinct whitish threads, glutenous to slimy, up to 7 cm diameter. Gills: cream to light yellow, crowded, broadly attached to decurrent. Stem: Concolorous with the cap or lighter, no or few yellowish scrobiculations, dry, slightly longer than cap diameter, cylindric, up to 1.7 cm wide. Flesh: white, cut surface quickly turns straw coloured, fruity smell, mild taste. Milk: white, turns lemon yellow immediately on exposure to air, mild to acidic taste. Sporeprint: pale cream. Spores: 7-8.5 x 6-6.5 μm, reticulate to ribbed, broadly ellipsoid. Habitat: calciphilic, in wet spruce forest. Fruits in August to September. Considered rare to uncommon in regions where it is known to fruit. Distribution: hitherto known from limestone in Scandinavia and Estonia; photos and description based on Estonian collections.

Similar species: *Lactarius scrobiculatus*, *L. leonis*, *L. tuomikoskii*. Distinguished by its prominent threads (wider than hairs) hanging from the cap edge.

Clavaria acuta Sowerby

Andrus Voitk

Clavaria acuta is a small, white, club-like *Clavaria* with a pointed tip, that usually fruits as a few scattered individuals. Its characterizing features are the relatively small size, pure white fertile surface, a grayish-translucent stem, and a non-cespitose mode of growth. The somewhat similar *C. vermicularis* and *C. fumosa* differ by being larger, having a hollow fruiting body, being without a transparent stem and growing in cespitose clusters. Microscopically these latter two are alike, but in colour *C. vermicularis* is white like *C. acuta* and *C. fumosa* is off-white, varying from gray, straw to pinkish tones. *Clavaria vermicularis* has not been recorded by FNL, nor was its DNA recorded in the soil study. This means that it may or may not exist in NL.

There were seven big (about 20 cm tall) and four small clubs in a lawn near the soil sampling site, but, of course, I had to photograph the only uncommon shape, the forked one, with the main branch broken. The common shape resembles single, pointed spindles, the pointed tip giving rise to the species epithet, acuta. Probably the species is commoner than thought, but not often recognized because of its small size and scattered growth habit. Finding at least one collection in the region of the soil samples increases the likelihood that this was a reliable finding.

Roger Smith's photo of *C. fumosa* comes from the fields around Killdevil Camp in Gros Morne National Park, site of our 2014 foray. It has also been collected from Sir Richard Squires Memorial Provincial Park, site of the 2014 mycoblitz. The cespitose growth pattern and off-white, smoky colour are evident. Comparing the moss on the two photos also gives an idea of the size difference between the two species, *C. fumosa* being at least three times taller. In addition, there are microscopic differences.

Russula griseascens (Bon & Gaugué) Marti

Andrus Voitk

Russula emetica is a common, relatively small, beautiful, red-capped *Russula* with a very acrid taste, famous for inducing vomiting in the mycophagist foolish enough to eat it. Although found in moist areas of coniferous forests, it is suspected to be mycorrhizal with birch, not conifers. It forms a species complex with several similar species. Among these we have identified *R. aquosa* and *R. nana*.

In 1975 Bon and Gaugué described *Russula emetica* var. *griseascens*, characterized by graying of the stem with age, trauma or handling. It was elevated to species status by Marti in 1984. Russulales news states that in the Alps this is the commonest species of the complex. At our foray, *R. griseascens* was identified from Fogo Island in 2013 because nuclear studies showed the closest match to a named species was at 97.5% to two Finnish collections identified as *R. griseascens* by Jukka Vauras. In the soil samples the third commonest identified species in our coniferous woods matched those same two Vauras collections at 99%. At the same time, there is no record of DNA marching *R. emetica*. Likely the soil fungus and the Fogo fungus are the same species, and provided Jukka Vauras identified the species of Bon and Gaugué, both are *R. griseascens*.

