

Identification of two genera of N₂-fixing cyanobacteria growing on three feather moss species in boreal forests of Quebec, Canada

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Abstract: The recent discovery that N₂ fixation rates by the feather moss carpet of boreal Scandinavian forests increases with stand maturity has put into question the paradigm that N₂ fixation is negligible in mature boreal forest. The N₂ fixation was attributed to a previously unknown association between *Nostoc* sp., a N₂-fixing cyanobacterium and *Pleurozium schreberi* (Brid.) Mitt., a feather moss that is abundant worldwide in the boreal forest. Here we report for the first time that this association also exists in the Canadian boreal forest. We discovered, however, that *Nostoc* was found growing not only on *Pleurozium* but also on two other moss species (*Hylocomnium splendens* (Hedw.) Br. Eur. and *Ptilium crista-castrensis* (Hedw.) De Not.). In addition, the N₂-fixing cyanobacterium *Stigonema* sp. was observed on the three moss species mentioned above, indicating the existence of six different associations. At least one of the six associations was found at 9 of 13 sites that are representative of a large area of the Quebec boreal forest. These findings suggest possibilities for further research, aimed at measuring the unaccounted for N₂-fixing potential of the feather moss carpet in Canadian boreal forests.

Key words: N₂-fixing cyanobacteria, feather mosses, boreal forest, *Nostoc*, *Stigonema*.

Résumé : La découverte récente que les taux de fixation de l'azote du couvert de mousse des forêts boréales de la Scandinavie augmente avec la maturation du peuplement remet en question le paradigme à savoir que la fixation de N₂ serait négligeable en forêts boréales matures. On a attribué la fixation de N₂ à une association jusqu'ici inconnue entre un *Nostoc* sp., une cyanobactérie fixatrice d'azote, et le *Pleurozium schreberi* (Brid.) Mitt., une mousse plumeuse qui abonde partout en forêt boréale. Les auteurs rapportent pour la première fois que cette association existe également dans la forêt boréale canadienne. Ils ont découvert que le *Nostoc* ne pousse pas seulement sur le *Pleurozium*, mais aussi sur deux autres espèces de mousses (*Hylocomnium splendens* (Hedw.) Br. Eur. et *Ptilium crista-castrensis* (Hedw.) De Not.), alors qu'on observe également la cyanobactérie fixatrice de N₂, *Stigonema* sp., sur les trois espèces de mousses mentionnées plus haut, ce qui indique la présence de 6 associations distinctes. On retrouve au moins une de ces six associations sur 9 de 13 sites représentatifs de vastes surfaces de la forêt boréale. Ces constatations suggèrent des possibilités pour de nouvelles recherches portant sur la mesure du potentiel de fixation de N₂ non pris en compte et impliquant les tapis de mousses plumeuses de la forêt boréale canadienne.

Mots clés : cyanobactérie fixatrice d'azote, mousses plumeuses, forêt boréale, *Nostoc*, *Stigonema*.

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Introduction

It is well established that the growth of temperate and boreal forests is limited by N availability (Vitousek et al. 2002). The atmosphere is the biosphere's largest reservoir of N (4×10^{21} g), while the soil and biomass contain $3 \times$

10^{17} g and 1.3×10^{16} g, respectively (Reeburgh 1997). In several ecosystems, the presence of microorganisms able to fix N₂ is thus an important adaptation to fulfill the need for N.

N₂-fixing rates by cyanobacteria of 1–41 kg·ha⁻¹·year⁻¹ have been reported for terrestrial environments (Cleveland et al. 1999). N₂-fixing cyanobacteria, either epiphytic or symbiotic with plants, are particularly abundant in tropical forests (Goosem and Lamb 1986; Sprent and Sprent 1990). Until recently, however, known associations in boreal forests were limited to the symbiosis of N₂-fixing cyanobacteria with peat bog mosses and lichens in the first stages of colonization on rocks or very poor soils, as well as in the Ant-arctic, Arctic, and tundra (Christie 1987; Chapin et al. 1991). These conditions have led to the recognition of the paradigm that N-fixing organisms are very abundant in tropical forests, while being almost nonexistent in advanced successional stages of boreal forests (Vitousek et al. 2002).

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Table 1. Presence (X) or absence of *Nostoc* sp. and *Stigonema* sp. on feather mosses at RESEF sites.

