



Cyanobacteria in lichens and mosses

Ósk Ukachi Uzondu Anuforo



**Líf- og umhverfisvíssindadeild
Háskóli Íslands
2012**

Cyanobacteria in lichens and mosses

Ósk Ukachi Uzondu Anuforo

42 eininga ritgerð sem er hluti af
Viðbótardiplóma gráðu í líffræði

Leiðbeinandi
Ólafur S. Andrésson

Líf- og umhverfisví sindadeild
Verkfræði- og náttúruvísindasvið
Háskóli Íslands
Reykjavík, Maí 2012

Cyanobacteria in lichens and mosses
Host speciation of *Nostoc*
42 eininga ritgerð sem er hluti af *Viðbótardiplóma* gráðu í líffræði

Höfundarréttur © 2012 Ósk Ukachi Uzondu Anuforo
Öll réttindi áskilin

Líf- umhverfisvísindadeild
Verkfræði- og náttúruvísindasvið
Háskóli Íslands
Askja, Sturlugata 7
107 Reykjavík

Sími: 525 4000

Skráningarupplýsingar:
Ósk Ukachi Uzondu Anuforo, 2012, *Cyanobacteria in lichens and mosses*,
Viðbótardiplóma ritgerð, Líf- umhverfisvísindadeild, Háskóli Íslands, 75 bls.

Prentun: Háskólaprent ehf.
Reykjavík, Maí 2012

Abstract

The *Nostoc* cyanobacteria which belong to the order *Nostocales* can be found living singly or in symbiotic associations. Here we identified and cultured *Nostoc* and other cyanobacteria from lichens and mosses and determined if they are of related or similar strains. 11 lichens of *Peltigera* and *Stereocaulon* species and 10 samples of 5 species of mosses were collected from different locations. DNA and cultures were taken for PCR and sequencing with rbcLX primers and 16S rDNA primers. We were able to identify and culture *Nostoc* from the *Peltigera* lichens and also determine that they are of related or similar strains to each other. However, no *Nostoc* was identified or cultured in the *Stereocaulon* lichens and mosses except from one moss sample. Cyanobacteria of different genera were identified by PCR from the *Stereocaulon* lichens and from mosses and it was found that for each group the cyanobacteria were closely related.

Blágrænbakteríur af ættkvíslinni *Nostoc* má finna víða, bæði útaf fyrir sig og í sambýli við aðrar lífverur. Í þessu verkefni voru greindar og ræktaðar *Nostoc* bakteríur og aðrar blkágrænbakteríur úr fléttum og mosum og kannað hvort þetta væru svipaðir eða skyldir stofnar. 11 fléttur af ættkvíslinni *Peltigera* og *Stereocaulon* ásamt 10 sýnum af 5 tegundum mosa var safnað á nokkrum stöðum. Erfðaefni var magnað og raðgreint úr DNA sýnum og bakteríuræktum með rbcLX og 16S rDNA vísum. Unnt var að rækta og greina *Nostoc* úr *Peltigera* sýnum og einnig að ákvarða skyldleika þeirra. Ekki var þó hægt að greina eða rækta *Nostoc* úr *Stereocaulon* eða mosasýnum neme í einu tilviki. Hægt var að nota PCR til að greina blágrænbakteríur af öðrum ættkvíslum úr *Stereocaulon* (grábreyksju) og úr mosasýnum og kom í ljós að fyrir hvorn hóp lífvera virtust blágrænbakteríurnar vera fremur einsleitar að skyldleika.

*For my mom and dad
Rebekka and Godson*

Table of content

Introduction	1
Lichens.....	1
Moss	3
<i>Nostoc</i>	4
Aims	5
Materials and methods.....	6
Sample collection.....	6
Sample processing	6
PCR and sequencing analysis	7
Sequencing results analysis	9
Cryopreservation of <i>Nostoc</i> in DMSO.....	9
Results.....	10
Morphology of lichens	10
Microscopic analysis of colonies from lichens and mosses	10
PCR results	11
Sequence analysis	13
Discussion	17
The lichens	17
<i>Nostoc</i> and cyanobacteria cultures.....	17
Cyanobacteria associations	17
Conclusion.....	20
References.....	21
Appendix	23

Figures

Figure 1.1: The thallus structure a) upper cortex, b) algal layer, c) medulla and d) lower cortex	2
Figure 1.2: Pictures of the growth forms of lichens a) crustose lichen b) foliose lichen c) frutose lichen	3
Figure 1.3: <i>Nostoc</i> filament. The arrow points to a heterocyst within the filament	4
Figure 3.1: Phylogenetic tree of <i>Nostoc</i> species isolated from the <i>Peltigera</i> species which were sequenced with CX primer	14
Figure 3.2: Phylogenetic tree of <i>Nostoc</i> species isolated from the <i>Peltigera</i> species which were sequenced with CX primer and their closest genetic relative identified from the BLAST results	14
Figure 3.3: Phylogenetic tree of bacteria/cyanobacteria species isolated from the <i>Stereocaulon</i> lichens and moss species which were sequenced with F850 primer	15
Figure 3.4: Phylogenetic tree of bacteria/cyanobacteria species isolated from the <i>Stereocaulon</i> lichens and moss species which were sequenced with F850 primer and their closest genetic relative identified from the BLAST results.....	16
Figure 4.1: Pictures of lichens a) <i>P. membranacea</i> & <i>P. islandica</i> , b) <i>P. islandica</i> , c) <i>P. neckeri</i> , d) <i>P. monticola</i> , and moss e) <i>Rhytidadelphus squarrorus</i> , f) <i>Hylocomium splendens</i> , g) <i>Racomitrium lanuginosum</i> , h) <i>Rhytidadelphus triquetrus</i> , and i) <i>Racomitrium canescens</i>	18

Tables

Table 2.1: List of lichen and moss samples collected and their locations.....	6
Table 2.2: List of primers used for the PCR.....	7
Table 3.1: Table showing the observed colonies from mosses cultured on BG-11 _o media.....	11
Table 3.2: Table showing PCR results from lichen DNA isolates	11
Table 3.3: Table showing PCR results from lichen colony cultures using CX/CW primers	12
Table 3.4: Table showing PCR results from lichen colony cultures using F850/CyaR1444 primers.....	12
Table 3.5: Table showing PCR results from moss DNA isolates.....	12
Table 3.6: Table showing PCR results from moss colony cultures using F850/CyaR1444 primers.....	13
Table 3.7: Top hits to the ITS sequences for lichen DNA	13
Table 3.8: Summary of BLAST results for the sequence analysis with the CX primer.....	14
Table 3.9: Summary of BLAST results for the sequence analysis with the F850 primer.....	15

Thanks

A special thanks to Sheeba Santhini Manoharan, who taught me all the proper laboratory procedures and everything about lichens and *Nostoc* cyanobacteria.

Thanks to Ingibjörg Svala Jónsdóttir, who helped me in identifying the moss species

Thanks to Starri Heiðmarsson, who helped me in identifying the *Stereocaulon* lichens

Introduction

Lichens

Lichens are fungi that are in a symbiotic relationship with certain species of algae or cyanobacteria (photobiont). The fungi (mycobiont) provide structural support and nutrients absorbed from the substrate and a stable microenvironment for the photobionts to exist. In turn the photobionts provide carbohydrates produced by photosynthesis. The mycobiont is most often an Ascomycete, less commonly a Basidiomycete, while the algal component is a chloophyte and the cyanobacteria are most commonly from the genera *Nostoc* and *Stigonema*. The establishment of the mycobiont- photobiont symbiosis is called lichenization. More than 30 species of photobionts have been identified in lichens. It is estimated that there are 15000 – 20000 species of lichens in the world, and about 750 known species in Iceland (Flora of Iceland. 2012). The names given to lichens come from the mycobiont component and thus lichens are usually grouped under the kingdom fungi. Lichens are able to survive in the harshest of conditions and therefore they are widespread around the world from the arid deserts to the Arctic and can grow on bare soil, tree trunks, rocks, fence post etc. (Vitt *et al.* 1988).

Structural components

The thallus of lichen is divided into three layers (Hale. 1974).

Cortex: This serves as the protective covering over the thallus. It is composed of compressed heavily gelatinized hyphae firmly held together. The upper cortex is about 10-15 μm thick with several layers of cells. The surface is covered with a very thin homogeneous cuticle or in some lichens a whitish pruina. The lower cortex is similar to the upper in thickness and structure in foliose lichens, but many lichens do not have a lower cortex. Some may have rhizines or other attachment structure (figure 1.1).

Medulla: Within the thallus the medullary tissue may be 500 μm thick. The hyphae are weakly gelatinized and have relatively large [inter cellular] cell lumina. There is little compression. The medulla has a greater water-holding capacity than the other lichen tissues and is the region for food storage (figure 1.1).

Algal layer: Lichen algae are surrounded by fungal tissue in the lichen and confined to a layer 10-15 μm thick between the upper cortex and medulla. This arrangement is called stratified or heteromerous. In foliose lichens the arrangement can also be unstratified or homoiomeric when there is no distinct differentiation between the algal layer and medulla (figure 1.1).

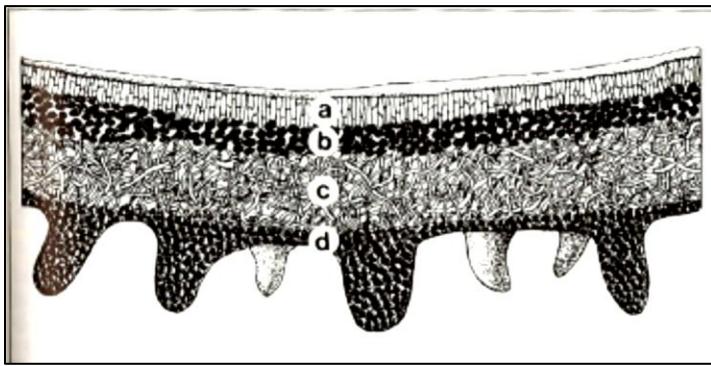


Figure 1.1: The thallus structure a) upper cortex, b) algal layer, c) medulla and d) lower cortex

Growth forms

Crustose lichens: They are flattened and adhere firmly to the substrate (figure 1.2a). They lack a lower cortex and most have an upper cortex, algal layer and medulla that penetrate the substrate. Endolithic lichens occur on rocks and have a thallus just beneath the surface of the rock with the fruiting bodies visible on the surface. Endophloic lichens occur on wood or bark and have their thallus contained entirely within the surface layer of the bark. Some crustose lichens have a thallus that is termed rimose or rimose-areolate. Here the lichen has a hypothallus, a thin layer of black nonlichenized hyphae which extends beyond the main thallus. Crustose lichens are termed squamulose when the thallus edges are free from the substrate. (Hale. 1974)

Foliose lichens: They are leaf-like and attached to the substrate by rhizines on the lower cortex (figure 1.2b). The algal layer is beneath the upper cortex and beneath the algal layer is the medulla composed of fungal hyphae which primarily determines the thickness of the lichen thallus. A lower cortex covers the lower surface of the foliose lichens. Some foliose lichens are gelatinous when wet and do not have an upper cortex and layered algae. They have a fungal hyphae and algae which are intertwined throughout the thallus. Some foliose lichens have the thallus attached to the substrate by a short stalk originating from a central point on the lower cortex. They are called the umbilicate (Hale. 1974).

Fruticose lichens: They are erect and often branched and bushy with a size range from 1 mm to 5 m long (figure 1.2c). They are radially symmetrical in structure with an outer layer of fungal cells that surrounds the algal layer, a medulla and a hollow centre or a centre filled with white cottony fungal hyphae. The thallus can be round or flattened, unbranched or branched. They are attached to the substrate by basal rhizoidal strands on the cortex although some do not have any attachment structure to the substrate (Hale. 1974).

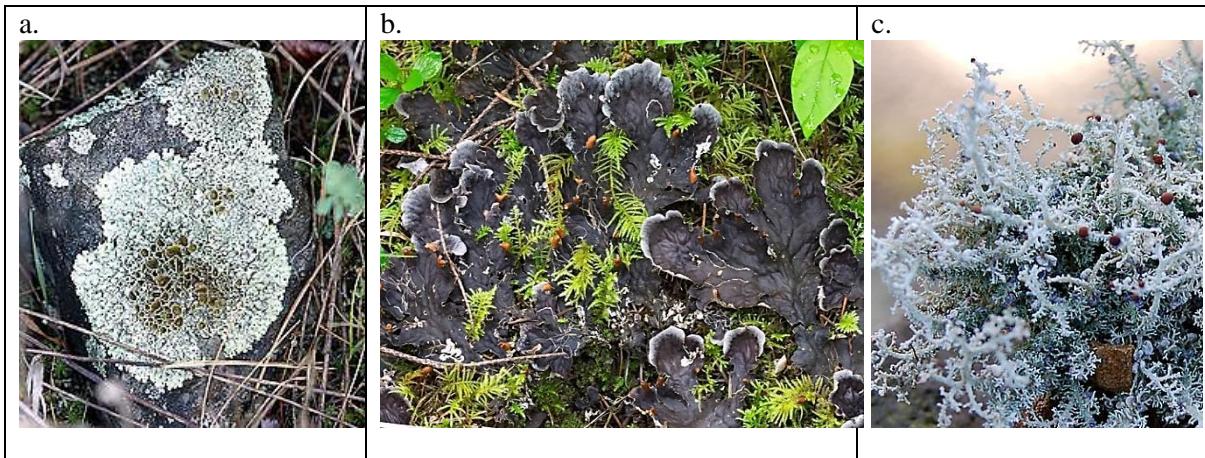


Figure 1.2: Pictures of the growth forms of lichens a) crustose lichen b) foliose lichen c) fruticose lichen

Moss

Mosses are the largest of the three groups in the phylum *Bryophyta*. It is estimated that there are 8000-9000 species of mosses in the world. They grow mostly in moist temperate and tropical habitats, some can be found in the Arctic and Antarctic, in dry habitats and a few in aquatic habitats. They have a leafy gametophyte that matures to produce the sex organs, antheridia (male) and archegonia (female). After fertilization, an erect sporophyte develops and permanently attaches to the gametophyte. The sporophyte contains a capsule in which meiosis occurs and spores are produced, which later develop into a new gametophyte in the following generation (Raven *et al.* 1999).

Structural features

A mature gametophyte of moss includes the following structures (Vitt *et.al.* 1988)

Stem: It consists of a central strand of narrow elongated cells which can either be present to develop and form a distinct central zone, or not present. They are dead cells and conduct water. Surrounding the central strand is the cortex consisting of broader and shorter living cells which become thicker walled and smaller closer to the stem surface. The stems usually branch, from buds or from beneath the sex organs.

Leaf: It consists of a lamina which is one layer of cells thick, and a costa, which is a central vein of several layers of differentiated cells. The leaves are attached to the stems by alar cells.

Gametangia: This is the sex organs antheridia and archegonia. The antheridia are multicellular, ovoid sacs produced terminally on stems or laterally along the stems and surrounded by differentiated leaves in structures called perigonia. Three to six antheridia and sterile hairs are contained in a perigonium. Sperm are produced in the antheridia and released through a terminal cap. The archegonia are the female sex organ and they are multicellular, flask shaped structures which are produced terminally or laterally in perichaetia. Several archegonia and some paraphyses are contained in each perichaetium. Eggs are produced in the archegonia. Fertilization occurs when a sperm fuses with the egg

by swimming down the archegonia neck canal. A zygote results and later differentiates into a foot which is embedded in the gametophyte, a stalk (seta) and a capsule.

Nostoc

Nostoc is a genus of cyanobacteria belonging to the order *Nostocales* and the family *Nostocaceae*. They can be found in a variety of environmental niches and they form colonies composed of filaments of moniliform cells in a gelatinous sheath (figure 1.3). *Nostoc* are characterised by forming unbranched filaments and producing three kinds of differentiated cells: heterocysts, hormogonia and akinetes.



Figure 1.3: Nostoc filament. The arrow points to a heterocyst within the filament

Heterocysts: These are cells with thick hyaline walls (figure 1.3, arrow). They are derived from ordinary vegetative cells and usually become larger than adjacent cells. They contain chlorophyll *a* and carotenoid pigment and therefore they are usually yellow-green in colour (Fogg *et.al.* 1973). They differentiate as a response due to lack of combined nitrogen in the environment and are the sites of nitrogen fixation. They are similar in shape to the cells which they are derived from and vary in size. They occur singly in an almost equidistant spacing pattern on the filament at a frequency of 3-10% of the total cells.

Hormogonia: These are short pieces of trichome, filamentous outgrowths that detach from the parent filament and move away with a gliding motion and later develop into a separate filament. In *Nostoc* they are formed by direct fragmentation of the trichome and rounding off of the end cells of the fragments (Fogg *et.al.* 1973).

Akinetes: They are spores which have developed from vegetative cells due to nutrient limitation. They are much larger than vegetative cells and range in shape from spherical to oblong. They are thick walled, brown or yellow and sometimes elaborate and sculptured in appearance. The protoplasm has a small amount of photosynthetic pigments but accumulates large quantities of cyanophycin granules that are proteinaceous reserves. They occur singly or in chains and originate next to or remote from the heterocysts. Germination of spores occurs rapidly in favourable conditions. The akinete wall gelatinizes and germination starts within the spore before the cell contents are extruded. It germinates either into a hormogonium or trichome (Fogg *et.al.* 1973).

Symbiosis

Nostoc can be found growing singly or in a symbiotic association. *Nostoc* forms a symbiotic relationship with a wide variety of organisms like fungi, bryophytes, gymnosperm and angiosperms. The *Nostoc* are housed in different structures depending on the host such as root in cycads, leaf in *Azolla* (fern), stem in *Gunnera* (angiosperm) and thallus in bryophytes and lichens. In lichens, as the fungal host is heterotrophic, it needs to obtain photosynthate as well as nitrogen from the *Nostoc*. The symbiont produces glucose, and other simple sugars, polysaccharides, nitrogenous products, thiamine, riboflavin, nicotinic acid, pantothenic acid and biotin which can be utilized by the host. Although the host provides protection from environmental extremes it is thought that this is not extremely beneficial to the *Nostoc* as they can survive extreme harsh conditions living singly. It is thought that in addition to the protection the symbiotic states allows for a favourable environment for cellular metabolism of the *Nostoc*. It has been shown that nitrogen fixing algae prefer conditions of low oxygen tension for nitrogen fixation and that high pO_2 levels inhibit activity. In the lichen thalli respiration by the mycobiont could prevent the accumulation of high oxygen level which could explain the high rates of nitrogen fixation and possibly other metabolic processes shown by the phycobiont in the symbiotic state (Fogg *et.al.* 1973). It has been shown that only one *Nostoc* type colonizes a thallus and there is a high degree of specificity. In addition, one *Nostoc* strain has been shown to colonize a lichen community from the same and different locations (Paulsrud & Lindblad, 1998).

In mosses, nitrogen fixing cyanobacteria especially *Nostoc* are hosted by several species of feather mosses like *Hylocomium splendens*, *Pleurozium schreberi*, *Ptilium cristaceum*, and *Sphagnum caprifolium* etc (Ininbergs *et.al.* 2011). The feather mosses are common mosses found mainly in boreal forests. The *Nostoc* occurs in mucilage-filled cavities on the undersurface or on the thallus surfaces of the mosses. Nitrogen fixation by the cyanobacterial associations with the mosses is very significant to the global N cycle as boreal forests occupy 11% of Earth's terrestrial forests. *Nostoc* fixes N_2 at a low temperature with temperature optima of 13°C (Gentili *et.al.* 2005). They have a very high degree of host specificity and it has been shown that two different mosses from the same site have different *Nostoc* cyanobionts (Zackrisson *et.al.* 2009).

Aims

1. To culture and identify *Nostoc* and other cyanobacterial symbionts in lichens and mosses.
2. To determine if they are of related or similar strains.

Materials and methods

Sample collection

Samples were collected from different locations as shown in table 2.1.

Table 2.1: List of lichen and moss samples collected and their locations

ID number	Identification name	Location
Lichens		
165	<i>Peltigera cf. islandica</i>	Öskjuhlíð, Reykavík
166	<i>Peltigera membranacea</i>	Öskjuhlíð, Reykavík
167	<i>Peltigera neckeri*</i>	Öskjuhlíð, Reykavík
168	<i>Peltigera canina*</i>	Öskjuhlíð, Reykavík
170	<i>Peltigera monticola</i>	Öskjuhlíð, Reykavík
171	<i>Stereocaulon cf. vesuvianum</i>	Öskjuhlíð, Reykavík
172	<i>Stereocaulon cf. vesuvianum</i>	Öskjuhlíð, Reykavík
173	<i>Peltigera monticola *</i>	Stöng, Þjórásdalur
174	<i>Peltigera canina*</i>	Stöng, Þjórásdalur
183	<i>Stereocaulon cf. vesuvianum</i>	Höfðabakki, Reykavík
184	<i>Stereocaulon cf. vesuvianum*</i>	Höfðabakki, Reykavík
Mosses		
1	<i>Rhytidadelphus squarrorus</i>	Öskjuhlíð, Reykavík
2	<i>Rhytidadelphus squarrorus</i>	Öskjuhlíð, Reykavík
3	<i>Rhytidadelphus squarrorus</i>	Öskjuhlíð, Reykavík
4	<i>Hylocomium splendens</i>	Öskjuhlíð, Reykavík
5	<i>Racomitrium lanuginosum</i>	Höfðabakki, Reykavík
6	<i>Rhytidadelphus triquetrus</i>	Höfðabakki, Reykavík
7	<i>Racomitrium canescens</i>	Höfðabakki, Reykavík
8	<i>Racomitrium canescens</i>	Höfðabakki, Reykavík
9	<i>Rhytidadelphus squarrorus</i>	Höfðabakki, Reykavík
10	<i>Hylocomium splendens</i>	Höfðabakki, Reykavík

*See ITS identification for confirmation

After collection, samples were taken back to the lab for cleaning and processing.

Sample Processing

Growing *Nostoc* colonies from samples (lichen and moss)

1. Homogenize the sample using a pellet pestle. For moss, the moss plant was divided into 3 parts: tip (tip of the moss), mid (stalk of the moss) and end (root of the moss), before they were homogenized.
2. Plate the homogenized sample using quadrant streaking on BG-11 agar.
3. Incubate inverted under light and monitor growth.
4. After some growth, identify colonies that are *Nostoc*-like and plate them on BG-11₀ agar (only *Nostoc* and other nitrogen fixing cyanobacteria will grow on the BG-11₀ agar). [See appendix A on how the plates are made].

DNA isolation from samples

The genomic DNA isolation method for lichen thallus was modified from Sinneman *et al.* (2000) [see Appendix A].

The CTAB method of extraction from plant tissues modified from Aldrich & Cullis (1993) was used to extract DNA from the whole moss (from tip to root) [see Appendix A].

PCR and sequencing analysis

All PCRs was done using the recipe for both DNA and colony isolates:

	1X (mL)	Final
10X Thermo pol buffer	2,5	1X
25 mM MgSO ₄	2,5	2,5 mM
dNTP (2,5 mM each)	2,5	0,25 mM each
F primer	0,5	0,2 µM
R primer	0,5	0,2 µM
Template (DNA/Colony)	2,5	
Taq polymerase	0,2	5,0 unit/mL
M.Q H ₂ O	13,8	
Total	25	

PCRs were repeated again with a higher concentration of MgSO₄. Primers used in the PCR are listed in table 2.2.

Table 2.2: List of primers used for the PCR

Locus	Primer Name	Primer sequence
ITS (fungal)	ITS1	TCCGTAGGTGAACCTGCGG (White <i>et al.</i> 1990)
ITS (fungal)	ITS4	TCCTCCGCTTATTGATATGC (White <i>et al.</i> 1990)
16S rRNA	9F	GAGTTTGATCCTGGCTCAG (Turner <i>et al.</i> 1999)
16S rRNA	R515d	GTATTACCGCGGCTGCTGGCAC (Lichen Genome Project. Unpublished)
16S rRNA	F850	AAACTCAAAGGAATTGACGG (Lichen Genome Project. Unpublished)
16S rRNA	Cya1444R	AAAGGAGGTGATCCAGCCAC (Lichen Genome Project. Unpublished)
rbcL (cyanobacterial)	CW	CGTAGCTTCCGGTGGTATCCACGT (Rudi <i>et al.</i> 1998)
rbcL (cyanobacterial)	CX	GGGGCAGGTAAGAAAGGGTTTCGTA (Rudi <i>et al.</i> 1998)
rbcL (cyanobacterial)	RbcLX F	CACACCCGATTACACACC (Lichen Genome Project. Unpublished)
rbcL (cyanobacterial)	RbcLX R	TCACCCATCACAAACCAAA (Lichen Genome Project. Unpublished)

PCR thermocycle:
 94°C – 5 min/2 min
 94°C – 20 sec
 55°C – 20 sec } x33
 72°C – 45 sec
 72°C – 10 min
 12°C - ∞

DNA sequencing

The exo sap and sequencing reaction was done using the recipe:

Exo Sap materials	1X (µL)
ddH ₂ O	3,7
Antarctic Phosphatase buffer	1
Antarctic phosphatase 0,2*5 U/µL = 1 U	0,2
Exo I 0,1*20 U/µL = 2 U	0,1
PCR product	5
Total	10

Exo SAP cycle
 38°C – 35 min
 80°C – 20 min
 12°C - ∞

Sequencing Reaction Materials	1X (µL)
ddH ₂ O	5,25
5X sequencing buffer	2,75
Big Dye(v3.1)	0,5
Primer (1,6 pmol/µL)	1,5
Exo SAP treated PCR product	5
Total	15

Sequencing cycle
 96°C – 10 sec
 96°C – 10 sec
 55°C – 5 sec } x24
 60°C – 2 min
 12°C – 7 min
 12°C - ∞

Precipitation protocol

After the sequencing reaction, precipitation of the samples was done using the protocol below:

For 24 samples

Material	Quantity (μ L)
Nuclease free water	1125
3M CH ₃ COONa	125
Glycogen	6,25
Total	1256,25

- Mix together 50 μ L of precipitation mix and 15 μ L of each sequencing reaction product sample. Then add 125 μ L of 96% EtOH (ice cold)
- Spin at 10500 rpm for 15 min at 4°C
- Take the liquid off with suction.
- Wash with 250 μ L of 70% EtOH. Spin at 10500 rpm for 5-6 min at 4°C. Take the liquid off with suction. Repeat this step.
- Air dry the samples in a dark place until dry. (Can be stored at 4°C)
- Add 12 μ L of Hi-Di (formamide) to the samples.
- Vortex, heat the samples at 92°C for 3 mins. Place on ice. Briefly spin, load 10 μ L of the sample into the sequencing tray.

Sequencing results analysis

After precipitation and loading of samples into the sequencing tray, the samples were analysed using the ABI3500XL Genetic Analyzer.

