Species concepts in the pixie cup lichens, *Cladonia pyxidata* and *Cladonia pocillum* (Cladoniaceae, Ascomycotina)

By

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A Thesis submitted to the Faculty of Graduate Studies of

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Abstract

Cladonia pyxidata and *Cladonia pocillum* are commonly referred to as "pixie cup" lichens. The species are mainly delimited by the morphology of the basal squamules, which are upright and separate or form a rosette-like pattern, respectively. *Cladonia pyxidata* is reported to prefer acidic soils, whereas *C. pocillum* grows exclusively on basic soils. By examining the morphological and genetic variation of *C. pyxidata* and *C. pocillum,* we assess their species status. We also examine whether the variation in morphology correlates with a change in soil pH. Samples from the *Cladonia pyxidata* group were collected from across Canada. Phylogenetic and population genetic analyses were conducted using 15 morphological characters (including soil pH and secondary metabolites identified by thin layer chromatography), two fungal datasets (nucleotide sequences of the fungal internal transcribed spacer (ITS) of nuclear ribosomal DNA (rDNA) and a polyketide synthase (PKS) gene), and one algal dataset (Restriction Fragment Length Polymorphisms (RFLPs) of the algal ITS rDNA). Three morphological species could be distinguished among the fungal partner. Soil pH values were significantly different for each species examined. Eight algal genotypes (A-H) were found to associate with three fungal species (*Cladonia pyxidata 1, C. pyxidata 2,* and *C. pocillum*). Members of the *C. pyxidata* group associate with multiple algae within the *Asterochloris* group. No geographical patterning was observed in either the fungal or algal symbionts.

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Introduction

Lichens exist in a wide array of marginal habitats, such as hot deserts, polar regions, and salty coasts. The symbiotic relationship between the mycobiont (fungus) and the photobiont (alga or cyanobacterium) is considered to be a highly specialized strategy that allows lichens to thrive in such habitats (Tehler 1982). The algae provide the heterotrophic fungal partner with a source of carbon and, in return, the algae are sheltered among the fungal hyphae from otherwise intolerable environments (Lewis 1973).

The dual nature of the lichen association makes it difficult to classify these organisms. One of the biggest problems is how to apply a species concept to a symbiotic relationship because the lichen may have a different morphology when the fungus associates with different algae. By convention, lichen classification is based on the fungal partner; however, the fungal partner does not always associate with only one algal partner. For example, in photosymbiodemes, the fungal partner takes up a new algal partner during its development (Armaleo & Clerc 1991, Yoshimura *et al.* 1993, Richardson 1999). Because of the inclusion of the new algal partner and the change in morphology of the lichen, both forms have been given names (Hawksworth 1976). Other common concerns related to lichen classification are the occurrence of species pairs and sibling species (Hawksworth 1976, Tehler 1982).

Lichen symbiosis

Although lichens had been recognized as organisms for quite some time, it wasn't until 1867, when Schwendener proposed his dual hypothesis of lichens, that the true nature of the lichen association began to emerge (Honegger, 2000). Schwendener's hypothesis, which at the time lacked experimental evidence, arose from his extensive analysis of the anatomy and development in lichens, algae, and fungi using a light microscope. Many of the leading lichenologists at the time, such as Crombie and Nylander, rejected Schwendener's hypothesis because the common consensus was that all living organisms were autonomous (Honegger 2000). Other prominent biologists, such as de Bary, Frank, and Hellriegel, were not so quick to reject Schwendener's ideas and the concept soon spread into other areas of study, such as microbial, plant, animal and human pathogens (Honegger 2000). When the complex relationships between pathogenic micro-organisms and their hosts were finally identified, which refutes the idea of holistic organisms, Schwendener's hypothesis began to gain real popularity. Further experimental proof of the dual nature of lichens was obtained when Thomas published his results in 1939 on the first successful re-synthesis experiment (Honegger, 2000). Today it is well established that organisms can exist in close association with one another.

Most lichen symbioses are bipartite associations between a mycobiont and a photobiont (Smith 1973, Honegger 1998, Richardson 1999, DePriest 2004). Eighty-five percent of all lichen-forming fungi associate with eukaryotic algae (phycobiont) and another ten percent associate with prokaryotic cyanobacteria (cyanobiont). The

remaining five percent contain both a phycobiont and a cyanobiont within the same lichen thallus thereby forming a tripartite lichen (Rikkinen 1995, DePriest 2004). In these tripartite lichens, the cyanobiont is generally confined to specialized structures called cephalodia, where conditions favourable for nitrogen fixation are maintained (Ott 1988, Rikkinen 1995, Büdel & Sdheidegger 1996, Richardson 1999).

Gargas *et al.* (1995) proposed that there were at least five independent origins of lichenization; three in the Basidiomycetes and at least two in the Ascomycetes. However, Lutzoni *et al.* (2000) indicate that lichenization probably evolved earlier and was followed by multiple independent losses. Some non-lichen-forming fungi may have secondarily lost the ability to form a lichen association. As a result, lichenization has been viewed as a highly successful nutritional strategy (Honegger 1998, Wedin *et al.* 2004). Even Schwendener recognized that the nutritional status of the fungus improved after forming an association with algal cells (Honegger 2000).

Of the approximate 13,500 lichen-forming fungal species, most belong to the Acsomycetes (Kirk *et al.* 2001, DePriest 2004). However, a few members are also found within the Basidiomycetes (≈50 sp.) and Deuteromycetes (≈200 sp.). By convention, species names that are applied to the lichen association refer to the fungal partners. The photosynthetic partners are given their own separate names. Approximately 100 species of photosynthetic partners from 40 genera and five distinct classes (prokaryotic – Cyanophyceae; eukaryotic – Tribophyceae, Phaephyceae, Chlorophyceae, and Pleurastrophyceae) have been found to associate with the lichen-forming fungi (Friedl &

Büdel 1996). *Trebouxia* Puymaly is the most common of these algae (Ahmadjian 1970, Honegger 1998), occurring in approximately 40% of all known lichen associations (Honegger 1991, Rikkinen 1995). Although not as common as *Trebouxia*, *Nostoc* Vaucher is the primary cyanobacterium found in lichen associations (Rikkinen 1995).

Like all fungi, the mycobionts in lichen associations are heterotrophic organisms that require an external supply of carbon (Lewis 1973, Rikkinen 1995, Honegger 1998). Whereas some fungi, such as mycorrhizae, saprobes and parasites (plant and animal), have exploited other sources of carbon, lichen-forming fungi exploit photosynthetic algae that are maintained within the thallus. Carbon, in the form of photosynthates, is transferred to the fungal partner. The type of photosynthate produced largely depends on the type of photobiont. Green algae (phycobionts) produce a number of sugar alcohols, or polyols, whereas cyanobacteria (cyanobionts) typically produce glucose (Smith 1973, Honegger 1991, Fahselt 1994, Rikkinen 1995, Richardson 1999). After the photosynthates are transferred to the fungal partner, they are rapidly converted to mannitol (Smith 1973). It has been hypothesized that the conversion of photosynthates to mannitol prevents most algae from using the stored photosynthates (Honegger 1991, Fahselt 1994).

The fungal partner in the lichen symbiosis is generally considered to be ecologically obligate (Honegger 1998). This means that although the algal and fungal partners may be cultured independently, both must be growing together to produce the distinct lichen morphology that is observed in nature (Honegger 1996). Establishment of

new lichen thalli is accomplished in at least one of two ways; germination of a lichen-forming ascospore followed by association with a compatible algal partner, or regeneration from vegetative propagules such as isidia, soredia, or lichen fragments (Büdel & Scheidegger 1996), which contain both symbionts. The recognition of compatible algal cells by lichen-forming fungi is thought to be facilitated by the production of a lectin, a potential recognition molecule (Richardson 1999). In the presence of compatible algae, mucilage is produced by the fungus and surrounds the algal cells. This begins the development of a new lichen thallus, as polarity is established and tissues begin to stratify. In some cases, specialized fungal hyphae, called haustoria, penetrate the algal cell walls but do not disrupt the plasma membrane (Smith 1973, Honegger 1991, Honegger 1998).

The lichen association is reported to have a number of effects on the photobiont. In the lichenized state, algal morphology is commonly altered, vegetative reproduction becomes controlled by the mycobiont, and conditions favourable for photosynthesis, such as the amount of H_2O, CO_2, and light, are maintained by the fungal partner (Ahmadjian 1987, Rikkinen 1995, Richardson 1999). The fungal-derived mucilage layer encloses the photobiont and restricts access to water or nutrients. The hydrophobic medullary hyphae help to position the algal cells directly below the upper cortex for optimal light conditions and gas exchange (Honegger 1991, Honegger 1998). In some cases the algal morphology is altered so dramatically that axenic culturing is the only means of accurate identification (Rikkinen 1995).

Three growth patterns have been observed in the lichen-forming fungi; apical or marginal growth, intercalary growth, and a combination of the two forms (Honegger 1991, 1996). In thalli showing the apical or marginal pattern, such as *Physia* (Schreber) Michaux, *Cladonia* P. Browne, and *Parmelia* Ach., growth of the lichen thallus occurs within the pseudomeristematic region located along the apices or margins (Honegger 1996). Cells in this region tend to be tiny and lack structure (Honegger 1991). However, as the thallus grows, the cells increase in size, the cortex begins to develop, and tissue stratification becomes apparent. In thalli showing intercalary growth, such as *Umbilicaria* Hoffm., lobes develop throughout the lichen thallus. As a result, younger lobes are often seen overgrowing older, senescing lobes (Honegger 1996). These two growth patterns are not exclusive. Species, such as *Lobaria pulmonaria* (L.) Hoffm., have been seen to posess both apical/marginal and intercalary growth (Honegger 1996).

Growth patterns have resulted in the formation of lichen thalli exhibiting one of three growth forms; crustose, foliose, or fruticose (Smith 1973, Büdel & Scheidegger 1996). Crustose lichens are generally tightly adpressed to, if not slightly embedded within, their substratum. Foliose lichens form leaf-like structures that are loosely attached to the substratum by attachment appendages, such as a holdfast in the Umbilicaraceae or rhizines in the Peltigerales. Both crustose and foliose lichens show a range of tissue differentiation from non-stratified homiomerous thalli to highly structured heteromerous thalli (Büdel & Scheidegger 1996). The homiomerous thallus consists of evenly distributed fungal and algal partners whereas the heteromerous thallus consists of a highly conglutinated upper cortex, a loosely woven hydrophobic

medullary region that contains the photobiont, which is positioned directly below the cortical layer for optimal gas exchange and illumination, and in some instances a conglutinated lower cortex (Honegger 1991, Büdel & Scheidegger 1996, Honegger 1998, Gaßmann & Ott 2000).

Fruticose lichens are attached to the substratum at a single point and are present in two forms, erect or pendulous (Smith 1973, Büdel & Scheidegger 1996). Thalli of erect fruticose lichens generally possess an upright stalk, which provides the structural support that allows the lichen thallus to maintain the vertical habit (Hammer 1993). The pendulous forms of fruticose lichens do not have the same structural requirements. Instead, they possess a variety of structural features ranging from a central elastic cord that allows the lichen thallus to stretch when disturbed to a loosely woven medulla providing less structural support (Büdel & Scheidegger 1996). The basic anatomy of fruticose lichens is otherwise the same as other lichen-forming fungi; however, because of the cylindrical nature of this growth form, an outer cortex is sometimes present and protects the photobiont in the medullary layer. In some cases, intermediates between crustose and fruticose lichens exist that possess both a horizontal (primary) and vertical (secondary) thallus. These lichens have been referred to as cladoniform lichens because the Cladoniaceae (including Stereocaulaceae at the time) exhibited this growth form (Ahti 1982). Although growth forms have been used to classify lichen-forming fungi in the past and present, recent molecular analyses indicate that these categories are not natural divisions (Stenroos & DePriest 1998, Wedin *et al.* 2000).

Species concepts and lichens

'Species' are the fundamental units of biodiversity, however, the nature of species and species concepts is constantly being debated (Bock 2004, Holynski 2005, Rieseberg *et al.* 2006). Our need to describe and classify the natural world has led to many differing opinions as to 'what is a species?'. Some authors have considered species to be individuals (Hull 1976, Sober 1984, Mayden 1997, Coleman & Wiley 2001) whereas others define them as sets, groups, or natural kinds (Kitcher 1984, Reydon 2003). In some cases, species and species concepts were even defined based on more operational approaches such as phenetics (Doyen & Slobodchikoff 1974). More recently, however, definitions of species and species concepts have expanded such that information into the evolutionary history of the species is also included in the definitions (Bock 2004, de Queiroz 2005a).

Regardless of the motivation behind the various debates, definitions and concepts about species, there appears to be an overall desire to arrive at a definition that is universally applicable (Cracraft 1997, de Queiroz 2005b). However, a single unified species concept is generally quite difficult to achieve due to the range of biodiversity that must be encompassed (Mayr 1957, Kitcher 1984, Hull 1997, Mayden 1997). The inability to develop a species concept that encompasses all the known biodiversity has resulted in a proliferation of species concepts within the literature (Hull 1997, Mayden 1997). Some of these include; the Biological Species Concept (Mayr 1957), Ecological Species Concept (van Valen 1976), Taxonomical or Morphological Species Concepts (Blackwelder 1967), Phenetic Species Concept (Sneath 1976),

Recognition Species Concept (Paterson 1993), Cohesion Species Concept (Templeton 1989), Flagship Species Concept (Caro *et al.* 2004), Evolutionary Species Concept (Simpson 1961, Wiley 1978, Wiley 1981, Holyński 2005), Unified Species Concept (de Queiroz 2005a) and a number of others (Grube & Kroken 2000).

All of the species concepts proposed to date have attempted to classify organisms in such a way that information can be inferred by examining closely related species. Over the years the kind of information deemed important in the classification of organisms has changed (Culberson CF 1986, Grube & Kroken 2000). However, three species concepts have been more commonly used than others; Morphological Species Concept, Biological Species Concept, and Evolutionary Species Concept. Historically, taxonomists classified species based on simple morphology (Morphological Species Concept). Over time, as the understanding of biological processes, such as reproductive isolation mechanisms, increased, numerous other concepts were proposed in an attempt to better differentiate among some of the variation observed (*e.g.* Biological Species Concept). More recent species concepts, such as the Evolutionary or Phylogenetic Species Concept, indicate a growing trend towards the identification of possible ancestral lineages within the classification system (Simpson 1961, Wiley 1978, Holynski 2005).

The Morphological (or Taxonomical) Species Concept (MSC) is likely the oldest species concept and it is still in use today. Early taxonomists commonly relied solely on the morphological characters of an organism to delimit species (Mayden 1997, Purvis

1997). Therefore, organisms that looked the same were generally considered to be the same species. However, morphological characters are not always homologous, which has lead to the artificial classification of many organisms. The MSC is particularly hard to apply to lichen-forming fungi because of the occurrence of photosymbiodemes, species pairs and sibling species (see next section), which make morphology an unreliable character if species concepts in lichens is to reflect a natural classification (Nourish & Oliver 1976, Park 1985, Ott 1988, Rogers 1989, Armaleo & Clerc 1991, Myllys *et al.* 2001).

The development of the Biological Species Concept (BSC) is largely attributed to Ernst Mayr (1957) and is probably the most well known and highly referenced of all the species concepts. According to Mayr (1957), species are naturally occurring populations that have the ability to interbreed. In order to implement the Biological Species Concept, however, an understanding of pre-zygotic (temporal or spatial) and post-zygotic (genetic) reproductive isolation mechanisms is required. The BSC works well for some organisms, but the existence of hybrids and asexual organisms pose a significant theoretical problem (Hull 1997). Hybrids are believed to represent a breakdown of isolation mechanisms, whereas asexual organisms, such as some commonly occurring lichen-forming fungi and many algae, do not have the ability to interbreed. In such cases, it would be inappropriate to apply the Biological Species Concept.

The Evolutionary Species Concept (ESC), which is rooted in the idea that species share a common ancestor, was proposed a number of years ago by Simpson (1961).

However, a reliable means of applying the ESC was missing at that time (Wiley 1978, Wiley 1981). With the recent advancements in equipment and techniques that allow for the phylogenetic analysis of organisms at the molecular level, an increase in the popularity of the Evolutionary (or Phylogenetic) Species Concept has been observed (Grube & Kroken 2000, Mishler & Theriot 2000, Holynski 2005). For example, molecular markers, which include such regions as the nuclear small subunit (nrSSU) rDNA and β-tubulin gene, have been found to contain enough genetic variation to examine evolutionary relationships among lichen-forming fungi at the species level (Stenroos & DePriest 1998, Grube & Kroken 2000, Lutzoni *et al.* 2001, Myllys *et al.* 2001, DePriest 2004, Molina *et al.* 2004, Thell *et al.* 2004).

Problems associated with species concepts in lichens

Photosymbiodemes, species pairs, and sibling species pose significant theoretical problems when attempting to identify lichen-forming fungal species (Tehler 1982, Purvis 1997). However, Grube and Kroken (2000) believe that phylogenetic analyses using nucleotide sequence data of the fungal partner will assist in understanding the evolutionary significance of these phenomena.

Photosymbiodemes are defined as lichen-forming fungi that may develop into a number of distinct morphologies depending on whether the photobionts associated with the fungi are green algae, cyanobacteria, or both (Purvis 1997). The morphotypes may be so distinct that they are each given species names and in some cases, placed in

completely different genera (Armaleo & Clerc 1991, Stenroos *et al.* 2003). However, this does not conform to the rules established by the International Code of Botanical Nomenclature (ICBN), which states that only one scientific name is to be applied to each organism recognized. Additionally, the placement of a single lichen-forming fungal species into two separate genera does not adequately represent the life history or evolutionary relationships that exist among the fungi of the photosymbiodemes (Armaleo & Clerc 1991, Roos 1995, Purvis 1997).

Species pairs refer to two species of lichens with identical morphologies, which are thought to differ only in reproductive strategy (Poelt 1970). The primary species of the species pair reproduces sexually through the production of ascospores in the apothecia while the secondary species reproduces only by means of soredia or other vegetative propagules (Poelt 1970, Purvis 1997). Tehler (1982) debated the evolutionary significance of recognizing each member of the species pair as a distinct species and suggested that this division may obscure the relationship among species. Tehler (1982) also suggested that the soredia produced by secondary species allow the lichen to expand into wider geographical ranges. Because soredia, which contain both partners of the lichen symbiosis, are thought to be genetically identical to the parent population, they are already adapted to the environment of the parental population (Culberson WL 1986, Purvis 1997). However, the lack of genetic flexibility in these clonal propagules consequently limits the ability of the organism to adapt to changing environments (Tehler 1982, Purvis 1997). On the other hand, algal switching can provide a mechanism

for the fungi and algae of vegetative propagules to change partners, ultimately providing flexibility (Piercey-Normore & DePriest 2001).

Studies on species pairs were expanded by Myllys *et al.* (2001), who examined the genetic variation of β-tubulin, ITS rDNA, and group I introns in the sexually reproducing *Physcia aipola* (Ehrh. ex Humb.) Fürnr. (primary species) and the asexual *Physcia caesia* (Hoffm.) Fürnr. (secondary species). Although the information obtained from the ITS and group I intron sequences was generally uninformative, β-tubulin sequences provided ample resolution by which to determine the relationship of *P. aipola* and *P. caesia*. The phylogeny produced from the combined dataset indicated that the two taxa were not monophyletic and suggested that *P. caesia* should be able to reproduce sexually. Based on these results, Myllys *et al.* (2001) concluded that *P. aipola* and *P. caesia* should not be recognized as separate species.

Sibling or 'cryptic' species were first recognized by amateur ornithologists in the 1700's who noticed that, although some species of birds appeared morphologically identical, they were unable to interbreed (Winker 2005). The subtle variations that were observed in behavioral characteristics, such as mating songs and nesting habits, were identified as reproductive barriers. Today, sibling species are recognized in a wide range of organisms, including lichen-forming fungi and mammals (Culberson WL 1986, Purvis 1997, Hawksworth 1976).

Two forms of sibling species are recognized among the lichen-forming fungi; ecotypes and chemotypes (Hawksworth 1976). Ecotype is the term applied to lichens

that shift phenotypes depending on the environment in which they grow. For example, *Acarospora smaragdula* (Wahlenb.) A. Massal. may be recognized as three separate species with a gray thallus, a green thallus, and one with no lichen secondary compounds depending on the copper content of the rock substratum (Purvis *et al.* 1985, 1987).

Chemotypes or chemospecies are lichens that are morphologically identical but differ in the type of secondary compounds that are produced in the thallus. A number of methods, such as thin-layer chromatography (TLC) (Culberson CF 1972, 1986), mass spectrometry, and high pressure liquid chromatography (HPLC) (Nourish & Oliver 1976), have been used to characterize these secondary compounds and identify commonly occurring chemotypes (Ahti 1966, Spier & Aptroot 2007). Although Coassini-Lokar *et al.* (1986) recognized *Cladonia chlorophaea* (Flörke ex Sommerf.) Sprengel, *C. grayi* G. Merr. ex Sandst., *C. cryptochlorophaea* Asahina and *C. merochlorophaea* Asahina by chemistry alone, Rogers (1989) argued that taxa should not be given species status based on variation observed in a single product, or even within the same biosynthetic pathway, since these products are not always genetically linked and may be influenced by the environment (Park 1985). Ahti (2000) also argued that chemical characters should not be used to determine species status because they cannot be readily determined in the field.

Phenotypic plasiticity

Species problems in lichens reflect the use of different phenotypic characters and may be explained by phenotypic plasticity. Phenotypic plasticity has been identified in a number of taxa from a wide range of phyla. The term has been used to identify genetically identical organisms with differing phenotypes depending on the nature of the environment in which the organism is growing (Behera 1997). Examples of phenotypic plasticity have been observed in a number of lichens; *Cladonia pocillum* (Ach.) Grognot (Gilbert 1977), *Catillaria corymbosa* (Hue) Lamb (Sojo *et al.* 1997), *Pseudevernia furfuracea* (L.) Zopf (Rikkinen 1997), and *Ramalina capitata* var. *protecta* (Magnusson) Nimis (Pintado *et al.* 1997). Wedin *et al.* (2004) expanded the concept to include the phenotypic plasticity observed in the *Stictis-Conotrema* complex, which consists of both lichenized and non-lichenized fungi. In their study, phenotypic plasticity was identified as a nutritional strategy when growing in unpredictable environments. In an attempt to determine the effect of phenotypic plasticity on the rate of evolution, Behera (1997) designed a model using genetic algorithms. In the model haploid population studied, phenotypic plasticity slowed down the rate of evolution but resulted in an organism that was subsequently better adapted to the environment.

Gilbert (1977) identified phenotypic plasticity within grassland populations of *Cladonia pocillum* (Ach.) Grognot. He observed that the basal squamules of thalli growing in grasslands, which were being grazed upon by sheep and rabbits, exhibited the typical rosette-like pattern. However, where grazing had been restricted due to fencing, vegetation was denser, and *C. pocillum* took on a slightly different morphology.

In this situation, the basal squamules did not form a simple rosette pattern but were observed growing in a more upright and dispersed manner, similar to that of *Cladonia pyxidata* (L.) Hoffm. The difference between the two *C. pocillum* morphotypes was attributed to the pH of the soil substratum. In grazed areas, the lichen thalli were attached directly to the substratum. However, in the ungrazed areas, lichen thalli grew among leaf litter that accumulates at the soil surface. This leaf litter lowered the pH of the substratum (<6.0), which was thought to alter the phenotypic expression of the basal squamules. As a result, Gilbert (1977) cautioned that the phenotypic plasticity observed in *C. pocillum* may result in the misidentification of morphotypes from grazed and ungrazed grasslands.

A more extensive study of phenotypic plasticity comparing morphology, anatomy, chlorophyll content, water relations and photosynthetic response, was conducted on *Catillaria corymbosa* (Hue) I. M. Lamb. from three different microhabitats near Risopatrôn base, Antarctica (Sojo *et al.* 1997). Typically, *C. corymbosa* is found growing on exposed rocks along the maritime coastline. However, it is sometimes found growing under shady overhangs or vertical fissures. The phenotypic plasticity, observed in *C. corymbosa* samples taken from the three microhabitats, was largely attributed to the environmental conditions to which the lichen was exposed at each of the three sites (Sojo *et al.* 1997). However, it is still unknown whether there is any genetic basis for the observed variation among the sites.

Sojo *et al.* (1997) determined that although there were no observable anatomical differences in *Catillaria corymbosa* between the exposed site and the protected site, morphological differences were common. Samples from exposed sites had whitish-gray, irregular thalli whereas thalli from protected sites were more compact, with shorter stalks and a much greener appearance; largely due to the occurrence of approximately three times the chlorophyll within the thallus. Thalli from protected sites also exhibited larger groups of soredia, lower water storage capacities, and larger water holding capacities than samples from the exposed sites. Sojo *et al.* (1997) proposed that the increased water holding capacity of *C. corymbosa* growing in the shady, protected sites was responsible for the higher values of photosynthesis observed when exposed to relatively high light levels. The increased water holding capacity also allowed for longer periods of metabolic activity in samples of *C. corymbosa* that were growing in protected rather than exposed sites (Sojo *et al.* 1997).

Phenotypic plasticity was also studied for populations of *Pseudevernia furfuracea* (L.) Zopf growing in a kettle hole (depression caused by subsurface ice melt) in central Finland (Rikkinen 1997). *Pseudevernia furfuracea* is a foliose lichen with an ascending to subpendulous thallus. The thallus is attached to the substate at the basal regions and follows a dichotomous branching pattern. Typically, *P. furfuracea* is found growing on the trunks of *Pinus sylvestris* L.. However, in this study, a habitat shift to the lower branches of *Picea abies* (L.) Karsten was observed in low-lying areas of the kettle hole, and on north-facing slopes.

Rikkinen (1997) attributed the habitat shift of *Pseudoevernia furfuracea* to the availability of photosynthetically active radiation (PAR) at various locations within the kettle hole. The aspect and inclination of the kettle hole play an important role in the amount of light available at each site. Aspect (north-facing *vs* south-facing slopes) affects the amount of direct solar radiation received, whereas, inclination (angle of the slope) affects the amount of incidental radiation. Therefore it would follow that the upper south-facing slopes of the kettle hole are generally exposed to higher light levels since both direct and reflected radiation is prevalent. The lower north-facing slopes, which rely primarily on indirect radiation, are exposed to less light because of the over-shading by dense canopy layers on the upper slopes. Shifting to the lower horizontal branches of *Picea abies* along the lower north-facing slopes provided *Pseudoevernia furfuracea* with a means to maintain appropriate light levels.

Morphological differences between populations of *Pseudoevernia furfuracea* growing along the upper south-facing slopes and lower north-facing slopes were also observed (Rikkinen 1997). Generally, thalli from the upper slopes were darker coloured (ash gray), more robust and had thick lobes covered with isidia and small lobuli. Thalli from the lower north-facing slopes were lighter coloured (pale gray to white), much smaller and had fewer isidia present. The morphological shift observed represents not only an adaptation to differences in amount of available light but can be related to evaporative stress as well. As the temperature and amount of available light decreases from the upper slopes towards the bottom of the kettle hole, the amount of available water increases, resulting in an excessively humid environment. The highly reduced

thalli with short, fine branches found growing on the lower slopes was therefore interpreted as a morphological shift, which promotes water loss and ensures adequate periods of dehydration (Rikkinen 1997).

Thalli of *Pseudevernia furfuracea* from the upper slopes are presented with a strikingly different problem. The direct radiation and high temperatures to which the lichen is exposed has a profound drying effect on the thalli. The robust thalli covered in dense isidia was seen by Rikkinen (1997) as a morphological adaptation that ensured metabolic activity for extended periods following precipitation events. It was advantageous in these conditions due to the large water holding capacity of the thallus (i.e. thicker medullary tissues and more external capillary spaces) and its ability to conduct water externally by capillary action, by means of ventral grooves, capillary spaces between isidia and protruding tips of cortical hyphae.

The external conduction of water is largely due to hygroscopic movements caused by the difference between the rate of water uptake and the swelling capacity of the thallus. Following a precipitation event, water uptake causes swelling of the cortical tissues that results in the reflexed margins of the thalli curling inwards. The dimensions of the ventral groove are altered such that the water potential inside the ventral groove is increased. Water, therefore, flows into the ventral groove causing the entrance to the groove to narrow and capillary tension to increase, thereby forcing water to advance along the groove to regions of apical growth (Rikkinen 1997).

On a smaller scale, Pintado *et al.* (1997) compared morphology, anatomy, water relations, and chlorophyll content between two populations of *Ramalina capitata* var. *protecta* growing on an exposed rock surface in Spain; thalli from the north-facing side of the rock and thalli from the south-facing side. Since the study was conducted in the Northern Hemisphere, objects on south-facing aspects would receive higher levels of irradiation and therefore would experience longer periods of evaporative stress. Pintado *et al.* (1997) hypothesized that optimal light harvesting mechanisms in north-facing populations of *R. capitata* var. *protecta* would have an advantage over south-facing populations. However, the phenotypic plasticity observed between the two populations could not be directly related to light alone. Relative humidity and thallus temperature were also identified as contributing factors in conjunction with the amount of available light. North-facing surfaces were shadier with lower temperatures and higher relative humidity than south-facing surfaces. The thalli growing on the north-facing surfaces were commonly pendulous due to the lower levels of evaporative stress to which this population was exposed (Pintado *et al.* 1997). However, south-facing populations had thicker, dense, erect thalli with shorter, wider lacinia (narrow, linear lobes) and fewer psuedocyphellae (pores through the upper cortex, which expose loosely woven medullary hyphae), which were seen as a method to increase the water retention capabilities of the thallus into dry periods. An increase in the percentage of algal cells within the thallus and a general increase in chlorophyll content in south-facing populations also provide evidence that the larger south-facing populations of *R. capitata*

var. *protecta* are structurally better adapted for the maximum usage of optimal PAR during the short periods of favorable growth (Pintado *et al.* 1997).

These studies (Gilbert 1977, Pintado *et al.* 1997, Rikkinen 1997, Sojo *et al.* 1997) indicate that habitat may influence morphology. Therefore, subtle differences in phenotype in response to variations within microhabitats could potentially result in the naming of different species.

Phylogenetics and population structure

The innovation of Polymerase Chain Reaction (PCR) and molecular markers, such as allozymes, Randomly Amplified Polymorphic DNA (RAPD), Restriction Fragment Length Polymorphisms (RFLPs), and DNA sequencing, have lead to new insights into the amount and pattern of variation that occurs in populations (Bridge & Hawksworth 1998). By assessing the degree and pattern of variation produced by molecular markers, information regarding evolutionary history, gene flow, and population genetics may be obtained. The symbiotic nature of the lichen thallus, in conjunction with the occurrence of sexual reproduction in some species, has lead to hypotheses regarding the amount and type of variation that occurs within and among lichen populations. Two types of variation are present among lichen populations; variation among symbionts (combinations of symbionts) and genetic variation within the genome of each of the symbionts. Dispersal of vegetative propagules, containing both symbionts, is believed to reduce levels of symbiont variation. However, dispersal of ascospores, which must re-

establish lichenization with a compatible algal cell, increases the level of symbiont variation. It is assumed that higher symbiont diversity improves the fitness of the population, allowing the species to survive under a wider range of conditions (Piercey-Normore & DePriest 2001, DePriest 2004)

Genetic variation in lichens

Over the last 15 years, the use of molecular markers to study population genetics in lichens has been increasing steadily (DePriest 2004). Some studies concentrate on the presence or absence of introns in the nrSSU rDNA (DePriest 1993, Beard & DePriest 1996; Robertson & Piercey-Normore 2007) or genetic fingerprints generated from Simple Sequence Repeats (Walser *et al.* 2003; Zoller *et al.* 1999), RFLPs and RAPDs (Murtagh *et al.* 1999; Dyer *et al.* 2001; Piercey-Normore 2006; Robertson & Piercey-Normore 2007). Studies have also examined genetic variation using DNA sequences (Printzen & Ekman 2003; Zoller & Lutzoni 2003, Thell *et al.* 2004). More recently, studies have focused on the ITS regions of both the fungal and algal partners and PKS genes (Bingle *et al.* 1999, Crespo *et al.* 1999, Helms *et al.* 2001, Miao *et al.* 2001, Molina *et al.* 2004, Cordeiro *et al.* 1995, Ohmura *et al.* 2006, Opanowicz *et al.* 2006, Beiggi & Piercey-Normore 2007).

Beard and DePriest (1996) examined the nrSSU rDNA from five geographically distinct populations of *Cladonia subtenuis* (Abbayes) Mattick in the southeastern United States, to determine the amount of genetic variation within and among populations.

PCR amplification of the nrSSU rDNA revealed fragments that were 200, 400, and 600 nucleotides longer than anticipated. These fragments, which were found to contain different sizes, positions, and number of inserts, were recognized as three size classes; Class I, Class II, and Class III. Class I was the largest fragment and contained two inserts; 400bp at position 1624 and 200bp at position 1777 of the nrSSU rDNA coding region. Class II also contained two inserts, 200bp at position 1624 and 200bp at position 1777, however the insert at position 1624 was a different length. Class III was the smallest of the three size classes and contained a single 200bp insertion at position 1777. These inserts were identified as putative group I introns (Beard & DePriest 1996, Piercey-Normore *et al.* 2004).

Variation in sequence length and consequently, intron presence or absence, was observed among the five geographic regions, as well as the five mats selected from one of these regions. However, no variation was observed within podetia from the same mat (Beard & DePriest 1996). These results indicate that although mats of *C. subtenuis* may consist of a single genetic entity, there are distinct populations in close proximity to each other. Beard & DePriest (1996) therefore concluded that the ability to detect variation with the nrSSU rDNA, such as intron presence/absence, was a variable marker for examining population structure.

