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# A Novel Isolate And Widespread Abundance Of The Candidate Alphaproteobacterial Order (Ellin 329), In Southern Appalachian Peatlands

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## Abstract

Peatlands of all latitudes play an integral role in global climate change by serving as a carbon sink and a primary source of atmospheric methane; however, the microbial ecology of mid-latitude peatlands is vastly understudied. Herein, next generation Illumina amplicon sequencing of small subunit rRNA genes was utilized to elucidate the microbial communities in three southern Appalachian peatlands. In contrast to northern peatlands, Proteobacteria dominated over Acidobacteria in all three sites. An average of 11 bacterial phyla was detected at relative abundance values >1%, with three candidate divisions (OP3, WS3 and NC10) represented, indicating high phylogenetic diversity. Physiological traits of isolates within the candidate alphaproteobacterial order, Ellin 329, obtained here and in previous studies indicate that bacteria of this order may be involved in hydrolysis of poly-, di- and monosaccharides. Community analyses indicate that Ellin 329 is the third most abundant order and is most abundant near the surface layers where plant litter decomposition should be primarily occurring. In sum, members of Ellin 329 likely play important roles in organic matter decomposition, in southern Appalachian peatlands and should be investigated further in other peatlands and ecosystem types.

**Suzanna L. Brauer**, Austin B. Harbison, Michael A. Carson, Louis J. Lamit, Nathan Basiliko (2016) "A Novel Isolate And Widespread Abundance Of The Candidate Alphaproteobacterial Order (Ellin 329), In Southern Appalachian Peatlands" *FEMS Microbiology Letters* Vol. 363 Issue 15 [DOI: 10.1093/femsle/fnw151] Version of Record Available from [www.oxfordjournals.org](http://www.oxfordjournals.org)

Wetlands account for approximately 23% of total atmospheric methane emissions (Gorham 1991; Mitsch and Gosselink 2000; Aronson et al. 2013), affecting the global climate system and playing important roles in biosphere feedbacks to climate and environmental changes. Mid- and southern latitude wetlands have been identified as the source of approximately 70% of the total methane released by wetlands (Walter, Heimann and Matthews 2001), due to longer and more productive growing seasons. As well as emitting methane, peat-forming wetlands

(peatlands) are also a substantial net carbon sink, having stored over one-third of global soil carbon over the Holocene as partially decomposed organic soil matter (Gorham 1991). A large portion of peatlands are *Sphagnum* dominated, characterized by acidic soils, small pools of available macro- and micronutrients, low water flow and associated low redox potentials (Wieder 1985; Benoit, Fitzgerald and Damman 1998; Aerts, Verhoeven and Whigham 1999; Moore and Basiliko 2006) and are prevalent throughout the northern parts of North America, Europe and Asia. Southern North American peatlands are less abundant than boreal counterparts but share many common traits, leading to increased interest in their importance in global climate studies.

Complex microbial processes determine rates of C loss from peatlands, yet the microbial ecology (Dedysh 2011) and key anaerobic degradation processes are vastly understudied. Studies have mainly focused on the archaea and bacteria involved in methane production or consumption (reviewed in Andersen et al. 2013; Bridgman et al. 2013; Mandic-Mulec et al. 2014). A few studies have examined overall microbial diversity in peatlands and found that the *Proteobacteria* and *Acidobacteria* are detected in the greatest abundance (Dedysh 2011). Other phyla almost always reported as non-rare (relative abundance values >1%) in peatlands include *Verrucomicrobia*, *Actinobacteria* and *Planctomycetes*; those sometimes considered non-rare have thus far included *Firmicutes*, *Bacteroidetes*, *Chloroflexi*, *Spirochaetes* (Dedysh 2011; Serkebaeva et al. 2013; Puglisi et al. 2014; Tsitko et al. 2014) *Chlamydiae* (Urbanová and Bárta 2014) and *Nitrospirae* (Urbanová and Bárta 2016). Members of *Proteobacteria* are very diverse organisms that can adapt to a wide variety of lifestyles (Serkebaeva et al. 2013) and environments. *Alphaproteobacteria* in particular have been isolated from environments worldwide, carrying out a range of ecological processes. In peatlands, *Alphaproteobacteria* are thought to be primarily involved in methane oxidation (Dedysh 2011; Tsitko et al. 2014), sugar fermentation and anaerobic respiration (Lipson et al. 2013; Tveit et al. 2013).

