

Corticolous Lichen Communities on Two Hemlock Populations

by

Bubba Pfeffer

Honors Thesis

Appalachian State University

Submitted to the Department of Biology

and the Honors College

in partial fulfillment to the requirements for the degree of

Bachelor of Science

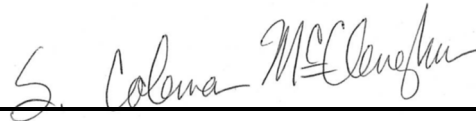
May, 2021

Approved by:



---

Michael Madritch, PhD., Thesis Director



---

Coleman McCleneghan, PhD., Thesis Director

---

Jefford Valhbusch, PhD., Dean, The Honors College

**Below-** Corticolous *Lepraria* on *Tsuga caroliniana*.



### **Abstract**

Epiphytic lichen communities on a population of *Tsuga caroliniana* on Bluff Mountain were surveyed and compared to the lichen communities surveyed on a population of *T. canadensis* in Appalachian State University's environmental studies area. A total of 12 species of corticolous lichens were found at Bluff Mountain and 14 species at the ASU environmental studies area. Only 3 lichens were found at both sites, and an analysis of similarities test (ANOSIM) confirms that these are distinct lichen communities.

## Introduction

Lichens are traditionally defined as the symbiotic relationship between a single fungal partner, or mycobiont, and one or more photosynthetic partners, or photobionts (Nash 2008). Though mycologists have detected fungi beyond the thallus-forming mycobiont, these fungi have long been assumed to be mycoparasites. Recent research that discovered the presence of basidiomycete with direct hyphal contact to the photobiont challenge this assumption and disprove the one lichen: one fungus paradigm (Tuovinen et al. 2019). Symbiotic strategies of life are important components of all ecosystems and remain poorly understood.

Lichens are important components of many ecosystems, contributing approximately 7% to global net primary production each year (Elbert et al. 2012). They are also effective indicators of certain types of air pollution (Hawksorth and Rose 1976). A study by Boch et al. (2013) including 631 European forest plots observed that old deciduous forests were richer in corticolous lichens than young ones, while old coniferous forests were poorer than young ones. Many dynamics between epiphytes and their host trees remain poorly understood. Little, possibly no research exists which describes the specific dynamics between *Tsuga caroliniana* and their associated corticolous lichen communities.

*Tsuga caroliniana* forests are an endangered ecosystem, geographically limited by high summer temperatures, fire, and limited availability of suitable cliff tops and rock outcrops (Jetton et al. 2008). The Hemlock Woolly Adelgid threatens *Tsuga* species, causing chlorosis, needle loss, bud loss, and tree mortality within 10 years of infection (Van de Gevel et al. 2016). Strategic implementation of insecticide treatments has shown to be effective at controlling the short-term impacts of the HWA and may be crucial to avoid the functional extinction of *T. caroliniana* (2016). *Tsuga canadensis* has a much more widespread distribution, frequently establishing itself in low-lying stream beds. The lower populations of *T. caroliniana* make it much more susceptible to extinction from HWA (2016).

The variable topography of the Southern Appalachians creates many microclimates upon which organisms adapt to. Rocky outcrops and cliffs represent highly diverse environments with many microclimates determined by a host of abiotic factors such as slope, rock type, aspect, latitude, and annual rainfall. Historically, cliffs and outcrops are areas of low disturbance and high climatic variability (Larson et al. 2000). The low disturbance of cliffs and outcrops has

made them excellent refugia habitats for vegetational communities that can withstand the harsh environmental conditions. As a result, cliffs and outcrops house many endemics and disjunct from far North species (Wiser et al 1996), including conifers such as *Tsuga*.

A total of 4 comprehensive floristic studies in the Amphibolites have reported unique natural communities, floristic composition, and high species diversity. These studies were performed by Tucker in 1972, Poindexter in 2006, Poindexter and Murrell in 2008, and Jenkins in 2011. 25 endangered vascular plant species have been documented within the Amphibolite mountains, as well as the discovery of a novelty lichen species, *Cladonia psoromica*, and the federally listed and endangered lichen *Cetradonia lineare*. Bluff mountain harbors several rare communities including a Carolina Hemlock Forest, a Southern Appalachian Fen, a dwarf red/white oak forest, as well as several distinct rock outcrops and cliff tops.