I reviewed all photographs of *R. emetica* from past forays. Five of nine photos showed mushrooms with some graying on the stem. The composite photo, above, shows the genetically matched *R. griseascens* from 2013, as well as three photos of *R.* "emetica" with similar graying stems. What does this mean?

Scientifically, nothing. All material needs to be sequenced, analyzed and matched (or not) with a reliable reference collection, before a definitive conclusion can be made. However, what is the likely implication? Given the prevalence of putative *R. griseascens* in our soil samples, the absence of *R. emetica* in the same samples, the genetically confirmed *R. griseascens* of 2013, and the presence of similar gray stems on photos of most collections labelled "R. emetica", there is a very real likelihood that *R. griseascens* grows in Newfoundland and Labrador. Our diversity here is low and usually we do not have several similar species occupying the same econiche. Therefore, *R. griseascens* may be our commonest member of the *R. emetica* complex, possibly hitherto misidentified as *R. emetica*, which may not even grow here. Of course, we may also have both, but our low diversity and the absence of DNA from *R. emetica* in the soil argues for one species only.

Until the question is confirmed, the above must be viewed as speculation only. As is the tenet that *R. emetica* grows here. To me the latter speculative position seems considerably less likely. Would we be more likely to be accurate, were we to list anything identified as *R. emetica* as *R. griseascens* until more clarity is brought to the question?

Morchella sp. Mel-19

Andrus Voitk

Photo: Vello Liiv

We have documented three morel species in Newfoundland and Labrador: *M. importuna* (the mulch morel), and two undescribed species, temporarily code named Mel-36 and Mel-19.[1] In addition, the DNA of *Morchella conica* was reported from the soil samples we submitted for study; this piqued our curiosity. *M. conica* is a European taxon, described by Persoon in 1818, reputedly not native to North America.[2] Current opinion holds that it is not a valid taxon at the species level.[3] Since *M. conica* is not a valid species and not found on this continent, and since we have not found other morel species after a decade and a half of collecting, it is most likely that the DNA found in our soil is that of one of the three species we have already identified.

DNA from soil is identified as a certain "species" by matching it up with DNA on deposit in depositories, where investigators have filed DNA sequences of collections they have identified. If soil DNA matches that of a collection, it is assumed to come from the same species as was identified by the depositor. The DNA from our soil matched that from a collection identified as *M. conica* that came from limestone barrens of an Estonian island. The photo in the title banner is of that collection, taken by Vello Liiv, whom foray veterans may remember as a member of our first faculty in 2003.

If *M. conica* does not exist here, there is reason to suspect that the reference collection may be misidentified. To test this, Kerry O'Donnell ran the DNA on file for the Estonian collection against known morel DNA sequences. On preliminary analysis, the Estonian collection matched Mel-19. Mel-19 is also known from Washington State, Newfoundland and Labrador, Scandinavia, The Netherlands, and various parts of China[4]. The match was so good, and the other evidence fit so well, that we predict that multilocus analysis will likely confirm Mel-19 in Estonia. Similar DNA was also recorded from another collection in the same area (photo, also by Vello, next page).

This fits well, because we know Mel-19 grows in Newfoundland and Labrador. Finding it in Estonia is also not surprising, because the species is known from nearby Scandinavia. Finding it on limestone barrens fits with our experience, because the majority of our collections of Mel-19 also come from this habitat.

This experience illustrates the difficulty of identifying an organism by matching its DNA with that on hand in gene depositories: the accuracy depends on the accuracy of the identification of the deposited collections. One review revealed that only 33% of morel collections that were deposited with a species name in GenBank were identified correctly.[5] In other words, if you were a betting woman, you could win 2:1, if you just bet that every named morel in GenBank is misidentified.