Robertson & Piercey-Normore (2007) also used the presence or absence of introns in the fungal nrSSU DNA to examine population structure among ten populations of *Cladonia arbuscula* (Wallr.) Flot within a 2 km range in northern Manitoba. By

comparing the genetic variation observed in the nrSSU of the fungal partner with the RFLP pattern obtained from the ITS rDNA of the algal partner, the primary dispersal method of *C. arbuscula* was inferred. If the genetic variation between the symbionts is correlated, it might indicate that the symbionts were dispersed together as vegetative propagules. Amplification of the fungal nrSSU revealed fragments that were 250, 500, and 750 base pairs longer than anticipated. From this data, eleven haploid genotypes were recognized; six of which were common and five were unique. Genotype A was the most common and contained all four introns. Analysis of molecular variance (AMOVA) of the fungal symbionts indicated significant population subdivision among the ten populations of *C. arbuscula* (Robertson & Piercey-Normore 2007).

Amplification of the algal ITS produced a single fragment 560 bp long (Robertson & Piercey-Normore 2007). Further analysis with RFLPs revealed two genotypes; Genotype I consisted of a single band at 200 bp and Genotype II consisted of two bands, one at 190 bp and the other at 200 bp. AMOVA of the algal symbionts from *C. arbuscula* showed no population subdivision, which suggests that gene flow was occurring among the algal populations. Since the fungal genotypes could not be correlated with the algal genotypes, it would suggest that the two symbionts were not dispersing together in the same propagule or the alga was not retained in the lichen after dispersal occurred (Robertson & Piercey-Normore 2007).

In an attempt to expand the number of markers that could be utilized in population studies, Murtagh *et al.* (1999) developed a protocol for using RAPDs to

genetically fingerprint the lichen-forming fungi. The benefits of the RAPD technique are that it is a quick, simple procedure that is more sensitive to variation than the commonly used rDNA sequencing analyses, and requires no *a priori* knowledge of the nucleotide sequence. A single arbitrary primer is used to amplify genomic DNA and produce fragments of varying lengths, which can be resolved by agarose gel electrophoresis.

In the study by Murtagh *et al.* (1999), PCR products ranging from 250 to 4000 bp in length were obtained. In total, six to nine reproducible RAPD bands were produced from the four random primers used. Although the protocol worked well for DNA extracted from axenic cultures of *Graphis scripta* (L.) Ach., *Graphis elegans* (Borrer ex Sm.) Ach. and *Phaeographis dendritica* (Ach.) Müll. Arg., which could be identified by their banding patterns, the DNA extracted from different parts of the whole thallus of *G. scripta* produced slightly different fingerprints. Murtagh *et al.* (1999) therefore cautioned against making interpretations from whole thalli due to the potential for the inclusion of contaminating DNA (*i.e.* algal DNA) or more than one fungal genotype making up the thallus.

Zoller *et al.* (1999) sequenced regions of the fungal Histone 3, β-tubulin, nrSSU, nrLSU, mtLSU, and mtSSU looking for variation in six populations of *Lobaria pulmonaria*, an epiphytic, endangered lichen of the European lowlands (Switzerland). Of the gene regions sequenced for this study, only ITS1 and nrLSU provided any variability. ITS1 was 550 bp long and contained three polymorphic sites. The nrLSU, however, was 400 bp

long and contained four variable nucleotides (2 indels) located in a small insertion that is 75-77 bp long. From the seven variable positions identified, a total of six genotypes were recognized. Two genotypes were the most common occurring genotypes and two others were only found in single populations.

Genetic variability within and among populations of *L. pulmonaria* was measured by Zoller *et al.* (1999) using the K_{ST} statistic (Hudson *et al.* 1992), which is a measure of genetic differentiation between geographical regions that is similar to y_{ST} (Nei 1982) and N_{ST} (Lynch & Crease 1990). Using the K_{ST} statistic, three of the six populations examined were reported to have high genetic diversity and the other three populations had low genetic diversity. No apothecia were observed in the populations with low genetic diversity; however, populations with high genetic diversity were seen to contain apothecia in 72% of the thalli examined. Of the six genotypes identified, only the rare AB genotype did not produce any apothecia at all. This study suggests that the sexually reproducing populations contain the most variability and therefore should exhibit improved fitness over populations that are not able to produce apothecia (Zoller *et al.* 1999).

The genus Cladonia

Cladonia is a genus of lichen-forming fungi within the family Cladoniaceae of the order Lecanorales (Ahti 2000, Miadlikowska *et al.* 2006). According to Esslinger (2008), there are currently 168 species of *Cladonia* found within North America. Many of these

have either a bipolar or cosmopolitan distribution (Andreev *et al.* 1996, Goward & Ahti 1997, Ahti 2000, Osyczka 2006). Within Manitoba alone, Piercey-Normore (2003) reported the occurrence of 49 species and 59 chemotypes of *Cladonia.* Unlike other members of the Lecanorales, such as Parmeliaceae and Physciaceae, which are found to associate with *Trebouxia* s.s. (Dahlkild *et al.* 2001, Helms *et al.* 2001), members of the Cladoniaceae associate with the coccoid green alga, *Asterochloris* (Rambold *et al.* 1998, DePriest 2004, Cordeiro *et al.* 2005, Miadlikowska *et al.* 2006). *Asterochloris* is similar to *Trebouxia* but does not contain the centrally located pyrenoid that is characteristic of *Trebouxia* s.s. (Hildreth & Ahmadjian 1981, Friedl 1989, Friedl & Rokitta 1997, Rambold *et al.* 1998). *Asterochloris* has an axial chloroplast that becomes parietal during certain stages of development (Friedl & Rokitta 1997).

Lichen thalli in the genus *Cladonia* have been referred to as cladoniform lichens and are generally considered to be a composite of the crustose and fruticose growth forms (Ahti 1982). The horizontal primary thallus, consisting of a crustose growth habit, produces a vertical secondary thallus, or podetium (Hammer 1993, Hammer 1995, Jahn *et al.* 1995, Osyczka 2006). As defined by Ahti (1982), "a podetium is a lichenized, stem-like portion (stipe, or discopodium) bearing the hymenial discs and sometimes conidiomata in a fruticose apotheci[um]." Although similar structures have been seen in such families as the Baeomycetaceae, Icmadophilaceae, Stereocaulaceae, and Siphulaceae, morphological (Ahti 1982), ontogenetic (Hammer 1993, Jahns *et al.* 1995), and molecular analyses (Stenroos & DePriest 1998, Wedin *et al.* 2000) indicate that the

origin of these structures is not homologous and does not provide taxonomic or phylogenetic information.

Ahti (2000) recognized seven taxonomic sections within the genus *Cladonia* (*Unciales, Cocciferae, Helopodium, Strepsiles, Perviae, Ascyphiferae*, and *Cladonia*) and three sections in the genus *Cladina* (*Cladina, Impexae*, and *Tenues*) based on gross morphology and secondary chemistry (taxonomic nomenclature following Ahti 2000). Members of the section *Unciales* generally contain usnic acid, are highly branched, have open axils, primary squamules that are evanescent and no soredia or podetial squamules. Members of the section *Cocciferae* also contain usnic acid but have closed axils. Members of the section *Helopodium* have large persistent basal squamules, sparsely produced clavate (club-shaped) podetia, and no cups or soredia. Members of the section *Strepsiles* also have a large persistent primary thallus (basal squamules) and no cups or soredia but the podetia are subclavate. Members of the section *Perviae* never contain atranorin and have branched podetia with perforated funnels, not cups. Members of the section *Ascyphiferae* have an evanescent primary thallus, branched subulate (tapering) podetia that are not perforated, and always contain fumarprotocetraric acid and atranorin. Members of the section *Cladonia* have a persistent primary thallus with unbranched to somewhat branched cup-forming podetia and closed axils. The placement of *Cladina* outside the genus *Cladonia* has been widely debated (Ahti 1984, Stenroos *et al.* 1997, Ahti 2000) and *Cladina* has recently been subsumed into the genus *Cladonia* (Ahti and DePriest 2001).

Stenroos *et al.* (2002) confirmed the inclusion of *Cladina* within the genus *Cladonia* (Ahti & DePriest 2001), in a phylogenetic study using a combined molecular, morphological, and chemical dataset. The analysis, which included representatives from each section of *Cladonia* and *Cladina*, did not support the sectional arrangement established by Ahti (2000). Instead, Stenroos *et al.* (2002) proposed that the genus *Cladonia* be divided into three Subdivisions (I, II, and III) representing four Supergroups (*Cladonia*, *Perviae*, *Cocciferae*, and *Crustaceae*; taxonomic nomenclature following Stenroos *et al.* 2002). Supergroup *Cladonia*, as recognized by Stenroos *et al.* (2002), included polyphyletic members from sections *Ascyphiferae*, *Helopodium*, and *Cladonia* (sensu Ahti). Supergroups *Perviae* and *Cocciferae*, corresponded to sections *Perviae* and *Cocciferae*, respectively, and a new Supergroup, Supergroup *Crustaceae*, was established which contained members from the genus *Cladina* and section *Unciales*.

Section *Cladonia* (Ahti 2000) was scattered throughout Subdivision II (Stenroos *et al.* 2002) and species complexes that are currently recognized within section *Cladonia*, such as the *C. verticillata, C. gracilis,* and *C. chlorophaea* species complexes, were reported to produce informal groupings. This raised a number of questions regarding the placement of certain taxa. For example, *C. pyxidata*, like many other cup-forming species, were scattered randomly within Subdivision II; one representative was seen to group with Subgroup *Graciles*, whereas another grouped with *C. furcata* (Hudson) Schrader and allies. Stenroos *et al.* (2002), therefore proposed that *C. pyxidata* is likely not a single species and that the inclusion of the closely related species, *C. pocillum,* would only complicate matters.

Beiggi & Piercey-Normore (2007) attempted to further our understanding of the phylogenetic relationships among the species complexes contained within *Cladonia* section *Cladonia* (*C. gracilis, C. chlorophaea, C. pyxidata,* and *C. verticillata* complexes) by examining the evolutionary histories of both the fungal and algal partner. Only three species, *Cladonia cervicornis* ssp. *verticillata* (Hoffm.) Ahti, *C. subulata* (L.) F. H. Wigg., and *C. merochlorophaea,* and none of the recognized species complexes examined were monophyletic. *Cladonia pyxidata* was again scattered throughout the tree and the inclusion of *C. pocillum*, as suggested by Stenroos *et al.* (2002), provided no resolution to the problem. It has therefore been proposed that the polyphyly observed among the species complexes within the genus *Cladonia* may be the result of phenotypic plasticity associated not only with changes in the environment but also changes among the algal partner (Beiggi & Piercey-Normore 2007).

While examining herbaria specimens, Aptroot *et al.* (2001) recognized that the *C. pyxidata* complex was more convoluted then originally thought and proposed a new species within Europe, *Cladonia monomorpha* Aptroot, Sipman & van Herk. This new species was based on the morphology of the basal squamules, cup features, and length of marginal proliferations. Aptroot *et al.* (2001) also indicated that *C. monomorpha* may be present in North America but that it may simply have been overlooked.

Objectives

The goal of this study was to examine the species designation between *Cladonia pyxidata* and *Cladonia pocillum* by examining the overall variation in association with environmental influences. The objectives of this study were;

1. To determine if *Cladonia pyxidata* and *C. pocillum* are separate phylogenetic species;
2. To test the correlation between fungal species and soil pH;
3. To examine the monophyly of the green algal partner, *Trebouxia (Asterochloris)* with respect to fungal species; and
4. To assess the algal population structure (i.e. gene flow) across Canada.

Based on current knowledge, the hypotheses for this study would be;

1. *C. pyxidata* and *C. pocillum* are separate species and will therefore form separate monophyletic groups within the phylogenetic trees;
2. Soil pH will be lower in *C. pyxidata* than *C. pocillum*;
3. Genotypes of the green algal partner will not be monophyletic but will be shared between the fungal species; and
4. Algal genotypes will show dispersal across the geographic region studied.

Materials & Methods

Study specimens and sampling location

Sample specimens consisting of *Cladonia pyxidata, Cladonia pocillum* and closely related pixie-cup species, such as *Cladonia fimbriata* (L.) Fr., *Cladonia grayi* G. Merr. ex Sandst., and *Cladonia chlorophaea,* were collected by myself and my advisor, Dr. Michele D. Piercey-Normore, from a number of locations across Canada during the summer of 2006 (Table 1). Lichen thalli, consisting of basal squamules and vertical podetia, were found growing on soil and humus along roadsides, at fire sites and in exposed areas of the boreal forest. A small sample of soil was collected with each specimen and soil pH was determined in the laboratory. A standardized water pH determination, as described in Jones (2001) was modified to obtain the pH value of the soil for each sample.

Morphological characters (Table 2) were examined for 141 samples using both compound and dissecting microscopes. Morphological characters selected were similar to those deemed important in Ahti (1966), Sipman (1973), Ahti (2000), Aptroot *et al.* (2001), Stenroos *et al.* (2002) and Osyczka (2006). A single podetium from each collection was selected, examined for discolouration, soil, or other contaminants, which were removed, and then placed in a 1.5 mL Eppendorf® tube. A selected number of these samples were further subjected to chemical (Thin Layer Chromatography) and molecular manipulations (PCR amplification, RFLPs, and sequencing).

Table 1. Collection sites of *Cladonia pyxidata 1* (pyx1), *Cladonia pyxidata 2* (pyx2), *Cladonia pocillum* (poc) and closely related species (*C. chlorophaea* - cch, *C. coccifera* - coc, *C. grayi* - gry, *C. pleurota* - cpl, and *C. fimbriata* - fim) from selected regions across Canada. Fungal ITS rDNA[1], fungal PKS[2], and/or algal ITS rDNA[3] sequence data was obtained from samples in **bold.** Collection numbers include collector's initials for reference (RK=Rhonda Kotelko; MPN=Michele Piercey-Normore).

Regions	Location	Site	Site description	GPS Co-ordinates	Elevation	Samples (Collection numbers)
Northern Yukon (N-YT)						
Dawson Rd (Hwy 2)	between Carmacks and Pelly Crossing	1	disturbed roadside	N62° 39' 42.1" W136° 47' 32.4"	570m	RK1073 (poc), RK1074 (pyx1)
	north of Pelly Crossing	2	black spruce, feathermoss	N62° 53' 41.8" W136° 30' 24.4"	602m	RK1070 (poc), RK1071 (pyx1), **RK1072 (poc)[1]**
	40km north of Stewart Crossing	3		N63° 32' 28.3" W137° 17' 20.8"	453m	**RK1064 (cch)[3], RK1065 (pyx2)[1,2]**
	50km south of junction with Hwy 5	4	black spruce, feathermoss, liverworts	N63° 49' 54.1" W137° 57' 47.9"	618m	RK1055 (poc)
	Dawson City - Dome Rd	5	exposed subalpine slope	N64° 04' 03.9" W139° 23' 48.9"	882m	RK1052 (poc)
Dempster Hwy (Hwy 5)	5km from junction with Hwy 2	6	black spruce, feathermoss; gravel pit	N63° 59' 38.6" W138° 39' 47.3"	495m	RK1032 (pyx1), **RK1033 (poc)[1]**
	65km from junction with Hwy 2	7	disturbed roadside; dwarf birch, *Ledum*	N64° 27' 55.6" W138° 12' 26.2"	990m	**RK1027 (poc)[1]**, RK1028 (poc)
	Mt Goldenside	8	subalpine/alpine tundra	N64° 31' 42.3" W138° 12' 08.0"	1490m	**RK999 (pyx1)[1,2]**
	Hart Pass	9	dwarf birch, willow	N64° 33' 26.2" W138° 14' 27.9"	1271m	RK1004 (coc)
Southern Yukon (S-YT)						
Alaska Hwy (Hwy 1)	8km west of Johnsons Crossing	10	disturbed roadside; black spruce	N60° 29' 38.5" W133° 24' 06.7"	797m	**RK905 (pyx1)[1]**, RK909 (pyx1), RK910 (pyx1)
	20km west of Jakes Corner	11		N60° 26' 27.9" W134° 12' 42.6"	742m	RK1077 (pyx1), **RK1078 (pyx2)[1]**, RK1079 (cpl), RK1080 (poc)
	Whitehorse - windmills	12	subalpine/alpine meadows; dwarf birch, *Ledum*	N60° 44' 56.1" W135° 13' 53.2"	1440m	RK912 (cch), RK916 (cch), **RK917 (poc)[1]**, RK922 (poc)
	Takini Burn (1958)	13	fire succession; aspen parkland	N60° 47' 02.3" W136° 01' 29.7"	700m	**RK945 (poc)[1]**
	8km east of Champagne	14		N60° 47' 57.6" W136° 21' 34.5"	717m	**RK946 (poc)[1,2]**, RK947 (poc), RK948 (poc)
	east of Aishihik Lake Rd	15		N60° 48' 54.8" W136° 51' 40.5"	700m	**RK974 (pyx1)[1]**, RK976 (cch)
	west of Aishihik Lake Rd	16		N60° 50' 26.5" W137° 16' 48.3"	688m	**RK969 (pyx1)[3]**, RK970 (poc), RK971 (poc), RK972 (poc)
	west of Haines Junction	17		N60° 51' 18.0" W137° 47' 36.9"	999m	RK957 (poc)
	Jenny Lake Rd	18	subalpine grasslands; disturbed roadside	N61° 03' 07.0" W138° 21' 29.0"	848m	RK961 (poc)
	Congdon Creek Campground	19		N61° 09' 07.3" W138° 33' 03.2"	800m	RK963 (poc), RK964 (poc), RK965 (poc), **RK966 (poc)[1]**, RK968 (poc)
	Destruction Bay	20		N61° 15' 21.2" W138° 48' 48.6"	802m	RK962 (poc)
Aishihik Lake Rd	south of campground	21	subalpine grasslands	N61° 04' 54.5" W136° 59' 41.2"	905m	RK949 (poc), **RK950 (pyx2)[1]**, **RK951 (poc)[1]**, RK952 (cch)
	north of campground	22	subalpine grasslands	N61° 14' 58.0" W136° 55' 24.8"	1018m	RK953 (pyx1), **RK954 (pyx1)[1]**, RK955 (pyx1)
Dawson Rd (Hwy 2)	Fox Lake	23		N61° 15' 47.6" W135° 29' 12.8"	793m	RK934 (poc), RK935 (cch), RK936 (poc), RK937 (cch), **RK938 (pyx2)[1]**
	Braeburn Burn (1958) Trail	24	fire succession; young black spruce and aspen	N61° 26' 00.7" W135° 46' 17.5"	728m	RK930 (poc), RK931 (pyx1)
	Braeburn Burn (1958)	25	fire succession; young black spruce and aspen	N61° 28' 08.5" W135° 46' 11.4"	730m	RK924 (poc), RK925 (poc), RK926 (poc)
Northern BC (N-BC)						
Alaska Hwy (Hwy 97)	between Wonowon and Pink Mountain	26		N56° 57' 12.7" W122° 05' 29.6"	960m	**RK862 (pyx1)[1,2,3]**
	55km north of Pink Mountain	27		N57° 25' 14.9" W122° 51' 59.4"	1132m	RK869 (poc), **RK870 (poc)[1,2]**, **RK871 (poc)[1]**
	15km east of Summit Lake	28		N58° 40' 03.0" W124° 24' 53.7"	975m	**RK879 (pyx1)[1]**
	2km west of Hyland River	29		N59° 57' 23.1" W128° 10' 52.5"	611m	**RK885 (poc)[1,2,3]**, RK888 (cch)
	80km west of Watson Lake, YT	30		N60° 11' 57.5" W130° 04' 16.4"	805m	RK1084 (cch)
Alberta/Saskatchewan (AB/SK)						
Turtle Lake, SK	Blueberry Golf Course	31	dry spruce/jackpine forest edges	N53° 35' 33.8" W108° 42' 36.9"	658m	**RK1091 (cch)[1,2,3]**
Hwy 2 (AB)	70km northwest of Athabasca, AB	32	sandy; black spruce	N55° 01' 26.4" W113° 59' 41.6"	649m	**RK1088 (poc)[1,2,3]**
Northern Manitoba (WNP)						
Wapusk National Park (WNP)	WNP	33	shallow *Carex* fen with hummocks	N58° 07' 48.9" W92° 51' 59.6"	3m	**MPN6171 (poc)[1,2,3]**
	WNP	34	lake edge with graminoids, hummocks	N58° 07' 48.9" W92° 51' 59.6"	3m	MPN6186 (pyx1), MPN6195 (poc)
	WNP	35	shallow *Carex* fen with hummocks; some dwarf birch	N58° 08' 10.6" W92° 52' 01.5"	2m	**MPN6232 (pyx1)[1,2,3]**, MPN6243 (poc)
	WNP	36	white spruce cluster on open tundra	N58° 06' 57.9" W92° 53' 13.3"	5m	MPN6358 (cch)
	WNP	37	open tundra on inland beach ridge, 6-7km from coast	N58° 07' 48.7" W92° 57' 54.9"	11m	MPN6395 (pyx1), **MPN6408 (gry)[1,2]**

Table 1 (continued). Collection sites of *Cladonia pyxidata 1* (pyx1), *Cladonia pyxidata 2* (pyx2), *Cladonia pocillum* (poc) and closely related species (*C. chlorophaea* - cch, *C. coccifera* - coc, *C. grayi* - gry, *C. pleurota* - cpl, and *C. fimbriata* - fim) from selected regions across Canada. Fungal ITS rDNA[1], fungal PKS[2], and/or algal ITS rDNA[3] sequence data was obtained from samples in **bold.** Collection numbers include collector's initials for reference (RK=Rhonda Kotelko; MPN=Michele Piercey-Normore).

Regions	Location	Site	Site description	GPS Co-ordinates	Elevation	Samples (Collection numbers)
Central Manitoba (MB)						
Long Point Rd	Long Point Rd	38	black spruce stand, some jack pine	N52° 56' 33.1" W98° 55' 45.0"	262m	**MPN6115 (pyx2)[1]**
	Long Point Rd	39	cedar, black spruce bog, some larch, *Sphagnum* hummocks	N52° 47' 09.6" W98° 48' 13.9"	263m	MPN6117 (pyx2), **MPN6118 (poc)[1]**, MPN6129 (cch), MPN6131 (poc)
	Long Point Rd	40	cedar-spruce stand, dry, open	N52° 56' 18.5" W98° 56' 18.9"	263m	MPN6143 (pyx1), MPN6147 (poc)
	12km east of Hwy 6	41	cedar, spruce, some birch	N52° 54' 12.8" W99° 00' 05.9"	250m	MPN5538a (poc), MPN5540 (cch), **MPN5541 (poc)[1]**, MPN5546 (poc), MPN5550 (pyx1), MPN5553 (poc), **MPN5556 (poc)[1]**
Hwy 6	90km north of Grand Rapids	42	open calcareous limestone table in disturbed jack pine forest	N53° 57' 57.6" W99° 12' 25.6"	-	**MPN5600 (fim)[1-3]**, MPN5618 (pyx1)
	55km south of Thompson	43	jack pine and poplar ridge, mix of granite and limestone	N55° 20' 13.4" W98° 21' 25.9"	267m	MPN5929 (pyx1)
Hwy 391	30km west of Thompson	44	black spruce bog, few willows, *Sphagnum* hummocks	N55° 53' 52.9" W98° 14' 38.8"	264m	MPN5624 (pyx1), MPN5638 (pyx1)
	11km east of Notigi	45	limestone ridge with black spruce, jack pine, alder (clearings)	N55° 52' 21.7" W99° 09' 11.1"	266m	MPN5707 (poc)
	11km west of Notigi	46	granite ridge with boulders, jack pine	N55° 50' 39.8" W99° 26' 02.2"	266m	**MPN5750 (pyx1)[1]**
	20km west of Notigi	47	dry black spruce bog	N55° 49' 45.6" W99° 31' 20.8"	282m	MPN5889 (cpl)
	50km south of Leaf Rapids	48	burned rock outcrop, jack pine; charred trees and debris	N56° 02' 37.2" W99° 53" 28.8"	294m	MPN5866 (pyx1), MPN5867 (pyx1)
Hwy 392 (Snow Lake)	25km north of Hwy 39	49	jack pine ridge	N54° 50' 47.8" W99° 56' 56.3"	238m	MPN5964 (pyx1)
Hwy 10 (The Pas)	20km north of The Pas	50	edge of black spruce forest	N53° 59' 14.3" W101° 15' 41.9"	314m	**MPN6081 (poc)[1]**
	20km south of The Pas	51	open area near spruce forest	N53° 41' 31.8" W101° 14' 25.7"	295m	MPN6083 (pyx1), MPN6084 (poc), **MPN6085 (poc)[1]**
	20km south of West Ray	52	open area near jack pine forest	N53° 25' 40.8" W101° 20' 18.5"	309m	**MPN6086 (pyx2)[1]**, MPN6089 (pyx2)
Hwy 60 (between Hwy 10 & 6)	1km east of Hwy 10	53	mossy clumps at edge of spruce forest	N53° 21' 08.7" W101° 03' 29.6"	300m	MPN6090 (poc)
	35km east of Hwy 10	54	spruce, cedar, larch bog	N53° 12' 49.5" W100° 42' 09.0"	306m	MPN6092 (pyx1)
	60km east of Hwy 10	55	edge of spruce-aspen forest	N53° 06' 09.9" W100° 23' 41.1"	305m	MPN6095 (cch), MPN6096 (poc)
	Mossy Portage Rd, 1km north of Hwy 60	56	edge of spruce-aspen forest	N53° 04' 37.6" W100° 07' 10.0"	292m	MPN6101 (pyx1), **MPN6102 (poc)[1]**, MPN6103 (pyx2), MPN6104 (cch)
	4km east of Hwy 327	57	spruce-*Hylocomium* forest	N52° 56' 16.1" W99° 42' 14.8"	297m	MPN6106 (pyx1)
	20km west of Hwy 6	58	black spruce forest	N52° 54' 25.1" W99° 27' 17.5"	309m	MPN6107 (pyx2)
	12km west of Hwy 6	59	cedar-aspen stand	N52° 55' 40.2" W99° 19' 51.7"	283m	MPN6108 (pyx2)
	Hwy 6, 13km south of Hwy 60	60	edge of black spruce/cedar stand	N52° 52' 14.5" W99° 01' 51.4"	263m	**MPN6112 (poc)[1]**, MPN6113 (poc)
Ontario/Quebec						
Northwestern Ontario (NW-ON)	east of Kenora; near jctn Hwy 17A & 658	61	roadside granite ridge with aspen	N49° 47' 56.5" W94° 28' 41.3"	6m	**MPN6576 (pyx1)[1-3]**, MPN6577 (cch), **MPN6578 (pyx1)[1]**
	Hwy 17; 13km east of Borups Corners	62	jack pine, granite ridge	N49° 32' 33.5" W92° 10' 26.8"	391m	**MPN6592 (coc)[1]**, MPN6598 (pyx1)
	Hwy 17; 5km west of Ignace	63	jack pine, sandy ridge	N49° 26' 07.1" W91° 42' 21.4"	439m	**MPN6623 (cch)[2]**
	Hwy 17; 7km east of Nipigon	64	wet cedar-spruce bog at roadside	N49° 00' 33.5" W88° 09' 36.4"	208m	**MPN7268 (cch)[1]**
Ontario-Quebec border (ON/QC)	Hwy 17; 1km west of Spanish	65	roadside rock outcrop; pine, white spruce	N46° 11' 58.5" W82° 21' 17.0"	201m	**MPN7209 (gry)[1]**, **MPN7214 (pyx2)[1]**
	Hwy 17; 3km east of Bissett Creek	66	roadside granite outcrop, Sumac shrubs	N46° 12' 39.3" W78° 00' 52.8"	192m	MPN7190 (pyx1)
	Hwy 66; 5km east of Virginiatown	67	granite ridge with spruce and jack pine	N48° 08' 32.7" W79° 32' 10.3"	331m	**MPN6776 (pyx1)[1-2-3]**, MPN6786 (pyx1)
	Hwy 117; 42km northwest of Le Domaine	68	rock outcrop on roadside	N47° 17' 32.6" W76° 51' 3.9"	-	**MPN6824 (pyx1)[1]**
Newfoundland						
	Hwy 13; Witless Bay Line	69	thick spruce-fir forest, top of ridge	N47° 20' 45.2" W52° 54' 04.5"	157m	**MPN6949 (poc)[1]**
	Hwy 1; 33km north of Badger	70	rock outcrop with birch, spruce, aspen	N49° 14' 14.8" W56° 07' 35.3"	124m	MPN7026 (cch)
	Hwy 430; 20km south of River of Ponds	71	open wet barrens	N50° 23' 32.8" W57° 31' 01.1"	-	**MPN7072 (pyx2)[1-2-3]**
	Hwy 430; 15km north of Eddies Cove West	72	open beach at coast	N50° 50' 28.1" W57° 02' 05.8"	26m	**MPN7104 (poc)[1]**
	Hwy 430 near Port aux Choix	73	black spruce bog	N50° 40' 59.6" W57° 13' 16.9"	55m	MPN7107 (pyx2)

Table 2. Morphological characters examined in samples *Cladonia pyxidata 1, C. pyxidata 2,* and *C. pocillum*.

Character	Character states
Basal squamules	
1) orientation of lobes	appressed (entire lobe is attached to substrate), upturned (tips of lobes are upright), upright (entire lobe is upright)
2) rosette pattern	yes/no
3) surface colour	green, gray/green, greenish/brown, browning, blackening
4) eroding	yes/no
Podetia	
5) surface features	areoles (> 100μm in diameter), granules (50-100μm), soredia (< 50μm)
6) podetial squamules	yes/no
Cups	
7) colour	green, gray/green, browning, brown, blackening
Areoles	
8) location	inside cup, outside cup, inside & outside cup, none
Granules	
9) location	inside, outside, inside & outside cup, upper podetia, none
Pycnidia	
10) location	cup margin, basal squamules (upper & lower), in cup, none
11) colour	brown, brown-black, black
Apothecia	
12) location	projection from cup margin, cup margin, center of cup, none
13) colour	brown, dark brown, black

Thin Layer Chromatography (TLC)

Secondary compounds were extracted from *Cladonia pyxidata, C. pocillum* and closely related species using a protocol modified from Culberson (1972). Secondary compounds were removed from each sample and transferred to a glass slide using two five minute acetone extractions, followed by a single 10 minute acetone extraction. The acetone was allowed to evaporate from the glass slide, leaving behind a concentrated film of secondary compounds on the glass microscope slide. Slides were stored in a slide box at room temperature until used.

Each extract was re-suspended in 3 or 4 drops of acetone and spotted onto two silica coated gel thin layer chromatography plates with fluorescent indicators (Fisher Scientific, Nepean, ON, Canada) using 25 µL glass capillary tubes. Secondary compounds from *Cladonia magyarica* (MS4553), known to contain atranorin and fumarprotocetraric acid was also spotted onto each plate and served as a reference control to indicate the *Rf* class of each of atranorin and fumarprotocetraric acid. One of the two plates was placed in a tank containing solvent system A (toluene-dioxane-acetic acid, 180:45:5) and the other was pre-treated with glacial acetic acid and then placed in a tank containing solvent system C (toluene-acetic acid, 200:30). The plates were removed from the tanks when the solvent front reached 1 cm from the top of the plate (approximately 10 min). Plates were then allowed to dry under a fume hood.

To determine the presence of secondary compounds within each sample, the plates were examined under both short (254 nm) and long-wave (365 nm) UV light.

Spots that were either fluorescent or were quenched by the UV light were marked and labelled accordingly (fl or q). The plates were then sprayed with 10% sulfuric acid and allowed to dry on a slide warmer. As the plates dried, the location of fatty acids appeared as opaque spots and were recorded. The plates were baked for 20 minutes at 80°C to complete the colour development of the samples. Any colour changes were subsequently reported. The *Rf* class of each substance was also determined by comparison with the reference control. Characteristics specific to known secondary compounds, such as colour, fluorescence, and *Rf* class, were used to identify the compounds present in each sample by comparing the observed characteristics with a spreadsheet of known characteristics (C.F. Culberson, unpublished).

DNA extraction

Total cellular DNA was extracted on the same podetia used for TLC following procedures modified from Grube *et al.* (1995). The selected podetia were ground to a fine powder in 1.5 mL Eppendorf® tubes using blue pestles. When sufficiently crushed, 500 µL of TES buffer [100mM Tris-HCl pH 8.0; 10mM EDTA (ethylenediaminetetraacetate); 2% SDS (sodium dodecyl sulfate)] was added and the tubes were vortexed to homogenize the mixture. NaCl, to a final concentration of 1.4M, and 0.1 volumes of 10% CTAB (cetyltrimethylammonium bromide) were then added. The tubes were vortexed briefly and placed in a 65°C water bath for one hour.

After this incubation period, the tubes were vortexed again and an equal volume of chloroform: isoamyl alcohol (24:1) was added. The tubes were mixed gently for 1 minute and centrifuged for 5 minutes at 5,000 rpm. The supernatant was collected and an equal volume of chloroform: isoamyl alcohol (24:1) was again added; the tubes were mixed gently and centrifuged for 5 minutes at 5,000 rpm. The supernatant was transferred to new Eppendorf® tubes and 0.2 volumes of 5M NaCl and 2.5 volume of 100% ethanol were added in order to precipitate the DNA. The tubes were mixed gently and left to stand at 4°C for 20 minutes, after which they were centrifuged for 10 minutes at 13,000 rpm. The supernatant was poured off and the DNA pellet was washed with 80% cold ethanol and left to air dry. Once the pellet was dry, the DNA was re-suspended in 50 mL sterile distilled water (sdH_2O) and stored in the freezer (-20°C) until needed.

Electrophoresis and Quantification of DNA extractions and purified PCR product

DNA extractions and RFLPs were quantified using horizontal gel electrophoresis. The gels contained 1-1.5% agarose in 1X TBE (0.089M Tris, 0.089M boric acid, 2mM EDTA) buffer and were stained with ethidium bromide (0.5 mg/mL). Samples were placed in bromophenol blue (BPB) to facilitate loading and transferred to wells within the prepared gel. The gel was run at 120V until the BPB was 1cm from the bottom (approximately 30 min) and then examined under UV light with an AlphaInnotech 2200 Gel Document System (Fisher Scientific, Nepean, ON, Canada).