The CLC Genomics Workbench computer program was used to evaluate which sequences are of good quality and could be useful for further analyses. Sequences were aligned to check for polymorphisms and errors in the sequence results. A phylogenetic tree was made with the aligned sequences to determine their genetic distances. The aligned sequences were further analysed using the BLAST at <http://blast.ncbi.nlm.nih.gov> to determine what known species are closest to the samples. From the BLAST results the closest named sequences was also aligned with the samples and a phylogenetic tree made to determine the genetic distance of the samples to its closest relative.

Cryopreservation of *Nostoc* in DMSO (see Appendix).

All cultured *Nostoc* samples where cryopreserved and stored in -150°C freezer.

Results

Morphology of lichens

Sample 165: A foliose lichen with broad and very bright green thalli. The lower cortex surface is whitish with simple rhizines.

Sample 166: A foliose lichen with broad, long and greyish green thalli. The lower cortex surface is whitish with brown vein with simple rhizines.

Sample 167: A foliose lichen with wide, long and bluish green thalli. The lower cortex is whitish towards the margin and has rhizines which are dichotomous.

Sample 168: A foliose lichen with wide, long and greyish green thalli. The lower cortex is whitish at the margins and brownish towards the centre. Has rhizines which are whitish near the margins and darker towards the centre.

Sample 170: A foliose lichen with small lobe like thalli that are dark green in colour. The margins are upturned and pruinose. Has pale rhizines on the lower cortex. Has apothecia which are reddish brown in colour.

Sample 171: A fruticose lichen with round, branched and whitish green branched thalli. Has basal rhizoidal strands from the cortex.

Sample 172: A fruticose lichen with round, branched and whitish green branched thalli. Has pseudopodetia rising from the thallus. Has basal rhizoidal strands from the cortex.

Sample 173: A foliose lichen with small lobe like thalli that are dark green in colour. The margins are upturned and pruinose. Has pale rhizines on the lower cortex. Has apothecia which are reddish brown in colour.

Sample 174: A foliose lichen with wide, long and bright green thalli. The lower cortex is whitish at the margins and brownish towards the centre. Has rhizines which are whitish near the margins and darker towards the centre.

Sample 183: A fruticose lichen with round, branched and whitish green branched thalli. Has pseudopodetia rising from the thallus. Has basal rhizoidal strands from the cortex.

Sample 184: A fruticose lichen with round, branched and whitish green branched thalli. Has pseudopodetia rising from the thallus. Has basal rhizoidal strands from the cortex.

Microscopic analysis of colonies from lichens and mosses

The colony isolates from the *Peltigera* lichens grew very well on the BG-11o media and *Nostoc* was isolated (see appendix D, figures 1 – 5). The *Stereocaulon* isolates did not grow very well on the BG-11o media and no *Nostoc* was isolated from them. Other unknown cyanobacteria and green algae were cultured from all *Stereocaulon* isolates (see appendix D, figures 6 – 8). The colony isolates from the mosses were isolated from the whole of the

moss for 2 samples and different parts of the mosses for the rest: tip of the moss, stalk of the moss and root of the moss. The isolates did not grow well on the BG-11o media. *Nostoc* was only isolated from moss 5 (*Racomitrium lanuginosum*), other unknown cyanobacteria and green algae were cultured from the other moss isolates (table 3.1) [Also see appendix D, figures 9 – 17 for some pictures of the isolates from mosses].

Table 3.1: Table showing the observed colonies from mosses cultured on BG-11_o media.

Sample Id	Name	Colonies											
		Whole of moss (all)		Tip of moss (tip)			Stalk of moss (mid)			Root of moss (end)			
		1	2	1	2	3	1	2	3	1	2	3	
1	<i>Rhytidadelphus squarrorus</i>	x	x	C	C/A	C	A	A	A	C	-	C	
2	<i>Rhytidadelphus squarrorus</i>	x	x	C	C	x	C	A	x	-	C	x	
3	<i>Rhytidadelphus squarrorus</i>	x	x	C/A	-	x	C	A	x	C/A	A	x	
4	<i>Hylocomium splendens</i>	x	x	A	C/A	x	A	A	x	C	A	x	
5	<i>Racomitrium lanuginosum</i>	x	x	A	A	x	x	x	x	N	A	x	
6	<i>Rhytidadelphus triquetrus</i>	x	x	C/A	C	x	C	A	x	C/A	A	x	
7	<i>Racomitrium canescens</i>	C/A	-	x	x	x	x	x	x	x	x	x	
8	<i>Racomitrium canescens</i>	C	C	x	x	x	x	x	x	x	x	x	
9	<i>Rhytidadelphus squarrorus</i>	x	x	C	C	X	A	x	x	C	-	x	
10	<i>Hylocomium splendens</i>	x	x	A	-	x	x	A	x	A	A	x	

C – Cyanobacteria (not *Nostoc*); A – algae; C/A – both cyanobacteria and algae N – *Nostoc*; - no growth; x – not cultured

PCR results

The PCR with the ITS primers gave very good results with all the DNA isolates from the samples it was used with (table 3.2). The PCR with the rbcL primers show very good results with the DNA and colony isolates from the *Peltigera* species (table 3.2 and 3.3). With the 16S rDNA primers the PCR results were also best with the DNA isolates from the *Peltigera* species and good with some of the DNA and colony isolates from the *Stereocaulon* species (table 3.2 and 3.3). [See appendix B for all gel pictures]

Table 3.2: Table showing PCR results from lichen DNA isolates

Sample id	Lichen	Primers				
		rbcLF & R	CX/C W	F850/CyaR 1444	F9/515 R	ITS
165	<i>P. cf. islandica</i>	+++	++	+++	x	++
166	<i>P. membranacea</i>	+++	+++	+++	+++	x
167	<i>P. neckeri</i>	+++	+	+++	x	+
168	<i>P. canina</i>	+++	+++	+++	x	++
170	<i>P. monticola</i>	++	++	-	x	+++
171	<i>Stereocaulon cf. vesuvianum</i>	-	-	+	+	x
172a	<i>Stereocaulon cf. vesuvianum</i>	-	-	+	+++	+++
172b	<i>Stereocaulon cf. vesuvianum</i>	-	-	-	-	x
173	<i>P. monticola</i>	++	++	+++	x	++
174	<i>P. canina</i>	++	+	++	x	++
183	<i>Stereocaulon cf. vesuvianum</i>	-	-	-	-	x
184	<i>Stereocaulon cf. vesuvianum</i>	-	-	+	++	++

+++ very strong band; ++ good band; + faint band; - no band; x PCR not done

Table 3.3: Table showing PCR results from lichen colony cultures using CX/CW primers

Sample id	Lichen	PCR - CX/CW				
		Colony 1	Colony 2	Colony 3	Colony 4	Colony 5
165	<i>P. cf. islandica</i>	+++	+++	-	x	x
166	<i>P. membranacea</i>	++	++	++	-	+
167	<i>P. neckeri</i>	+	+++	+++	+	-
168	<i>P. canina</i>	-	+++	+	+++	x
170	<i>P. monticola</i>	x	x	x	x	x
171	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	x	x
172a	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	x	x
172b	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	x	x
173	<i>P. monticola</i>	-	-	+	x	x
174	<i>P. canina</i>	-	-	-	+	-
183	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	x	x
184	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	x	x

+++ very strong band; ++ good band; + faint band; - no band; x PCR not done

Table 3.4: Table showing PCR results from lichen colony cultures using F850/CyaR1444 primers

Sample id	Lichen	F850/CyaR1444			
		Colony 1	Colony 2	Colony 3	Colony 4
165	<i>P. cf. islandica</i>	x	x	x	x
166	<i>P. membranacea</i>	x	x	x	x
167	<i>P. neckeri</i>	x	x	x	x
168	<i>P. canina</i>	x	x	x	x
170	<i>P. monticola</i>	x	x	x	x
171	<i>Stereocaulon cf. vesuvianum</i>	++	x	x	x
172a	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	x
172b	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	x
173	<i>P. monticola</i>	x	x	x	x
174	<i>P. canina</i>	x	x	x	x
183	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	x
184	<i>Stereocaulon cf. vesuvianum</i>	x	x	x	+

+++ very strong band; ++ good band; + faint band; - no band; x PCR not done

PCR with the rbcL primers showed no results with the DNA isolates from the mosses, but the 16S rDNA primers showed good results using on the DNA and colony isolates from the mosses (Table 3.5 and 3.6).

Table 3.5: Table showing PCR results from moss DNA isolates

Sample id	Moss	PCR			
		rbcLF & R	CX/CW	F850/Cyar1444	F9/515R
1	<i>Rhytidadelphus squarrorus</i>	-	-	++	+
2	<i>Rhytidadelphus squarrorus</i>	-	-	-	++
3	<i>Rhytidadelphus squarrorus</i>	-	-	+	+
4	<i>Hylocomium splendens</i>	-	-	-	++
5	<i>Racomitrium lanuginosum</i>	x	x	-	+
6	<i>Rhytidadelphus triquetrus</i>	x	x	++	+
7	<i>Racomitrium canescens</i>	x	x	++	+
8	<i>Racomitrium canescens</i>	x	x	+	+
9	<i>Rhytidadelphus squarrorus</i>	x	x	+	+
10	<i>Hylocomium splendens</i>	x	x	++	+

+++ very strong band; ++ good band; + faint band; - no band; x PCR not done

Table 3.6: Table showing PCR results from moss colony cultures using F850/CyaR1444 primers

Sample id	Moss	F850/Cyar1444					
		Tip 1	Tip 3	Mid 1	Mid 2	End 1	End 3
1	<i>Rhytidadelphus squarrosus</i>	++	++	x	x	-	++
2	<i>Rhytidadelphus squarrosus</i>	x	x	x	x	x	x
3	<i>Rhytidadelphus squarrosus</i>	x	x	x	x	x	x
4	<i>Hylocomium splendens</i>	x	x	x	x	x	x
5	<i>Racomitrium lanuginosum</i>	x	x	x	x	x	x
6	<i>Rhytidadelphus triquetrus</i>	x	x	x	x	x	x
7	<i>Racomitrium canescens</i>	x	x	x	x	x	x
8	<i>Racomitrium canescens</i>	x	x	x	x	x	x
9	<i>Rhytidadelphus squarrosus</i>	x	x	x	x	x	x
10	<i>Hylocomium splendens</i>	x	x	x	x	x	x

+++ very strong band; ++ good band; + faint band; - no band; x PCR not done

Sequence analysis

The BLAST results of the lichen samples with the ITS primers identified all the samples with 100% identity and 0% gaps (Table 3.7). [See appendix C for all BLAST results]

Table 3.7: Top hits to the ITS sequences for lichen DNAs

Sample Id	Primer	Description	Length	Score	Expect	Identities	Gaps	Strand
167	ITS1	<i>Peltigera neckeri</i> strain HOB040605-12-4	642	606 bits (328)	3e-170	328/328 (100%)	0/328 (0%)	Plus/Plus
168	ITS1	<i>Peltigera canina</i> strain UK133	528	592 bits (320)	7e-166	320/320 (100%)	0/320 (0%)	Plus/Plus
173	ITS4	<i>Peltigera rufescens</i>	510	603 bits (326)	3e-169	326/326 (100%)	0/326 (0%)	Plus/Minus
174	ITS1	<i>Peltigera canina</i> strain UK133	528	592 bits (320)	7e-166	320/320 (100%)	0/320 (0%)	Plus/Plus
184	ITS4	<i>Astrochloris photobiont</i> ITS	514	628 bits (340)	6e-177	340/340 (100%)	0/340 (0%)	Plus/Minus

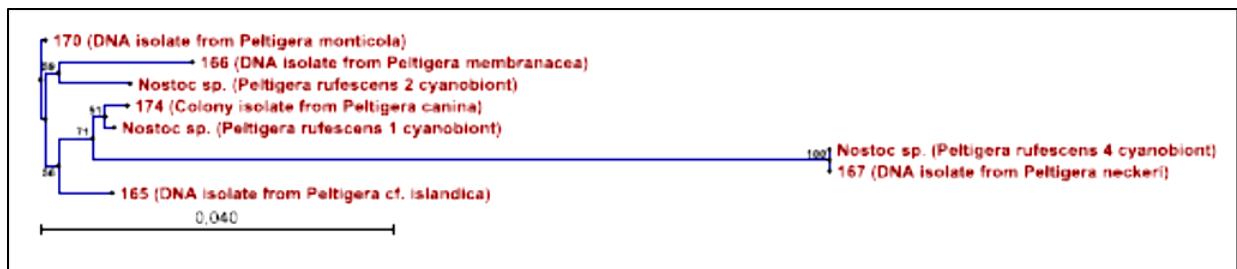
The BLAST results on the samples sequenced with the CX primer identified the samples to all contain *Nostoc* with 98-100 % identities and 0% gaps (Table 3.8). Figures 3.1 and 3.2 show the genetic distances between the *Nostoc* species identified in the samples and also their genetic distance to their closest genetic relative obtained from the BLAST results respectively.

Table 3.8: Summary of BLAST results for the sequence analysis with the CX primer

Sample Id	Isolate type	Description	Length	Score	Expect	Identities	Gaps	Strand
165	DNA	<i>Nostoc sp. 'Peltigera rufescens 1 cyanobiont'</i>	826	1062 bits (575)	0.0	583/587 (99%)	0/587 (0%)	Plus/Minus
166	DNA	<i>Nostoc sp. 'Peltigera rufescens 2 cyanobiont'</i>	824	1251 bits (677)	0.0	712/729 (98%)	2/729 (0%)	Plus/Minus
167	DNA	<i>Nostoc sp. 'Peltigera rufescens 4 cyanobiont'</i>	872	1434 bits (776)	0.0	776/776 (100%)	0/776 (0%)	Plus/Minus
170	DNA	<i>Nostoc sp. 'Peltigera rufescens 1 cyanobiont'</i>	826	1291 bits (699)	0.0	721/731 (99%)	3/731 (0%)	Plus/Minus
174	Colony	<i>Nostoc sp. 'Peltigera rufescens 1 cyanobiont'</i>	826	1332 bits (721)	0.0	728/731 (99%)	1/731 (0%)	Plus/Minus



*Figure 3.1: Phylogenetic tree of *Nostoc* species isolated from the *Peltigera* species which were sequenced with CX primer*



*Figure 3.2: Phylogenetic tree of *Nostoc* species isolated from the *Peltigera* species which were sequenced with the CX primer and their closest genetic relative identified from the BLAST results*

The BLAST results on the samples sequenced with the F850 primer identified the samples to contain either proteobacteria or cyanobacteria with 99-100 % identities and 0% gaps (Table 3.9). Figures 3.3 and 3.4 shows the genetic distances between the bacterium/cyanobacterium species identified in the samples and also the genetic distance with their closest genetic relative obtained from the BLAST results respectively.

Table 3.9: Summary of BLAST results for the sequence analysis with the F850 primer

Sample Id	Isolate type	Description	Length	Score	Expect	Identities	Gaps	Strand
171	DNA	<i>Curvibacter sp.</i> S2H46	973	569 bits (308)	8e-160	308/308 (100%)	0/308 (0%)	Plus/Plus
172	DNA	Uncultured bacterium clone Bas-7-83	1331	584 bits (316)	3e-164	318/319 (99%)	0/319 (0%)	Plus/Plus
184	Colony	<i>Sphingomonas sp.</i> S6-259	1427	571 bits (309)	2e-160	313/315 (99%)	0/315 (0%)	Plus/Plus
1	Colony	<i>Agrobacterium sp.</i> BE516	1451	721 bits (390)	0.0,	390/390 (100%)	0/390 (0%)	Plus/Plus
3	DNA	Uncultured <i>Chroococcidiopsis sp.</i> clone QB23	1319	569 bits (308)	8e-160	308/308 (100%)	0/308 (0%)	Plus/Plus
6	DNA	Uncultured <i>Chroococcidiopsis sp.</i> clone QB23	1319,	569 bits (308)	8e-160	308/308 (100%)	0/308 (0%)	Plus/Plus
7	DNA	Uncultured <i>Chroococcidiopsis sp.</i> clone QB23	1319,	569 bits (308)	8e-160	308/308 (100%)	0/308 (0%)	Plus/Plus
8	DNA	Uncultured <i>Chroococcidiopsis sp.</i> clone QB23	1319	571 bits (309)	2e-160	309/309 (100%)	0/309 (0%)	Plus/Plus
9	DNA	Uncultured cyanobacterium clone OCLS087	876,	470 bits (254)	7e-130	254/254 (100%)	0/254 (0%)	Plus/Plus

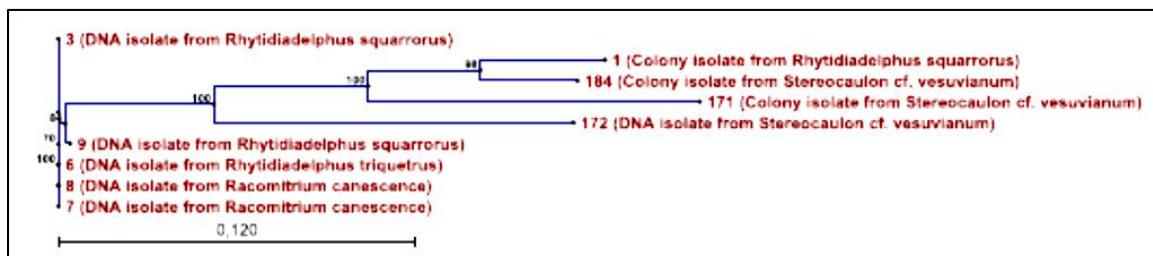
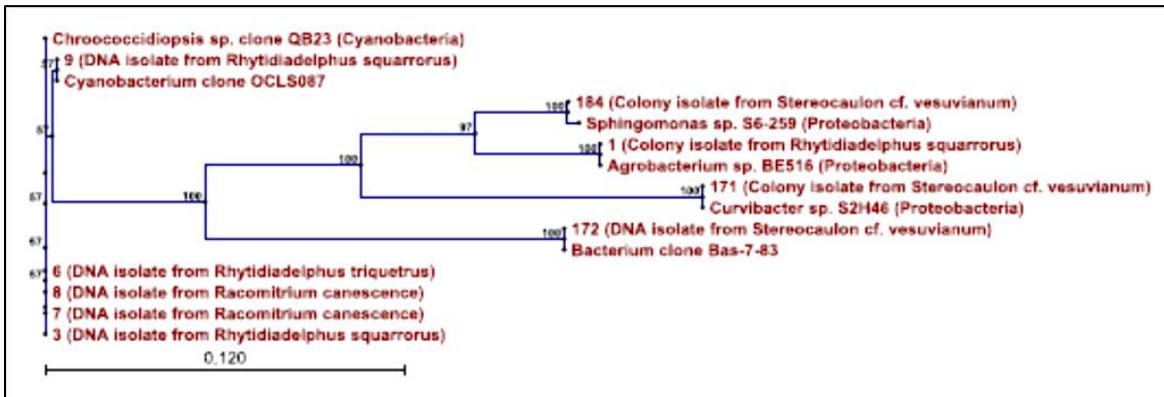


Figure 3.3: Phylogenetic tree of bacteria/cyanobacteria species isolated from the *Stereocaulon* lichens and moss species which were sequenced with F850 primer



*Figure 3.4: Phylogenetic tree of bacteria/cyanobacteria species isolated from the *Stereocaulon* lichens and moss species which were sequenced with the F850 primer and their closest genetic relative identified from the BLAST results*

Discussion

The lichens

From the morphological structure and the ITS sequencing analysis, it was identified that the lichens collected includes *Peltigera cf. islandica* (sample 165), however this is a new species of lichen that has recently been discovered in Iceland and it has not yet been identified and recorded as a new species (figure 4.1a and b). The other samples include *Peltigera membranacea* (166), which was identified from its morphological structure alone (figure 4.1a). *Peltigera neckeri* (167) identified from its morphological structure (figure 4.1c) and confirmed using the ITS sequencing analysis. *Peltigera canina* (168 and 174) identified from its morphological structure and confirmed using the ITS sequencing analysis. *Peltigera monticola* (170) identified from its morphological structure (figure 4.1d). Sample 173 was originally identified as *P. monticola* however using the ITS sequencing analysis it was identified as *Peltigera rufescens*. Samples 171, 172, 183 and 184 were identified by their morphological structure to be *Stereocaulon vesuvianum* and sample 184 using the ITS sequencing analysis showed the presence of *Astrochloris photobiont*, a photobiont which has been found to associate with the *Stereocaulon* genus (Peska & Skaloud. 2011)

The moss samples were identified from its morphological structures alone to be *Rhytidadelphus squarrosus* (samples 1, 2, 3 and 9), *Hylocomium splendens* (samples 4 and 10), *Racomitrium lanuginosum* (5), *Rhytidadelphus triquetrus* (sample 6), and *Racomitrium canescens* (samples 7 and 8) (figure 4.1e – i).

***Nostoc* and cyanobacteria cultures**

Nostoc was only successfully cultured from the *Peltigera* lichens. However, only cyanobacteria and no *Nostoc* were cultured from the *Stereocaulon* lichens and mosses except from one moss species *Racomitrium lanuginosum* (moss 5). It is thought that during the cleaning process of the *Stereocaulon* lichens and moss samples, the *Nostoc* symbionts were washed away.

Cyanobacteria associations

The PCR results on the DNA and colony isolates from the *Peltigera* lichens revealed that *Nostoc* is associated with the lichens. However, the sequencing analysis with the CX primer was not successful on all DNA and colony isolates. Out of all *Peltigera* isolates only 5 isolates in total (both DNA and colony) from *P. islandica*, *P. membranacea*, *P. neckeri*, *P. monticola*, and *P. canina* were of good quality and were of use for further sequencing analysis. The BLAST analysis on the *Peltigera* lichens confirmed that they are associated with the *Nostoc*. The *Nostoc* isolated from *P. islandica*, *P. monticola*, and *P. canina* all share similar genetic information as the *Nostoc* from *P. rufescens* cyanobiont 1. From the phylogenetic tree we see that they are genetically related to each other (figure 3.1), however the *Nostoc* isolated from *P. canina* is more closely related to the *Nostoc*

from *P. rufescens* cyanobiont 1 compared to the other 2 (figure 3.2). The *Nostoc* isolated from *P. membranacea* is more genetically related to *Nostoc* from *P. rufescens* cyanobiont 2 (figure 3.2). The *Nostoc* isolated from *P. neckeri* is a more distant relative from the other



Figure 4.1: Pictures of lichens a) P. membranacea & P. islandica, b) P. islandica, c) P. neckeri, d) P. monticola, and moss e) Rhytidiodelphus squarrosus, f) Hylocomium splendens, g) Racomitrium lanuginosum, h) Rhytidiodelphus triquetrus, and i) Racomitrium canescens.

Nostoc isolates (figure 3.1). This is due to a large intron inserted within its rbcLX sequence (see appendix C, figure 1) and its BLAST results show that it has greatest homology to *Nostoc* from *P. rufescens* cyanobiont 4 (figure 3.2). Sequencing analysis with the CW

primer resulted in only isolates from 3 of the *Peltigera* species being of good quality, however they were not used for further sequencing analysis.

The PCR with the rbcLX primers on the DNA and colony isolates from the *Stereocaulon* lichens and mosses showed no results but showed good results with 16S rDNA primers. This shows that some cyanobacteria are associated with them however not directly confirmed to be *Nostoc*. Sequencing analysis of the F850-CyaR1444 PCR products with only the F850 primer was done on the *Stereocaulon* lichens and mosses that showed clear PCR bands, however only 9 isolates in total from 3 *Stereocaulon* lichens and 6 moss species showed sequences of good quality. The phylogenetic tree shows the DNA/colony isolates from the *Stereocaulon* lichens to be closely genetically related to each other along with one of the mosses (figure 3.3), however the BLAST results revealed them to be genetically close to *Curvibacter* (171), a bacteria (172), *Sphingomonas* (184) and *Agrobacterium* (moss 1) (figure 3.4). The phylogenetic tree shows the isolates from the rest of the mosses to be more genetically related with each other and the BLAST results revealed them to be genetically related to *Chroococcidiopsis* (mosses 3, 6, 7 and 8) and a cyanobacteria (moss 9) (figure 3.4).

Although no *Nostoc* was identified from the *Stereocaulon* lichens and mosses, the different species of cyanobacteria observed in the cultures and identified from the sequencing analysis is of great interest. Further analysis with other primers like the tRNA^{Leu} (UAA) group I intron, *trnL* primers should identify the different species or strains of these cyanobacteria. The group I intron is conserved in the tRNA^{Leu} (UAA) gene located at a very specific insertion site and highly conserved across the cyanobacteria (Brien *et.al.* 2005).

Conclusion

In conclusion, the cyanolichens are associated with the *Nostoc* species and they were successfully cultured and identified. They were also determined to be of related or similar strains to each other. The *Stereocaulons* lichens are associated with mostly a bacteria and some other cyanobacteria symbiont. However, although they were cultured they were not positively identified or determined to be of related or similar strains to each other. Further research is needed probably with different primers, to positively identify these cyanobacterial symbionts and to determine their phylogeny. The mosses are associated with bacteria and cyanobacteria except one moss where *Nostoc* was found. The cyanobacteria, although determined to be genetically related, they were not positively identified and further research is needed to positively identify them.

We could utilize these to further understand the host specialization of cyanobacteria especially *Nostoc* within lichens and mosses. Furthermore, to better understand the symbiotic relationship between the host species and the symbiont.

References

- Brien, H.E., Miadlikowska, J. and Lutzoni, F. 2005. *Assessing host specialization in symbiotic cyanobacteria associated with four closely related species of lichen fungus Peltigera*. European Journal of Phycology. **60**: 310-315.
- Fogg, G.E., Stewart, W.D.P., Fay, P. and Walsby, A.E. 1973. *The blue-green algae*. Academic press Inc. Ltd. London.
- Gentili, F., Nilsson, M., Zackrisson, O., DeLuca, T.H., and Sellstedt, A. 2005. *Physiological and molecular diversity of feather moss associative N₂-fixing cyanobacteria*. Journal of Experimental Botany. Vol 56, No. 422: 3121-3127.
- Hale, M.E. 1974. *The biology of lichens*. 2nd edition. Edward Arnold (publishers) Ltd. London.
- Ininbergs, K., Bay, G., Rasmussen, U., Wardle, D.A., and Nilsson, M. 2011. *Composition and diversity of nifH genes of nitrogen-fixing cyanobacteria associated with boreal forest feather mosses*. New Phytologist. **192**: 507-517.
- Paulsrud, P. and Lindblad, P. 1998. *Sequence variation of the tRNA^{Leu} intron as a marker for genetic diversity and specificity of symbiotic cyanobacteria in some lichens*. Applied and Environment Microbiology. **64**: 310-315.
- Peska, O. and Skaloud, P. 2011. *Do photobionts influence the ecology of lichens? A case study of environmental preferences in symbiotic green alga Asterochloris (Trebouxiophyceae)*. Molecular Ecology. **20**: 3936-3948.
- Raven, P.H., Evert, R.F. and Eichhorn, S.E. 1999. *Biology of plants*. 6th edition. W.H. Freeman and company. New York.
- Rudi, K., Skulberg, O.M. and Jakobsen, K.S. 1998. *Evolution of cyanobacteria by exchange of genetic material among phyletically related strains*. Journal of Bacteriology. 3453-3461.
- Turner, S., Pryer, K.M., Miao, V.P.W. and Palmer, J.D. 1999. *Investigating deep phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis*. Journal of Eukaryotic Microbiology **46**: 327–338.
- Vitt, D.H., Marsh, J.E. and Bovey, R.B. 1988. *Mosses, lichens and ferns of northwest North America*. Lone Pine publishing. Canada
- White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. *Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics*. In: Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White, T.J., eds. *PCR Protocols: a guide to methods and applications*. Academic Press. New York, USA. 315–322.
- Zackrisson, O., Deluca, T.H., Gentili, F., Sellstedt,A., and Jaderlund, A. 2009. *Nitrogen fixation in mixed Hylocomium splendens moss communities*. Oecologia. **160**:309-319.

