Non-Vascular

Plant Inventory

In Langley BC, Canada: Glen Valley Blaauw ECO Forest



Beth Guirr April 23, 2015 Dr. Clements Directed Study

Abstract

This project examined the pattern of nonvascular plant diversity, with more attention to bryophytes, within Blaauw Eco Forest, Glen Valley, British Columbia, Canada. The study provides a better understanding of the inner distribution ecology of bryophytes, and the relationship between sensitive species and their habitat specifications; which may offer insight that can be used to minimize the impact of forestry and peat mining operations on microbiological diversity. Patterns of non-vascular species were observed according to different locations within the forest. It was observed that many factors affected the pattern growth within the varying areas of Blaauw Eco Forest. At a small scale, the type and number of microhabitats were an important predictor for the type of species present. The detailed examination of bryophyte identification and habitat observation represent an ecosystem health at a microscopic level. The evaluation of water quality, habitat species, and bark pH throughout different sects of the forest showed the importance of microhabitat requirements for nonvascular diversity. This project complemented previous work of Curtis Abney in identifying vascular plants in the Blaauw Eco Forest, and continues to contribute towards a knowledge of the forests flora and fauna distribution. By also observing and identifying vascular plants, animal species, and mapping coordinates, this project provides a new insight into the basic ecology of Blaauw Eco Forest. With the already observed findings, this forest has already been saved from peat mining, and Northern Red Legged frog (Rana aurora) protection; and hopefully will assist future research.

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Introduction

The Coastal Western Hemlock Zone (CWH) stretches along the coastal mountains of British Columbia, all the way from Alaska to Washington, Oregon; and eastward into the valleys of the inner coastline (Pojar *et al.*, 1991). British Columbia has over 850 species of mosses and hepatics, thereby making it one of the greatest range of bryoflora in North America (Arsenault, 2000). Its vast diversity in non-vascular plants makes it the richest in endemic species and genera; with some only found in these areas of the world (Arsenault, 2000). The CWH zone of British Columbia, is generally the rainiest biogeoclimatic zone in the province (Pojar *et al.*, 1991). The climate ranges through cool summers (with periodic hot and dry spells), and mild winters (Pojar *et al.*, 1991). The average yearly temperature for the entire CWH zone is 8°C, with annual precipitation at 2.23m (Pojar *et al.*, 1991). These climate characteristics provide the preferred ecosystem for western hemlock, sparse undergrowth and herbs, and a large variety of moss species (Pojar *et al.*, 1991).

With climate changes comes the change of chemical and biological differences in biota (Turetsky, 2003). Plants are critical as regulators within our biosphere; they are primary producers of which control the exchange and absorption of gases, and further affect the distribution of energy to higher levels of trophic organisms (Turetsky, 2003). Non-vascular plants are a very important aspect of a forests biodiversity. Bryophytes may appear to be small in significance when compared to vascular plants, but in actuality they are roughly equal; in any ecoystems the biomass of non-vascular plants may be equivalent to, or even exceed that of vascular plants (Reader and Steward, 1972; Epstein *et al.,* 2008). As a result of their unique

physiology and ecology requirements, bryophytes are separated from vascular plants due to their processing cycles for energy and water (Turetsky, 2003). Bryophytes lack a vascularized system, which ultimately influences their ability to handle water stress- where they can rehydrate and dry out faster than vascular plants (Turetsky, 2003). The entire plant surface therefore absorbs solutes and water with ease. This ultimately allows bryophytes to act as an effective trap for water and nutrients, but makes them incredibly sensitive to a change in atmosphere and environment (Turetsky, 2003). Further, they have the ability to tolerate a range of temperatures in both terrestrial and aquatic locations (Fogg, 1998; Seppelt 1995). They lack a root system, which allows them to colonize a variety of habitats, such that of rock and wood (Turetsky, 2003). The ability to colonize on relatively any surface enables them to stabilize soil deposits and further prevent erosion (Martinez & Maun, 1999). This ability to grow within an extensive range of habitats, makes non-vascular plant presence critical within every ecosystem.

Non-vascular plants partake in an important role for terrestrial and aquatic habitats (Belnap and Lange, 2001; Eldridge *et al.*, 2003). They frequently act as pioneering species, where they help protect and strengthen the integrity of soil- particularly after disturbances such as flooding or fires (Eldridge *et al.*, 2003). Bryophytes and liverworts are significant for fixing atmospheric and soil compounds, such that of nitrogen, carbon and phosphorus (Eldridge *et al.*, 2003). Non-vascular plants moderate the levels of ground moisture, and thus influence the potentiality of germination and vascular plant establishment (Eldridge, *et al.*, 2003). This ability to balance soil moisture and chemical component creates and establishes desirable habitats for a range of invertebrates (Eldridge *et al.*, 2003).

While bryophytes and liverworts maintain the structure and moisture levels of soil, fungi are important for processing and decomposing organic matter. Forest management is very dependent on the fungal community because they largely influence soil nutrients (Tanesaka *et al.*, 1993). Fungi recycle carbon in the process of decomposing wood, tree litter, or through a mycorhizal symbiont (Tanesaka *et al.*, 1993). The role of fungi are essential for ecosystem maintenance; within the soil, they promote water infiltration, nutrient exchange, nitrogen fixation, increase water-holding capacity, and create a habitat for mycorrhizae relationships (Tanesaka *et al.*, 1993).

Despite the evident importance of non-vascular plants, there has been little to no attempts of surveying their population and species when monitoring environmental ecosystems (Eldridge *et al.*, 2003). Unfortunately the reason for this is due to plant size; identifying them requires taxonomic microscopy and chemical tests (Eldridge *et al.*, 2003). The purpose of this study is to examine the diversity and any relevant patterns in the non-vascular community in at Blaauw Eco Forest.

This forest runs along the Fraser River in Glen Valley from Langley British Columbia, Canada. Blaauw Eco Forest is a thirty acre parcel donated from the Blaauw family in memory of Mr. Thomas Blaauw to Trinity Western University. It is a mixed coniferous and deciduous forest, with ponds, springs and a bog. Trinity Western University research within the forest began in 2013 when Abney (2014) and Loubser (2014) completed undergraduate thesis projects. Abney (2014) focussed on vertebrate animals and vascular plants, and only inventoried select nonvascular plants; Loubser (2014) studied two species blue-listed in British Columbia, the Northern Red Legged Frog (*Rang aurora*) and Pacific sideband snail (*Monadenia fidelis*). While the bio inventory of the forest by Abney (2014) encompassed some of the biodiversity, it is critical to also study non-vascular plants including bryophytes, liverworts, slime molds, and fungal species. In the present study, these different divisions of non-vascular species were observed for different substrates (rocks, trees, ground, etc.) and different areas within the forest. Special attention will focus on the bog, as the environment is different with the variance in pH, plant species, and nutrient availability. Within this project, the goal is to identify as many non-vascular plants (with more focus to bryophytes) as possible. The study will examine nonvascular plant habitat; and specific ecosystem requirements of various nonvascular plants to determine how well the Blaauw Eco Forest is providing for these specific niches. This study will also develop insight into human impact upon forests, and methods of decreasing potential future human invasion.

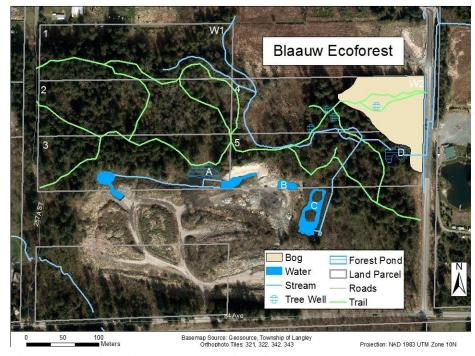
General Objectives:

- Develop non-vascular species inventory list within the forest, according to different macro and microhabitats.
- Observe the patterns flora and fauna diversity (with more focus on nonvascular plants), throughout the different regions of the forest.
- 3. Compare flora composition and affinity for species-habitat relationships

Materials and Methods

Field Experiment:

This experiment ran for the duration of three consecutive semesters and three months of the summer (2014-2015); with at least five hours a week spent on the research. The field experiment consists of qualitative analysis and in the future, quantitative





analysis. The focus is on the non-vascular plant inventory throughout the entirety of Blaauw ECO forest. Samples of moss and liverwort have been collected into brown paper bags; and the surrounding micro and macrohabitat are noted, along with where in the forest it was found, date, temperature, canopy coverage, and general surrounding environment. These samples will then be taken to lab to dry out and package into a mini herbarium, and later identified. Samples will be taken from both ground and trees, and as extensive as possible. A photo of the sample will be taken so it can be compiled into a document later, so it can be seen in its natural habitat.

Fungal samples were taken from the forest, wrapped in parchment paper, and placed into a paper bag (procedure in appendix). The habitat, location within the forest, date, and

temperature were noted, along with any key species nearby. These were immediately taken to lab where they can be further analysed.

Slime molds were also observed as extensively as possible through qualitative observations, along with photos for further identifications. If possible, samples will be taken to lab for spore observation.

Further animal observation were studied through the use of animal trap cameras set up weekly throughout different places in the forest. As well, non-invasive forest maintenance proceeded throughout the journey of this research. This is a public forest, for the community to walk about and enjoy. Therefore, basic trail clearing, invasive species removal, and trash removal was a mandatory phase in the research.

Water quality tests were observed with specifics to pH, turbidity, dissolved oxygen, nitrate and phosphates tests, and temperature. Five samples were taken per location, which provided a standard error bar. Water pH was tested in the summer of 2014, winter of 2014 and spring of 2015.

