The Lichenologist (2021), **53**, 215–220
doi:10.1017/S0024282921000062

CAMBRIDGE UNIVERSITY PRESS

Short Communication

Stigonema associated with boreal *Stereocaulon* possesses the alternative vanadium nitrogenase

J. C. Villarreal A.[1,2,3*], M. Renaudin[4,*], A. Beaulieu-Laliberté[1] and J. P. Bellenger[4]

[1]Department of Biology, Université Laval, Québec G1V 0A6, Canada; [2]Smithsonian Tropical Research Institute, Panama City, Panama; [3]Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec G1V 0A6, Canada and [4]Centre Sève, Département de Chimie, Université de Sherbrooke, Sherbrooke J1K 2R1, Canada

(Accepted 23 November 2020)

Reindeer lichens from the genus *Cladonia* and snow lichens from the genus *Stereocaulon* are components of spruce lichen woodland, which is one of the most extensive ecosystems in Eastern Canada (Payette & Delwaide 2018). Unlike reindeer lichens, *Stereocaulon* species have a mutualistic association with cyanobacteria, mainly from the genus *Stigonema,* which are located in cephalodia (Huss-Danell 1977, 1979; Kershaw 1978; Kytöviita & Crittenden 2002; Lavoie *et al.* 2020). In Canada, acetylene reduction assays on *Stereocaulon* cyanobacteria have demonstrated their critical contribution to the nitrogen (N) budget (Crittenden & Kershaw 1978; Kershaw 1978). Cyanobacteria use the nitrogenase enzymatic complex to reduce atmospheric N_2 into bioavailable ammonium. Thus, understanding the biology and efficacy of the nitrogenase, which reduces atmospheric N_2 into bioavailable ammonium, is crucial to obtaining a clearer picture of nutrient flow in boreal forests.

Nitrogenase comes in three main isoforms, characterized by different metal cofactors in their active site (Eady & Leigh 1994): molybdenum (Mo)-nitrogenase, present in all N_2 fixers, and at least two 'complementary nitrogenases' in which vanadium (V) or iron (Fe) replace Mo in the cofactor (V- and Fe-nitrogenase) (Eady 1996). Recent studies on species of *Nostoc* associated with *Peltigera* revealed that V-nitrogenase is present and provides 15–50% of N fixed by these cyanolichens in boreal forests (Darnajoux *et al.* 2019). However, whether V-nitrogenase is present in other boreal or arctic cyanolichens remains unknown. Evaluating the presence of V-nitrogenase in other cyanolichens is essential to establish whether this trait is ubiquitous or limited to a small number of cyanobacterial clades.

The contribution of V-nitrogenase to N_2 fixation is strongly controlled by Mo availability. Darnajoux *et al.* (2019) reported that, in *Peltigera* species, V accumulation in cephalodia is controlled by Mo availability and identified a Mo threshold of ~250 pbb ($ng_{Mo} \cdot g^{-1}_{thallus}$) below which V-nitrogenase also contributes to N_2 fixation (Darnajoux *et al.* 2017, 2019). Another potentially important environmental factor affecting V-nitrogenase contribution to N_2 fixation is temperature. *In vitro* studies showed that the regulation of V-nitrogenase genes by Mo disappears at temperatures below 15 °C and at these lower temperatures V-nitrogenase, which is less efficient than its Mo-based counterpart at high temperatures (> ~20 °C), achieves similar or even higher activity to that of Mo-nitrogenase (Miller & Eady 1988; Walmsley & Kennedy 1991). This suggests that V-nitrogenase could significantly contribute to N_2 fixation in cold and Mo-poor habitats, such as the boreal forest (Bellenger *et al.* 2020). Indeed, Darnajoux *et al.* (2019) reported an increasing contribution of V-nitrogenase to N_2 fixation with latitude in *Peltigera* species in Eastern Canada, which was primarily attributed to increased Mo stress but could also reflect decrease in average temperature (Darnajoux *et al.* 2019).

The main objective of this study was to determine the presence of the complementary V-nitrogenase in *Stigonema*, from several *Stereocaulon* accessions in a latitudinal gradient in Eastern Canada (Fig. 1), using phylogenetic analyses. Additionally, using metal analyses (i.e. Mo and V) we also evaluated if V accumulation by *Stereocaulon* is indicative of a potential use of this metal to cope with Mo limitation of N_2 fixation as observed with *Peltigera* species. Sequence analyses targeting the vnfD region confirmed the presence of V-nitrogenase in *Nostoc* strains associated with *Peltigera* collected both in North America and Northern Europe (Hodkinson *et al.* 2014; Darnajoux *et al.* 2017), and here we report similar findings in *Stigonema* for the first time. Metal analyses (i.e. Mo and V) of *Stigonema*-containing cephalodia confirmed that V accumulation in *Stereocaulon* responds to Mo availability, strongly suggesting that V-nitrogenase is used by *Stereocaulon*, at least in part, to cope with Mo limitation as observed in *Peltigera* (Darnajoux *et al.* 2017, 2019).