Corticolous lichens grow epiphytically on the bark of woody shrubs and trees. Their growth responds to a variety of conditions including bark microtopography, texture, and pH (Brodo 1973), nutrient availability (Hawksworth 1975), light levels (Gauslaa et al. 2006, 2007), and relative humidity (Frahm 2003). The study of epiphytic lichens on conifers, particularly those on *Tsuga*, remains under-researched. This study aims to describe the diversity of lichens growing on Bluff Mountain's *T. caroliniana* population and compare it with the lichen diversity found on *T. canadensis* in the ASU environmental studies area.

## **Methods**

Each site, at Bluff Mountain and the Appalachian State Natural Area, was comprised of 3 clusters of 3 trees. Trees were selected based on availability of suitable hemlocks with a DBH>10cm in the case of the ASU natural area. In the case of Bluff Mountain, trees were selected with the intention of describing all lichen diversity found on this population of *T. caroliniana*. Trees close to the edge of Perkin's Rock's cliff were excluded from the survey. For each tree, circumference was measured at breast height and at the base of the tree. GPS coordinates were recorded for each cluster of trees.

After trees were selected and clusters established, lichen presence was recorded. Trees were divided into two plots, each 50cm in height. At the base of the tree and at breast height, each plot was further divided into 4 subplots based on cardinal direction. Using a tape measure,

compass, and thumb tacks, lichen presence was recorded in binary for each subplot. Collections were made for all lichens found at Bluff Mountain for identification. Lichens at the ASU site that were morphologically similar to Bluff Mountain lichens were collected and compared under a microscope to determine identity.

Photographs were taken using a Nikon D3100 digital SLR camera with a NIKKOR 40mm f/2.8G lens. The camera was stationed on a tripod, and a wire-connected shutter release was utilized to prevent shaking of the camera during long-exposure pictures. ISO was set to 800 and images were saved in a RAW format for maximum quality. Images were processed in photoshop. Images were cropped as needed, light levels were adjusted for clarity, and images were converted to PNG files.

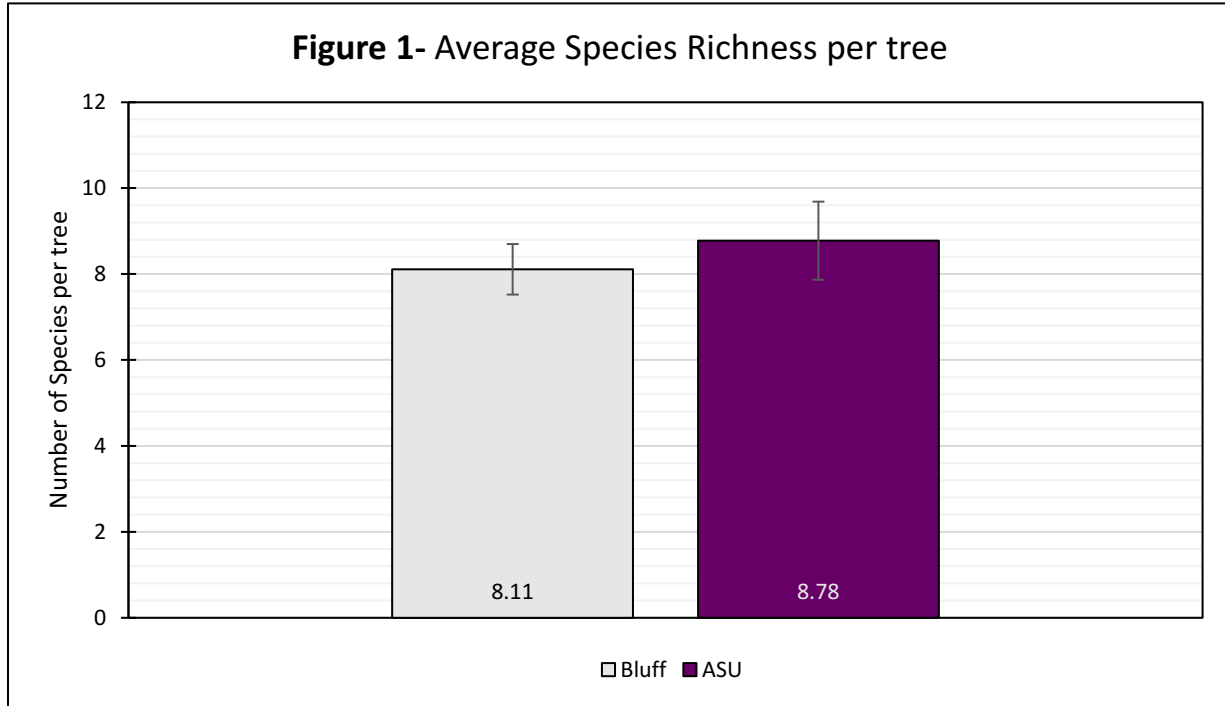
All lichens collected from Bluff Mountain were identified using standard lab stereoscopic microscopes and confirmed by Coleman McCleneghan. For chemical tests, A 10% KOH solution was used to run K tests, household bleach to run C tests, and para-phenylenediamine dissolved in ETOH to run PD tests. *Macrolichens of New England* by Hinds and Hinds was used to identify macrolichens. *Field Guide to the Lichens of Great Smoky Mountains National Park* by Tripp and Lendemer was used for crustose and powder lichens. Any lichens that could not be keyed out in either of these two books were identified using *Lichens of North America* by Erwin Brodo.