The YSI Model 55 Handheld Dissolved Oxygen System measured the amount of gaseous oxygen that is dissolved in the water. Oxygen concentration can be increased through plant photosynthetic processes where they produce oxygen during the day, but retain and consume oxygen during the night to breathe (Bainard *et al.* 2012). In order to test oxygen content in water the meter has a probe that is immersed in the water and produces an electric current that reacts with oxygen. The oxygen diffuses into the probe and allows readings to be taken for concentration in that given area. Turbidity is the amount of suspended particles that are found in the water; whether these particles are sediments, microscopic organisms, or pollutants. When there is a large amount of turbidity, the light penetration through water lessens and can affect photosynthetic organisms; which constitutes water quality (Bainard *et al.* 2012). To calculate turbidity, water samples were taken in a vile and placed in a Hach Model 2100P Turbidity Meter. When the water sample is measured, light passes through the sample and calculates how much light scatters and is absorbed by the floating materials.

When determining what a healthy stream qualifies for, pH is a large factor and can express what organisms can be supported in the area. Typically a pH between 6.0 and 8.5 is a range where aquatic organisms prefer (Bainard *et al.* 2012). The pH in a stream can be affected by agricultural surroundings, mineral content, vegetation, algal blooms and precipitation (Bainard *et al.* 2012). The instrument used to collect pH samples was Hach HQ30D pH meter and a PHC101 pH probe. Connected to the meter is a probe that is held in the water, and uses an electrical current to determine the hydrogen ion activity in the sample (Bainard *et al.* 2012). There is a bulb at the end of the probe that is rinsed with deionized water before taking a sample, as to ensure accurate results.

Lab Work:

The moss and liverwort that has been dried out, were rehydrated by using a beaker filled with tap water and a hot plate to heat the water. The sample was placed into the beaker and rehydrated for approximately fifteen seconds. After it has been removed from the beaker it will be placed onto a glass slide where it can be viewed under a stereoscope. Distinctive features were noted. Then a single leaflet (frond/blade) is removed from the sample, placed onto a second slide with water, and a coverslip placed on top. Then utilizing a compound microscope, the sample is observed for distinctive features to help identify. A photo is taken, to be used for reference for its identification. Using dichotomous keys and several identification guides, the sample is identified through its appearance. See appendix for further details of identification.

Fungal identification will begin with basic morphology, size, and smell analysis. Then the cap is placed on a glass slide, with the gills faced down. This will allow the spores to fall out in a pattern that can be observed for colour, size and further microscopic details. Using a dichotomous key (with specifics to <u>http://s158336089.onlinehome.us/lan/</u>) to finalize the identified species. See appendix for further details for taxonomizing and collecting.

Further water quality was tested using nitrate and phosphate tests. Nitrate is an inorganic form of nitrogen and is found in decaying biomass, calcareous sedimentary landforms, human sewage and fertilizers (Bainard *et al.* 2012). Typically nitrogen cycles are influenced by bacterial, fungal and microbial metabolism (Bainard *et al.* 2012). This study used a cadmium-reduction method of determining the nitrate water quality. Samples were taken from the water bed and brought back to the laboratory. Using a Hach DR/2400 spectrophotometer, light passes through the samples at specific wavelengths and calculates the proportional absorption to the colored reaction products that are pre measured and given. Before testing the sample, shaking the liquids together is important to make sure that they emulsify into one complete mixture. After mixing the sample is to sit for five minutes, while the

spectrophotometer calculates a control vial. As the control is calculated the nitrate sample is recorded and calculated by subtracting any colour or turbidity from the test reading.

Phosphate is a dissolved inorganic component of phosphorus called orthophosphate which is found in organic matter like bogs, vertebrate waste, and phosphatic rock (Bainard *et al.* 2012). It is unnaturally found in urban and industrial areas through fertilizers, sewage and cleaning detergents (Bainard *et al.* 2012). To calculate phosphate levels, a Hach DR/2400 Spectrophotometer was used by an ascorbic acid-molybdate method. Similar to nitrate testing, water samples were taken and returned to the laboratory and mixed with a prepared reagent packet, within a 10mL of water sample in a glass vial; which provided phosphate levels. Analysis:

Using a template created for identifications, details of each sample will be noted and compiled. A dropbox account will be made so that all information can be placed into files that can be accessible to any computer or individual working on this project. Photos will be added to these documents for future reference of species identification, and as a comparison for other samples in the future. A document will be formed at the end with all the species identified. The template for identification is found in the appendix.

Results:

Table 1: The total number of different species representing each taxonomic group of nonvascular plants, along with the total number of nonvascular flora discovered to date

Non-Vascular Plant/Organism	Number of Species Identified
True Moss	41
Liverwort	12
Lichen	5
Fungi	22
Slime Mold	6
TOTAL COUNT:	86

True Moss: Genus	, species
1. Antrichum selwynii	2. Leucolepis acanthoneuron
3. Antrichum undulatum	4. Metaneckera menziesii
5. Atrichum undulatum	6. Mnium rostratum
7. Buckiella undulate (Abney, 2014)	8. Neckera douglasii
9. Brachythecium asperrimum	10. Orthotrichum lyellii
11. Calypogeia muelleriana	12. Plagiochila porelloides
13. Claopodium crispifolium	14. Plagiothecium undulatum
15. Dichodontium pellucidum (Abney, 2014)	16. Plagomnium insigne
17. Dicranum scoparium	18. Platydictya jungermannioides
19. Dicranum tauricum	20. Rhizomnium glabrscens
21. Ditrichum heteromalla	22. Rhizomnium gracile
23. Eurhynchium praelonga	24. Rhizomnium magnifolium
25. Eurhynchium oreganum	26. Rhytidiadelphus loreus
27. Hookeria lucens	28. Rhytidiadelphus triquetrus
29. Hylocomnium splendens	30. Sphagnum palustre
31. Hypnum cupressiforme	32. Sphagnum squarrosum
33. Hypnum revolutum	34. Sphagnum tenellum
35. Hypnum subimponens	36. Sphagnum fuscum
37. Isothecium stolonifera	38. Sphagnum capillifolium
39. Kindbergia praelonga	40. Sphagnum angustifolium
41. Aulacomnium androgynum	42. Isothecium myosuroides

Table 2: List of bryophyte species documented in the Blaauw ECO Forest, according to their respective taxa



Figure 2: Sphagnum squarrosum



Figure 3: Rhizomnium gracile

Table 3: List of liverwort species documented in the Blaauw ECO Forest, according to their respective taxa

1. Bazzania Gray	2. Pellia neesiana
	2. / Сла несэнана
3. Bazzania trilobata	4. Plagiochila porelloides
5. Calyogeia sphagnicola	6. Porella navicularis
7. Conocephalum conicum	8. Preissia quadrata
9. Calypogeia muelleroama	10. Pseudotaxiphyllum elegans
11. Lepidozia reptans	12. Scapania bolendari



Figure 4: Porella navicularis



Figure 5: Porella navicularis

Table 4: List of lichen species documented in the Blaauw ECO Forest, according to their respective taxa

Lichen: Genus, species				
1. Cetrelia cetrarioides	2. Parmelia flaventior			
3. Cladonia chlorophaea	4. Parmelia sulcata			
5. Leparia incana				





Figure 7: Cladonia chlorophaea

Figure 6: Leparia incana

Table 5: List of slime mould species documented in the Blaauw ECO Forest, according to their respective taxa

Slime Mould: Genus, species				
1. Fuligo septica	2. Ceratiomyxa fruticulosa			
3. Stemonitis fuscka	4. Physarum polycephalum			
5. Arcyris denudata	6. Metatrichia sp.			



Figure 8: Stemontitis fuscka



Figure 9: Metatrichia sp.

Table 6: List of fungi species documented in the Blaauw ECO Forest, according to their respective taxa

Fungi: Genus, species					
1. Basidioradulum radula	12. Lycoperdon pyriforme				
2. Chlorociboria aeruginascens	13. Morchella esculenta				
3. Clavicorona pyxidata	14. Nidula candida				
4. Clavuina cristata	15. Pholiota Squarrosoides				
5. Clitocybe fragrans	16. Pleurocybella porrigens				
6. <u>Cyathus striatus</u>	17. Pseudohydnum gelatinosum				
7. Dacrymyces palmatus	18. Ramaria formosa				
8. Daldinia concentrica	19. Trametes versicolor				
9. Exidia recisa	20. Tremella mesenterica				
10. <u>Fomes fomentarius</u>	21. Tremiscus helvelloides				
11. <u>Galerina pumila</u>	22. Xylaria hypoxylon				





Figure 10: Exidia recisa

Figure 11: Nidula candida

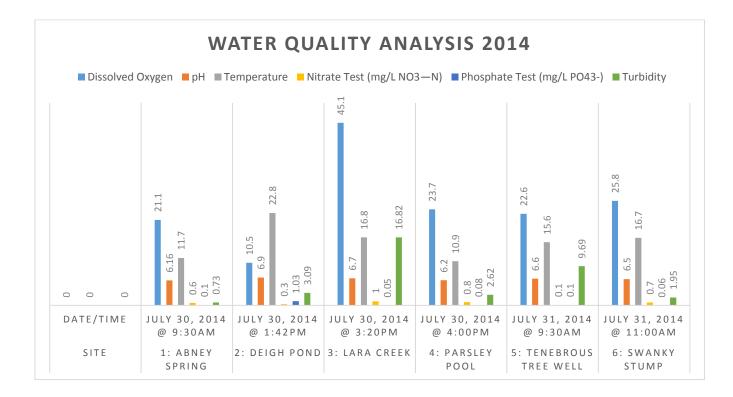


Figure 12: Water quality analysis of the Blaauw ECO Forest in late summer.

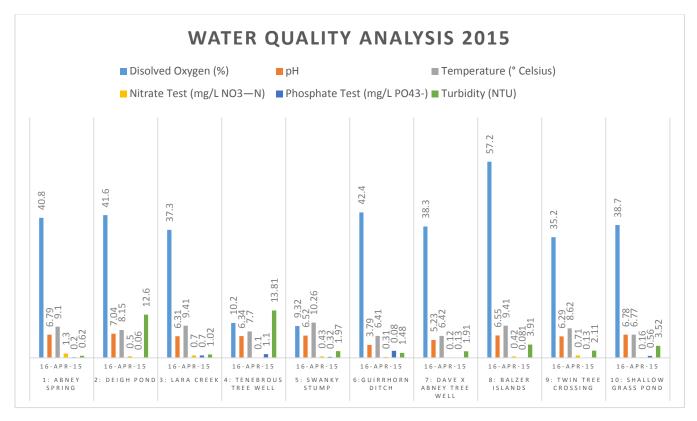


Figure 13: Water quality analysis of the Blaauw ECO Forest in early spring.

