A Preliminary Report on the Aerophytic Cyanoprokaryota and Algae from the Great Smoky Mountains National Park, USA

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Abstract: An annotated list is provided for 38 species of aerophytic algae (31 cyanobacteria, six Chlorophyta, and one Bacillariophyta) that were recovered from eight soil study sites (in coniferous and hardwood forests) collected during surveys carried out in the Great Smoky Mountains National Park (GSMNP) in August 2010. The cyanobacterium *Leptolyngbya foveolarum* was the single most frequently encountered species recovered from all eight study areas. Most species (68% of the total) were found at only one or two sampling sites. There were 32 new species records in the GSMNP.

Keywords: Algae, Cyanoprokaryota, Aerophytic, Great Smoky Mountains National Park, Biogeography, Tennessee, North Carolina, Aerial, Terrestrial.

INTRODUCTION

The Great Smoky Mountains National Park (GSMNP) straddles the border between the states of Tennessee and North Carolina (Figure **1**). This park has a total area of 211,415 ha and is one of the most visited parks within the National Park system, with more than 11 million visitors during 2017. The highest elevation in the park is 2,025 m at the summit of Clingmans Dome and the lowest elevation is 267 m.

GSMNP receives an average of 140 cm of rainfall each year at lower elevations, while the highest peaks can get around 220 cm per year [12]. The park is almost 95 percent forested, and almost 36 percent (76,000 ha) is estimated by the Park Service to be old-growth forest, with many trees that predate European settlement of the area [24].

There are over 1,400 species of flowering plants and at least seven different types of forests within the park boundaries (beech forest, cove hardwood forest, eastern hemlock forest, mixed hardwood forest, northern hardwood forest, pine-oak forest and spruce-fir forest). The forest distribution is influenced by elevation, with hardwoods generally occurring at lower elevations and the very highest elevations being dominated by conifers [24, 31, 32].

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There have been a number of previous studies of algae in the GSMNP. Most of the work on algae that has been carried out has focused on freshwater habitats [2, 3, 6, 9, 14-18, 21, 23, 25-28, 30]. There have only been a few studies that focused on aerial or terrestrial habitat [10, 13, 22], and none of these looked at forest soils.

The purpose of the study reported herein was to document the aerophytic algae and cyanoprokaryota associated with sample material collected from eight study sites in GSMNP). As the terms are used in this paper, algae are defined as eukaryotic organisms containing chlorophyll “a” in the Kingdom *Protoctista*, whereas cyanoprokaryota are prokaryotic organisms in the Kingdom *Prokaryotae* containing chlorophyll “a” and phycobilins as photosynthetic pigments.

METHOD AND MATERIALS

A total of eight study areas were established throughout GSMNP during the 2010 field season (Figure **2**). These study areas encompassed forests dominated by red spruce (*Picea rubens* Sarg.) at 1618m), American beech (*Fagus grandifolia* Ehrh.) at 1577 m, northern hardwoods at 1502 m, cove hardwoods at 965 m, eastern hemlock (*Tsuga canadensis* [L.] Carriére) at 734 m, mixed hardwoods with a high proportion of oak at 636 m, *Lirodendron tulipera* L. (an early successional community) at 622 m and pine (Pinus rigida *Mill.* and Pinus virginiana *Mill.*) at 568 m (Table **1**). Samples were collected for the laboratory isolation of algae (both eukaryotic and

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Figure 1: Great Smoky Mountains National Park location in USA.

Figure 2: Great Smoky Mountains National Park with sampling localities identified.

prokaryotic [*i.e.*, cyanobacteria]) from the soil/humus-litter interface zone. To the best of our knowledge, the present study represented the first substantial attempt to study these organisms from this microhabitat within the GSMNP.