Presence data was imported into R studios and analyzed using the Vegan package. Using a Constrained Community Analysis (CCA), ordination graphs were generated, comparing the study sites, cardinal direction, and base/breast plots. Statistical tests were run for each of these using an Analysis of Similarities (ANOSIM) using the Bray distance method.

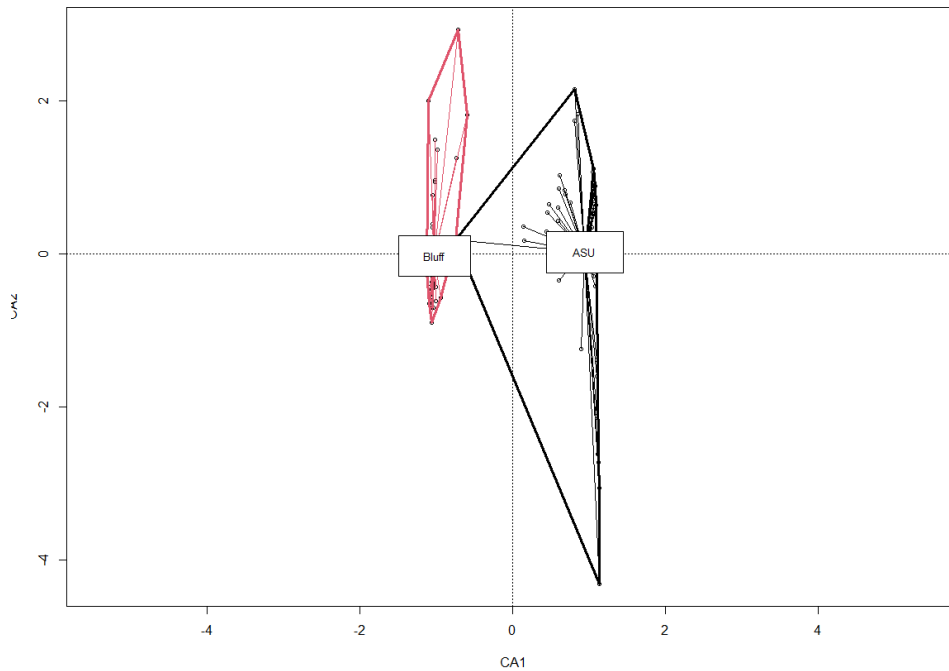
## **Results**

A total of 12 corticolous lichens on *Tsuga* were found on Bluff Mountain, including 4 members of the *Lepraria* genus complex, and 2 reproductive members of the *Cladonia* complex: *Cladonia turgida* and *Cladonia incrassata*. A total of 14 lichens were found at the Appalachian State Environmental Studies Area. Species richness per tree was not significantly different between Bluff and ASU study sites (Fig 1).