Discussion:

This study has shown that Blaauw ECO Forest sustains a large variety of non-vascular plants. Totalling 86 species of non-vascular plants for 35 acres of land. This is quite substantial for the size and region of this forest. Including past research for vascular plant, vertebrates, and macroinvertebrates by Abney (2014) the total inventory sits at 337 species. This is comparable to the forest inventory of Vancouver's Stanley Park, where a 2-day excursion of specialists observed 395 species (BioBlitz Summary Report 2011). A more extensive inventory of Stanley Park would no doubt yield more species than the 2-day BioBlitz, but this points out that the number of species at the Blaauw ECO Forest is substantial. The Blaauw ECO Forest inventory list continues to fill up over time as different species are observed. Micro-invertebrates have yet to be taxonomized and added to the inventory, along with algae, migratory animals, and more fungal species.

The high diversity in species types suggests that Blaauw ECO Forest houses a healthy ecosystem, which allows a variety of different organisms to grow and flourish. Blaauw ECO Forest contains several ecosystem types within a relatively small area. The front of the forest is mixed coniferous and deciduous trees; while the back near the bog is all coniferous. Coniferous forests are composed of evergreen trees that bear cones, such as hemlocks, cedars, pines, and firs. These tree types eventually shed their needles, which develops a springy mat on the forest floor. Pine needles do not decompose easily, so fungi help break down the needles which provides nutrients back to the trees roots (Anonymous 2014; WWF 2015). Due to the slow process of decomposing pine needles, the soils in coniferous forests become acidic and poor in minerals, organic material, and number of invertebrate species, which influences the depth tree roots (WWF 2015). Mixed forests contain tree types such as maple, birch, oak, and etcetera. This kind of forest contains four layers: a canopy, shrub layer, grasses, and other herbaceous plants (WWF 2015). These forests are the richest because of the biodiversity patterns that reflect different ecoregions that may harbour special and exotic flora and fauna species (WWF 2015). Within these varying habitats of the Blaauw Eco Forest, there are swamps, bogs, ponds, springs, decaying logs, and other habitat features that allow organisms to grow and flourish (Tamme et al., 2010).

With this vast diversity in microhabitat, a range of non-vascular plants were identified as seen in Tables 2-6. While the current total of 40 bryophyte species seems low when comparing

to all of Canada at 965 species, this forest is vastly rich in species types (Ireland *et al.*, 1987). Though the Blaauw ECO Forest is only 35 acres it contains 1/20 of all the moss species found in over 9 million square kilometres of Canada (Schofield 1990). While the identified moss species (table 2) are large in variety, they are also large in population density. British Columbia has the largest bryoflora in North America, including rare and endangered species (Schofield 1972). Though none of the identified species in this study appeared to be at risk or endangered, there is a possibility that the Blaauw ECO forest has some.

While the Blaauw Eco Forest seems healthy, it has experieced major ecological disturbances that could eventually risk the biodiversity. The property is surrounded by residential roads and houses, a liquid dumping site, a recovering peat mining plot, and continuous land development. The human impact from these areas effect the forest via chemical and noise pollutants. Therefore studying the quality of water provides small insight into the levels of pollution that have entered the waterways of the forest. While being limited to five different tests (dissolved oxygen, nutrient tests, pH, temperature, and turbidity), the different bodies of water seemed relatively normal in test results. The bog showed relatively low numbers of nutrient tests (N: 0.031mg/L; P: 0.08mg/L), which coincides with the slow process of decomposing organics due to a low pH (3.79). Slightly higher nutrient values (N: 0.7mg/L; P: 0.7mg/L) were obtained in the more deciduous/coniferous mixed forest area of Lara Creek. This indicates that the forests ability to decompose and recycle organic and/or inorganic compounds hasn't been negatively impacted. With human impact being a concern, it appears that the forest continues to thrive.

Study Limitations

While this study has undergone extensive work in identifying non-vascular plants, there still is so much to be done. Despite the effort put into classifying the non-vascular plants according to accepted techniques for identifications, looking at microscopic structures, the lack of expertise made identifying taxa more difficult. Therefore, some species may have been identified incorrectly.

Future Studies

For future studies, the continuation of identifications for non-vascular is highly recommended. More extensive collection of fungal and lichen taxa would further improve the biological inventory. During the warmer time of the year, algae should be identified and analysed in the different bodies of water throughout the Blaauw ECO Forest. Verification of the species identified by experts in the various taxonomic groupings is recommended. In terms of fauna, it is important to focus on invertebrate species identification at this point, while continuing to look for additional vertebrate species.

To protect non-vascular plants that grow near trail edges, it is imperative that the forest trails are maintained. A forest is always chaotic with trees falling and changing the microecosystems, and with a public forest, it is important that trails remain accessible. Due to the bog encroaching into the forest, trees are losing their root holding, and have completely blocked a trail at present. Continued upkeep in these areas is important so that people don't start to make their own trails and accidentally damage a sensitive species.

Conclusion

While the Blaauw ECO Forest is surrounded by a mixture of human development and agriculture, the diverse flora and fauna makes it clear the forest is a reservoir of native species amidst in the increasingly depauperate surrounding landscape. With the total count of 86 non-vascular plants in the forest, including bryophytes, liverworts, fungi, lichen, and slime molds, the supports a substantial level of biological diversity even in the context of British Columbia as a whole. The 40 were identified in an 11-month span, comprises 1/20th of all of Canadian identified bryophytes. In order to continue protecting the biological diversity of the Blaauw ECO Forest, continued conservation work is strongly encouraged.

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Appendix:

Site	Date/Time	Dissolved Oxygen (%)	рН	Temperature (° Celsius)	Nitrate Test (mg/L NO ₃ N)	Phosphate Test (mg/L PO4 ³⁻)	Turbidity (NTU)
1: Abney Spring	July 30, 2014 @ 9:30am	21.1	6.16	11.7	0.6	0.10	0.73
2: Deigh Pond	July 30, 2014 @ 1:42pm	10.5	6.9	22.8	0.3	1.03	3.09
3: Lara Creek	July 30, 2014 @ 3:20pm	45.1	6.7	16.8	1.0	0.05	16.82

Table Appendix 1: Summer 2014 Water Quality Analysis

4: Parsley Pool	July 30, 2014 @ 4:00pm	23.7	6.2	10.9	0.8	0.08	2.62
5: Tenebrous Tree Well	July 31, 2014 @ 9:30am	22.6	6.6	15.6	0.1	0.10	9.69
6: Swanky Stump	July 31, 2014 @ 11:00am	25.8	6.5	16.7	0.7	0.06	1.95

Table Appendix 2: Spring 2015 Water Quality Analysis

Site	Date/Time	Disolved Oxygen (%)	рН	Temperature (° Celsius)	Nitrate Test (mg/L NO ₃ N)	Phosphate Test (mg/L PO4 ³⁻)	Turbidity (NTU)
1: Abney Spring	April 16, 2015	40.8	6.79	9.10	1.30	0.20	0.62
2: Deigh Pond	April 16, 2015	41.6	7.04	8.15	0.50	0.06	12.6
3: Lara Creek	April 16, 2015	37.3	6.31	9.41	0.70	0.70	1.02
4: Tenebrous Tree Well	April 16, 2015	10.2	6.34	7.70	0.10	1.10	13.81
5: Swanky Stump	April 16, 2015	9.32	6.52	10.26	0.43	0.32	1.97
6:Guirrhorn Ditch	April 16, 2015	42.4	3.79	6.41	0.31	2.08	1.48
7: Dave x Abney Tree Well	April 16, 2015	38.3	5.23	6.42	0.12	0.13	1.91
8: Balzer Islands	April 16, 2015	57.2	6.55	9.41	0.42	0.081	3.91
9: Twin Tree Crossing	April 16, 2015	35.2	6.29	8.62	0.71	0.13	2.11
10: Shallow Grass Pond	April 16, 2015	38.7	6.78	6.77	0.16	0.56	3.52

Specimen Nº: 000

DATE COLLECTED:	DATE IDENTIFIED:
DATE COLLECTED.	DATE IDENTITIED:

IDENTITY:

LOCATION AND HABITAT:

PICTURES:

Collected and Identified by:

PROCEDURE FOR MOSS IDENTIFICATION

- Sign in to dropbox. The email is ______ and the password is ______
- Heat the water to just below boiling and add a small branch of the sample of the moss to be rehydrated. Leave in water for 30 – 60 seconds.
- 3. Examine under stereoscope and confirm whether it is a bryophyte or a hepatophyte.
 - The "leaves" of bryophytes have pointed ends whereas the <u>hepatophyes</u> are rounded
- 4. Take a picture to be uploaded to dropbox into the file "stereoscope pictures"
 - Name file as: preliminary identification sample# mm.dd.yy.jpg for example bryophyte 0001 01.29.14.jpg
- Use a scalpel to remove <u>several</u> leaves by scraping tip→stem (against the direction of growth), it may also work to cut the leaves off
- Make a wet mount and view under the compound microscope (and camera)

 View under the different magnifications
- 7. Take a picture with the camera
 - Plug it in, press "snap" (screen will blink) on the remote and the picture will save to the camera's SD card
- Begin the identification process using the book: Some common mosses of British Columbia
 - At the beginning of the book is a dichotomous key, follow the questioning process until you reach an identification and then double check by googling a picture
- After identification please highlight the sample in the lab notebook (big blue book we recorded everything in) to indicate that it has been identified

 Specimen N°: 0002

 DATE COLLECTED: 06/11/12
 DATE IDENTIFIED: 30/1/14

 DESCRIPTION: Green/yellow, transparent, central vein on leaflets, large leaflets, visible
 hexagonal cells in even straight patterns; outer ridge is pointed, lancelet shaped, radial growth at irregular intervals, long individual growth strands; brown stem; huge leaflets; leaflet shot is using 3x; serrated edging

 IDENTITY: cinclidium stygium (rare)

LOCATION AND HABITAT: Glen Valley, surrounded by lots of ferns open canopy

PICTURES:



Collected by: Christina, Jasmine, Karen Identified by: Beth Guirr and Karen Eenkhoorn

 DATE COLLECTED: 06/11/13
 DATE IDENTIFIED: 01/30/14

 DESCRIPTION: leaflets are in rows of two; bright yellow/green; large; flattened; undulating edges; smooth edges; cells are elongated at 10x; multiple cells thick; double veined/ribbed; directional cells; photo taken of leaflet at 3x

 IDENTITY: Plagiothecium undulatum

LOCATION AND HABITAT: Growing on a dead tree branch, mixed forest Glen Valley at the base of a tree.