DNA was quantified by comparing the intensity of the bands within the samples to the intensity of the 1650 bp band in the 1 kb Plus DNA Ladder (Invitrogen, Burlington, ON, Canada). The 1650 bp band contains 8% of total mass loaded. Therefore if 1 µg of 1 kb DNA ladder is added, then 0.08 µg of DNA is within the 1650 bp band. Bands of similar intensity will contain the equivalent amount of DNA. The 1 kb Plus DNA ladder, which ranges from 100 bp to 12,000 bp, was also used to determine the approximate length of the fragments generated by RFLPs.

DNA Amplification using Polymerase Chain Reaction

Three gene regions, two from the fungal partner and a third from the algal partner, were selected for amplification by polymerase chain reaction (PCR). ITS rDNA and the keto-synthase (KS) domain of a PKS gene complex of the fungal partner were amplified using the fungal specific primer, 1780f-5', and a universal primer, ITS4-3', for the ITS region and newly designed primers, PKS1F-5' and PKS2R-3', for the PKS gene region (Table 3). These newly designed primers were designed from conserved regions within sequences obtained from degenerate primers, LC1 and LC2c (Bingle *et al.* 1999). The ITS rDNA of the algal partner was amplified using an algal specific primer, Al1700f-5', and the same universal primer, ITS4-3'.

Table 3. Primers, primer sequence and source for the amplification of fungal ITS rDNA, PKS, and algal ITS rDNA.

Primer	Sequence	Reference	Primer Function
1780f-5'	CTG CGG AAG GAT CAT TAA TGA G	Piercey-Normore and DePriest 2001	forward primer for fungal ITS
Al1700f-5'	CCC ACC TAG AGG AAG GAG	Helms *et al*. 2001	forward primer for algal ITS
ITS4-3'	TCC TCC GCT TAT TGA TAT GC	White *et al*. 1990	reverse primer for algal and fungal ITS
PKS1F-5'	TAC GAA GCC CTA GAA ATG GC	this lab	forward primer for PKS
PKS2R-3'	ACG TTT GGC AGT TTC CTG TC	this lab	reverse primer for PKS
LC1	GAY CCI MGI TTY TTY AAY ATG	Bingle *et al.* 1999	forward primer for PKS (primer design)
LC2c	GTI CCI GTI CCR TGC ATY TC	Bingle et al. 1999	reverse primer for PKS (primer design)
ITS2-5'	GCT GCG TTC TTC ATC GAT GC	White *et al*. 1990	reverse internal primer for sequencing
ITS3-3'	GCA TCG ATG AAG AAC GCA GC	White et al. 1990	forward internal primer for sequencing

Amplification reactions followed that of Beiggi & Piercey-Normore (2007) and were prepared in 0.2 mL PCR tubes on ice. Preliminary screening of each sample and amplification of the algal ITS rDNA for RFLPs was conducted in 20 µL reaction volumes and contained approximately 10-50 ng DNA/reaction. Amplification for sequencing was carried out in eight 50 µL reaction volumes and contained approximately 25-100 ng DNA/reaction. A mastermix was prepared containing sterile distilled water (sdH_20) to final volume, 1X PCR buffer (500 µM KCl, 100 µM Tris-HCl pH 8.3), 0.2 µM of each dNTP, 2.0 mM $MgCl_2$, 0.5 µM of each primer (2 primers per reaction; Table 3) and 2 units of recombinant Pfu DNA polymerase per sample. Recombinant Pfu DNA polymerase was cloned and purified from *E. coli* in Dr. Loewen's laboratory (Department of Microbiology, University of Manitoba, MB, Canada). One drop of diluted DNA (containing 10-50 ng) was added to the bottom of each tube. The mastermix was briefly mixed and added to each tube.

PCR reactions were carried out in a Techne Genius thermal cycler (Fisher Scientific, Nepean, ON, Canada). Amplification conditions were optimized for each gene region amplified. Fungal ITS rDNA was amplified using touchdown PCR consisting of initial denaturation conditions of 94°C for 5 min, followed by two cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1.5 min. The annealing temperature was then decreased by two degrees after two cycles until reaching the final annealing temperature of 54°C. Once the final annealing temperature was reached, 30 more cycles of denaturation, annealing and extension were performed.

Fungal PKS and algal ITS rDNA were amplified by standard PCR. Amplification conditions for the PKS were initial denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 58°C for 1 min and extension at 72°C for 1 min. Amplification conditions for the algal ITS rDNA were initial denaturation at 94°C for 5 min, followed by 33 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 1.5 min. All samples were finally stored at 4°C.

Samples amplified for PCR and RFLPs were then quantified using 1 kb Plus DNA ladder and horizontal gel electrophoresis, as previously described (see *Electrophoresis and Quantification of DNA extractions and purified PCR product)*. Amplification products to be used for sequencing were combined in a 1.5 mL Eppendorf® tube (400 µL) and precipitated using 0.2 volumes 5M NaCl and 2.5 volumes 100% ethanol (ethanol). Samples were mixed gently and incubated at 4°C for 20 min. They were then centrifuged at 13,000 rpm for 10 min and the supernatant was discarded. The remaining pellet was washed with cold 80% ethanol and allowed to air dry. Samples were then re-suspended in 20 µL sdH_2O.

Gel purification

PCR products were purified by horizontal gel electrophoresis in 1% agarose in 1X TBE buffer, stained with ethidium bromide, as previously described. Bands were visualized with a light box under short wave (254 nm) UV light, excised from the agarose gel, and placed in 1.5 mL Eppendorf® tubes.

One of two methods was then used to complete the purification of PCR products from gel slices for cycle sequencing. The first method involved freezing the gel slice overnight. The frozen block was then crushed in a folded strip of paraffin film and the resulting liquid, containing PCR product, was pipetted back into the Eppendorf® tube. Samples were precipitated by adding 0.2 volumes 5M NaCl and 2.5 volumes 100% ethanol, mixing gently, and incubating at 4°C for 20 min. Tubes were then centrifuged for 10 min at 13,000 rpm. The supernatant was poured off and the remaining pellet was washed with 200 µL cold 80% ethanol. Tubes were inverted and allowed to air dry. When dry, pellets were re-suspended in 20 µL sdH_2O.

The other method followed a column purification protocol established for the Wizard SV Gel and PCR Clean-Up System (Promega Corporation, Madison, WI, United States). Samples were re-suspended in either 25 µL or 50 µL sdH_2O depending on the concentration of PCR product during the initial stages of gel purification. Once re-suspended, PCR products, obtained from either method, were quantified for cycle sequencing using horizontal gel electrophoresis, as previously described.

Cycle sequencing

Cycle sequencing reactions were set up in 0.2 mL PCR tubes on ice. Approximately 20-50 ng of PCR product, sdH_2O to a total volume of 20 µL, 3.2 pmol primer (one/reaction; Table 3), 0.5X sequencing buffer and BigDye Terminator v3.1®

(Applied Biosystems, Foster City, CA, United States), were contained in each 20 µL reaction volume.

Cycle sequencing reactions were conducted in a Techne Genius thermal cycler (Fisher Scientific, Nepean, ON, Canada). Amplification conditions were initial denaturation at 96°C for 2 min, followed by 25 cycles of denaturation at 96°C for 10 sec, annealing at 50°C for 5 sec, and extension at 60°C for 4 min (according to manufacturer's instructions; Applied Biosystems, Foster City, CA, United States). Samples were then stored at 4°C.

Post reaction clean-up and Sequencing

Following the cycle sequencing reactions, unincorporated fluorescent dyes were removed according to the manufacturer's instructions (Applied Biosystems, Foster City, CA, United States) by precipitating the DNA with 0.25 volumes 125 mM EDTA and 3 volumes 100% ethanol to each tube. Tubes were inverted four times to mix the samples, incubated at room temperature for 15 min, and then centrifuged at 4,000 rpm for 45 min. The supernatant was pipetted off and 80% cold ethanol was added to rinse the pellet. Tubes were then centrifuged at 4,200 rpm for 15 min and the supernatant was pipetted off again. The samples were then place in the DNA120 SpeedVac (Thermo Savant, Holbrook, NY, USA) for 20 min to dry.

To denature the final product for sequencing, 20 µL Hi-Di Formamide (Applied Biosystems, Foster City, CA, United States) was added to each tube and incubated at

95°C for 5 min. Following this incubation, tubes were immediately placed on ice before being loaded into a Genetic Analyser 3130 (Applied Biosystems, Foster City, CA, United States) for sequencing.

Restriction Fragment Length Polymorphisms (RFLPs)

RFLPs were generated by digesting the algal ITS rDNA PCR product with the restriction enzymes, MseI ($T^{\downarrow}TAA$) and HhaI ($GCG^{\downarrow}C$; Invitrogen, Burlington, ON, Canada). Approximately 100 ng of PCR product was digested in 20 µL reaction volumes containing sdH_2O to volume, 1X each of the manufacturer's buffers, REACT 1 and REACT 2, and 2 units of each MseI and HhaI. To ensure the complete digestion of the PCR product, samples were left overnight at 37°C.

Banding patterns were visualized using horizontal gel electrophoresis, as previously described. Fragment lengths were estimated by comparison with the 1 kb Plus DNA ladder (Invitrogen, Burlington, ON, Canada) and confirmed by sequencing representative samples of each different algal RFLP genotype.

Data analysis

Morphological characters (areoles and basal squamules) were used to distinguish among the fungal species examined. Secondary compounds were used to confirm

identifications. Soil pH values were compared between fungal species using Analysis of Variance (ANOVA) in Microsoft Excel 2007.

Fungal ITS rDNA, fungal PKS, and algal ITS rDNA sequences were assembled and edited in Sequencher 4.6 (Gene Codes Corp., Ann Arbor, MI, United States) and then aligned manually in Se-Al v2.0 (Rambaut 2001). Additional sequences, including outgroups (*Cladonia cenotea* – fungal ITS rDNA and PKS; *Trebouxia erici* – algal ITS rDNA), were retrieved from NCBI GenBank. Accession numbers are indicated in phylogenies (Figures 3, 4, 5, & 6).

Aligned fungal ITS, PKS, and algal ITS sequences were subjected to phylogenetic analyses using PAUP* 4.0b10 (Swofford 2003). Analyses were conducted on four sets of data; the fungal ITS rDNA, the KS domain of a fungal PKS gene, a combined fungal ITS and PKS dataset, and the algal ITS rDNA. Maximum parsimony was performed using the options tree bisection and reconnection (TBR) branch swapping, collapse zero length branches, and acctran character-state optimization. Heuristic searches were conducted using 1000 random addition replicates with a limit of 1000 trees per search and bootstrap searches of 100 re-samplings (Felsenstein 1985). Phylogenetic trees were midpoint rooted except for the algal ITS data, which was presented as an unrooted cladogram.

Incongruence tests were performed for tree topologies obtained from the fungal ITS and PKS data. Tree topologies were compared using the Kishino-Hasagawa (K-H) test performed in PAUP (Kishino & Hasagawa 1989). The partition homogeneity test was also

implemented in PAUP and was used for the Incongruence Length Difference (ILD) test (Farris *et al.* 1994).

Distribution of algal genotypes among geographical regions and fungal species was examined by Analysis of Molecular Variance (AMOVA) using GenAlEx 6.0 (Peakall & Smouse 2005). ϕPT (Peakall & Smouse 2005) is a measure of population differentiation analogous to F_{ST} (Weir & Cockerham 1984) and is calculated as the proportion of variance among populations (V_{AP}) relative to the total variance ($V_{AP}+V_{WP}$). Populations were defined based on geographical regions and fungal species.

Results

Morphological variation and secondary chemistry

Morphology was examined for all samples (n=141) and species were delimited on the basis of the presence or absence of aereoles, morphology of the basal squamules, and secondary chemistry (Table 2). *Cladonia pyxidata* and *C. pocillum* were distinguished from closely relates species, such as *C. chlorophaea*, by the presence of aereoles within the cups. The morphology of the basal squamules was used to further delimit *C. pyxidata* from *C. pocillum*. *Cladonia pyxidata* had separated upright basal squamules, whereas the basal squamules of *C. pocillum* formed a distinct rosette-like pattern, which was tightly appressed to the substratum. There also appeared to be a second morphotype of *C. pyxidata*. Therefore *C. pyxidata* was separated into two morphotypes; *Cladonia pyxidata 1* and *C. pyxidata 2*. *Cladonia pyxidata 1* had the typical upright basal squamules used to describe *Cladonia pyxidata*. *Cladonia pyxidata 2* had the same upright basal squamules but they formed the rosette-like pattern characteristic of *C. pocillum* instead. A total of 63 samples of *C. pocillum,* 39 of *C. pyxidata 1*, 14 of *C. pyxidata 2*, 18 of *C. chlorophaea*, two each of *C. grayi, C. pleurota*, and *C. coccifera,* and a single sample of *C. fimbriata* were identified from 141 specimens collected across Canada.

TLC was used to identify secondary compounds in all samples (Figure 1). Fumarprotocetraric acid was detected in all but six samples; *C. pleurota* (MPN5889), *C.*

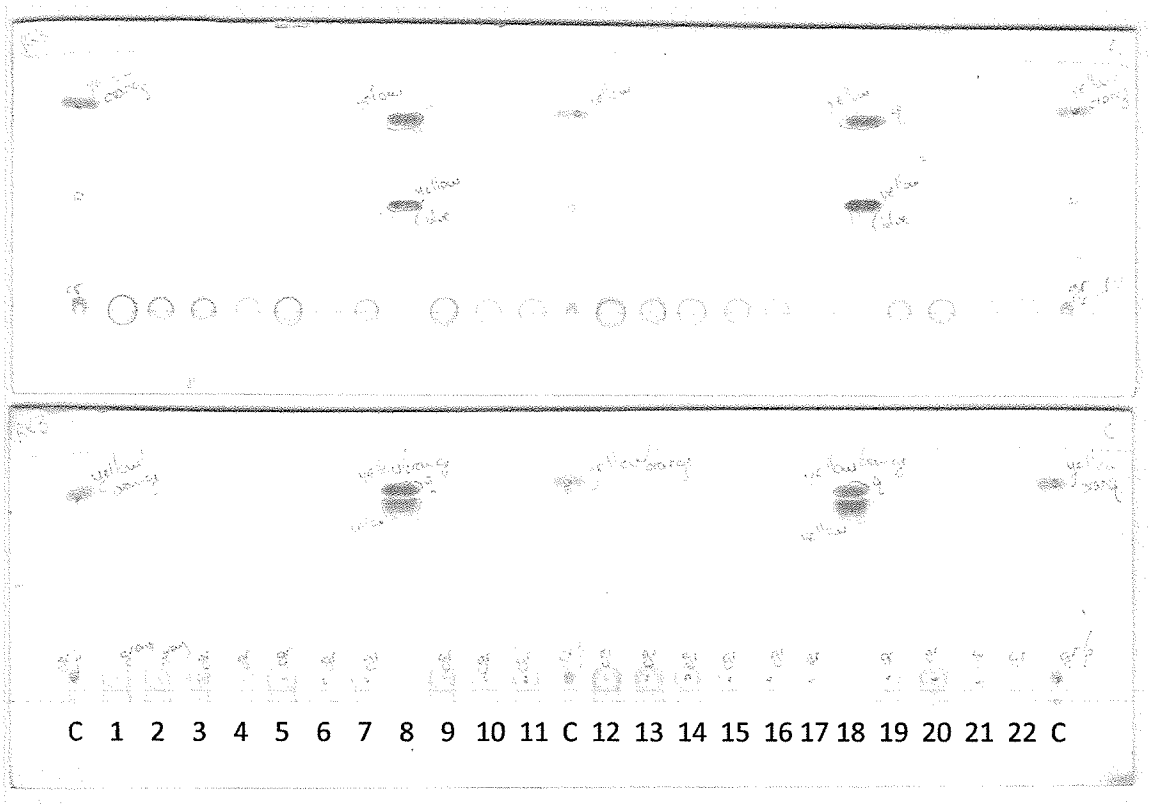

Figure 1. TLC plates showing presence of secondary compounds in solvent systems A and C for 22 samples (1-22) and three controls (C).

pleurota (RK1079), *C. chlorophaea* (MPN6129), *C. coccifera* (MPN6592), *C. coccifera* (RK1004), and *C. grayi* (MPN7209). *Cladonia chlorophaea* (MPN6129) was unique in being the only sample to contain no secondary compounds detectable by TLC. Atranorin was detected in a single sample, *C. pocillum* (RK885). Two samples of *C. pleurota* (MPN5889 and RK1079) contained the diagnostic compounds, usnic and barbatic acids, and two samples of *C. coccifera* (MPN6592 and RK1004) contained usnic acid and zeorin. The two samples of *C. grayi* (MPN6408 and MPN7209) also contained grayanic acid; however, *C. grayi* (MPN6408) contained fumarprotocetraric acid in addition to grayanic acid. Four other samples, *C. chlorophaea* (MPN6623), *C. chlorophaea* (RK912), *C. pyxidata* (RK879), and *C. pyxidata* (RK922), were found to contain quantities of either unknown, trace, or contaminating compounds. Two samples of *Cladonia chlorophaea* (MPN6623 and RK912) both contained an unknown trace compound (Solvent A – *Rf* class 4 and Solvent A – *Rf* class 2, respectively). Two unknown compounds (Solvent A – *Rf* classes 4 and 7) were also observed in *C. pyxidata* (RK879). The presence of thamnolic and baeomycesic acid in *C. pyxidata* (RK922) were thought to be the result of transfer from a sample of *Thamnolia* sp. in the same packet.

Trends in soil pH

Soil pH was determined for 46 samples corresponding to the samples selected for sequencing of the fungal ITS rDNA and PKS gene regions. Overall soil pH values ranged from 6.44 to 7.84 (Figure 2). Soil pH values for samples of *C. pocillum* ranged from 6.90 to 7.84, *C. pyxidata 1* ranged from 6.76 to 7.33, and *C. pyxidata 2* ranged from 6.91 to 7.82. pH was significantly different among the three species ($F(2, 38) = 5.85$, $p<0.01$). *Cladonia grayi* was found on soil at significantly lower pH levels (6.44 and 6.53). In some cases, thalli of *C. pocillum* (MPN6949, MPN6118, MPN5541, MPN6112, MPN6081, MPN6085, MPN7104), *C. pyxidata 1* (MPN6232), *C. pyxidata 2* (MPN6086, MPN6115, MPN7214), and *C. grayi* (MPN6408), were found growing on a layer of moss, which was used instead of soil to determine the pH of the substratum. Soil pH could not be obtained for *C. fimbriata* (MPN5600) because it was growing among leaf litter and pine needles from which the basal squamules could not be separated.

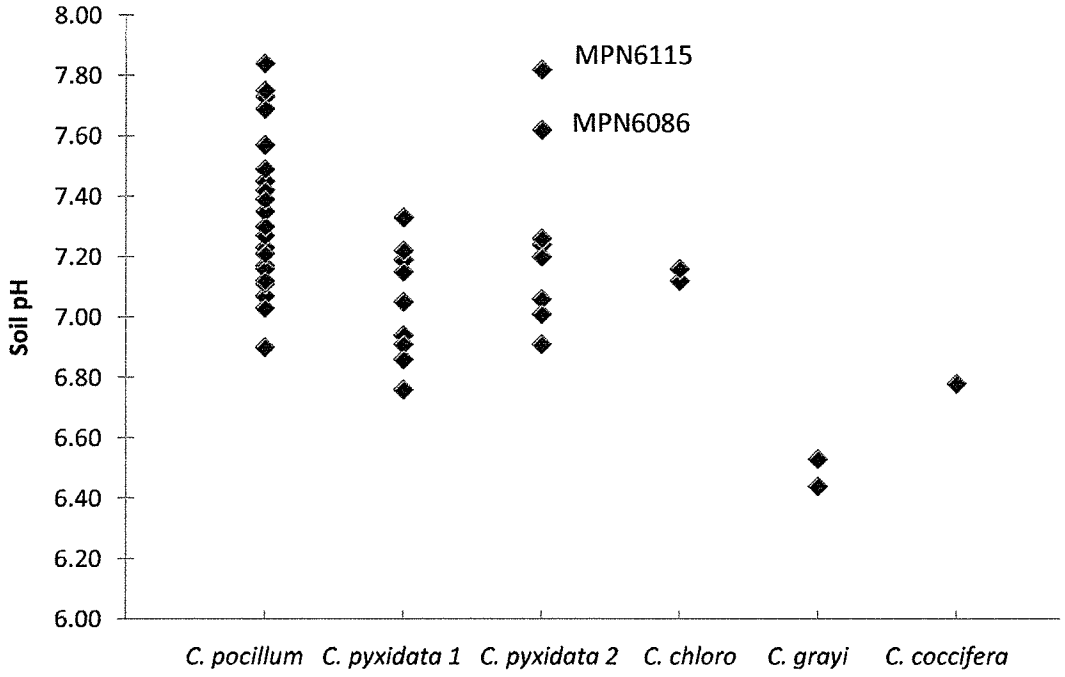

Figure 2. Soil pH values for samples of *C. pyxidata, C. pocillum,* and closely related species from across Canada. Two samples of *Cladonia pyxidata 2* (MPN6115, MPN6086) were considered outliers and not included in statistical analyses. *Cladonia chlorophaea* is abbreviated as *C. chloro* in the graph.

Evolution of the fungal partner

The fungal ITS rDNA was sequenced for 48 samples collected from across Canada and *C. magyarica* from Finland. Nine sequences were also included from GenBank (*C. pyxidata*, EU034665; *C. pyxidata*, DQ534463; *C. pyxidata*, DQ530199; *C. pyxidata*, AF455223; *C. pocillum*, DQ530205; *C. pocillum*, DQ530198; *C. pocillum*, DQ530209; and *C. pocillum*, DQ530204), including *C. cenotea*, AF457900, which was selected as the outgroup. Raw sequences ranged from 512bp to 618bp in length, and the aligned dataset was 645bp long. One hundred and fifty-five of these characters were polymorphic but only 83 were parsimony-informative. One of 47 most parsimonious trees, with a length of 262 steps, was displayed (Figure 3).

All species were polyphyletic, however two poorly supported (<60%) clades, A and B, were observed (Figure 3). Clade A contained representatives of *C. pocillum, C. chlorophaea, C. fimbriata,* and three samples of *C. pyxidata 1* (MPN6824, MPN6576, and RK879) that were in a mixed sample with *C. chlorophaea*. Clade B contained *C. pyxidata 1, C. pyxidata 2, C. grayi,* and *C. magyarica*. GenBank samples were found scattered throughout the tree. No pattern was observed when soil pH or geographic regions were mapped onto the tree. Algal genotypes were also mapped onto the tree; algal genotype A was predominately present in Clade B, whereas algal genotypes A, B, C, D, E, G, and H were present in Clade A.

A portion of the KS region of a fungal PKS gene was sequenced for 16 samples collected from across Canada as well as *C. monomorpha* from The Netherlands and *C.*

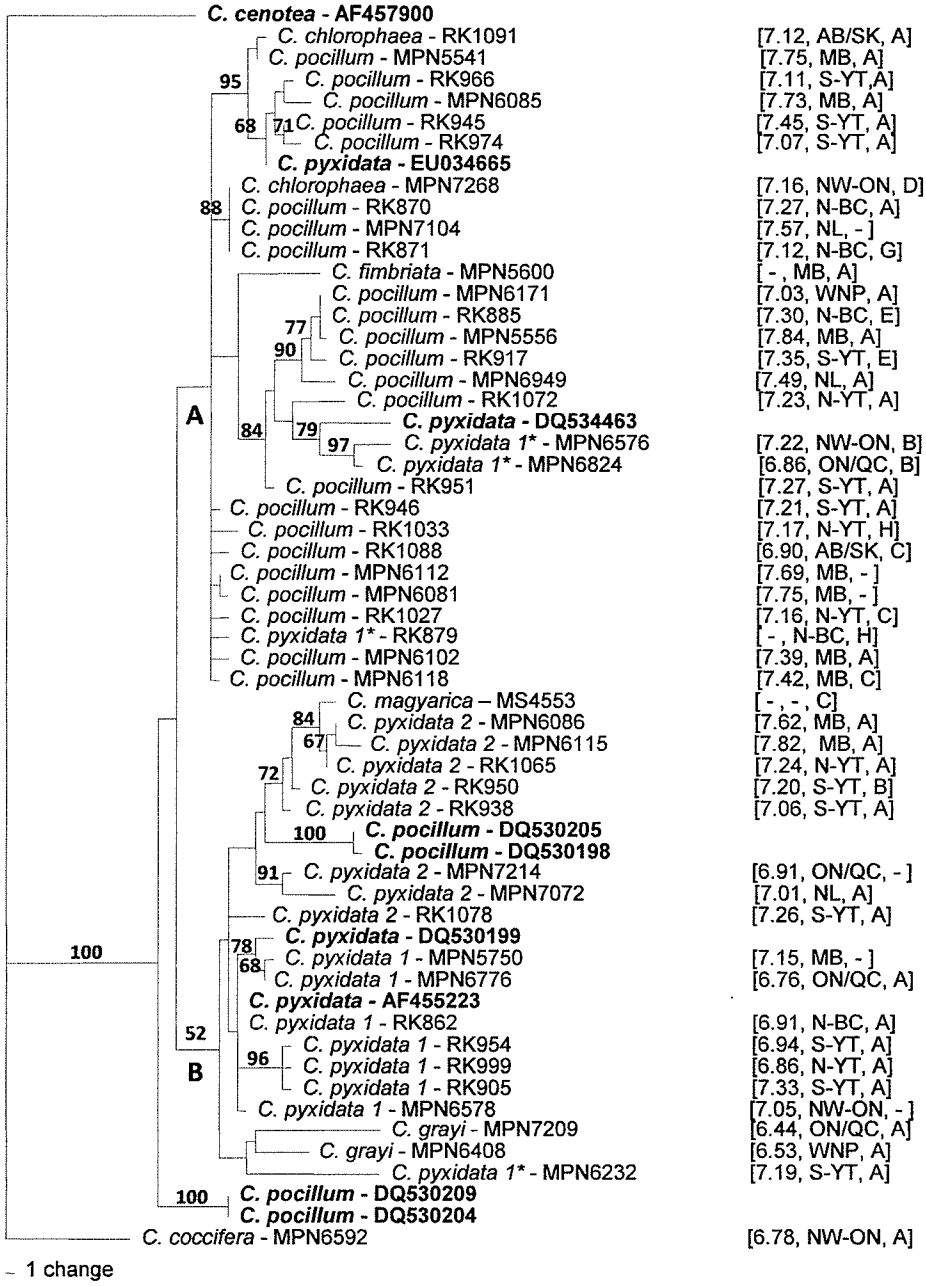

Figure 3. One of 47 most parsimonious trees obtained from DNA sequence data of the fungal ITS rDNA. Soil pH, geographical region, and algal genotype are reported in parentheses following the sample number. Bootstrap values (>65%) are above branches. CI=0.721, RI=0.840, HI=0.279. Samples with asterisks indicate that the sample was in a mixture with *Cladonia chlorophaea*. Samples in **bold** are from GenBank and samples with collection numbers RK or MPN are from this study.

magyarica from Finland. *Cladonia cenotea* (EF363874) was obtained from GenBank as an outgroup. Aligned sequences were 421bp long and ranged from 419bp to 421bp in actual length. Seventy-three nucleotide sites were polymorphic but only 27 were parsimony-informative. MP analysis revealed six most parsimonious trees with a branch length of 93 steps (Figure 4).

Cladonia pyxidata 1, C. pyxidata 2, and *C. pocillum* were not monophyletic; however, the clade containing *C. pocillum* was nested within *C. pyxidata 1* in the PKS tree. *Cladonia magyarica* and *C. monomorpha* grouped with samples of *C. pyxidata 1* and *C. pyxidata 2* at low bootstrap support (63% and 65%, respectively), while *C. fimbriata* (MPN5600) and *C. chlorophaea* (RK1091 and MPN6623) formed a highly supported clade (99%) with *C. pocillum*.

Sequence data from the fungal ITS rDNA and PKS gene regions for 15 samples collected from across Canada and *C. magyarica* were combined and analyzed (Figure 5). Aligned sequences contained 1069 characters, of which 60 were parsimony-informative. Fifty-four MP trees were obtained with a branch length of 238 steps. Species were not monophyletic; however, *C. pocillum, C. chlorophaea,* and *C. fimbriata* formed a highly supported clade (92%). *Cladonia magyarica* grouped with *C. pyxidata 2* (98%). Phylogenetic trees from each of the ITS and PKS genes were not congruent; p-values for Kishino-Hasegawa (K-H) and Incongruency Length Difference (ILD) tests were p=0.0004 and p=0.01, respectively.

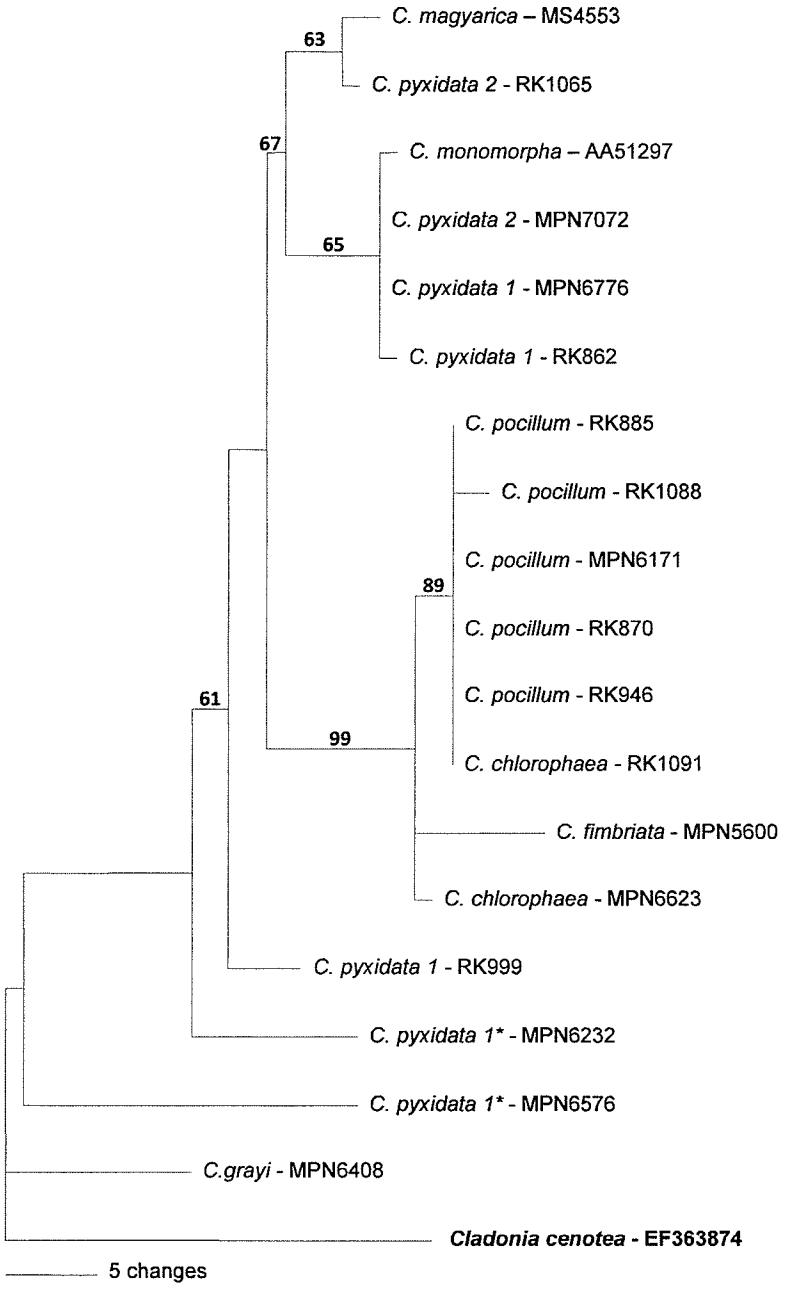

Figure 4. One of six most parsimonious trees obtained from PKS sequence data. Bootstrap values (>60%) are above branches. CI=0.882, RI=0.892, HI=0.118. Samples with asterisks are a mixture with *Cladonia chlorophaea*. Sample in **bold** is from GenBank.