Material and Methods

A molecular marker to test the presence of the alternative V-nitrogenase

To determine the presence of the alternative V-nitrogenase and the phylogenetic affinities of the cyanobionts found in *Stereocaulon*, six specimens representing four morphospecies from the boreal biome were used (*S.* cf. *dactylophyllum, S.* cf. *paschale, S.* cf. *saxatile, S.* cf. *tomentosum*) (Fig. 1, Table 1). Accessions were sampled from New Brunswick (Fundy National Park), southern Québec (Forêt Montmorency-Charlevoix,

Author for correspondence: J. C. Villarreal A. E-mail: juan-carlos.villarreal-aguilar@bio.ulaval.ca

*Equal contribution

Cite this article: Villarreal A. JC, Renaudin M, Beaulieu-Laliberté A and Bellenger JP (2021) *Stigonema* associated with boreal *Stereocaulon* possesses the alternative vanadium nitrogenase. *Lichenologist* **53**, 215–220. https://doi.org/10.1017/S0024282921000062

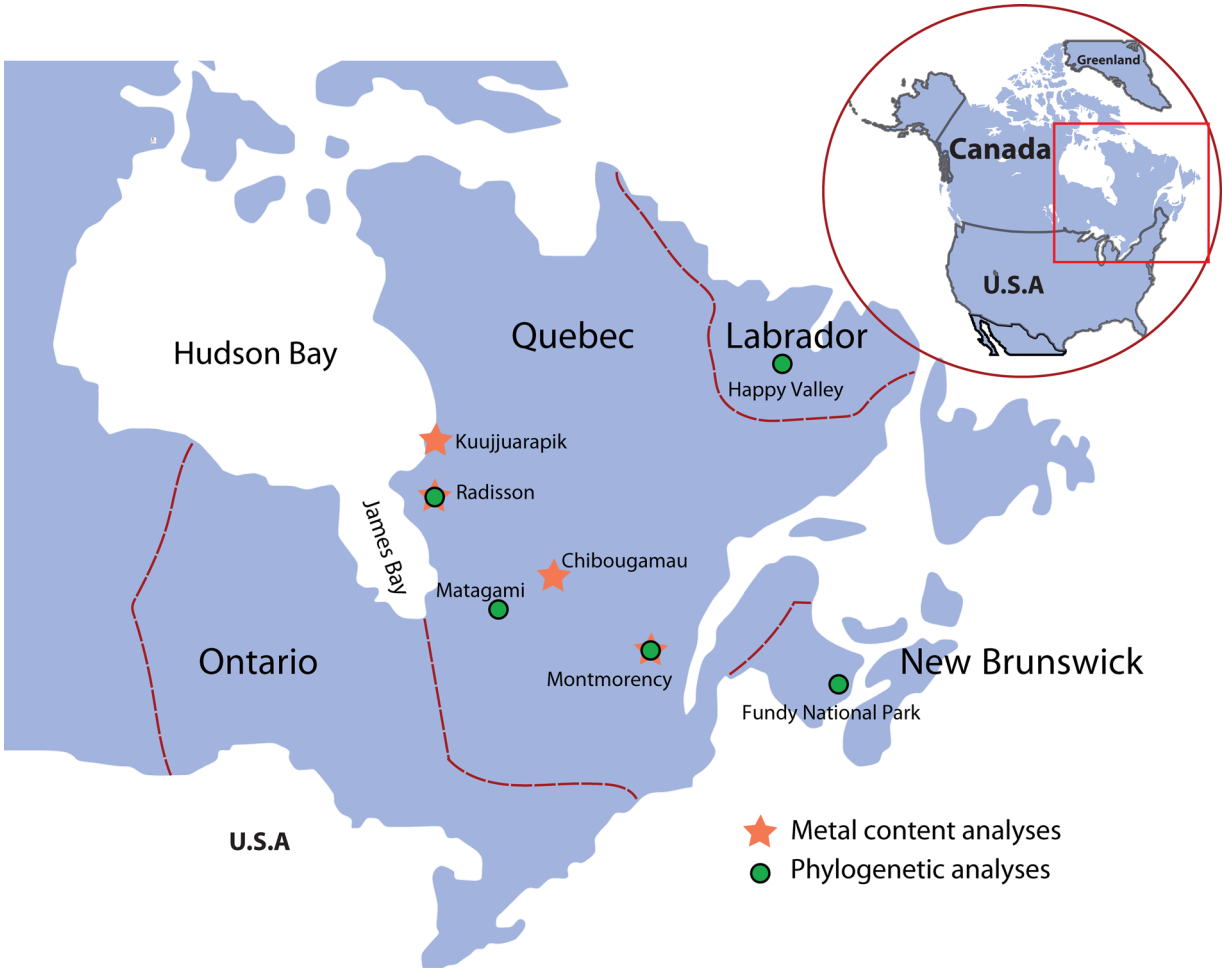

Fig. 1. Map of eastern North America showing collection points of *Stereocaulon* samples. Sites with samples used for the metal analyses (i.e. molybdenum and vanadium) of *Stigonema*-containing cephalodia, are indicated by stars; those sites sampled for phylogenetic analyses of *Stigonema* are indicated by filled circles. Note that sampling for both analyses is undertaken at some sites. In colour online.

hereafter referred to as 'Montmorency') and the Québec boreal zone (Happy Valley in Labrador, Matagami, Radisson at James Bay) (shown as filled circles in Fig. 1). The samples for the phylogenetic analyses differ from those of the metal analyses (see below), except those from the Montmorency Forest and Radisson (Table 1). Sanger sequencing was performed with new primers designed using Geneious 9.0.5 (Biomatters Ltd), targeting the coding region of the vanadium cluster (vnf), specifically the vnfDG region (Hodkinson *et al.