PICTURES:



Collected by: Christina, Karen, Jasmine

Identified by: Beth Guirr

DATE COLLECTED: 06/11/13	DATE IDENTIFIED: 30/01/14
	t green leaflets with one midrib of a red/brown colour, w, slightly cupped sporangium angled downwards; growth
IDENTITY: <u>Bhizomoium aracile</u>	
LOCATION AND HABITAT: found grov of a dead tree	wing on a dead branch in a mixed forest in Glen Valley at the base
PICTURES:	
collected by: Christina, Karen, Jasmine	e Identified by: Karen <u>Eenkhoorn</u> and Beth Guirr

Collected by: Christina, Karen, Jasmine

Identified by: Karen <u>Eenkhoorn</u> and Beth Guirr

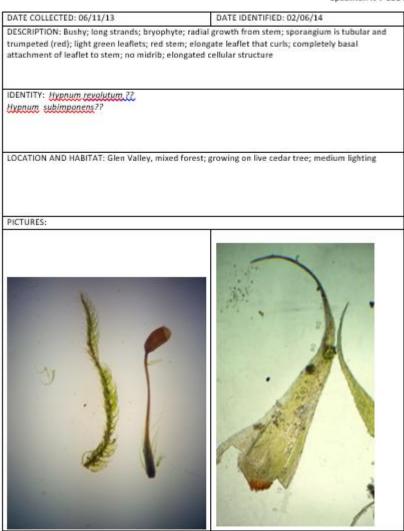
DATE COLLECTED: 06/11/13	DATE IDENTIFIED: 31/01/14
stem; very small; width of leaflet is equa	led branches; leaflets are spaced; leaflets grow radially from I to thickness of stem; very sparse branching; fine tipped cells in an offset manner; large green midrib reaching d
IDENTITY: Isothecium staloniferum?	
LOCATION AND HABITAT: hanging off the	e branch of a salmon berry tree, mixed forest, glen valley
open canopy.	
PICTURES:	

Collected by: Christina, Jasmine, Karen Identified by: Karen, Eenkhoorn and Beth Guirr

DATE COLLECTED: 11/06/13	DATE IDENTIFIED: 02/06/14
	atophyte: red midrib; smooth edging of leaflet; flowered end
	ells with clear centers; curved leaflets; clustered leaflets with
arge gaps of non-growth in between br	ranching
IDENTITY: Rhizomnium glabrescens	
OCATION AND HABITAT: Glen Valley, n	nixed forest; growing on dead tree found on ground
PICTURES:	80 C
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	and the second sec
Hannah han Phylatian Manage According	

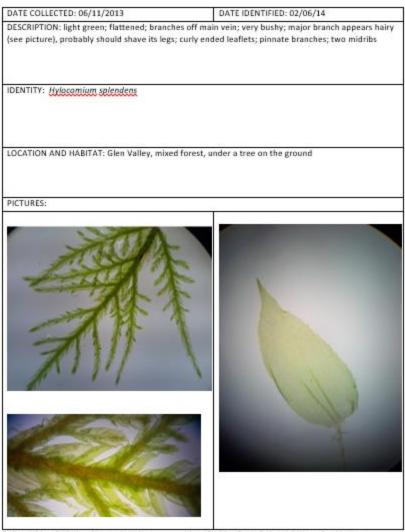
Collected by: Christina, Karen, Jasmine

Identified by: Beth Guirr and Karen Eenkhoorn



Collected by: Chris, Karen, Jasmine

dentified by: Beth Guirr and Karen Leokhoom



Collected by: Christina, Karen, Jasmine Identified by: Beth Guirr and Karen Eenkhoorg



Collected by: Christina, Karen, Jasmine

Identified by: Beth Guirr and Karen Eenkhopm



Collected by: Chris, Karen and Carson

Identified by: Beth Guirr

DATE COLLECTED: 08/11/2013 DATE IDENTIFIED: 02/12/14
DESCRIPTION: Bryophyte; yellow/green; long spindly growth; many branches; bunched growth;
random branching patterns; brownish/red stem; pleated leaflets; lacking midrib; very opaque; small;
slender leaflet point; cells are elongated

IDENTITY: Rhytidiadelphus lareus

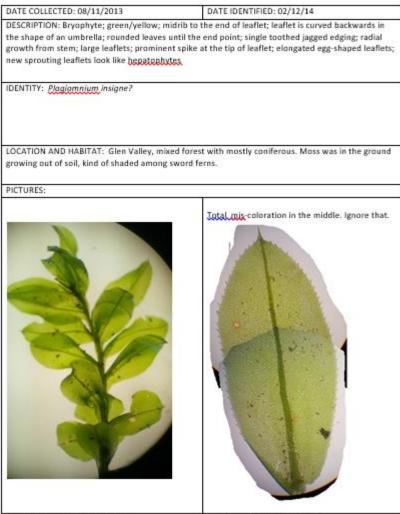
LOCATION AND HABITAT: Glen Valley, mixed forest with mostly coniferous trees; growing on soil, near sword ferns, in a moist area that is partially shaded

PICTURES:

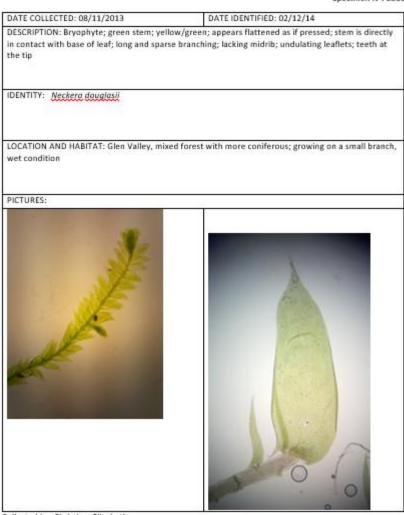


Collected by: Christina, Elizabeth

Identified by: Beth Guirr and Karen Eepkhooro

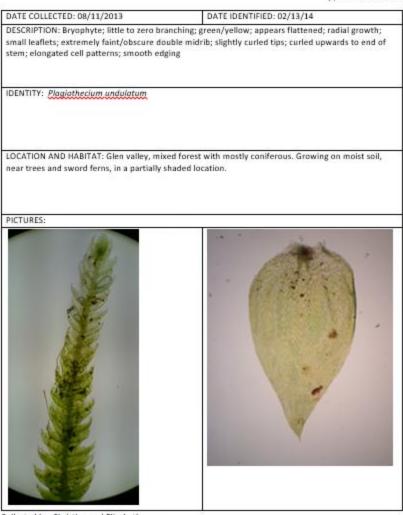


Collected by: Chris Hall, Christina

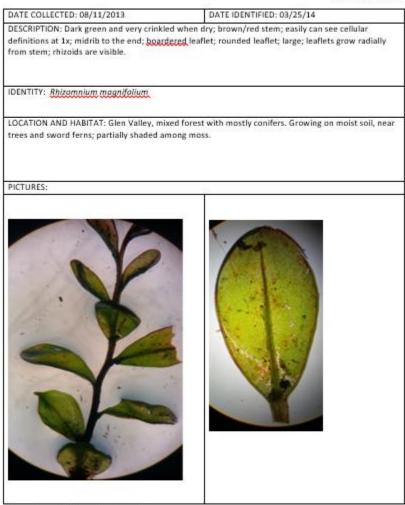


Collected by: Christina, Elizabeth

Identified by: Beth Guirr and Karen Eenkhoorn

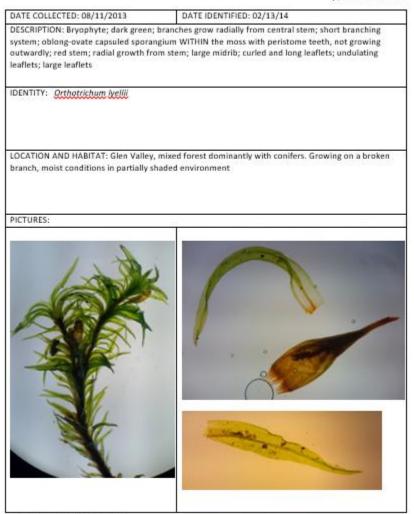


Collected by: Christina and Elizabeth



Collected by: Christina, Karen, Jasmine

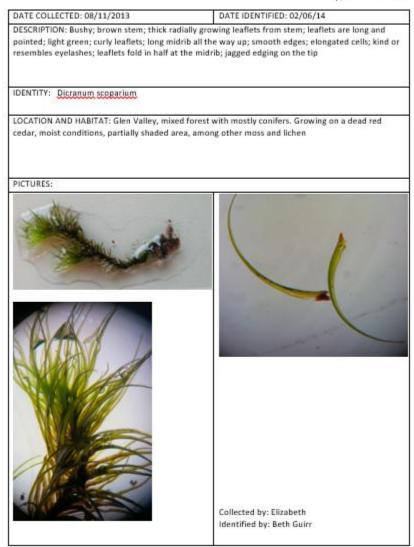
Identified by: Beth Guirr and Karen Eenkhoorn