Website reference

www.floraislands.is. Visited June 2012

www.ncbi.nlm.nih.gov/pubmed. Visited May 2012

Appendix

Appendix A

Complete protocol procedures.

Procedure for BG-11 medium

Stock solutions for BG-11:

Stock 1:

Na ₂ MG EDTA	0,1g/L
Ferric ammonium citrate	0,6 g/L
Citric acid·1H ₂ O	0,6 g/L
CaCl ₂ ·2H ₂ O	3,6 g/L

Filter sterilize into a sterile bottle or autoclave.

Stock 2:

MgSO ₄ ·7H ₂ O	7,5 g/L
--------------------------------------	---------

Filter sterilize into a sterile bottle or autoclave.

Stock 3:

K ₂ HPO ₄ ·3H ₂ O	4,0 g/L
or K ₂ HPO ₄	3,05 g/L

Filter sterilize into a sterile bottle or autoclave.

Stock 5 (Microelements):

H ₃ BO ₃	2,86 g/L
MnCl ₂ ·4H ₂ O	1,81 g/L
ZnSO ₄ ·4H ₂ O	0,222 g/L
CuSO ₄ ·5H ₂ O	0,079 g/L
COCl ₂ ·6H ₂ O	0,050 g/L
NaMoO ₄ ·2H ₂ O	0,391 g/L
or MoO ₄ (85%)	0,018 g/L

For basic BG-11 medium combine the following stock solutions:

<u>Stock solution</u>	<u>Per litre of medium</u>
Stock 1	10 ml
Stock 2	10 ml
Stock 3	10 ml
Na ₂ CO ₃	0,02 g
Stock 5	1,0 ml
NaNO ₃	1,5 g

Combine stocks and chemicals except stock 5 and adjust pH to 7,5 (use 1,0 M HCl).

Aliquot into flasks (50 ml/125 ml flask) with cotton stoppers on top and autoclave. After autoclaving and cooling the pH is about 7,1. Add stock 5 after autoclaving. For solid media add 1% noble agar or Difco Bacto-Agar. For BG-11₀ do not add NaNO₃.

Genomic DNA isolation from lichen sample (whole thallus)

Method modified from Sinneman *et al.* (2000).

1. Collect the samples and process the samples.
2. Set the water bath at 65°C.
3. Grind the sample (2 g) to a powder with liquid nitrogen in a pre-chilled mortar and pestle. (Mortar + liquid nitrogen + add samples, wait till the nitrogen stops churning and then start homogenising).
4. Using pre-chilled metal spatula scrape the powdered tissue to the premeasured 25 ml lysis buffer (temperature of buffer 65°C).
5. Incubate at 65°C for 20 min. (Mix the contents of the tube during the incubation).
6. Centrifuge at 5000 rpm for 15 min or 14000 rpm for 4 mins.
7. Transfer the supernatant to a new tube and add 8,56 ml of 10,5 M ammonium acetate (i.e. 8,56 ml for 20 ml & 545µl for 1,25 ml).
8. Mix the contents and incubate on ice for 20 mins.
9. Centrifuge at 5000 rpm for 15 mins or 4000 rpm for 4 mins.
10. Transfer the supernatant to a new tube, discard the pellet and add 12,8 ml of isopropanol. (i.e. 12,8 ml for 20 ml & 800µl for 1,25 ml).
11. Mix the contents and incubate on ice for 20 mins.
12. Centrifuge at 5000 rpm for 15 mins or 14000 rpm for 4 mins.
13. Discard the supernatant.
14. Add TE buffer and RNase A to the pellet. (1 ml TE + 4 µl of RNase A).
15. Incubate the tubes at 50°C for 20-60 mins till the pellet is dissolved. (Gently tap the tube to dissolve the pellet during incubation).
16. Centrifuge briefly (short spin) and discard the supernatant.
17. Add equal volume of phenol:choloroform:isoamyl alcohol (25:24:1).
18. Vortex briefly.
19. Centrifuge at 500 rpm for 15 mins or 14000 rpm for 4 mins.
20. Transfer the aqueous phase to a fresh tube without disturbing the interphase.
21. Add equal volume of saturated choloroform.
22. Vortex briefly.
23. Centrifuge at 5000 rpm for 15 mins or 14000 rpm for 4 mins.
24. Transfer the aqueous phase to a fresh tube without disturbing the interphase.
25. Add 1/10th volume of 3 M sodium acetate.
26. Add twice the volume of 100% EtOH.
27. Keep the tubes on ice.
28. Centrifuge at 14000 rpm for 4 mins and discard the supernatant.
29. Add ~500 µl of 70% EtOH.
30. Centrifuge at 14000 rpm for 4 mins.
31. Discard the supernatant.
32. Air dry the pellet.
33. Add 500 µl of TE buffer (to dissolve the pellet keep at 50°C for 2-5mins, gently tap during incubation)
34. Load in 0,6% agarose gel.

DNA Isolation from moss

Method modified from Aldrich & Cullis (1993).

Reagents and solutions: CTAB extraction buffer

Final concentration	Solution/Reagent	For 100 ml	Stock
2% (1%)	CTAB	2 g(1g)	
2% (1%)	PVP (MV 40000)	2 g (1g)	
1,4 M	NaCl	28 mL	5 M
20 mM	ECTA pH 8	4 mL	0,5 M pH 8
100 mM	TrisHCl pH 8	10 mL	1 M pH 8
2% (1%)	2-mercaptoethanol	2 mL (1 mL)	

Chloroform: Isoamyl alcohol – 24:1 v/v

Isopropanol – 100%

WB Wash Buffer – 76% ethanol 38 mL

10 mM ammonium acetate 47,6 µL

Make up volume to 50 mL with d.d H₂O

TE (1X) - 10 mM TrisHCl pH 8

1 mM EDTA pH 8

RNase – 10 mg/mL

5M NaCl – 292,2 g/L

Ethanol – 100% and 70%

Method

1. Prepare extraction buffer and incubate at 65°C.
2. Get 2 mL eppendorf tubes (50 mL tubes) and label.
3. Flash freeze 0,4 g (3–5 g) of fresh leaves in liquid nitrogen and grind to fine powder.
4. Transfer to tubes and add 2 mL (15 mL) if pre-warmed extraction buffer.
5. Mix well and place in 65°C incubator. Agitate every 10mins and keep in incubator for at least an hour. Longer incubation results in more products.
6. Add an equal volume of chloroform:isoamyl alcohol and vortex briefly.
7. Centrifuge at 7000 rpm for 10 mins.
8. Transfer the upper phase to new tubes and add equal volume of chloroform:isoamyl alcohol.
9. Vortex and centrifuge for 10 mins at 7000 rpm.
10. Transfer supernatant to new tubes.
11. Precipitate the DNA with 2X volume of ice cold 96% ethanol. Mix gently by turning upside down several times and then place in fridge (or freezer) for 1hr.
12. Centrifuge at 14000 rpm for 10 mins to get the pellet.
13. Discard the supernatant.
14. Wash twice with wash buffer (WB). Add 400 µL of WB mix carefully. Centrifuge at 14000 rpm for 3-5 mins and remove WB. Repeat washing once more.
15. Let DNA pellet air dry.
16. Add 50–300 µL of TE buffer and let pellet dissolve.

RNase treatment of moss sample

1. Add 1µL of 10 g/mL RNase enzyme per sample and incubate at 37°C for 30 mins.
2. Add 120 µL of 5M NaCl.
3. Add 2X volume of 96% ethanol.
4. Put in freezer for ~30 mins.
5. Centrifuge at 14000 rpm for 10 mins and discard supernatant
6. Keep pellet and wash once with 70% (0,5 mL) cold alcohol. Centrifuge at maximum for 3 mins.
7. Discard supernatant and air dry. Dissolve in 100 µL TE.

Cryopreservation of *Nostoc* in DMSO

1. Grow the desired strain in liquid until a fairly dense, but healthy culture is achieved.
2. Add 5% DMSO in a sterile manner to the cell culture and mix. (9,5 ml of culture + 0,5 ml of DMSO).
3. Place 1,0 ml of the culture into sterile freezing (cryo-) vials.
4. Place tubes in -150°C freezer.
5. To revive a previously frozen culture, simply thaw the tube at room temperature. As soon as the sample is liquid, pipette the cells into growth medium immediately. Place the culture under standard growth conditions.

Appendix B

Pictures of PCR gels

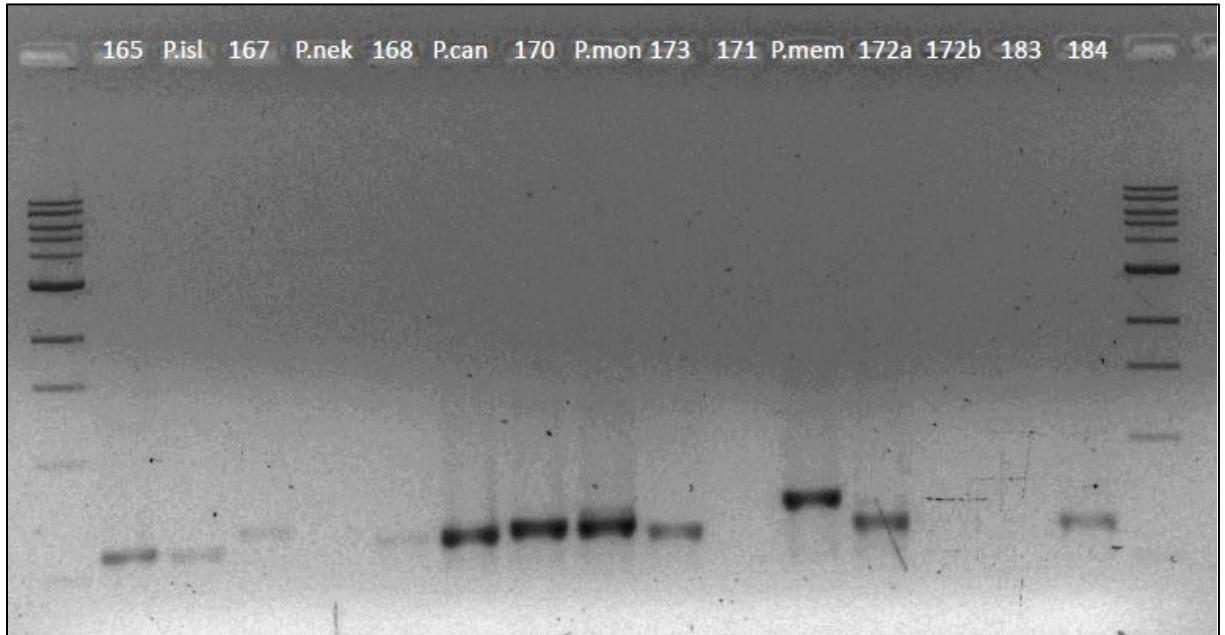


Figure 1: PCR gel with ITS 1 and 4 primers of the DNA isolates from 165 (*P. islandica*), P.isl (positive control for *P. islandica*), 167 (*P. neckeri*), P.nek (positive control for *P. neckeri*), 168 (*P. canini*), P.can (positive control for *P. canini*), 170 (*P. monticola*), P.mon (positive control for *P. monticola*), 173 (*P. rufescens*), 171, 172a, 172b, 183, 184 (*Stereocaulon vesuvianum*), P.mem (*P. membranacea* – positive control for all)

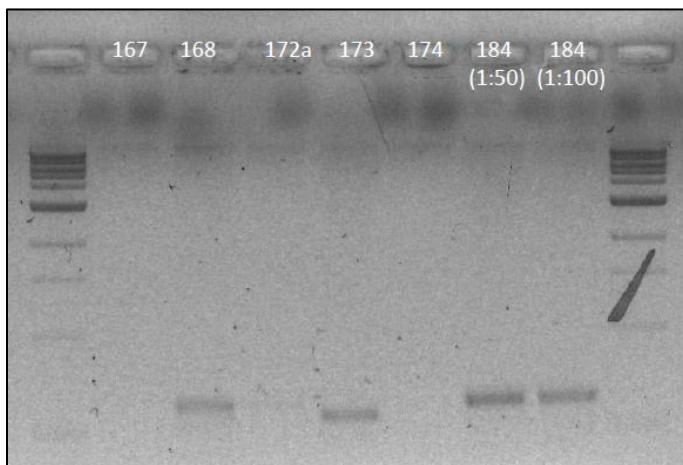


Figure 2: PCR gel with ITS 1 and 4 primers of the DNA isolates from 167 (*P. neckeri*), 168 & 174 (*P. canini*), 172a(*Stereocaulon vesuvianum*), 173(*P. rufescens*), 184 (*Stereocaulon vesuvianum*) in 1:50 and 1:100 dilutions

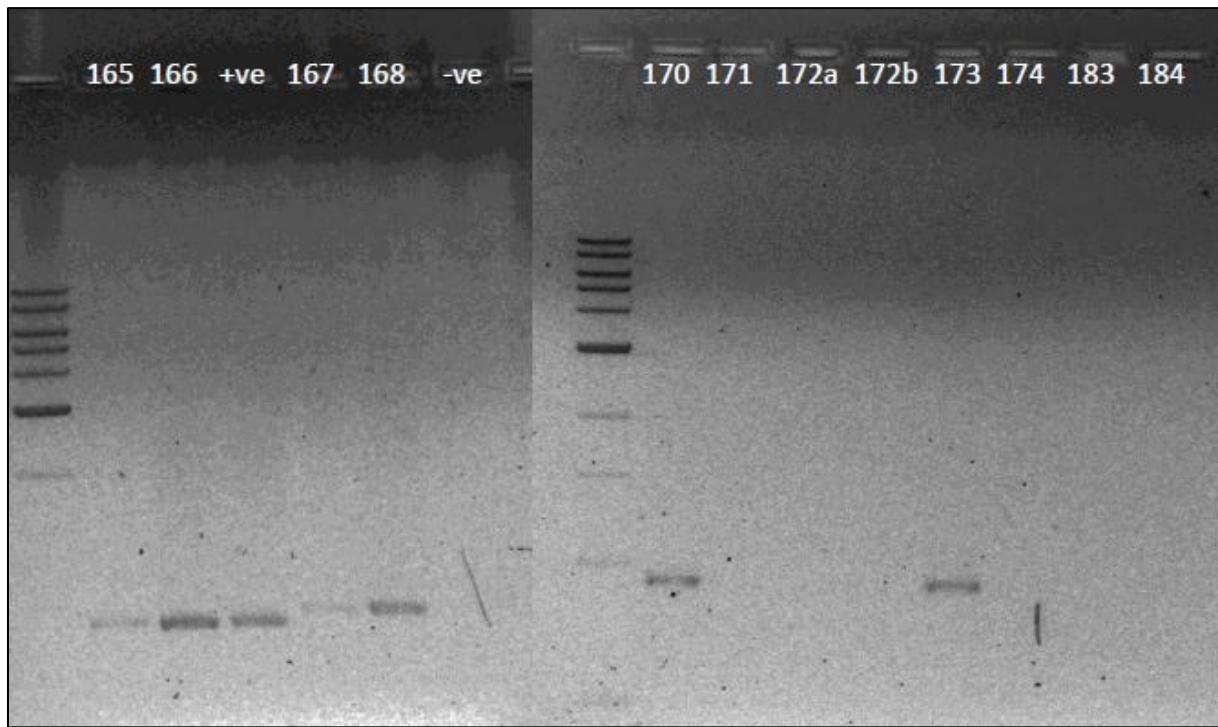


Figure 3: PCR gel with the rbcL (CX/CW) primers of the DNA isolates from 165 (*P. islandica*), 166 (*P. membranaceae*), 167(*P. neckeri*), 168 & 174(*P. canini*), 170(*P. monticola*), 173 (*P. rufescens*), 171, 172a, 172b, 183 & 184 (*Stereocaulon vesuvianum*), +ve (Positive control – *Nostoc* DNA), -ve (negative control – ddH₂O)

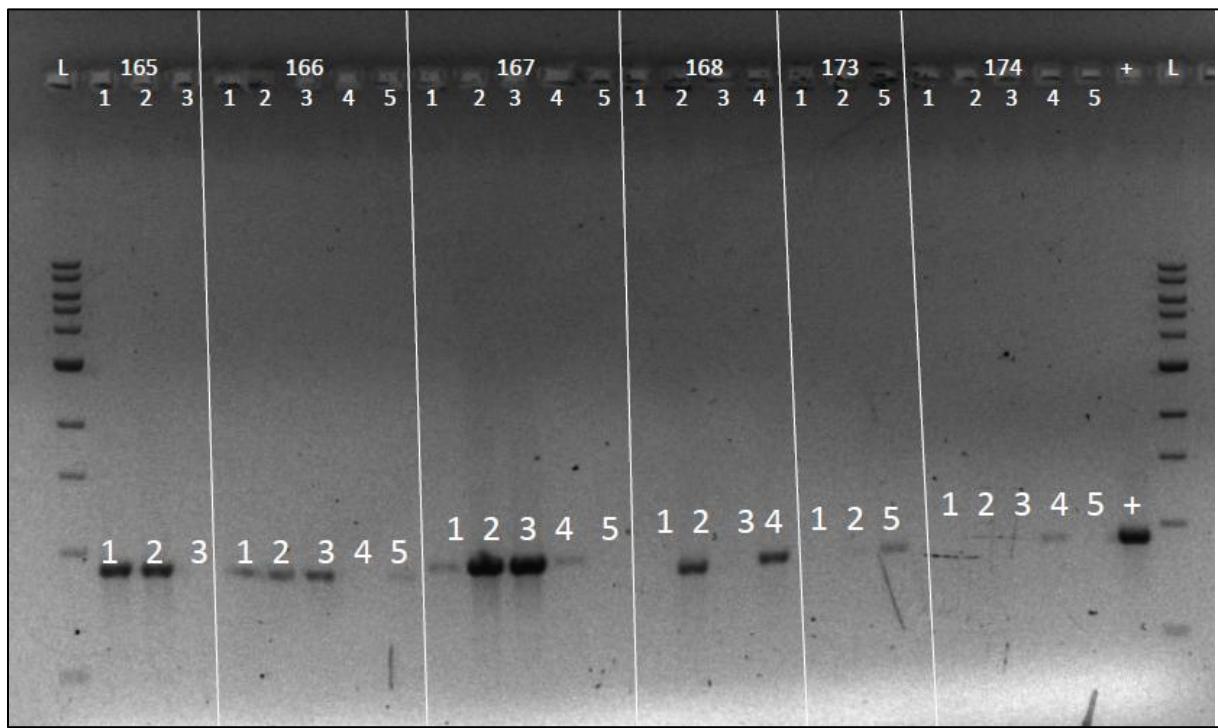


Figure 4: Colony PCR gel with the rbcL (CX/CW) primers of the colony isolates from 165 (*P. islandica*), 166 (*P. membranaceae*), 167(*P. neckeri*), 168 & 174(*P. canini*), 173 (*P. rufescens*), +ve (Positive control – *Nostoc* DNA). Number 1-5 indicate colony number.

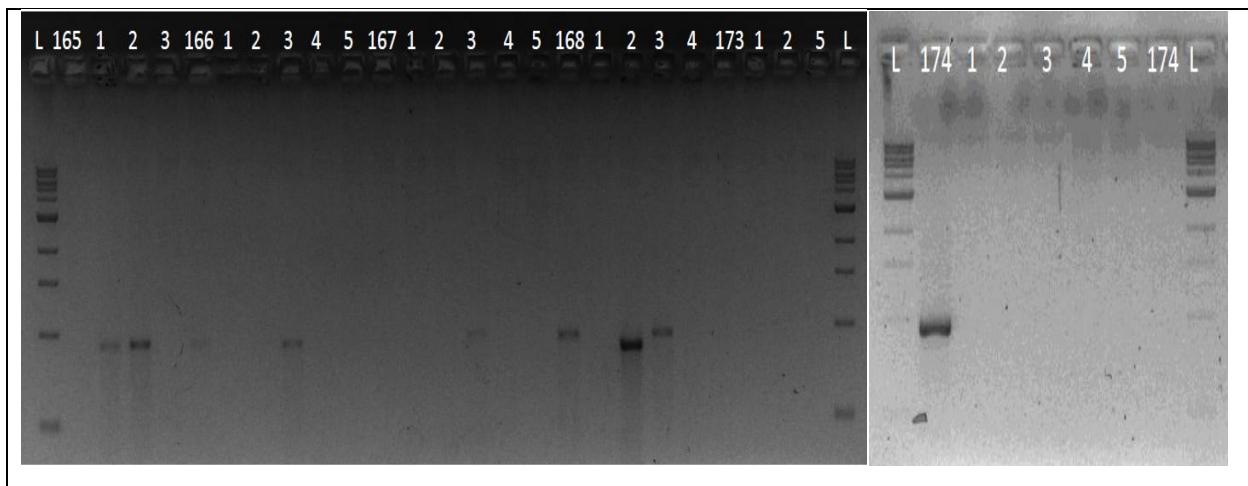


Figure 5: PCR gel with *rbcL* (CX/CW) primers comparing the DNA and colony isolates from 165 (*P. islandica*), 166 (*P. membranacea*), 167(*P. neckeri*), 168 & 174(*P. canini*), 173 (*P. rufescens*), +ve (Positive control – *Nostoc* DNA). Number 1-5 indicate colony number.

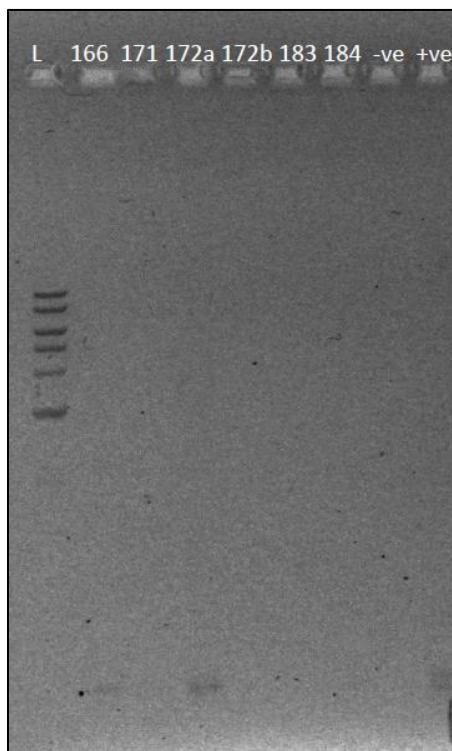


Figure 6: PCR gel with 16s (805F/CyaR1444) primers of DNA isolates from 166 (*P. membranacea*), 171, 172a, 172b, 183 & 184 (*Stereocaulon vesuvianum*), +ve (Positive control – *Nostoc* DNA), -ve (negative control – ddH₂O)

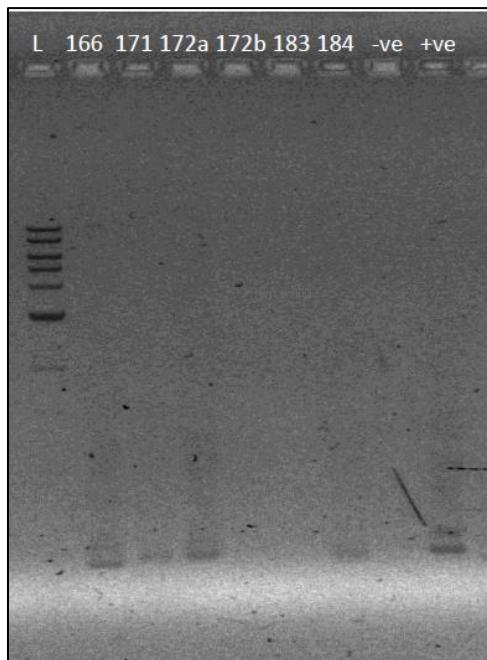


Figure 6: PCR gel with 16s (9F/515R) primers of DNA isolates from 166 (*P. membranacea*), 171, 172a, 172b, 183 & 184 (*Stereocaulon vesuvianum*), +ve (Positive control – *Nostoc* DNA), -ve (negative control – ddH₂O)