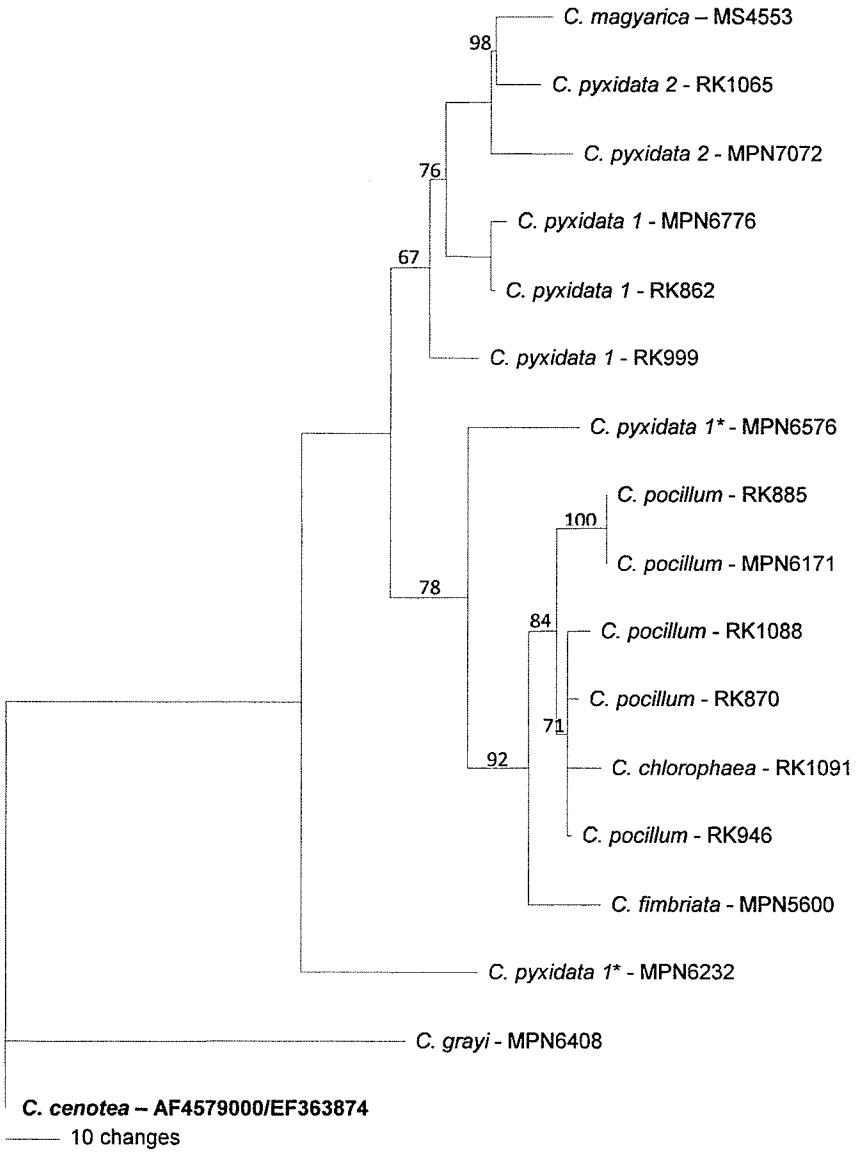

Figure 5. One of 54 most parsimonious trees obtained from the combined fungal ITS rDNA and PKS dataset. Bootstrap values (>60%) are recorded above the branches. CI=0.824, RI=0.777, HI=0.176. Samples with asterisks are a mixture with *Cladonia chlorophaea.* Sample in **bold** is from GenBank.

Evolution and dispersal in the algal partner

The algal ITS rDNA was sequenced from 12 samples collected from across Canada. Fourteen sequences were obtained from GenBank, eight samples of uncultured *Trebouxia (Asterochloris)* and six from cultured representatives of *T. magna* (AF345423), *T. pyriformis* (AF345407), *T. glomerata* (AF345404), *T. irregularis* (AF345411), *T. excentrica* (AF345433), and the outgroup *T. erici* (AF345440). *Trebouxia erici* was selected as an outgroup based on the isolated position of *T. erici* in the 26S phylogeny produced by Friedl & Rokitta (1997). Aligned sequences contained 560 characters, 29 of which were parsimony-informative. Fifty-six most parsimonious trees were obtained with a branch length of 66 steps (Figure 6). The most commonly occurring algal genotype, Genotype A (see Algal RFLPs), which is found in most species examined, founded a highly supported clade (99%) with *T. irregularis, T. pyriformis,* and *T. glomerata*. The algal ITS rDNA from *C. magyarica*, Genotype C, was more closely related to *T. magna* (79%).

Amplification of the algal ITS rDNA in *C. chlorophaea* (RK1064; Genotype F) produced two bands (670bp and 850bp), which were excised from an agarose gel and sequenced independently. A BLAST search in NCBI GenBank of the shorter fragment placed the sequence with *Trebouxia phycobiontica* (AM900491). A BLAST search of the longer fragment placed the sequence with *T. usneae* (AJ249573), *T. corticola* (AJ249566), and *T. incrustata* (AJ293795). The e-value for all three was $3x10^{-71}$ and the maximum identities were 94%, 94%, and 92%, respectively.

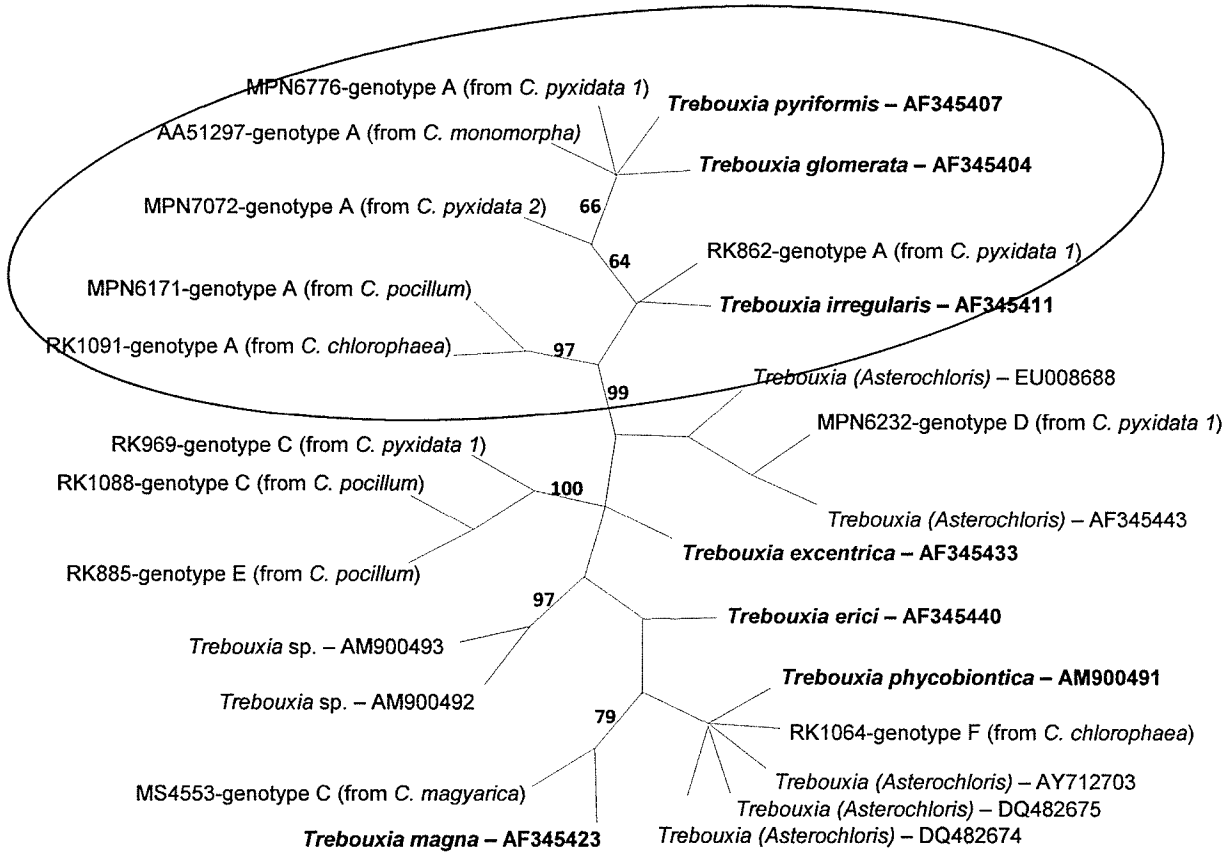

Figure 6. One of 56 unrooted most parsimonious cladograms obtained from sequence data from the algal ITS rDNA. Samples containing the most common algal genotype, genotype A, are circled. Bootstrap values (>60%) are next to the branches. CI=0.803, RI=0.884, HI=0.197. Samples with accession numbers are from GenBank and samples with collection numbers are from this study. Sequences obtained from cultured material in **bold**.

Digestion of the algal ITS rDNA with restriction enzymes, HhaI and MseI, revealed eight different banding patterns (Figure 7), which were interpreted as genotypes A to H (Figure 8). All algal ITS rDNA PCR products were approximately 670bp long. Twelve samples could not be amplified for digestion or contained a second fragment, which was approximately 850bp in length. Genotype A, which was detected in 93 samples and contained two bands, one at 380bp and the other at 140bp (fragments <100bp were not recorded), was the most commonly occurring genotype (Table 4). Genotype B, which contained three bands (430bp, 380bp, and 140bp), was found in 19 samples. Genotype C also had three bands (380bp, 170bp, and 100bp) and was detected in seven of the samples analyzed.

The remaining four genotypes were rare, occurring in only one to four of the samples collected. Two samples contained Genotype D, which had two bands, one at 380bp and the other at 280bp. Two samples were assigned Genotype E, which had four bands; 430bp, 380bp, 170bp, and 100bp. There was a single occurrence of Genotype F, which contained four bands; 380bp, 300bp, 170bp, and 140bp. Genotype G contained two bands (430bp and 380bp) and was detected in only two samples. Genotype H, which was found in four samples, produced three bands at 670bp, 380bp, and 140bp.

Since the sequences were obtained for a representative of algal Genotypes A, C, D, E, and F, exact fragment lengths were determined to be 377bp, 140bp, and 136bp for Genotype A (MPN7072), 377bp, 170bp, and 107bp for Genotype C (RK1088), and 382bp and 276bp for Genotype D (MPN6232). Algal Genotype E (RK885) may be an incomplete

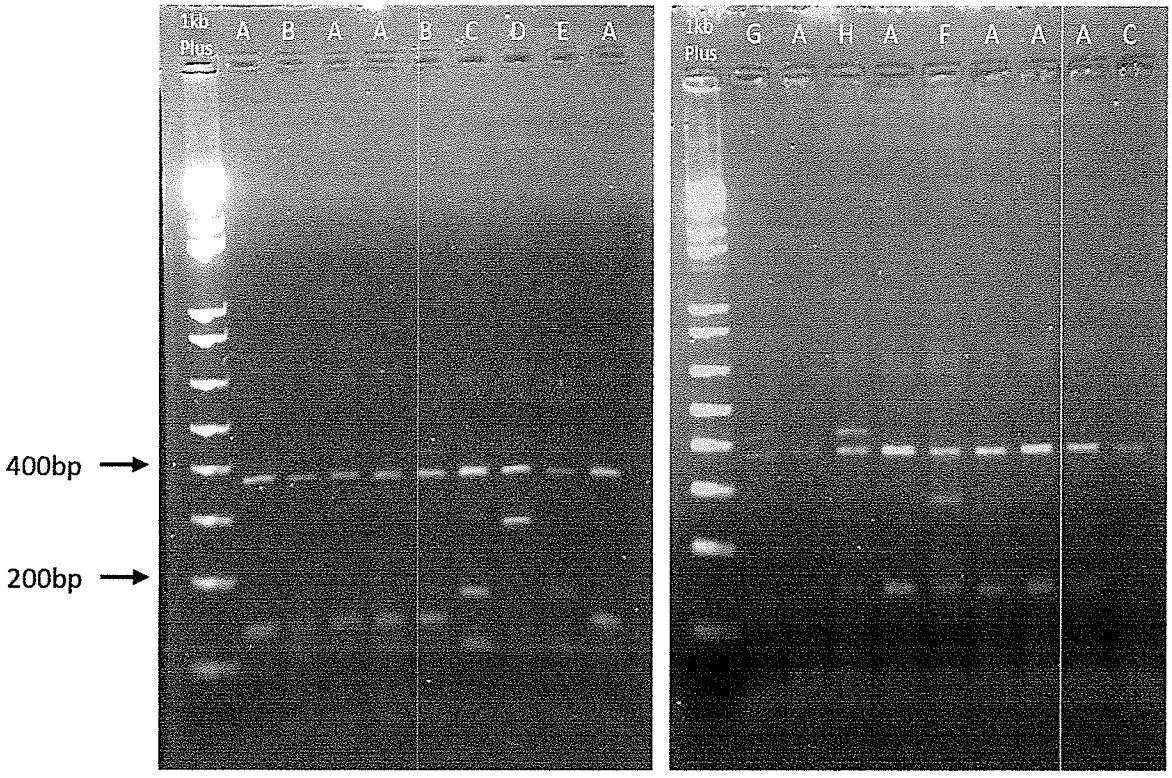

Figure 7. Agarose gels showing the eight algal genotypes (A-H) identified by RFLPs of PCR amplified algal ITS rDNA. Samples were digested with restriction enzymes, HhaI and MseI. A 1kb Plus DNA ladder was used to estimate fragment lengths. Bands less than 100bp in length were not recorded.

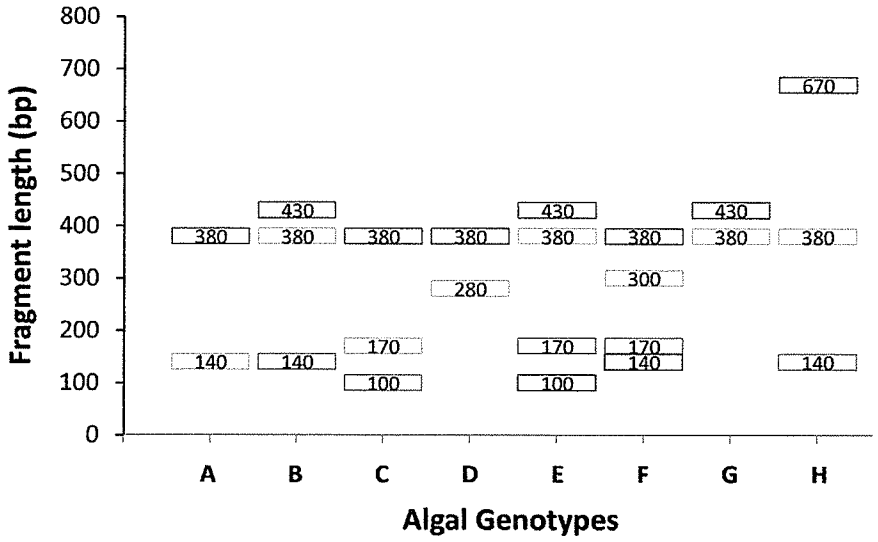

Figure 8. Digest pattern and fragment lengths used to identify the eight algal genotypes (A-H) generated from RFLPs of the PCR amplified algal ITS rDNA.

Table 4. Geographical distribution of eight algal genotypes (A-H) as identified by RFLPs of the algal ITS region in *C. pyxidata 1, C. pyxidata 2, C. pocillum* and closely related species from across Canada showing actual number of samples collected (n=141). "No data" indicates the number of samples for which RFLP data could not be obtained.

Regions	A	B	C	D	E	F	G	H	No Data
Northern Yukon (N-YT)	9	3	1	0	0	1	0	1	0
Southern Yukon (S-YT)	35	5	2	0	1	0	0	2	1
Northern B.C. (N-BC)	3	1	1	0	1	0	1	1	0
Alberta/Sask (AB/SK)	1	0	1	0	0	0	0	0	0
Wapusk NP - MB (WNP)	5	1	1	1	0	0	0	0	0
Manitoba (MB)	31	5	1	0	0	0	1	0	6
Northwestern Ontario (NW-ON)	3	2	0	1	0	0	0	0	1
(ON/QC)	2	1	0	0	0	0	0	0	3
Newfoundland (NL)	3	1	0	0	0	0	0	0	1
Totals	92	19	7	2	2	1	2	4	12

digestion of Genotype C, since the exact fragment lengths were 377bp, 170bp, and 107bp. The observed digest pattern in Genotype F (RK1064) appears to be a combination of the two fragments amplified by PCR. Exact fragment lengths for the shorter fragment were 374bp, 142bp, and 140bp and 308bp, 166bp, 140bp, and 109bp for the longer fragment.

There was no significant population subdivision of algal genotypes across Canada ($\Phi PT=0.004$, $p=0.28$). However, pairwise comparisons showed significant ($p<0.05$) population subdivision between Southern Yukon and Northern B.C. and between Manitoba and Northern B.C. (Table 5).

Table 5. Pairwise ϕPT values for algal genotypes across geographical regions. Site abbreviations correspond to those used in Tables 1&4. Significant ϕPT values ($p<0.05$) are reported in **bold**.

Regions	N-YT	S-YT	N-BC	AB/SK	WNP	MB	NW-ON	ON/QC
S-YT	0.007							
N-BC	0.000	**0.128**						
AB/SK	0.000	0.101	0.000					
WNP	0.000	0.000	0.000	0.000				
MB	0.029	0.000	**0.180**	0.191	0.008			
NW-ON	0.000	0.086	0.000	0.000	0.000	0.116		
ON/QC	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
NL	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Diversity among combinations of fungal and algal symbionts

Combinations of fungal and algal symbionts in lichen thalli were determined by assigning numbers (1-10) to the fungal species and combining them with the algal genotype (A-H) determined by RFLPs (Figure 9). Algal genotype A was the most common and associated with all fungal species examined except *C. pleurota*. Algal genotype B was the next most common and was not detected in association with samples of *C. grayi, C. fimbriata, C. monomorpha,* or *C. magyarica*. The remaining algal genotypes were only detected within a few fungal species and showed no pattern in their distribution. The combined fungal and algal genotypes also showed no pattern across geographical regions (Figure 10).

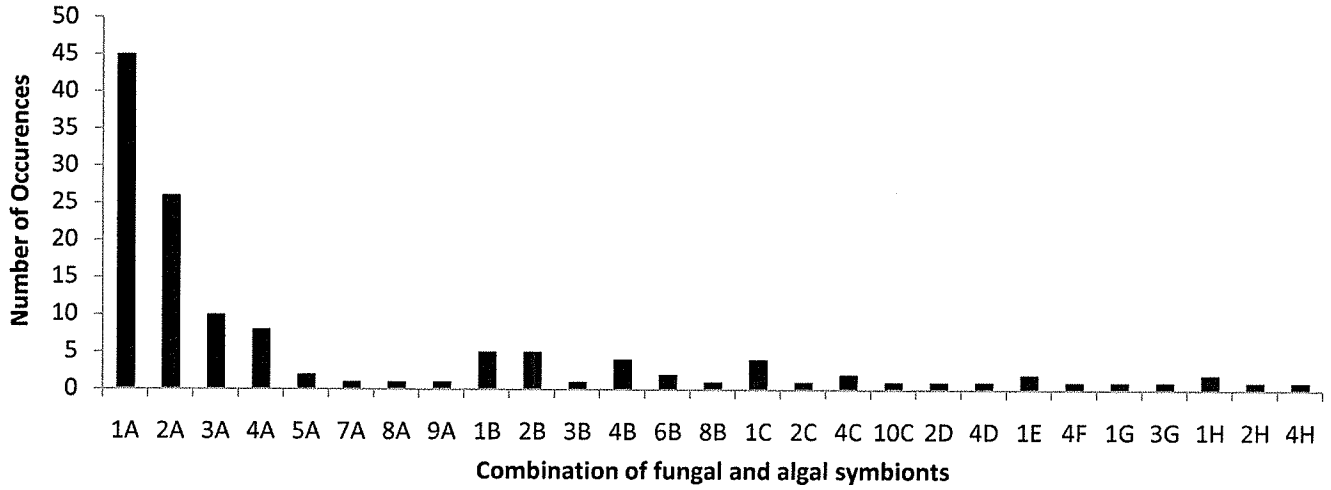

Figure 9. Histogram showing the occurrence of algal genotypes (A-H) associated with fungal species (1-10; 1=*C. pocillum*, 2=*C. pyxidata 1*, 3=*C. pyxidata 2*, 4=*C. chlorophaea*, 5=*C. grayi*, 6=*C. pleurota*, 7=*C. fimbriata*, 8=*C. coccifera*, 9=*C. monomorpha*, 10=*C. magyarica*).

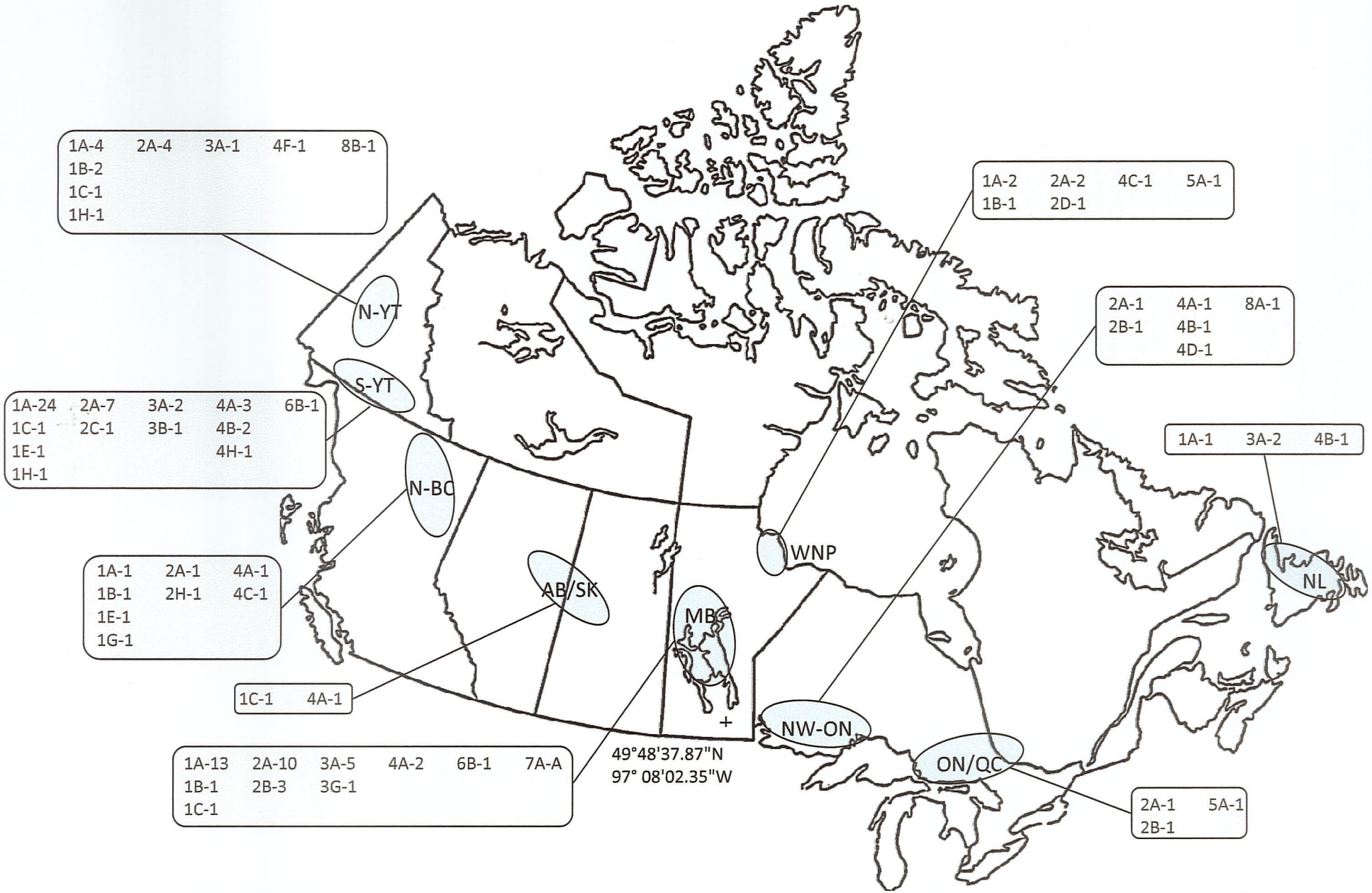

Figure 10. Distribution of the combined fungal (1-8; 1=*C. pocillum*, 2=*C. pyxidata 1*, 3=*C. pyxidata 2*, 4=*C. chlorophaea*, 5=*C. grayi*, 6=*C. pleurota*, 7=*C. fimbriata*, 8=*C. coccifera*) and algal (A-H) genotypes for 129 samples collected from across Canada. N-YT = northern Yukon, S-YT = southern Yukon, N-BC = northern British Columbia, AB/SK = Alberta/Saskatchewan, MB = Manitoba, WNP = Wapusk National Park, NW-ON = north-western Ontario, ON/QC = Ontario/Quebec, NL = Newfoundland.

Discussion

There are three phylogenetic species in the Cladonia pyxidata *group*

Two taxa, *Cladonia pyxidata* and *C. pocillum*, are commonly recognized within the *C. pyxidata* species group (Ahti 2000). Morphological examination of herbaria specimens by Aptroot (2001) revealed the presence of a third species, *C. monomorpha*, among European collections. When the morphology of the basal squamules was mapped onto the phylogenetic tree obtained from fungal ITS rDNA sequence data in this study (Figure 3), a third phylogenetic species, referred to here as *C. pyxidata 2*, also became apparent. When morphology was re-examined, *Cladonia pyxidata 2* had similar upright basal squamules as *C. pyxidata 1* but instead of growing as separate squamules on the substratum, the squamules form a rosette-like pattern typical of *C. pocillum*. The rosette-like pattern of the basal squamules in combination with an upright habit leads to the recognition of *C. pyxidata 2.*

Cladonia pyxidata 2 is easily mistaken for *C. pocillum* if the rosette-like pattern is used as the key character in the delimitation of species. This intergrading character may explain the placement of GenBank sequences, *C. pocillum* (DQ530205 and DQ530198), with *C. pyxidata 2* in the fungal ITS rDNA tree. All other samples of *C. pyxidata 2* and *C. pocillum* formed separate clades (Figures 3). *Cladonia pyxidata 2* may be synonymous with *C. monomorpha*, as suggested by the placement of *C. monomorpha* with *C. pyxidata 2* (MPN7072) in the PKS tree (Figure 4). However, due to the poor quality of the basal squamules within the single sample of *C. monomorpha* examined, the low

resolution of the PKS tree, and absence of sequence data for the fungal ITS rDNA, further studies are required to determine if *C. pyxidata 2* and *C. monomorpha* can be referred to as the same phylogenetic species.

Cladonia magyarica, which was once considered a chemical strain of *C. pyxidata* since they are morphologically indistinguishable (Ahti 1966), was also nested within *C. pyxidata 2* with high (84%) bootstrap support in the ITS tree (Figure 3) and low (63%) bootstrap support in the PKS tree (Figure 4). The presence of atranorin in addition to fumarprotocetraric acid is diagnostic of *C. magyarica* (Ahti 1966) but none of the samples of *C. pyxidata 2* examined contained atranorin. *Cladonia magyarica* and *C. pyxidata 2* would not be considered synonymous using a chemospecies concept. Further analyses with a larger number of samples of *C. magyarica* would help to understand the relationship between *C. magyarica* and *C. pyxidata 2*.

Cladonia pocillum may be interpreted to be a recent rapid radiation from *C. pyxidata.* The interpretation of a rapid radiation is supported by short branches and the recent timing of the radiation is supported by high bootstrap support for *C. pocillum* nested among members of *C. pyxidata 1* and *C. pyxidata 2* in both phylogenetic trees (Figures 3 & 4). *Cladonia pocillum* is recognized as a distinct species in many studies (Ahti 1966, Ahti 2000, Aptroot *et al.* 2001, Osyczka 2006). However, the *C. pocillum* clade (Clade A) of the fungal ITS rDNA tree also contained members of other species such as *C. chlorophaea, C. fimbriata,* and *C. pyxidata* (Figure 3). Analysis of a more variable DNA region may resolve these closely related species.

Members of the closely allied *C. chlorophaea* species complex were scattered throughout the phylogenetic trees produced in this study. Two members of *Cladonia chlorophaea* s.s. and one specimen of *C. fimbriata* were nested among members of *C. pocillum* in all trees, whereas *C. grayi* grouped with *C. pyxidata 1* in the fungal ITS tree and took on a more basal position in both the PKS and combined trees (Figures 3, 4, & 5). The separation of *C. chlorophaea* s.s. from the morphologically identical *C. grayi,* was not entirely unexpected (DePriest 1994, Stenroos *et al.* 2002). A study by DePreist (1994) showed that the *C. chlorophaea* chemotype containing grayanic acid (*C. grayi*), was the only chemotype within the *C. chlorophaea* complex that could be distinguished by a unique rDNA restriction-fragment pattern, which suggests that *C. grayi* is a distinct species separate from *C. chlorophaea* s.s.

To determine if geographical distance had any influence on the lack of monophyly of *C. pyxidata*, geographical regions were mapped onto the fungal ITS rDNA tree (Figure 3). The clusters of *C. pocillum, C. pyxidata 1,* and *C. pyxidata 2* were not consistent with geographical regions. Similarly, geographic region did not influence the monophyly of ten species of *Cladonia* examined by Beiggi & Piercey Normore (2007). Since no geographical pattern was observed, it is possible that these species may represent ecotypes based on microhabitats within the geographic regions examined as suggested by Gilbert (1977), Coassini-Lokar *et al.* (1986), and Beiggi & Piercey-Normore (2007). Environmental conditions have been shown to influence morphological features (Rikkinen 1997) and production of polyketides (Culberson & Armaleo 1992, Hawksworth 1976, Leuckert *et al.* 1990, Stocker-Wörgötter 2001, Oksanen 2006).

Environmental influences on the lichen association

Although there was an overlap in the range of pH values obtained from *C. pocillum, C. pyxidata 1,* and *C. pyxidata 2* (Figure 2), the significantly different soil pH for each species suggests that the environment may have an effect on morphology. It is well known that *C. pocillum* prefers basic soils, whereas *C. pyxidata* grows on more acidic soils (Sipman 1973, Gilbert 1977, Ahti 2000). Samples of *C. pocillum* that were collected from across Canada were found on soil or moss with a significantly higher pH than that of *C. pyxidata 1. Cladonia pyxidata 2* was also found on soil with a slightly higher soil pH than that of *C. pyxidata 1.* The rosette-like pattern seen in the basal squamules of both *C. pocillum* and *C. pyxidata 2* may therefore be a phenotypic response to changes in the soil pH. Gilbert (1977) came to a similar conclusion when examining the morphology of the basal squamules of *C. pocillum* growing in grazed and ungrazed grasslands. Samples growing in the ungrazed grasslands produced squamules similar to that of *C. pyxidata 1.* Gilbert (1977) attributed this shift in morphology to an accumulation of leaf litter, which caused a decrease in soil pH. Numerous studies have suggested that the morphological variation observed within some lichen-forming fungi may be a phenotypic response to a change in the environment (Gilbert 1977, Pintado *et al.* 1997, Rikkinen 1997, Sojo *et al.* 1997, Beiggi & Piercey-Normore 2007). It has been proposed that even subtle differences in habitats, such as soil pH or available algae, may also influence the lichen association (Hawksworth 1976). For example, Coassini-Lokar *et al.* (1986) suggest that *C. pocillum* is merely a morphotype of *C. pyxidata* that is being influenced by ecological factors.

Other chemospecies, *C. polycarpia* and *C. polycarpoides,* in the genus *Cladonia* have also been reported on specific soil types (Park 1985). Therefore, soil pH was determined for *C. chlorophaea, C. grayi, C. fimbriata* and *C. coccifera* in this study. However, due to the small sample size ($n \leq 2$) of each of these species examined, only general comments can be made relating to soil pH. The soil beneath the single sample of *C. coccifera* examined in this study produced a pH reading of 6.78, which is lower then most other samples analyzed. No results were obtained for *C. fimbriata* because the primary thallus was growing on pine needles and leaf litter, which did not allow for an accurate pH reading. Samples of *C. chlorophaea,* which were generally found on wood, had pH values similar to that of the *C. pyxidata* species complex (pH 6.76 to 7.84). *Cladonia grayi,* which is morphologically indistinguishable from *C. chlorophaea* s.s., was found on soils with significantly lower pH values (pH 6.44 to 6.53). The occurrence of *C. grayi* on acidic substrata ($pH < 6.0$) is well documented in the literature (Ahti 1966, Sipman 1973, Coassini-Lokar *et al.* 1986, Ahti 2000) suggesting that the production of grayanic acid in *C. grayi* may be related to the low soil pH on which *C. grayi* grows. If production of grayanic acid depends on pH, perhaps the production of other polyketides in the *C. chlorophaea* species complex also relies on pH. Further analysis is needed to confirm this hypothesis.

Studies have shown that lichen morphology can be influenced by association with different algal partners especially in the case of photosymbiodemes where the same fungus associates with cyanobacterium in one habitat and with primarily green algae in another habitat (Ott 1988, Armaleo and Clerc 1991, Yoshimura *et al.* 1993,

Stenroos *et al.* 2003). However, the algal genotypes identified in this study were all within the genus *Trebouxia*, a green algal partner, and were randomly scattered throughout the fungal ITS rDNA tree (Figure 3). The random pattern may suggest that the changes in the algal environment in this study were too minor taxonomically to have any influence on the fungal morphology of *C. pocillum, C. pyxidata 1,* or *C. pyxidata 2* or that the sample size was too small to detect a pattern.

Multiple algal species associate with the Cladonia pyxidata *group*

Five species of algae can be inferred to associate with members of the *Cladonia pyxidata* group. Algal genotype A, as defined by RFLP of the ITS rDNA, was the most common genotype associated with *Cladonia pyxidata 1, C. pyxidata 2, C. pocillum,* and *C. monomorpha.* The DNA sequence of genotype A was most similar to the species, *Trebouxia pyriformis, T. glomerata,* and *T. irregularis*. Samples of *C. pyxidata* and *C. pocillum* that associate with algal genotypes C, D, and E were poorly resolved in the algal ITS tree but may represent the species *T. excentrica*. A study by Piercey-Normore & DePriest (2001) suggested that *C. pyxidata* associates with *T. excentrica* using phylogenetic inference. Beiggi & Piercey-Normore (2007) also detected sequences significantly similar to that of *T. excentrica* in association with both *C. pyxidata* and *C. pocillum*. However, studies by Yahr *et al.* (2004) and Cordeiro *et al.* (2005) determined that the position of the alga that associated with *C. pyxidata* was largely unresolved.