Collected by: Christina, Elizabeth

나는 것 같아요. 그 가슴 집안 다 있는 것이 가슴 것이 좋아 있는 것 같아요. 것이 있는 것이 같아.	tight knit cluster; very small; leaflets look like bear claws {
ancient accorned to the stem, pare green	as the individual ones on the branch; base of leaflet
IDENTITY: Lepidozia reptans	
LOCATION AND HABITAT: Glen Valley, mixed (potentially dead cedar?), moist conditions,	d forest with mostly conifers; growing on a red cedar , in a partially shaded location.
PICTURES:	

Collected by: Elizabeth



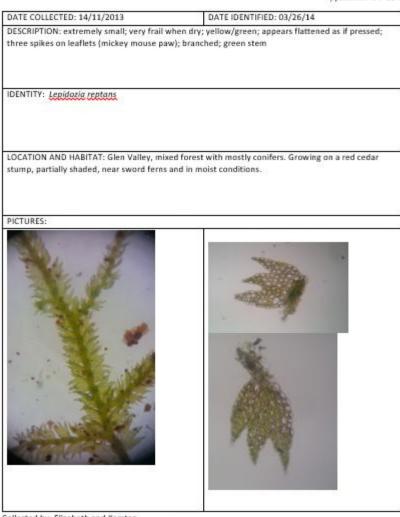
ATE COLLECTED: 08/11/2013	DATE IDENTIFIED: 03/25/14
	; really soft and squishy like cotton; underneath is soft an
vhite; looks like cauliflower; crustose; spo	
DENTITY: Lepraia incana	
OCATION AND HABITAT: Glen Valley, mix edar, in moist conditions and partially sha	ed forest with mostly conifers. Growing on a dead red aded
ICTURES:	<u>8</u> :

Collected by: Elizabeth

DATE COLLECTED: 14/11/2013	DATE IDENTIFIED: 02/13/14
DESCRIPTION: Bryophyte; branched; small toothed edged leaflets; midrib reaches % u	leaflets; green stem; appears pressed as if flattened; up of leaflet;
IDENTITY: Isothecium stoloniferum	
LOCATION AND HABITAT: Glen Valley, mixe sword ferns, partially shaded and moist co	ed forest with mostly conifers. Growing on a rock near nditions
PICTURES:	
10 Martin	

Collected by: Elizabeth and Karston

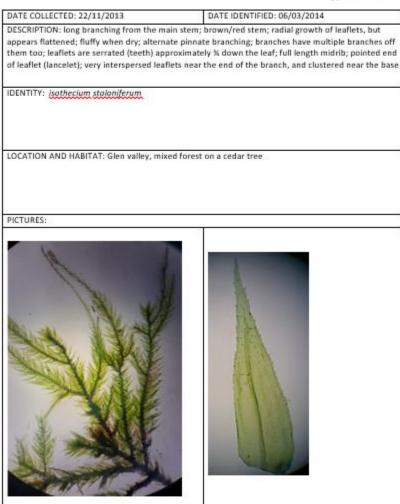
Identified by: Beth Guirr and Karen Eenkhoorn



Collected by: Elizabeth and Karston

Identified by: Beth Guirr

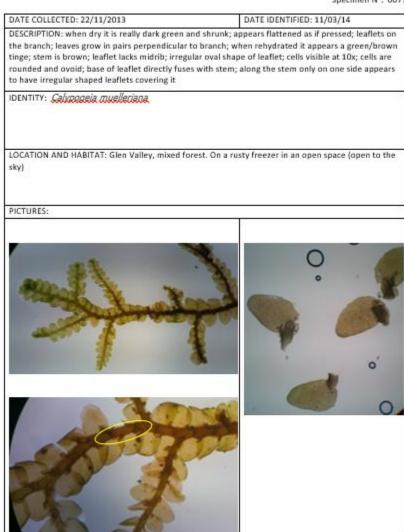
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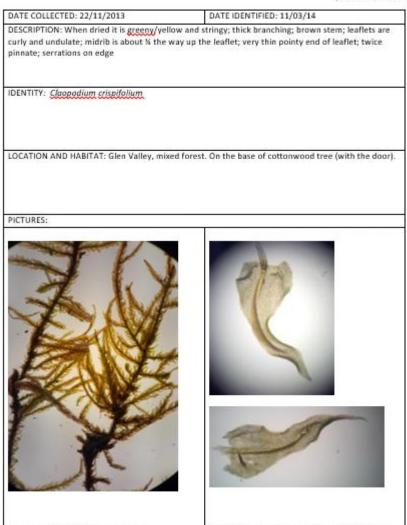
Collected by: Christina, Karen, Elizabeth

Identified by: Beth Guirr and Karen Eenkhoorn

_



Collected by: Christina, Karen, Elizabeth



Collected by: Carston, Karen, Jasmine

Identified by: Beth Guirr and Karen Eenkhoorn

DATE IDENTIFIED: 11/03/14
es arranged radially and flattened, leaves rounded at the tions across the leaves, edges curling inwards (appear
on the trunk of a big leaf maple? Cottonwood? (the tree

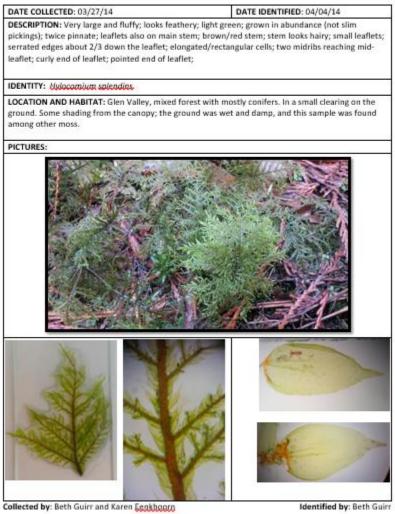
Collected by: Christina, Karen, Jasmine

Identified by: Beth Guirr and Karen Eenkhoorn

DATE COLLECTED: 22/11/2013	DATE IDENTIFIED: 03/19/14
curled up towards the end of stem; leaflet	green leaves; green stem; radial leaflet growth; leaflets is are sharp points; single midrib nearly the entire length o id and rectangular; visible rhizoids on moss; clustered t
IDENTITY: Aulacomnium androgynum	
LOCATION AND HABITAT: Glen Valley, Mix	ed forest. On a maple tree (the one with the door)
PICTURES:	
MAN N	

DATE COLLECTED: 22/11/2013	DATE IDENTIFIED: 03/19/14
	fy; feathery; green stem; weird brown/red spots on leaflets n; midrib midway up leaflet; serrated edging; elongated cells end of stem
IDENTITY: Isothecium stalanilerum	
LOCATION AND HABITAT: Glen valley, m	ixed forest; big maple tree {with the door}
PICTURES:	
2	1 miles
-	- State - Stat
- The	
-	
The second secon	
Collected by: Christina, Karen, Jasmine	

Identified by: Beth Guirr and Jasmine Bepapel



DATE COLLECTED: 27/03/14 DATE IDENTIFIED: 08/04/14 DESCRIPTION: When dry its very shriveled and dark green; when rehydrated the leaflets are huge; very clustered leaflet growth; radial leaflet growth around stem; long shoots of stem; serrated edging around entire leaflet; approximately 11mm average; large midrib reaches end-to-end of leaflet; ovoid cells, easily seen at 1x; looks like a mixed species of *glagomnium* and *dichodontium*

IDENTITY: Plagomnium undulatum

LOCATION AND HABITAT: Glen Valley, mixed forest with mostly conifers. <u>Blaaux ecoforest</u>, On a rotten log in a small clearing, among other moss. Some shaded area due to the partial covering canopy, and very wet.

PICTURES:





Collected by: Beth Guirr and Karen Eenkhoorn

DATE COLLECTED: 27/03/14 DESCRIPTION: When dry it is dark green and shriveled leaflets; when hydrated the leaflets are rounded with a large red midrib; at the point of the leaflet there is a little spike; red stem; radial growth around stem; cells easily seen at 1x; Tylenol pill used for scale (didn't have a dime...)

IDENTITY: Rhizomnium aracile.

LOCATION AND HABITAT: Glen valley, mixed forest with mostly conifers \rightarrow Blaaux property. On a rotten log in a partial clearing, with some shade due to canopy. Plenty of decaying matter around, lots of ferns, damp conditions

PICTURES:



Collected by: Beth Guirr and Karen Eenkhoorn

DATE COLLECTED: 27/03/14 DATE IDENTIFIED: 08/04/14
DESCRIPTION: When dry it is light green and feels fuzzy; pinnate branching; red/brown stem; radial
leaflet growth from stem; irregular branching; very dense leaflet growth; Tylenol pill for scale (didn't
have a dime); undulate base of leaflet; curled tip of leaflet; lacking midrib; elongated cellular
structures; minor/no serrated edges;

IDENTITY: rhytidiadelphus loreus.

LOCATION AND HABITAT: Glen Valley, mixed forest with mostly conifers. On a rotten log surrounded by ferns and other moss, partially shaded and in a low clearing.