Why such seeming unreliability? For one thing, Mel-19, although known from around the world, has not been described or named yet. Thus, most workers are not able to consider it in their identification. As for the described species, identifying often relies on a morphological species concept. This does not work

well with the genetically discovered morel species: with a few distinctive exceptions, most members of the Elata clade resemble each other morphologically both on the macro- and microscopic level, and cannot be separated morphologically. Multilocus genetic analysis is required to identify them reliably, not available to everybody collecting and depositing material.

The species concept we prefer today rests on evolutionary genetic lineage. We have no idea of the genetic sequences of the specimen Persoon held in his hand in 1818, when he described *Morchella conica* as a new morphological taxon (or the one Fries held in his hand when he described *Morchella elata* as a new morphological species four years later). Those type specimens are too old to yield useful DNA for the sophisticated analyses required to determine this. Genetic research over the past two decades, correlating morphologic and other characters with gene sequences, has culminated in a detailed study, where species have been described again and new type collections with good genetic material have been "appointed" to support them.[3] This work should go a long way to stabilize the situation for future investigators. Thew study was unable to define the *M. elata* of Fries, and work to elucidate this is still going on. It is not impossible that our Mel-19 could be defined as that species.

Meanwhile, to our knowledge, all morel species are desirable edibles. For the morelophagist, identification of morels to exact species may be irrelevant.

Unless she becomes curious, when edibility may become irrelevant.

Acknowledgments

I thank Kerry O'donnell for the DNA matching described above, as well as review of the MS, and Vello Liiv for permission to use his beautiful photographs.

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Seeking the elusive Newfoundland truffle

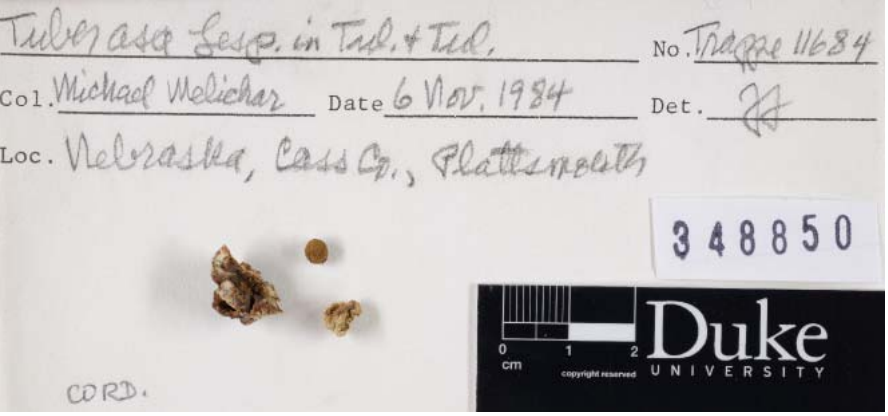
Andrus Voitk

Photo: Maria Voitk

Among interesting fungal DNA samples recovered from our soil were two undescribed species of *Tuber*, *Tuber* sp. 16 from our coniferous woods and *Tuber* sp. 19 from our birchwoods. *Tuber* is a genus of hypogeous, or underground ectomycorrhizal ascomycetes, including the famous and expensive gourmet truffles, as well as some 100 species world-wide, about half in North America.[1] Here the famed edible ones are few, mostly limited to the Pacific coast, although *T. lyonii* s.l. and *T. canaliculatum* are of some commercial interest in the east.

Many of the others are only known from genetic material recovered in environmental samplings, and the fruiting organism has not been seen. Not only are they underground, many are also very small, so they go unnoticed by most hypergeous observers of macrofungi. Therefore, there are many genetic species that have not been seen to describe them, and no doubt many additional species remain to be uncovered.