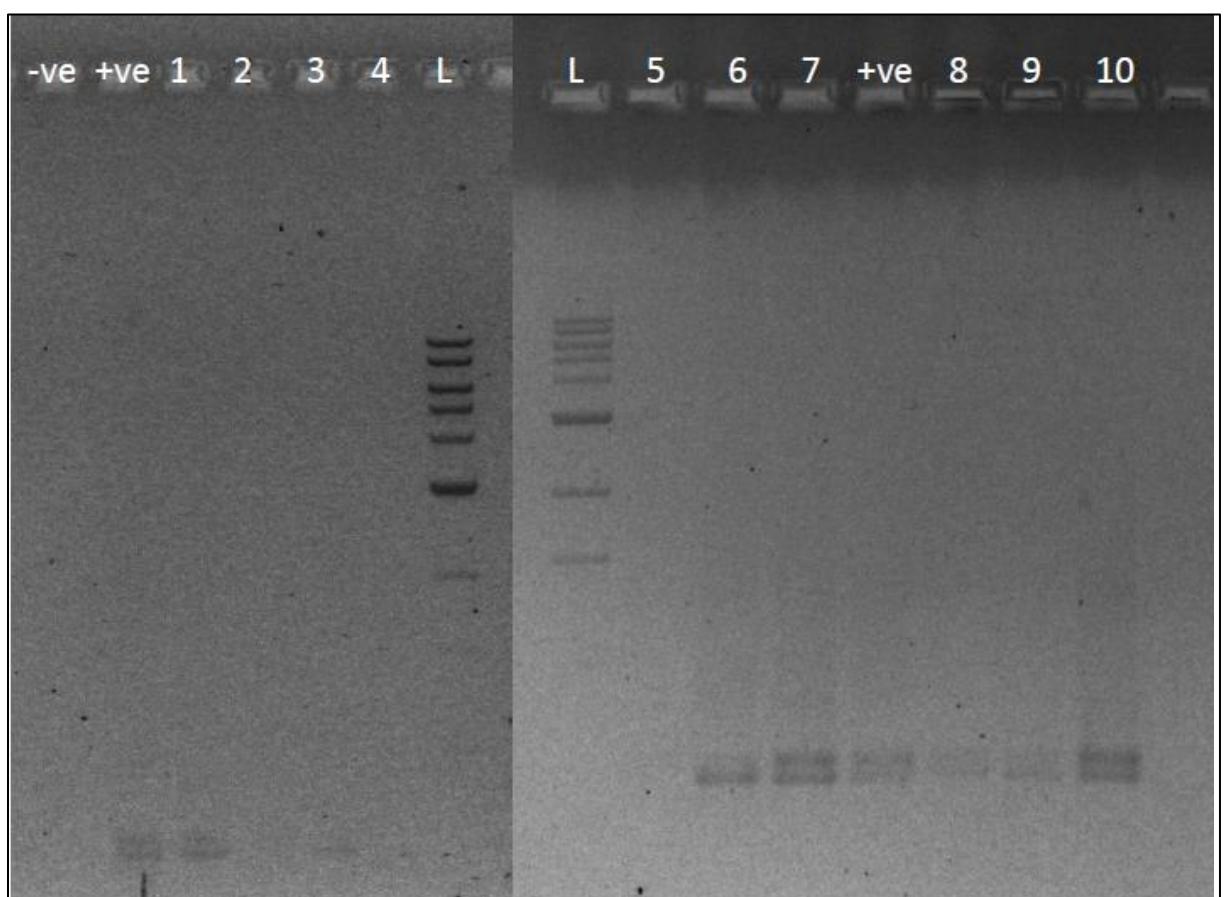


Figure 7: PCR gel with 16s (805F/CyaR1444) primers of DNA isolates from mosses 1, 2, 3 & 9 (*Rhytidadelphus squarrorus*), 4 & 10 (*Hylocomium splendens*), 5 (*Racomitrium lanuginosum*), 6 (*Rhytidadelphus triquetrus*), 7 & 8 (*Racomitrium canescens*)

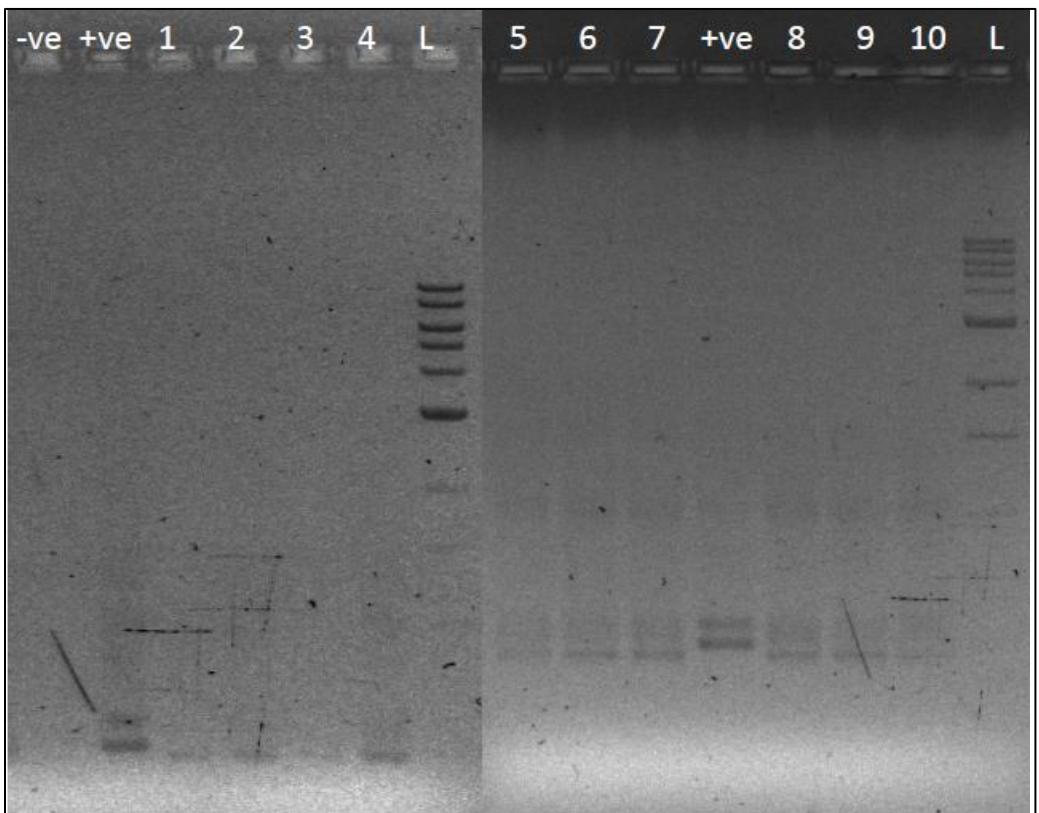


Figure 8: PCR gel with 16s (9F/515R) primers of DNA isolates from mosses 1, 2, 3 & 9 (*Rhytidadelphus squarrorus*), 4 & 10 (*Hylocomium splendens*), 5 (*Racomitrium lanuginosum*), 6 (*Rhytidadelphus triquetrus*), 7 & 8 (*Racomitrium canescens*)

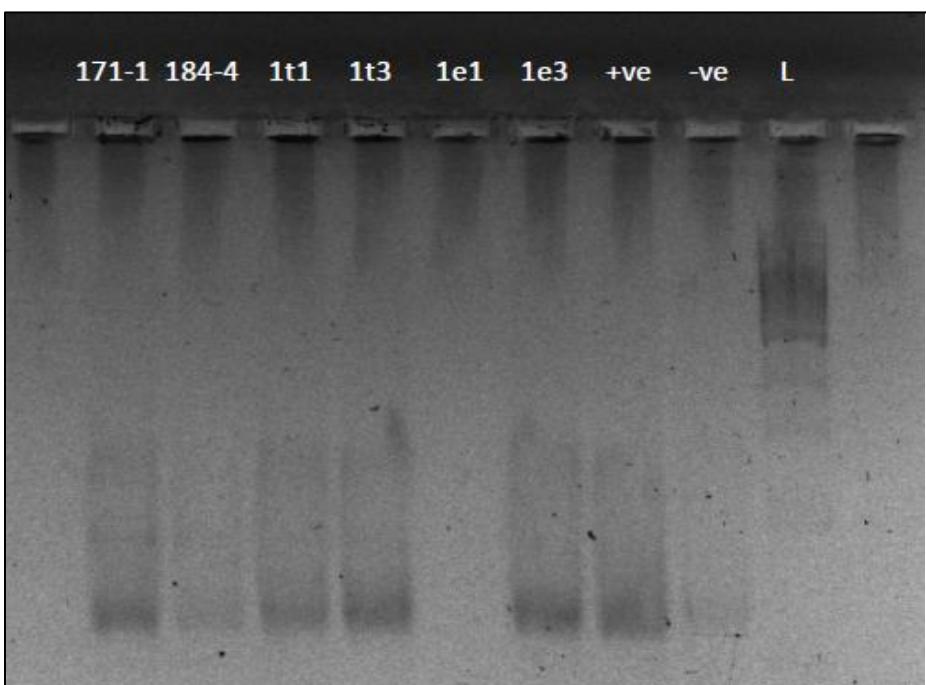


Figure 9: PCR gel with 16s (805F/CyaR1444) primers of colony isolates from 171 & 184 (*Stereocaulon vesuvianum*) and moss 1(*Rhytidadelphus squarrorus*), +ve (Positive control – *Nostoc* DNA), -ve (negative control – ddH₂O). Numbers 1 - 4 indicate colony numbers. t indicates tip of moss, e indicate root of moss.

Appendix C

BLAST results of the sequence analysis of samples with the different primers. Shown are the sequences of the first/the next named taxa hit of the BLAST results. The tables show the first 20 species hits of the BLAST results.

ITS primer

167: *Peltigera neckeri* from DNA isolate sequenced with ITS1 primer

gb|FJ708929.1| *Peltigera neckeri* strain HOB040605-12-4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence
Length=642, Score = 606 bits (328), Expect = 3e-170, Identities = 328/328 (100%), Gaps = 0/328 (0%), Strand=Plus/Plus

```

Query 1   TGCCACAATTTAAAATTCTAGTAAATATGATGTCGaaaaaaaaATTAAAAATAAGCAAAACTTCAACACGGATCTTGGTCCGGCATCGATGAAGAACGCAGCGAAATGCGA 120
Sbjct 165  TGCCACAATTTAAAATTCTAGTAAATATGATGTCGaaaaaaaaATTAAAAATAAGCAAAACTTCAACACGGATCTTGGTCCGGCATCGATGAAGAACGCAGCGAAATGCGA 284
Query 121  TAAGTAATGTGGACCGCAGTACTCAGCGACTCATCGAATCTTGAACGCATATTGCGCCCCCTGGTATCCAAGGGGCACACCTGTCCGAGCGTCATTATACATCTCATCTAGCCTGGCT 240
Sbjct 285  TAAGTAATGTGGACCGCAGTACTCAGCGACTCATCGAATCTTGAACGCATATTGCGCCCCCTGGTATCCAAGGGGCACACCTGTCCGAGCGTCATTATACATCTCATCTAGCCTGGCT 404
Query 241  TGCTAAGTCTTGCGGGCTTGGTTTGGGCTCCATCTGCTTAACGTAGAGGTCTAAAGTCAGTGGCGGATCAATTAGCGTTCCAGC 328
Sbjct 405  TGCTAAGTCTTGCGGGCTTGGTTTGGGCTCCATCTGCTTAACGTAGAGGTCTAAAGTCAGTGGCGGATCAATTAGCGTTCCAGC 492

```

Table 1: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 167 sequenced with ITS1 primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
FJ708929.1	<i>Peltigera neckeri</i> strain HOB040605-12-4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	606	606	98%	3,00E-170	100%
FJ708928.1	<i>Peltigera neckeri</i> strain HOB040605-13-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	606	606	98%	3,00E-170	100%
AF075725.1	<i>Peltigera neckeri</i> internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	606	606	98%	3,00E-170	100%
GQ292453.1	<i>Peltigera collina</i> voucher Hur 060789 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	601	601	98%	1,00E-168	99%
GQ292452.1	<i>Peltigera collina</i> voucher Hur 060816 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	601	601	98%	1,00E-168	99%
AY257963.1	<i>Peltigera neckeri</i> internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	601	601	98%	1,00E-168	99%
AF074978.1	<i>Peltigera collina</i> specimen-voucher Park 2581 (DUKE) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	601	601	98%	1,00E-168	99%
FJ527275.1	<i>Peltigera polydactyloides</i> voucher Killmann, KOB internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	593	593	98%	2,00E-166	99%
AY257967.1	<i>Peltigera polydactyloides</i> internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	588	588	98%	9,00E-165	99%
FJ708927.1	<i>Peltigera collina</i> strain HOB040605-7-3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	566	566	98%	4,00E-158	98%
FJ708926.1	<i>Peltigera collina</i> strain HOB040605-7-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	566	566	98%	4,00E-158	98%

FJ708925.1	Peltigera collina strain HOB040605-6-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	566	566	98%	4,00E-158	98%
FJ708924.1	Peltigera collina strain HOB040605-5-3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	566	566	98%	4,00E-158	98%
FJ708923.1	Peltigera collina strain HOB040605-4-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	566	566	98%	4,00E-158	98%
AF074976.1	Peltigera collina specimen-voucher Goward 91-2074 (UBC) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	566	566	98%	4,00E-158	98%
AY257969.1	Peltigera collina internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	560	560	98%	2,00E-156	98%
AF108142.1	Peltigera collina specimen-voucher Hafellner & Hafellner (Duke) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	560	560	98%	2,00E-156	98%
AF074977.1	Peltigera collina specimen-voucher Davis 2278 (UBC) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	556	556	98%	3,00E-155	97%
AY257968.1	Peltigera phyllidiosa internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	525	525	98%	8,00E-146	96%
AF074975.1	Peltigera phyllidiosa internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	525	525	98%	8,00E-146	96%

168: *Peltigera canina* from DNA isolate sequenced with ITS1 primer

gb|HM448796.1| *Peltigera canina* strain UK133 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence

Length=528, Score = 592 bits (320), Expect = 7e-166, Identities = 320/320 (100%), Gaps = 0/320 (0%), Strand=Plus/Plus

```

Query   1  TGCCACAGTTAAAATCTGGTTAAATACGGTGTGAGCAAATTATTTAAATAAGCAAACCTTCACAAACGGATCTTGGTCCGGCATCGATGAAGAACGCAGCGAAATGCGA  120
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  114  TGCCACAGTTAAAATCTGGTTAAATACGGTGTGAGCAAATTATTTAAATAAGCAAACCTTCACAAACGGATCTTGGTCCGGCATCGATGAAGAACGCAGCGAAATGCGA  233
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query  121  TAAGTAATGTGGACCGCAGTACTCAGCGACTCATCGAATCTTGAAACGCACATTGCGCCCCCTGGTGTCCCCAAAGGGCACACCTGTTGAGCGTCATCATACATTGCACTAGGCTAAGG  240
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  234  TAAGTAATGTGGACCGCAGTACTCAGCGACTCATCGAATCTTGAAACGCACATTGCGCCCCCTGGTGTCCCCAAAGGGCACACCTGTTGAGCGTCATCATACATTGCACTAGGCTAAGG  353
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Query  241  GCCCGGCTTGCCTTGGCTTTCTATCTATGGAAGGTCTAAAATCAGTGGCTGTTAAGTTAGGCATTCCAGCGTAGT  320
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  354  GCCCGGCTTGCCTTGGCTTTCTATCTATGGAAGGTCTAAAATCAGTGGCTGTTAAGTTAGGCATTCCAGCGTAGT  433
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

```

Table 2: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 168 sequenced with ITS1 primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
HM448796.1	Peltigera canina strain UK133 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448795.1	Peltigera canina strain UK121 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448794.1	Peltigera canina strain UK132 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448793.1	Peltigera canina strain UK123 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448792.1	Peltigera canina strain UK127 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448791.1	Peltigera canina strain UK118 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and	592	592	100%	7,00E-166	100%

173: *Peltigera rufescens* from DNA isolate sequenced with ITS4 primer

gb|AY257927.1| *Peltigera rufescens* internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence

Length=510, Score = 603 bits (326), Expect = 3e-169, Identities = 326/326 (100%), Gaps = 0/326 (0%), Strand=Plus/Minus

Query 1 ACTACGCTGGAAATGTCTAACCTAACCGTCACTGACTTTAGACTTCATATAAGCAGACAAAGGCCAATTGCAAGCCGGCGCCAGGCTGATTCGATGTATGACGCTCGAAC 120

Figure 100.

Grade Level	Percentage (%)
1	10
2	12
3	15
4	18
5	20
6	22
7	25
8	28
9	30
10	32
11	34
12	36

Query 121 GGTGTCGCCCTTGGTATAACCAAGGGCGCAATGTGCGTTCAAAGATTGATGAGTCGCTGAGTACTGCCTTCCACATTACTATCGATTGCTCGCTTCTCATCGATGCCGGAAC 240

Sequence: 241 - AGCGATGCCATTGTCAGATTTTCTGTGTTAATAATTTCTTGACAGATTAACTGCTGTTTACCGATTTCCTTAATCTA - 266

Query 241 AAAGAGATCCGTTGGAAAGTTTGCCTTGTTAAAATAATTCACTTCAGACATTAATTGTTAAGGATTTGTAAAGTGA 326

Table 3: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 173 sequenced with ITS4 primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
AY257927.1	Peltigera rufescens internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	603	603	100%	3,00E-169	100%
AY257926.1	Peltigera rufescens internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	603	603	100%	3,00E-169	100%
AY257925.1	Peltigera rufescens internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	603	603	100%	3,00E-169	100%
AY257918.1	Peltigera neorufescens internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	603	603	100%	3,00E-169	100%
AY257917.1	Peltigera neorufescens internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	603	603	100%	3,00E-169	100%
FJ527245.1	Peltigera papuana voucher H:Koponen 31592 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	542	542	97%	7,00E-151	97%
FJ527252.1	Peltigera papuana voucher LG:Serusiaux 13655 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	540	540	97%	3,00E-150	97%
FJ527251.1	Peltigera papuana voucher B:Sipman 38336 note duplicate voucher internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	540	540	97%	3,00E-150	97%
FJ527250.1	Peltigera papuana voucher B:Sipman 35270 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	540	540	97%	3,00E-150	97%
FJ527248.1	Peltigera papuana voucher B:Sipman 38336 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	540	540	97%	3,00E-150	97%
FJ527249.1	Peltigera papuana voucher Diederich 10581, herb. Diederich internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	536	536	97%	3,00E-149	97%
AY257921.1	Peltigera lepidophora internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	536	536	97%	3,00E-149	97%
FJ527247.1	Peltigera papuana voucher LG:Serusiaux 14106 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	534	534	97%	1,00E-148	97%
FJ527246.1	Peltigera papuana voucher LG:Serusiaux 13656 note holotype internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	534	534	97%	1,00E-148	97%
AY257920.1	Peltigera lepidophora internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	525	525	97%	7,00E-146	97%
FJ527257.1	Peltigera dolichorrhiza isolate 2 voucher LG:Serusiaux s.n. internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	518	518	97%	1,00E-143	96%
FJ527253.1	Peltigera dolichorrhiza isolate 1 voucher LG:Serusiaux s.n. internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	518	518	97%	1,00E-143	96%
FJ527255.1	Peltigera granulosa voucher LG:Serusiaux 15150 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	516	516	89%	4,00E-143	99%
FJ527254.1	Peltigera granulosa voucher H:Koponen 33267 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	516	516	89%	4,00E-143	99%
FJ527256.1	Peltigera granulosa voucher B:Sipman 39313 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	503	503	97%	3,00E-139	95%

174: *Peltigera canina* from DNA isolate sequenced with ITS1 primer

gb|HM448796.1| *Peltigera canina* strain UK133 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence

Length=528, Score = 592 bits (320), Expect = 7e-166, Identities = 320/320 (100%), Gaps = 0/320 (0%), Strand=Plus/Plus

Query	1	TGCCACAGTTAAAATCTGGTAAATCGGTGATGTCGAGCAAATTATAAAGCAAAACTTCAACAAACGGATCCTGGTCCGGCATCGATGAAGAACGCAAGCGAAATCGA	120
Sbjct	114	TGCCACAGTTAAAATCTGGTAAATCGGTGATGTCGAGCAAATTATAAAGCAAAACTTCAACAAACGGATCCTGGTCCGGCATCGATGAAGAACGCAAGCGAAATCGA	231

```

Query 121 TAAGTAATGTGGACCGCAGTACTCAGCGACTCATCGAATCTTGAAACGCACATTGCGCCCTTGGTGTCCCCAAAGGGCACACCTGTCGAGCGTCATCATACATTGCACTCAGGCTAAGG 240
Sbjct 234 TAAGTAATGTGGACCGCAGTACTCAGCGACTCATCGAATCTTGAAACGCACATTGCGCCCTTGGTGTCCCCAAAGGGCACACCTGTCGAGCGTCATCATACATTGCACTCAGGCTAAGG 353

Query 241 GCGCGGCTTGCCTGGCTTTCTATGGAAAGGTCTTAAAATCAGTGGCTGTTAAGTTAGGCATTCAGCGTAGT 320
Sbjct 354 GCGCGGCTTGCCTGGCTTTCTATGGAAAGGTCTTAAAATCAGTGGCTGTTAAGTTAGGCATTCAGCGTAGT 433

```

Table 4: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 174 sequenced with ITS1 primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
HM448796.1	Peltigera canina strain UK133 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448795.1	Peltigera canina strain UK121 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448794.1	Peltigera canina strain UK132 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448793.1	Peltigera canina strain UK123 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448792.1	Peltigera canina strain UK127 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448791.1	Peltigera canina strain UK118 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448788.1	Peltigera canina strain UK111 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448789.1	Peltigera canina strain UK109 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
HM448790.1	Peltigera canina strain UK126 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708891.1	Peltigera canina strain HOB030611-5-10-6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708890.1	Peltigera canina strain HOB020708-4-5-1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708889.1	Peltigera canina strain HOB030611-10-5-5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708888.1	Peltigera canina strain HOB030611-10-5-2 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708887.1	Peltigera canina strain HOB030611-10-10-4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708886.1	Peltigera canina strain HOB030611-10-0-6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708885.1	Peltigera canina strain HOB030611-10-0-5 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708884.1	Peltigera canina strain HOB030611-10-0-3 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708883.1	Peltigera canina strain HOB030611-0-10-4 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166	100%
FJ708882.1	Peltigera canina strain HOB030611-0-0-7 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA	592	592	100%	7,00E-166	100%

	gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence				
FJ708881.1	Peltigera canina strain HOB030611-0-0-6 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 26S ribosomal RNA gene, partial sequence	592	592	100%	7,00E-166

184: *Stereocaulon vesuvianum* from DNA isolate sequenced with ITS4 primer

emb|FM945376.1| *Astrochloris photobiont* ITS1, 5.8S rRNA gene and ITS2, strain Backor 29

Length=514, Score = 628 bits (340), Expect = 6e-177, Identities = 340/340 (100%), Gaps = 0/340 (0%), Strand=Plus/Minus

```

Query  1  ACCAAGCTGGAGTCCGATTAACGGTCCCTGCAGTGTCACTGAATTTCGGCCAAGTGGCGCAGCACAGACCACAACCTTCCGATCCACTATATGTAAAGGGGGAGAGGGGGTAAAC  120
|||||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Sbjct  433  ACCAAGCTGGAGTCCGATTAACGGTCCCTGCAGTGTCACTGAATTTCGGCCAAGTGGCGCAGCACAGACCACAACCTTCCGATCCACTATATGTAAAGGGGGAGAGGGGGTAAAC  314
|||||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Query 121  AGACGCTGAGGCAGACATGCCCTGGCCGAGGCCCTGGGGCGAATATGCCCTAAAGATTCGATGGTTACCGAATTCTGCAATTCAACTACCTATCGCATTTCGTGGTTCTTCAT  240
|||||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Sbjct 313  AGACGCTGAGGCAGACATGCCCTGGCCGAGGCCCTGGGGCGAATATGCCCTAAAGATTCGATGGTTACCGAATTCTGCAATTCAACTACCTATCGCATTTCGTGGTTCTTCAT  194
|||||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Query 241  CGTTGCGGGAGCCAAGATAATCGCTTGTGAGAGTTGCTTTGTTAGTCGCCGCTCGTGTGAGCAAGCAATTGCCCTCAGATATTGGTTGAATGGGAGAA  340
|||||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Sbjct 193  CGTTGCGGGAGCCAAGATAATCGCTTGTGAGAGTTGCTTTGTTAGTCGCCGCTCGTGTGAGCAAGCAATTGCCCTCAGATATTGGTTGAATGGGAGAA  94
|||||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||

```

Table 5: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 184 sequenced with ITS4 primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
FM945376.1	<i>Astrochloris photobiont</i> ITS1, 5.8S rRNA gene and ITS2, strain Backor 29	628	628	100%	6,00E-177	100%
FM945347.1	<i>Astrochloris photobiont</i> ITS1, 5.8S rRNA gene and ITS2, strain Backor 13	628	628	100%	6,00E-177	100%
AM906000.1	<i>Astrochloris irregularis</i> ITS1, 5.8S rRNA gene, ITS2 and 26S rRNA gene (partial), strain Backor 13	628	628	100%	6,00E-177	100%
AM905999.1	<i>Astrochloris irregularis</i> ITS1, 5.8S rRNA gene, ITS2 and 26S rRNA gene (partial), strain STER 1	628	628	100%	6,00E-177	100%
DQ229881.1	Trebouxia (<i>Astrochloris</i>) photobiont 103 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	628	628	100%	6,00E-177	100%
DQ229880.1	Trebouxia (<i>Astrochloris</i>) photobiont 101 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	628	628	100%	6,00E-177	100%
AF345413.1	Trebouxia (<i>Astrochloris</i>) photobiont MN097 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AY622825.1 Trebouxia (<i>Astrochloris</i>) photobiont MN433 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AY622834.1 Trebouxia (<i>Astrochloris</i>) photobiont MN354 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	628	628	100%	6,00E-177	100%
AF345408.1	Trebouxia (<i>Astrochloris</i>) photobiont MN106 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AF345409.1 Trebouxia (<i>Astrochloris</i>) photobiont MN101 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AF345410.1 Trebouxia (<i>Astrochloris</i>) photobiont MN102 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AF345411.1 Trebouxia irregularis internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequences >gb AY622821.1 Trebouxia (<i>Astrochloris</i>) photobiont MN751 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AY622823.1 Trebouxia (<i>Astrochloris</i>) photobiont MN749 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AY622827.1 Trebouxia (<i>Astrochloris</i>) photobiont MN356 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AY622828.1 Trebouxia (<i>Astrochloris</i>) photobiont MN352 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence >gb AY622829.1 Trebouxia (<i>Astrochloris</i>) photobiont MN350 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	628	628	100%	6,00E-177	100%

FM945392.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain Backor 09	623	623	100%	3,00E-175	99%
FM945388.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain CLAD 15	623	623	100%	3,00E-175	99%
FM945372.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain Backor 06	623	623	100%	3,00E-175	99%
FM945371.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain Backor 28	623	623	100%	3,00E-175	99%
FM945370.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain Backor 18	623	623	100%	3,00E-175	99%
FM945368.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain Backor 05	623	623	100%	3,00E-175	99%
FM945367.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain Backor 01	623	623	100%	3,00E-175	99%
FM945364.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain CLAD 05(A)	623	623	100%	3,00E-175	99%
FM945357.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain CLAD 08	623	623	100%	3,00E-175	99%
FM945353.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain Backor 03	623	623	100%	3,00E-175	99%
FM945352.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain CLAD 07	623	623	100%	3,00E-175	99%
FM945351.1	Astrochloris photobiont ITS1, 5.8S rRNA gene and ITS2, strain CLAD 06	623	623	100%	3,00E-175	99%

CX primer

165: *Peltigera islandica* from DNA isolate sequenced with CX primer

gb|DQ185274.1| *Nostoc sp. 'Peltigera rufescens 1 cyanobiont'* ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds Length=826, Score = 1062 bits (575), Expect = 0.0, Identities = 583/587 (99%), Gaps = 0/587 (0%), Strand=Plus/Minus