* 2014). Cyanobiont DNA was extracted using the Qiagen DNeasy Plant Kit (Qiagen, Hilden, Germany). The vnfDG region was amplified by PCR using the new primers: VN 60F (forward), CGTTGT CCGAGAGAGGA TGC; VN 807R (reverse), CGCAATCCTTCTTTGGCATAGT. Each 25 µl PCR reaction included 25 µg BSA, 0.626 U Taq DNA polymerase (Qiagen, Hilden, Germany), 1.5 mM $MgCl_2$, dNTPs (0.2 mM each), 0.5 µM forward and reverse primers and 1× PCR buffer, with 1 µl of DNA template. The thermocycler temperature profile for the locus vnfDG was an annealing temperature set at 56 °C and a 95 °C denaturation temperature for 5 min, followed by 35 cycles at 95 °C for 45 s, 56 °C for 30 s and 72 °C for 1 min 30 s, with a final extension of 72 °C for 10 min. Sequencing was carried by the Genomic Analysis Platform of the Institut de biologie integrative et des systems, Université Laval.

We analyzed a total of 24 accessions, including our six newly sequenced cyanobiont samples. The dataset used was obtained from Hodkinson *et al.* (2014) and includes 15 sequences from the vnfD-like region across Archaea, Bacteroidetes (Chlorobi), Proteobacteria, Firmicutes and Cyanobacteria, with four *Nostoc* sequences associated with *Peltigera* species. The outgroup sequences were three accessions of the anfD (Fe-nitrogenase) region from *Clostridium pasteurianum*, *Chloroherpeton thalassium* and *Rhodobacter capsulatum* (Hodkinson *et al.* 2014). We used Geneious 9.0.5 (Biomatters Ltd) to align the nucleotide sequences, with manual verification of the resulting alignment. Sequences have been deposited in GenBank (Table 1) and the alignment is available upon request.

Phylogenetic analyses

The vnfDG coding region was analyzed under the maximum likelihood (ML) criterion using the GTR-CAT model approximation implemented in RAxML (Stamatakis 2014) with 500 bootstrap replicates (MLB). Basic statistics on the dataset were retrieved using PAUP* (Swofford 2002) (Table 2). Bayesian analyses of the genomic region were conducted in MrBayes 3.2 (Ronquist *et al.* 2012), using the default two runs and four chains, with

https://doi.org/10.1017/S0024282921000062

Table 1. Specimens of *Stereocaulon* used in the present study, including area of collection, voucher information and GenBank Accession numbers for sequences of the vnfDG region from associated cyanobacteria.