Our truffle species known hitherto, both real and false, have been reviewed before.[2,3] Species of *Tuber* were not among them, and to our knowledge species of this genus have not been recorded for the province. Therefore, finding the DNA of two *Tuber* species in our soil was definitely of interest. Checking with Greg Bonito, who reviewed the genus and its phylogeny world-wide,[1] we learned that fruiting bodies of these two numbered species have not been seen, preventing formal descriptions. The DNA of *Tuber* sp. 16 has been recovered from environmental samples in Québec, New York State, Michigan, and California; the Michigan sample came from the roots of *Epipactis helleborine*.[4]



Above: Photo of the only known collection of Tuber *sp. 19, with permission from MycoPortal and Duke University, Rytas Vilgalys. Below: Close-up and inflorescence of* Epipactis helleborine *in our birchwoods, where this introduced species has made itself quite at home. Not as invasive here as in parts of the mainland, it may serve as an indicator of some of our underground* Tuber *species.*

The DNA of *Tuber* sp. 19 has been recovered from *Epipactis dunensis* in Austria, and identified in other environmental samples from Germany, Lithuania, Sweden, England, New England, California and New Zealand.[4] An unidentified collection of tiny truffles from Nebraska was identified by sequencing to be this species (illustration).

The association of *Tuber* with *Epipactis* has been noted regularly. *Epipactis helleborine* (illustration) is a European orchid, introduced to North America, where it seems to have established itself quite well. A decade ago, in Newfoundland it was only known from a site in St. John's, but now it has spread; there are several pockets in the birchwoods near our house, where it thrives happily. Orchids have such small seeds, consisting of genetic material only, that to grow they need to get outside nourishment. This is supplied by a mycorrhizal fungus, and many are quite specific. One of our soil samples with *Tuber* DNA came from the birchwoods where *Epipactis* has settled.

Jess Rumburg, one of Tom Horton's students, showed that *Tuber* sp. are the commonest mycorrhizal fungi found on *Epipactis helleborine* in New York State.[5] With this in mind, before the 2014 Foray we explored the earth around one patch of *E. helleborine*. The digging was done by lichenologists Michele Piercy-Normore, Teuvo Ahti and Chris Deduke. No luck. Lacking a proper search image, finding 4 mm brown pebbles in duff may be an unfair task even for non-lichenologists.

This article serves as a tribute to Teuvo (Ted) Ahti on the occasion of his 80th birthday a few months before the title banner photo was taken. The much younger Chris Deduke seems to have completely collapsed in the background, toes up, but, like the Eveready bunny, Teuvo just smiles and keeps on truffling.

Congratulations, Teuvo!

Acknowledgments

I thank Greg Bonito for reviewing the MS.