Query	1	CTGGGGTTAGATGTC TCACTTGTGTGATCGCTCTAAATGCTGGCGACGCTGTTCCATTGGCTGCTGAATGCCAGTGCACCATTCGGTAAAAATTCTGCAATTCTTC	120
Sbjct	731	CTGGGGTTAGATGTC TCACTTGTGTGATCGCTCTAAATGCTGGCGACGCTGTTCCATTGGCTGCTGAATGCCAGTGCACCATTCGGTAAAAATTCTGCAATTCTTC	612
Query	121	GCAATGTGTTCTCTGACAGTCATGATTCGCAAAGCCAATCTGGTTTCTCGAACAGTTGCTCAAGGAATGACTCACCAATTGAAATTGCCAGACGAAAAGTTATGCAACCAAAGT	240
Sbjct	611	GCAATGTGTTCTCTGACAGTCATGATTCGCAAAGCCAATCTGGTTTCTCGAACAGTTGCTCAAGGAATGACTCACCAATTGAAATTGCCAGACGAAAAGTTATGCAACCAAAGT	492
Query	241	TCTAATGGCGGATTAGTTCGCCTAGCTGTGCCAATACTGTCTTAGAGCCTGATAAGTCAGATAGCTTGGAGAGTTGGCTGTGCTTCGCAATTGCTTAAGATTCTGCTTGAC	360
Sbjct	491	TCTAATGGCGGATTAGTTCGCCTAGCTGTGCCAATACTGTCTTAGAGCCTGATAAGTCAGATAGCTTGGAGAGTTGGCTGTGCTTCGCAATTGCTTAAGATTCTGCTTGAC	372
Query	361	CCCAGCCCCGAAAGAGTTATGAGTTATGAGTTATGAGTTATGAAATTGCAATCTAACCTTGACAGACGGATTCTCGCTCTACTCTAACCTTAACTCTAACGTAAAC	480
Sbjct	371	CCCAGCCCCGAAAGAGTTATGAGTTATGAGTTATGAGTTATGAAATTGCAATCTAACCTTGACAGACGGATTCTCGCTCTACTCTAACCTTAACTCTAACGTAAAC	252
Query	481	CCAGATCAGACGGTATCCATTGCTTCAAACCTCGAACATTGATTTCTTCCACAGTTGCGAACAGCCAGTTGCGAGCTTCAGGAGACCCTTGGCAGCTTCGGGATGATTC	587
Sbjct	251	CCAGATCAGACGGTATCCATTGCTTCAAACCTCGAACATTGATTTCTTCCACAGTTGCGAACAGCCAGTTGCGAGCTTCAGGAGACCCTTGGCAGCTTCGGGATGATTC	145

Table 6: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 165 sequenced with CX primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
DQ185274.1	Nostoc sp. 'Peltigera rufescens 1 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1062	1062	99%	0.0	99%
DQ185277.1	Nostoc sp. 'Peltigera didactyla 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1029	1029	99%	0.0	98%
DQ185272.1	Nostoc sp. 'Peltigera rufescens 3 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1029	1029	99%	0.0	98%
DQ185287.1	Nostoc sp. 'Peltigera canina 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1026	1026	99%	0.0	98%

EU877530.1	Nostoc sp. 'Leptogium scharderii' (151) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1022	1022	99%	0.0	98%
DQ185266.1	Nostoc sp. 'Mollenhauer 1:1-106b2' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1020	1020	99%	0.0	98%
DQ185275.1	Nostoc sp. 'Peltigera rufescens 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1014	1014	99%	0.0	98%
DQ185271.1	Nostoc sp. 'Mollenhauer 94.1' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1011	1011	99%	0.0	98%
AJ632064.1	Nostoc calcicola VI partial rbcL gene for RuBisCo large subunit and rbcX gene, strain VI	998	998	99%	0.0	97%
AJ632063.1	Nostoc calcicola III partial rbcL gene for RuBisCo large subunit and rbcX gene, strain III	998	998	99%	0.0	97%
EU877481.1	Nostoc sp. 'Collema tenax' (246) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	983	983	99%	0.0	97%
DQ185314.1	Nostoc punctiforme SAG 65.79 isolation-source lichen specimen voucher SAG 65.79 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	983	983	99%	0.0	97%
DQ185273.1	Nostoc sp. 'Collema crispum cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	983	983	99%	0.0	97%
DQ185267.1	Nostoc sp. 'Mollenhauer 1:1-108' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	983	983	99%	0.0	97%
EF102336.1	Nostoc sp. 'Peltigera neopolydactyla cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	981	981	99%	0.0	97%
DQ185307.1	Nostoc sp. 'Peltigera membranacea 4 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	976	976	99%	0.0	97%
Z94889.1	Nostoc sp. rbcL and rbcX genes (strain NIVA-CYA 124)	976	976	99%	0.0	97%
EU877477.1	Nostoc sp. 'Collema polycarpon' (889) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and chaperonin-like protein (rbcX) genes, partial cds	968	968	99%	0.0	96%
DQ185291.1	Nostoc sp. 'Lobaria hallii cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	965	965	99%	0.0	96%
EU877469.1	Nostoc sp. 'Collema furfuraceum' (297) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	948	948	99%	0.0	96%

166: *Peltigera membranacea* from DNA isolate sequenced with CX primer

gb|DQ185275.1| Nostoc sp. 'Peltigera rufescens 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds
Length=824, Score = 1251 bits (677), Expect = 0.0, Identities = 712/729 (98%), Gaps = 2/729 (0%), Strand=Plus/Minus

```

Query  1  CTGGGGTTAGATCGCTATTCTGTGATGCCCTAAATGCTGGCAGCGCTTCCATGTT-GCTTTGCTGAATGCCAGTGGAACCATTCGGTAAAAATTCCGTTATTCTCCGC  119
Sbjct  728  CTGGGGTTAGATGTGCTATTCTGTGATGCCCTAAATGCTGGCAGCGCTTCCATGTTGGC-TTGCTGAATGCCAGTGGAACCATTCGGTAAAAATTCCGCAATTCTCCGC  610

Query 120  AATGTGTTCTCTGACAGTCATGATTCGCAAAGCCAATCTGGTTTCTCTCAGCAGTGCTCAATGAATGACTCACCATTTGAATTTCGCCAGACGAAAAGTTATGCAACCAAAGTC  239
Sbjct 609   AATGTGTTCTCTGACAGTCATGATTCGCAAAGCCAATCTGGTTTCTCGCAACAGTGCTCAATGACTCACCATCTGAATTTCGCCAGACGAAAAGTTATGCAACCAAAGTC  490

Query 240  TAATGGCGGATTGTTGCCCTAGCTGTGCCAATACTGTCCTTAGAGCCTGATAAGTCAGATAGCTTGAGAGTTTGGCTGTGTCCTTCGCAATTGCTTAAGATTGCTGACCC  359
Sbjct 489   TAATGGCGGATTGTTGCCCTAGCTGTGCCAATACTGTCCTTAGAGCCTGATAAGTCAGATAGCTTGAGAGTTGGCTGTGTCCTTCGCAATTGCTTAAGATTGCTGACCC  370

Query 360  CAGCCCTGAAAGAGTTATGAGTTATGAGTTATGAGTTATGAAATTATGCAAATCCTAACCTCTGTACAGACGCGATTATCGCGTCTACTCTAACCTTAACCTTAACGTAAACCC  479
Sbjct 369   CAGCCCTGAAAGAGTTATGAGTTATGAGTTATGAGTTATGAAATTATGCAAATCCTAACCTCTGTACAGACGCGATTATCGCGTCTACTCTAACCTTAACGTAAACCC  250

```

```

Query 480 AGATCAGACGGTATCCATTGCTTCAAACCTGAACCTGATTCTTCACAGTTGCAAGCAACAGCCAGTCAGGAGACCACCTGGCTGCTTCGGGATGATATCGTTACCTTCAGGAGC 599
Sbjct 249 AGATCAGACGGTATCCATTGCTTCAAACCTGAACCTGATTCTTCACAGTTGCAAGCAACAGCCAGTCAGGAGACCACCTGGCTGCTTCGGGATGATATCGTTACCTTCAGGAGC 130
Query 600 CAAGTTACGGCCTTCGTTACGAGCTTGAAACAACGCGTTCAAGGCAGCGGTTAGCGGTGACCAGGAGCGTTACCCAGGGTGTCCCAGAGTACCAACCCGAATTGTAGTACGGAGTCATCACC 728
Sbjct 129 CAAGTTACGGCCTTCGTTACGAGCTTGAAATAACGGCTTCAAGGCAGCGGTTAGCGGTGACCAGGAGCGTTACCCAGGGTGTCCCAGAGTACCAACCCGAATTGTAGTACAGAGTCATCACC 1

```

Table 7: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 166 sequenced with CX primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
DQ185275.1	Nostoc sp. 'Peltigera rufescens 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1251	1251	90%	0.0	98%
DQ185266.1	Nostoc sp. 'Mollenhauer 1:1-106b2' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1240	1240	90%	0.0	97%
AJ632063.1	Nostoc calcicola III partial rbcL gene for RuBisCo large subunit and rbcX gene, strain III	1238	1238	91%	0.0	97%
AJ632064.1	Nostoc calcicola VI partial rbcL gene for RuBisCo large subunit and rbcX gene, strain VI	1227	1227	91%	0.0	97%
DQ185274.1	Nostoc sp. 'Peltigera rufescens 1 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1225	1225	90%	0.0	97%
DQ185291.1	Nostoc sp. 'Lobaria hallii cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1223	1223	90%	0.0	97%
DQ185277.1	Nostoc sp. 'Peltigera didactyla 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1219	1219	90%	0.0	97%
DQ185273.1	Nostoc sp. 'Collema crispum cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1219	1219	90%	0.0	97%
DQ185272.1	Nostoc sp. 'Peltigera rufescens 3 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1219	1219	90%	0.0	97%
DQ185267.1	Nostoc sp. 'Mollenhauer 1:1-108' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1219	1219	90%	0.0	97%
Z94889.1	Nostoc sp. rbcL and rbcX genes (strain NIVA-CYA 124)	1218	1218	91%	0.0	96%
EF102336.1	Nostoc sp. 'Peltigera neopolydactyla cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1214	1214	89%	0.0	97%
EU877493.1	Nostoc sp. Leptogium corticola (299) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1208	1208	90%	0.0	96%
DQ185307.1	Nostoc sp. 'Peltigera membranacea 4 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1206	1206	90%	0.0	97%
EU877476.1	Nostoc sp. 'Collema polycarpon (247) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1205	1205	89%	0.0	97%
EU877469.1	Nostoc sp. 'Collema furfuraceum (297) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1203	1203	90%	0.0	96%
DQ185314.1	Nostoc punctiforme SAG 65.79 isolation-source lichen specimen voucher SAG 65.79 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1201	1201	90%	0.0	96%
DQ185271.1	Nostoc sp. 'Mollenhauer 94.1' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1199	1199	90%	0.0	96%
EU877477.1	Nostoc sp. 'Collema polycarpon (889) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and chaperonin-like protein (rbcX) genes, partial cds	1182	1182	90%	0.0	96%
EU877530.1	Nostoc sp. 'Leptogium scharderrii (151) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1179	1179	89%	0.0	96%

167: *Peltigera neckeri* from DNA isolate sequenced with CX primer

gb|DQ185278.1| Nostoc sp. 'Peltigera rufescens 4 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds Length=872, Score = 1434 bits (776), Expect = 0.0, Identities = 776/776 (100%), Gaps = 0/776 (0%), Strand=Plus/Minus

Query 1	CTGGGGTTAGATGTCTACTACTTGTGATGCCCTCAAATGCTGGCACGTTGCTCATATTGCCCTGCTGAATGCCAGTGCACCAACCATTCCGGTAAAATTCTGCAATTCTTCG	120
Sbjct 776	CTGGGGTTAGATGTCTACTACTTGTGATGCCCTCAAATGCTGGCACGTTGCTCATATTGCCCTGCTGAATGCCAGTGCACCAACCATTCCGGTAAAATTCTGCAATTCTTCG	657
Query 121	GCAATGTGTTCTGACAGTCATGATTGCAAAGCCAATCTGGTTTCTCGAACAGTTGCTCAATGAATGACTCACCATTTGAATTGGCAGACGAAAAGTTATGCAACCAAAGT	240
Sbjct 656	GCAATGTGTTCTGACAGTCATGATTGCAAAGCCAATCTGGTTTCTCGAACAGTTGCTCAATGAATGACTCACCATTTGAATTGGCAGACGAAAAGTTATGCAACCAAAGT	537
Query 241	TCTAATGGTGGATTCTGCTTAGCTGCAAATACTGTCCTAGAGCCTGATAAGTCAGATAGCTTGGAGAGTTTGCTGTCCTTCGCAATTGCTTAAGATTGCTTGAC	360
Sbjct 536	TCTAATGGTGGATTCTGCTTAGCTGCAAATACTGTCCTAGAGCCTGATAAGTCAGATAGCTTGGAGAGTTTGCTGTCCTTCGCAATTGCTTAAGATTGCTTGAC	417
Query 361	CCCAGCCTGAAGAGTTATGAGTTATGAGTTATGAAATTAGCAAATCCTAACTCTTGACAGACGGATTATGAGCCAGCGCTTGGGGGGTCCCCCGTGTAGGGAC	480
Sbjct 416	CCCAGCCTGAAGAGTTATGAGTTATGAGTTATGAAATTAGCAAATCCTAACTCTTGACAGACGGATTATGAGCCAGCGCTTGGGGGGTCCCCCGTGTAGGGAC	297
Query 481	TGGCGTCGCTCTACTTCAACTCTAACTGTTAACCTAGATCAGACGGTATCCATTGCTCAAACCTGAACCTGATTCTTCACAGTCGAAGCAACAGCCAGTTCA	600
Sbjct 296	TGGCGTCGCTCTACTTCAACTCTAACTGTTAACCTAGATCAGACGGTATCCATTGCTCAAACCTGAACCTGATTCTTCACAGTCGAAGCAACAGCCAGTTCA	177
Query 601	GGAGACCACTTGCAGCTTCGCGATGATATGTTACCTTACGGAGCAAGTTACGGCTTACGAGCTTGAACAACGGCTTCAAGGCAGCGGGTTAGCGGTTGCACCAGGAGCG	720
Sbjct 176	GGAGACCACTTGCAGCTTCGCGATGATATGTTACCTTACGGAGCAAGTTACGGCTTACGAGCTTGAACAACGGCTTCAAGGCAGCGGGTTAGCGGTTGCACCAGGAGCG	57
Query 721	TTACCCCAAGGGTGTCCCAGAGTACCAACCCGAATTGTTAGTACGGAGTCATCACC	776
Sbjct 56	TTACCCCAAGGGTGTCCCAGAGTACCAACCCGAATTGTTAGTACGGAGTCATCACC	1

Table 8: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 167 sequenced with CX primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
DQ185278.1	Nostoc sp. 'Peltigera rufescens 4 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1434	1434	95%	0.0	100%
DQ185308.1	Nostoc sp. 'Peltigera rufescens 5 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1378	1378	95%	0.0	99%
DQ185296.1	Nostoc sp. 'Peltigera canina 3 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1378	1378	95%	0.0	99%
DQ185279.1	Nostoc sp. 'Peltigera canina 4 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1378	1378	95%	0.0	99%
DQ185276.1	Nostoc sp. 'Peltigera rufescens 3 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1367	1367	95%	0.0	98%
CP001037.1	Nostoc punctiforme PCC 73102, complete genome	1351	1707	100%	0.0	100%
DQ185268.1	Nostoc sp. 'Mollenhauer 1:1-115' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1303	1303	95%	0.0	97%
DQ185316.1	Nostoc punctiforme SAG 69.79 isolation-source lichen specimen voucher SAG 69.79 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1297	1297	95%	0.0	97%
DQ185280.1	Nostoc commune 'O'Brien 02011101' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-	1286	1286	93%	0.0	97%

	like protein (rbcX) gene, complete cds					
AJ632065.1	Nostoc edaphicum X partial rbcL gene for RuBisCo large subunit and rbcX gene, strain X	1245	1245	96%	0.0	95%
EU877515.1	Nostoc sp. 'Leptogium magnussonii' (318) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene, partial cds; rbcL gene, partial sequence; and chaperonin-like protein (rbcX) gene, complete cds	1240	1240	95%	0.0	96%
EU877521.1	Nostoc sp. 'Leptogium pseudofurfuraceum' (238) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1230	1230	94%	0.0	96%
EU877520.1	Nostoc sp. 'Leptogium pseudofurfuraceum' (233) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and chaperonin-like protein (rbcX) genes, partial cds	1170	1170	89%	0.0	96%
EU877522.1	Nostoc sp. 'Leptogium pseudofurfuraceum' (263) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1168	1168	91%	0.0	95%
EU877464.1	Nostoc sp. 'Collema fasciculare' (212) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1155	1155	94%	0.0	94%
EF102299.1	Nostoc sp. 'Lobaria pulmonaria cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1151	1151	97%	0.0	93%
EU877474.1	Nostoc sp. 'Collema nigrescens' (243) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and chaperonin-like protein (rbcX) genes, partial cds	1142	1142	94%	0.0	94%
DQ266018.1	Nostoc sp. 'Pseudocyphellaria mallota cyanobiont' clone SS5556R ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and ribulose-1,5-bisphosphate carboxylase/oxygenase small subunit (rbcX) genes, partial sequence	1138	1138	96%	0.0	93%
EF102295.1	Nostoc sp. 'Degelia plumbea cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1123	1123	95%	0.0	93%
EU877470.1	Nostoc sp. 'Collema furfuraceum' (D28) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1114	1114	90%	0.0	94%

170: *Peltigera monticola* from DNA isolate sequenced with CX primer

gb|DQ185274.1| *Nostoc* sp. 'Peltigera rufescens 1 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds Length=826. Score = 1291 bits (699). Expect = 0.0. Identities = 721/731 (99%). Gaps = 3/731 (0%). Strand=Plus/Minus

Query 1	CTGGGGTTAGATGTCATA-T-TCGTGATGCCGTTCAATGCGCAGCTGTCATGGCTGCGAATGCCAGTGCACCCATTCCGGTAAATTCGCAATTCTTC	117
Sbjct 731	CTGGGGTTAGATGTCACTACTTGCGATGCCGTTCAATGCGCAGCTGTCATGGCTGCGAATGCCAGTGCACCCATTCCGGTAAATTCGCAATTCTTC	612
Query 118	GCAATGTTCTCTGACAGTCATGATGCCAACGCCAATCTGGTTTCTCGCAACAGTGCATGACTCACCATTTGAATTGGCAGACGAAAAGTTATGCAACCAAAGT	237
Sbjct 611	GCAATGTTCTCTGACAGTCATGATGCCAACGCCAATCTGGTTTCTCGCAACAGTGCATGACTCACCATTTGAATTGGCAGACGAAAAGTTATGCAACCAAAGT	492
Query 238	TCTAATGGGGATTAGTTTCGCTAGCTGCGAACATGTCCTAGAGCCTGATAAGTCAGATAGCTGGAGAGTTGGCTGTGCTCTCCGAAATTGCTTAAGATTGATGAC	357
Sbjct 491	TCTAATGGCGGATTAGTTCCCTAGCTGCGAACATGTCCTAGAGCCTGATAAGTCAGATAGCTGGAGAGTTGGCTGTGCTCTCCGAAATTGCTTAAGATTGATGAC	372
Query 358	CCCCAGCCCCTGAAAGACTTATGAGTTATGAGTTATGAAATTAAATGCCAACCTTAACTCTTGTACAGACGCCATTGATCGCGTCTACTCTAACCTTAACTCTAAC	477
Sbjct 371	CCCCAGCCCCTGAAAGACTTATGAGTTATGAGTTATGAAATTAAATGCCAACCTTAACTCTTGTACAGACGCCATTGATCGCGTCTACTCTAACCTTAACTCTAAC	252
Query 478	CCAGATCAGACGGTATCCATTGCTCAAACCTGAACTGATTCTTCACAGTGCAGCAAGCACGCCAGTTCAGGAGACCACTGGCAGCTCGCGATGATATCGTACCTCACGA	597
Sbjct 251	CCAGATCAGACGGTATCCATTGCTCAAACCTGAACTGATTCTTCACAGTGCAGCAAGCACGCCAGTTCAGGAGACCACTGGCAGCTCGCGATGATATCGTACCTCACGA	132
Query 598	GCCAAGTTACGGCTTCGTTAGAGCTTGAACAACAGCTTAAGCGACGCCAGTTCAGGAGACCACTGGCAGCTCGCGATGATATCGTACCTCACCC	728
Sbjct 131	GCCAAGTTACGGCTTCGTTAGAGCTTGAACAACAGCTTAAGCGACGCCAGTTCAGGAGACCACTGGCAGCTCGCGATGATATCGTACCTCACCC	1

Table 9: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 170 sequenced with CX primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
DQ185274.1	Nostoc sp. 'Peltigera rufescens 1 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1291	1291	89%	0.0	99%
DQ185266.1	Nostoc sp. 'Mollenhauer 1:1-106b2' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1284	1284	89%	0.0	98%
DQ185275.1	Nostoc sp. 'Peltigera rufescens 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1279	1279	89%	0.0	98%
AJ632064.1	Nostoc calcicola VI partial rbcL gene for RuBisCo large subunit and rbcX gene, strain VI	1266	1266	90%	0.0	98%
AJ632063.1	Nostoc calcicola III partial rbcL gene for RuBisCo large subunit and rbcX gene, strain III	1260	1260	90%	0.0	98%
Z94889.1	Nostoc sp. rbcL and rbcX genes (strain NIVA-CYA 124)	1256	1256	91%	0.0	97%
DQ185277.1	Nostoc sp. 'Peltigera didactyla 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1253	1253	89%	0.0	98%
DQ185273.1	Nostoc sp. 'Collema crispum cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1253	1253	89%	0.0	98%
DQ185272.1	Nostoc sp. 'Peltigera rufescens 3 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1253	1253	89%	0.0	98%
DQ185267.1	Nostoc sp. 'Mollenhauer 1:1-108' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1253	1253	89%	0.0	98%
DQ185314.1	Nostoc punctiforme SAG 65.79 isolation-source lichen specimen voucher SAG 65.79 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1245	1245	89%	0.0	97%
DQ185291.1	Nostoc sp. 'Lobaria hallii cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1245	1245	89%	0.0	98%
DQ185271.1	Nostoc sp. 'Mollenhauer 94.1' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1243	1243	89%	0.0	97%
EF102336.1	Nostoc sp. 'Peltigera neopolydactyla cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1236	1236	89%	0.0	97%
EU877530.1	Nostoc sp. 'Leptogium scharderrii (151) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1234	1234	88%	0.0	97%
DQ185307.1	Nostoc sp. 'Peltigera membranacea 4 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1234	1234	89%	0.0	97%
DQ185287.1	Nostoc sp. 'Peltigera canina 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1234	1234	85%	0.0	99%
EU877469.1	Nostoc sp. 'Collema furfuraceum (297) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1225	1225	90%	0.0	97%
EU877477.1	Nostoc sp. 'Collema polycarpon (889) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and chaperonin-like protein (rbcX) genes, partial cds	1221	1221	89%	0.0	97%
EU877493.1	Nostoc sp. 'Leptogium corticola (299) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1208	1208	90%	0.0	96%

174: *Peltigera canini* from colony isolate sequenced with CX primer

gb|DQ185274.1| *Nostoc* sp. 'Peltigera rufescens 1 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds
Length=826, Score = 1332 bits (721), Expect = 0.0, Identities = 728/731 (99%), Gaps = 1/731 (0%), Strand=Plus/Minus

```

Query   3  CTGGGGTTAGATGTGCTACTACTTGTGATGCCCTCTAAATGCTGGCAGCGTTGCTCATATTGCCCTGCTGAATGCCAGTGCGAACCATTCGGTAAAAATTCTGCAATTCTTCC  122
Sbjct  731  CTGGGGTTAGATGTGCTACTACTTGTGATGCCCTCTAAATGCTGGCAGCGTTGCTCATATTGCCCTGCTGAATGCCAGTGCGAACCATTCGGTAAAAATTCTGCAATTCTTCC  612

Query  123  GCAATGTTCTCTGACAGTCAGTCAAGTCCAAAGCCAATCTGGTTTCTCGCAACAGTTGCTCARGRATGACTCACCATTTGAATTGGCCAGACGCAAAGTTATGCAACCAAAGT  242
Sbjct  611  GCAATGTTCTCTGACAGTCAGTCAAGTCCAAAGCCAATCTGGTTTCTCGCAACAGTTGCTCAAGGAATGACTCACCATTTGAATTGGCCAGACGCAAAGTTATGCAACCAAAGT  492

Query  243  TCTAATGGCGGATTAGTTGCCCTAGCTGTGCCAATACTGTCTTAGGCCGATAGCTTGTGCTTAGAGCCTGATAAGTCAGATAGCTTGGAGAGTTGGCTGTGCTTCGAATTGCTTAAGATTGCTTGC  362
Sbjct  491  TCTAATGGCGGATTAGTTGCCCTAGCTGTGCTTAGAGCCTGATAAGTCAGATAGCTTGGAGAGTTGGCTGTGCTTCGAATTGCTTAAGATTGCTTGC  372

Query  363  CCCAGCCCTGAAGAGTTATGAGTTATGAGTTATGAGTTATGAAATTAACTGCAAACTCTAACTCTGTACAGACCGATTATGCCGCTCTACTCTAACTCTTAACTCTTAACTGTTAAC  482
Sbjct  371  CCCAGCCCTGAAGAGTTATGAGTTATGAGTTATGAGTTATGAAATTAACTGCAAACTCTAACTCTGTACAGACCGATTATGCCGCTCTACTCTAACTCTTAACTGTTAAC  252

Query  483  CCAGATCAGACGGTATTGCTCAAACCTGAACTTGTGATTTCCACAGTTGCAAGCA-CAGCCAGTTCAAGGAGACCACTTGGCAGCTCGGGATGATATGTTACCTTCACGA  601
Sbjct  251  CCAGATCAGACGGTATTGCTCAAACCTGAACTTGTGATTTCCACAGTTGCAAGCAACAGCCAGTTCAAGGAGACCACTTGGCAGCTCGGGATGATATGTTACCTTCACGA  132

Query  602  GCCAAGTTACGGCTTCGTTACGAGCTTGAACAACAGCTTCAAGGGACCCGGTTAGCGGTTGACCAGGAGCGTTACCCAAAGGGTGTCTAAAGTACCCACCCGAATTGTAGTACGGAGTCATCACC  732
Sbjct  131  GCCAAGTTACGGCTTCGTTACGAGCTTGAACAACAGCTTCAAGGGACCGGGTTAGCGGTTGACCAGGAGCGTTACCCAAAGGGTGTCTAGAGTACCCACCCGAATTGTAGTACGGAGTCATCACC  1