Herein, we employed Illumina Miseq amplicon sequencing technology to elucidate the microbial assemblages involved in southern Appalachian peatlands. Additionally, using peat collected from Pineola Bog, we obtained a novel isolate, strain CS4, within the candidate alphaproteobacterial order, Ellin 329. The few known cultured (Sait, Hugenholtz and Janssen 2002; Ueki et al. 2010; Kodama and Watanabe 2011; this publication) and uncultured members (Verastegui et al. 2014) of Ellin 329 have been implicated in degradation of sugars and more complex plant di- and polysaccharides such as cellobiose, xylan and/or other compounds. Culture-independent Illumina data further demonstrated the prevalence, and potential importance, of members of the Ellin 329 order.

## MATERIALS AND METHODS

### Site description

The three peatland study sites in north-western North Carolina, USA, have been described in detail by Hawkins, Johnson and Bräuer (2014). The sites represent a nutrient gradient from poor to intermediate fens, each having at least partial *Sphagnum* moss cover at each of three within-site sampling locations. Sugar Mountain Bog had an average pore water pH of 4.9 (range 4.6–5.4) and the water table depths at time of sampling ranged from 8 to 22 cm below the surface; Pineola Bog had an average pore water pH of 5.1 (range 4.9–5.2) and the water table depths at time of sampling ranged from 17 to 38 cm; Tater Hill Bog is a

more minerotrophic site with numerous *Carex* species, an average pore water pH of 6.1 (range 6.0–6.2) and the water table depths at time of sampling ranged from 0 to 14 cm below the surface. All three sites are between 1080 and 1300 m elevation.

### Sampling method

Peat samples were collected on 12 September 2013 from Pineola Bog at three locations within the bog at a depth of 25–30 cm (Pineola 2013, P.13. or P.25). Samples for a collaborative US Department of Energy's Joint Genome Institute (JGI, Walnut Creek, California) study collected on 19 and 20 June 2014, from all three sites, denoted as 2014, were collected in triplicate at a depth of 10–20 cm, and 30–40 cm from all sites and additionally, at a depth of 60–70 cm from Pineola Bog (Pineola 2014), since this was the only site containing peat below 45 cm. Pineola 2013 samples were collected in airtight jars, while the 2014 JGI samples were collected using an 11 cm diameter PVC corer and stored in Ziplock bags. Samples for molecular work were frozen at  $-80^{\circ}\text{C}$  until further use and samples for culturing were taken directly to the glove box.

### Community analysis

Samples collected in 2013 were processed for community sequencing as follows. DNA was extracted from the peat material using the MoBio Laboratories PowerSoil® DNA Isolation Kit according to the manufacturer's instructions. Extracted DNA was quantified using a nanodrop and stored at  $-4^{\circ}\text{C}$  until further use. PCR amplification was performed in a 25  $\mu\text{l}$  reaction containing 10  $\mu\text{l}$  PCR master mix (Q5 Hot start High Fidelity 2X Master Mix, New England BioLabs), 1  $\mu\text{l}$  of 50  $\mu\text{M}$  primers modified with Illumina adapters (515F 5' GTGCCAGCMGCCGCGGTAA and 806R 5' GGACTACVSGGGTATCTAAT), forward and reverse, respectively, PCR water to bring the volume to 25  $\mu\text{l}$  and DNA to equal approximately 15 ng of DNA per reaction. The PCR protocol was executed using the following parameters:  $94^{\circ}\text{C}$  denaturation for 3 min, then 35 cycles of a  $94^{\circ}\text{C}$  denaturation for 45 s,  $55^{\circ}\text{C}$  primer annealing for 60 s and  $72^{\circ}\text{C}$  extension for 90 s. The last step was a  $72^{\circ}\text{C}$  extension for 10 min followed by a hold at  $10^{\circ}\text{C}$  until storage. After PCR purification, samples were sent to West Virginia University's Genomic Core Facility (Morgantown, WV) for pair-end sequencing on an Illumina MiSeq system. 2013 sequencing data are accessible via the NCBI database (<http://www.ncbi.nlm.nih.gov/sra>) as project number SRP070579.

Samples collected in 2014 were processed as follows. Samples were sent to the US Forest Service Northern Research station (Houghton, Michigan) for DNA extraction and purification. Approximately 10  $\text{cm}^3$  of collected peat were subsampled, placed in a 50 ml falcon tube with twenty 3.2 mm chrome-steel beads and pulverized for 2 min in a modified mini-beadbeater-96 (Biospect Products, Bartlesville, OK, USA). DNA was then extracted from 0.5 g of the pulverized peat using a MoBio PowerSoil DNA Isolation Kit following the manufacturer's instructions, amended with an additional 30 min incubation at  $65^{\circ}\text{C}$  following the addition of the C1 lysis buffer and 10 min of vortexing. DNA was cleaned with a MoBio PowerClean® Pro DNA Clean-Up Kit and quantified with a Qubit Fluorometer (Invitrogen). DNA extracts were then sent to the US Department of Energy JGI where they were subjected to PCR amplification with the primers 515F and 806R fitted with adaptors and unique 11 bp barcode sequences. Amplified DNA was then sequenced on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA)

using 2×250 chemistry. One subsample from the Sugar 30–40 cm range failed to sequence and is excluded from analyses. Data are accessible via the JGI portal under the DOE contract number: DE-AC02-05CH11231 (<http://genome.jgi.doe.gov/>).