Cladonia magyarica, which also contained algal genotype C but was not collected in North America, was the only sample that associated with *T. magna.*

The five species of *Trebouxia* that have been inferred to associate with members of the *Cladonia pyxidata* group are thought to make up the *Asterochloris* group within the genus *Trebouxia* (Rambold *et al.* 1998, Piercey-Normore and DePriest 2001, Yahr *et al.* 2004, Cordeiro *et al.* 2005, Yahr *et al.* 2006). Members of the *Asterochloris* group are morphologically similar to *Trebouxia* (Friedl 1989); however, the *Asterochloris* group has chloroplasts that exhibit a parietal position during certain stages of cell development (Friedl & Rokitta 1997), no distinct centrally located pyrenoid (Friedl 1989), and ITS rDNA sequences that cannot be aligned with sequences from *Trebouxia* s.s. (Piercey-Normore & DePriest 2001, Cordeiro *et al.* 2005). Studies have suggested that although members of the genus *Cladonia* are specific for algal species within the *Asterochloris* group (Ahmadjian 1970, Rambold *et al.* 1998, DePriest 2004), selectivity for a specific species or clade of algae can range from low, in which a single fungal species will associate with multiple algae, to high, in which the fungal species will select for a single species or genotype of *Asterochloris* (Piercey-Normore 2004, Yahr *et al.* 2004).

Results obtained from this study suggest that members of the *Cladonia pyxidata* species complex have low selectivity for algae within the *Asterochloris* group. Algal DNA sequences from samples of *Cladonia chlorophaea* were most similar to sequences from four algal species, *T. phycobiontica, T. pyriformis, T. glomerata,* and *T. irregularis.* Piercey-Normore & DePriest (2001) and Yahr *et al.* (2004) also placed the algal

sequences from *C. chlorophaea* in a poorly resolved clade with *T. excentrica*. The four species of algae that are inferred to associate with *C. chlorophaea* would also suggest low selectivity of members of the *Asterochloris* group by *C. chlorophaea*.

Amplification of the algal ITS rDNA in *C. chlorophaea* (RK1064), produced two bands suggesting that both *Asterochloris* and *Trebouxia* s.s. were associating with the fungus (Robertson & Piercey-Normore 2007). These fragments were sequenced separately and revealed two disparate groups of algae. The shorter fragment was most similar to *T. phycobiontica* (a member of *Asterochloris*) and a BLAST search in NCBI GenBank suggested the longer fragment represents *T. usnea, T. corticola* or *T. incrustata,* which belong to *Trebouxia* s.s. These species are previously unreported in not only *C. chlorophaea* but the entire genus *Cladonia*.

Twenty samples produced multiple bands when amplifying the algal ITS region of the *Cladonia pyxidata* group and may explain the RFLP patterns observed in algal genotypes B, F, G, and H. These genotypes may be combinations of more than one algal genotype; members of *Asterochloris* with shorter ITS rDNA regions and members of *Trebouxia* s.s. with longer ITS rDNA regions. Further studies are required to determine if these genotypes represent multiple algal genotypes occurring within the same fungal thallus or if they are generated by some other contaminating source.

Geographic patterns within the lichen association

Results obtained in this study suggest that there is a wide availability of both the fungal and algal partners across Canada (Table 1 & 4; Figure 10). *Cladonia pocillum* and *C. pyxidata*, which are reported to have a circumpolar distribution (Goward & Ahti 1997, Ahti 2000), were identified from most regions within Canada, albeit no representatives of *C. pocillum* were collected in NW-ON or ON/QC and *C. pyxidata 1* was not collected from NL. Overall, a larger number of samples of *C. pocillum* than *C. pyxidata* were collected from western Canada, while a larger number of samples of *C. pyxidata* and *C. chlorophaea* than *C. pocillum* were collected from eastern Canada. This is likely the result of different sampling techniques between RK and MPN, since Hammer (1995) stated that *C. pyxidata*, which is generally considered common, was in fact rare in western United States. *Cladonia pyxidata 2* was not found at all sites but was identified among samples collected from N-YT, S-YT, MB, and NL, which suggests that *C. pyxidata 2* is not restricted in its geographical distribution. As previously discussed (see Discussion; *There are three phylogenetic species in the* Cladonia pyxidata *group*), further studies are required to determine if *C. pyxidata 2* corresponds to the European species, *C. monomorpha* (Aptroot *et al.* 2001).

Algal genotypes identified within the samples examined did not correspond to specific fungal species (Figure 3 & 10). The low level of selectivity by the fungal species for a specific species of *Asterochloris* may reflect the finding that dispersal of the algal partners was occurring across the geographical range examined (Table 4). The most common algal genotypes, genotypes A and B, were detected within samples collected

from across Canada, whereas rare algal genotypes (n≤4) occurred primarily in the west, except for genotype D, which was only detected in samples from WNP and NW-ON. Similarily, Romeike *et al.* (2002) reported a high level of diversity among the algal partners associated with four species of *Umbilicaria* collected in Antarctica. Three of the four species of *Umbilicaria* examined associated with more than one algal partner, which suggested that the selection of the algal partner, more closely reflects the availability of the photobiont and not the selectivity of the mycobiont. The low level of selectivity by the mycobiont was interpreted as an advantageous adaptation to the harsh environmental conditions. By being less selective of its algal partner, the mycobiont can potentially colonize otherwise unavailable habitats.

Yahr *et al.* (2004, 2006) examined the algal diversity that associated with species of *Cladonia* over both small and large geographical scales. Within the Florida shrub, six species of *Cladonia*, including *C. subtenuis*, were identified as "photobiont specialists" and two species were considered "photobiont generalists" (Yahr *et al.* 2004). Photobiont specialists were defined as "showing specificity for a single clade of algae" and photobiont generalists "associat[ed] with divergent clades of photobiont genotypes" (Yahr *et al.* 2004). However, when algal diversity was examined for *C. subtenuis* over a larger geographical range (eastern United States), the algal specificity was reported to be significantly lower (Yahr *et al.* 2006). Contrary to the results obtained in this study (ϕPT=0.004, p=0.28), Yahr *et al.* 2006 reported that population structure was evident among the algal symbionts, with significant differences seen between samples collected inland and those from the southern coastal plain. Further

analysis by Yahr *et al.* (2006) revealed that geographical position and habitat account for >40% of the genetic variation detected within the algal partner.

Conclusions

Phylogenetic analyses suggest that the *Cladonia pyxidata* species group in Canada consists of, not two (*C. pyxidata* and *C. pocillum*), but three separate species (*C. pyxidata 1, C. pyxidata 2,* and *C. pocillum*), which show small variations in the morphology of their basal squamules. *Cladonia pyxidata 1* has separate upright squamules; *C. pyxidata 2* also has upright margins but forms a rosette-like pattern; and *C. pocillum* has appressed squamules that form a distinct rosette. As reported in the literature, *C. pyxidata 1* was found on soils with significantly lower pH than *C. pocillum*. *Cladonia pyxidata 2* was also found on soils with significantly lower pH than *C. pocillum* but not as low as that of *C. pyxidata 1*. Therefore the morphological variation observed in the basal squamules of *C. pyxidata 1, C. pyxidata 2,* and *C. pocillum* may reflect a phenotypic response to a change in the soil pH. However, further analysis is required since no pattern was observed when pH was mapped onto the phylogenetic tree and the range of actual pH values overlapped even though the mean soil pH values for each species were significantly different.

Morphological variation between *C. pyxidata 1, C. pyxidata 2,* and *C. pocillum* was not influenced by the algal partner. Algal genotypes identified by RFLPs were randomly dispersed throughout the phylogenetic trees and did not correspond to fungal species. ITS rDNA sequence data from representatives of select algal genotypes suggests that members of the *Cladonia pyxidata* species group associate with multiple algal species (*Trebouxia pyriformis, T. glomerata, T. irregularis, T. excentrica,* and *T. magna*) within the *Asterochloris* group. The lack of population subdivision of algal genotypes

among geographical regions suggests that gene flow (i.e. dispersal) is occurring among the sites studied. The lack of geographic pattern among the combined fungal and algal genotypes suggests that members of the *Cladonia pyxidata* species group have low selectivity for their algal partners.

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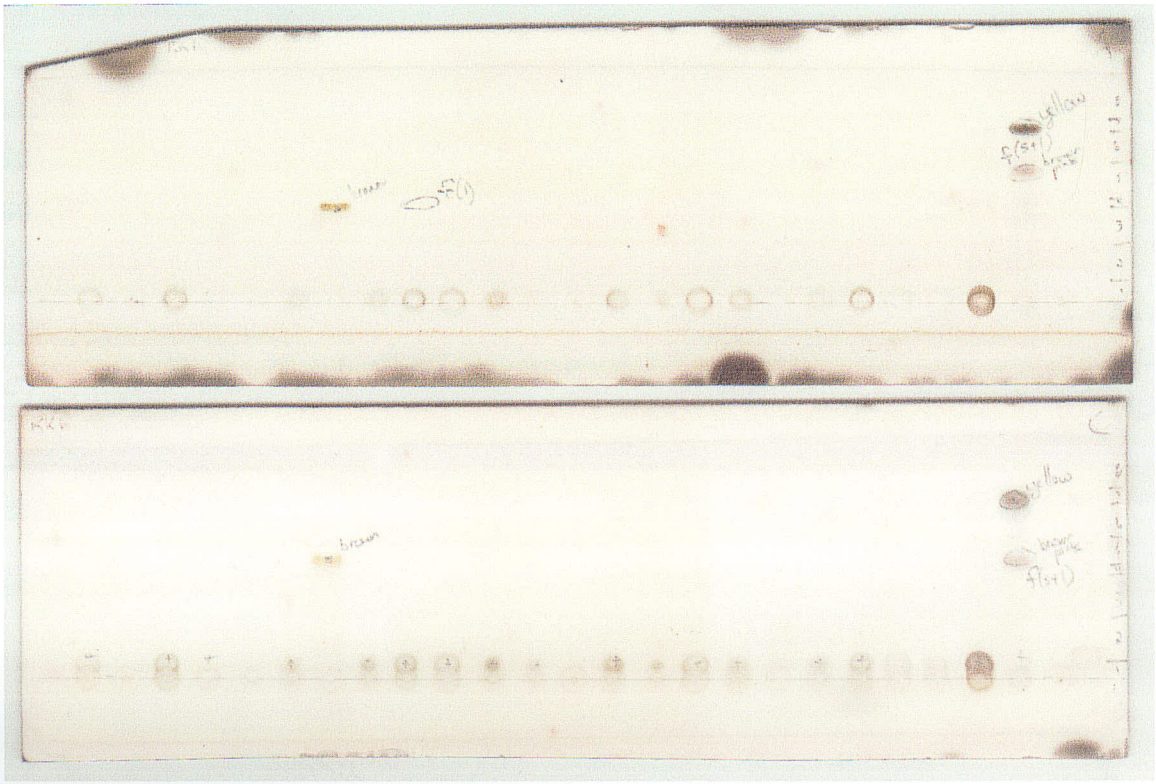
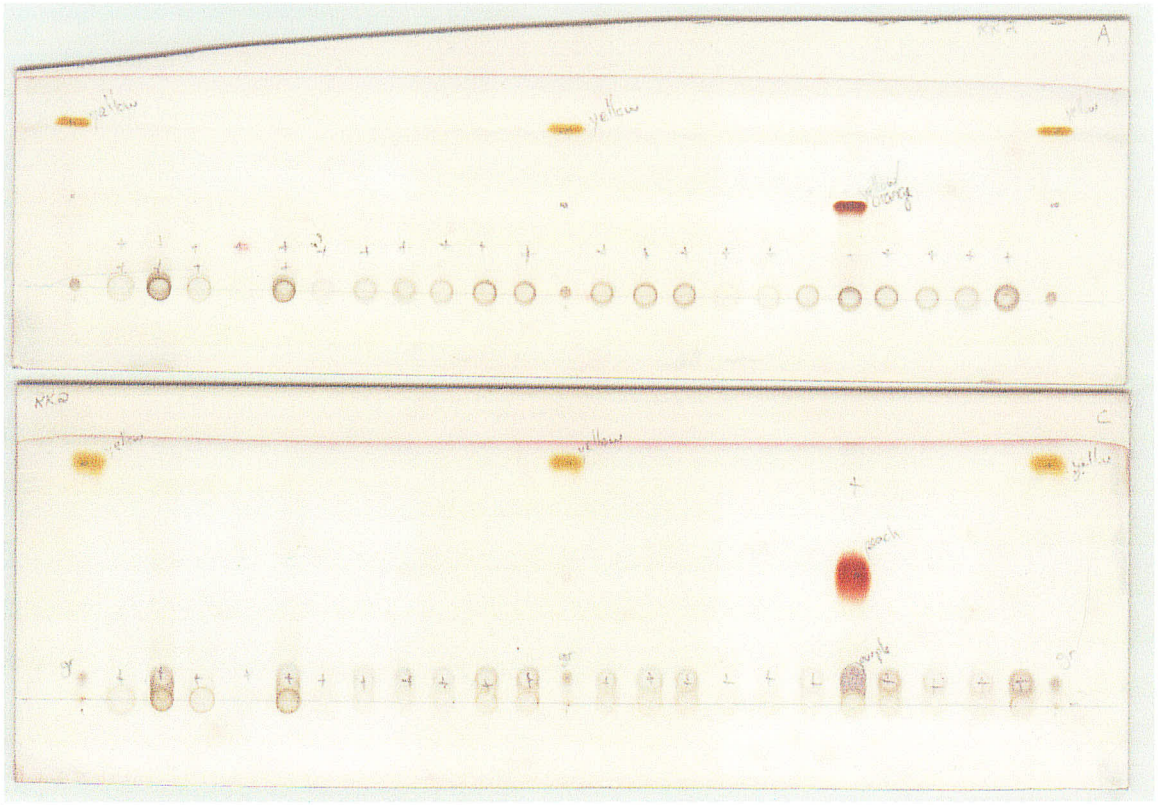
Appendix I

Seven sets of TLC plates showing characteristics of secondary compounds extracted from the 141 samples collected across Canada. Upper plates were run in Solvent A; lower plates were run in Solvent B.

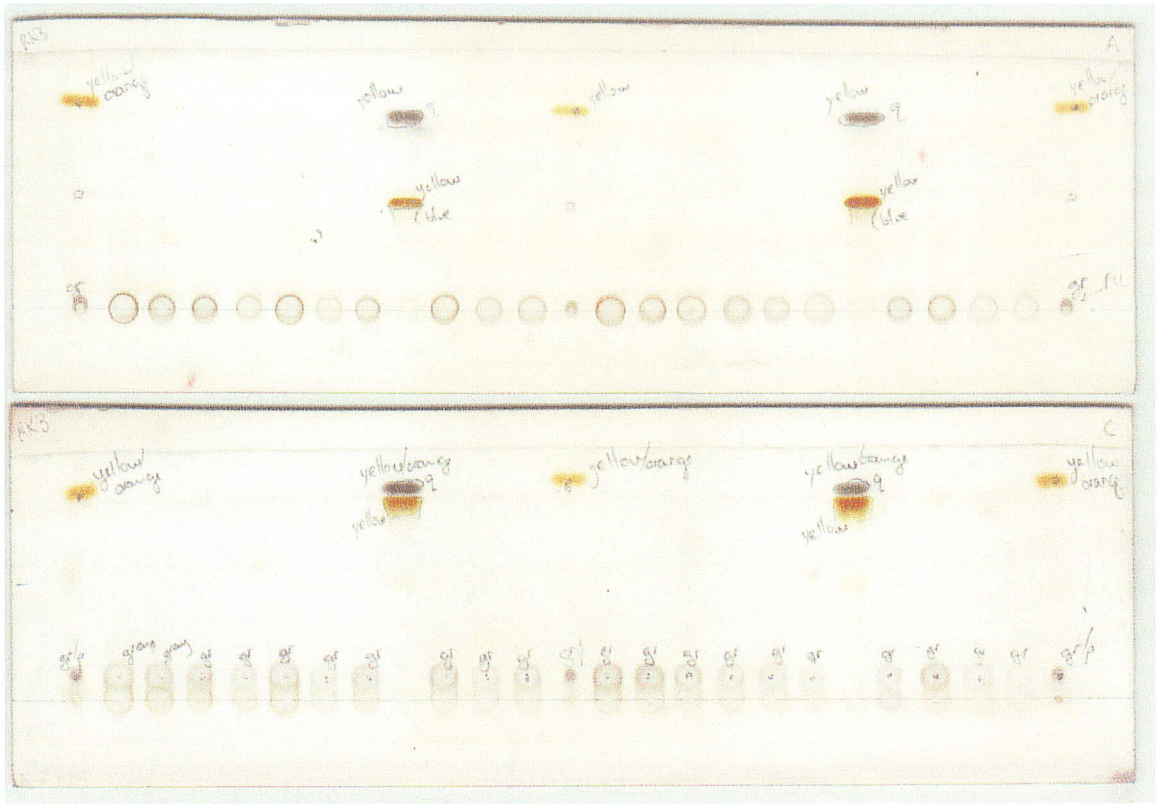
TLC plates – RK1; Samples in order from left to right are – control (*Cladonia perforata*), MPN7268, MPN7104, MPN7190, MPN7107, MPN7214, MPN7209, MPN6598, MPN6623, MPN6776, MPN6786, MPN6824, control, MPN6949, MPN7026, MPN7072, MPN6195, MPN6232, MPN6243, MPN6358, MPN6576, MPN6577, MPN6578, MPN6592, control.



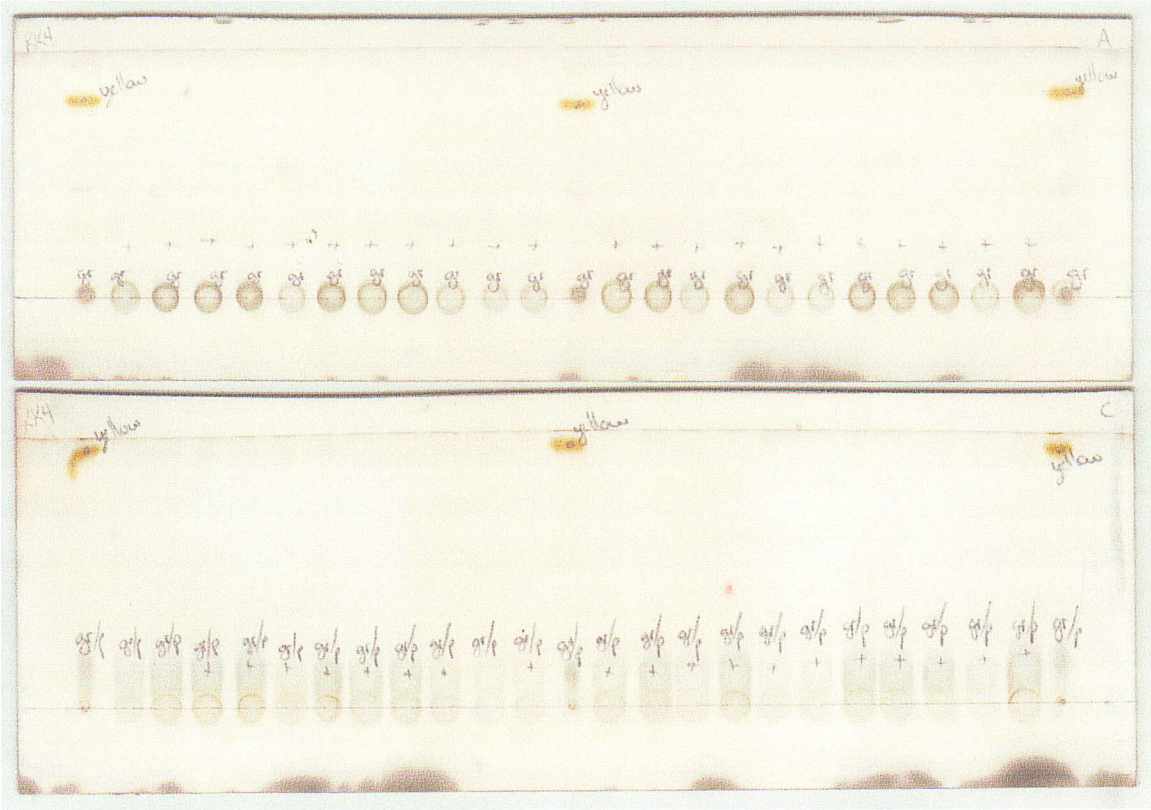
TLC plates – RK2; Samples in order from left to right are – control (*Cladonia magyarica*), MPN6232, MPN6786, MPN7214, MPN6129, MPN6131, MPN6143, MPN6147, MPN6171, MPN6186, MPN6395, MPN6106, control, MPN6107, MPN6108, MPN6112, MPN6113, MPN6115, MPN6117, MPN6408, MPN6118, MPN5964, MPN5638, MPN5750, control.



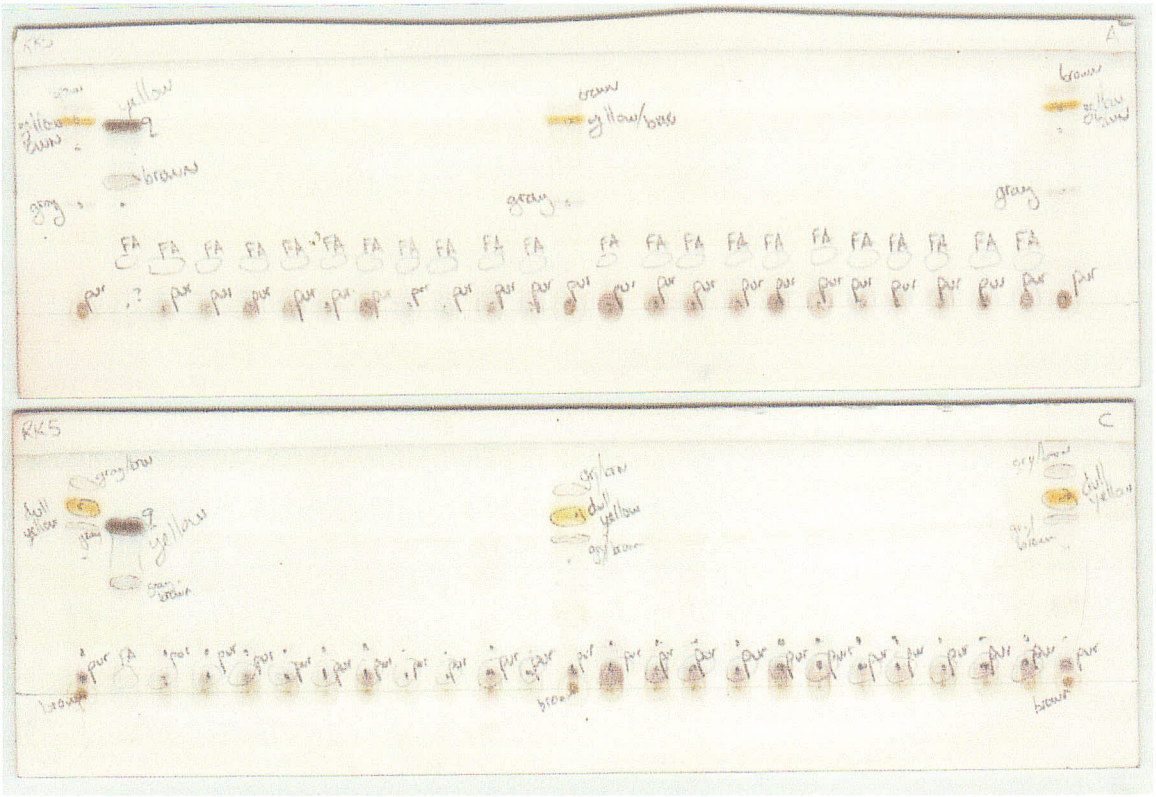
TLC plates – RK3; Samples in order from left to right are – control (*C. magyarica*), MPN5867, MPN6101, MPN6102, MPN6103, MPN6104, MPN6090, MPN6092, MPN5889, MPN6081, MPN6083, MPN6084, control, MPN5866, MPN5929, RK1088, RK1091, RK1084, RK1080, RK1079, RK1078, RK1077, RK1074, RK1073, control.



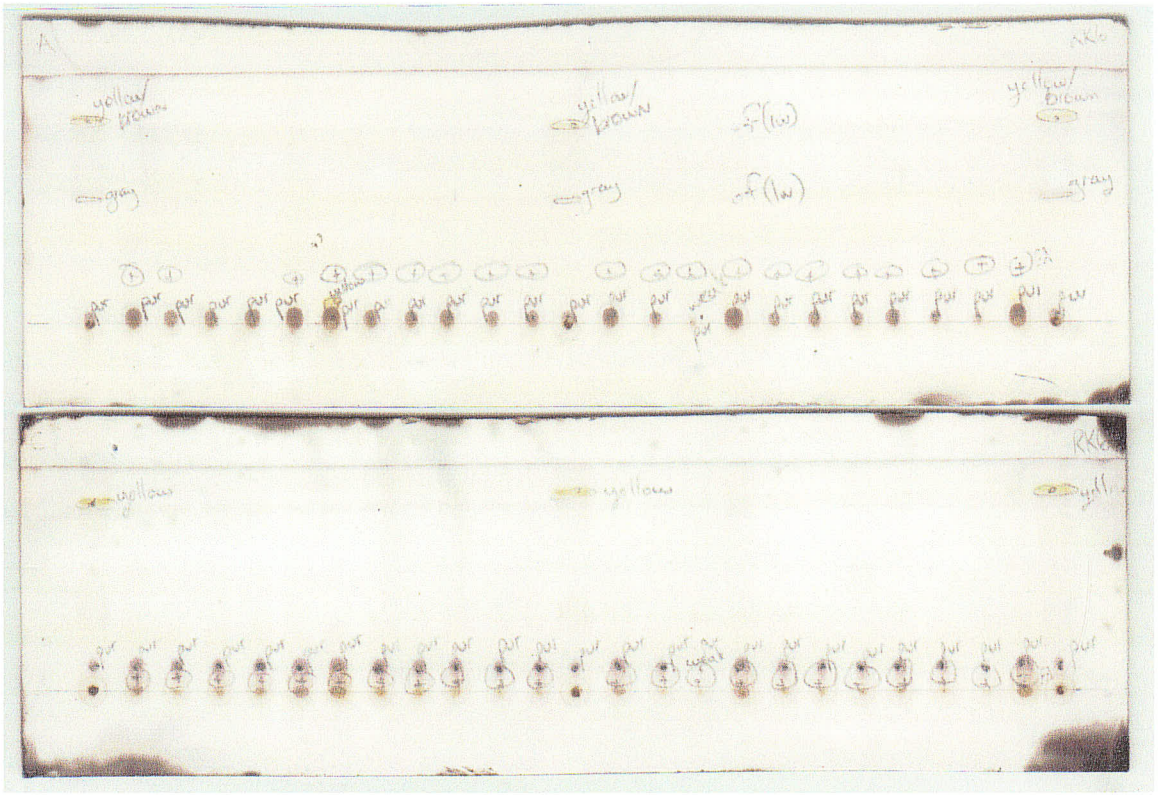
TLC plates – RK4; Samples in order from left to right are – control (*C. magyarica*), RK947, RK946, RK938, RK1064, RK1032, RK1033, RK1052, RK1055, RK1065, RK1070, RK971, control, RK972, RK1071, RK1072, RK945, RK961, RK934, RK931, RK930, RK926, RK1028, RK1027, control.



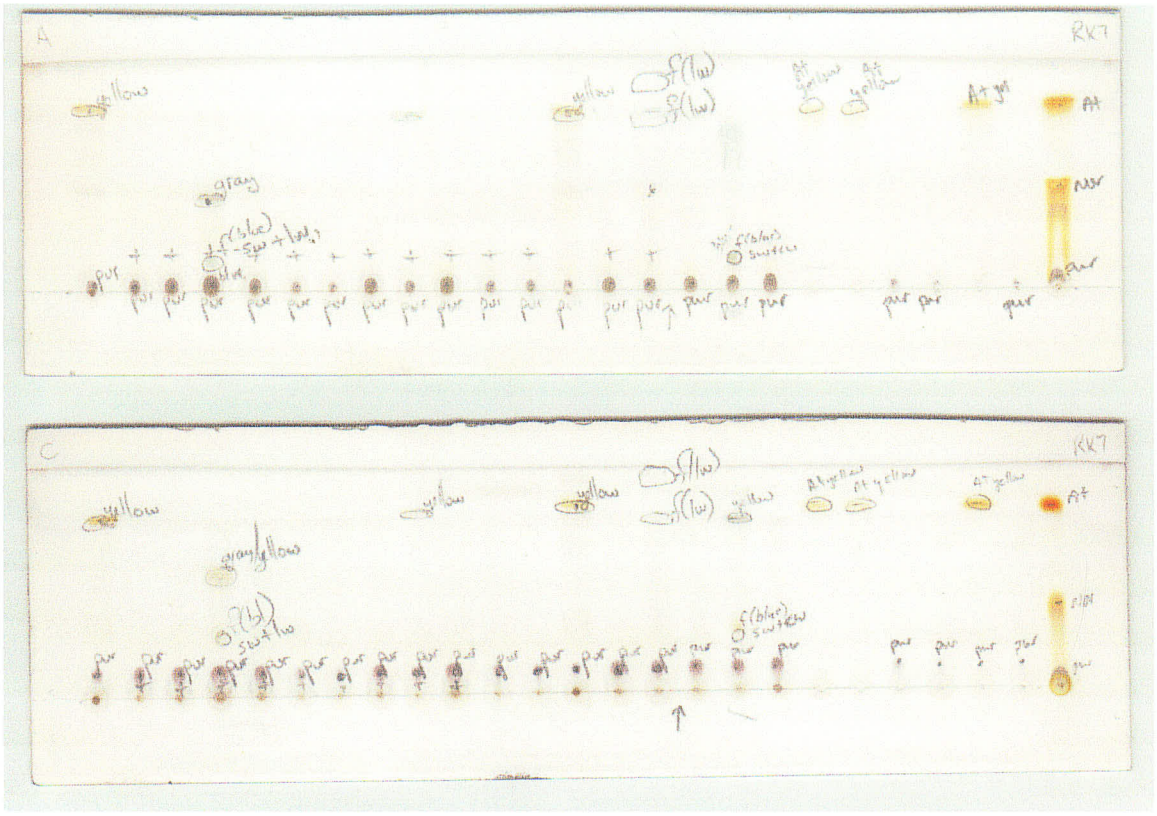
TLC plates – RK5; Samples in order from left to right are – control (*C. magyarica*), RK1004, RK999, RK976, RK974, MPN5553, MPN5556, MPN5707, MPN5541, MPN5546, MPN5550, MPN5618, control, MPN5600, MPN6095, MPN5624, MPN6096, MPN6085, MPN6086, MPN6089, MPN5538a, MPN5540, RK951, RK949, control.



TLC plates – RK6; Samples in order from left to right are – control (*C. magyarica*), RK950, RK948, RK937, RK916, RK888, RK912, RK964, RK963, RK962, RK957, RK955, control, RK954, RK953, RK952, RK879, RK871, RK870, RK869, RK862, RK968, RK966, RK965, control.



TLC plates – RK7; Samples in order from left to right are – control (*C. magyarica*), RK925, RK924, RK922, RK917, RK910, RK909, RK905, RK885, RK970, RK969, RK935, control (*C. magyarica*), RK936, Aptroot 51297, nine additional samples not included in this study, control (*C. perforata*)



Appendix II

Character states of morphological characters examined in 141 samples collected from across Canada. Numbers correspond to characters listed in Table 2.