To inoculate the Bold's Basal Medium (BBM) [1] liquid media, small amounts of sample material were placed into test tubes for culturing. Cultures were maintained at room temperature (22 °C). The cultures were examined after four weeks. Although BBM is the standard medium used for culturing algae, it is possible that other media might have yielded at least a few additional taxa. Semipermanent slides were prepared with distilled water and sealed with epoxy [29]. The keys were used to identify species and to determine the appropriate nomenclature were used [1, 5, 4, 7, 8, 19, 20]. To update the nomenclature, the

Table 1: Sampling Localities in the Great Smoky Mountains National Park

Site	Date Collected	Latitude	Longitude	Forest Type	Elevation (m)
1	8/14/2010	35.607867	-83.447067	Red Spruce	1618
2	8/14/2010	35.641359	-83.48315	Cove Hardwood	965
3	8/15/2010	35.607494	-83.790525	Pine	568
4	8/15/2010	35.67031	-83.593559	Hemlock	734
5	8/16/2010	35.56248	-83.164135	Beech Gap	1577
6	8/16/2010	35.521808	-83.308833	Oak Dominated Mixed Hardwoods	636
7	8/17/2010	35.659588	-83.520966	Early Successional Tulip Tree Dominated Site	622
8	8/17/2010	35.586333	-83.074807	Northern Hardwoods	1502

Algaebase.org website was used [11]. Therefore, all names are currently accepted taxonomically. To determine similarity between forest type and site location, the Jaccard Index was used.

RESULTS AND DISCUSSION

Thirty-eight species were identified from the eight sites (Table **2**). Cyanoprokaryota were the dominant group, represented by 31 species (81.6% of the total species reported), while six species (15.8%) of Chlorophyta (green algae) and one diatom (2.6%) were identified. Most of the species (32 or 86.5% of the species identified) were new records for GSMNP, with the Cyanoprokaryota with 28 new records and the green algae with four new records.

As a general observation, the GSMNP study sites were not very diverse and algal species richness was low. There was an average of 4.1 species per study site. Species tended to be site specific. On average, each species was found at 1.38 study sites.

The cyanobacterium *Leptolyngbya foveolarum* was the most frequently encountered species recovered from all eight study areas. Most species (68.4% of the total) were found at only one or two study sites. Cyanoprokaryota had the largest number of species identified, and this group had the largest number of species (71%) found at one or two sites. The Chlorophyta was represented by six species and 50% of the species were found infrequently. There was only one diatom (*Navicula veneta*) recovered, and it was found at only one study site.

The Shannon Diversity Index ranged from a lowest value (H´ = 1.2) for the oak-dominated mixed hardwoods study site to the highest value (H´ = 2.3) for the northern hardwoods study site (Table **3**). The cove hardwoods study site and the Eastern hemlock study site contained the most dissimilar assemblages of species (Jaccard Index Similarity value = 0.071), whereas the pine study site and the eastern hemlock study sites were characterized by the most similar assemblages (Jaccard Index Similarity value = 0.50).

Elevations of the study sites ranged from 622 m to 1618 m in the GSMNP. There was not a significant correlation between elevation and the total number of species recovered at a particular study site, but there was a positive trend (Figure **3A**) with respect to elevation and the total number of species for the Cyanoprokaryota and a negative trend (Figure **3B**) with respect to the relationship between elevation and the total number of species of green algae. Consequently, there was a slightly higher number of species identified at higher elevations.

There were five study sites dominated by angiosperms (hardwoods in Sites #2, 5, 6, 7 & 8) and three study sites dominated by conifers (Sites #1, 3 & 4). The angiosperm study sites had 30 species identified, with the Cyanoprokaryota representing, the dominant group (26 species) and the green algae and diatoms representing three and one species, respectively. The conifer-dominated study sites yielded 19 identified species (Cyanoprokaryota with 15 species, green algae at four and no diatoms observed). The average number of species identified per study site was 11 for the angiosperm study sites and 10 for the conifer study sites (Figure **4**). The average number of species present was the Cyanoprokaryota at 9.4, the green algae at 1.4 and diatoms with 0.2 species identified per angiosperm site. At the conifer study

sites, the averages were Cyanoprokaryota 8.7 species

Table 2: Annotated Taxonomic list with their Locality of the Species of Algae and Cyanoprokaryota Recovered from Samples Collected from Great Smoky Mountains National Park

