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Antarctic Cyanobacteria Biodiversity Based on ITS and *Trn*L Sequencing and Its Ecological Implication

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Abstract

Antarctic cyanobacteria biodiversity was investigated by simultaneous sequencing of the nuclear ribosomal internal transcribed spacer (ITS flanked by partial 16S and 23S), and Chloroplast tRNA^{Leu} UAA intron (*Trn*L), exploring whether such morphotypes constitute distinct species and explaining their current distribution. We identified *Nostocales*, *Chrococcales* and *Oscillatoriales* species, collected in different habitats (soil, algal mats, lake sediments, ice-water) after their growth in cultures. By comparative sequence analyses available in Genbank, our results proved to be mostly in agreement with both *Trn*L and ITS, in the identification of the strains, particularly for *Nostocales*. Although ITS demonstrated more usefully than *Trn*L did in identifying *Oscillatoriales* and *Chroococcales*, due to the frequent lack of the intron in these groups, our results lead us to support an independent phylogenetic dataset of ITS and *Trn*L (the latter based on conserved regions) producing not only concordant clusters but also a secondary structure. Specific assignments of the secondary structure evidenced by different cyanobacteria groups, especially the D1-D1' region of ITS and the P6b region of *Trn*L. For the latter region, the sequences analyzed for *Nostoc* species could be divided into the two classes previously identified, on the basis of different heptanucleotide repeats in P6b which were not found in *Oscillatoriales*, which instead showed marked sequence length variation in region P5. A correlation between the two P6b intron classes and their ecological role is suggested for free-living Antarctic *Nostoc*, as it was previously found in symbiontic cyanobacteria from other regions.

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Keywords

Antarctic Cyanobacteria, Biodiversity, ITS, tRNA^{Leu} UAA, Secondary Structure

1. Introduction

Biodiversity and evolutionary origins of Antarctic microbial ecosystems have recently stimulated interest since providing useful models of evolutionary ecology [1] [2]. In fact the Antarctic ice sheet provides unique, natural culture for study of microorganisms which have been isolated from the global gene pool over timescales of evolutionary significance. In this context cyanobacteria prove good bio-indicators since they adapt their growth to the dynamic interface between ice and water. Polar marine ecosystems are particularly sensitive to climatic change and small temperature differences can have large effects on the extent and thickness of sea ice [3]. Therefore Antarctic cyanobacteria diversity could be used as a possible biological indicator of global climate change. Cyanobacteria belong to an ancient group of oxygenic, photosynthetic organisms with a cosmopolitan distribution in many extreme environments [4]. They are characterized by physiological versatility and ample ecological tolerance, which allow them to compete successfully with other organisms in aquatic and terrestrial environments [5]. They are considered valid bio-indicators for their capacity to adapt to various stress environments, playing important roles in carbon and nitrogen cycles and modifying morphology, metabolism and light-harvesting systems.

Phylogenetic relationships among cyanobacteria are still not fully resolved [6]. At present three taxonomic schemes are used for cyanobacteria [7]-[11] which give varying emphasis to morphological, biochemical and genetic aspects of both wild and cultivated strains. Among these molecular markers are potentially most promising, especially in the identification of field samples without culturing [12]. So far, 16S rDNA has been widely used to infer phylogenetic relationships between species and strains as a single marker or combined with traditional techniques [13] [14].

An increasing number of molecular markers have been proposed to provide a robust phylogeny such as the more variable ITS, the transcribed spacer between 16S and 23S [15] whilst another candidate is the chloroplast tRNA^{Leu} (UAA) intron (*Trn*L) by which one region is shown to be particularly useful for detecting species level variation [16]-[18]. The *Trn*L gene, including the intron, has been used as a phylogenetic marker for cyanobacteria and appears to be in agreement with phylogeny estimated using rRNA (e.g. 16S, [19]). Moreover tRNA^{Leu} intron contains regions which have been highly conserved over a billion years of chloroplast evolution [20] [21].

However, molecular markers are potentially useful in cyanobacteria, not only for genus and species identification, but also to better characterize different ecotypes. In fact recent studies revealed that cyanobacteria adapt physiologically to different environments, producing secondary active biological molecules such as fatty acids which are potentially useful for human health [22]-[25]. Some authors suggest correlations between *Trn*L variation, and the physiological role of various organisms such as nitrogen-fix, photosynthetic organisms and symbionts [26]-[30].

The objective of this research was to analyze the genetic diversity of 13 strains of clonal filamentous cyanobacteria, coming from different locations of the Antarctic region of south polar lake (sediments, soil, algal mats and water), exploring whether such morphotypes constitute distinct species, comparing them to Mediterranean strains (fresh water and sea) and also to those available in Genbank. To this aim two regions were sequenced: internal transcribed spacers ITS (flanked by partial 16S and 23S rRNA), and part of the *Trn*L gene (including the intron). In a previous study, Pushparaj *et al.* [24] found that the fatty acid composition varied between these Antarctic strains, with relatively high quantities of polyunsaturated fatty acids, as well as the total lipid content (from 13% to 9%) and the C/N ratio (3.7 to 11.2). In the present study, the further aim was to identify the genetic features of these individual strains; therefore the utility of the two markers for phylogenetic inference and ecotype characterization, including secondary structure of *Trn*L, will be discussed.