All samples were aligned with PANDAseq (Masella et al. 2012), processed with QIIME (Caporaso et al. 2010) and USEARCH (Edgar 2010) (OTU assignment at a 97% cut-off value), and taxonomically identified via Greengenes database (DeSantiz et al. 2006) using the RDP method, described below. Pineola 2013 data were received as forward and reverse read sample pairs, while JGI data were rearranged and quality filtered from raw read data using the BMap package (Bushnell 2015). A total of 187 645 reads were entered for Pineola 2013, having a total of 16 567 unique reads. After analysis, a total of 30 843 singletons and chimeric sequences were removed. A total of 1786 853 sequences were entered for JGI, having a total of 103 187 unique reads. After analysis, 211 793 singletons and chimeric sequences were removed, resulting in 15 200 total OTUs.

Sequences in both the 2013 and 2014 datasets identified as Ellin 329 by the Greengenes database were collected. Additional unclassified sequences within *Alphaproteobacteria* were analyzed using RDP classifier and RDP Seq Match (Cole et al. 2005), and any sequences with *Rhizomicrobium* spp. top hits were included in the analysis (88%–94% ID to strain CS4; 89%–96% ID to Bacterium Ellin329 accession number: AF498711.1). To eliminate repetitive OTUs between the two datasets, DOTUR was used to reassign OTUs using a 97% cut-off value. The Ellin 329 sequences, the sequence for the novel isolate CS4 (Sanger sequencing) and top hit sequences from the NCBI database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>), were aligned using the SILVA aligner of the ARB program (<http://www.arb-silva.de/aligner/>). Aligned sequences were edited utilizing the BioEdit software (Ibis Biosciences, Carlsbad, CA). Phylogenetic trees were constructed with the neighbor-joining (Saitou and Nei 1987) and maximum-likelihood methods using the PHYLIP package (<http://evolution.gs.washington.edu/phylip.html>). Sequences for Ellin 329 representative OTUs were deposited in GenBank with accession numbers KU705479–KU705504, and the sequence for strain CS4 with accession number KU738893.

### Culture growth and isolation

Cells were isolated in PM1 medium, prepared in anaerobic balch tubes as described in Bräuer et al. (2006), and flushed with ultrahigh purity N<sub>2</sub> gas. Post sterilization, the following additions were made (final concentrations): ca. 0.5 mM Ti(III) Nitrilotriacetate (TiNTA) (preparation described in Bräuer et al. 2006), 20 mM MES (pH 5.7), Vitamin Solution (0.2, 1.0, 0.5 or 0.01 mg L<sup>-1</sup> of each vitamin as described in Bräuer et al. 2006 and Balch et al. 1979), 40 mg L<sup>-1</sup> yeast extract, 1 g L<sup>-1</sup> glycerol and 10 mg L<sup>-1</sup> rifampicin. Tubes were inoculated with 0.5 ml of 0.45 μm filtrate from the 2013 collection of peat from Pineola Bog. Inoculated media was incubated in the dark at 28°C without shaking, and growth was monitored by checking for turbidity in the liquid media by use of a spectrophotometer. Once isolated, cells were also grown in PM1 medium containing the addition of 500 mg L<sup>-1</sup> peptone and/or with 500 mg L<sup>-1</sup> sucrose or dextrose substituted for glycerol.

Colonies were isolated on 1.5% agar plates supplemented with glucose and peptone, streaked aerobically, but incubated anaerobically, in a mason jar with an N<sub>2</sub> headspace. After approximately 1 week of growth single colonies were transferred to new plates. Colonies were restreaked three times to ensure

purity and axenic colonies were transferred back to liquid media in the anaerobic chamber. Purity was visually inspected using fluorescence microscopy on an Olympus BX51 using acridine orange with a FITC filter. DNA was extracted, PCR amplified and sent to Beckman-Coulter Genomics (Danvers, MA) for Sanger sequencing.