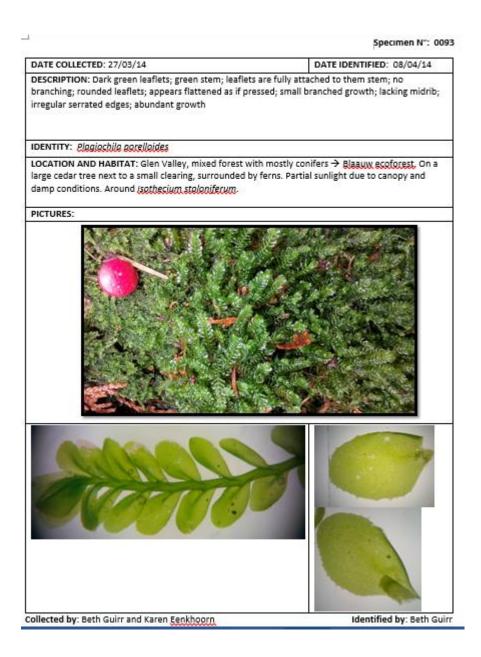
PICTURES:

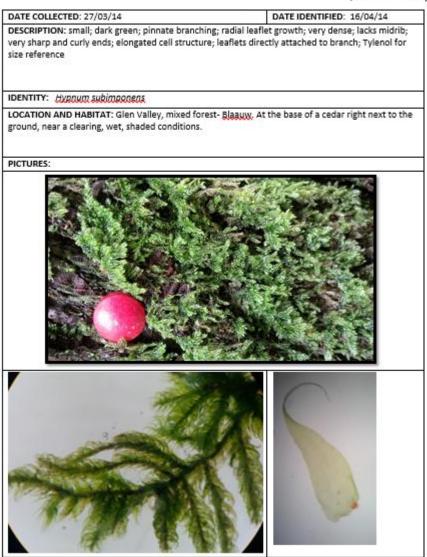


Collected by: Beth Guirr and Karen Eenkhoorn

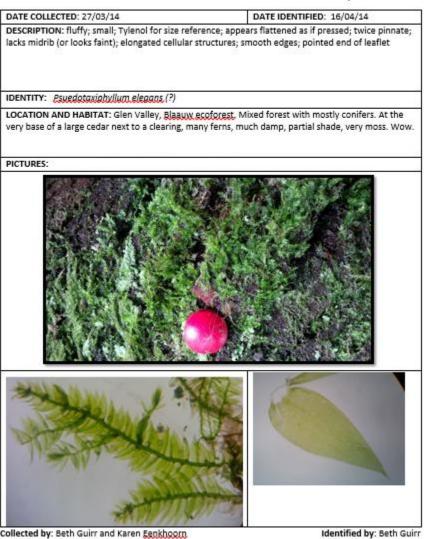
_ Specimen Nº: 0091 DATE COLLECTED: 27/03/14 DATE IDENTIFIED: 08.04.14 DESCRIPTION: Light green; fluffy; minor branching; appears flattened; dense growth; Tylenol pill for size reference (didn't have a dime); green stem; double connected midrib; undulated tip of leaflet; pointed end of leaflet; **IDENTITY:** Plagiothecium undulatum LOCATION AND HABITAT: Glen Valley, mixed forest with mostly conifers -> Blaguw forest. On a rotten cedar log, in a low clearing surrounded by ferns and conifers. Very wet and damp !!! PICTURES: Collected by: Beth Guirr and Karen Eenkhoorn Identified by: Beth Guirr

DATE COLLECTED: 27/03/14	DATE IDENTIFIED: 08/04/14
leaflets grow radially from stem; very small; po	h; long stranded ends of stems; stems are green; vinted end of leaflet; single midrib reaching 2/3 up the g; Tylenol for size reference (didn't have a dime);
IDENTITY: Isothecium stoloniferum	
	orest with mostly conifers
PICTURES:	
ollected by: Beth Guirr and Karen Eenkhoorn	Identified by: Beth Gui

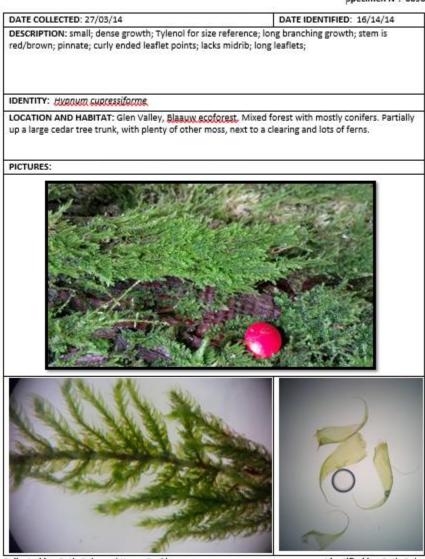




Collected by: Beth Guirr and Karen Eenkhoorn



Collected by: Beth Guirr and Karen Eenkhoorn



Collected by: Beth Guirr and Karen <u>Eenkhoorn</u>



Collected by: Beth Guirr and Karen Eenkhoorn



Collected by: Beth Guirr and Karen <u>Eenkhoorn</u>

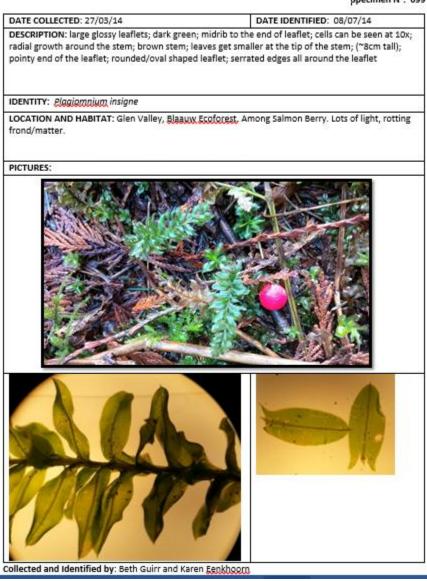
Specimen N°: 0098b



Collected by: Beth Guirr and Karen Eenkhoorn

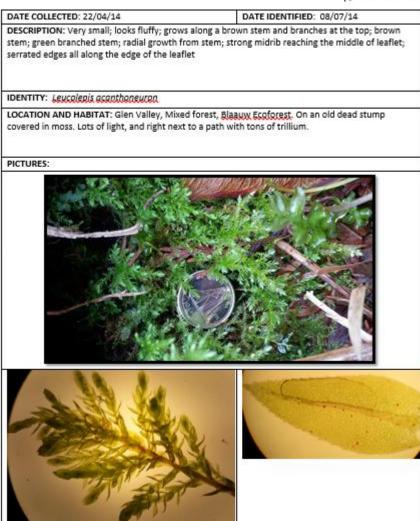
-

Specimen N°: 099

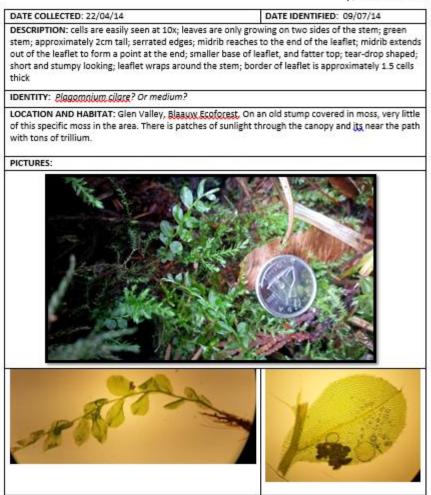




Collected and Identified by: Beth Guirr and Karen Eenkhoorn



Collected and Identified by: Beth Guirr and Karen Eenkhoorn



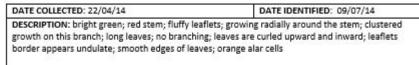
Collected and Identified by: Beth Guirr and Karen Eenkhoorn



Collected and Identified by: Beth Guirr and Karen Eenkhoorn



Collected and Identified by: Beth Guirr and Karen Eenkhoorn



IDENTITY: Ditrichum heteromallum

LOCATION AND HABITAT: Glen Valley, Blaauw Ecoforest. On a fallen branch next to the path, with lots of light and leaf litter. A lot of trillium and bleeding heart growing near the path.

PICTURES:





DATE COLLECTED: 22/04/14 DATE IDENTIFIED: 09/07/14
DESCRIPTION: flowering leaflets from birds eye view; leaflets curl out and down; midrib extends all
the way to the end and then beyond the border of the leaflet causing a point; radial growth around
the stem; brown stem; dark green; thinner leaf, but long

IDENTITY: Plagomnium insigne?

LOCATION AND HABITAT: Glen Valley, mixed forest, Blaauw Ecoforest. On a very rotten log/stump that's covered in moss. There are sword ferns and lady ferns around the area. Base of a sloping hill.

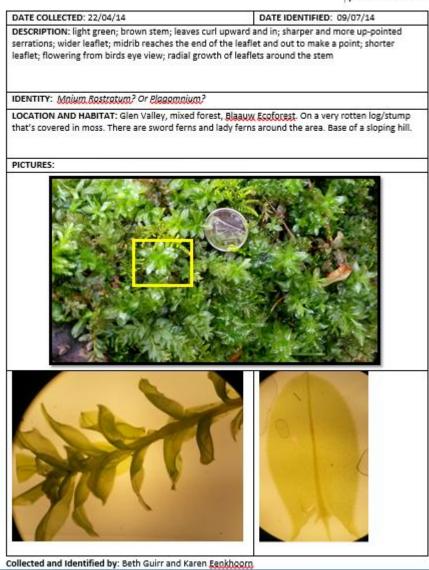
PICTURES:



Collected and Identified by: Beth Guirr and Karen Eenkhoorn

1

Specimen N°: 0108b



Specimen Nº: 0109

DATE COLLECTED: 22/04/14 DATE IDENTIFIED: 09/07/14 DESCRIPTION: Orange sporophytes; dark green leaflets; radial growth around the stem; no branching; leaves are translucent; sporophyte has a twisted end before the head; tubular head; very long leaves; serrated edges; midrib to the end; pointed end of leaflet; leaves grow radially from stem; undulate leaves

IDENTITY: Atrichum undulatum

LOCATION AND HABITAT: Glen Valley, Mixed forest, <u>Blaauw Ecoforest</u>. On a very rotten log/ground with lots of light due to partially open canopy. On a slope of a hill with lots of leaf litter.

PICTURES:



Collected and Identified by: Beth Guirr and Karen Eenkhoorn

DATE COLLECTED: 22/04/14 DATE IDENTIFIED: 09/07/14
DESCRIPTION: stem is red at the top and brown and the bottom; light green/yellow leaflets; radial
growth around the stem; once pinnate; irregular branching; double midrib; narrow heart-shaped
leaflet; slightly wrinkled; edges are wavy; tip is pointy

IDENTITY: Rhytidiadelahus triavertrus

LOCATION AND HABITAT: Glen Valley, Mixed forest, <u>Blaaux Ecoforest</u>. On the ground next to the path with plenty of light due to partial opened canopy. Trillium, bleeding heart and salmon berry are abundant.