			Sample information		
Fungal morphospecies host species	Biome or ecoregion	Location	GenBank Accession no.	Voucher accession no.	Collector
Stereocaulon cf. *dactylophyllum*	Boreal/Acadian Forest	Fundy National Park, New Brunswick, Canada	MK040915	QFA600843	*C. Roy*
S. cf. *paschale*	Boreal	Forêt Montmorency, Québec, Canada	MK040912	CA-17-1676	*J. C. Villarreal A.*
S. cf. *paschale*	Boreal	Happy Valley, Labrador, Canada	MK040914	17157	*R. T. McMullin*
S. cf. *paschale*	Taiga	Radisson, James Bay, Québec, Canada	MK040921	CA-17-1735	*J. C. Villarreal A.*
S. cf. *saxatile*	Boreal	Forêt Montmorency, Québec, Canada	MK040913	PA-CAN-029	*P. A. Picord*
S. cf. *tomentosum*	Boreal	Matagami, Québec, Canada	MK040916	CA-17-1677B	*J. C. Villarreal A.*

default priors on most parameters and a model chosen by MrModeltest (Nylander 2004). To assess burn-in and convergence, we compared bipartitions across the two runs and stationarity in the chain using Tracer (Rambaut *et al.* 2018). Convergence was achieved in MrBayes after 2 million generations, with trees sampled every 10 000th generation for a total length of 20 000 000 generations; 25% of each run were discarded and the runs were pooled. All analyses were carried out on the CIPRES platform (Miller *et al.* 2010).

Element analyses: molybdenum, vanadium and phosphorus

Samples of *Stereocaulon paschale* were collected from the southern Québec black spruce forest (Montmorency, $n = 3$), the northern Québec boreal forest (Chibougamau, $n = 2$ and Radisson, $n = 4$) and the forest tundra (Kuujjuarapik, Hudson Bay, $n = 3$) (shown as stars in Fig. 1). Sampling was carried out at least 20 m from roads and buildings to avoid the influence of human activities. To determine Mo, V and phosphorus (P) concentrations in cephalodia, these structures were dissected under a stereomicroscope using needles and forceps. The cephalodia were macroscopically free of large visible fragments of the main fungal host, however, fungal hyphae are intrinsically part of the structure, along with bacteria. Therefore, it is possible that some epiphytic bacteria (even cyanobacteria) were included in the cephalodia dissected out from the pseudopodetia. The samples were oven dried (50 °C for 48 h) and weighed. Samples were digested for 4 h in 1 ml of nitric acid (trace metal grade, Fisher Scientific) mixed with 0.2 ml of hydrogen peroxide (trace metal grade, Sigma-Aldrich) using a heating block digestion system (DigiPREP Jr, SCP Sciences) as previously described (Darnajoux *et al.* 2014). Mo, V and P levels in the cephalodia were determined by inductively coupled mass spectrometry (ICP-MS, X-series II, Thermo Fisher Scientific) using the XSeries PlasmaLab 2.6 software. Results are presented as Mo:P and V:P (mol:mol) ratios and as element concentration in cephalodium in ppb ($ng_{element} \cdot g^{-1}_{dry\ weight}$). Statistical analyses were performed using R v. 3.4.3 (R Core Team 2017). Data normality was tested using the Shapiro test. The nonparametric Kruskal-Wallis test followed by the post hoc Dunn test, were used to test for significant differences ($\alpha < 0.05$) among the Mo:P and V:P ratios at the different collecting sites.

Results and Discussion

In this study, we confirm the presence of the alternative vanadium nitrogenase in *Stigonema,* as our newly designed primers successfully amplified a portion of the vnfDG region (Hodkinson *et al.* 2014). This suggests that cyanobionts associated with *Stereocaulon*, an important component of temperate, boreal and tundra ecosystems (Crittenden & Kershaw 1978; Kershaw 1978), are able to use both nitrogenases (Mo- and V- nitrogenases). Recent molecular analyses have shown an extremely low genetic diversity in *Stigonema* across temperate, boreal and tundra biomes (Lavoie *et al.* 2020), without mycobiont specificity. The vnfDG sequences from *Stigonema* form a monophyletic group (Fig. 2) sister to *Nostoc* from *Peltigera* species (*P. malacea, P. membranacea, P. neopolydactyla*). Although reported in *Peltigera* species (Hodkinson *et al.* 2014), the complementary V-nitrogenase is not ubiquitous across the bacterial and archaeal world (Harwood 2020). In *Peltigera*, it contributes significantly to N_2 fixation by allowing species to cope with Mo limitation (Darnajoux *et al.* 2019). The presence and activity of V-nitrogenase in other cyanolichen genera remains to be determined. The presence of the alternative V-nitrogenase in *Stigonema* associated with *Stereocaulon*, although not entirely surprising, is a major finding because *Stereocaulon* is widespread in boreal forests. Estimates from *Peltigera* species suggests that V-nitrogenase provides 15–50% of fixed N by *Peltigera* in boreal forests (Darnajoux *et al.* 2019). *Stereocaulon* species are more locally abundant than *Peltigera* in boreal forests and, therefore, we expect the contribution by *Stigonema* to the fixed N budget to be much higher, with a considerable input from V-nitrogenase.