Our research is part of a multidisciplinary study of PNRA (Programma Nazionale Ricerche in Antartide) that included morphological identification, RAPD genetic diversity [31] and fatty acid composition [24] of Antarctic cyanobacteria.

2. Materials and Methods

2.1. Sampling and DNA Extraction

The cyanobacteria specimens were collected in the Antarctic region around Italian station, Mario Zucchelli, (ex-BTN, Baia Terranova), during various Italian expeditions in the years 2003 to 2005 (Austral summers) organized by the Programma Nazionale di Ricerche in Antartide (PNRA). Collecting data and geographical coordinates are given in **Table 1**. The strains were grown in laboratory conditions [24] and their morphological and biomass analyses (g/mL) were performed before RAPD genetic studies [31].

DNA extraction was carried out, using the *Magna Rack* method (Kit of Invitrogen SpA, Italy) with some modifications involving reaction times. Briefly, fresh cells were harvested during exponential phase by centrifugation (10,000 × g for 10 min) then they were washed in buffer (10 mM Tris-HCl, 1 mM EDTA pH 8.5) and subsequently they were incubated for ten minutes at 37˚C in 500 µL Lysis Buffer containing 10 µL proteinase K and 5 µL lysozyme (a fresh solution of 50 µg/mL in water Sigma Aldrich L7651). Subsequently, the cells were incubated for 1 hour at 80˚C, before to start the followed re-suspending steps of the *Charge Switch Magnetic Beads* method (Invitrogen SpA, Italy). The supernatant containing the genomic DNA, was washed twice and eluited in a 200 µL of Tris-HCl buffer (pH 8.5). By spectrophotometric analyses (Perkin Elmer) the DNA content was determined (qualitatively and quantitatively).

Table 1. Cyanobacteria strains, geographical coordinate of sampling sites and substrate.

STRAINS*	Morphotype	Geographicalcoordinates of sampling	Substrate
*N*0 *EPN*16*bS*01	*Nostoc commune*	Edmonson Point North, Antarctic 74˚19'55.19"S; 165˚07'45.26"E	Soil
*N*1 *KP* 1*aS*01	*Nostoc commune*	Kar Plateau, Antarctic 76˚54'00"S; 162˚32'00"E	Algal mat
*N*5 = *LBT* 88 *S*01	*Nostoc commune*	Cape King, Antarctic 73˚35'22.54"S; 166˚33'55.25"E	Soil, water
*N*8 8 *S*01	*Nostoc commune*	Icaro, Antarctic 74˚42'54.18"S; 164˚06'31.76"E	Dry moss
*N*43 *S*01	*Nostoc commune*	Tarn flat, Antarctic 75˚00'27.34"S; 162˚15'46.97"E	Soil
*N*117 *S*01	*Nostoc commune*	Harrow Peak, Antarctic 74˚05'00,00"S; 164˚49'13.75"E	Moss
*L CK*338*bS*01	*Leptolyngbya* sp. *Pseudophormidium* sp.	Cape King, Antarctic 73˚35'22.54"S; 166˚33'55.25"E	Moss
LY TN 1*bS*04	*Leptolyngbya* sp.	Ten Nunatak, Antarctic 74˚50'21.14"S; 162˚33'45.14"E	Water, sediment
*L*4 *CK* 344*b*	*Phormidium* sp.	Cape King, Antarctic 73˚35'22.54"S; 166˚33'55,25"E	Musk
*L*5 *BTNS*01	*Leptolyngbya* sp.	Mario Zucchelli Station Baia Terranova Antarctic 74˚41'44.84"S; 164˚06'39.97"	Soil
*L*11 *S*01	*Leptolyngbya* sp.	Icaro, Antarctic 74˚42'54.18"S; 164˚06'31.76"E	Dry moss
*P KP*1*aS*04	(*Plectonema* sp.). *Pseudophormidium* sp.	Kar Plateau, Antarctic 76˚54'00"S; 162˚32'00"E	Algae
*P*2 *El* 1*cS*01	(*Plectonema* sp.) *Pseudophormidium* sp.	Enigma lake, Antarctic 74˚43'18.06"S; 163˚55'49.12"E	Algae
*LM*** 2*LT*2*S*	*Leptolyngbya* sp.	Lake of Trasimeno, Northern Italy 43˚08'22"N 12˚06'27"E	Fresh water
*N*6** *BB*14	*Nostoc* sp.	Lake of Bubano, Northern Italy 44˚24'40"N; 11˚46'53"E	Fresh water
*NM***	*Nostoc* sp.	S. Maria di Leuca, South Italy. Mediterranean 39˚47'0"N; 18˚19'0"E	Sea water
*G*3**** 3*Be S*65	*Gloeocapsa*	Belize 17˚28'N ; 88˚10'W	Water

*(Codes in this paper, above, and Pushparaj *et al.*, 2008, below); **Strains coming from Italian lakes and Mediterranean sea for comparison purpose; ***Strain used as outgroup.

2.2. *Trn*L and 16S-ITS-23S Amplification and Data Analyses

The amplification products were obtained by a thermal cycler (Perkin Elmer/Cetus 2400) and visualized after 1.4% agarose gel-electrophoresis (BIORAD, power pack 300, Italy).