### SEM imaging

Strain CS4 was grown anaerobically on agar plates supplemented with the aforementioned media additions excluding TiNTA. Sterile 0.2 μm filters were cut into quarters and placed on the agar prior to inoculation with cells grown in liquid culture. After 2 weeks of growth in an anaerobic jar, the filter was removed and soaked in 2.5% glutaraldehyde for 2.5 h. The filter was then dehydrated by soaking in a series of 50%, 75%, 85% 90% and 95% ethanol-water solutions, followed by soaking two times in 100% ethanol for a minimum of 1 h per soak, the final soak overnight. The filter was then critically point-dried using liquid CO<sub>2</sub> in a Tousimis 931 CP drier. The dried filter was mounted on a metal stub with adhesive and gold coated. Images were collected at 25 kV with a 4 μm spot size.

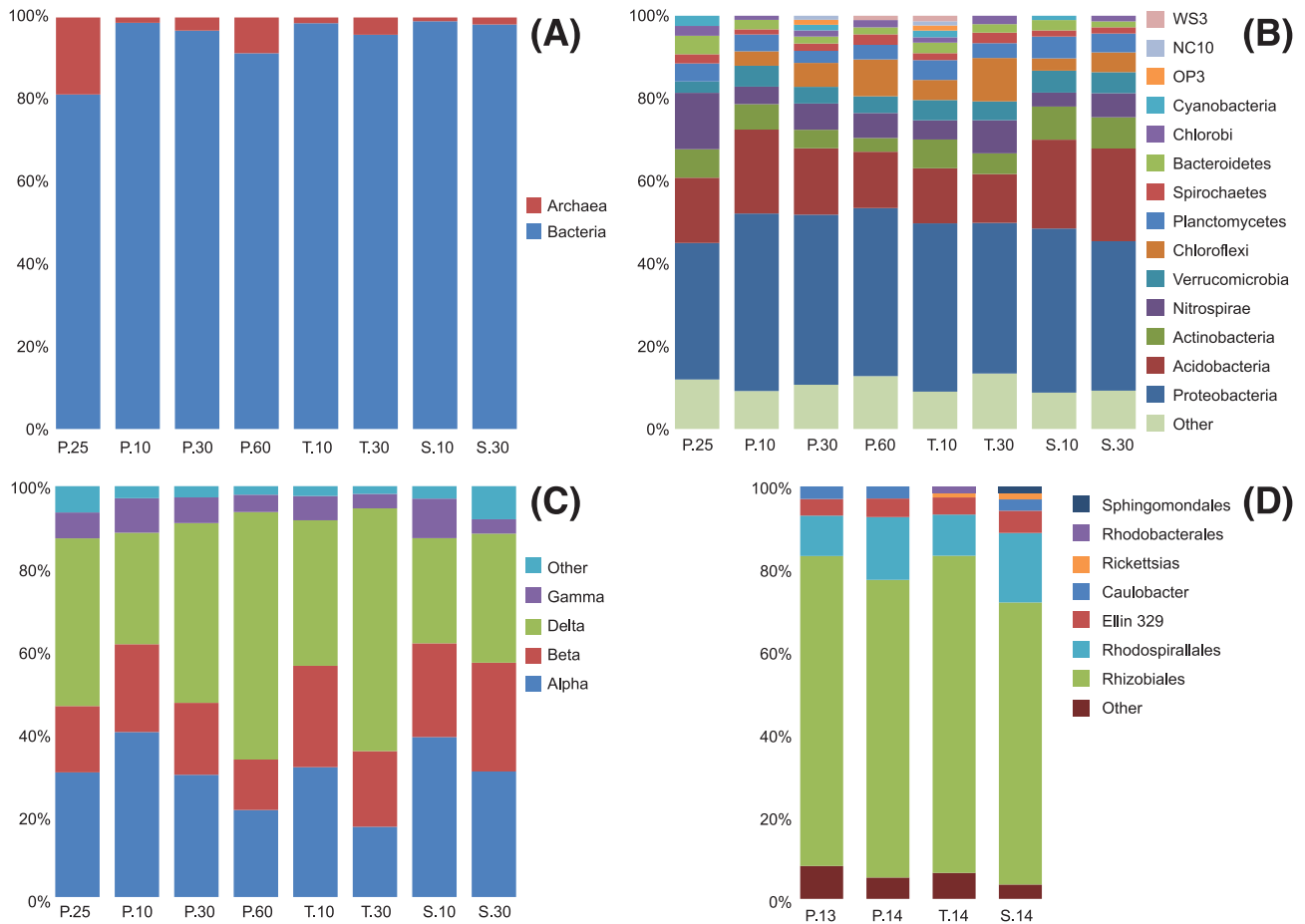
## RESULTS

### Bacterial and Archaeal communities

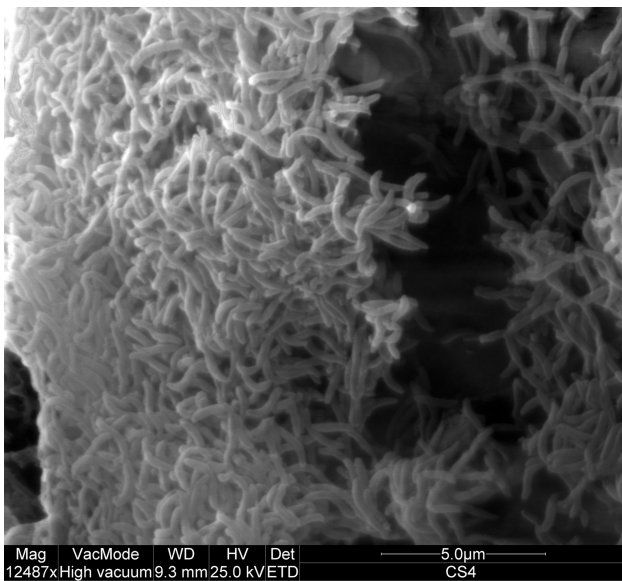
Bacterial sequences had the highest relative abundance, although relative abundance of archaeal sequences increased with depth. Archaeal sequences ranged from approximately 1% in the 10–20 cm depths to 3% in the 30–40 cm depths and 8.7% in the Pineola 60–70 cm depth. The highest archaeal relative abundance (18.7%) was detected in the Pineola 2013 dataset in the 25–30 cm depth (Fig. 1A). Among bacterial phyla, *Proteobacteria* had the highest abundance (33%–42%), with *Acidobacteria*, *Actinobacteria*, *Nitrospirae*, *Verrucomicrobia*, *Chloroflexi*, *Planctomycetes*, *Spirochaetes* and *Bacteroidetes* also present in all sample locations and dates (Fig. 1B). Within *Proteobacteria*, the alphaproteobacterial and deltaproteobacterial sequences had the greatest abundance. *Alphaproteobacteria* were the most prevalent in the 10–20 cm depth in Pineola and Sugar Mountain Bog sites (40% and 39%, respectively) with