Site	Latitude, longitude	Main tree species	Stand age	Moss species	<i>Nostoc</i>	<i>Stigonema</i>
104	48°21'43"N, 67°00'52"W	<i>A. balsamea</i>	65	<i>Pleurozium</i>	X	
				<i>Ptilium</i>		
202	48°14'08"N, 70°20'54"W	<i>P. mariana</i>	85	<i>Pleurozium</i>		
				<i>Hylocomnium</i>		
203	48°48'41"N, 72°46'03"W	<i>P. mariana</i>	85	<i>Pleurozium</i>		
				<i>Ptilium</i>		
				<i>Hylocomnium</i>		
204	49°12'27"N, 73°38'38"W	<i>P. mariana</i>	70	<i>Pleurozium</i>		X
				<i>Ptilium</i>	X	X
303	47°19'41"N, 71°07'37"W	<i>A. balsamea</i>	55	<i>Pleurozium</i>		
				<i>Ptilium</i>		X
403	47°37'30"N, 72°19'15"W	<i>A. balsamea</i>	70	<i>Ptilium</i>	X	
				<i>Hylocomnium</i>		
404	47°54'12"N, 74°37'48"W	<i>P. mariana</i>	60	<i>Pleurozium</i>		
				<i>Ptilium</i>		
801	48°22'03"N, 77°07'28"W	<i>P. mariana</i>	60	<i>Pleurozium</i>	X	X
				<i>Ptilium</i>	X	X
901	49°45'00"N, 68°41'12"W	<i>A. balsamea</i>	120	<i>Pleurozium</i>		
				<i>Ptilium</i>	X	X
				<i>Hylocomnium</i>		X
902	48°52'41"N, 69°05'05"W	<i>P. mariana</i>	70	<i>Pleurozium</i>		
				<i>Ptilium</i>	X	X
				<i>Hylocomnium</i>		X
903	50°08'21"N, 67°08'35"W	<i>P. mariana</i>	60	<i>Pleurozium</i>		
				<i>Ptilium</i>	X	X
				<i>Hylocomnium</i>	X	X
1001	49°49'40"N, 74°57'15"W	<i>P. mariana</i>	60	<i>Pleurozium</i>	X	X
				<i>Ptilium</i>		X
				<i>Hylocomnium</i>	X	X
1101	48°56'11"N, 66°03'00"W	<i>A. balsamea</i>	65	<i>Pleurozium</i>		

This paradigm was, however, thoroughly questioned recently: a new association between a N-fixing cyanobacterium (*Nostoc* sp.) and the moss *Pleurozium schreberi* (Brid.) Mitt. was reported in the boreal forests of Finland and Sweden (DeLuca et al. 2002). The cyanobacterium is an epiphyte, living on the shoots of mosses. N₂ fixation begins under snow during spring and lasts until November, despite nocturnal temperatures below 0 °C (DeLuca et al. 2002). The N₂-fixing rate attributed to *Nostoc* was between 1.5 and 2 kg·ha⁻¹·year⁻¹ (DeLuca et al. 2002). Atmospheric inputs of N in Quebec are about 7–10 kg·ha⁻¹·year⁻¹ in the south, but only 2–3 kg·ha⁻¹·year⁻¹ in the north (Duchesne and Houle 2006), which is typical of N depositions in black spruce (*Picea mariana*) – jack pine (*Pinus banksiana*) boreal ecosystems. An input of 1.5–2 kg·ha⁻¹·year⁻¹ resulting from N₂-fixing cyanobacteria would thus be very significant for the N supply of these forests. The large abundance of *Pleurozium schreberi* in the Canadian boreal forest suggests that the association described above should also exist there. However, despite its potential importance for N-input, the existence of this association is unknown to date in Canada. The objective of this research was to qualitatively assess the existence of associations between N-fixing cyanobacteria and mosses in the Quebec boreal forest.

Methods

Site description and sampling

Mosses were collected at 13 coniferous sites in the boreal forest of Quebec, which are part of the forest ministry's RESEF monitoring network (Réseau québécois d'étude et de surveillance des écosystèmes forestiers; Quebec Forest Ecosystem Research and Monitoring Network). These unperturbed sites are dominated by 55- to 120-year-old balsam fir (*Abies balsamea*) or black spruce trees (Table 1). Each site was visited once during the September and October 2003 sampling period. Existing data report that *Nostoc* N-fixing activity is at its highest in the autumn (DeLuca et al. 2002; Zackrisson et al. 2004).