Then sequencing was carried out by ABI automated dye-terminator system (Macrogen).

2.2.1. *Trn*L Amplification and Sequencing

For *Trn*L amplification, we used 5'-GGGGRTRTGGYGRAAT-3' as forward primer, and 5'-GGGGRYRGRGGGACTT-3' as reverse primer [12]. Amplification L volume containing 50 ng DNA in a mixer of reaction was performed in 50 Applied Biosystem reagents (1.25 U Taq polymerase, 0.2 mM dNTP, 2.5 mM $MgCl_2$ and 1× buffer HCL). The cycling parameters for PCR were: initial denaturation at 95˚C for 5 min, then cycles repeated 35 times of 94˚C for 45', 53˚C for 45' annealing temperature, 72˚C for 1 min; finally one cycle at 72˚C for 10 min. The sequences were obtained with the primers: 5'-GGTAGACGCWRCGGACTT-3' and 5'-TWTACARTCRACGGATTTT-3'.

2.2.2. 16S-ITS-23S Amplification and Sequencing

For both the amplification and sequencing of 16S-ITS-23S region, we used Wilmotte's universal 322 and 340 primers [15], respectively: 5'-TGTACACACCGCCCGTC-3' and 5'-CTCTGTGTGCCTAGGTATCC-3'. L mixer (Applied Biosystem reagents) The reaction was obtained in a 50 containing 50 ng DNA (1.25 U Taq polymerase, 0.2 mM dNTP, 2.5 mM $MgCl_2$ and 1× buffer HCL, 0.2 mM primers). PCR conditions were: initial denaturation at 95˚C for 5 min, then 35 cycles repeated at 94˚C for 45", 50˚C annealing temperature for 45", 72˚C for 1.20 min; finally one cycle at 72˚C for 10 min.

The obtained sequences were aligned using the program SEAVIEW version 2.4 [32], which implements MUSCLE (multiple sequence comparison by log expectation) [33], and by hand, also taking into account the secondary structures. Comparisons with sequences available in Genbank database were performed, using MEGABLAST version 3.1 available from http://www.ncbi.nlm.nih.gov/BLAST/. Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4 [34]. Phylogenetic trees for the two regions were obtained using Maximum parsimony method, Neighbour Joining and Maximum Likelihood, with Bootstrap analysis. Predictions regarding secondary structures for the *Trn*L intron were made using the program RNA Structure version 4.6 [35].

3. Results

3.1. Molecular Identification

Overall, the ITS types observed differed from those identified by [36] in a study of Antarctic cyanobacteria, thereby confirming the variability of this region. **Figure 1** (left) and **Figure 1** (right) show the best hits found in Gene-Bank database for the sequences obtained for ITS and *Trn*L respectively for the samples studied.

For *Nostocaceae,* identifications based on molecular markers were generally in close agreement with morphological ones: N0, N1, NM, corresponded to *Nostoc* sp. with both ITS and *Trn*L, and N5, N6 and N8 corresponded to *Nostoc commune* with both markers; N43 and N117 were successful sequenced only by *Trn*L and were related to *N. sphaericum* and *N. commune*, respectively.

In the case of *Oscillatoriales*, L, L5, LY and LM were identified by ITS as *Leptolyngbya*, and L4 as *Phormidium*, in agreement with morphological analysis.

The sequences obtained for *Trn*L region ranged from 272 bp (NM) to 389 bp (L, LY). In particular, in the *Trn*L region, a band of low molecular weight was consistently amplified, and a very short region sequenced with the forward primer (especially for L4, L5, G3, P2). This suggests a lack of the intron, as frequently observed in cyanobacteria [19]. Details of secondary structure are given below.

For the 16S-ITS-23S region, the obtained sequences ranged from 338 bp (N0) to 495 bp (LM). Amplification and sequencing of 3 strains (N43, N117, L11) were successful identified by only *Trn*L (**Figure 1**, right).

A single RNA operon, including both tRNA-Ile and tRNA-Ala, was found for L, LY, L4, L5, LM, P, P2, and G3, whereas a short one, not including these tRNAs, was amplified for N0, N1, N5, N6 and N8. Instead, two different operons were found for the Mediterranean strain NM: a short one, without the two tRNAs, and a longer one which included at least part of tRNA-Ile (**Figure 1**, right). *Trn*L provided less clear-cut identification for L, LY and LM, mainly corresponding to *Oscillatoriales* (**Figure 1**, right).