alphaproteobacterial and betaproteobacterial relative abundance decreasing with depth (Fig. 1C). *Deltaproteobacteria* became the most prevalent class in deep samples from Tater Hill and Pineola. Within *Alphaproteobacteria*, sequences related to *Rhizobiales* dominated in all sites, ranging from 68% to 77%, with sequences related to *Rhodospirillales* and the rarely cultivated candidate order, Ellin 329, the second and third most abundant (Fig. 1D).

### Candidate order Ellin 329

A novel, acid-tolerant, fermentative, obligate anaerobe, designated strain CS4, was isolated from Pineola Bog. The culture contained Gram-negative, curved rods (~0.25 μm diameter and ~3.0 μm long; Fig. 2). Growth was initiated between pH 5 and 6.8; however, actively growing cultures continued to grow down to pH 4. Growth was also observed between 15°C and 35°C and with less than 100 mM NaCl. Phylogenetic analysis indicated that strain CS4 clusters with OTUs identified as Ellin 329 in the Greengenes database, and alphaproteobacterial order *incertae sedis* in RDP. Isolates *Rhizomicrobium palustre*, *R. electricum*, Ellin 329, Ellin 332 and Ellin 5086 among others (Fig. 3) also clustered within this novel order. Clear clustering can be seen defining Ellin 329 from



**Figure 1.** (A–D) Relative abundance values for sequences detected in samples collected at 10–20, 25–30, 30–40 and/or 60–70 cm depths in Pineola in 2013 (P.25 or P.13), Pineola in 2014 (P.10, P.30 and P.60, or P.14), Tater in 2014 (T.10 and T.30, or T.14) and Sugar in 2014 (S.10 and S.30, or S.14). Relative abundance values of domain-level (A), bacterial phylum-level (B) and proteobacterial class-level (C) sequences at every depth, in all sites examined. Relative abundance values for sequences within the alphaproteobacterial order (D), averaged across all depths in all sites examined.