The sampling protocol was as follows: within a circle with a radius of approximately 3 m, five bunches of mosses were randomly collected and pooled in a plastic bag. Between three and five samples were obtained at each site. An effort was made to qualitatively reflect the relative abundance of three moss species (*Pleurozium schreberi*, *Hylocomnium splendens* (Hedw.) Br. Eur., and *Ptilium crista-castrensis* (Hedw.) De Not.), which are widely distributed in the eastern Canadian boreal forest. Care was taken to ensure that samples of the same moss species were placed in a

Fig. 1. (A) A 400× view of a strand of *Stigonema* sp. under epifluorescence (A, fluorescent active cells; H, heterocyst). (B) The same strand viewed with bright field. (C) A larger and branched strand of a *Stigonema* sp. under epifluorescence with multiple intercalary heterocysts. (D) A similar strand under phase contrast with detailed cells.

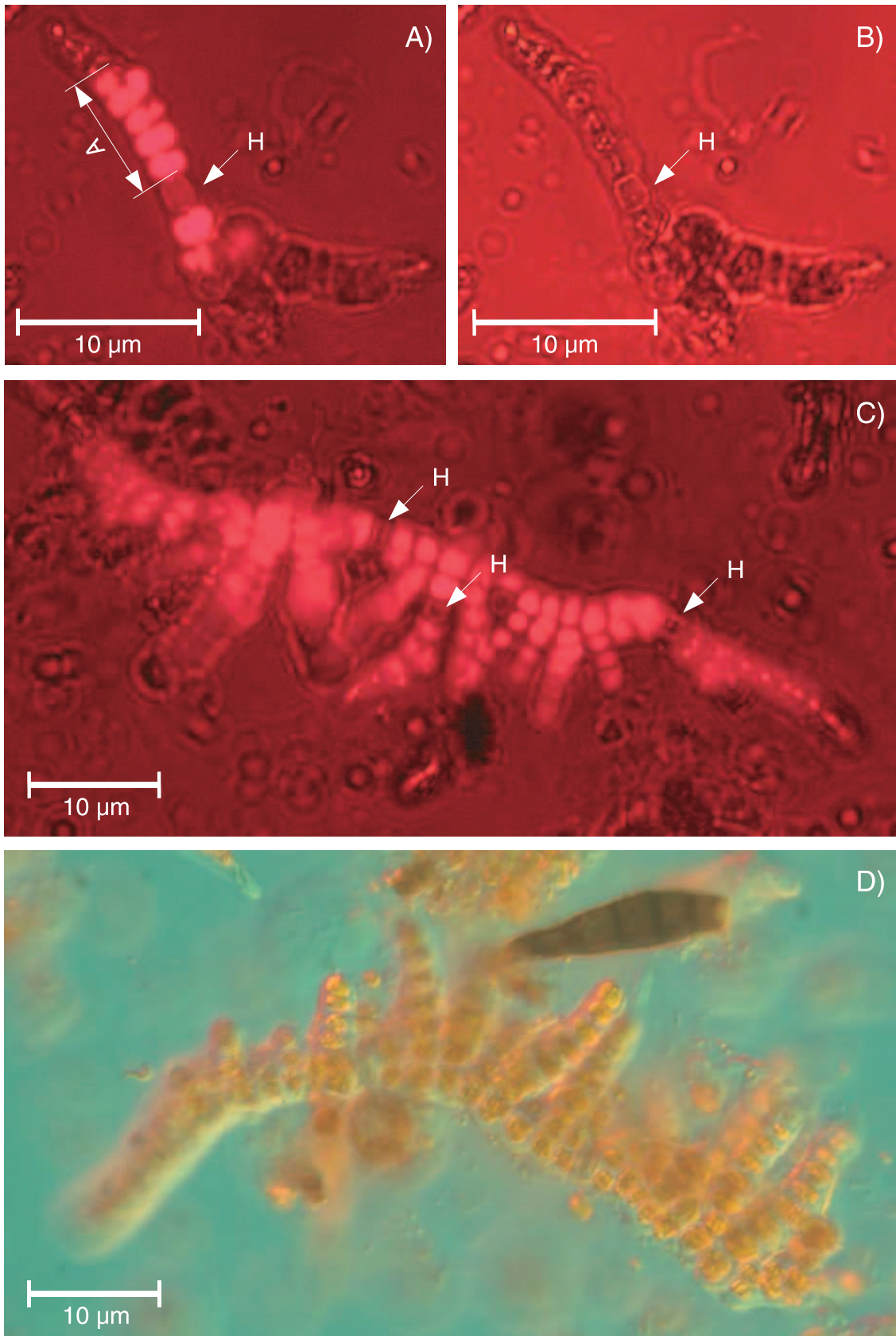
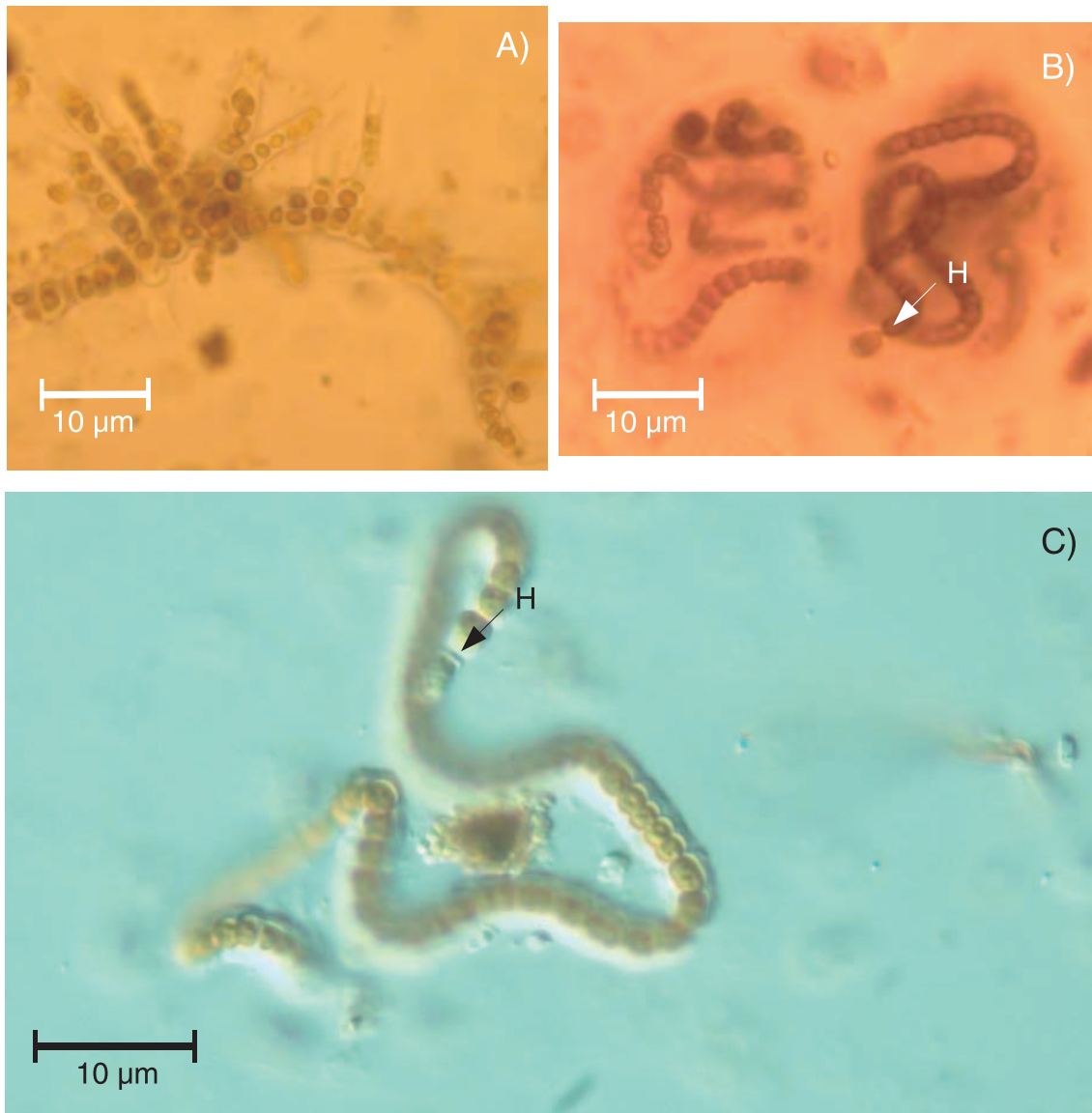


Fig. 2. (A) Regular bright field view of a *Stigonema* sp. strand. (B) *Nostoc* sp. small colony with a heterocyst (H) under bright field view. (C) Another *Nostoc* sp. isolated strand under phase contrast.



given bag, so as to prevent potential cross contamination of cyanobacteria among moss types. Shortly after collection, the samples were sent to the laboratory and examined over the following days.