**Figure 1- Average Species Richness per tree**



**Figure 2- CCA Site Ordination**



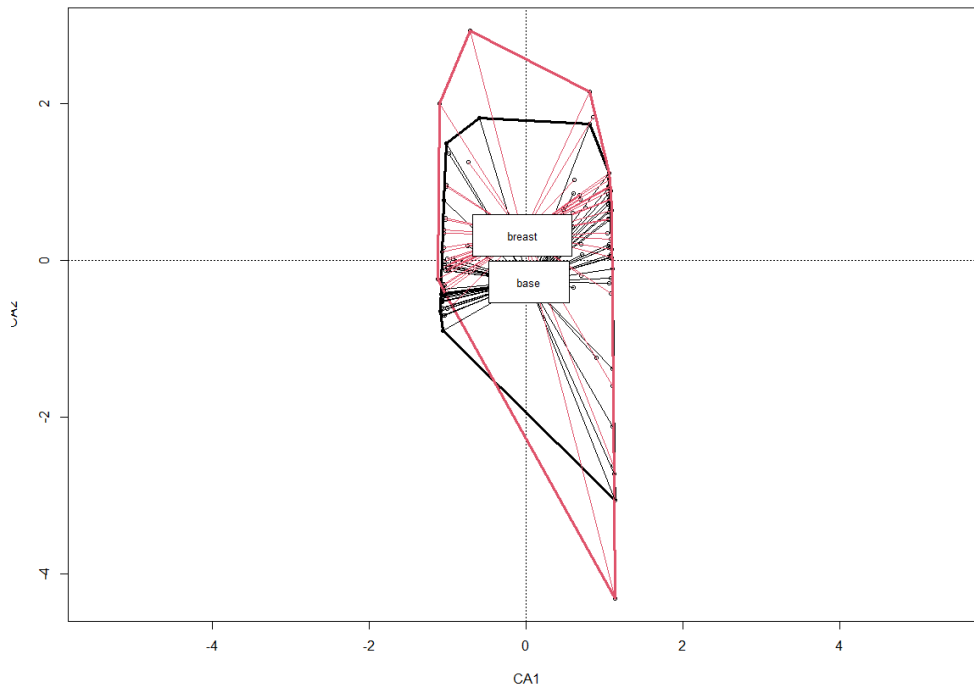
Lichen distribution varied between the two study sites ( $R = 0.8912$ ) and this difference is statistically significant ( $P = 0.001$ ). The ASU study site displays a greater distribution spread

than the Bluff site (Fig. 2). Only 3 lichens were found at both study sites. Of these three, one was *Parmelia saxitalis* (Fig. 3), and the other two were members of the *Lepraria* genus complex.

**Figure 3- *Parmelia saxitalis***

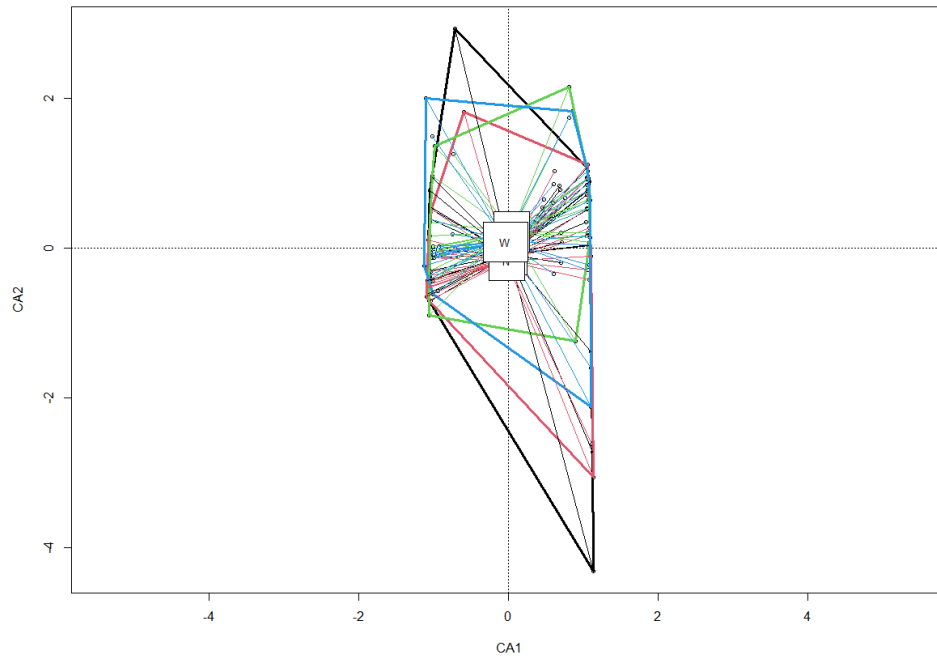


**Figure 4- CCA Plot Ordination**



Lichen distribution was not significantly different for base and breast plots (Fig 4,  $R = 0.00912$ ,  $P = 0.147$ ).