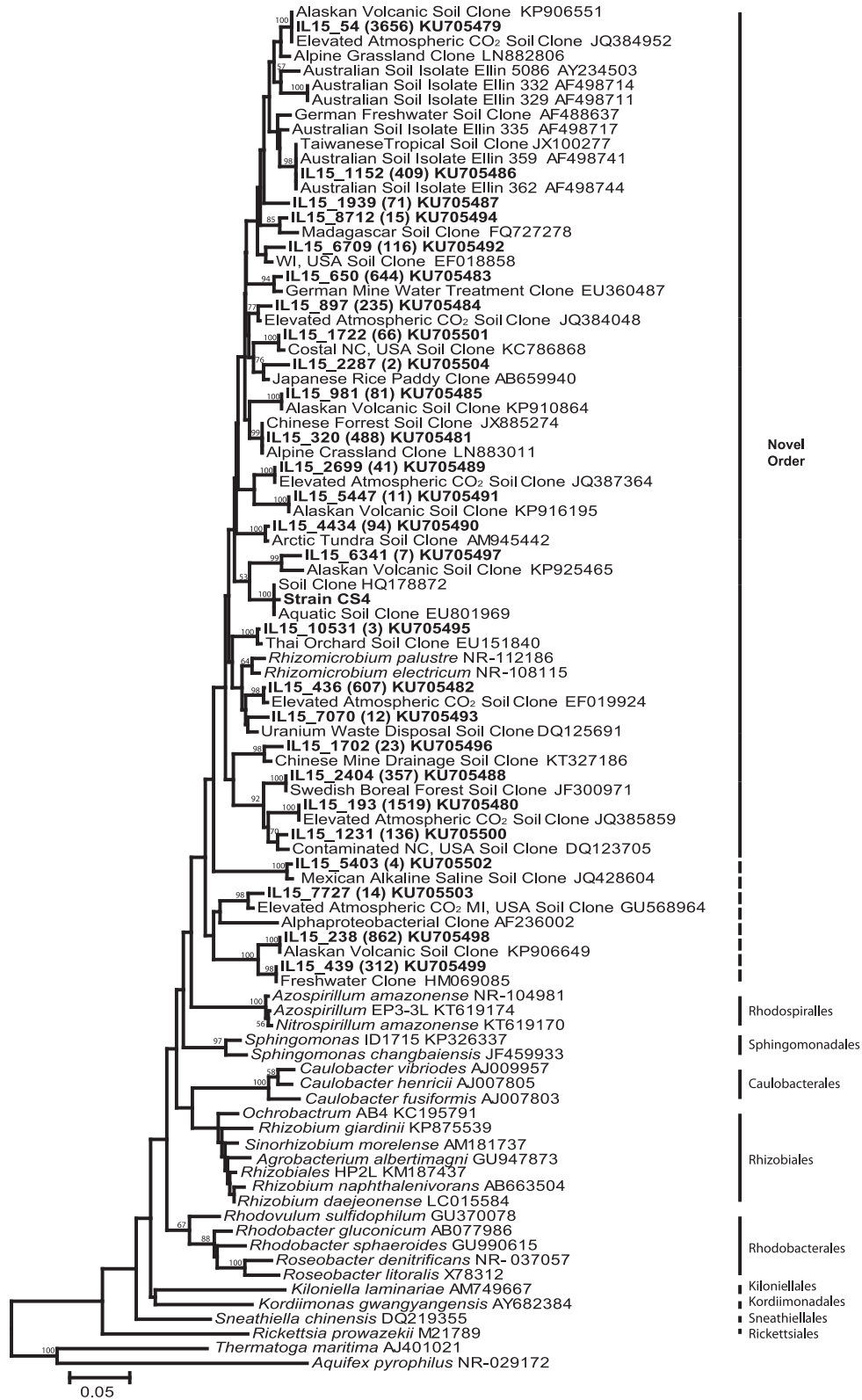


**Figure 2.** Scanning electron micrograph of strain CS4. Image contrast and brightness were edited using Photoshop.

other well-characterized orders, including *Rhizobiales*, *Caulobacterales* and *Sphingomonadales*. From the illumina sequencing data, 26 unique OTUs were retrieved, 22 of which clearly clustered within Ellin 329. Four of the OTUs, 5403, 7727, 238 and 439, may represent another novel order or a candidate family within Ellin 329 (Fig. 3, dashed line). Abundance of Ellin 329 sequences ranged from 4% to 5% across all sites, and decreased in abundance with depth even as a percentage of alphaproteobacterial reads (Table 1).