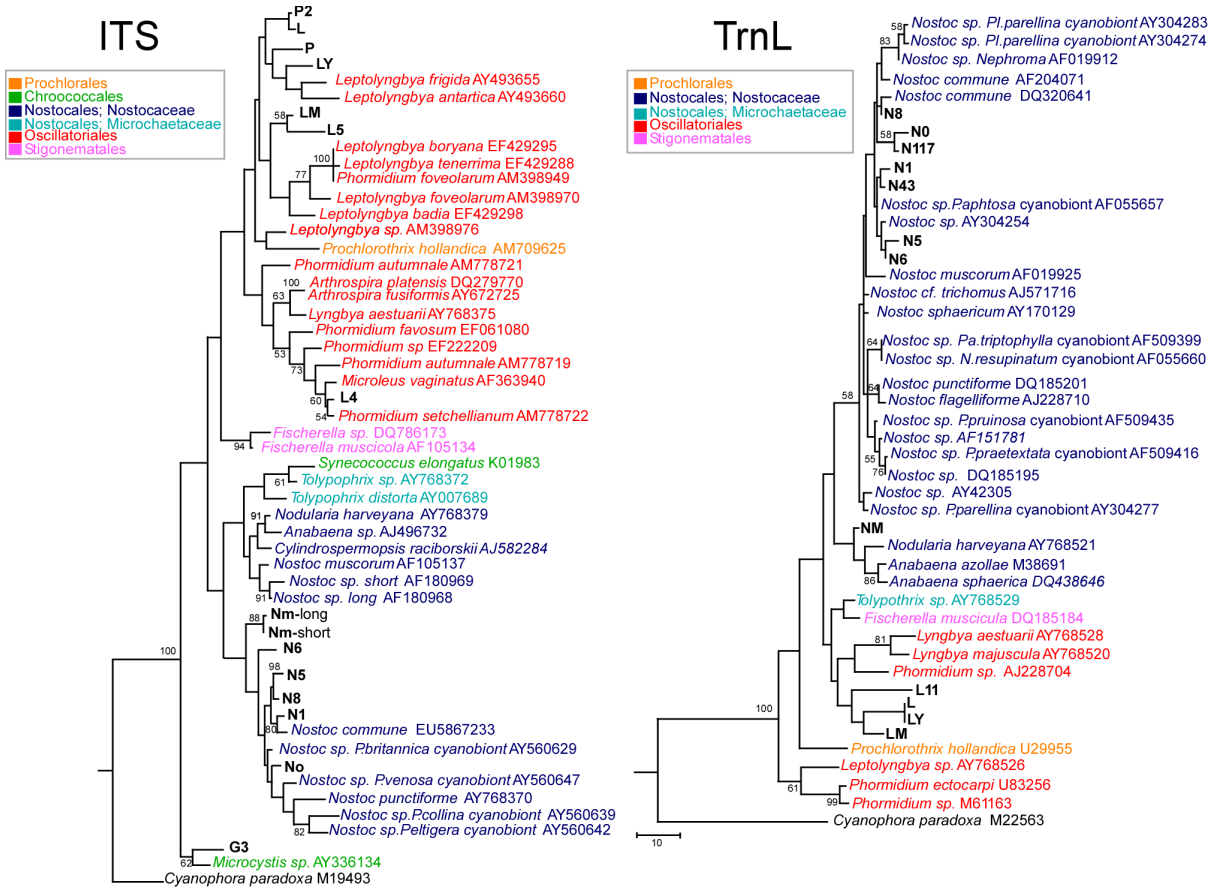


Figure 1. Left—ITS. Maximum Parsimony tree of ITS region for the samples studied, compared to taxa in Genbank. One of 12 most parsimonious trees is shown, with bootstrap values above 50% over 500 replicates indicated (final dataset of 576 bp, of which 244 parsimony informative). Tree drawn to scale, with branch lengths calculated using the average pathway method, as implemented in MEGA 4. Right—*Trn*L. Maximum Parsimony tree of *Trn*L region for the samples studied, compared to taxa in Genbank. Bootstrap consensus tree is shown, with values above 50% over 500 replicates (final dataset, of which 77 parsimony informative). Tree drawn to scale, with branch lengths calculated using the average pathway method, as implemented in MEGA 4. culated using the average pathway method, as implemented in MEGA 4. P. = Peltigera; N. = Nephroma; Pa. = Palmeliella; Pl. = Placopsis.

In the case of P, P2, the molecular identifications contrasted with those based on morphology that identified them as *Pseudophormidium* sp., whilst by ITS they proved related to *Leptolyngbya*. No *Trn*L intron was apparently recovered for these two samples according to the other Antarctic *Oscillatoriales*. Sample G3, identified morphologically as *Gloeocapsa* (*Chroococcales*), proved related to *Microcystis* (*Chroococcales*) by ITS and it was used as outgroup.

3.2. Phylogenetic Relationships

Evolutionary distances among the strains were estimated by Kimura-2-parameter.

In one case a same *Trn*L haplotype was shared by Antarctic samples geographically far apart (*Leptolyngbya sp:* L-Ly). A close relationship was detected at ITS between L and P2, also from different locations. The range of distance values (by pairwise deletion method) for the cyanobacteria studied at ITS were: 0.031 (N0-N8) - 0.100 (N5-NM) within *Nostocales*; 0 (L-P2) - 0.129 (L4-L, LY) within *Oscillatoriales*, and 0.102 (N6-LM) - 0.22602 (N1-L5) between the two groups.

The outgroup G3 (*Chroococcales*) showed values from 0.135 (with L) to 0.178 (with N1).