Mo and V content as well as ratios (Mo:P and V:P) in cephalodia suggest not only that genes coding for V-nitrogenase are present in the genome of *Stigonema*, but that both Mo and V contribute to N_2 fixation by *Stereocaulon* species. Mo concentration in cephalodia ranged from 182 to 412 ppb ($ng_{element} \cdot g^{-1}_{dry\ weight}$) (Fig. 3A). Samples collected in the most southern site (Montmorency, near Québec City) had significantly higher Mo concentrations (412 ± 42 ppb) than other sites located in less densely

https://doi.org/10.1017/S0024282921000062

Table 2. Basic statistics and informative sites for the vnfDG locus analyzed using the GTR + Γ model chosen by MrModeltest (Nylander 2004).

Locus	No. of taxa	Total length	No. of excluded positions	No. of included positions	No. of uninformative characters	No. of informative characters
VnfDG region	24	876	0	876	54	518

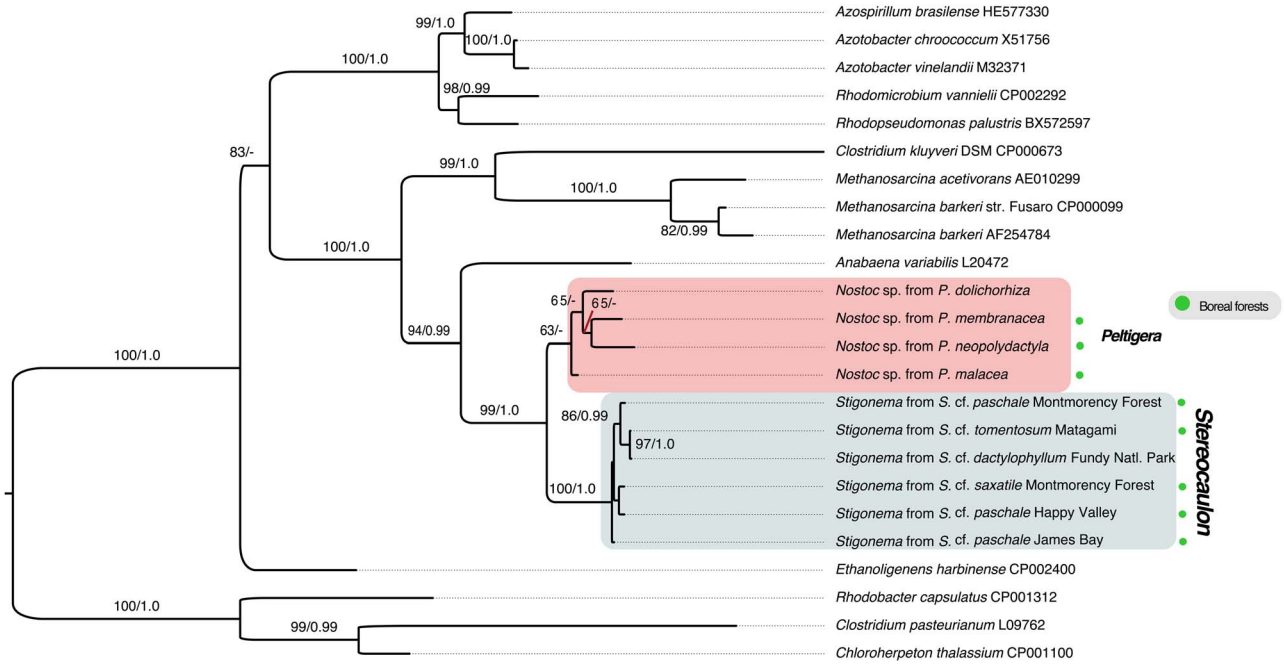

Fig. 2. Majority-rule consensus tree (MJR) of the vnfDG region (including the vnf-like regions), displaying the posterior probabilities (PP) and maximum likelihood bootstrap (MLB) values from each clade indicated above the branches (MLB/PP). The analyses included six samples from the present study representing several species of *Stigonema* associated with *Stereocaulon* (see Table 1), as well as selected bacterial and archaeal strains, and cyanobionts associated with four species of *Peltigera* (Hodkinson *et al*. 2014). The outgroup sequences were three accessions of the anfD (Fe-nitrogenase) region from *Clostridium pasteurianum*, *Chloroherpeton thalassium* and *Rhodobacter capsulatum* (Hodkinson *et al*. 2014). In colour online.