## DISCUSSION

Previous studies have shown that *Acidobacteria* dominate over the second most abundant phylum, *Proteobacteria*, in northern oligotrophic peatlands (Costello and Schmidt 2006; Pankratov et al. 2011; Serkebaeva et al. 2013). However, our study shows that *Proteobacteria* were the dominant bacterial phyla in all sites, potentially the result of higher pH (4.6–6.2) and nutrient content of the sites studied here, compared to many northern peatlands. At least one study has reported that abundance of *Acidobacteria* and *Proteobacteria* were inversely proportionate to each other (Urbanová and Bárta 2014) with acidobacterial abundance greater in bog environments with lower pH and lower



**Figure 3.** Neighbor-joining tree of select SSU rRNA gene sequences within several alphaproteobacterial orders. Representative OTUs (in bold) and top blast hits for sequences clustering in the Ellin 329 order that were retrieved from Pineola Bog, Tater Hill Bog, Sugar Mountain Bog and the sequence for strain CS4 (GenBank accession number KU738893, also in bold). The number of sequences represented by each OTU (97%) is indicated in parenthesis. The solid line indicates clusters containing sequences clearly identified as Ellin 329 in the Greengenes database. The dashed line indicates more divergent sequences that may represent novel order(s) or (a) novel family/families, within Ellin 329. *Thermatoga maritima* and *Aquifex pyrophilus* were used as an outgroup. Nodal support was determined using bootstrapping values of 100 replicates, in neighbor joining, and values are displayed only for those nodes with support values >50% that were also supported by maximum-likelihood analyses.

**Table 1.** Relative abundance of Ellin 329, as a percentage of total bacterial reads (% Total), and as a percentage of alphaproteobacterial reads (% Alphas), by depth for samples collected in Pineola Bog in 2013, as well as Pineola Bog, Tater Hill Bog and Sugar Mountain Bog in 2014. Samples were collected from 10–20, 30–40 and 60–70 cm depths, except for Pineola 2013 where samples were collected from a depth of 25–30 cm (30\*). Read totals represent triplicate field replicates (n = 3), except for Sugar 30 which had a failed sequencing reaction (n = 2).

Site	Pineola 2013			Pineola 2014			Tater 2014			Sugar 2014		
	10	30*	60	10	30	60	10	30	60	10	30	60
% Total	–	0.4	–	0.98	0.48	0.27	0.72	0.23	–	0.93	0.51	–
% Alphas	–	4.1	–	5.7	3.8	3.3	5.5	3.8	–	6	4.6	–
Total reads	–	126707	–	244705	257117	154164	237402	236826	–	228133	162712	–
Alpha reads	–	12695	–	42223	32193	13113	31384	14224	–	35288	1976	–
Ellin 329 reads	–	516	–	2407	1237	427	1709	545	–	2118	828	–

nutrient concentrations (Lin et al. 2014). This trend is echoed in the relatively high microbial diversity shown here where each site has an average of 11 phyla (range 10–15) in contrast to northern bogs which showed an average of eight phyla (range 6–9; as reviewed by Dedysh 2011) calculated for phyla with a relative abundance greater than 1% in both cases. This corroborates other wetland studies that have shown a positive correlation between pH and both bacterial diversity (Dorador et al. 2013) and archaeal diversity (Kotsyurbenko 2010). Indeed, even in a range of non-saturated soils, Fierer and Jackson (2006) showed that bacterial diversity and richness increased along a spectrum of acidic to more neutral conditions. Overall, the sites analyzed here display high bacterial diversity, as seen by the 15 non-rare phyla detected in total across all three sites (Fig. 1B).

Most notably, representatives of *Cyanobacteria* (Pineola 2014 30–40 cm, Sugar 2014 and Tater 2014 10–20 cm) and *Chlorobi* (Pineola 2013, Tater 2014, Sugar 2014; increasing in abundance with depth), both phototrophic organisms (Gupta 2004), were not only detected in the top layer but also in deeper layers. Although *Cyanobacteria* are not commonly detected in peatland soils, they have been detected in sphagnum moss (Berg, Danielsson and Svensson 2013). Interestingly, a number of rarely detected groups, candidate divisions WS3, NC10 and OP3 were detected in our sites, and they have been detected in previous analyses of high elevation wetlands (Dorador et al. 2013). Candidate division WS3, originally detected in a methanogenic aquifer (Dojka et al. 1998), represented (1.21%) in Tater 2014 at both depths, though it has previously only been detected in deeper peatland depths (Kirkpatrick et al. 2006). Candidate division NC10 was detected in Pineola 2014, 60–70 cm and Tater 2014, 30–40 cm, and this group has been previously implicated in anaerobic methane oxidation coupled to denitrification (Ettwig et al. 2009). Candidate division OP3 was originally retrieved from the Obsidian Pool hot spring in Yellowstone National Park, later detected in flooded patty soils (Derakshani, Lukow and Liesack 2001) and peatlands (Serkebaeva et al. 2013), and may have similar metabolic capabilities to Deltaproteobacteria (Glöckner et al. 2010). OP3 was detected in our sites in Pineola 2014, 60–70 cm and Tater 2014, 30–40 cm.