	Taxon	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Site 8
	Cyanoprokaryota								
1	**+Anathece minutissima* (West) Komárek, Kastovsky & Jezberová			X	X				
2	**Aphanocapsa biformis* A.Braun in P.Richter		X	X		X		X	X
3	*#Aphanocapsa fuscolutea* Hansgirg						X	X	
4	**#Aphanocapsa grevillei* (Berkeley) Rabenhorst								X
5	*#Aphanothece saxicola* Nägeli								X
6	**#Chroococcus bituminosus* (Bory) Hansgirg						X	X	X
7	*#Chroococcus pallidus* Nägeli						X		
8	**Chroococcus rufescens* (Kützing) Nägeli	X	X			X	X	X	X
9	**+Chroococcus turgidus* (Kützing) Nägeli				X				
10	**Chroococcus varius* A.Braun in Rabenhorst	X		X	X				X
11	**+Dactylococcopsis fascicularis* Lemmermann	X		X					
12	**#Desmonostoc muscorum* (C.Agardh ex Bornet & Flahault) Hrouzek & Ventura in Hrouzek *et al.*							X	
13	**Gloeobacter violaceus* Rippka, J.B.Waterbury & Cohen-Bazire			X	X				X
14	**Gloeocapsa biformis* Ercegovic	X	X			X	X		X
15	**Gloeocapsopsis pleurocapsoides* (Novácek) Komárek & Anagnostidis ex Komárek	X		X	X		X		X
16	**Hormothece rupestris* C.-C.Jao	X							X
17	**#Jaaginema kuetzingianum* (Nägeli ex Gomont) Anagnostidis & Komárek						X		
18	*Leptolyngbya foveolara* (Gomont) Anagnostidis & Komárek	X	X	X	X	X	X	X	X
19	**Myrmecia globosa* Printz	X							X
20	**#Nostoc calcicola* Brébisson ex Bornet & Flahault							X	
21	**#Nostochopsis radians* Bharadwaja		X						
22	**+Pleurocapsa concharum* Hansgirg			X					
23	**#Radiosphaera dissecta* (Korshikov) Starr							X	
24	**#Rhabdoderma compositum* (G.M.Smith) Fedorov								X
25	**#Rhabdogloea ellipsoidea* Schröder		X						X
26	**#Rhabdogloea hungarica* (Kol) Hindák								X
27	**#Rhabdogloea linearis* (Geitler) Komárek								X
28	**+Symploca muscorum* Gomont ex Gomont	X							
29	**Synechococcus elongatus* (Nägeli) Nägeli	X		X	X	X		X	X
30	**#Synechocystis aquatilis* Sauvageau								X
31	**#Synechocystis primigenia* N.L.Gardner					X			
	Bacillariophyta								
32	*#Navicula veneta* Kützing							X	
	Chlorophyta								
33	*#Asterocapsa divina* Komárek					X	X	X	X
34	**Chlorococcum infusionum* (Schrank) Meneghini			X	X				X
35	**+Chlorosarcina brevispinosa* S.Chantanachat & Bold				X				
36	*#Oedogonium* sp1 Link ex Hirn					X	X		
37	**+Oocystis pusilla* Hansgirg			X					
38	**+Scenedesmus ecornis* (Ehrenberg) Chodat			X					
	Total Species Identified	**10**	**9**	**12**	**9**	**8**	**10**	**11**	**21**

* New species identified to GSMNP; +Species only identified at conifer sites; # Species only identified at angiosperm sites.

and the green algae at 1.7 species. There was no significant difference between algal group and angiosperm and conifer forest preference.

Table 3: Shannon Diversity Index and Jaccard Index of Similarity between the Sampling Localities (#1-8) from Great Smoky Mountains National Park

Sites	S1	S2	S3	S4	S5	S6	S7	S8		H'
S1	1									1.8
S2	0.231	1								1.4
S3	0.235	0.125	1							2.1
S4	0.267	0.071	0.500	1						1.7
S5	0.286	0.400	0.176	0.133	1					1.7
S6	0.250	0.231	0.100	0.118	0.385	1				1.2
S7	0.167	0.214	0.150	0.111	0.357	0.313	1			1.5
S8	0.364	0.238	0.280	0.261	0.273	0.250	0.240	1		2.3