populated areas (Chibougamau, Radisson and Kuujjuarapik: 209 ± 37 ppb, 210 ± 46 pbb and 182 ± 6 ppb, respectively). This trend in Mo content in *Stereocaulon* is consistent with atmospheric metal deposition measured using *Peltigera* species in this region (Darnajoux *et al.* 2015). Darnajoux *et al.* (2015) reported a sharp decrease in atmospheric metal deposition with latitude. Metal depositions above the 48th parallel (slightly below the Chibougamau site) were low and comparable to deposition in remote area such as the Himalayas, Greenland and Antarctica. Mo:P ratios in cephalodia were similar among sites and maintained within a narrow range (0.76×10^{-4} to 1.76×10^{-4} $mol_{Mo}.mol_{P}^{-1}$; Fig. 3B). These ratios were close to, but below, the minimal cellular Mo requirement (between $2–6 \times 10^{-4}$ mol_{Mo} mol_{P}^{-1}; grey area in Fig. 3B) for N_2 fixation reported in cyanobacteria and other cyanolichens (Darnajoux *et al.* 2014).

The consistency of Mo:P ratios in samples collected along the 1000 km latitudinal gradient, characterized by a sharp latitudinal drop in metal deposition (between the site below and the sites above the 48th parallel; Darnajoux *et al.* 2015), reflects a tight homeostatic control of Mo accumulation in *Stereocaulon*, a phenomenon common among N_2 fixers. However, data suggest that there might not be enough Mo accumulated in cephalodia to fully support N_2 fixation, especially at the most northern site (Kuujjuarapik) where cephalodial Mo concentrations are particularly low ($0.76 \pm 0.04 \times 10^{-4}$ mol_{Mo} mol_{P}^{-1}; Fig. 3B). It appears that V can be used to cope with Mo limitation in N_2 fixers possessing the alternative V-nitrogenase. In *Peltigera* species, V accumulation in response to Mo availability and independent of atmospheric sources, has been shown to be a strong indicator of V use for N_2 fixation to cope with Mo limitation (Darnajoux *et al.* 2017).

In our study, V concentrations ranged from 313 to 1434 ppb ($ng_{element} \cdot g_{dry\ weight}^{-1}$) with no clear latitudinal pattern, suggesting that atmospheric deposition is not the primary driver of V accumulation in *Stereocaulon*. The highest V concentrations were found at Chibougamau and Radisson (1434 ± 425 ppb and 482 ± 104 ppb, respectively), while concentrations at Montmorency (the southernmost site) and Kuujjuarapik (the northernmost site) were low and comparable (337 ± 145 ppb and 313 ± 21 ppb, respectively) (Fig. 3A). V:P ratios in cephalodia of *Stereocaulon* specimens also varied significantly (by up to 7-fold, from 2.45×10^{-4} to 1.73×10^{-3}) among sites (Fig. 3B). These patterns in cephalodia V concentrations and V:P ratios along the latitudinal gradient are unlikely to be explained by passive accumulation, which would reflect deposition and thus result in lower V concentrations in Chibougamau, Radisson and Kuujjuarapik (sites above the 48th parallel, where atmospheric metal deposition is low) than in Montmorency (Darnajoux