**Figure 5- CCA Cardinal Subplot Ordination**



Lichen distribution was unaffected by aspect on the hemlocks ( $R = -0.01773$ ,  $P = 0.977$ ). The center of the ordination plots for each of the four cardinal directions were precisely similar (Fig 5).

### **Discussion & Observations**

Many variables differ between the ASU and Bluff Mountain study sites. The Bluff site is situated on top of Perkin's rock on a mountain with amphibolite bedrock. The ASU site is situated in a valley within a nature preserve. The surrounding forests and plant communities are different. The *Tsuga* population at ASU has experienced extensive mortality, so the population is mostly made up of juvenile trees next to dead, old growth Hemlocks. Two old growth hemlocks from the ASU site were alive, although unhealthy, and included in the community analysis. Additionally, the ASU site is situated close to Boone, North Carolina, and so there are many urban lichen spores and propagules in the environment. Lastly, the Bluff site population is composed of *T. caroliniana* while the ASU site is composed of *T. canadensis*. As a result of these many differences, this study cannot explain the reason for the difference between the two sites (Fig 2).

The Bluff Mountain *Cladonia* complex was made up of *C. turgida*, *C. incrassata*, and a network of their associated squamules. The squamules are often congregated in dense clusters,



frequently co-occurring with moss (Fig. 6). Some *Cladonia* squamules grew up the base of the tree on shallow accumulated soil (Fig 6), but others grew directly on the bark of hemlocks (Fig. 7).

**Figure 6 (Left)-** *Cladonia* spp. Growing with moss on Bluff Mountain at the Base of a Hemlock

**Figure 7 (Right)-** *Cladonia* spp. Growing corticolously, directly on Hemlock Bark.



Members of the *Cladonia* genus complex found on hemlocks were distinct from nearby terricolous *Cladonia*. *Cladonia cornuta* was abundant on the forest floor in the Bluff Mountain site. It was not observed growing on the base of *Tsuga caroliniana* trees. *C. cornuta* may be restricted to environments with enough soil to sustain its high biomass, whereas *C. turgida* and *C. incrassata* may be adapted to the shallow soils and bark found on trees. Members of the *Cladonia* genus complex display a diverse array of substrate living conditions and reproductive strategies. Their podetia possess sexual reproductive structures, while their squamules possess vegetative reproductive structures (Fig. 7).

*Lepraria* represents one of the most cryptic genera of lichens. *Lepraria* is a highly nondescript genus of sterile, leprose lichens which require TLC procedures to identify to species.

Four unidentified species of *Lepraria spp.* were found on Bluff Mountain. One such *Lepraria spp.* was present on every Bluff Mountain subplot but two. As such, *Lepraria* represents a highly abundant component of Bluff Mountain's *Tsuga caroliniana* corticolous lichen ecosystem.

Photography is a useful tool for communicating and contextualizing research. It allows researchers to display the study organisms in their natural habitat. The increasing popularity of citizen-science internet tools for sharing pictures of living things, such as iNaturalist, show potential. These technologies are still young and have several problems. Misidentification makes their use for research limited, and attention biases make it so that non-charismatic organisms like lichens are often not included. These sites often incorporate machine-learning technology to assist in identification, and the inclusion of high-quality images with correctly identified names incrementally improves the effectiveness of those technologies.

The exclusion of nonvascular plants limits this study's effectiveness at comparing the community composition at the base and breast height of trees, because mosses and liverworts make up such a substantial amount of the biomass at the base of many old growth hemlock trees (Fig. 4). Additionally, the use of binary data, while increasing the speed and efficiency of surveying, reduces the descriptive power of the data. Future studies that survey a transitional gradient from the base of the tree to breast height could better illuminate some of the complexities of corticolous lichen communities.

Much of the specifics underlying lichen ecology and physiology remain understudied. Specifically, more research is needed on the ecological interactions between lichens and other poikilohydric organisms, like mosses and liverworts. Is this a strictly competitive interaction, or does growing with a large biomass of moss also assist in the lichen's poikilohydrology? The inclusion of more abiotic factors such as light, moisture, and quantification of micro-topography into the community analysis would improve the understanding of corticolous lichen communities on *Tsuga* populations. Additionally, more research illuminating the ecology and systematics of *Lepraria spp.* is essential to understanding the community dynamics of the corticolous lichens *Tsuga*. Lastly, research is needed to quantify the difference between corticolous lichen communities in forested and urban ecosystems.