Collection #	orientation 1	rosette 2	colour 3	eroding 4	surface features 5	podetial squam 6	cup colour 7	aereoles 8	granules 9	pycnidia 10	colour 11	apothecia 12	colour 13	pH 14	Identification
MPN5540	2	1	4	1	1,2	0	3	1	2	4	3	1	1	-	*C. pocillum*
MPN5541	1	1	3	1	1,2	0	2	1	4	1	3	1	1	7.73	*C. pocillum*
MPN5546	2	1	3	0	1,2	0	3	1	4	0	0	1	1	-	*C. pocillum*
MPN5550	2	0	3	1	1	0	4	1	2	4	3	1	1	-	*C. pyxidata 1*
MPN5553	1	1	3	1	1,2	0	4	1	4	4	3	0	0	-	*C. pocillum*
MPN5556	1	1	3	1	1	0	4	1	0	1	3	1	1	7.84	*C. pocillum*
MPN5600	2	0	1	1	3	1	1	0	0	1	2	0	0	-	*C. fimbriata*
MPN5618	3	0	2	0	1	0	3	1	0	4	2	1	1	-	*C. pyxidata 1*
MPN5624	3	0	2	1	1	0	2	1	3	1	2	1	1	-	*C. pyxidata 1*
MPN5638	0	0	0	0	1, 2, 3	0	3	1	4	1	2	-	0	-	*C. pyxidata 1*
MPN5707	2	1	4	1	1	0	4	1	0	4	3	1	1	-	*C. pocillum*
MPN5750	3	0	4	1	1	0	2	3	0	1	3	0	0	7.15	*C. pyxidata 1*
MPN5866	0	0	0	0	1	0	5	1	-	-	0	1	1	-	*C. pyxidata 1*
MPN5867	3	0	0	0	1, 2	0	0	1	4	4	3	-	0	-	*C. pyxidata 1*
MPN5889	3	0	0	0	1	0	0	1	0	1	0	-	0	-	*C. pleurota*
MPN5929	0	0	4	0	1	0	0	1	0	0	0	1	1	-	*C. pyxidata 1*
MPN5964	0	0	4	0	1	0	3	1	0	0	0	1	1	-	*C. pyxidata 1*
MPN6081	1	1	3	1	1,2	0	3	1	2	1	3	1	1	7.75	*C. pocillum*
MPN6083	0	1	0	0	1	0	3	1	0	0	0	0	0	-	*C. pyxidata 1*
MPN6084	0	1	0	0	1	0	3	1	0	1	1	0	0	-	*C. pocillum*
MPN6085	1	1	3	0	1,2	0	3	1	2	1	3	1	1	7.73	*C. pocillum*
MPN6086	2	1	3	1	1	0	2	1	0	4	3	1	1	7.62	*C. pyxidata 2*
MPN6089	2	1	4	1	1	0	3	1	0	1	3	1	1	-	*C. pyxidata 2*
MPN6090	1	1	4	1	1	0	4	1	0	1	3	1	1	-	*C. pocillum*
MPN6092	3	0	2	0	1	1	5	1	0	1	2	0	0	-	*C. pyxidata 1*
MPN6095	3	1	2	0	2	0	3	0	3	1	3	0	0	-	*C. chlorophaea*
MPN6096	2	1	2	0	1,2	0	2	1	3	1	2	1	1	-	*C. pocillum*
MPN6101	2	0	2	0	1	0	3	1	0	1	3	1	1	-	*C. pyxidata 1*
MPN6102	1	1	3	0	1,2	0	3	1	2	1	3	1	1	7.39	*C. pocillum*
MPN6103	2	1	3	0	1,2	0	3	1	2	1	3	1	1	-	*C. pyxidata 2*
MPN6104	0	0	0	1	1	0	3	1	-	1	3	1	1	-	*C. chlorophaea*
MPN6106	0	0	3	0	1	0	0	1	0	1	3	1	1	-	*C. pyxidata 1*
MPN6107	0	0	1, 3	0	1	0	3	1	0	1	3	1	1	-	*C. pyxidata 2*
MPN6108	2	0	2	0	1	0	0	1	0	1	2	1	1	-	*C. pyxidata 2*
MPN6112	1	1	3	1	1,2	0	3	1	2	1	3	1	1	7.69	*C. pocillum*
MPN6113	0	3	1	0	1	0	5	1	0	1	3	1	1	-	*C. pocillum*
MPN6115	2	1	3	1	1	0	5	1	0	1	3	0	0	7.82	*C. pyxidata 2*
MPN6117	0	0	3	1	1	0	5	1	0	1	3	-	0	-	*C. pyxidata 2*
MPN6118	1	1	3	1	1,2	0	2	1	2	4	3	1	1	7.42	*C. pocillum*
MPN6129	0	0	1	1	1, 2	0	0	1	2	1	3	1	1	-	*C. chlorophaea*
MPN6131	0	1	3	0	1	0	0	1	0	1	3	1	1	-	*C. pocillum*
MPN6143	0	0	1, 3	0	1	0	0	1	0	1	3	1	1	-	*C. pyxidata 1*
MPN6147	2	1	2	0	2	0	2	0	2	1	3	1	2	-	*C. pocillum*
MPN6171	1	1	3	1	1	0	3	3	0	1	3	0	0	7.03	*C. pocillum*
MPN6186	1	0	3	1	1,2	0	3	1	2	1	3	1	1	-	*C. pyxidata 1*
MPN6195	2	0	3	0	1, 2	0	0	1	2	1	3	1	1	-	*C. pocillum*
MPN6232	3	0	2	0	1,2	1	5	1	2	1	3	0	0	7.19	*C. pyxidata 1*
MPN6243	0	0	3	0	1, 2	0	3	1	2	1	3	-	0	-	*C. pocillum*
MPN6358	2	1	3	0	2	0	3	1	1	4	3	2	1	-	*C. chlorophaea*
MPN6395	0	0	1, 3	0	1	0	3	3	-	1	3	-	0	-	*C. pyxidata 1*
MPN6408	2	0	3	0	2	0	3	0	3	1	2	2	2	6.53	*C. grayi*
MPN6576	2	0	2	0	1,3	1	3	1	0	1	3	0	0	7.22	*C. pyxidata 1*
MPN6577	3	0	0	0	3	0	3	-	-	1	2	-	0	-	*C. chlorophaea*
MPN6578	3	0	2	0	1	1	3	1	0	1	3	1	1	7.05	*C. pyxidata 1*
MPN6592	2	0	2	0	2,3	0	2	0	3	1	2	2	1	6.78	*C. coccifera*
MPN6598	2	0	2, 3	0	1	0	3	1	-	1	3	1	1	-	*C. pyxidata 1*
MPN6623	0	0	0	0	2, 3	0	0	-	3	-	0	-	0	-	*C. chlorophaea*
MPN6776	3	0	5	1	1,2	0	5	3	2	1	3	0	0	6.76	*C. pyxidata 1*
MPN6786	2	0	1, 3	0	1	0	3	1	-	1	3	1	1	-	*C. pyxidata 1*
MPN6824	2	0	2	0	1,2	0	3	1	2	1	3	0	0	6.86	*C. pyxidata 1*
MPN6949	2	1	3	0	1,2	0	3	3	3	1,3	3	1	2	7.49	*C. pocillum*
MPN7026	2	0	0	0	1	0	0	3	-	1	3	1	1	-	*C. chlorophaea*
MPN7072	2	0	3	1	1,2	0	4	1	2	1	3	0	0	7.01	*C. pyxidata 2*
MPN7104	1	1	3	1	1,2	0	3	1	3	1	3	0	0	7.57	*C. pocillum*
MPN7107	0	1	3	0	1, 2	0	3	1	1	1	2	-	0	-	*C. pyxidata 2*
MPN7190	3	0	4	0	1	0	4	1	0	4	3	0	0	-	*C. pyxidata 1*
MPN7209	3	0	2	0	1,2	1	2	1	3	1	3	1	1	6.44	*C. grayi*
MPN7214	3	1	3	0	1	1	4	3	0	1	3	0	0	6.91	*C. pyxidata 2*
MPN7268	2	1	2	1	4	0	1	1	3	1	3	0	0	7.16	*C. chlorophaea*
MPN5538a	1	1	3	1	1,2	0	3	1	4	1	3	1	1	-	*C. pocillum*
RK862	2	0	3	1	1	0	3	1	0	1	3	0	0	6.91	*C. pyxidata 1*

Collection #	orientation 1	rosette 2	colour 3	eroding 4	surface features 5	podetial squam 6	cup colour 7	aereoles 8	granules 9	pycnidia 10	colour 11	apothecia 12	colour 13	pH 14	Identification
RK869	0	0	3	0	1, 2	0	0	1	0	1	3	-	0	-	*C. pocillum*
RK870	2	1	3	1	1	0	4	3	0	1	3	1	1	7.27	*C. pocillum*
RK871	1	1	4	1	1,2	0	5	3	2	1	3	0	0	7.12	*C. pocillum*
RK879	0	0	0	0	1	0	0	1	0	1	3	1	0	-	*C. pyxidata 1*
RK885	1	0	3	1	1	0	5	1	0	1	3	0	0	7.30	*C. pocillum*
RK888	2	0	1	0	2	0	2	0	3	1	1	0	0	-	*C. chlorophaea*
RK905	3	0	4	1	1,2	0	4	1	3	1	3	1	1	7.33	*C. pyxidata 1*
RK909	0	0	3	1	1	0	5	1	0	1	3	-	0	-	*C. pyxidata 1*
RK910	0	1	3	1	1	0	0	1	0	1	2	-	0	-	*C. pyxidata 1*
RK912	3	0	2	0	2	1	2	0	3	1	2	0	0	-	*C. chlorophaea*
RK916	0	0	1	1	1, 2	0	3	1	3	1	2	1	1	-	*C. chlorophaea*
RK917	2	1	4	1	1	0	4	3	0	1	3	0	0	7.35	*C. pocillum*
RK922	1	1	3	1	1,2	0	4	1	2	1	3	0	0	-	*C. pocillum*
RK924	0	1	3	1	1	0	0	1	0	1	3	-	0	-	*C. pocillum*
RK925	0	1	0	0	1, 2	0	0	1	4	-	0	-	0	-	*C. pocillum*
RK926	0	1	3	0	1	0	0	1	0	1	3	-	0	-	*C. pocillum*
RK930	0	0	0	0	1	0	0	1	0	1	3	-	0	-	*C. pocillum*
RK931	2	0	0	0	1	0	0	1	0	1	1	-	0	-	*C. pyxidata 1*
RK934	2	1	0	0	1	0	0	1	0	1	3	-	0	-	*C. pyxidata 2*
RK935	0	0	3	1	1	0	0	1	0	1	1	-	0	-	*C. chlorophaea*
RK936	2	0	3	0	1, 2	0	5	1	2	1	1	-	0	-	*C. pocillum*
RK937	0	0	1	1	1,2,3	1	3	1	2	1	2	0	0	-	*C. chlorophaea*
RK938	1	1	4	1	1	0	3	1	0	1	3	1	1	7.06	*C. pyxidata 2*
RK945	2	1	3	1	1, 2	0	3	1	2	1	3	0	0	7.45	*C. pocillum*
RK946	2	1	4	1	1, 2	0	5	3	4	1	3	1	1	7.21	*C. pocillum*
RK947	1	1	4	1	1, 2	0	5	1	4	1	3	2	1	-	*C. pocillum*
RK948	2	1	4	1	1,2	0	5	1	2	1	3	1	2	-	*C. pocillum*
RK949	3	1	3	0	1,2	1	1	3	2	1	3	1	1	-	*C. pocillum*
RK950	2	1	2	1	1	0	1	3	0	1	2	0	0	7.20	*C. pyxidata 2*
RK951	1	1	2	0	1, 2	1	1	1	2	1	3	1	1	7.27	*C. pocillum*
RK952	0	0	0	1	1	0	4	0	0	1	3	-	0	-	*C. chlorophaea*
RK953	2	0	4	1	4	0	3	1	2	1	3	2	1	-	*C. pyxidata 1*
RK954	3	0	4	1	1	0	3	1	0	1	3	1	2	6.94	*C. pyxidata 1*
RK955	3	0	4	1	1	0	3	3	0	-	0	-	0	-	*C. pyxidata 1*
RK957	0	1	4	0	1	0	0	1	0	1	3	-	0	-	*C. pocillum*
RK961	0	1	3	0	1	0	3	1	0	1	3	1	1	-	*C. pocillum*
RK962	0	1	3	1	1, 2	0	0	3	2	1	3	-	0	-	*C. pocillum*
RK963	0	1	1, 3	0	1	0	0	1	0	4	3	1	1	-	*C. pocillum*
RK964	0	1	3	0	1	0	0	1	0	1	3	-	0	-	*C. pocillum*
RK965	0	1	1, 3	0	1, 2	0	0	1	?	1	3	1	1	-	*C. pocillum*
RK966	1	1	3	1	1,2	0	2	1	2	1	3	0	0	7.11	*C. pocillum*
RK968	0	1	1, 3	0	1, 2	0	0	1	0	1	3	-	0	-	*C. pocillum*
RK969	3	1	4	1	4	0	3	3	4	4	2	1	2	-	*C. pyxidata 1*
RK970	2	1	3	1	4	1	3	3	4	1	3	1	2	-	*C. pocillum*
RK971	2	1	4	1	1, 2	0	3	1	4	1	3	2	1	-	*C. pocillum*
RK972	2	1	4	1	1	1	3	1	0	1	2	1	1	-	*C. pocillum*
RK974	1	1	3	0	1, 2	0	3	1	4	1	3	2	1	7.07	*C. pocillum*
RK976	1	0	3	0	1, 2	0	3	1	4	1	2	1	2	-	*C. chlorophaea*
RK999	3	0	4	1	1	1	3	3	0	1	3	1	2	6.86	*C. pyxidata 1*
RK1004	2	0	1	0	1, 2	0	1	3	3	1	3	0	0	-	*C. coccifera*
RK1027	2	1	4	0	1, 2	0	3	1	4	1	3	0	0	7.16	*C. pocillum*
RK1028	1	1	4	0	1, 2	0	3	3	4	1	3	1	2	-	*C. pocillum*
RK1032	1	1	4	1	1	0	3	3	0	1	3	1	1	-	*C. pyxidata 1*
RK1033	2	1	4	1	1, 2	0	5	1	3	4	3	1	1	7.17	*C. pocillum*
RK1052	1	1	4	1	1	0	3	1	0	1	3	2	1	-	*C. pocillum*
RK1055	2	0	3	0	1	0	3	1	0	1	3	1	1	-	*C. pocillum*
RK1064	2	0	3	0	1, 2	0	3	1	3	-	0	-	0	-	*C. chlorophaea*
RK1065	2	1	2	0	1	0	3	1	0	1	3	0	0	7.24	*C. pyxidata 2*
RK1070	0	0	0	0	1	0	0	1	0	1	3	1	1	-	*C. pocillum*
RK1071	0	0	0	1	1	0	0	1	0	1	3	1	1	-	*C. pyxidata 1*
RK1072	1	1	3	1	1,2	0	3	1	2	1	3	1	1	7.23	*C. pocillum*
RK1073	0	1	1, 3	0	1	0	3	1	0	1	3	1	1	-	*C. pocillum*
RK1074	2	0	1, 3	0	1, 2	0	0	1	2	1	3	1	1	-	*C. pyxidata 1*
RK1077	2	0	3	0	1	0	5	3	0	1	3	-	0	-	*C. pyxidata 1*
RK1078	2	1	4	1	1,2	0	4	1	2	1	3	0	0	7.26	*C. pyxidata 2*
RK1079	3	0	3	0	1	0	3	3	0	1	3	1	1	-	*C. pleurota*
RK1080	1	1	5	1	1, 2	0	3	3	3	1	3	2	1	-	*C. pocillum*
RK1084	1	1	3	1	1, 2	0	3	3	3	1	3	2	1	-	*C. chlorophaea*
RK1088	1	1	3	1	1	0	3	1	0	1	3	1	1	6.90	*C. pocillum*
RK1091	3	1	2	0	2	0	2	0	3	1	3	1	1	7.12	*C. chlorophaea*

Appendix III

Overview of geographical region, species identifications, TLC, RFLP, pH, and sequence data. See Table 1 in Methods and Figures 8 and 9 in Results for abbreviations.

Region	Area	Site	Coll#	Identification	Secondary compounds (TLC)	RFLPs	combined	pH	FgITS (bp)	PKS (bp)	Alg ITS (bp)
N-YT	Dawson Rd	1	RK1073	*C. pocillum*	FU (Fumarprotocetraric acid)	B	1B				
			RK1074	*C. pyxidata 1*	FU	A	2A				
		2	RK1070	*C. pocillum*	FU	A	1A				
			RK1071	*C. pyxidata 1*	FU	A	2A				
			RK1072	*C. pocillum*	FU	A	1A	7.23	608		
		3	RK1064	*C. chlorophaea*	FU	F	4F				F
			RK1065	*C. pyxidata 2*	FU	A	3A	7.24	611	420	
		4	RK1055	*C. pocillum*	FU	A	1A				
		5	RK1052	*C. pocillum*	FU	A	1A				
	Dempster Hwy	6	RK1032	*C. pyxidata 1*	FU	A	2A				
			RK1033	*C. pocillum*	FU	H	1H	7.17	605		
		7	RK1027	*C. pocillum*	FU	C	1C	7.16	608		
			RK1028	*C. pocillum*	FU	B	1B				
		8	RK999	*C. pyxidata 1*	FU	A	2A	6.86	604	420	
		9	RK1004	*C. coccifera*	usnic acid + zeorin	B	5B				
S-YT	Alaska Hwy	10	RK905	*C. pyxidata 1*	FU	A	2A	7.33	605		
			RK909	*C. pyxidata 1*	FU	B	2A				
			RK910	*C. pyxidata 1*	FU	no data	-				
		11	RK1077	*C. pyxidata 1*	FU	A	2A				
			RK1078	*C. pyxidata 2*	FU	A	3A	7.26	614		
			RK1079	*C. pleurota*	usnic + barbatic acid	B	6B				
			RK1080	*C. pocillum*	FU	H	1H				
		12	RK912	*C. chlorophaea*	FU + unknown	A	4A				
			RK916	*C. chlorophaea*	FU	B	4B				
			RK917	*C. pocillum*	FU	E	1E	7.35	612		
			RK922	*C. pocillum*	thamnolic + baeomycesic acids (contaminants)	A	1A				
		13	RK945	*C. pocillum*	FU	A	1A	7.45	608		
		14	RK946	*C. pocillum*	FU	A	1A	7.21	607	421	
			RK947	*C. pocillum*	FU	A	1A				
			RK948	*C. pocillum*	FU	C	1C				
		15	RK974	*C. pocillum*	FU	A	1A	7.07	608		
			RK976	*C. chlorophaea*	FU	A	4A				
		16	RK969	*C. pyxidata 1*	FU	C	2C				C
			RK970	*C. pocillum*	FU	A	1A				
			RK971	*C. pocillum*	FU	A	1A				
			RK972	*C. pocillum*	FU	A	1A				
		17	RK957	*C. pocillum*	FU	A	1A				
		18	RK961	*C. pocillum*	FU	A	1A				
		19	RK963	*C. pocillum*	FU	A	1A				
			RK964	*C. pocillum*	FU	A	1A				
			RK965	*C. pocillum*	FU	A	1A				
			RK966	*C. pocillum*	FU	A	1A	7.11	608		
			RK968	*C. pocillum*	FU	A	1A				
		20	RK962	*C. pocillum*	FU	A	1A				
	Aishihik Lake Rd	21	RK949	*C. pocillum*	FU	A	1A				
			RK950	*C. pyxidata 2*	FU	B	3B	7.20	564		
			RK951	*C. pocillum*	FU	A	1A	7.27	608		
			RK952	*C. chlorophaea*	FU	B	4B				
		22	RK953	*C. pyxidata 1*	FU	A	2A				
			RK954	*C. pyxidata 1*	FU	A	2A	6.94	604		
			RK955	*C. pyxidata 1*	FU	A	2A				
	Dawson Rd	23	RK934	*C. pocillum*	FU	A	1A				
			RK935	*C. chlorophaea*	FU	A	4A				
			RK936	*C. pocillum*	FU	A	1A				
			RK937	*C. chlorophaea*	FU	H	4H				
			RK938	*C. pyxidata 2*	FU	A	3A	7.06	609		
		24	RK930	*C. pocillum*	FU	A	1A				
			RK931	*C. pyxidata 1*	FU	A	2A				
		25	RK924	*C. pocillum*	FU	A	1A				
			RK925	*C. pocillum*	FU	A	1A				
			RK926	*C. pocillum*	FU	A	1A				
N-BC	Alaska Hwy	26	RK862	*C. pyxidata 1*	FU	A	2A	6.91	611	420	A
		27	RK869	*C. pocillum*	FU	B	1B				
			RK870	*C. pocillum*	FU	A	1A	7.27	607	421	
			RK871	*C. pocillum*	FU	G	1G	7.12	608		
		28	RK879	*C. pyxidata 1*	FU + 2 trace compounds (likely contaminants)	H	2H		609		
		29	RK885	*C. pocillum*	Atranorin + FU	E	1E	7.30	609	421	E
			RK888	*C. chlorophaea*	FU	A	4A				
		30	RK1084	*C. chlorophaea*	FU	C	4C				
AB/SK	SK	31	RK1091	*C. chlorophaea*	FU	A	4A	7.12	608	421	A
	AB	32	RK1088	*C. pocillum*	FU	C	1C	6.90	518	421	C

Region	Area	Site	Coll#	Identification	Secondary compounds (TLC)	RFLPs	combined	pH	FgITS (bp)	PKS (bp)	Alg ITS (bp)
WNP		33	MPN6171	*C. pocillum*	FU	A	1A	7.03	609	421	A
		34	MPN6186	*C. pyxidata 1*	FU	A	2A				
			MPN6195	*C. pocillum*	FU	B	1B				
		35	MPN6232	*C. pyxidata 1*	FU	D	2D	7.19	618	420	D
			MPN6243	*C. pocillum*	FU	A	1A				
		36	MPN6358	*C. chlorophaea*	FU	C	4C				
		37	MPN6395	*C. pyxidata 1*	FU	A	2A				
			MPN6408	*C. grayi*	grayonic + FU	A	5A	6.53	614	419	
MB	Long Point Rd	38	MPN6115	*C. pyxidata 2*	weak FU	A	3A	7.82	568		
		39	MPN6117	*C. pyxidata 2*	FU	A	3A				
			MPN6118	*C. pocillum*	FU	C	1C	7.42	574		
			MPN6129	*C. chlorophaea*	no compounds	no data	-				
			MPN6131	*C. pocillum*	FU	A	1A				
		40	MPN6143	*C. pyxidata 1*	weak FU	A	2A				
			MPN6147	*C. pocillum*	FU	B	1B				
		41	MPN5538a	*C. pocillum*	FU	A	1A				
			MPN5540	*C. pocillum*	FU	A	1A				
			MPN5541	*C. pocillum*	FU	A	1A	7.75	568		
			MPN5546	*C. pocillum*	FU	A	1A				
			MPN5550	*C. pyxidata 1*	FU	A	2A				
			MPN5553	*C. pocillum*	FU	A	1A				
			MPN5556	*C. pocillum*	FU	A	1A	7.84	610		
	Hwy 6	42	MPN5600	*C. fimbriata*	FU	A	7A		604	421	
			MPN5618	*C. pyxidata 1*	FU	B	2B				
		43	MPN5929	*C. pyxidata 1*	FU	A	2A				
	Hwy 391	44	MPN5624	*C. pyxidata 1*	FU	B	2B				
			MPN5638	*C. pyxidata 1*	FU	A	2A				
		45	MPN5707	*C. pocillum*	FU	A	1A				
		46	MPN5750	*C. pyxidata 1*	FU	no data	-	7.15	608		
		47	MPN5889	*C. pleurota*	Usnic + barbatic acid	B	6B				
		48	MPN5866	*C. pyxidata 1*	FU	A	2A				
			MPN5867	*C. pyxidata 1*	FU	A	2A				
	Snow Lake	49	MPN5964	*C. pyxidata 1*	FU	A	2A				
	The Pas	50	MPN6081	*C. pocillum*	FU	A	1A	7.75	570		
		51	MPN6083	*C. pyxidata 1*	FU	B	2B				
			MPN6084	*C. pocillum*	FU	no data	-				
			MPN6085	*C. pocillum*	FU	A	1A	7.73	610		
		52	MPN6086	*C. pyxidata 2*	FU	A	3A	7.62	550		
			MPN6089	*C. pyxidata 2*	FU	A	3A				
	Hwy 60	53	MPN6090	*C. pocillum*	FU	A	1A				
		54	MPN6092	*C. pyxidata 1*	FU	A	2A				
		55	MPN6095	*C. chlorophaea*	FU	A	4A				
			MPN6096	*C. pocillum*	FU	no data	-				
		56	MPN6101	*C. pyxidata 1*	FU	A	2A				
			MPN6102	*C. pocillum*	FU	A	1A	7.39	512		
			MPN6103	*C. pyxidata 2*	FU	G	3G				
			MPN6104	*C. chlorophaea*	FU	A	4A				
		57	MPN6106	*C. pyxidata 1*	FU	A	2A				
		58	MPN6107	*C. pyxidata 2*	FU	A	3A				
		59	MPN6108	*C. pyxidata 2*	FU	no data	-				
		60	MPN6112	*C. pocillum*	FU	no data	-	7.69	608		
			MPN6113	*C. pocillum*	weak FU	A	1A				
NW-ON		61	MPN6576	*C. pyxidata 1*	FU	B	2B	7.22	608	420	
			MPN6577	*C. chlorophaea*	weak FU	B	4B				
			MPN6578	*C. pyxidata 1*	FU	no data	-	7.05	611		
		62	MPN6592	*C. coccifera*	usnic acid + zeorin	A	8A	6.78	614		
			MPN6598	*C. pyxidata 1*	FU	A	2A				
		63	MPN6623	*C. chlorophaea*	FU + unknown trace compound (contaminant?)	A	4A			421	
		64	MPN7268	*C. chlorophaea*	FU	D	4D	7.16	607		
ON/QC		65	MPN7209	*C. grayi*	grayanic acid	A	5A	6.44	612		
			MPN7214	*C. pyxidata 2*	FU	no data	-	6.91	619		
		66	MPN7190	*C. pyxidata 1*	weak FU	no data	-				
		67	MPN6776	*C. pyxidata 1*	FU	A	2A	6.76	608	420	A
			MPN6786	*C. pyxidata 1*	FU	no data	-				
		68	MPN6824	*C. pyxidata 1*	weak FU	B	2B	6.86	609		
NL		69	MPN6949	*C. pocillum*	FU	A	1A	7.49	607		
		70	MPN7026	*C. chlorophaea*	FU	B	4B				
		71	MPN7072	*C. pyxidata 2*	FU	A	3A	7.01	614	420	A
		72	MPN7104	*C. pocillum*	FU	no data	-	7.57	608		
		73	MPN7107	*C. pyxidata 2*	weak FU	A	3A				
Cladonia monomorpha			AA51297	*C. monomorpha*	FU + 2 unknown compounds	A	9A			420	A
Cladonia magyarica			MS4553	*C. magyarica*	Atranorin + FU	C	10C		539	420	C

Appendix IV

Aligned fungal ITS rDNA sequences from in this study. Dots indicate the occurrence of the same nucleotide as the reference sequence. Dashes represent gaps or missing data.

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                                  1          11         21         31         41         51
                                  |          |          |          |          |          |
'RK870- C. pocillum'              ATGAGTTCGG GGGCCTA-GC CCCCAGCGGC GAGTGTC-GT T-GAGTCCCC C-GGGACTCA
'RK946 - C. pocillum'             .......... .......... .......... .......... .........T ..........
'RK966 - C. pocillum'             .......... ......-G.. .......... .......... .......... ..........
'RK1033 - C. pocillum '           .......... .......... .......A.. .......... .......... ..........
'RK1088 - C. pocillum'            ---------- -------.-- ---------- -------.-- -.-------- -.-.......
'MPN6171 - C. pocillum'           .......... .......... .......... .......... .T........ ..A.......
'MPN7104 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK885 - C. pocillum'             .......... .......... .......... .......... .T........ ..A.......
'MPN6949 - C. pocillum'           .......... .......... .......... .......... A......... ..A.......
'RK917 - C. pocillum'             .......... .......... .......... .......... .T........ ..A.......
'MPN6085 - C. pocillum'           .......... ......-G.. .......... .......... .C........ ..........
'MPN6112 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'MPN5556 - C. pocillum'           .......... .......... .......... .......... .T........ ..A.......
'RK945 - C. pocillum'             .......... ......-G.. .......... .......... .......... ..........
'RK1027 - C. pocillum'            .......... .......... .......... ....C..... .......... ..........
'RK1072 - C. pocillum'            .......... .......... .......... .......... G..C...... .........C
'RK951 - C. pocillum'             .......... .......... .......... .......... .......... ..........
'RK871 - C. pocillum'             .......... .......... .......... .......... .......... ..........
'RK974 - C. pocillum'             .......... ......-G.. .......... ........A. .......... ..........
'MPN6081 - C. pocillum'           ---------- ----...... .......... .......... .......... ..........
'MPN6102 - C. pocillum'           ---..--... -----..... .......... .......... .......... ..........
'MPN5541 - C. pocillum'           ---------- -------... .......... .......... .......... ..........
'MPN6118 - C. pocillum'           ---------- -.-..... ....-..... .......... .......... ..........
'RK862 - C. pyxidata 1'           .......... .......A.. .......... .......... .C........ .........G
'RK954 - C. pyxidata 1'           .......... .......... .......... .......... .C........ .........G
'RK999 - C. pyxidata 1'           .......... .......... .......... .......... .C........ .........G
'RK905 - C. pyxidata 1'           .......... .......... .......... .......... .C........ .........G
'MPN6232 - C. pyxidata 1'         .......... ...T...... .......... .......... .C...C..G. TC...G...G
'MPN6578 - C. pyxidata 1'         .......... .......AA. .......... .......... .C........ .........G
'MPN5750 - C. pyxidata 1'         .......... .......A.. .......... .......... .C........ .........G
'MPN6776 - C. pyxidata 1'         .......... .......A.. .......... .......... .C........ .........G
'MPN6576 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..A.....T.
'MPN6824 - C. pyxidata 1'         .......... .......G.. .......... .......... .......... ........T.
'RK879 - C. pyxidata 1'           .......... .......... .......... .......... .......... ..........
'MPN6086 - C. pyxidata 2'         .......... ...T...... .......... .......... .C........ ..A......G
'RKRK1065 - C. pyxidata 2'        .......... .......... .......... .......... .C........ ..A......G
'MPN6115 - C. pyxidata 2'         ---------- -------... .......... .......... .C...C.... ..A......G
'MPN7214 - C. pyxidata 2'         .......... .......A.. .......... .......... .T..A..... .........G
'RK1078 - C. pyxidata 2'          .......... .......... .......... .......... .CA....... .........G
'MPN7072 - C. pyxidata 2'         T..-...-.. ....-..A.. .......... ......-... .T........ .....T...G
'RK950 - C. pyxidata 2'           ---------- -------.-- ---------- -------.-- -.--...... ..A......-
'RK938 - C. pyxidata 2'           .......... ....T..... .......... .......... .......... ..A......G
'MS4553 - C. magyarica'           .......... .......... .........G .......... .T........ ..A......G
'RK1091 - C. chlorophaea'         .......... ......-G.. .......... .......... .......... ..........
'MPN7268 - C. chlorophaea'        .......... .......... .......... .......... .......... ..........
'MPN6592 - C. coccifera'          .......G.. .......... .......... .G.....T.. .C........ ..A......G
'MPN5600 - C. fimbriata'          .......... .......... .......... .......... .......... ..........
'MPN6408 - C. grayi'              .......... .......... .......... .G........ .C........ .....G.C.G
'MPN7209 - C. grayi'              .......... .......... .......... .G........ .CA.....TT T....G...G
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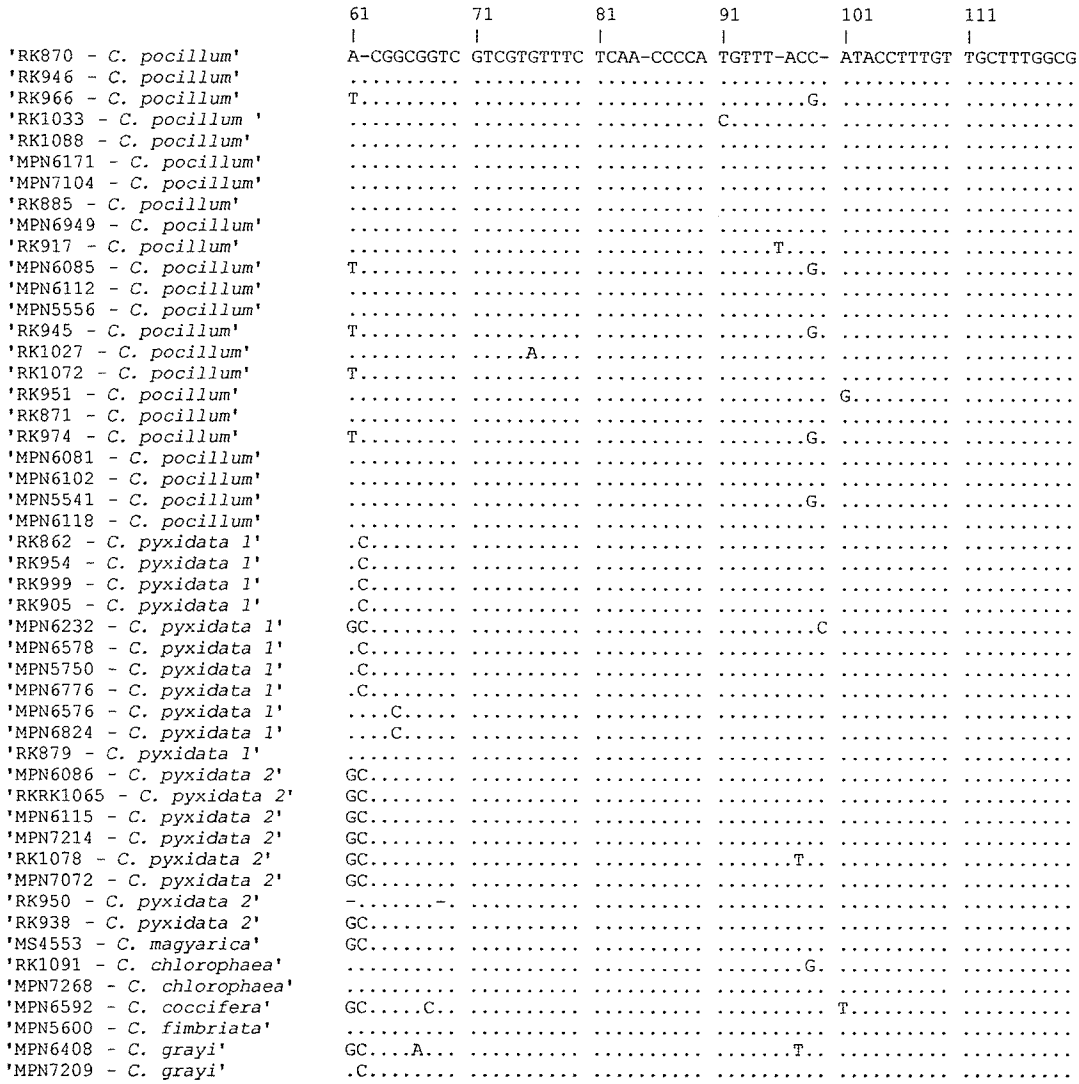
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'RK946 - C. pocillum'
'RK966 - C. pocillum'
'RK1033 - C. pocillum '
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'MPN6171 - C. pocillum'
'MPN7104 - C. pocillum'
'RK885 - C. pocillum'
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'RK945 - C. pocillum'
'RK1027 - C. pocillum'
'RK1072 - C. pocillum'
'RK951 - C. pocillum'
'RK871 - C. pocillum'
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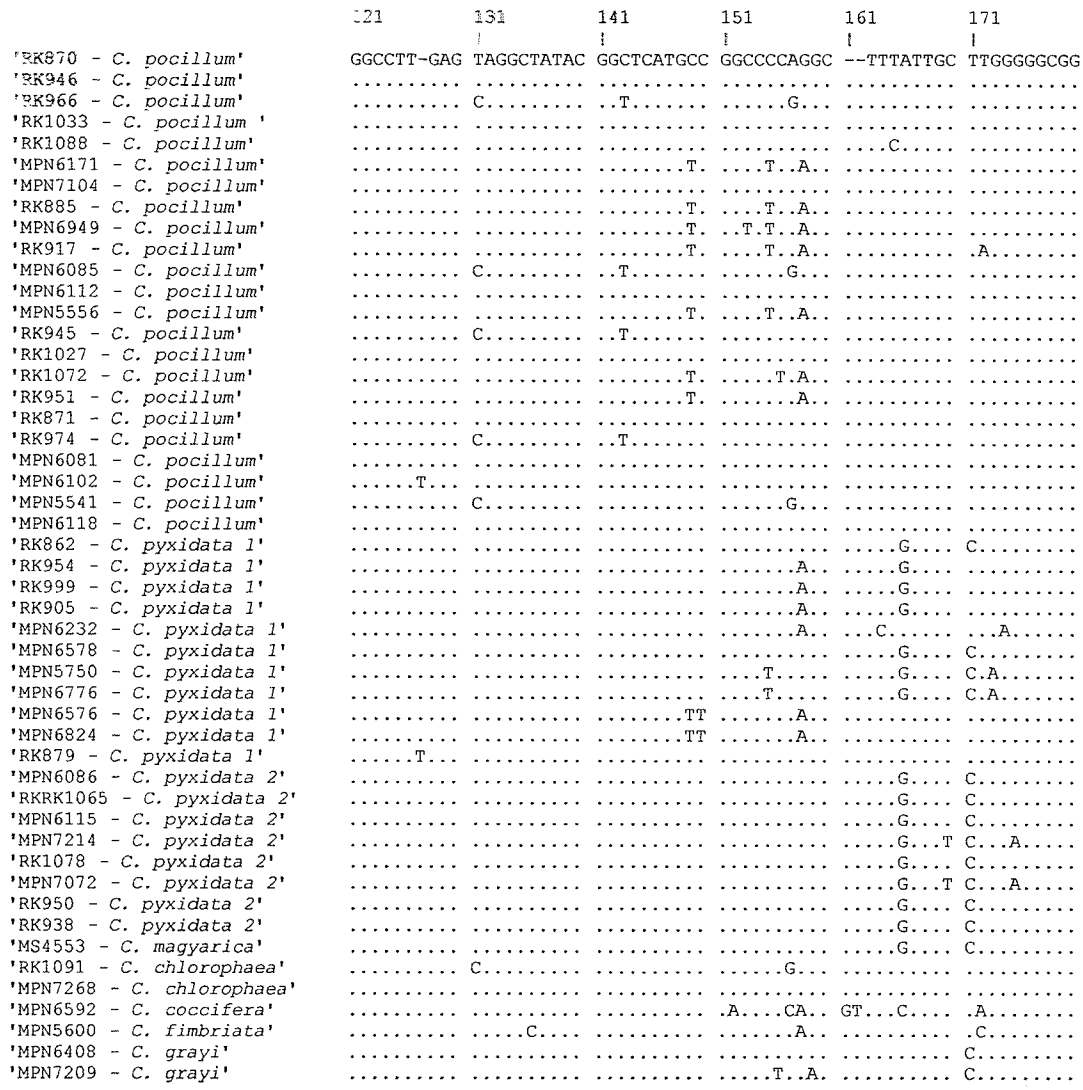
'MPN6081 - C. pocillum'
'MPN6102 - C. pocillum'
'MPN5541 - C. pocillum'
'MPN6118 - C. pocillum'
'RK862 - C. pyxidata 1'
'RK954 - C. pyxidata 1'
'RK999 - C. pyxidata 1'
'RK905 - C. pyxidata 1'
'MPN6232 - C. pyxidata 1'
'MPN6578 - C. pyxidata 1'
'MPN5750 - C. pyxidata 1'
'MPN6776 - C. pyxidata 1'
'MPN6576 - C. pyxidata 1'
'MPN6824 - C. pyxidata 1'
'RK879 - C. pyxidata 1'
'MPN6086 - C. pyxidata 2'
'RKRK1065 - C. pyxidata 2'
'MPN6115 - C. pyxidata 2'
'MPN7214 - C. pyxidata 2'
'RK1078 - C. pyxidata 2'
'MPN7072 - C. pyxidata 2'
'RK950 - C. pyxidata 2'
'RK938 - C. pyxidata 2'
'MS4553 - C. magyarica'
'RK1091 - C. chlorophaea'
'MPN7268 - C. chlorophaea'
'MPN6592 - C. coccifera'
'MPN5600 - C. fimbriata'
'MPN6408 - C. grayi'
'MPN7209 - C. grayi'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'RK946 - C. pocillum' ....
'RK966 - C. pocillum' ..... C..... ..T..... .....G... ....
'RK1033 - C. pocillum ' ....
'RK1088 - C. pocillum' ....C..... .....
'MPN6171 - C. pocillum' ....T. ....T..A.. ....
'MPN7104 - C. pocillum' ....
'RK885 - C. pocillum' ....T. ....T..A.. ....
'MPN6949 - C. pocillum' ....T. ..T.T..A.. ....
'RK917 - C. pocillum' ....T. ....T..A.. ..... .A.....
'MPN6085 - C. pocillum' ..... C..... ..T..... .....G... ....
'MPN6112 - C. pocillum' ....
'MPN5556 - C. pocillum' ....T. ....T..A.. ....
'RK945 - C. pocillum' ..... C..... ..T..... ....
'RK1027 - C. pocillum' ....
'RK1072 - C. pocillum' ....T. .....T.A.. ....
'RK951 - C. pocillum' ....T. .....A.. ....
'RK871 - C. pocillum' ....
'RK974 - C. pocillum' ..... C..... ..T..... ....
'MPN6081 - C. pocillum' ....
'MPN6102 - C. pocillum' .....T... ....
'MPN5541 - C. pocillum' ..... C..... ....G... ....
'MPN6118 - C. pocillum' ....
'RK862 - C. pyxidata 1' ....G.... C.....
'RK954 - C. pyxidata 1' ....A.. .....G.... .....
'RK999 - C. pyxidata 1' ....A.. .....G.... .....
'RK905 - C. pyxidata 1' ....A.. .....G.... .....
'MPN6232 - C. pyxidata 1' ....A.. ...C..... ...A.....
'MPN6578 - C. pyxidata 1' ....G.... C.....
'MPN5750 - C. pyxidata 1' ....T..... .....G.... C.A.....
'MPN6776 - C. pyxidata 1' ....T..... .....G.... C.A.....
'MPN6576 - C. pyxidata 1' ....TT ......A.. ....
'MPN6824 - C. pyxidata 1' ....TT .....A.. ....
'RK879 - C. pyxidata 1' .....T... ....
'MPN6086 - C. pyxidata 2' ....G.... C.....
'RKRK1065 - C. pyxidata 2' ....G.... C.....
'MPN6115 - C. pyxidata 2' ....G.... C.....
'MPN7214 - C. pyxidata 2' ....G...T C...A.....
'RK1078 - C. pyxidata 2' ....G.... C.....
'MPN7072 - C. pyxidata 2' ....G...T C...A.....
'RK950 - C. pyxidata 2' ....G.... C.....
'RK938 - C. pyxidata 2' ....G.... C.....
'MS4553 - C. magyarica' ....G.... C.....
'RK1091 - C. chlorophaea' ..... C..... ....G... ....
'MPN7268 - C. chlorophaea' ....
'MPN6592 - C. coccifera' .... .A....CA.. GT...C.... .A.....
'MPN5600 - C. fimbriata' ....C.... ....A.. ..... .C.....
'MPN6408 - C. grayi' .... C.....
'MPN7209 - C. grayi' ....T..A. ..... C.....