PICTURES:



Collected and Identified by: Beth Guirr and Karen Eenkhoorn

Specimen Nº: 0112

DATE COLLECTED: 22/04/14 DATE IDENTIFIED: 10/06/14
DESCRIPTION: Light green and brown and the base; very fluffy; shallow root systems; very thin stem; bowled shaped leaflets; curved upward; pointed end; short and stumpy; fat and clustered at the end (looks like a rose); growth is in small hummocks which are not connected (various groups).

IDENTITY: Sphagnum tenellum?

LOCATION AND HABITAT: Glen Valley, mixed forest in the <u>Blaauw ecoforest</u>. In a boggy area at the back of the property. The ground was bouncy and moist. It's a shaded area with little-to-no canopy openings. There is other moss around this one, and a lot of pine needles.

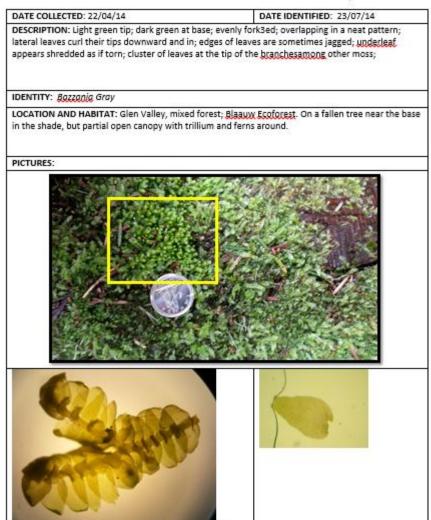
PICTURES:





Collected by: Beth Guirr and Karen Eenkhoorn

Identified by: Beth Guirr and Karen Eenkhoorn

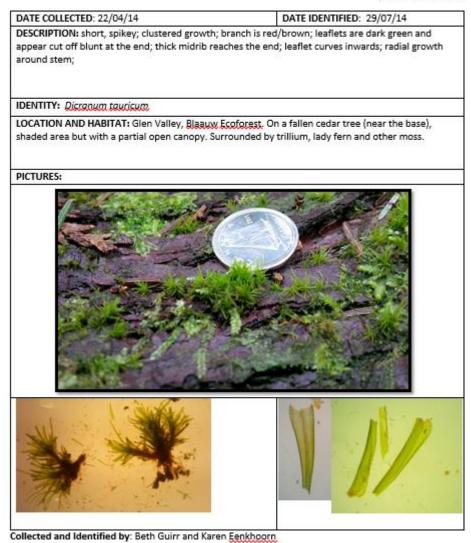


Collected and Identified by: Beth Guirr and Karen Eenkhoorn

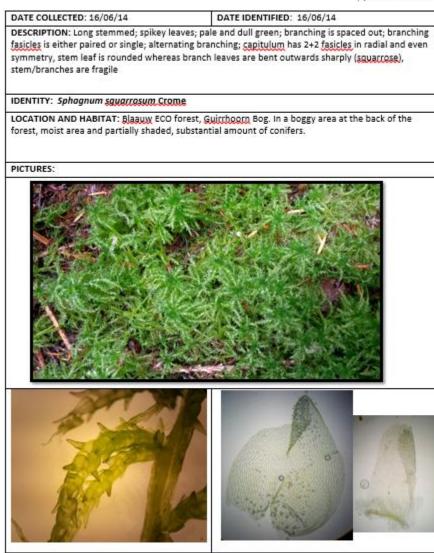
Specimen N°: 0113d



Specimen Nº: 0114

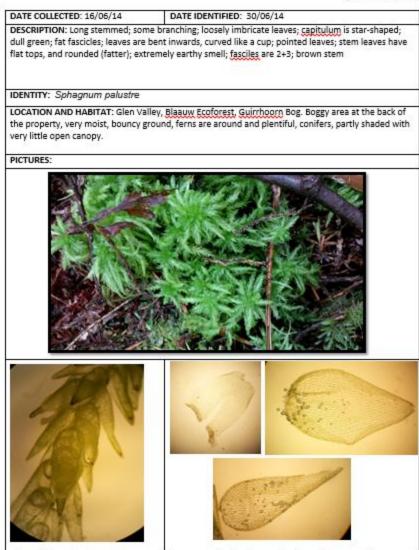


Specimen Nº: 0148



Collected by: Beth Guirr and Karen Eenkhoorn

Identified by: Karen Eenkhoorn and Beth Guirr



Collected by: Beth Guirr and Karen Eenkhoorn

Identified by: Beth Guirr and Karen Eenkhoorn

4



Collected by: Beth Guirr and Karen Eenkhoorn

Identified by: Beth Guirr and Karen Eenkhoorn

Fungi Identification Procedure Guide

Trinity Western University

Supervisor: Chris Hall

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Fungi Tissue Collection	11
Dry & Store Fungi	12

Purpose: This outline provides as a procedural guideline as to the process of collecting, testing and identifying the various fungi species that exist in the Blaauw Forest.

Tools for Mushroom Collection:

- Pocket Knife
- Roll of wax paper/wax bags
- Label paper
- Twist ties
- Permanent Marker
- Measuring Tape
- Zip-lock bags
- Collection Basket (prevents mushrooms from getting squished from a backpack)

Identification Tools:

- 90% Alcohol
- Melzer's reagent
- Stain (Safranin, Congo Red or Lactophenol cotton blue)
- Cover Slips
- Slides
- Oil (for oil immersion)
- Lens paper

Optional Chemical's for Tests:

- KOH
- Ammonia
- Iron Salts

How to Collect Mushrooms:

- 1) Find Mushroom
- 2) Identify observable characteristics
 - a. Habitat & Environment
 - b. Was it growing from a tree?
 - c. What are the surrounding plants?
- 3) Be careful we want to maintain key characteristics of the mushroom, so use caution
- 4) Take Picture
 - a. It is key to take numerous pictures
 - i. 1 from a distance taking in the surrounding botany
 - ii. 1 with a full view of the mushroom
 - iii. 1 with a view of the underside of the cap (checks to see the pores or gills)
 - iv. 1 aireal view of the mushroom cap
- 5) Dig the mushroom out, the whole stem (a key characteristic is found at the base of the stem)
- 6) Wrap the mushroom up (not tightly) in wax paper for preservation and twist ends

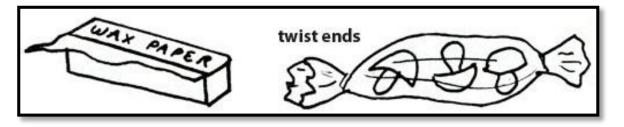


Figure 1: Process of collecting fungi

Image from http://urbanmushrooms.com/index.php?id=69

- 7) Make further notes:
 - a. Where the mushroom was found?
 - b. Was it shaded?
 - c. Was it growing from grass?
 - d. What was the formation it was growing in?
 - * Write these notes on the template posted

PHYSICAL IDENTIFICATION:

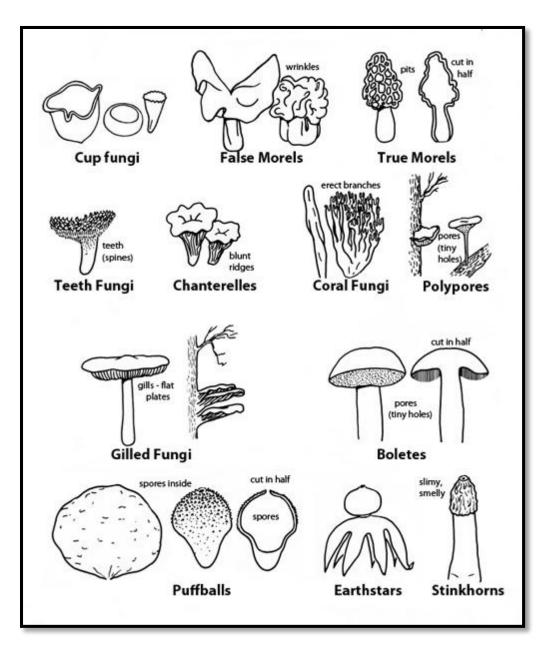


Figure 2: Basic fungi outline to identify the easiest mushrooms (not all fungi will fit into these categories)

Image from http://urbanmushrooms.com/index.php?id=69

Physical Components of Mushroom:

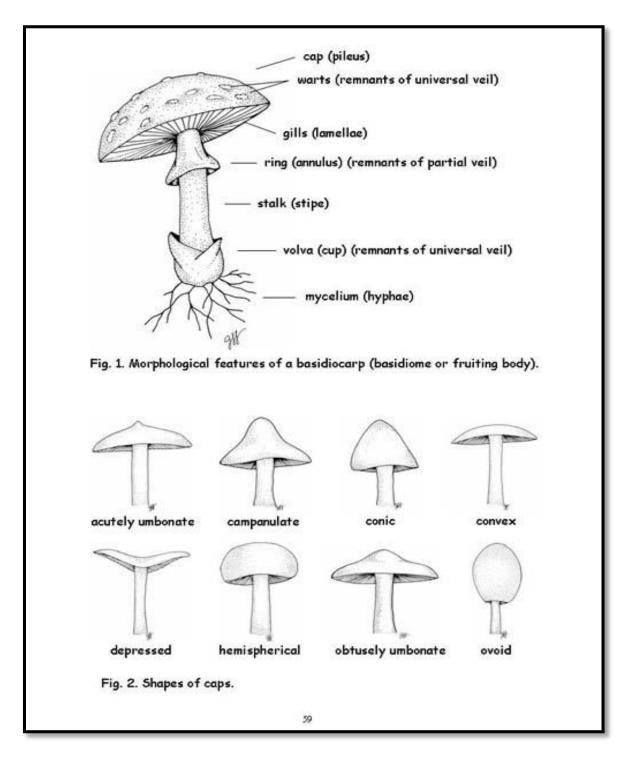


Figure 3: Basic physical fungi characteristics to describe the mushroom (not all features are presented in this image and descriptions vary per identifier)