```

Table 10: Table showing the first 20 hits of the BLAST results for the colony isolate from sample 174 sequenced with CX primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
DQ185274.1	<i>Nostoc</i> sp. 'Peltigera rufescens 1 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1332	1332	89%	0.0	99%
DQ185277.1	<i>Nostoc</i> sp. 'Peltigera didactyla 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1282	1282	89%	0.0	98%
DQ185272.1	<i>Nostoc</i> sp. 'Peltigera rufescens 3 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1282	1282	89%	0.0	98%
DQ185271.1	<i>Nostoc</i> sp. 'Mollenhauer 94.1' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1273	1273	89%	0.0	98%
EU877530.1	<i>Nostoc</i> sp. 'Leptogium scharderii (151) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1264	1264	88%	0.0	98%
DQ185266.1	<i>Nostoc</i> sp. 'Mollenhauer 1:1-106b2' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1247	1247	89%	0.0	98%
DQ185275.1	<i>Nostoc</i> sp. 'Peltigera rufescens 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1219	1219	89%	0.0	97%
AJ632064.1	<i>Nostoc calcicola</i> VI partial rbcL gene for RuBisCo large subunit and rbcX gene, strain VI	1218	1218	90%	0.0	96%
AJ632063.1	<i>Nostoc calcicola</i> III partial rbcL gene for RuBisCo large subunit and rbcX gene, strain III	1212	1212	90%	0.0	96%
DQ185314.1	<i>Nostoc punctiforme</i> SAG 65.79 isolation-source lichen specimen voucher SAG 65.79 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1208	1208	89%	0.0	96%
Z94889.1	<i>Nostoc</i> sp. rbcL and rbcX genes (strain NIVA-CYA 124)	1208	1208	90%	0.0	96%
DQ185273.1	<i>Nostoc</i> sp. 'Collema crispum cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1205	1205	89%	0.0	96%

DQ185267.1	Nostoc sp. 'Mollenhauer 1:1-108' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1205	1205	89%	0.0	96%
DQ185287.1	Nostoc sp. 'Peltigera canina 2 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1197	1197	85%	0.0	98%
DQ185291.1	Nostoc sp. 'Lobaria hallii cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1186	1186	89%	0.0	96%
EU877469.1	Nostoc sp. 'Collema furfuraceum (297) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1177	1177	89%	0.0	96%
EF102336.1	Nostoc sp. 'Peltigera neopolydactyla cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1177	1177	89%	0.0	96%
DQ185307.1	Nostoc sp. 'Peltigera membranacea 4 cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1175	1175	89%	0.0	96%
EU877477.1	Nostoc sp. 'Collema polycarpon (889) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) and chaperonin-like protein (rbcX) genes, partial cds	1173	1173	89%	0.0	96%
EU877493.1	Nostoc sp. 'Leptogium corticola (299) cyanobiont' ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds; and chaperonin-like protein (rbcX) gene, complete cds	1160	1160	89%	0.0	95%

805F primer

171: *Stereocaulon vesuvianum* from colony isolate sequenced with 805F

emb[HE814761.2] *Curvibacter* sp. S2H46 partial 16S rRNA gene, isolate S2H46

Length=973, Score = 569 bits (308), Expect = 8e-160, Identities = 308/308 (100%), Gaps = 0/308 (0%), Strand=Plus/Plus

```

Query  1  ACAGGTGCTGCATGGCTGTCGTCAAGTCGTCGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCAATTAGTGCTACATTCAAGTTGGGACTCTAAATGAGACTGCCGGT  120
|||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||
Sbjct  556  ACAGGTGCTGCATGGCTGTCGTCAAGTCGTCGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTCAATTAGTGCTACATTCAAGTTGGGACTCTAAATGAGACTGCCGGT  675
|||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||
Query 121  GACAAACCGGAGGAAGGTGGGATGACGTCAGTCCTCATGCCCTTATAGGTGGGCTACACAGTCATACAATGGCTGGTACAAAGGGTTGCCAACCCCGAGGGGGAGCCAATCCCA  240
|||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||
Sbjct 676  GACAAACCGGAGGAAGGTGGGATGACGTCAGTCCTCATGCCCTTATAGGTGGGCTACACAGTCATACAATGGCTGGTACAAAGGGTTGCCAACCCCGAGGGGGAGCCAATCCCA  795
|||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||
Query 241  TAAAAACCAGTCGTAGTCGGATCGCAGTCGCAACTCGACTCGCTGAAGTCGAATCGCTAGTAATCG  308
|||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||||...|||
Sbjct 796  TAAAACCAGTCGTAGTCGGATCGCAGTCGCAACTCGACTCGCTGAAGTCGAATCGCTAGTAATCG  863

```

Table 11: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 171 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
HE814761.2	<i>Curvibacter</i> sp. S2H46 partial 16S rRNA gene, isolate S2H46	569	569	100%	8,00E-160	100%
HE600662.1	Limnohabitans sp. 2KL-5 genomic DNA containing partial 16S rRNA gene, 16S rRNA-23S rRNA IGS and partial 23S rRNA gene, strain 2KL-5	569	569	100%	8,00E-160	100%
JF174621.1	Uncultured bacterium clone ncd2013f02c1 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HQ453886.1	Uncultured bacterium clone Beta_38_inf_48 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM130006.1	Uncultured bacterium clone SINO942 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129997.1	Uncultured bacterium clone SINO933 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129993.1	Uncultured bacterium clone SINO924 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129968.1	Uncultured bacterium clone SINO892 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129950.1	Uncultured bacterium clone SINO866 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%

HM129912.1	Uncultured bacterium clone SINO800 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129889.1	Uncultured bacterium clone SINO759 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129887.1	Uncultured bacterium clone SINO753 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129878.1	Uncultured bacterium clone SINO743 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129877.1	Uncultured bacterium clone SINO742 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129814.1	Uncultured bacterium clone SINO632 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129812.1	Uncultured bacterium clone SINO628 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129783.1	Uncultured bacterium clone SINO592 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129769.1	Uncultured bacterium clone SINO574 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129727.1	Uncultured bacterium clone SINO510 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
HM129679.1	Uncultured bacterium clone SINO433 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%

172: *Stereocaulon vesuvianum* from colony isolate sequenced with 805F

gb|HM241099.1| Uncultured *Chroococcidiopsis* sp. clone LIM31 16S ribosomal RNA gene, partial sequence

Length=1413, Score = 422 bits (228), Expect = 2e-115, Identities = 293/323 (91%), Gaps = 10/323 (3%), Strand=Plus/Plus

Table 12: Table showing the first 20 hits of the BLAST results for the DNA isolate from sample 172 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
GQ495439.1	Uncultured bacterium clone Bas-7-83 16S ribosomal RNA gene, partial sequence	584	584	99%	3,00E-164	99%
HM641471.1	Uncultured bacterium clone 1026B47 16S ribosomal RNA gene, partial sequence	449	449	100%	1,00E-123	93%
JQ677860.1	Uncultured bacterium clone 09A_H1_09 16S ribosomal RNA gene, partial sequence	433	433	100%	1,00E-118	91%
HM973263.1	Uncultured cyanobacterium clone GE7GXPU01C5016 16S ribosomal RNA gene, partial sequence	431	431	100%	4,00E-118	92%
JQ300239.1	Uncultured bacterium clone SeCo-50 16S ribosomal RNA gene, partial sequence	425	425	100%	2,00E-116	91%
FJ516979.1	Uncultured cyanobacterium clone TDNP_USbc97_68_2_157 16S ribosomal RNA gene, partial sequence	425	425	100%	2,00E-116	91%
HM241099.1	Uncultured Chroococcidiopsis sp. clone LIM31 16S ribosomal RNA gene, partial sequence	422	422	100%	2,00E-115	91%
HM127752.1	Uncultured bacterium clone SINP841 16S ribosomal RNA gene, partial sequence	418	418	100%	3,00E-114	90%
FN811192.1	Uncultured cyanobacterium partial 16S rRNA gene, clone UMAB-cl-8	416	416	100%	1,00E-113	90%
EF019002.1	Uncultured Oxalobacteraceae bacterium clone Amb_16S_1527 16S ribosomal RNA gene, partial sequence	416	416	100%	1,00E-113	90%
EF018791.1	Uncultured Oxalobacteraceae bacterium clone Amb_16S_1256 16S ribosomal RNA gene, partial sequence	416	416	100%	1,00E-113	90%
JQ300416.1	Uncultured bacterium clone SeRi-58 16S ribosomal RNA gene, partial sequence	414	414	100%	4,00E-113	91%
JN014532.1	Uncultured bacterium clone H1860_02 16S ribosomal RNA gene, partial sequence	411	411	100%	5,00E-112	90%
FR848788.1	Uncultured bacterium partial 16S rRNA gene, clone Limnopolar-4.2-B9	411	411	100%	5,00E-112	90%
FR848768.1	Uncultured bacterium partial 16S rRNA gene, clone Limnopolar-4-F4	411	411	100%	5,00E-112	90%
JQ407502.1	Uncultured cyanobacterium clone p660_S5 16S ribosomal RNA gene, partial sequence	409	409	100%	2,00E-111	90%

HQ622719.1	Uncultured bacterium clone IC4001 16S ribosomal RNA gene, partial sequence	409	409	100%	2,00E-111	90%
HQ595228.1	Uncultured bacterium clone IC3146 16S ribosomal RNA gene, partial sequence	409	409	100%	2,00E-111	90%
FN862168.1	Uncultured bacterium partial 16S rRNA gene, clone A330506F02	409	409	100%	2,00E-111	90%
FN862127.1	Uncultured bacterium partial 16S rRNA gene, clone A330506B03	409	409	100%	2,00E-111	90%

184: *Stereocaulon vesuvianum* from colony isolate sequenced with 805F

gb|JQ660208.1| *Sphingomonas* sp. S6-259 16S ribosomal RNA gene, partial sequence

Length=1427, Score = 571 bits (309), Expect = 2e-160, Identities = 313/315 (99%), Gaps = 0/315 (0%), Strand=Plus/Plus

```

Query  1  ACAGGTGCTGCATGGCTGCGTCAGCTCGTGTGAGATTTGGGTAAGTCCCGAACGAGCGAACCCCTGCCCTTAGTTACCATTCAGTTGGGACTCTAAAGGAACGCCGG  120
Sbjct  963  ACAGGTGCTGCATGGCTGCGTCAGCTCGTGTGAGATTTGGGTAAGTCCCGAACGAGCGAACCCCTGCCCTTAGTTACCATTCAGTTGGGACTCTAAAGGAACGCCGG  1082
Query 121  TGATAAGCCGAGGAAGGTGGGATGACGTCAAGTCCTCATGCCCTTACGCCCTGGCTACACGTGCTACAATGGCGTGACAGTGGCAGCAACCCCGGAGGGTAGCTC  240
Sbjct 1083  TGATAAGCCGAGGAAGGTGGGATGACGTCAAGTCCTCATGCCCTTACGCCCTGGCTACACGTGCTACAATGGCGTGACAGTGGCAGCAACCCCGGAGGGTAGCTC  1202
Query 241  CAAAAGCCGCTCAGTCGGATTGTTCTCTGCTACTCGAGAGCATGAAGGCGGAATCGCTAGTAATCGGGATCA  315
Sbjct 1203  CAAAAGCCGCTCAGTCGGATTGTTCTCTGCAAGGAGCATGAAGGCGGAATCGCTAGTAATCGGGATCA  1277

```

Table 13: Table showing the first 20 hits of the BLAST results for the colony isolate from sample 184 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
JQ660208.1	<i>Sphingomonas</i> sp. S6-259 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
JQ660128.1	<i>Sphingomonas</i> sp. S3-190 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
JF175076.1	Uncultured bacterium clone ncd2021a06c1 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
GU980225.1	<i>Sphingomonas</i> sp. CCGE4037 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
HM185849.1	Uncultured bacterium clone HDB_SIOH1081 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
HM275488.1	Uncultured bacterium clone ncd505e07c1 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
EU977595.1	<i>Sphingomonas</i> melonis strain 1P04ME 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
GQ228589.1	<i>Sphingomonas</i> sp. H4TB9 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
FJ192534.1	Uncultured <i>Sphingomonas</i> sp. clone GI5-14-G05 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
AB434710.1	<i>Sphingomonas</i> sp. LP7A gene for 16S ribosomal RNA, partial sequence	571	571	99%	2,00E-160	99%
DQ129619.1	Uncultured bacterium clone AKIW901 16S ribosomal RNA gene, partial sequence	571	571	99%	2,00E-160	99%
HE815158.2	Uncultured endophytic bacterium partial 16S rRNA gene, clone Dkk5F05.M13-F	566	566	99%	1,00E-158	99%
HE815152.2	Uncultured endophytic bacterium partial 16S rRNA gene, clone Dkk5E11.M13-F	566	566	99%	1,00E-158	99%
HE815108.2	Uncultured endophytic bacterium partial 16S rRNA gene, clone Dkk5B01.M13-F	566	566	99%	1,00E-158	99%
HE814795.2	<i>Sphingomonas</i> sp. S3H38b partial 16S rRNA gene, isolate S3H38b	566	566	99%	1,00E-158	99%
HE814791.2	<i>Sphingomonas</i> sp. S3H35a partial 16S rRNA gene, isolate S3H35a	566	566	99%	1,00E-158	99%
HE814672.2	<i>Sphingomonas</i> sp. J3U2 partial 16S rRNA gene, isolate J3U2	566	566	99%	1,00E-158	99%
HE814908.2	Uncultured endophytic bacterium partial 16S rRNA gene, clone DKK2H08.M13-F	566	566	99%	1,00E-158	99%
HE815093.2	Uncultured endophytic bacterium partial 16S rRNA gene, clone Dkk4H10.M13-F	566	566	99%	1,00E-158	99%
HE814659.1	<i>Sphingomonas</i> sp. S2U11 partial 16S rRNA gene, isolate S2U11	566	566	99%	1,00E-158	99%

1: *Rhytidadelphus squarrorus* from colony isolate sequenced with 805F primer

gb|JQ764998.1| *Agrobacterium* sp. BE516 16S ribosomal RNA gene, partial sequence
Length=1451, Score = 721 bits (390), Expect = 0.0, Identities = 390/390 (100%), Gaps = 0/390 (0%), Strand=Plus/Plus

```

Query  1  ACAGGTGGCATGGCTGTCAGCTCGTGGAGATGTTGGGTTAAGTCCCGAACGAGCGAACCCCTCGCCCTAGTTGCCAGCATTCAAGGGACTGCCGG  120
          ||||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  982  ACAGGTGGCATGGCTGTCAGCTCGTGGAGATGTTGGGTTAAGTCCCGAACGAGCGAACCCCTCGCCCTAGTTGCCAGCATTCAAGGGACTGCCGG  1101
          ||||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Query  121  TGATAAGCGAGAGGAAGGTGGGATGACGTCAGCTCTCATGGCCCTACGGGCTGGGCTACACAGTGCACATGGTGTGACAGTGGCGAGACCGGAGGTCGAGCTATCT  240
          ||||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1102  TGATAAGCGAGAGGAAGGTGGGATGACGTCAGCTCTCATGGCCCTACGGGCTGGGCTACACAGTGCACATGGTGTGACAGTGGCGAGACCGGAGGTCGAGCTAATCT  1221
          ||||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Query  241  CCAAAAGCCATCTCAGTCGGATTGCACTCGAAGTGCTGAAGTTGGAATCGCTAGTAATCGCAGATCAGCATGCTGCGGTGAATACGTTCCGGGCTTGTACACACCGCCCGTACACCATGGGAGTTGGTTTACCGAA  390
          ||||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1222  CCAAAAGCCATCTCAGTCGGATTGCACTCGAAGTGCTGAAGTTGGAATCGCTAGTAATCGCAGATCAGCATGCTGCGGTGAATACGTTCCGGGCTTGTACACACCGCCCGTACACCATGGGAGTTGGTTTACCGAA  1371
          ||||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

```

Table 14: Table showing the first 20 hits of the BLAST results for the colony isolate from moss sample 1 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
JQ764998.1	Agrobacterium sp. BE516 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
JQ396566.1	Rhizobium sp. MN6-12 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
JQ396565.1	Rhizobium sp. MN6-11 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
JQ396533.1	Rhizobium sp. MN2-11 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
JQ342849.1	Rhizobium giardinii strain DSS9 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
JN648903.1	Rhizobium sp. BLR12 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
JN204133.1	Uncultured bacterium clone CR16_1 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
HQ121108.1	Uncultured bacterium isolate 1112863845162 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
AB680780.1	Agrobacterium vitis gene for 16S rRNA, partial sequence, strain: NBRC 15143	721	721	100%	0.0	100%
AB680777.1	Agrobacterium vitis gene for 16S rRNA, partial sequence, strain: NBRC 15140	721	721	100%	0.0	100%
AB680778.1	Agrobacterium vitis gene for 16S rRNA, partial sequence, strain: NBRC 15141	721	721	100%	0.0	100%
AB680779.1	Agrobacterium vitis gene for 16S rRNA, partial sequence, strain: NBRC 15142	721	721	100%	0.0	100%
AB682469.1	Rhizobium giardinii gene for 16S rRNA, partial sequence, strain: NBRC 107135	721	721	100%	0.0	100%
JN662528.1	Rhizobium sp. A21.1 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
AB636290.1	Rhizobium sp. Rb122 gene for 16S rRNA, partial sequence	721	721	100%	0.0	100%
JN622152.1	Mesorhizobium sp. CCNWGS0174 16S ribosomal RNA (rrs) gene, partial sequence	721	721	100%	0.0	100%
JN367022.1	Uncultured alpha proteobacterium clone SeqEEZR227 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
JN366966.1	Uncultured alpha proteobacterium clone SeqEEZR169 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
HQ218484.1	Uncultured bacterium clone N-41 16S ribosomal RNA gene, partial sequence	721	721	100%	0.0	100%
FN546874.1	Rhizobium sp. ORAII 13a2 partial 16S rRNA gene, strain ORAII 13a2	721	721	100%	0.0	100%

3: *Rhytidadelphus squarrorus* from DNA isolate sequenced with 805F primer

gb|FJ790603.1| Uncultured *Chroococcidiopsis* sp. clone QB23 16S ribosomal RNA gene, partial sequence
Length=1319, Score = 569 bits (308), Expect = 8e-160, Identities = 308/308 (100%), Gaps = 0/308 (0%), Strand=Plus/Plus

```

Query  1  ACAGGTGGCATGGCTGTCAGCTCGTGGCTAAGGTGGGTTAAGTCCCGAACGAGCGAACCCCTCGTGTAGTTGCCATCATTAGTTGGAACCTAAACAGACTGCCGG  120
          ||||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct  973  ACAGGTGGCATGGCTGTCAGCTCGTGGCTAAGGTGGGTTAAGTCCCGAACGAGCGAACCCCTCGTGTAGTTGCCATCATTAGTTGGAACCTAAACAGACTGCCGG  1092
          ||||||||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

```

```

Query 121 TGATAAGCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCTTACGCCCTGGCGACACACGTCTACAATGGCGGGACAAAGGGTTGCAGCCCGGAGGGCAAGCTAACCTC 240
|||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Sbjct 1093 TGATAAGCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCTTACGCCCTGGCGACACACGTCTACAATGGCGGGACAAAGGGTTGCAGCCCGGAGGGCAAGCTAACCTC 1212
|||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Query 241 AAAAACCGGCCCTAGTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGAATCGCTAGTAATC 308
|||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Sbjct 1213 AAAAACCGGCCCTAGTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGAATCGCTAGTAATC 1280
|||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||

```

Table 15: Table showing the first 20 hits of the BLAST results for the DNA isolate from moss sample 3 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
AB696414.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN42	569	569	100%	8,00E-160	100%
AB696411.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN38	569	569	100%	8,00E-160	100%
AB696409.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN36	569	569	100%	8,00E-160	100%
AB696402.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN27	569	569	100%	8,00E-160	100%
AB696395.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN19	569	569	100%	8,00E-160	100%
AB696381.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN1	569	569	100%	8,00E-160	100%
AB696365.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN36	569	569	100%	8,00E-160	100%
AB696355.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN26	569	569	100%	8,00E-160	100%
AB696345.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN16	569	569	100%	8,00E-160	100%
AB696343.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN14	569	569	100%	8,00E-160	100%
AB696335.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN5	569	569	100%	8,00E-160	100%
AB696334.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN4	569	569	100%	8,00E-160	100%
AB696315.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN42	569	569	100%	8,00E-160	100%
AB696311.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN38	569	569	100%	8,00E-160	100%
AB696310.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN37	569	569	100%	8,00E-160	100%
AB696308.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN35	569	569	100%	8,00E-160	100%
AB696307.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN34	569	569	100%	8,00E-160	100%
JQ696629.1	Uncultured bacterium clone 4783682 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
GQ495441.1	Uncultured bacterium clone Bas-7-85 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%
FJ790603.1	Uncultured Chroococcidiopsis sp. clone QB23 16S ribosomal RNA gene, partial sequence	569	569	100%	8,00E-160	100%

6: *Rhytidadelphus triquetrus* from DNA isolate sequenced with 805F primer

gb|FJ790603.1| Uncultured *Chroococcidiopsis* sp. clone QB23 16S ribosomal RNA gene, partial sequence
Length=1319, Score = 569 bits (308), Expect = 8e-160, Identities = 308/308 (100%), Gaps = 0/308 (0%), Strand=Plus/Plus

```

Query 1 ACAGGTGGTGCATGGCTGCGTCAGCTCGTCCGTAAGGTGTTGGTTAAGTCCCACAGCGCAACCCCTCGTGTAGTTGCCATCATTAGTTGGAACCTAAACAGACTGCCG 120
|||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Sbjct 973 ACAGGTGGTGCATGGCTGCGTCAGCTCGTCCGTAAGGTGTTGGTTAAGTCCCACAGCGCAACCCCTCGTGTAGTTGCCATCATTAGTTGGAACCTAAACAGACTGCCG 1092
|||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Query 121 TGATAAGCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCTTACGCCCTGGCGACACACGTCTACAATGGCGGGACAAAGGGTTGCAGCCCGGAGGGCAAGCTAACCTC 240
|||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||||.....|||
Sbjct 1093 TGATAAGCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCTTACGCCCTGGCGACACACGTCTACAATGGCGGGACAAAGGGTTGCAGCCCGGAGGGCAAGCTAACCTC 1212
|||.....|||||.....|||||.....|||||.....|||||.....|||
Query 241 AAAAACCGGCCCTAGTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGAATCGCTAGTAATC 308
|||.....|||||.....|||||.....|||||.....|||
Sbjct 1213 AAAAACCGGCCCTAGTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGAATCGCTAGTAATC 1280
|||.....|||||.....|||

```

Table 16: Table showing the first 20 hits of the BLAST results for the DNA isolate from moss sample 6 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identity
AB696414.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN42	564	564	100%	4,00E-158	99%
AB696411.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN38	564	564	100%	4,00E-158	99%
AB696409.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN36	564	564	100%	4,00E-158	99%
AB696402.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN27	564	564	100%	4,00E-158	99%
AB696395.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN19	564	564	100%	4,00E-158	99%
AB696381.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN1	564	564	100%	4,00E-158	99%
AB696365.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN36	564	564	100%	4,00E-158	99%
AB696355.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN26	564	564	100%	4,00E-158	99%
AB696345.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN16	564	564	100%	4,00E-158	99%
AB696343.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN14	564	564	100%	4,00E-158	99%
AB696335.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN5	564	564	100%	4,00E-158	99%
AB696334.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN4	564	564	100%	4,00E-158	99%
AB696315.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN42	564	564	100%	4,00E-158	99%
AB696311.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN38	564	564	100%	4,00E-158	99%
AB696310.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN37	564	564	100%	4,00E-158	99%
AB696308.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN35	564	564	100%	4,00E-158	99%
AB696307.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN34	564	564	100%	4,00E-158	99%
JQ696629.1	Uncultured bacterium clone 4783682 16S ribosomal RNA gene, partial sequence	564	564	100%	4,00E-158	99%
JF776939.1	Uncultured bacterium clone QZ-J51 16S ribosomal RNA gene, partial sequence	564	564	100%	4,00E-158	99%
GQ495441.1	Uncultured bacterium clone Bas-7-85 16S ribosomal RNA gene, partial sequence	564	564	100%	4,00E-158	99%
FJ790603.1	Uncultured Chroococcidiopsis sp. clone QB23 16S ribosomal RNA gene, partial sequence	564	564	100%	4,00E-158	99%

7: *Racomitrium canescens* from DNA isolate sequenced with 805F primer

gb|FJ790603.1| Uncultured *Chroococcidiopsis* sp. clone QB23 16S ribosomal RNA gene, partial sequence
Length=1319, Score = 569 bits (308), Expect = 8e-160, Identities = 308/308 (100%), Gaps = 0/308 (0%), Strand=Plus/Plus

Query	1	ACAGGTGGTGCATGGCTGTCGTCAGCTCGGCCGAAGGGTTAAGTCCCAACGAGCGAACCTCGTGTAGTGCATCTTGGAAACCTAAACAGACTGCCGG	120
Sbjct	973	ACAGGTGGTGCATGGCTGTCGTCAGCTCGGCCGAAGGGTTAAGTCCCAACGAGCGAACCTCGTGTAGTGCATCTTGGAAACCTAAACAGACTGCCGG	1092
Query	121	TGATAAGCCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCTTAACGGCTGGCGACACAGTGTACAATGGCCGGACAAGGGTTGCACCCGGAGGGCAAGCTAACCTC	240
Sbjct	1093	TGATAAGCCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCTTAACGGCTGGCGACACAGTGTACAATGGCCGGACAAGGGTTGCACCCGGAGGGCAAGCTAACCTC	1212
Query	241	AAAAACCCGCCCTCAGTCGATTGCAAGGCTGCAACTCGCTGCATGAAGCCGGAATCGCTAGTAACTC	308
Sbjct	1213	AAAAACCCGCCCTCAGTCGATTGCAAGGCTGCAACTCGCTGCATGAAGCCGGAATCGCTAGTAACTC	1280

Table 17: Table showing the first 20 hits of the BLAST results for the DNA isolate from moss sample 7 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identity
AB696414.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN42	571	571	100%	2,00E-160	100%
AB696411.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN38	571	571	100%	2,00E-160	100%
AB696409.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN36	571	571	100%	2,00E-160	100%
AB696402.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN27	571	571	100%	2,00E-160	100%
AB696395.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN19	571	571	100%	2,00E-160	100%