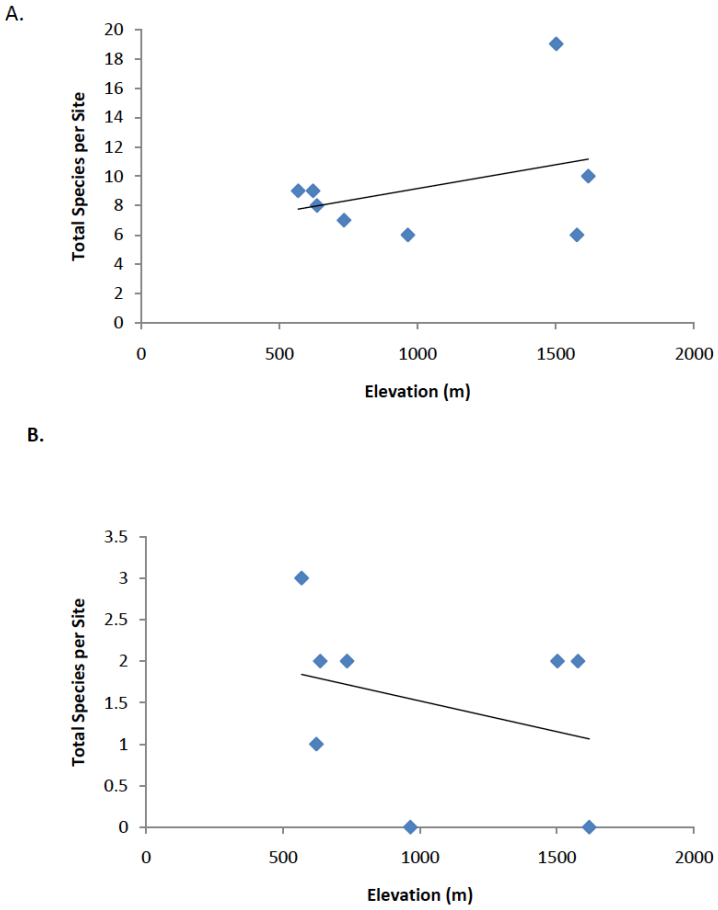

Figure 3: The total number of Cyanoprokaryota (**A**) and Chlorophyte (**B**) species identified per site and the altitude (m) with trend line for Great Smoky Mountains National Park.

Since only a limited number of samples were examined in the present study, both the lack of diversity and low species richness are not surprising. However, our data do suggest that particular species tend to be site specific and possibly forest habitat specific. There were 19 species (16 Cyanoprokaryota, 2 green algae and 1 diatom) found only in the angiosperm sites, while 8 species (5 Cyanoprokaryota and 3 green algae) were only found in conifer sites (Table **2**). The forest and soil habitats are potentially species rich with new species identified for the park. In the future, additional samples would need to be obtained to generate a more complete annotated species list for the GSMNP.

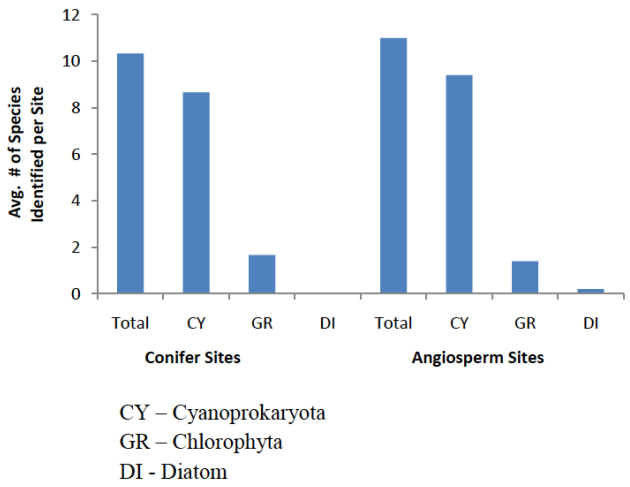

Figure 4: The average number of Cyanoprokaryota, Chlorophyta, and Diatom species identified per conifer study sites and angiosperm study sites for Great Smoky Mountains National Park.

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