The difference between archaeal sequence abundance in the 25–30 cm Pineola 2013 samples versus the 30–40 cm Pineola 2014 samples is striking. This difference may be attributed to the fact that 25 cm was previously identified as the zone of maximum potential for methane production for Pineola Bog (Hawkins, Johnson and Bräuer 2014), and may correlate with a greater abundance of methanoarchaeal sequences. In addition, this difference may be due to seasonal factors between sampling dates (Sun et al. 2012).

### A novel isolate from the poorly described, candidate alphaproteobacterial order Ellin 329

Phylogenetic analysis of the SSU rRNA gene of strain CS4 places it within a novel alphaproteobacterial order first recognized in a study of Australian pasture soils (Sait, Hugenholtz and Janssen 2002) from which several isolates were also obtained (Ellin329, Ellin332, Ellin335, Ellin359 and Ellin362, included in Fig. 2) on low nutrient, 5% xylan agar. Two additional cultured strains, *Rhizomicrobium electricum* and *R. palustre*, (Ueki et al. 2010; Kodama and Watanabe 2011) also fall within this order, along with the 26 unique OTUs retrieved here in our study by Illumina sequencing (Fig. 3). According to the literature, the minimum sequence identity for order classification is 83.55% (Yarza et al. 2014), supporting the proposition that all clones collected in this study (89%–96% ID to *Bacterium* Ellin329) are members of the order, Ellin 329. Members of Ellin 329 are diverse in nature originating from many different sites such as rice paddies (Kodama and Watanabe 2011; Itoh et al. 2013), tropical soils (Ducey et al. 2013), volcanic soils (Zeglin et al. 2016), tundra soils (Männistö, Tiirola and Häggblom 2009), freshwater (Bruns, Hoffelner and Overmann 2003) grassland soils (GenBank accession number LN8828806) and other soils (Sait, Hugenholtz and Janssen 2002; Lesaulnier et al. 2008; Dunbar et al. 2012). Interestingly, 44% of sequences identified in our sites as Ellin 329 (OTUs 54, 981, 5447, 6341) showed high identity to clones derived from soil samples collected on Kasatochi Island in Alaska (Zeglin et al. 2016). The isolates described by Sait, Hugenholtz and Janssen (2002) and the isolate *R. palustre* (Ueki et al. 2010) have been shown to utilize xylan as a growth substrate among other carbon substrates. Additionally, *R. electricum* was isolated from a cellulose-fed microbial fuel cell (Kodama and Watanabe 2011), suggesting that it is involved in polysaccharide degradation, at least indirectly. Strain CS4, cultured here, is adapted to relatively low pH (6) and oligotrophic environments ( $\text{Na}^+ < 50 \text{ mM}$ ), and utilizes both simple and more complex saccharides. Overall, the collective cultured strains indicate that members of Ellin 329 may be involved in primary plant litter decomposition, as well as sugar fermentation. Corroborating this idea, Ellin 329 was found to thrive in the shallow (10–20 cm) depths of all three peatlands, where fresh plant litter and exudates would be most prevalent. Our results show that representatives of Ellin 329 were present in moderately high abundance in these three peatlands, which vary in pH, hydrology and nutrient concentrations. This finding, combined with data in the literature demonstrating Ellin 329 sequence presence in diverse sites globally, suggests that Ellin 329 may play an integral role in C cycling at global scales. As such, further studies should be conducted to examine the presence

and functions of Ellin 329 in wetlands, and perhaps other soil types worldwide.

## ACKNOWLEDGEMENTS

The authors would like to thank Dr Mike Madritch for assistance with Illumina sequencing preparation. Mara Cloutier for field work assistance, Illumina sequencing, and sequence analysis and submission. Laiken Price for laboratory assistance. Dr Sarah Carmichael for guidance with electron imaging.

## FUNDING

Partial support from Appalachian Women Scientists, Appalachian State University Office of Student Research and the Cratis D. Williams Graduate School. LJ Lamit and processing of the 2014 samples were funded in-part by the United States Forest Service Northern Research Station, Houghton, Michigan. Sequencing of the 2014 samples was supported by the United States Department of Energy Joint Genome Institute. The work conducted by the US Department of Energy Joint Genome Institute, a Department of Energy Office of Science User Facility, is supported by the Office of Science of the United States Department of Energy under Contract No. DE-AC02-05CH11231.

**Conflict of interest.** None declared.

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