AB696381.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN1	571	571	100%	2,00E-160	100%
AB696365.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN36	571	571	100%	2,00E-160	100%
AB696355.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN26	571	571	100%	2,00E-160	100%
AB696345.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN16	571	571	100%	2,00E-160	100%
AB696343.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN14	571	571	100%	2,00E-160	100%
AB696335.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN5	571	571	100%	2,00E-160	100%
AB696334.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN4	571	571	100%	2,00E-160	100%
AB696315.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN42	571	571	100%	2,00E-160	100%
AB696311.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN38	571	571	100%	2,00E-160	100%
AB696310.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN37	571	571	100%	2,00E-160	100%
AB696308.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN35	571	571	100%	2,00E-160	100%
AB696307.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN34	571	571	100%	2,00E-160	100%
JQ696629.1	Uncultured bacterium clone 4783682 16S ribosomal RNA gene, partial sequence	571	571	100%	2,00E-160	100%
GQ495441.1	Uncultured bacterium clone Bas-7-85 16S ribosomal RNA gene, partial sequence	571	571	100%	2,00E-160	100%
FJ790603.1	Uncultured Chroococcidiopsis sp. clone QB23 16S ribosomal RNA gene, partial sequence	571	571	100%	2,00E-160	100%

8: *Racomitrium canescens* from DNA isolate sequenced with 805F primer

gb|JF790603.1| Uncultured *Chroococcidiopsis* sp. clone QB23 16S ribosomal RNA gene, partial sequence
Length=1319, Score = 571 bits (309), Expect = 2e-160, Identities = 309/309 (100%), Gaps = 0/309 (0%), Strand=Plus/Plus

Query	1	ACAGGTGGTGCATGGCTGTCGCTCAGCTCGTGGCGTAAGGTGGTTAAGTCCCCAACGAGCGAACCCCTCGTGTAGTTGCCCACATTTAGTTGGAAACCTAAACAGACTGCCGG	120
Sbjct	973	ACAGGTGGTGCATGGCTGTCGCTCAGCTCGTGGCGTAAGGTGGTTAAGTCCCCAACGAGCGAACCCCTCGTGTAGTTGCCCACATTTAGTTGGAAACCTAAACAGACTGCCGG	1092
Query	121	TGATAAGCCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCTTACGCCCTGGCGACACAGTGTACAATGCCGGGACAAGGGTTGCGACCCCGGAGGGCAAGCTAACCTC	240
Sbjct	1093	TGATAAGCCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCTTACGCCCTGGCGACACAGTGTACAATGCCGGGACAAGGGTTGCGACCCCGGAGGGCAAGCTAACCTC	1212
Query	241	AAAAACCCGCCCTCAGTCGATTGCAAGGCTGCAACTCGCCCTGCAAGGCCGGATCGCTAGTAATCG	309
Sbjct	1213	AAAAACCCGCCCTCAGTCGATTGCAAGGCTGCAACTCGCCCTGCAAGGCCGGATCGCTAGTAATCG	1281

Table 18: Table showing the first 20 hits of the BLAST results for the DNA isolate from moss sample 8 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
AB696414.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN42	571	571	100%	2,00E-160	100%
AB696411.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN38	571	571	100%	2,00E-160	100%
AB696409.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN36	571	571	100%	2,00E-160	100%
AB696402.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN27	571	571	100%	2,00E-160	100%
AB696395.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN19	571	571	100%	2,00E-160	100%
AB696381.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN1	571	571	100%	2,00E-160	100%
AB696365.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN36	571	571	100%	2,00E-160	100%
AB696355.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN26	571	571	100%	2,00E-160	100%
AB696345.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN16	571	571	100%	2,00E-160	100%
AB696343.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN14	571	571	100%	2,00E-160	100%
AB696335.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN5	571	571	100%	2,00E-160	100%
AB696334.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN4	571	571	100%	2,00E-160	100%
AB696315.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN42	571	571	100%	2,00E-160	100%

AB696311.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN38	571	571	100%	2,00E-160	100%
AB696310.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN37	571	571	100%	2,00E-160	100%
AB696308.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN35	571	571	100%	2,00E-160	100%
AB696307.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN34	571	571	100%	2,00E-160	100%
JQ696629.1	Uncultured bacterium clone 4783682 16S ribosomal RNA gene, partial sequence	571	571	100%	2,00E-160	100%
GQ495441.1	Uncultured bacterium clone Bas-7-85 16S ribosomal RNA gene, partial sequence	571	571	100%	2,00E-160	100%
FJ790603.1	Uncultured Chroococcidiopsis sp. clone QB23 16S ribosomal RNA gene, partial sequence	571	571	100%	2,00E-160	100%

9: *Rhytidadelphus squarrorus* from DNA isolate sequence with 805F primer

gb|EF522448.1| Uncultured cyanobacterium clone OCLS087 16S ribosomal RNA gene, partial sequence
Length=876, Score = 470 bits (254), Expect = 7e-130, Identities = 254/254 (100%), Gaps = 0/254 (0%), Strand=Plus/Plus

```
Query   6  TGTTCGTCAGCTCGTCCGTAAGGTGGTTAAGTCCCGAACGAGGCCAACCTCGTGTAGTTGCATCATTTAGTTGGAACCTAAACAGACTGCCGTGATAAGCCGGAGGAA  125
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  537  TGTCGTCAGCTCGTCCGTAAGGTGGTTAAGTCCCGAACGAGGCCAACCTCGTGTAGTTGCATCATTTAGTTGGAACCTAAACAGACTGCCGTGATAAGCCGGAGGAA  656
Query  126  GGTGAGGATGACGTCAGTCAGCATGCCCTTACGCCCTGGCGACACAGTGTACAATGGCGGGACAAAGGGTTGCGACCCCGCAGGGGAAGCTAACCTAAAACCGGCCCTAGTCGGATTGCAGGC  259
|||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  657  GGTGAGGATGACGTCAGTCAGCATGCCCTTACGCCCTGGCGACACAGTGTACAATGGCGGGACAAAGGGTTGCGACCCCGCAGGGGAAGCTAACCTAAAACCGGCCCTAGTCGGATTGCAGGC  790
```

Table 19: Table showing the first 20 hits of the BLAST results for the DNA isolate from moss sample 9 sequenced with 805F primer

Accession	Description	Max score	Total score	Query coverage	E value	Max identities
EF522448.1	Uncultured cyanobacterium clone OCLS087 16S ribosomal RNA gene, partial sequence	470	470	94%	7,00E-130	100%
AB696414.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN42	464	464	94%	3,00E-128	99%
AB696411.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN38	464	464	94%	3,00E-128	99%
AB696409.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN36	464	464	94%	3,00E-128	99%
AB696402.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN27	464	464	94%	3,00E-128	99%
AB696395.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN19	464	464	94%	3,00E-128	99%
AB696390.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN14	464	464	94%	3,00E-128	99%
AB696381.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L3CLN1	464	464	94%	3,00E-128	99%
AB696373.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN44	464	464	94%	3,00E-128	99%
AB696372.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN43	464	464	94%	3,00E-128	99%
AB696365.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN36	464	464	94%	3,00E-128	99%
AB696355.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN26	464	464	94%	3,00E-128	99%
AB696348.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN19	464	464	94%	3,00E-128	99%
AB696345.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN16	464	464	94%	3,00E-128	99%
AB696343.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN14	464	464	94%	3,00E-128	99%
AB696335.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN5	464	464	94%	3,00E-128	99%
AB696334.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L2CLN4	464	464	94%	3,00E-128	99%
AB696315.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN42	464	464	94%	3,00E-128	99%
AB696311.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN38	464	464	94%	3,00E-128	99%
AB696310.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN37	464	464	94%	3,00E-128	99%
AB696308.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN35	464	464	94%	3,00E-128	99%
AB696307.1	Uncultured bacterium gene for 16S rRNA, partial sequence, clone: L1CLN34	464	464	94%	3,00E-128	99%
JQ696629.1	Uncultured bacterium clone 4783682 16S ribosomal RNA gene, partial sequence	464	464	94%	3,00E-128	99%
JF776939.1	Uncultured bacterium clone QZ-J51 16S ribosomal RNA gene, partial sequence	464	464	94%	3,00E-128	99%

HQ197648.1	Uncultured bacterium clone 3-1AU1C1 16S ribosomal RNA gene, partial sequence	464	464	94%	3,00E-128	99%
GQ495441.1	Uncultured bacterium clone Bas-7-85 16S ribosomal RNA gene, partial sequence	464	464	94%	3,00E-128	99%
FJ790603.1	Uncultured Chroococcidiopsis sp. clone QB23 16S ribosomal RNA gene, partial sequence	464	464	94%	3,00E-128	99%

The figures show the alignment of the sequences for the samples isolates.

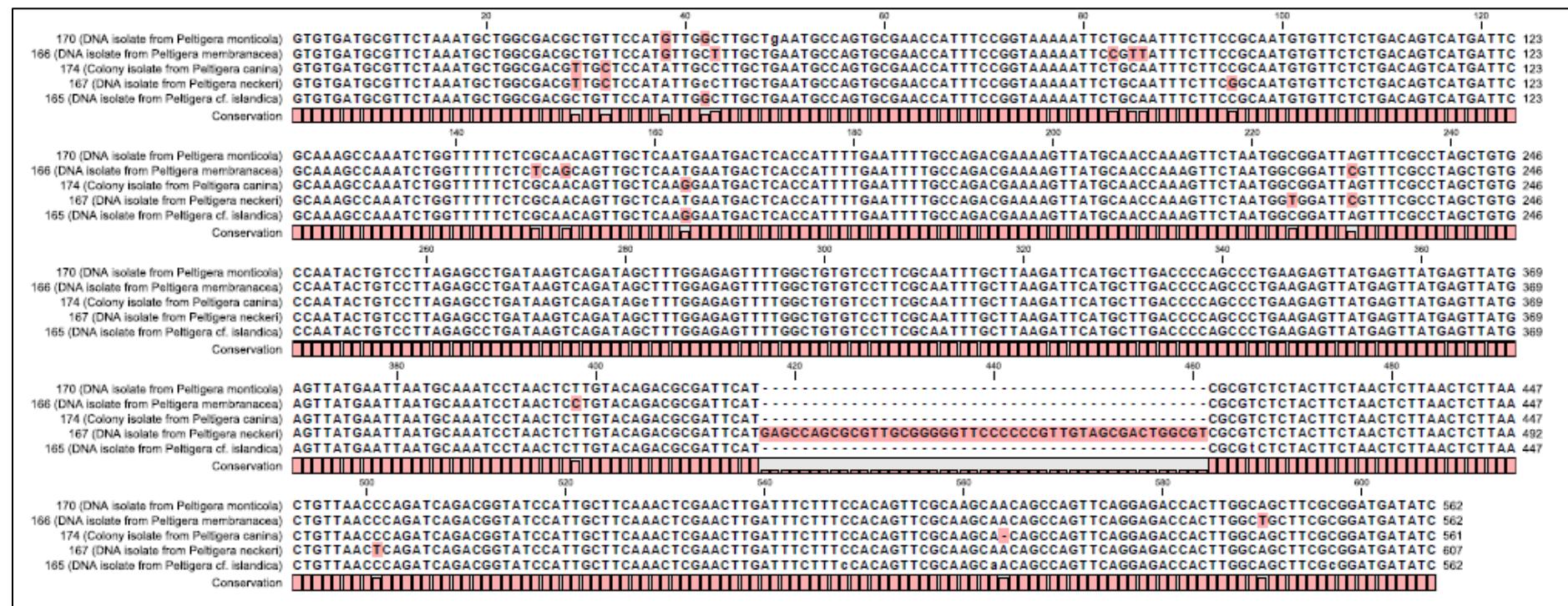


Figure 1: Aligned sequences of the isolates from samples 165, 166, 167, 173 and 174

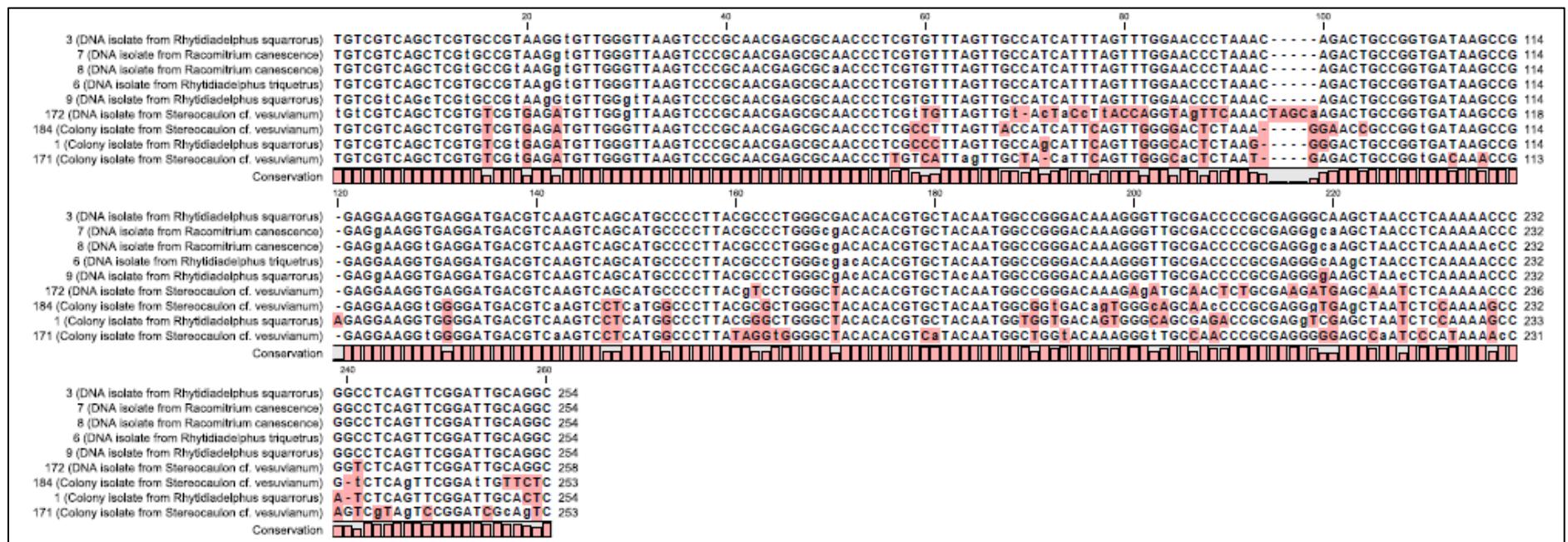


Figure 2: Aligned sequences of the isolates from lichen samples 171, 172 and 184 and the moss samples 1, 3, 6, 7, 8 and 9

Appendix D

Pictures colonies from lichen annd moss samples



Figure 1: Colony isolates from *Peltigera canina*



Figure 2: Colony isolates from *Peltigera neckeri*

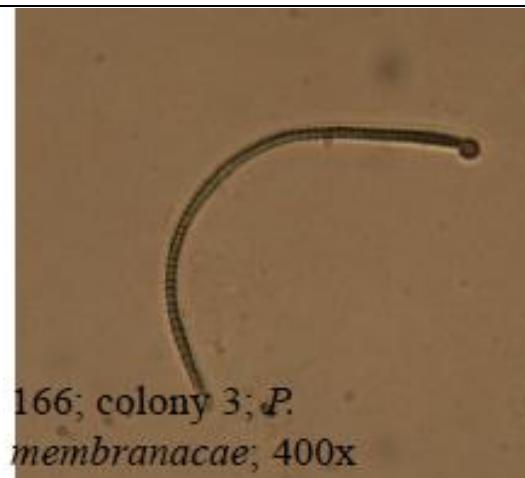


Figure 3: Colony isolates from *Peltigera membranacea*

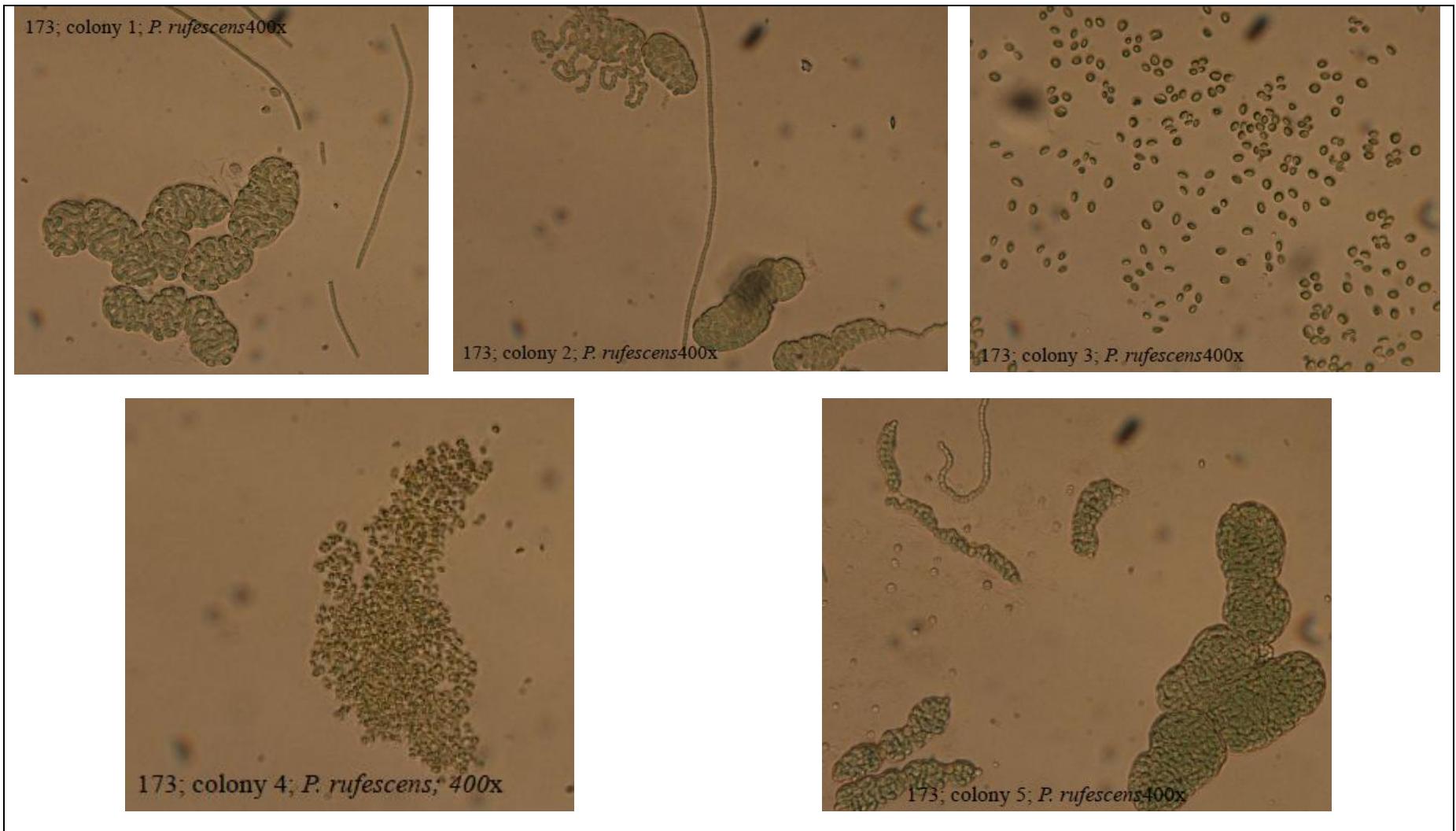


Figure 4: Colony isolates from *Peltigera rufescens*

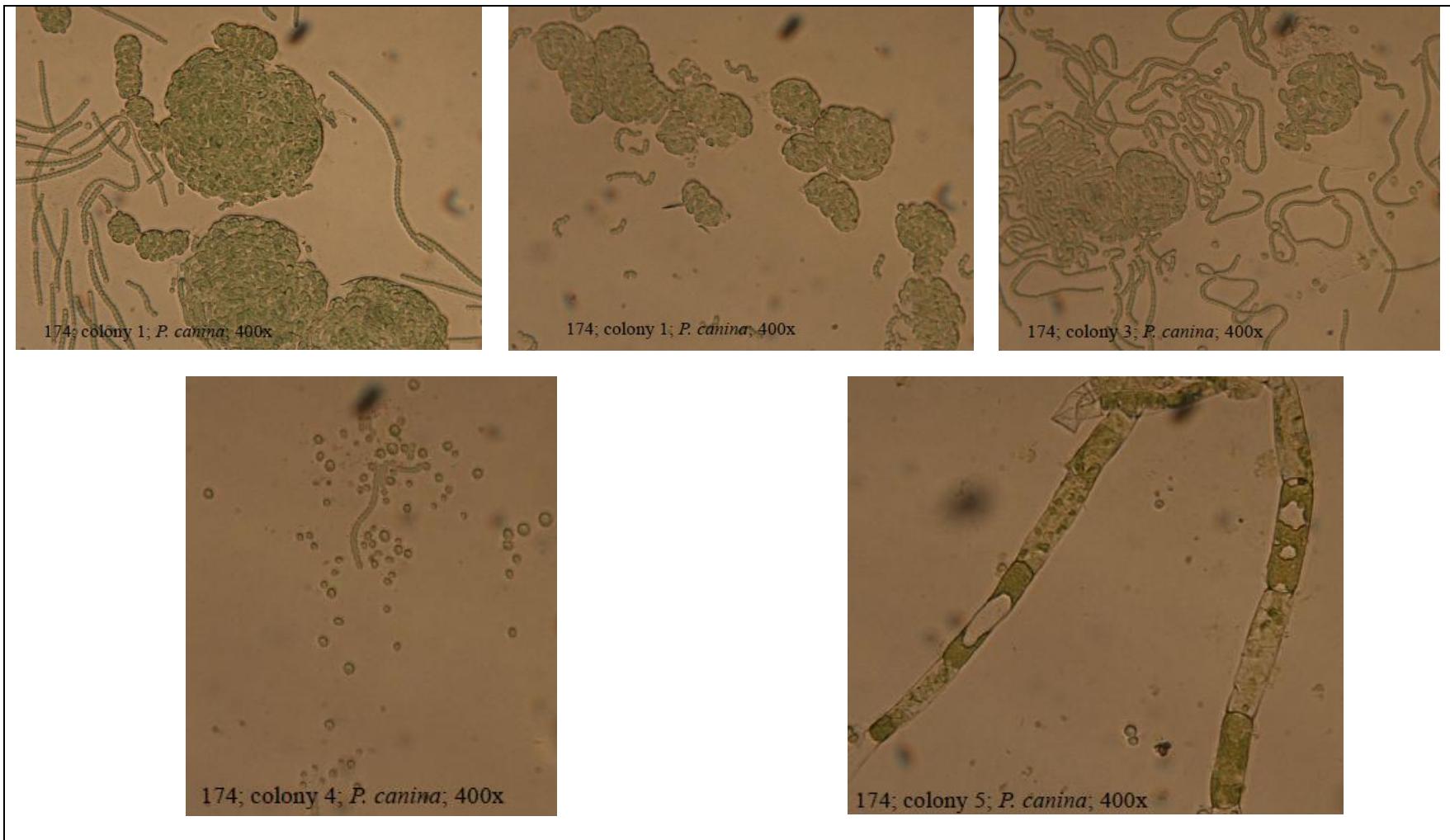
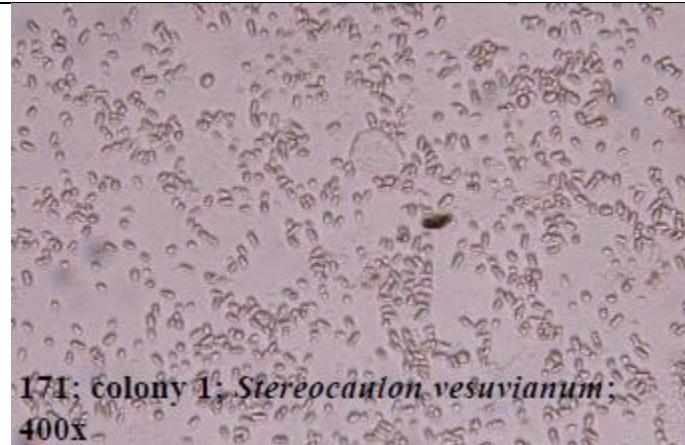
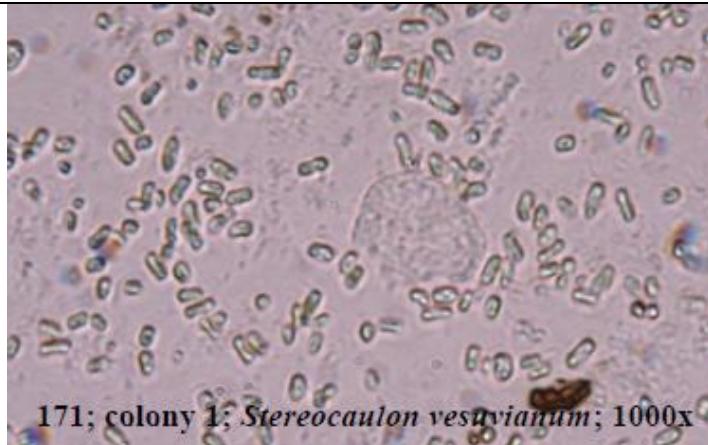


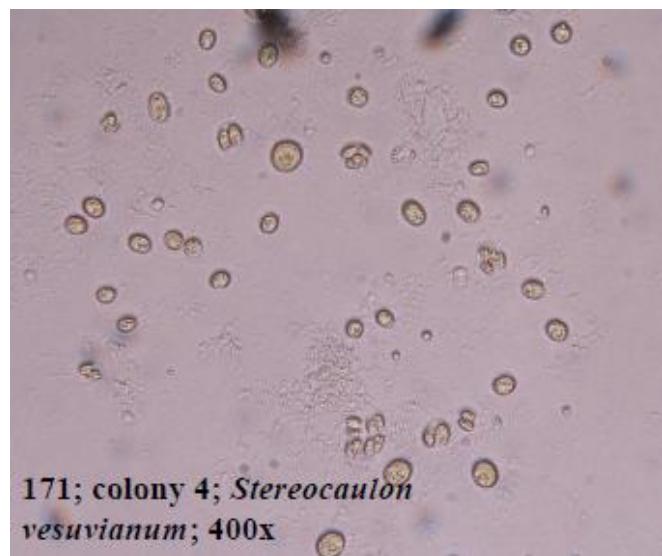
Figure 5: Colony isolates from *Peltigera canina*



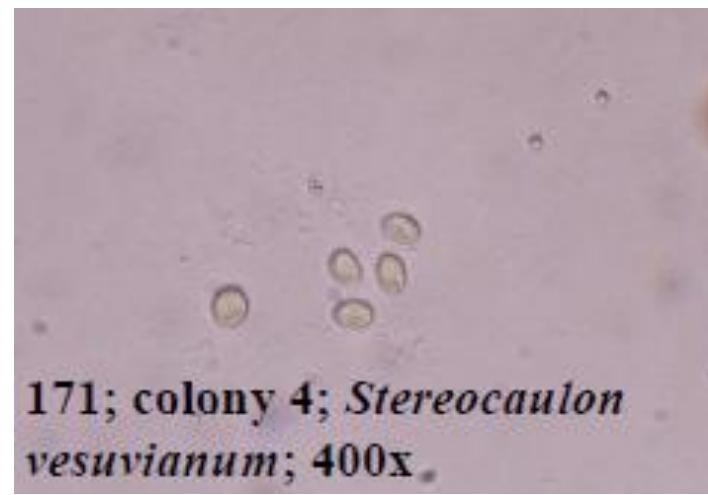
171; colony 1; *Stereocaulon vesuvianum*; 400x