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                                181         191         201         211         221         231
                                |           |           |           |           |           |
'RK870 - C. pocillum'           CTCGCGCCCG CCAGAGGTTT -AA-TCAAAT CCTATTT-AT TAGTGATGTC TGAGTAAATA
'RK946 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK966 - C. pocillum'           .......... .......... ....C..... .......... ......C... ......T...
'RK1033 - C. pocillum '         .......... .......... .......... .......... .......... ..........
'RK1088 - C. pocillum'          .......... .......... .......... .......... .......... ..........
'MPN6171 - C. pocillum'         .......... .......... .......... .......... A......... .......T..
'MPN7104 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK885 - C. pocillum'           .......... .......... .......... .......... A......... .......T..
'MPN6949 - C. pocillum'         .......... .......... .......... .......... A......... .......T..
'RK917 - C. pocillum'           .......... .......... .......... .......... A......... .......T..
'MPN6085 - C. pocillum'         .......... .......... ....C..... .......... .......... ..........
'MPN6112 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN5556 - C. pocillum'         .......... .......... .......... .......... A......... .......T..
'RK945 - C. pocillum'           .......... .......... ....C..... .......... ......C... ..........
'RK1027 - C. pocillum'          .......... .......... .......... .......... .......... ..........
'RK1072 - C. pocillum'          .......... .......... ....C..... .......... A......... .......T..
'RK951 - C. pocillum'           .......... .......... .......... .......... A......... ..........
'RK871 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK974 - C. pocillum'           .......... .......... ....C..... .......... ......C... ..........
'MPN6081 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN6102 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN5541 - C. pocillum'         .......... .......... ....C..... .......... ......C... ..........
'MPN6118 - C. pocillum'         .......... .......... .......... .......... .......... C.........
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'RK954 - C. pyxidata 1'         .......... .......... ...TC..... .......... C......... ....C.....
'RK999 - C. pyxidata 1'         .......... ........C. ...TC..... .......... C......... ....C.....
'RK905 - C. pyxidata 1'         .......... .......... ...TC..... .......... C......... ....C.....
'MPN6232 - C. pyxidata 1'       .......... .........C ...AC..... .......... C......... ....C.....
'MPN6578 - C. pyxidata 1'       .......... .......... ...AC..... .......... C......... ....C.....
'MPN5750 - C. pyxidata 1'       .......... .......... ...AC..T.. .......... C......... ....C.....
'MPN6776 - C. pyxidata 1'       .......... .......... ...AC..T.. .......... C......... ....C.....
'MPN6576 - C. pyxidata 1'       .......... .......... ....C..... T......... A......... ....C..T..
'MPN6824 - C. pyxidata 1'       .......... .......... ....C..... T......... A......... ....C..T..
'RK879 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
'MPN6086 - C. pyxidata 2'       .......... .......... ...AC..... ...C...T.. C......... ....C.....
'RKRK1065 - C. pyxidata 2'      .......... .......... ...AC..... ...C...T.. C......... ....C.....
'MPN6115 - C. pyxidata 2'       .......... .......... ...AC..... ...C...... C......... ....C.....
'MPN7214 - C. pyxidata 2'       .......... .......... T..AC..... .......... C......... ....C.....
'RK1078 - C. pyxidata 2'        .......... .......... .......... .......... C......... ....C.....
'MPN7072 - C. pyxidata 2'       .......... .......... ...AC....C .......... C......... ....C..C..
'RK950 - C. pyxidata 2'         .........C .......... ...AC..... .......... C......... ....C.....
'RK938 - C. pyxidata 2'         .......... .......... ...AC..... .......... C......... ....C.....
'MS4553 - C. magyarica'         .......... .......... ...AC..... ...C...T.. C......... ....C.....
'RK1091 - C. chlorophaea'       .......... .......... ....C..... .........A ......C... ..........
'MPN7268 - C. chlorophaea'      .......... .......... .......... .......... .......... ..........
'MPN6592 - C. coccifera'        .......... ..G......C ....C..... T..G...... ......A... C.....G.A.
'MPN5600 - C. fimbriata'        .......... .......... .....T...C .......... .G........ ....C.....
'MPN6408 - C. grayi'            .......... .......... ....C..... .......... C.T..... ....C.....
'MPN7209 - C. grayi'            .......... .......... ....C..... T.....-... C......... ....C.....
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                                241         251         261         271         281         291
                                |           |           |           |           |           |
'RK870 - C. pocillum'           TTAAATAA-T CAAAACTTTC AACAACGGAT CTCTTGGTTC TGGCATCGAT GAAGAACGCA
'RK946 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK966 - C. pocillum'           .......... .......... .......... .......... .......... ..........
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'RK1088 - C. pocillum'          .......... .......... .......... .......... .......... ..........
'MPN6171 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN7104 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK885 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'MPN6949 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK917 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'MPN6085 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN6112 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN5556 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK945 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK1027 - C. pocillum'          .......... .......... .......... .......... .......... ..........
'RK1072 - C. pocillum'          .......... .......... .......... .......... .......... ..........
'RK951 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK871 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK974 - C. pocillum'           .......... .......... .......... .......... .......... ..........
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'MPN6102 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN5541 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN6118 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK862 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
'RK954 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
'RK999 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
'RK905 - C. pyxidata 1'         .C........ .......... .......... .......... .......... ..........
'MPN6232 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'MPN6578 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'MPN5750 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'MPN6776 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'MPN6576 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'MPN6824 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'RK879 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
'MPN6086 - C. pyxidata 2'       ..C....... .......... .......... .......... .......... ..........
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'MPN5600 - C. fimbriata'        .......... .......... .......... .......... .......... ..........
'MPN6408 - C. grayi'            .......... .......... .......... .......... .......... ..........
'MPN7209 - C. grayi'            ..C....... .......... .......... .......... .......... ..........
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                                301         311         321         331         341         351
                                |           |           |           |           |           |
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'RK946 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK966 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK1033 - C. pocillum '         .......... .......... .......... .......... .......... ..........
'RK1088 - C. pocillum'          .......... .......... .......... .......... .......... ..........
'MPN6171 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN7104 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK885 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'MPN6949 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK917 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'MPN6085 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN6112 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'MPN5556 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK945 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK1027 - C. pocillum'          .......... .......... .......... .......... .......... ..........
'RK1072 - C. pocillum'          .......... .......... .......... .......... .......... ..........
'RK951 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK871 - C. pocillum'           .......... .......... .......... .......... .......... ..........
'RK974 - C. pocillum'           .......... .......... .......... .......... .......... ..........
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'MPN6118 - C. pocillum'         .......... .......... .......... .......... .......... ..........
'RK862 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
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'RK999 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
'RK905 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
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'MPN6578 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'MPN5750 - C. pyxidata 1'       .......... .......... .......... ....T..... .......... ..........
'MPN6776 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'MPN6576 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'MPN6824 - C. pyxidata 1'       .......... .......... .......... .......... .......... ..........
'RK879 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
'MPN6086 - C. pyxidata 2'       .......... .......... .......... .......... .......... ..........
'RKRK1065 - C. pyxidata 2'      .......... .......... .......... .......... .......... ..........
'MPN6115 - C. pyxidata 2'       .......... .......... .......... .......... .......... ..........
'MPN7214 - C. pyxidata 2'       .......... .......... .......... .......... .......... ..........
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'MPN7072 - C. pyxidata 2'       .......... .......... .......... .......... .......... ..........
'RK950 - C. pyxidata 2'         .......... .......... .......... .......... .......... ..........
'RK938 - C. pyxidata 2'         .......... .......... .......... .......... .......... ..........
'MS4553 - C. magyarica'         .......... .......... .......... .......... .......... ..........
'RK1091 - C. chlorophaea'       .......... .......... .......... .......... .......... ..........
'MPN7268 - C. chlorophaea'      .......... .......... .......... .......... .......... ..........
'MPN6592 - C. coccifera'        .......... .......... .......... .......... .......... ..........
'MPN5600 - C. fimbriata'        .......... .......... .......... .......... .......... ..........
'MPN6408 - C. grayi'            .......... .......... .......... .......... .......... ..........
'MPN7209 - C. grayi'            .......... .......... .......... .......... .......... ..........
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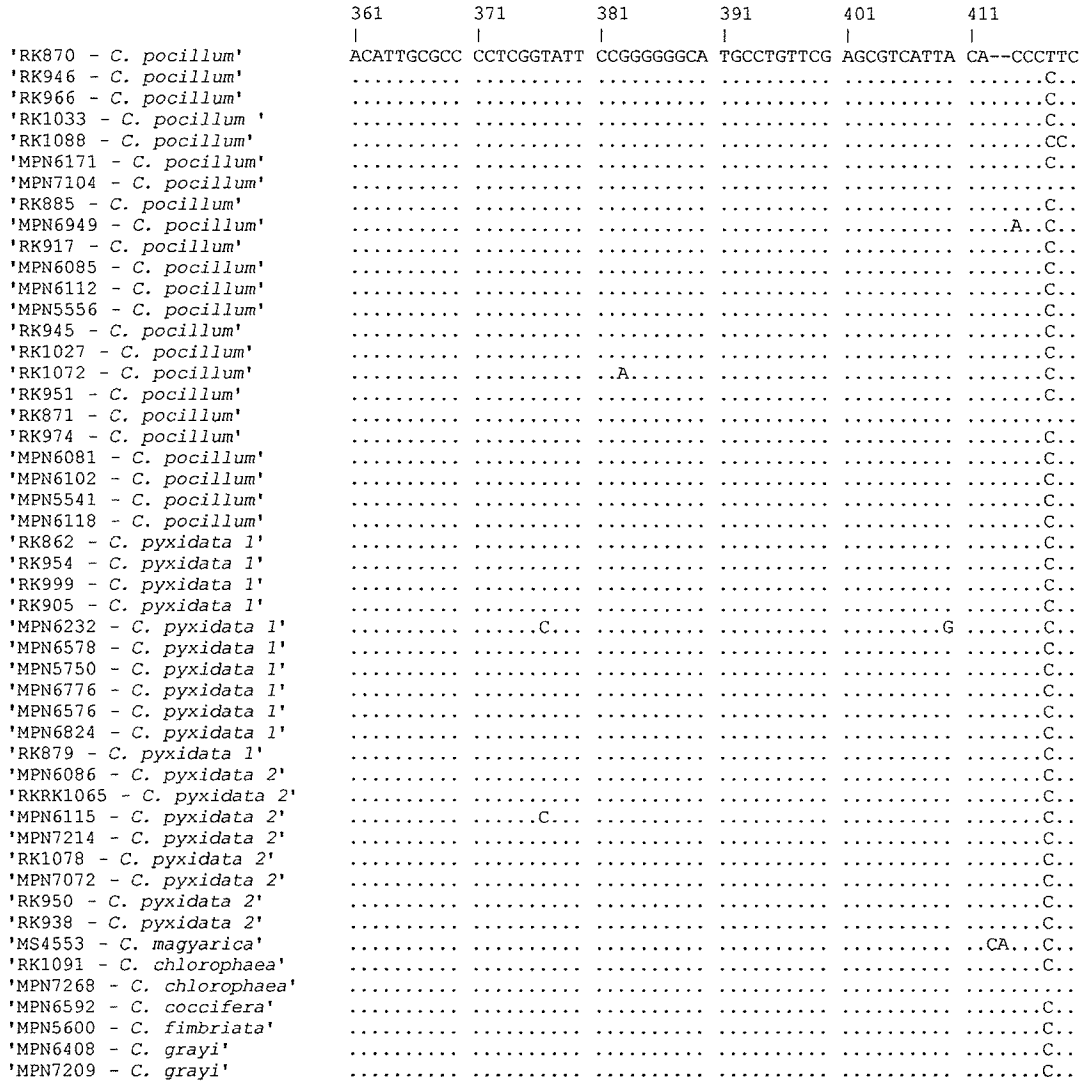
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'RK870 - C. pocillum' ACATTGCGCC CCTCGGTATT CCGGGGGGCA TGCCTGTTCG AGCGTCATTA CA--CCCTTC
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'RK966 - C. pocillum' .....C..
'RK1033 - C. pocillum ' .....C..
'RK1088 - C. pocillum' .....CC.
'MPN6171 - C. pocillum' .....C..
'MPN7104 - C. pocillum' .....
'RK885 - C. pocillum' .....C..
'MPN6949 - C. pocillum' ....A..C..
'RK917 - C. pocillum' .....C..
'MPN6085 - C. pocillum' .....C..
'MPN6112 - C. pocillum' .....C..
'MPN5556 - C. pocillum' .....C..
'RK945 - C. pocillum' .....C..
'RK1027 - C. pocillum' .....C..
'RK1072 - C. pocillum' ..A..... .....C..
'RK951 - C. pocillum' .....C..
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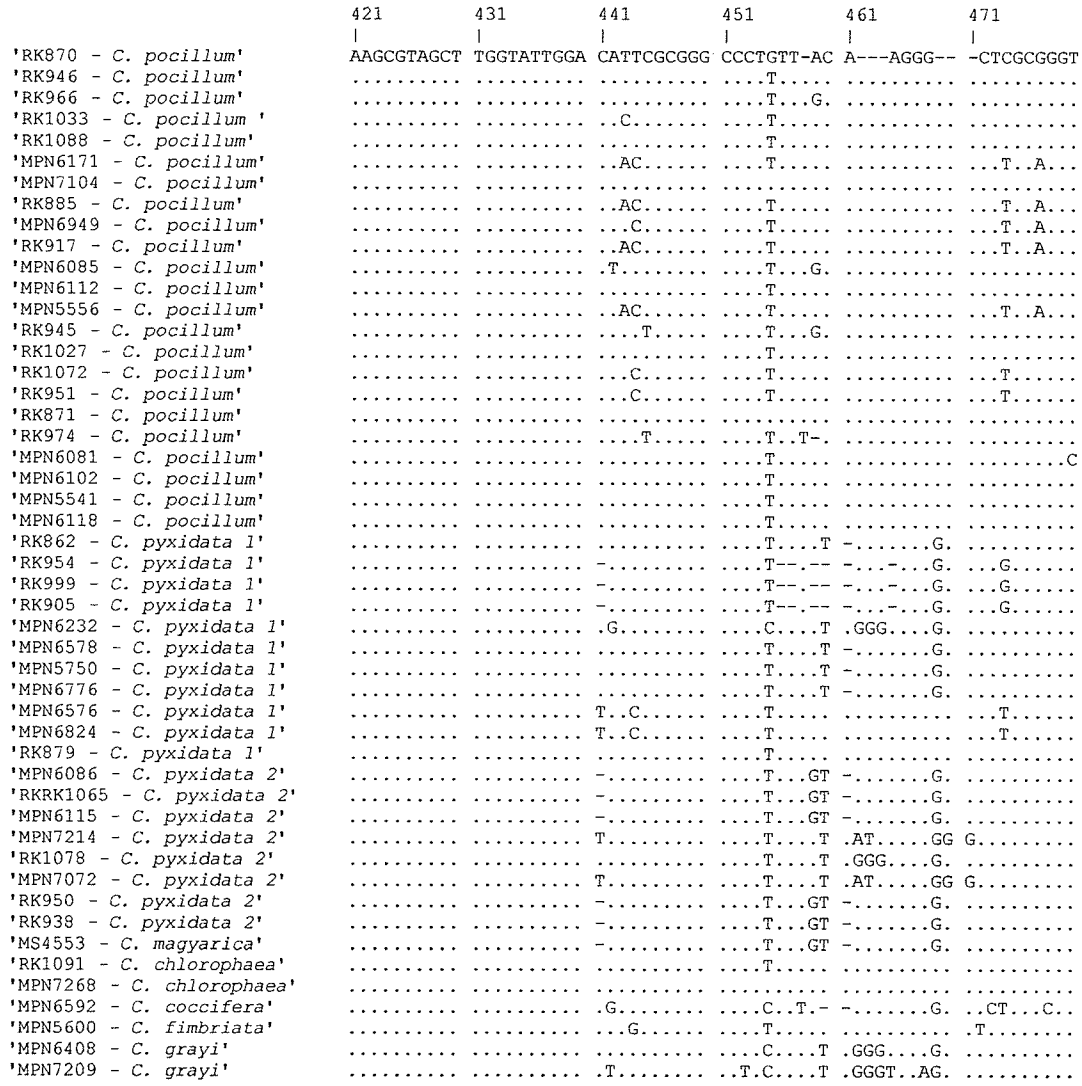
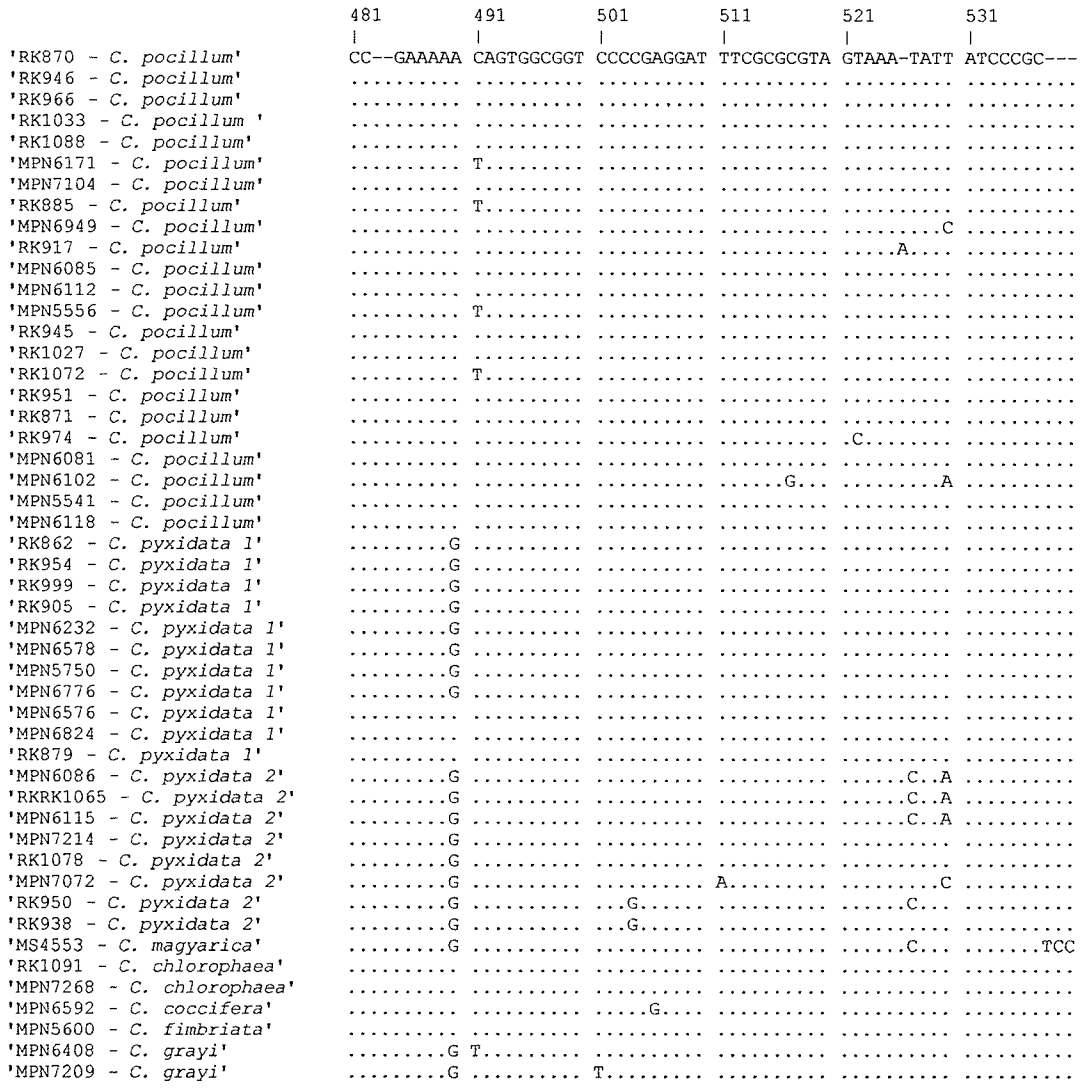
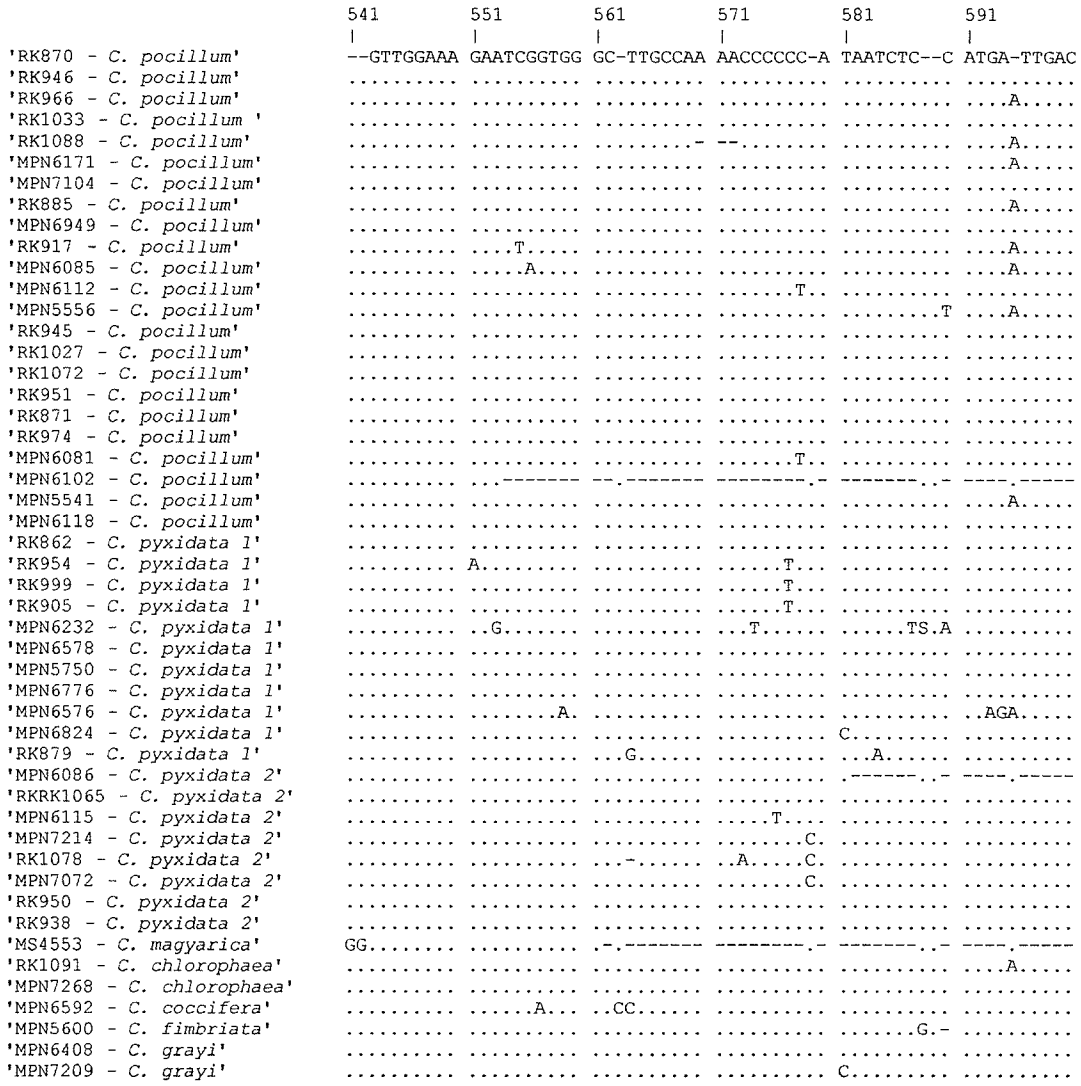
'MPN6102 - C. pocillum' .....C..
'MPN5541 - C. pocillum' .....C..
'MPN6118 - C. pocillum' .....C..
'RK862 - C. pyxidata 1' .....C..
'RK954 - C. pyxidata 1' .....C..
'RK999 - C. pyxidata 1' .....C..
'RK905 - C. pyxidata 1' .....C..
'MPN6232 - C. pyxidata 1' ......C... .....G .....C..
'MPN6578 - C. pyxidata 1' .....C..
'MPN5750 - C. pyxidata 1' .....C..
'MPN6776 - C. pyxidata 1' .....C..
'MPN6576 - C. pyxidata 1' .....C..
'MPN6824 - C. pyxidata 1' .....C..
'RK879 - C. pyxidata 1' .....C..
'MPN6086 - C. pyxidata 2' .....C..
'RKRK1065 - C. pyxidata 2' .....C..
'MPN6115 - C. pyxidata 2' ......C... .....C..
'MPN7214 - C. pyxidata 2' .....C..
'RK1078 - C. pyxidata 2' .....C..
'MPN7072 - C. pyxidata 2' .....C..
'RK950 - C. pyxidata 2' .....C..
'RK938 - C. pyxidata 2' .....C..
'MS4553 - C. magyarica' ..CA...C..
'RK1091 - C. chlorophaea' .....C..
'MPN7268 - C. chlorophaea' .....
'MPN6592 - C. coccifera' .....C..
'MPN5600 - C. fimbriata' .....C..
'MPN6408 - C. grayi' .....C..
'MPN7209 - C. grayi' .....C..