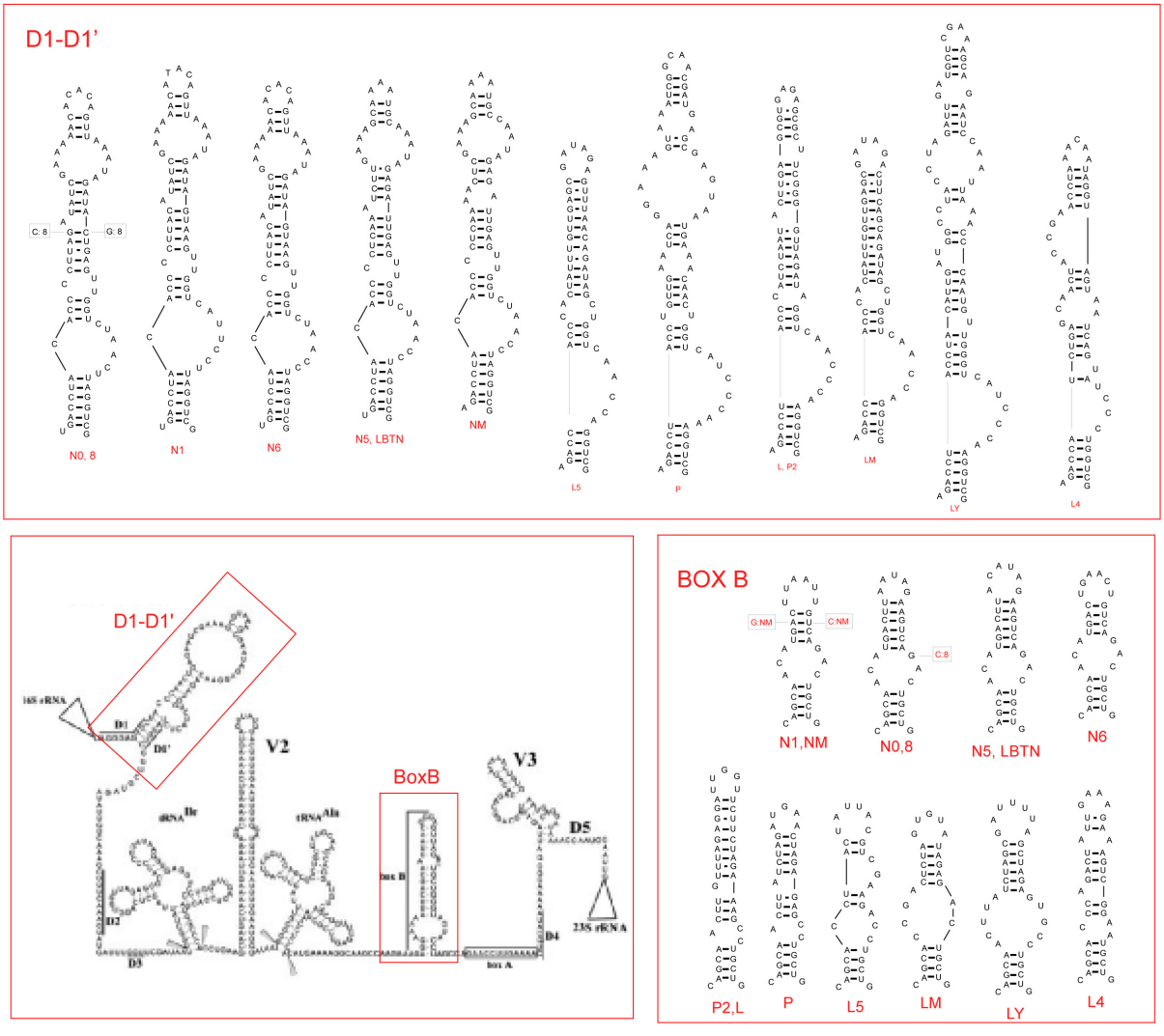
At T*rn*L the ranges of K-2 values were: 0.015 (N0-N117) - 0.112 (NM-N117) within *Nostocales*, 0 (L-LY) - 0.098 (L, LY-L11) within *Oscillatoriales* and 0.072 (L, LY-NM) - 0.152 (N5- LM) between the two groups.

Evolutionary relationships among the Antarctic and Mediterranean cyanobacterial strains studied are summarized in **Figure 1** (left) and **Figure 1** (right), which show trees for the ITS and *Trn*L regions respectively, obtained by the maximum parsimony method.