https://doi.org/10.1017/S0024282921000062

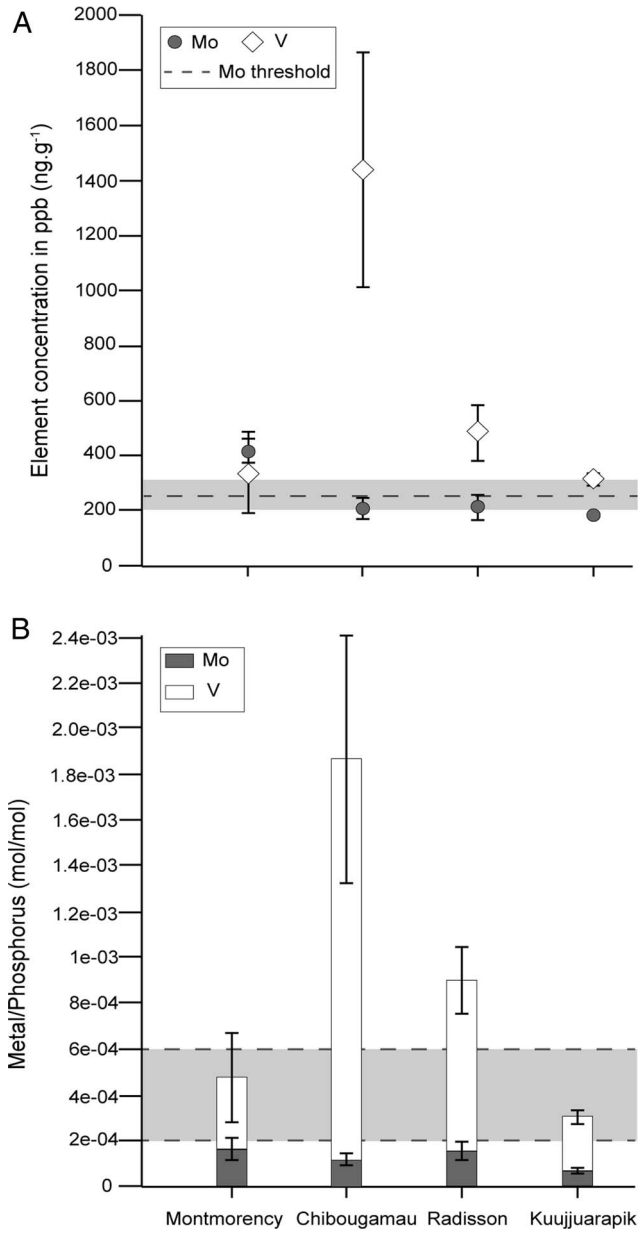

Fig. 3. Molybdenum (Mo) and vanadium (V) content in cephalodia of *Stereocaulon* in four different areas ranging from southern Québec (e.g. Montmorency, 47°N, 71°W) and northern Québec (Chibougamau (49°N, 74°W), Radisson (53°N, 77°W) and Kuujjuarapik (55°N, 77°W)). A, molybdenum (grey circles) and vanadium (white diamonds) concentrations in ppb. The dashed grey line represents the average value and the grey shaded area represents the molybdenum threshold (250 ± 50 ppb) for the contribution of V-nitrogenase to N_2 fixation reported in *Peltigera* species by Darnajoux *et al.* (2019). B, molybdenum:phosphorous (grey bars) and vanadium:phosphorus (white bars) ratios (mol:mol). The grey shaded area represents the minimum Mo:P and V:P ratio for N_2 fixation reported in cyanobacteria and cyanolichen species (Darnajoux *et al.* 2014). Error bars in parts A and B represent standard errors.

et al. 2015). This suggests a biological control over V accumulation in cephalodia, possibly in response to Mo stress (i.e. Mo limitation of N_2 fixation) as reported in *Peltigera* species (Darnajoux *et al.* 2014, 2017, 2019). The minimum V cellular requirement for N_2 fixation reported for cyanobacteria is similar to the Mo cellular requirement ($2–6 \times 10^{-4}$ mol_V mol_P^{-1}; Darnajoux *et al.* 2014). Thus, in all our sites, there was enough V in cephalodia to support N_2 fixation with the V-nitrogenase alone.

Since V can be toxic at high concentrations, this large accumulation of V in cephalodia probably reflects a role in N_2 fixation. Indeed, one of the best-known biological functions of V in terrestrial ecosystems is N_2 fixation (see also Rehder 2013). Considering the strong similarities in V and Mo homeostasis among *Stereocaulon* species (this study) and *Peltigera* species (Darnajoux *et al.* 2014, 2017, 2019), we hypothesize that the higher concentration of V in cephalodia of *Stereocaulon* with low Mo content (below 250 ppb) reflects a higher physiological V demand to cope with Mo limitation of N_2 fixation. The decrease in V content with latitude within sites located above the 48th parallel (Chibougamau, Radisson and Kuujjuarapik) probably reflects the decrease in V availability (atmospheric deposition) with latitude reported in this area (Darnajoux *et al.* 2015). Further research is required to confirm V-nitrogenase activity and fully characterize the spatiotemporal contribution of V-nitrogenase to N_2 fixation by *Stereocaulon*. Nonetheless, our study provides evidence of the presence of a complementary nitrogenase in *Stigonema* associated with the lichen *Stereocaulon* and gives a new dimension to studies on nitrogen cycling in temperate, boreal and tundra ecosystems.

Acknowledgements. We thank Camille Lavoie and Adriel Sierra for their help in the laboratory, T. McMullin for providing specimens and the Villarreal Lab for discussions on cyanobacterial symbiosis.

Author ORCIDs. Juan Carlos Villarreal A., 0000-0002-0770-1446; Marie Renaudin, 0000-0001-6907-2596; Jean-Phillipe Bellenger, 0000-0001-6842-4561.

References

Bellenger JP, Darnajoux R, Zhang X and Kraepiel AML (2020) Biological nitrogen fixation by alternative nitrogenases in terrestrial ecosystems: a review. *Biogeochemistry* **149**, 53–73.