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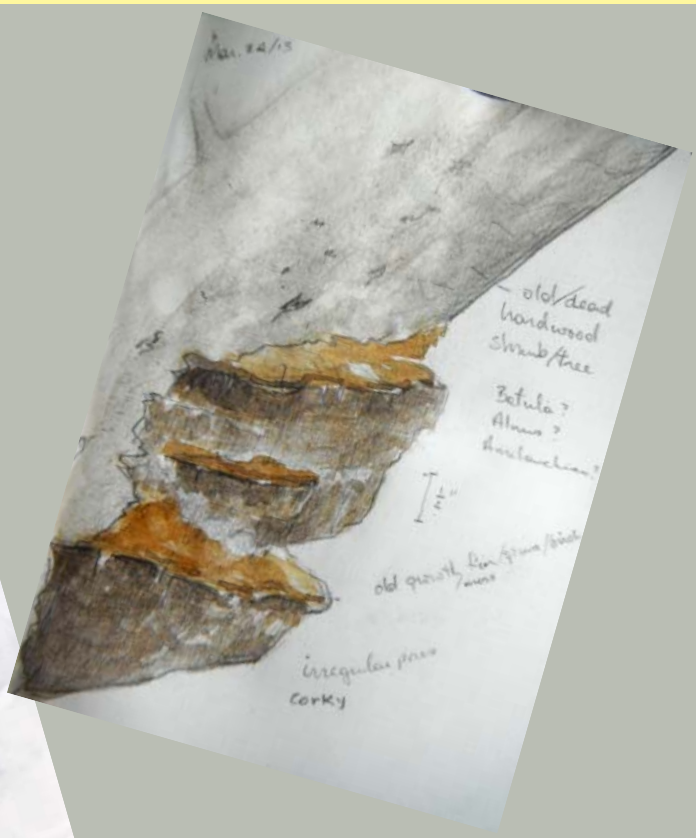
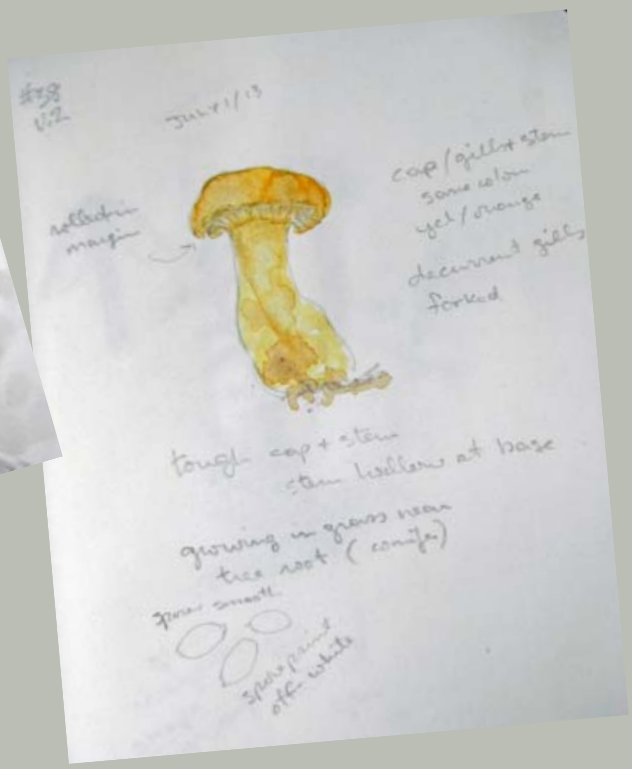
The Bishop's Sketchbook





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Today (Nov 28, 2014) is the 10-th anniversary of the day when my wife Oluna Ceska started her macrofungal survey on Observatory Hill (Victoria, Vancouver Island, British Columbia). After 10 years and close to 400 visits to "The Hill", Oluna recorded 1,298 species from an area of about 75 ha (approx. 185 acres). A part of those collections (several hundred earlier collections of *Cortinarius*, *Inocybe*, *Russula* and some others) have been DNA sequenced and deposited in GenBank, and some were studied and published. Observatory Hill collections of several species have been directly cited in the original descriptions of some of the species listed here: http://mushroomobserver.org/species_list/show_species_list/708. The majority of the Observatory Hill collections were donated to the UBC herbarium where the collections have been accessioned or are awaiting accessioning. Many of those collections have been already been included in the UBC Fungi Herbarium database http://www.beatymuseum.ubc.ca/herbarium/index.html, and mirrored in the Consortium of the Pacific Northwest Herbaria http://www.pnwherbaria.org/ "Virtual herbarium", created in Mushroom Observer. It contains photos, microphotos, and drawings of about one third of all our Observatory Hill specimens: http://mushroomobserver.org/species_list/show_species_list/227. Partial results of the survey have been summarized in annual reports deposited on the GOERT web site: http://www.goert.ca/activities/2013/05/macrofungi-observatory-hill/. This project was partially supported by the Herzberg Institute of Astrophysics, GOERT (Garry Oak Ecosystem Restoration Team), and private donors. Cataloguing of the collections has been supported by the Canadian Wildlife Service. Our special thanks go to Dr. Paul Feldman, HIA astronomer, who initiated the project and provided all the help we needed.

Adolf & Oluna Ceska, Victoria, British Columbia, Canada
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Ed comment: Congratulations on a phenomenal team effort! A suitable contrast to seeking fungi in soil samples…

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