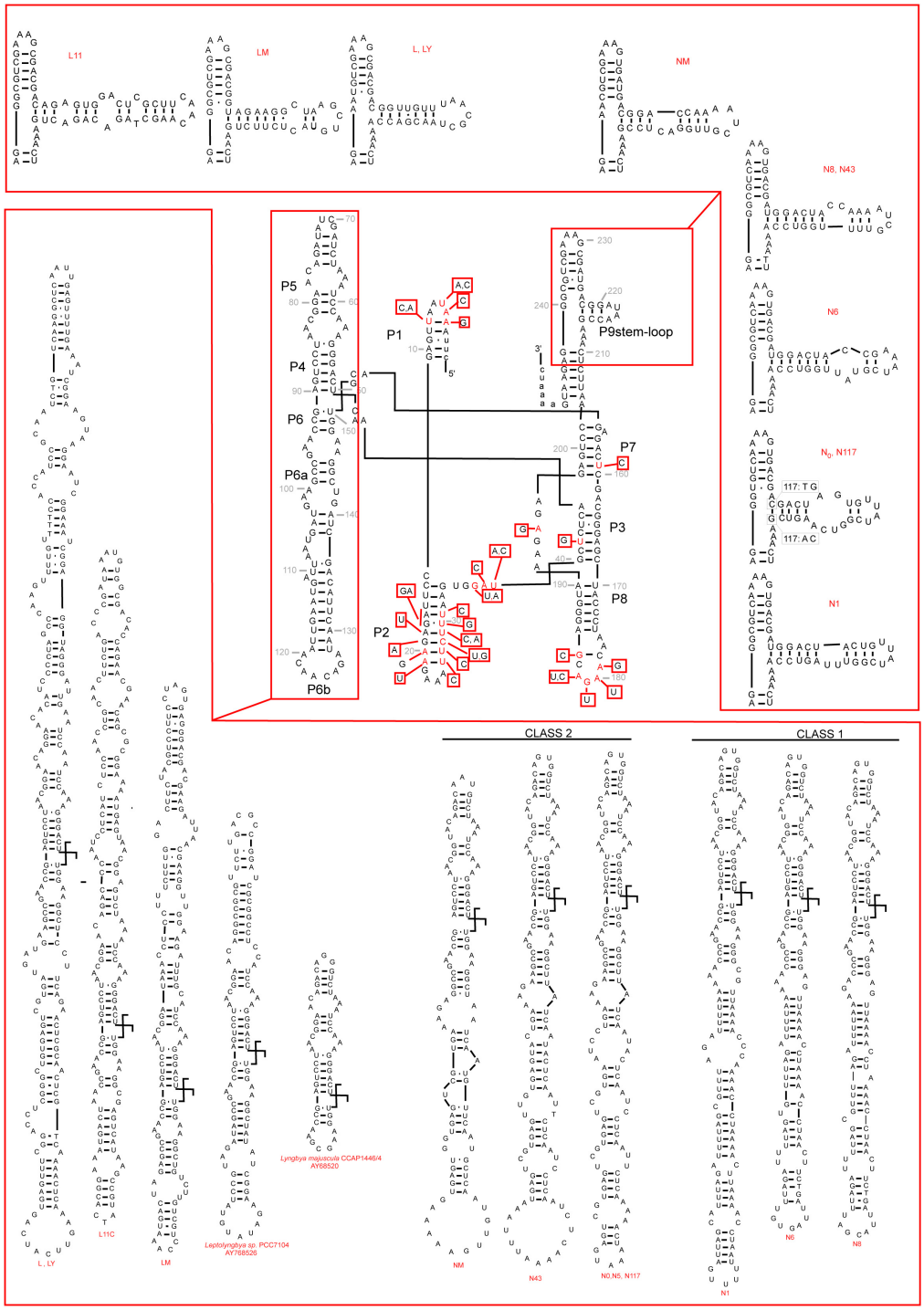
A number of cyanobacteria sequences available in Genbank were included for comparison: photosynthetic cyanelle of *Cyanophora paradoxa*, available for both regions, was used as outgroup.

At ITS (**Figure 1**, left), *Chroococcales* (G3 and *Microcystis* sp.) form a basal well supported clade; in which two main clusters include respectively: 1) *Nostocales*, with *Nostoc* spp. (including all our *Nostoc* sp. samples) grouping together in a distinct subcluster; 2) *Oscillatoriales*, together with *Stigonematales*, and *Prochlorales* (*Prochlorothrix hollandica*). Within the latter cluster, three subclusters include: 1) *Fischerella* sp. (*Stigonematales*); 2) mainly *Leptolyngbya* spp., with L, LY, P, P2 related to other Antarctic *Leptolyngbya* strains, LM and L5 more related to *Leptolyngbya faveolarum* and *L. badia*; this subcluster also includes *Prochlorothrix hollandica* (*Prochlorales*); c) mainly *Phormidium* spp. and *Microcoleus vaginatus*, grouping with L4; this subcluster also includes *Arthrospira* spp. and *Lyngbya estuarii*.

*Trn*L phylogenetic analysis was carried out, excluding from analysis the most variable regions (loop sequences in P6b, P9, P5), as suggested by [6] and [19] (cf. **Figure 2(b)**). As shown in **Figure 1** (right) (at *Trn*L) *Nostocales* form a distinct clade, whereas the relationships among *Oscillatoriales*, *Stigonematales* and *Prochlorales*, are less resolved, possibly due to the few sequences available: only one GenBank sequence of *Leptolyngbya* (well separated from the others with ITS) could be included.



(a)



(b)

Figure 2. (a) Putative secondary structure of the 16S rRNA-23S rRNA ITS region in *Anabaena* PCC 7120 (central box, from Iteman *et al.*, 2000) and details of the regions D1-D1' and BoxB in the studied cyanobacteria (inserts). (b) Putative secondary structure of the *Trn*L region in the studied cyanobacteria. In the central part, variable sites are shown in red, compared to the model structure *Anabaena* sp.PCC7120 (modified from Paulsrud and Lindblad, 1998). Details of variable parts P6b and P9 stem-loops, are shown for each specimen in the inserts. Secondary structures of Structures of *Leptolyngbya* sp. PCC7104 (AY768526) and *Lyngbya majuscula* CCAP1446/4 (AY68520) have been added for comparison. Class 1 and class 2 introns follow Paulsrud and Lindblad, 1998.