Image from http://urbanmushrooms.com/index.php?id=69

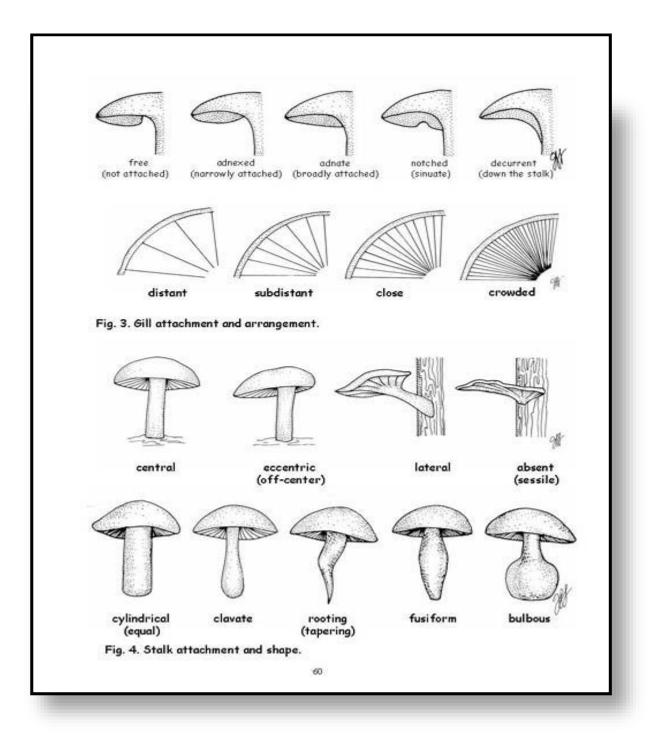


Figure 4: Basic physical stalk and gill attachment characteristics to describe the fungi (not all features are presented in this image and descriptions vary per identifier)

Image from http://urbanmushrooms.com/index.php?id=69

CHEMICAL TESTS:

Important Information:

- All tests must be conducted on fresh mushrooms
- It is only necessary to add a single drop onto the mushroom

A. Ammonia Test (Identifies boletes)

- 1) Place a drop of ammonia on a fresh cap, stem, sliced flesh and pore surface
- 2) Identify any color change (some change into multiple colors and others only one)

B. Potassium Hydroxide Test (KOH) (2-5% aqueous solution) (Identifies boletes, polypores and gilled mushrooms)

- This will have to be purchased online

Boletes:

- 1) For boletes, place a drop of KOH on the cap, stem, sliced flesh, and pore surface
- 2) Note color changes (if any)

Polypores:

- 1) For polypores, apply the KOH to the flesh and the cap surface
- 2) Note color changes (if any)

Gilled mushrooms:

- 1) For gilled mushrooms, place a drop on the cap surface
- 2) Note color changes (if any)

C. Iron Salts (FeSO₄) (Identifies boletes and russulas)

Boletes:

1) For boletes, place a drop on the cap, stem, sliced flesh, and pore surface

Russulas:

1) For russulas, place a drop on the stem surface

D. Melzer's Reagent (Safety Procedures)

Melzer Reagent is a highly dangerous substance due to the addition of the chloral hydrate, which is a medically controlled sedative and hypnotic (Leonard 2006).

Hazards:

Melzer Reagent is toxic is swallow and can potentially cause skin irritation and serious eye irritation.

Preventative Measures:

- Wash hands thoroughly after handling
- Avoid consumption at all costs
- Wear protective gloves and safety glasses at all times during chemical usage
- 1) Extract spores from either a spore print or from asci
 - a. Extract spores from the asci via slicing a very thin surface piece. If...
 - i. Morel, extract from surface of pit
- 2) Place spores on slide
- 3) Add a single drop of Melzer's reagent
- 4) Place cover slip over spores/Melzer's reagent
 - a. If extracting spores from asci apply slight pressure to flatten specimen
- 5) Remove excess stain with tissue and wait a few minutes for stain to permeate the specimen
- 6) Observe color change and associate with reaction type. Certain reactions can take up to 20 minutes.
- 7) Observe and record color, shape and size of spores

Table 1.1: Color change associated with Reaction Name

Color Change	Reaction Name	
Blue to Black	Amyloid or Melzer's-positive reaction	
Brown to Reddish-Brown	Pseudoamyloid or Dextrinoid reaction	
No Color Change or faintly Yellow to Brown	Inamyloid or Melzer's-negative	

The Amyloid reaction can be further isolated into two additional reactions upon addition of KOH.

KOH Present	Color Change	Reaction Name
No	Blue	Euamyloid Reaction
Yes	Blue	Hemiamyloid Reaction*

*No reaction with just Melzer's reagent and turns red in Lugol's solution

SPORE PRINT:

A spore print can only be completed on mature mushrooms and is completed to determine spore color.

- 1) Remove stock from smaller mushrooms and place cap, gills or pores downward on a piece of paper. The best paper utilized for a spore print has two colors (so as to prevent color misinterpretation)
 - For larger mushrooms slice off a section of the cap

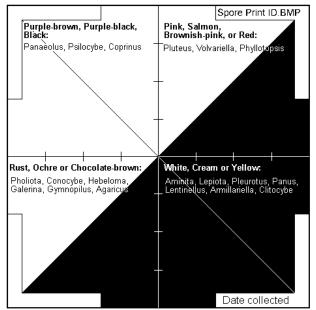


Figure 5: Ideal paper for spore print (Extracted from http://en.wikipedia.org/wiki/File:Spore_Print_ID.gif)

- 2) Place cup or glass upside down on top of mushroom to keep air currents away
- 3) Leave spore sprints overnight (approx. 24 hours) and do not move
- 4) Identify color of spore print

If Ascomycetes (morels & false morels)

- 5) Place piece of cap on glass or paper
- 6) Spores will show up around mushroom section

Note: Color

SPORE COLLECTION:

A spore collection is conducted on a mature mushroom on a slide for analysis of shape.

- 1. Place cap of mushroom downward onto the slide (fertile side down) and wait 1-2 hours until spore dust is present on the slide
- 2. Add a single drop of...
 - a. Colored spore = DI water or soapy water
 - b. White/clear spore = Melzer's reagent
- 3. Place cover slip over the spore/aqueous mixture
- 4. Identify key characteristics of spore
 - a. Shape
 - b. Size
 - c. Spore surface

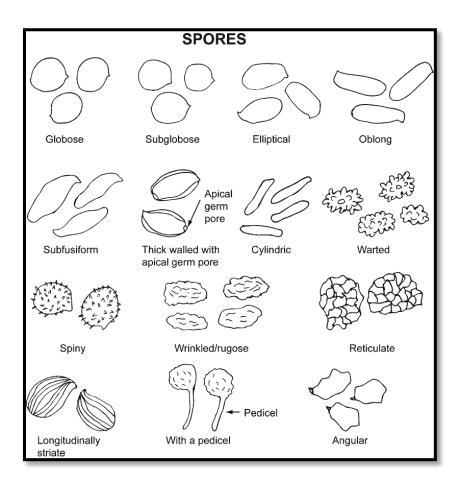


Figure 6: Potential spore shapes from spore collection (Extracted from http://www.toxinology.com/generic_static_files/images_generic/MD-spores1.gif)

* Not exhaustive

KEY SPORE TERMS:

- 1. Amyloid if they turn a blue-black colour.
- 2. Dextrinoid if they turn a reddish-brown colour.
- 3. Inamyloid (or negative) if they merely turn yellowish or do not change at all.

FUNGAL TISSUE COLLECTION:

A fungal tissue collected is conducted to determine key characteristics of the hyphae. Proper collections are difficult to extract because the tissue tends to be too thin. The hypha analysis tends to be conducted on the gills (or pores).

- 1. Cut a thin sliver of the gill or other portion from the fungi using a sharp razorblade
- 2. Place specimen on slide, add a few drops of stain and place cover slip on top and apply pressure to flatten the tissue

- 3. Remove excess stain using absorbent tissue and wait 5 minutes to ensure the stain has properly permeated tissue specimen
- 4. Observe tissue structure!

MICROSCOPIC IDENTIFICATION OF DRY SPECIMEN:

- Completed after drying to reuse specimen

- 1) Break off small piece of dried specimen's cap
- 2) Let specimen soak in 90% alcohol for a few minutes
- 3) Transfer specimen to tap-water dish & let it soak (few minutes)
- 4) Blot specimen with paper towel
- 5) Roll up specimen tightly so gills run lengthwise
- 6) With sharp razor blade slice very thin cross-section
- 7) Transfer cross-section to slide and add medium (usually 2% KOH with Phloxine stain)

SAVE MUSHROOMS (DRYING THEM OUT):

- Drying can take up to two days or longer depending on the size of the mushroom
- 1) Food Dehydrator

OR

- 2) Pinned in a Paper Towel
 - a. Place mushrooms in paper towel loosely
 - b. Fold paper towel over
 - c. Pin paper towel to a wall near lamp (dried within one or two days)

- 3) Over a Lamp
 - a. Put a wire mesh over a lamp
 - b. Place paper towel over wire mesh
 - c. Place mushroom on top of paper towel

STORE MUSHROOMS:

- 1) Place mushrooms in sturdy plastic zip-lock bags or acid-free paper
- 2) Place identification note in/attached to bag Note:
 - Identified Species:
 - Identification ID:
 - Location:
 - Date of Collection:
 - Name of Identifier:
- 3) Place in cardboard box
- 4) Place cardboard box in a dry location

FINAL IDENTIFICATION PROCESS:

Collect all of the spore, tissue, and identification images collected throughout procedure and use characteristics to identify fungi via Dichotomous key (Mushrooms Demystified or *MushroomExpert.Com* website recommended).

Major Sources for Procedure Guide and Species Identification:

Arora, David. 1986. Mushrooms Demystified. Ten Speed Press. Berkeley, California.

Kuo, M. (2006, February). Studying Mushrooms. Retrieved on October 1, 2014 from the *MushroomExpert.Com* Web site: <u>http://www.mushroomexpert.com/microscope.html</u>.

O'Reilly, Pat. 2014. Fascinated by Fungi. Retrieved on November 13, 2014 from <u>http://www.first-nature.com/fungi/facts/microscopy.php</u>.