## Literature Cited

- Austin, D. A., Van de Gevel, S. L., and Soule, P. T. 2016. Forest dynamics and climate sensitivity of an endangered Carolina hemlock community in the southern Appalachian Mountains. *Botany*. 94, 301-309.
- Boch, S. Prati, D., Hessenmoller, D., Schulze, E. D., Fischer, M. 2013. Richness of Lichen Species, especially of threatened ones, is promoted by Management Methods Furthering Stand Continuity. *PLOS One*. 8(1)
- Brodo, I. M. 1973. Substratum Ecology. *The Lichens*. Academic Press, New York, pp. 401-436.
- Elbert, W., Weber, B., Burrows, S., Steinkamp, J., Budel, B., Andreae, M. O., Poschel, U. 2012. Contributions of Cryptogamic covers to the global cycles of carbon and nitrogen. *Nat. Geosci.* 5, 459-462.
- Gauslaa, Y., Lie, M., Solhaug, Ohlson, M. 2006. Growth and ecophysiological acclimation of foliose lichen *Lobaria pulmonaria* in forests with contrasting light climates. *Oecologia* 147, 406-416. t
- Gauslaa, Y., Palmqvist, K., Solhaug, K. A., Holien, H., Hilmo, O., Hybakken, L., Myhre, L.C., Ohlson, M. 2007. Growth of epiphytic old forest lichens across climatic and successional gradients. *Can. J. For Res.* 37, 1832-1845
- Harper, C. J., Walker, C., Schwendemann, A. B., Kerp, H., Krings, M., 2020. *Archaeosporites rhyniensis* gen. et sp. nov (Glomeromycota, Archaeosporaceae) from the Lower Devonian Rhynie chert: a fungal lineage morphologically unchanged for more than 400 million years. *Annals of Botany*. 5, 915-928.
- Hawksworth, D. L. 1975. Lichens – New Introductory, matter and supplementary. Index by Smith A. L. 1921. The Richmond Publishing CO, Cambridge. (462p).
- Hawksworth, D. L., and Rose, F. 1976. Lichens as Pollution monitors. Arnold Publishers, London.
- Jenkins, D. 2011. The Vascular Flora of Three Top Mountain Game Land Preserve, Ashe County, North Carolina. M.S. Thesis, Appalachian State University, Boone, North Carolina.
- Jetton, R.M., William, S.D., and Whittier, W.A. 2008. Ecological and genetic factors that define the natural distribution of Carolina hemlock in the southeastern United States and their role in ex site conservation. *For. Ecol. Manage.* 255. 654–661
- Larson, D. W., Matthews, U., Kelly, P. E. 200. Cliff ecology : pattern and process in cliff ecosystems. Cambridge University Press, 2000.
- Lutzoni, F., Pagel, M., and Reeb, V. 2001. Major Fungal Lineages are derived from lichen symbiotic ancestors. *Nature*. 411, 937-940.

- Nash, T. H., 2008. *Lichen Biology: Second Edition*. Cambridge University Press.
- Poindexter, D. B. 2006. *Vascular Flora of Mount Jefferson State Park and surrounding environs*. M.S. Thesis, Appalachian State University, Boone, North Carolina.
- Poindexter, D. B. and Murrell, Z. E. 2008. *Vascular Flora of Mount Jefferson State Natural area and environs, Ashe County, North Carolina*. *Castanea* 73, 283-327.
- Tucker, G. E. 1972. *The vascular flora of Bluff Mountain, Ashe County, North Carolina*. *Castanea* 37, 2-26.
- Tuovinen, V., Spribille, T., Johannesson, H., Ekman, S., Thor, G., and Vanderpool, D. 2019. *Two Basidiomycete Fungi in the Cortex of Wolf Lichens*. *Current Biology*. 29, 476-483.
- Wiser, S. K., Peet, R. K., and White, P. S. 1996. *High-elevation rock outcrop vegetation of the Southern Appalachian Mountains*. *Journal of Vegetation Science*. 7, 703-722.