For *Trn*L, all *Nostoc* sp. samples form a cluster with *Nostoc commune*, whilst the Mediterranean strain NM clusters with *Anabaena* spp. and *Tnodularia harveyana.* The strains L, LY (with the same haplotype), L11 and the Mediterranean LM clustering together within *Oscillatoriales*.

3.3. Secondary Structure

3.3.1. ITS Region

Two semi-conserved regions of the internal transcribed spaces of the rRNA operon were analyzed: 1) the stem included between the conserved domains D1 (immediately following the 16S RNA) and D1'; and 2) the Box B stem, preceding the conserved anti-terminator Box A sequences, involved in preventing a premature termination of transcription (cf. [15]-[37]). The putative secondary structures of the samples studied are shown in **Figure 2(a)**, together with that of the 16S-23S rRNA region for *Nostoc* PCC 7120 (data from [15]). The D1-D1' region shows marked differences between *Nostocales* and *Oscillatoriales*, with characteristic one-sided loops in the basal part of the stem and allows a ready distinction of the two groups. Box B appears to be more variable among both *Nostocales* and *Oscillatoriales*, but also for this region the two groups can be distinguished. The patterns observed are consistent with previous findings in *Nostocales* [38] and *Leptolyngbia* [39].

3.3.2. *Trn*L Intron Region

The predicted secondary structures of the *Trn*L intron for the Antarctic and Mediterranean cyanobacteria studied, are given in **Figure 2(b)**, and compared to the model structure proposed by [40] for *Nostoc* (*Anabaena*) *PCC* 7120. Our data confirm that the *Trn*L intron core structure is highly conserved, with variability restricted to some regions, especially P6b, P9, P5 loops, characterized by striking sequence length variation [19]. In the *Nostoc* sequences analyzed we could recognize the two classes, identified by [26], characterized by different heptanucleotide repeats in P6b: class 1, shared by N1, N6 and N8, and class 2, shared by N0, N5, N43, N177 and NM. This was not shown by *Oscillatoriales*, which instead showed marked sequence length variation in region P5, from a very short sequence e.g. in *Lyngbya majuscula* CCAP1446/4 (Gene-bank accession AY68520T), to a very long one in L1 and LY (so far not observed elsewhere). Such differences in the regions P6b and P5 might be useful in distinguishing between *Nostocaceae* and *Oscillatoriales*, if confirmed in a higher number of strains.

4. Discussion

Comparative sequence analyses of Antarctic cyanobacteria proved very useful for identifying species of *Nostocales*, *Chrococcales* and *Oscillatoriales*, and for analyzing their genetic diversity, especially when these strains were sequenced at ITS and *Trn*L regions. Molecular identifications were generally in agreement with both *Trn*L and ITS, particularly for *Nostocales*. ITS proved more useful than *Trn*L for the assignments of *Oscillatoriales* and *Chroococcales*, due to the frequent lack of the intron in these groups (e.g. [19]). On the other hand, by microscope similar morphologies we found for genetically different strains, e.g. P and P2 that at first showed the highest similarity to *Pseudophormidium* sp. in *Oscillatoriales*, but subsequently they were recognized as *Leptolyngbia* by ITS.

Our data confirm the need for multiple taxonomic criteria, including molecular markers, for a successful classification of cyanobacteria. In particular our findings support the validity of the *Trn*L intron as a phylogenetic marker for cyanobacteria and especially for *Nostocale*s. Independent analyses of ITS and *Trn*L datasets (the latter based on conserved regions) produced concordant clusters, especially when the most variable regions of the intron (parts of the helix P6, P9, P5) were excluded, as suggested by [19] and [6]. For both markers, the Antarctic strains studied in the present paper proved related, not only to Antarctic species, but also to those from other parts of the world, including the Mediterranean region [36]-[41]. The analysis of *Trn*L secondary structure in the cyanobacteria studied confirmed the presence of two classes in *Nostoc* sp., characterized by different heptanucleotide repeats in the region P6b, shared by Antarctic and Mediterranean species. Interestingly, class 2 was observed in strains from three Antarctic samples isolated from soil, moss, and a Mediterranean strain from seawater; whereas class 1 was detected in Antarctic strains isolated from algal mats, moss, and fresh water. These findings support the correlation found by [26] between the two intron classes and physiological roles in symbiotic cyanobacteria, particularly nitrogen fixation (class 2), or both photosynthesis and nitrogen fixation (class 1). Previous analysis also suggests differences in haplotypes and other characteristics, such as C/N ratios and fatty

acid composition; these were found to vary among Antarctic strains, including those studied in the present paper [24]. Interestingly, strains L and Ly, which shared the same *Trn*L haplotype, both showed high values of palmitoleic oil (16:01) and only traces of 17.04 (IUPAC), in contrast to P and P2 (possibly intronless), which showed values more similar to *Nostocales*. A correlation between fatty acid composition and RAPD genetic diversity at genera level was detected by [31].

Moreover in further *ad hoc* studies we found a correlation between cyanobacteria haplotypes and their eco-physiological characteristics on the basis of sequences available in GenBank, as shown for *Nostoc* species in the **Figure 3**.