Microscopic observations

For each fresh moss sample, five to seven strands were randomly chosen. From each strand, one branch was separated and all the leaves on that branch were placed on a microscope slide with a few drops of distilled water. The number of leaves prepared depended on the species of moss, but was typically between 50 and 100. The mounted samples were observed under a Leitz (Labovert FS) epifluorescence microscope equipped with a green filter to detect the presence of cyanobacteria living cells (DeLuca et al. 2002). Bright-field or phase-contrast were also occasionally used for further observations. If the target cyanobacteria (*Nostoc* and *Stigonema*) were detected on the first micro-

scope slide, additional slides were not prepared for that particular site; in the case where no target cyanobacteria were found, up to five additional slides were prepared for further observation for a given site. If positive results were not obtained using this procedure, the site was declared as having no N_2 -fixing cyanobacteria. Two references (Prescott 1962; Bourelly 1985) were used to identify the cyanobacteria genera found on the moss samples.

Results and discussion

Observations of the mosses collected at the 13 study sites (Table 1) revealed the presence of two genera of N-fixing cyanobacteria, *Nostoc* and *Stigonema*, that were each associated with three moss species (*Pleurozium schreberi*, *Hylocomium splendens*, and *Ptilium crista-castrensis*), leading to six different associations.

To date, the only reported association was between *Pleu-*

roizium schreberi and *Nostoc* (DeLuca et al. 2002) in the boreal forests of Scandinavian countries. To our knowledge, this is the first time the cyanobacterium–moss association has been studied in Canada, with the result that we can report the existence of six cyanobacteria–moss association types in mature Canadian boreal forests. It clearly suggests that both cyanobacteria genera are not host specific. Hormogonia were also frequently observed, but it was not possible to determine the cyanobacterium genus (*Stigonema*, *Nostoc*, or both). DeLuca et al. (2002) also reported the frequent presence of *Nostoc* hormogonia on *Pleurozium*. Overall, the presence of at least one of the six N₂-fixing associations was observed at 9 of 13 sites (Table 1). The simultaneous presence of both *Nostoc* and *Stigonema* was observed at 8 of 13 sites. The high frequency of N₂-fixing cyanobacteria, as well as the variety of associations, is surprisingly high given the fact that each site was visited only once.

Given the relatively high rates of N₂ fixation (1.5–2.0 kg·ha⁻¹·year⁻¹) reported for mature forests in Sweden and Finland (DeLuca et al. 2002), and the low N atmospheric deposition (2–3 kg·ha⁻¹·year⁻¹) on the boreal forest of the Canadian Shield (Duchesne and Houle 2006), the potential contribution of N fixation by cyanobacteria would represent a significant up-to-now unaccounted for N input. Although no N₂ fixing rates were measured in this study, the presence of heterocysts (Figs. 1A–1C, 2B, and 2C), the specialized algal cells in which N₂ fixation takes place, on almost all specimens at each site where cyanobacteria were present indicated that they were likely fixing N.

Ptilium crista-castrensis was the most common host of cyanobacteria, particularly for *Nostoc*, at seven of the eight sites where *Nostoc* was found. *Nostoc* was found on *Pleurozium schreberi* at the other site. *Hylocomium splendens* was associated with either only *Stigonema* (two of four sites) or *Stigonema* and *Nostoc* (two of four sites). *Pleurozium* seems to be a less frequent cyanobacterium host than the other two moss species: only three of the eight sites had cyanobacteria present on *Pleurozium*. This is surprising, given that so far only the *Pleurozium*–*Nostoc* association has been reported in Scandinavia (DeLuca et al. 2002). Given the relatively low number of samples observed, conclusions cannot be made concerning host preference by the cyanobacteria. However, they clearly show that future attempts to measure N₂-fixing rates and to identify moss – N₂-fixing cyanobacteria associations in mature boreal forest stands should not be restricted to *Pleurozium schreberi* and *Nostoc*. The results of this study suggest many avenues for further research

to measure the unaccounted N-fixing potential of the moss carpet in Canadian boreal forests.

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