171; colony 1; *Stereocaulon vesuvianum*; 1000x



171; colony 4; *Stereocaulon vesuvianum*; 400x



171; colony 4; *Stereocaulon vesuvianum*; 400x

Figure 6: Colony isolates from *Stereocaulon vesuvianum*

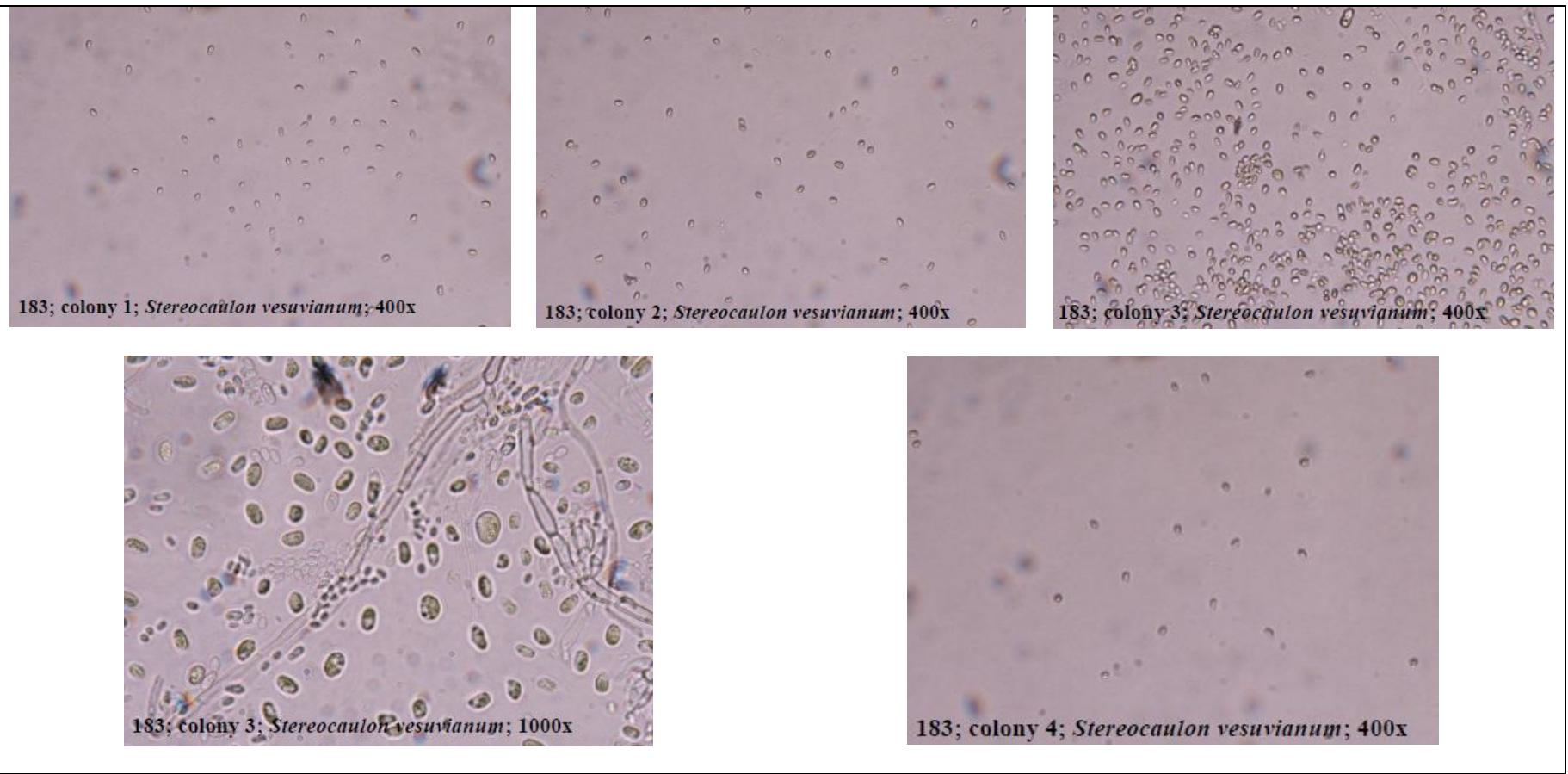
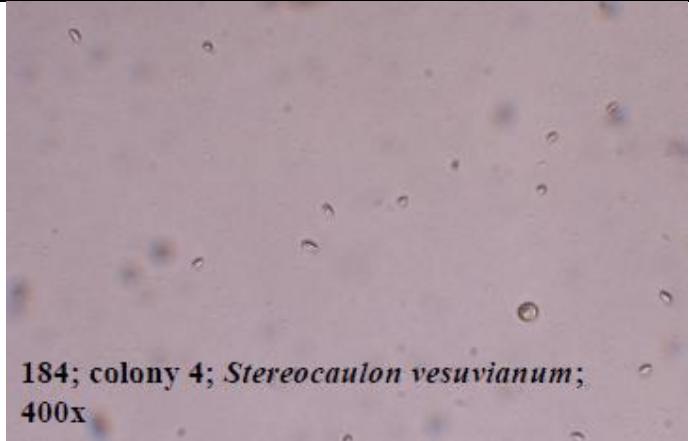


Figure 7: Colony isolates from *Stereocaulon vesuvianum*

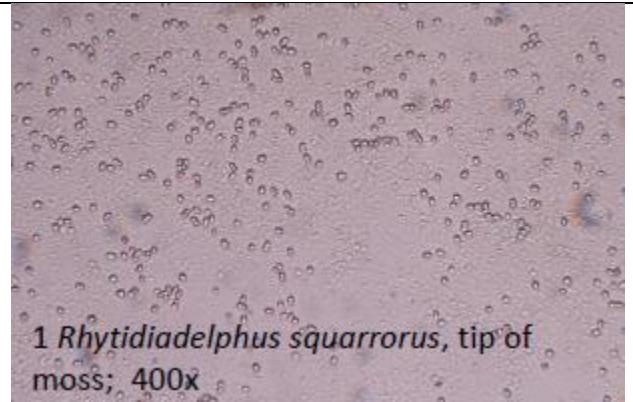


184; colony 1; *Stereocaulon vesuvianum*;
400x

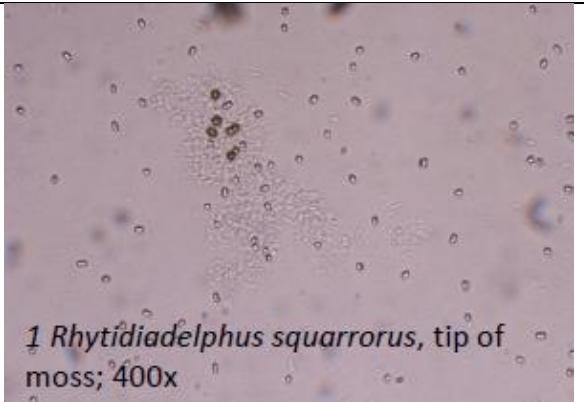


184; colony 4; *Stereocaulon vesuvianum*;
400x

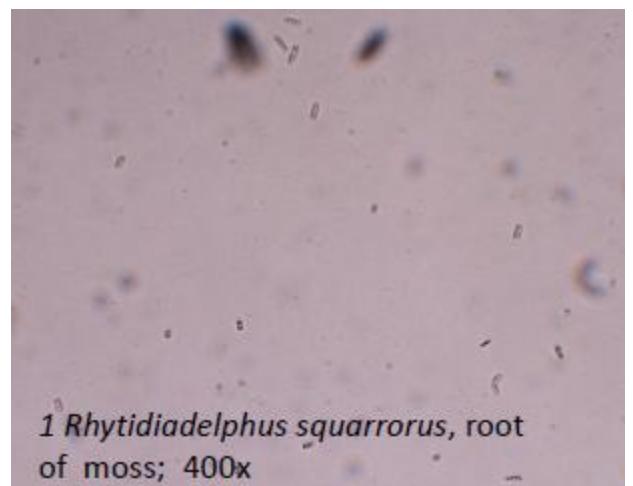
Figure 8: Colony isolates from *Stereocaulon vesuvianum*



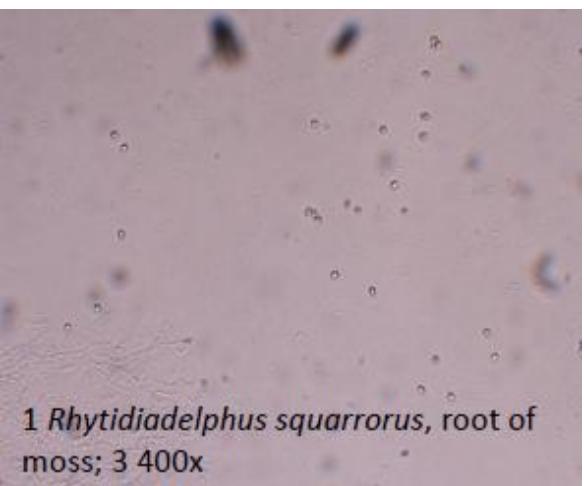
1 *Rhytidadelphus squarrorus*, tip of
moss; 400x



1 *Rhytidadelphus squarrorus*, tip of
moss; 400x



1 *Rhytidadelphus squarrorus*, root
of moss; 400x



1 *Rhytidadelphus squarrorus*, root of
moss; 3400x

Figure 9: Colony isolates from *Rhytidadelphus squarrorus*

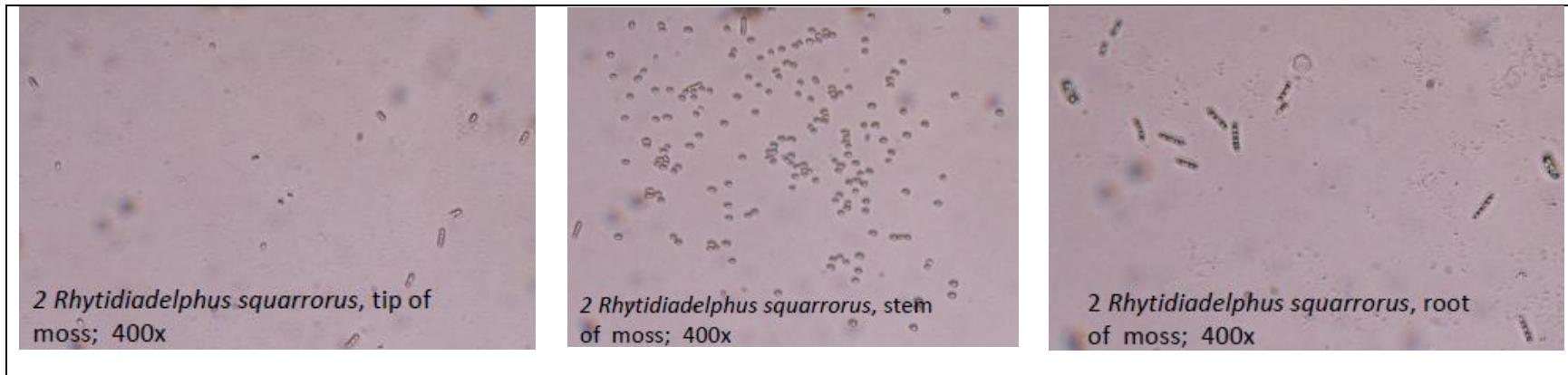


Figure 10: Colony isolates from *Rhytidadelphus squarrorus*

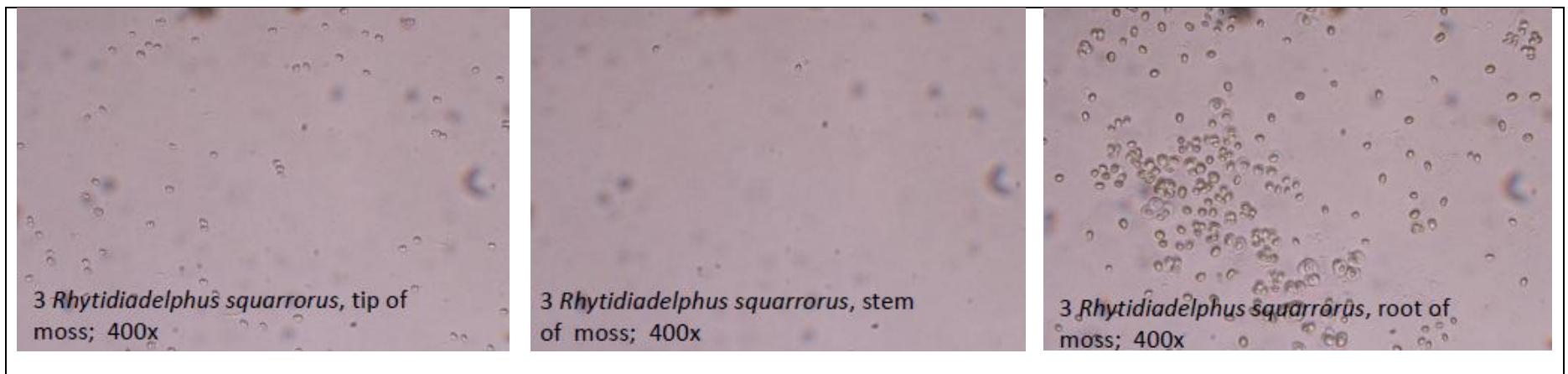


Figure 11: Colony isolates from *Rhytidadelphus squarrorus*

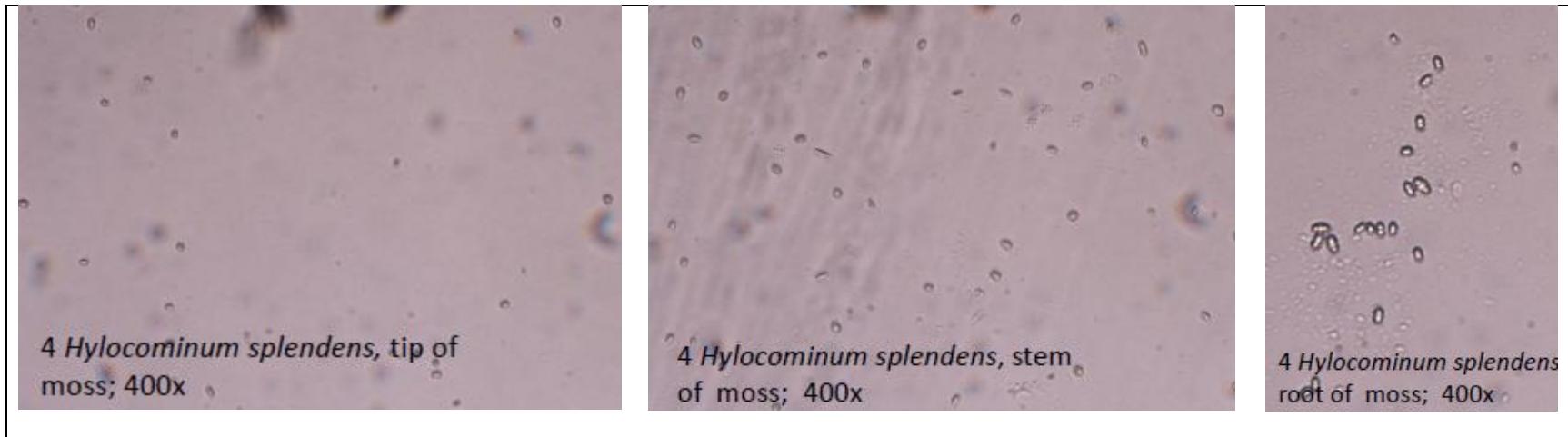


Figure 12: Colony isolates from *Hylocomium splendens*

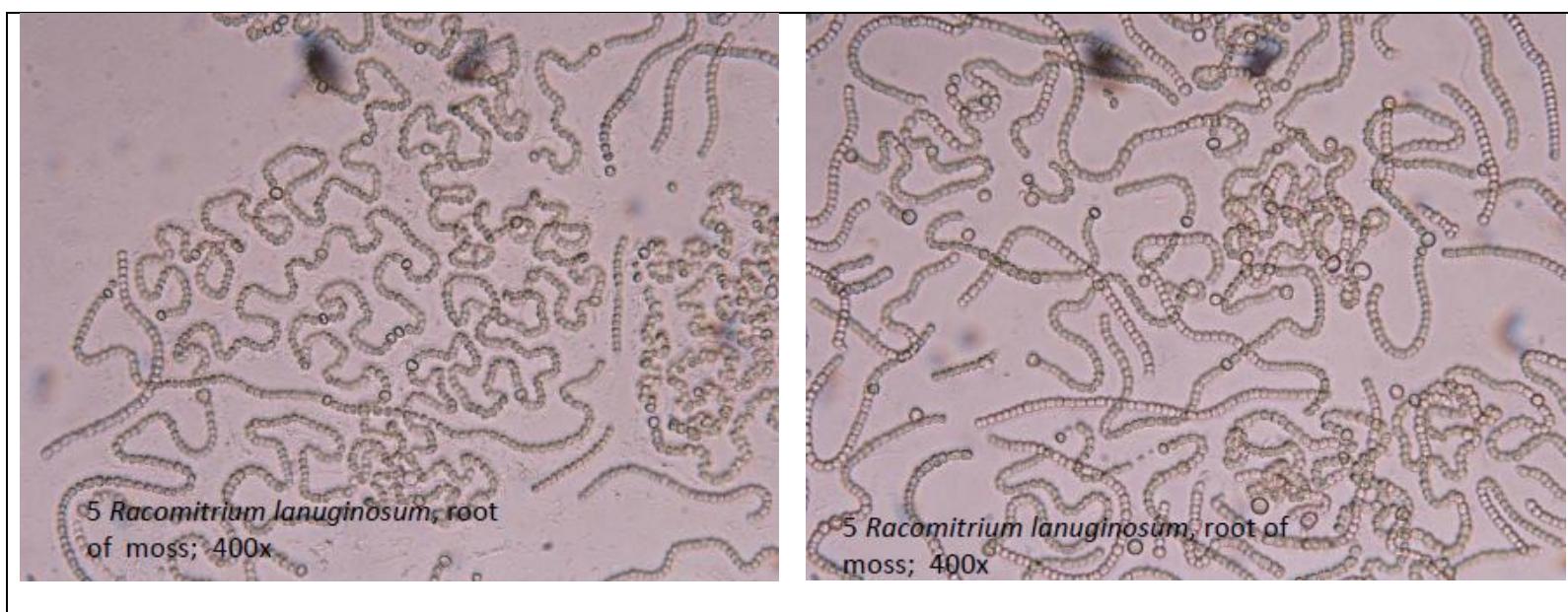


Figure 13: Colony isolates from *Racomitrium lanuginosum*

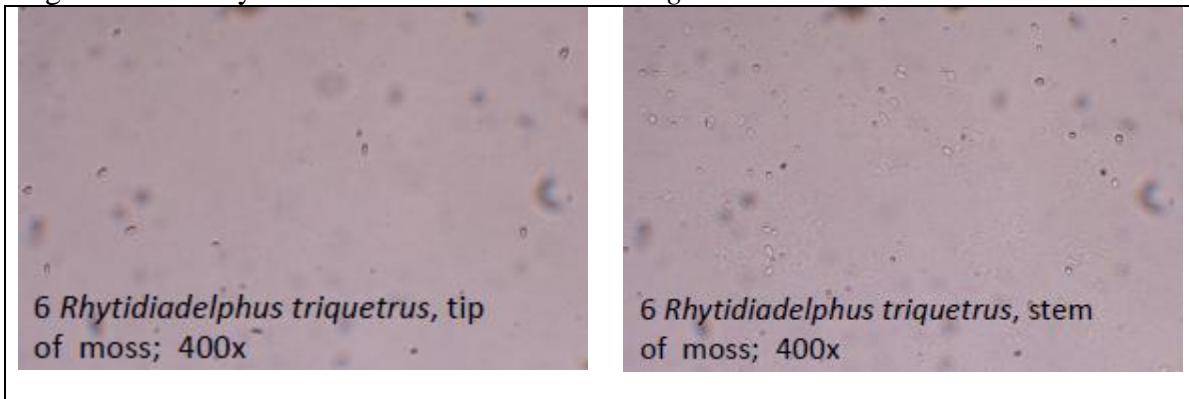


Figure 14: Colony isolates from *Rhytidadelphus triquetrus*

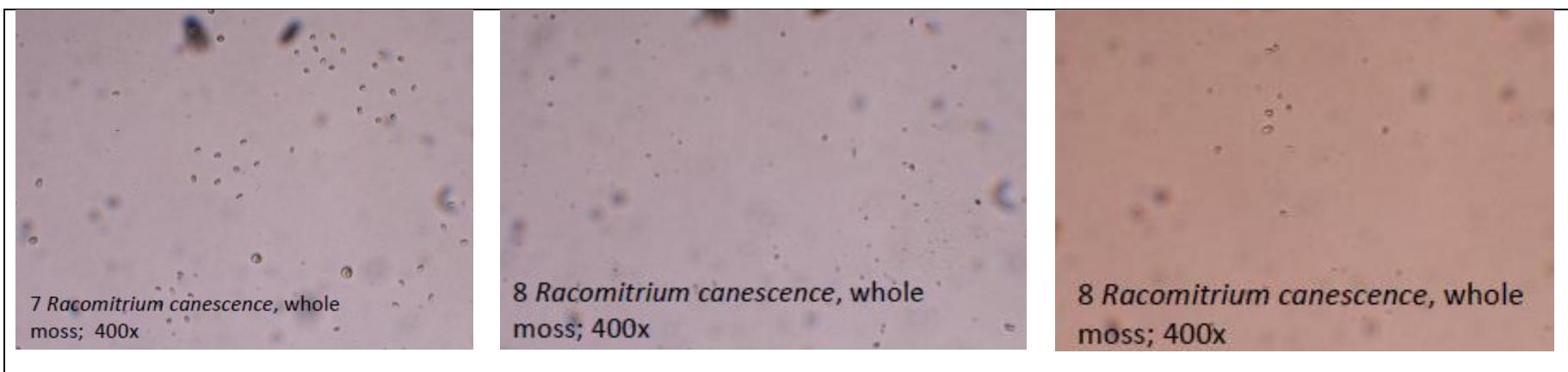


Figure 15: Colony isolates from *Racomitrium canescens*

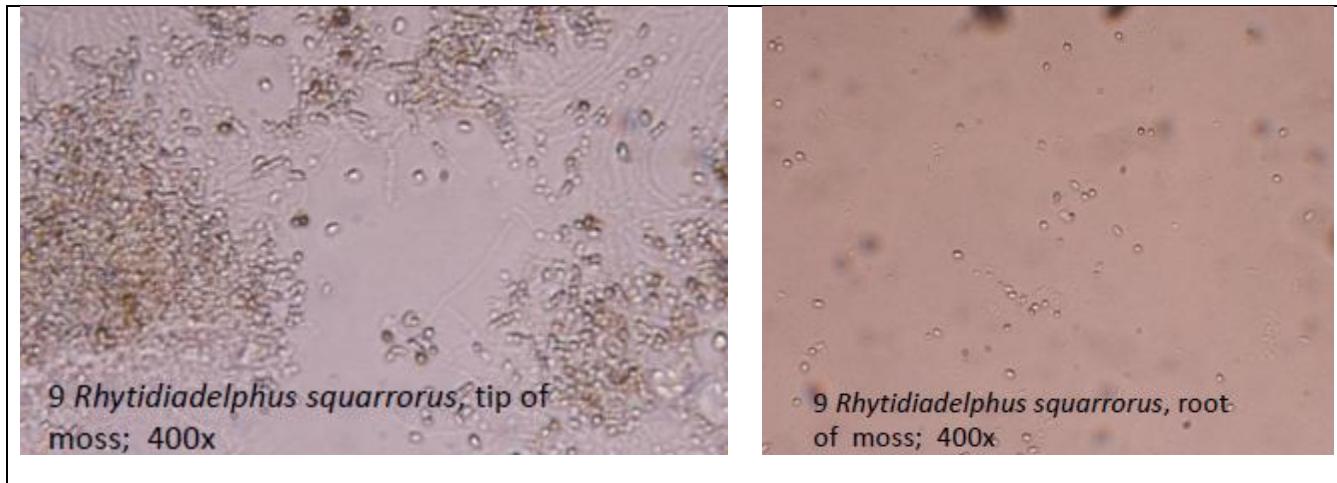


Figure 16: Colony isolates from *Rhytidadelphus squarrorus*

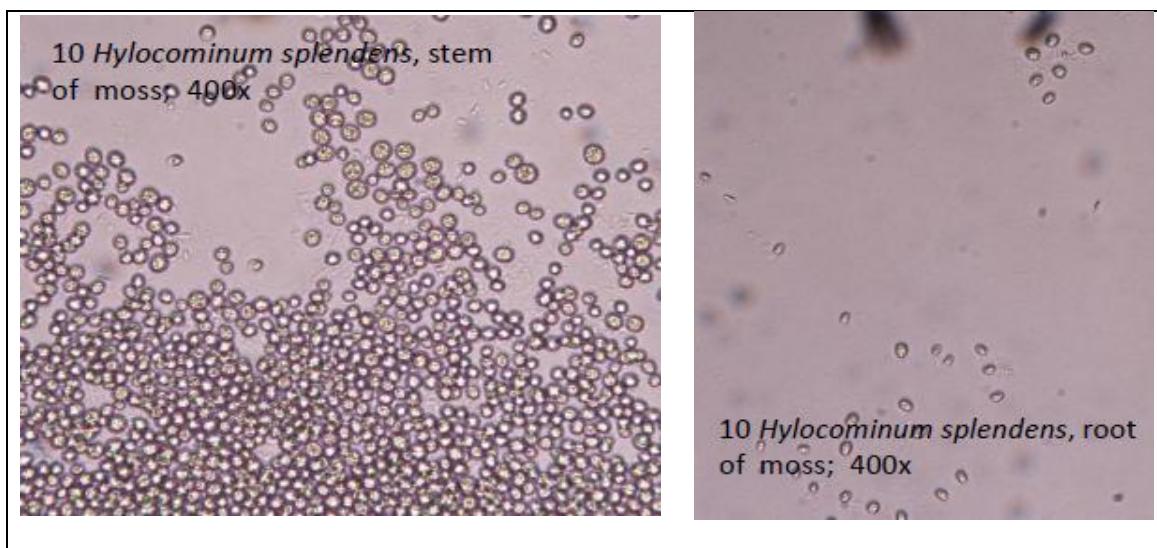


Figure 17: Colony isolates from *Hylocomium splendens*