We can conclude that the combined use of ITS and *Trn*L markers has proved useful to identify Antarctic cyanobacterial samples. The secondary structure of the D1-D1' region appears particularly promising for a rapid routine distinction between *Nostocales* and *Oscillatoriales*, whereas the secondary structure of the P6b region can provide insight into the functional role in *Nostocales*. Such tools can contribute to the build-up of a database

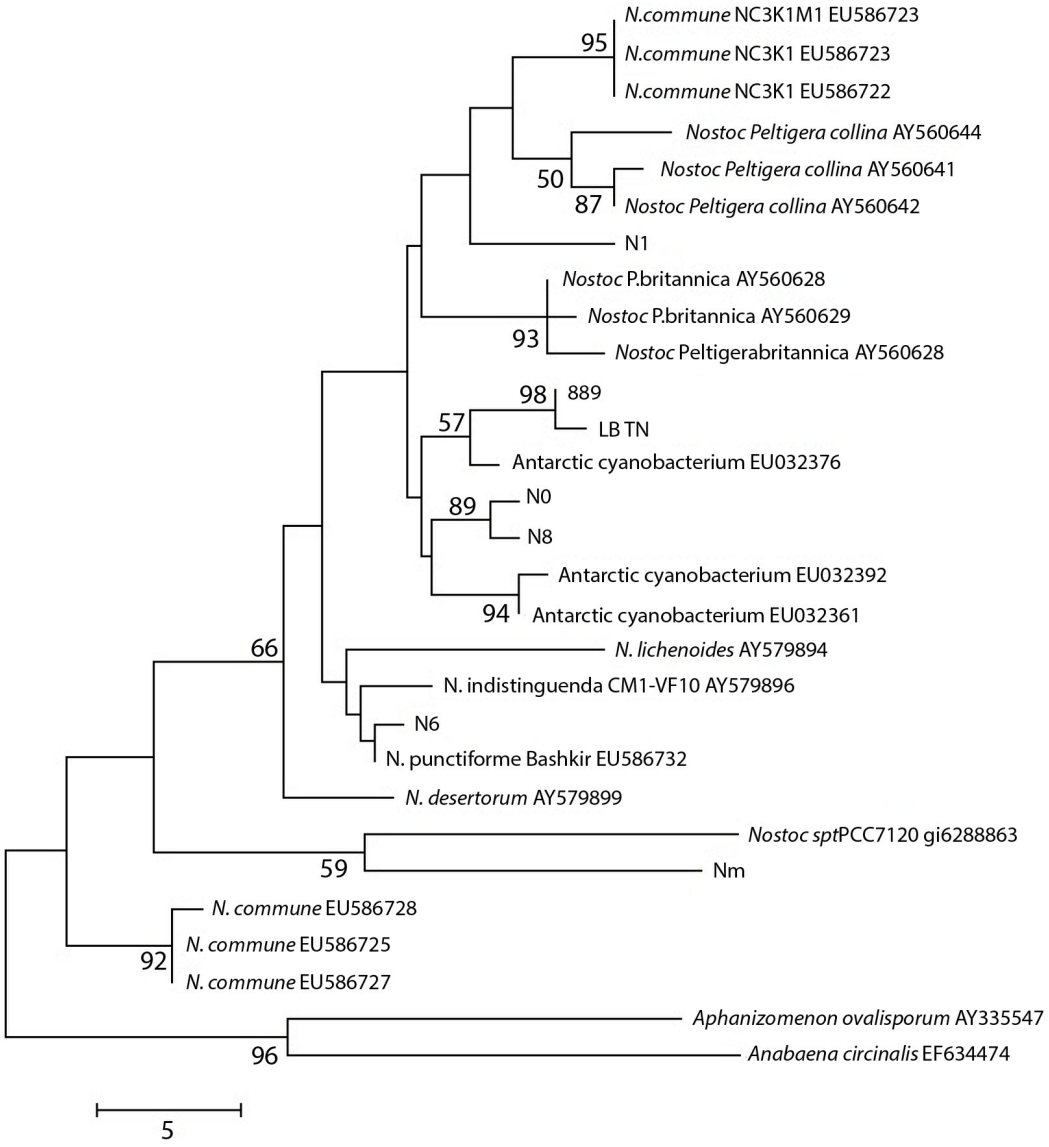


Figure 3. Maximum Parsimony tree of ITS region for the samples studied, compared to taxa in GenBank. Consensus tree (from 28 most parsimonious trees) is shown, with bootstrap values above 50% over 100 replicates. Tree drawn to scale (branch lengths calculated using the average pathway method, as implemented in MEGA 4; complete deletion; 248 bp of which 48 were parsimony informative).

of genetic diversity of cyanobacteria in the still relatively undisturbed Antarctic ecosystem. This will make it possible to monitor and track changes in genetic diversity, and relate them to local, regional and global climate changes habitat disturbance. With increasing concern regarding global climate change, evaluation of biodiversity (genetic, species, ecosystem), is necessary in order to track changes within the climate system: it affects interpretations of future global, and especially polar climate change. In this context cyanobacteria diversity monitoring could be important as a possible biological indicator, in particular regarding diversity and connectivity between diverse habitats across a range of geographic scales.

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