Crittenden PD and Kershaw KA (1978) Discovering the role of lichens in the nitrogen cycle in boreal-arctic ecosystems. *Bryologist* **81**, 258–267.

Darnajoux R, Constantin J, Miadlikowska J, Lutzoni F and Bellenger JP (2014) Is vanadium a biometal for boreal cyanolichens? *New Phytologist* **202**, 765–771.

Darnajoux R, Lutzoni F, Miadlikowska J and Bellenger JP (2015) Determination of elemental baseline using peltigeralean lichens from north-eastern Canada (Québec): initial data collection for long term monitoring of the impact of global climate change on boreal and subarctic area in Canada. *Science of the Total Environment* **533**, 1–7.

Darnajoux R, Zhang X, McRose DL, Miadlikowska J, Lutzoni F, Kraepiel AML and Bellenger JP (2017) Biological nitrogen fixation by alternative nitrogenases in boreal cyanolichens: importance of molybdenum availability and implications for current biological nitrogen fixation estimates. *New Phytologist* **213**, 680–689.

Darnajoux R, Magain N, Renaudin M, Lutzoni F, Bellenger JP and Zhang X (2019) Molybdenum threshold for ecosystem scale alternative vanadium nitrogenase activity in boreal forests. *Proceedings of the National Academy of Sciences of the United States of America* **116**, 24682–24688.

Eady RR (1996) Structure-function relationships of alternative nitrogenases. *Chemical Reviews* **96**, 3013–3030.

Eady RR and Leigh GJ (1994) Dalton perspectives. Metals in the nitrogenases. *Journal of the Chemical Society, Dalton Transactions* **1994**, 2739–2747.

Harwood CS (2020) Iron-only and vanadium nitrogenases: fail-safe enzymes or something more? *Annual Review of Microbiology* **74**, 247–266.

Hodkinson BP, Allen JL, Forrest LL, Goffinet B, Sérusiaux E, Andrésson ÓS, Miao V, Bellenger JP and Lutzoni F (2014) Lichen-symbiotic cyanobacteria associated with *Peltigera* have an alternative vanadium-dependent nitrogen fixation system. *European Journal of Phycology* **49**, 11–19.

Huss-Danell K (1977) Nitrogen fixation by *Stereocaulon paschale* under field conditions. *Canadian Journal of Botany* **55**, 585–592.

Huss-Danell K (1979) The cephalodia and their nitrogenase activity in the lichen *Stereocaulon paschale*. *Zeitschrift für Pflanzenphysiologie* **95**, 431–440.

https://doi.org/10.1017/S0024282921000062

Kershaw KA (1978) The role of lichens in boreal tundra transition areas. *Bryologist* **81**, 294–306.

Kytöviita MM and Crittenden PD (2002) Seasonal variation in growth rate in *Stereocaulon paschale*. *Lichenologist* **34**, 533–537.

Lavoie C, Renaudin M, McMullin RT, Gagnon J, Roy C, Beaulieu M-E, Bellenger JP and Villarreal JC (2020) Extremely low genetic diversity of *Stigonema* associated with *Stereocaulon* in eastern Canada. *Bryologist* **123**, 188–203.

Miller MA, Pfeiffer W and Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In *Proceedings of the Gateway Computing Environments Workshop (GCE), 14 November 2010, New Orleans, Louisiana*, pp. 1–8.

Miller RW and Eady RR (1988) Molybdenum and vanadium nitrogenases of *Azotobacter chroococcum*. Low temperature favours N_2 reduction by vanadium nitrogenase. *Biochemical Journal* **256**, 429–432.

Nylander JAA (2004) *MrModeltest v2*. Program distributed by the author. Evolutionary Biology Centre, Uppsala University.

Payette S and Delwaide A (2018) Tamm review: the North-American lichen woodland. *Forest Ecology and Management* **417**, 167–183.

R Core Team (2017) *R: a Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. [WWW resource] URL http://WWW.R-project.org/.

Rambaut A, Drummond AJ, Xie D, Baele G and Suchard MA (2018) Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology* **67**, 901–904.

Rehder D (2013) The future of/for vanadium. *Dalton Transactions* **42**, 11749–11761.

Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA and Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology* **61**, 539–542.

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**, 1312–1313.

Swofford DL (2002) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods), Version 4*. Sunderland, Massachusetts: Sinauer Associates.

Walmsley J and Kennedy C (1991) Temperature-dependent regulation by molybdenum and vanadium of expression of the structural genes encoding three nitrogenases in *Azotobacter vinelandii*. *Applied and Environmental Microbiology* **57**, 622–624.

https://doi.org/10.1017/S0024282921000062