421 431 441 451 461 471
'RK870 - C. pocillum' AAGCGTAGCT TGGTATTGGA CATTCGCGGG CCCTGTT-AC A---AGGG-- -CTCGCGGGT
'RK946 - C. pocillum'
'RK966 - C. pocillum'
'RK1033 - C. pocillum '
'RK1088 - C. pocillum'
'MPN6171 - C. pocillum'
'MPN7104 - C. pocillum'
'RK885 - C. pocillum'
'MPN6949 - C. pocillum'
'RK917 - C. pocillum'
'MPN6085 - C. pocillum'
'MPN6112 - C. pocillum'
'MPN5556 - C. pocillum'
'RK945 - C. pocillum'
'RK1027 - C. pocillum'
'RK1072 - C. pocillum'
'RK951 - C. pocillum'
'RK871 - C. pocillum'
'RK974 - C. pocillum'
'MPN6081 - C. pocillum'
'MPN6102 - C. pocillum'
'MPN5541 - C. pocillum'
'MPN6118 - C. pocillum'
'RK862 - C. pyxidata 1'
'RK954 - C. pyxidata 1'
'RK999 - C. pyxidata 1'
'RK905 - C. pyxidata 1'
'MPN6232 - C. pyxidata 1'
'MPN6578 - C. pyxidata 1'
'MPN5750 - C. pyxidata 1'
'MPN6776 - C. pyxidata 1'
'MPN6576 - C. pyxidata 1'
'MPN6824 - C. pyxidata 1'
'RK879 - C. pyxidata 1'
'MPN6086 - C. pyxidata 2'
'RKRK1065 - C. pyxidata 2'
'MPN6115 - C. pyxidata 2'
'MPN7214 - C. pyxidata 2'
'RK1078 - C. pyxidata 2'
'MPN7072 - C. pyxidata 2'
'RK950 - C. pyxidata 2'
'RK938 - C. pyxidata 2'
'MS4553 - C. magyarica'
'RK1091 - C. chlorophaea'
'MPN7268 - C. chlorophaea'
'MPN6592 - C. coccifera'
'MPN5600 - C. fimbriata'
'MPN6408 - C. grayi'
'MPN7209 - C. grayi'


481 491 501 511 521 531
'RK870 - C. pocillum' CC--GAAAAA CAGTGGCGGT CCCCGAGGAT TTCGCGCGTA GTAAA-TATT ATCCCGC---
'RK946 - C. pocillum'
'RK966 - C. pocillum'
'RK1033 - C. pocillum '
'RK1088 - C. pocillum'
'MPN6171 - C. pocillum'
'MPN7104 - C. pocillum'
'RK885 - C. pocillum'
'MPN6949 - C. pocillum'
'RK917 - C. pocillum'
'MPN6085 - C. pocillum'
'MPN6112 - C. pocillum'
'MPN5556 - C. pocillum'
'RK945 - C. pocillum'
'RK1027 - C. pocillum'
'RK1072 - C. pocillum'
'RK951 - C. pocillum'
'RK871 - C. pocillum'
'RK974 - C. pocillum'
'MPN6081 - C. pocillum'
'MPN6102 - C. pocillum'
'MPN5541 - C. pocillum'
'MPN6118 - C. pocillum'
'RK862 - C. pyxidata 1'
'RK954 - C. pyxidata 1'
'RK999 - C. pyxidata 1'
'RK905 - C. pyxidata 1'
'MPN6232 - C. pyxidata 1'
'MPN6578 - C. pyxidata 1'
'MPN5750 - C. pyxidata 1'
'MPN6776 - C. pyxidata 1'
'MPN6576 - C. pyxidata 1'
'MPN6824 - C. pyxidata 1'
'RK879 - C. pyxidata 1'
'MPN6086 - C. pyxidata 2'
'RKRK1065 - C. pyxidata 2'
'MPN6115 - C. pyxidata 2'
'MPN7214 - C. pyxidata 2'
'RK1078 - C. pyxidata 2'
'MPN7072 - C. pyxidata 2'
'RK950 - C. pyxidata 2'
'RK938 - C. pyxidata 2'
'MS4553 - C. magyarica'
'RK1091 - C. chlorophaea'
'MPN7268 - C. chlorophaea'
'MPN6592 - C. coccifera'
'MPN5600 - C. fimbriata'
'MPN6408 - C. grayi'
'MPN7209 - C. grayi'


541 551 561 571 581 591
'RK870 - C. pocillum' --GTTGGAAA GAATCGGTGG GC-TTGCCAA AACCCCCC-A TAATCTC--C ATGA-TTGAC
'RK946 - C. pocillum'
'RK966 - C. pocillum'
'RK1033 - C. pocillum '
'RK1088 - C. pocillum'
'MPN6171 - C. pocillum'
'MPN7104 - C. pocillum'
'RK885 - C. pocillum'
'MPN6949 - C. pocillum'
'RK917 - C. pocillum'
'MPN6085 - C. pocillum'
'MPN6112 - C. pocillum'
'MPN5556 - C. pocillum'
'RK945 - C. pocillum'
'RK1027 - C. pocillum'
'RK1072 - C. pocillum'
'RK951 - C. pocillum'
'RK871 - C. pocillum'
'RK974 - C. pocillum'
'MPN6081 - C. pocillum'
'MPN6102 - C. pocillum'
'MPN5541 - C. pocillum'
'MPN6118 - C. pocillum'
'RK862 - C. pyxidata 1'
'RK954 - C. pyxidata 1'
'RK999 - C. pyxidata 1'
'RK905 - C. pyxidata 1'
'MPN6232 - C. pyxidata 1'
'MPN6578 - C. pyxidata 1'
'MPN5750 - C. pyxidata 1'
'MPN6776 - C. pyxidata 1'
'MPN6576 - C. pyxidata 1'
'MPN6824 - C. pyxidata 1'
'RK879 - C. pyxidata 1'
'MPN6086 - C. pyxidata 2'
'RKRK1065 - C. pyxidata 2'
'MPN6115 - C. pyxidata 2'
'MPN7214 - C. pyxidata 2'
'RK1078 - C. pyxidata 2'
'MPN7072 - C. pyxidata 2'
'RK950 - C. pyxidata 2'
'RK938 - C. pyxidata 2'
'MS4553 - C. magyarica'
'RK1091 - C. chlorophaea'
'MPN7268 - C. chlorophaea'
'MPN6592 - C. coccifera'
'MPN5600 - C. fimbriata'
'MPN6408 - C. grayi'
'MPN7209 - C. grayi'

```
                                601         611         621         631         641
                                |           |           |           |           |
'RK870 - C. pocillum'           CTCGGATCAG GTAGGGATAC CCGCTGAACT TAAGCATATC AATA-
'RK946 - C. pocillum'           .......... .......... .......... .......... .....
'RK966 - C. pocillum'           .......... .......... .......... .......... .....
'RK1033 - C. pocillum '         .......... .......... .......... .......... ..--.
'RK1088 - C. pocillum'          ......---- ---------- ---------- ---------- ----.
'MPN6171 - C. pocillum'         .......... .......... .......... .......... .....
'MPN7104 - C. pocillum'         .......... .......... .......... .......... ....A
'RK885 - C. pocillum'           .......... .......... .......... .......... .....
'MPN6949 - C. pocillum'         .......... .......... .......... .......... .....
'RK917 - C. pocillum'           .......... .......... .......... .......... ....A
'MPN6085 - C. pocillum'         .......... .......... .......... .......... ....A
'MPN6112 - C. pocillum'         .......... .......... .......... .......... ....A
'MPN5556 - C. pocillum'         .......... .......... .......... .......... ....A
'RK945 - C. pocillum'           .......... .......... .......... .......... ....A
'RK1027 - C. pocillum'          .......... .......... .......... .......... ....A
'RK1072 - C. pocillum'          .......... .......... .......... .......... ....A
'RK951 - C. pocillum'           .......... .......... .......... .......... ....A
'RK871 - C. pocillum'           .......... .......... .......... .......... ....A
'RK974 - C. pocillum'           .......... .......... .......... .......... ....A
'MPN6081 - C. pocillum'         .......... .......... .--------- ---------- ----.
'MPN6102 - C. pocillum'         ---------- ---------- ---------- ---------- ----.
'MPN5541 - C. pocillum'         .......... .......... .--------- ---------- ----.
'MPN6118 - C. pocillum'         .......... .......... ....------ ---------- ----.
'RK862 - C. pyxidata 1'         .......... .......... .......... .......... .....
'RK954 - C. pyxidata 1'         .......... .......... .......... .......... .....
'RK999 - C. pyxidata 1'         .......... .......... .......... .......... .....
'RK905 - C. pyxidata 1'         .......... .......... .......... .......... ....A
'MPN6232 - C. pyxidata 1'       .......... .......... .......... .......... ....A
'MPN6578 - C. pyxidata 1'       .......... .......... .......... .......... .....
'MPN5750 - C. pyxidata 1'       .......... .......... .......... .......... .---.
'MPN6776 - C. pyxidata 1'       .......... .......... .......... .......... .---.
'MPN6576 - C. pyxidata 1'       .......... .......... .......... .......... .....
'MPN6824 - C. pyxidata 1'       .......... .......... .......... .......... ....A
'RK879 - C. pyxidata 1'         .......... .......... .......... .......... ....A
'MPN6086 - C. pyxidata 2'       ---------- ---------- ---------- ---------- ----.
'RKRK1065 - C. pyxidata 2'      .......... .......... .......... .......... ....A
'MPN6115 - C. pyxidata 2'       .......... .......... ---------- ---------- ----.
'MPN7214 - C. pyxidata 2'       .......... .......... .......... .......... ....A
'RK1078 - C. pyxidata 2'        .......... .......... .......... .......... ....A
'MPN7072 - C. pyxidata 2'       .......... .......... .......... .......... ....A
'RK950 - C. pyxidata 2'         .......... .......... .......... .......... ....A
'RK938 - C. pyxidata 2'         .......... .......... .......... .......... ....A
'MS4553 - C. magyarica'         ---------- ---------- ---------- ---------- ----.
'RK1091 - C. chlorophaea'       .......... .......... .......... .......... .....
'MPN7268 - C. chlorophaea'      .......... .......... .......... .......... .....
'MPN6592 - C. coccifera'        .......... .G........ .......... .......... ....A
'MPN5600 - C. fimbriata'        .......... .G........ .......... .......... .---.
'MPN6408 - C. grayi'            .......... .......... .......... .......... ....A
'MPN7209 - C. grayi'            .......... .......... .......... .......... .....
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Appendix V

Aligned fungal PKS sequences obtained in this study. Ambiguity codes used include R (A or G), M (A or C), and Y (C or T). Dots indicate the occurrence of the same nucleotide as the reference sequence. Dashes represent gaps or missing data.

```
                                  1          11         21         31         41         51
                                  |          |          |          |          |          |
'MS4553 - C. magyarica'           ---------- ---------- CTAGAAATGG CGGGCTATGT CCCAAATCGA ACGCCCTCCA
'AA51297 - C. monomorpha'         .......... .......... .......... .A........ .......... ..........
'MPN6232 - C. pyxidata 1'         .......... .......... .......... .A........ .......... ..........
'MPN7072 - C. pyxidata 2'         .......... .......... .......... .A........ .......... ..........
'MPN6776 - C. pyxidata 1'         .......... .......... .......... .A........ .......... ..........
'RK862 - C. pyxidata 1'           .......... .......... .......... .A........ .......... ..........
'RK1065 - C. pyxidata 2'          .......... .......... .......... .A........ .......... ..........
'RK999 - C. pyxidata 1'           .......... .......... .......... .A........ .......... ..........
'MPN6576 - C. pyxidata 1'         .......... .......... .......... .A........ .......... ..T.....T.
'RK885 - C. pocillum '            .......... .......... .......... .A........ .......... ..........
'RK1088 - C. pocillum'            .......... .......... .......... .A........ .......... ..........
'MPN6171 - C. pocillum'           .......... .......... .......... .A........ .......... ..........
'RK870 - C. pocillum'             .......... .......... .......... .A........ .......... ..........
'RK946 - C. pocillum'             .......... .......... .......... .A........ .......... ..........
'MPN5600 - C. fimbriata'          .......... .......... .......... .A........ .......... ..........
'RK1091 - C. chlorophaea'         .......... .......... .......... .A........ .......... ..........
'MPN6623 - C. chlorophaea'        .......... .......... .......... .A........ .......... ..........
'MPN6408 - C. grayi'              .......... .......... .......... .A.....C.. .......... .....T..T.

                                  61         71         81         91         101        111
                                  |          |          |          |          |          |
'MS4553 - C. magyarica'           CGAAACTTGA CAGAATTGGC ACGTTCTACG GTCAGACGAG CGACGACTGG CGGGAAATTA
'AA51297 - C. monomorpha'         .......... .......... .......... .......... .......... ..........
'MPN6232 - C. pyxidata 1'         ...G...... .......... .......... .......... .......... ..A..G....
'MPN7072 - C. pyxidata 2'         .......... .......... .......... .......... .......... ..........
'MPN6776 - C. pyxidata 1'         .......... .......... .......... .......... .......... ..........
'RK862 - C. pyxidata 1'           .......... .......... .......... .......... .......... ..........
'RK1065 - C. pyxidata 2'          .......... .......... .......... .......... .......... ..........
'RK999 - C. pyxidata 1'           .......... .......... .......... .......... .......... ..........
'MPN6576 - C. pyxidata 1'         ....G..... .......... .......... .......... .......... A.A..G..C.
'RK885 - C. pocillum '            .......... T......... .......... .......... .......... ..........
'RK1088 - C. pocillum'            .......... T......... .......... .......... .......... .....G....
'MPN6171 - C. pocillum'           .......... T......... .......... .......... .......... ..........
'RK870 - C. pocillum'             .......... T......... .......... .......... .......... ..........
'RK946 - C. pocillum'             .......... T......... .......... .......... .......... ..........
'MPN5600 - C. fimbriata'          .......... .......... ..C....... .......... .......... ..A.......
'RK1091 - C. chlorophaea'         .......... T......... .......... .......... .......... ..........
'MPN6623 - C. chlorophaea'        .......... .......... .......... .......... .......... ..........
'MPN6408 - C. grayi'              ...G...... .......... .......... ....A..C.. T.....T... ..A..G..C.

                                  121        131        141        151        161        171
                                  |          |          |          |          |          |
'MS4553 - C. magyarica'           ATGAAGCTCA GGATATCGAC ACCTACTTCA TTACCGCTGG AGTTAGAGCA TTCGCACCAG
'AA51297 - C. monomorpha'         .......... .......... .......... ....T..... .......... ..........
'MPN6232 - C. pyxidata 1'         .C........ .......... ..A....... .......... .......... ..........
'MPN7072 - C. pyxidata 2'         .......... .......... .......... ....T..... .......... ..........
'MPN6776 - C. pyxidata 1'         .......... .......... .......... ....Y..... .......... ..........
'RK862 - C. pyxidata 1'           .......... .......... .......... ....Y..... .......... ..........
'RK1065 - C. pyxidata 2'          .......... .......... .......... .......... .......... ..........
'RK999 - C. pyxidata 1'           ....R..... .......... ..M....... .......... .......... ..........
'MPN6576 - C. pyxidata 1'         ....G..... .......... ..A....... .......... .......... ..........
'RK885 - C. pocillum '            ....G..... .......... ..A....... .......... .......... ..........
'RK1088 - C. pocillum'            ....G..... .......... ..A....... .......... .......... ..........
'MPN6171 - C. pocillum'           ....G..... .......... ..A....... .......... .......... ..........
'RK870 - C. pocillum'             ....G..... .......... ..A....... .......... .......... ..........
'RK946 - C. pocillum'             ....G..... .......... ..A....... .......... .......... ..........
'MPN5600 - C. fimbriata'          ....G..... .......... ..A....... .......... .......... ..........
'RK1091 - C. chlorophaea'         ....G..... .......... ..A....... .......... .......... ..........
'MPN6623 - C. chlorophaea'        ....G..... .......... ..A....... .......... .......... ..........
'MPN6408 - C. grayi'              .......... .......... ..T....... .......... .......... ..........
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                                  181         191         201         211         221         231
                                  |           |           |           |           |           |
'MS4553 - C. magyarica'           TGAGCAACAT  TTTTCGCCCA  GAATATTCCT  TAGGATCT-G  CTAACAGAAT  ATCAGGGTAG
'AA51297 - C. monomorpha'         ..........  ..........  ..........  ..........  ....T.....  ..........
'MPN6232 - C. pyxidata 1'         ........G.  ..C.......  ..........  ..........  ....T.....  ..........
'MPN7072 - C. pyxidata 2'         ..........  ..........  ..........  ..........  ....T.....  ..........
'MPN6776 - C. pyxidata 1'         ..........  ..........  ..........  ..........  ....T.....  ..........
'RK862 - C. pyxidata 1'           ..........  ..........  ..........  ..........  ....T.....  ..........
'RK1065 - C. pyxidata 2'          ..........  ..........  ..........  ..........  ....Y....Y  ..........
'RK999 - C. pyxidata 1'           ..........  ..........  ...Y......  ..........  ....T....Y  ..........
'MPN6576 - C. pyxidata 1'         ........C.  ..........  ..........  ..........  ....T.....  .AT.......
'RK885 - C. pocillum '            ..........  ..........  ....G.....  CC......T.  ....T.....  ..........
'RK1088 - C. pocillum'            ..........  ..........  T...G.....  CC......T.  ....T.....  ..........
'MPN6171 - C. pocillum'           ..........  ..........  ....G.....  CC......T.  ....T.....  ..........
'RK870 - C. pocillum'             ..........  ..........  ....G.....  CC......T.  ....T.....  ..........
'RK946 - C. pocillum'             ..........  ..........  ....G.....  CC......T.  ....T.....  ..........
'MPN5600 - C. fimbriata'          .......G..  ..........  ..........  CC......T.  ....T.....  ..T.......
'RK1091 - C. chlorophaea'         ..........  ..........  ....G.....  CC......T.  ....T.....  ..........
'MPN6623 - C. chlorophaea'        ..........  ..........  ..........  CC......T.  ....T.....  ..........
'MPN6408 - C. grayi'              ..........  .C..T.....  C..C......  -.........  ......C...  ..........

                                  241         251         261         271         281         291
                                  |           |           |           |           |           |
'MS4553 - C. magyarica'           GATCAATTAC  TATTTTAAAT  TTAGCGGGCC  GAGTTTTAGC  ATCGATACTG  CCTGCTCTTC
'AA51297 - C. monomorpha'         ..........  ..........  ..........  ..........  ..........  ..........
'MPN6232 - C. pyxidata 1'         .........T  ..........  ..........  ..........  ..........  ..........
'MPN7072 - C. pyxidata 2'         ..........  ..........  ..........  ..........  ..........  ..........
'MPN6776 - C. pyxidata 1'         ..........  ..........  ..........  ..........  ..........  ..........
'RK862 - C. pyxidata 1'           ..........  .....C....  ..........  ..........  ..........  ..........
'RK1065 - C. pyxidata 2'          ..........  ..........  ..........  ..........  ..........  ..........
'RK999 - C. pyxidata 1'           ..........  .....C....  ..........  ..........  ..........  ..........
'MPN6576 - C. pyxidata 1'         ..........  ..........  ..........  ..........  ..........  ..........
'RK885 - C. pocillum '            ..........  ..........  ..........  ..........  ..........  ..........
'RK1088 - C. pocillum'            ..........  ..........  ..........  ..........  ..........  ..........
'MPN6171 - C. pocillum'           ..........  ..........  ..........  ..........  ..........  ..........
'RK870 - C. pocillum'             ..........  ..........  ..........  ..........  ..........  ..........
'RK946 - C. pocillum'             ..........  ..........  ..........  ..........  ..........  ..........
'MPN5600 - C. fimbriata'          A.........  ..........  ..........  ..........  ..........  ..........
'RK1091 - C. chlorophaea'         ..........  ..........  ..........  ..........  ..........  ..........
'MPN6623 - C. chlorophaea'        ..........  ..........  ..........  ..........  ..........  ..........
'MPN6408 - C. grayi'              ..........  ..........  ..........  ..........  ..........  ..........

                                  301         311         321         331         341         351
                                  |           |           |           |           |           |
'MS4553 - C. magyarica'           AAGTGCCGCA  GCTTTACAGC  TCGCATGCAC  GTCCTTGTGG  GCTGGTGACT  GCGATACAGC
'AA51297 - C. monomorpha'         ..........  ..........  ..........  ..........  ..........  ..........
'MPN6232 - C. pyxidata 1'         G.........  ..........  ..........  ..........  ..........  ..........
'MPN7072 - C. pyxidata 2'         ..........  ..........  ..........  ..........  ..........  ..........
'MPN6776 - C. pyxidata 1'         ..........  ..........  ..........  ..........  ..........  ..........
'RK862 - C. pyxidata 1'           ..........  ..........  ..........  ..........  ..........  ..........
'RK1065 - C. pyxidata 2'          ..........  ..........  ..........  ..........  ..........  ..........
'RK999 - C. pyxidata 1'           R.........  ..........  ..........  ..........  ..........  ....C.....
'MPN6576 - C. pyxidata 1'         G.........  ..........  ..........  ..........  ..........  ..........
'RK885 - C. pocillum '            G.........  .....G....  ..........  ..........  ..C.......  ..........
'RK1088 - C. pocillum'            G.........  .....G....  ..........  ..........  ..C.......  ..........
'MPN6171 - C. pocillum'           G.........  .....G....  ..........  ..........  ..C.......  ..........
'RK870 - C. pocillum'             G.........  .....G....  ..........  ..........  ..C.......  ..........
'RK946 - C. pocillum'             G.........  .....G....  ..........  ..........  ..C.......  ..........
'MPN5600 - C. fimbriata'          G.........  .....G....  ..........  ..........  ..C.......  ..........
'RK1091 - C. chlorophaea'         G.........  .....G....  ..........  ..........  ..C.......  ..........
'MPN6623 - C. chlorophaea'        G.........  .....G....  ..........  ..........  ..C..G....  ..........
'MPN6408 - C. grayi'              T.........  ..C.......  ..........  ..........  ..........  ..........
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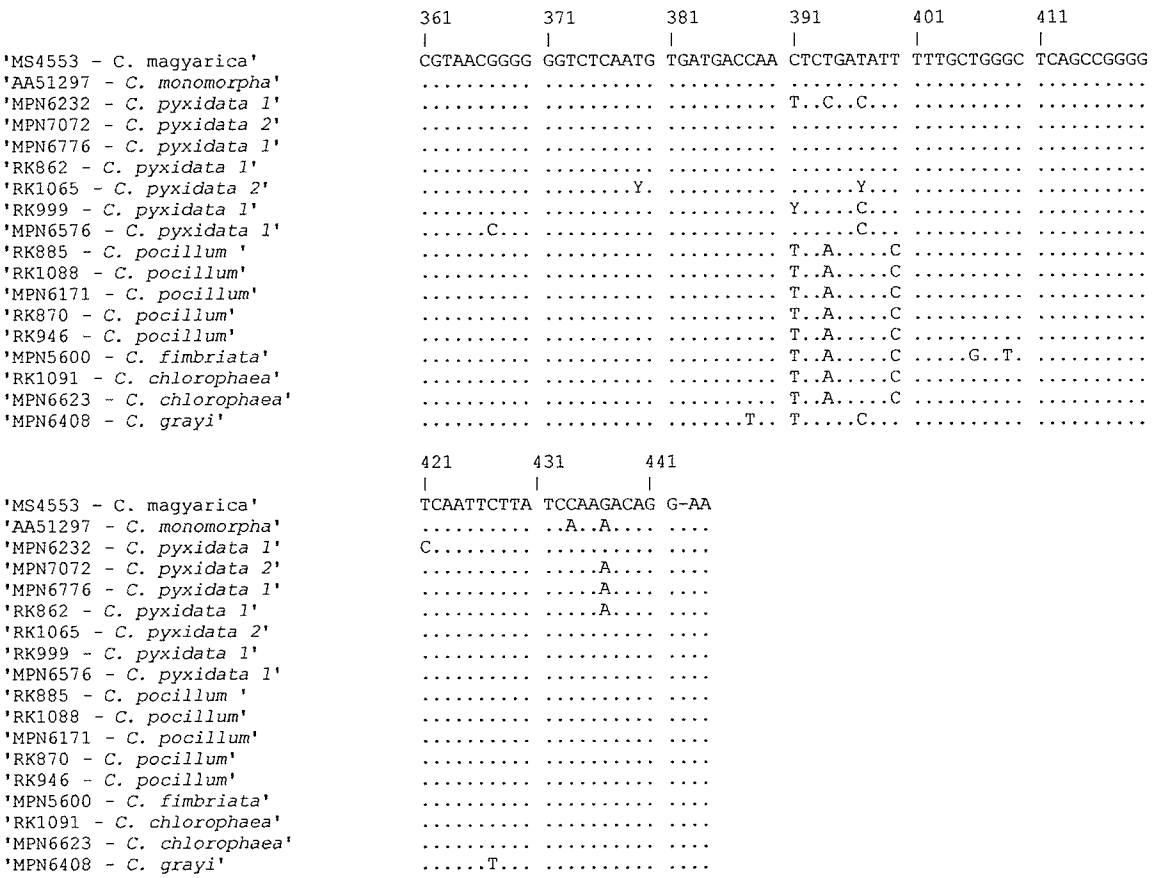
361 371 381 391 401 411
'MS4553 - C. magyarica' CGTAACGGGG GGTCTCAATG TGATGACCAA CTCTGATATT TTTGCTGGGC TCAGCCGGGG
'AA51297 - C. monomorpha' .....
'MPN6232 - C. pyxidata 1' .... T..C..C... ....
'MPN7072 - C. pyxidata 2' .....
'MPN6776 - C. pyxidata 1' .....
'RK862 - C. pyxidata 1' .....
'RK1065 - C. pyxidata 2' ....Y. ....Y... ....
'RK999 - C. pyxidata 1' .... Y.....C... ....
'MPN6576 - C. pyxidata 1' ......C... ....C... ....
'RK885 - C. pocillum ' .... T..A.....C ....
'RK1088 - C. pocillum' .... T..A.....C ....
'MPN6171 - C. pocillum' .... T..A.....C ....
'RK870 - C. pocillum' .... T..A.....C ....
'RK946 - C. pocillum' .... T..A.....C ....
'MPN5600 - C. fimbriata' .... T..A.....C .....G..T. .....
'RK1091 - C. chlorophaea' .... T..A.....C ....
'MPN6623 - C. chlorophaea' .... T..A.....C ....
'MPN6408 - C. grayi' ....T.. T.....C... ....
421 431 441
'MS4553 - C. magyarica' TCAATTCTTA TCCAAGACAG G-AA
'AA51297 - C. monomorpha' ....A..A.... ....
'MPN6232 - C. pyxidata 1' C..... ....
'MPN7072 - C. pyxidata 2' ....A.... ....
'MPN6776 - C. pyxidata 1' ....A.... ....
'RK862 - C. pyxidata 1' ....A.... ....
'RK1065 - C. pyxidata 2' ....
'RK999 - C. pyxidata 1' ....
'MPN6576 - C. pyxidata 1' ....
'RK885 - C. pocillum ' ....
'RK1088 - C. pocillum' ....
'MPN6171 - C. pocillum' ....
'RK870 - C. pocillum' ....
'RK946 - C. pocillum' ....
'MPN5600 - C. fimbriata' ....
'RK1091 - C. chlorophaea' ....
'MPN6623 - C. chlorophaea' ....
'MPN6408 - C. grayi' ......T... ....

Appendix VI

Aligned algal ITS rDNA sequences from this study. Dots indicate the occurrence of the same nucleotide as the reference sequence. Dashes represent gaps or missing data.

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                                  1          11         21         31         41         51
                                  |          |          |          |          |          |
'MPN6171 (from C. pocillum)'      ATTCCATCGT ATCCACACCG AGAACAACCC CATGTTGGCC TGGCTGCCAA AGATTCACCC
'RK1088 (from C. pocillum)'       .......... .......... .......... .......... ....C..... ..G.-.-...
'RK885 (from C. pocillum)'        .......... .......... .......... .......... ....C..... ..G.-.-...
'MPN6232 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..G..T....
'MPN6776 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..G.-.-...
'RK969 (from C. pyxidata 1)'      .......... .......... .......... ....AA.... ....C..... ..G.-.-...
'RK862 (from C. pyxidata 1)'      .......... .......... .......... .......... .......... ..G.-.-...
'MPN7072 (from C. pyxidata 2)'    .......... .......... .......... .......... .......... ..G.-.-...
'AA51297 (from C. monomorpha)'    .......... .......... .......... .......... .......... ..G.-.-...
'MS4553 (from C. magyarica)'      .......... .......... .......... .......... .......... ..G.......
'RK1064 (from C. chlorophaea)'    .......... .......... .......... .......... ....C..... ..G.......
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........

                                  61         71         81         91         101        111
                                  |          |          |          |          |          |
'MPN6171 (from C. pocillum)'      TTT-GGCGGT CACCTGCAGC CCGGCTGGCT TGCCAGCCCG ACTGTGGGGG CCGGGTTCAC
'RK1088 (from C. pocillum)'       .......... .......... .......... .......... .......... ..........
'RK885 (from C. pocillum)'        .......... .......... .......... .......... .......... ..........
'MPN6232 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..........
'MPN6776 (from C. pyxidata 1)'    ...T...... .........G .......T.. .......... .......... ..........
'RK969 (from C. pyxidata 1)'      .......... .......... .......... .......... .......... ..........
'RK862 (from C. pyxidata 1)'      .......... .........G .......... .......... .......... ..........
'MPN7072 (from C. pyxidata 2)'    .......... .........G .......T.. .......... .......... ..........
'AA51297 (from C. monomorpha)'    ...T...... .........G .......T.. .......... .......... ..........
'MS4553 (from C. magyarica)'      .......... .......... .......... .......... .......... ..........
'RK1064 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........

                                  121        131        141        151        161        171
                                  |          |          |          |          |          |
'MPN6171 (from C. pocillum)'      ACCTGGCCGG CTGTTTCTCC CACTCAAACC AATATATGAA GGCAATTGCT TGCTCACACG
'RK1088 (from C. pocillum)'       .......... .C........ ..T....... .....C.... .......... ..........
'RK885 (from C. pocillum)'        .......... .C........ ..T....... .....C.... .......... ..........
'MPN6232 (from C. pyxidata 1)'    .......... .......... ..T....... .....C.... .......... ..........
'MPN6776 (from C. pyxidata 1)'    .......... .......... ..T....... .....C.... .......... ..........
'RK969 (from C. pyxidata 1)'      .......... .C........ ..T....... .....C.... .......... ..........
'RK862 (from C. pyxidata 1)'      .......... .......... G.T....... .....C.... .......... ..........
'MPN7072 (from C. pyxidata 2)'    .......... .......... ..T....... .....C.... .......... ..........
'AA51297 (from C. monomorpha)'    .......... .......... ..T....... .....C.... .......... ..........
'MS4553 (from C. magyarica)'      .......... .......... ..T....... .....T.... .......... ..........
'RK1064 (from C. chlorophaea)'    .......... .......... ..T....... .....T.... .......... ..........
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........

                                  181        191        201        211        221        231
                                  |          |          |          |          |          |
'MPN6171 (from C. pocillum)'      AGCGGCGACT AACAAAGACA ACTCTCAACA ACGGATATCT TGGCTCCCGC AACGATGAAG
'RK1088 (from C. pocillum)'       .......... .......... .......... .......... .......... ..........
'RK885 (from C. pocillum)'        .......... .......... .......... .......... .......... ..........
'MPN6232 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..........
'MPN6776 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..........
'RK969 (from C. pyxidata 1)'      .......... .......... .......... .......... .......... ..........
'RK862 (from C. pyxidata 1)'      .......... .......... .......... .......... .......... ..........
'MPN7072 (from C. pyxidata 2)'    .......... .......... .......... .......... .......... ..........
'AA51297 (from C. monomorpha)'    .......... .......... .......... .......... .......... ..........
'MS4553 (from C. magyarica)'      ......T... .......... .......... .......... .......... ..........
'RK1064 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........
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                                  241         251         261         271         281         291
                                  |           |           |           |           |           |
'MPN6171 (from C. pocillum)'      AACGCAGCGA AATGCGATAC GTAGTGTGAA TTGCAGAATT CCGTGAACCA TCGAATCTTT
'RK1088 (from C. pocillum)'       .......... .......... .......... .......... .......... ..........
'RK885 (from C. pocillum)'        .......... .......... .......... .......... .......... ..........
'MPN6232 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..........
'MPN6776 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..........
'RK969 (from C. pyxidata 1)'      .......... .......... .......... .......... .......... ..........
'RK862 (from C. pyxidata 1)'      .......... .......... .......... .......... .......... ..........
'MPN7072 (from C. pyxidata 2)'    .......... .......... .......... .......... .......... ..........
'AA51297 (from C. monomorpha)'    .......... .......... .......... .......... .......... ..........
'MS4553 (from C. magyarica)'      .......... .......... .......... .......... .......... ..........
'RK1064 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........

                                  301         311         321         331         341         351
                                  |           |           |           |           |           |
'MPN6171 (from C. pocillum)'      GAACGCATAT TGCGCCCCAC GGCCTCGGCC CAGGGCATGT CTGCCTCAGC GTCTGTTTAC
'RK1088 (from C. pocillum)'       .......... .......... .......... .......... .......... ..........
'RK885 (from C. pocillum)'        .......... .......... .......... .......... .......... ..........
'MPN6232 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..........
'MPN6776 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... ..........
'RK969 (from C. pyxidata 1)'      .......... .......... .......... .......... .......... ..........
'RK862 (from C. pyxidata 1)'      .......... .......... .......... .......... .......... ..........
'MPN7072 (from C. pyxidata 2)'    .......... .......... .......... .......... .C........ ..........
'AA51297 (from C. monomorpha)'    .......... .......... .......... .......... .......... ..........
'MS4553 (from C. magyarica)'      .......... .......... .......... .......... .......... ..........
'RK1064 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........

                                  361         371         381         391         401         411
                                  |           |           |           |           |           |
'MPN6171 (from C. pocillum)'      CCCCTCTTCC CCCTTTCACA TATTG-TGTG AAATC-GGGA AGGTTGTGGT CTTGTGCTGC
'RK1088 (from C. pocillum)'       .......... .......... ..C.....-- .....G.... .......... ..........
'RK885 (from C. pocillum)'        .......... .......... ..C.....-- .....G.... .......... ..........
'MPN6232 (from C. pyxidata 1)'    .......C.. .......... ..C.....-- -...TG.... .......... ..........
'MPN6776 (from C. pyxidata 1)'    .......C.. .......... ...A....G. --........ .......... ..........
'RK969 (from C. pyxidata 1)'      .......... .......... ..C.....-- .....G.... .......... ..........
'RK862 (from C. pyxidata 1)'      .......C.. .......... ...A....G. --........ .......... ..........
'MPN7072 (from C. pyxidata 2)'    .......C.. .......... ...A....G. --........ .......... ..........
'AA51297 (from C. monomorpha)'    .......C.. .......... ...A....G. --........ .......... ..........
'MS4553 (from C. magyarica)'      .......C.. ....C..... ..CACA..-- .G........ .......... ..........
'RK1064 (from C. chlorophaea)'    .......C.. ....C..... ..CC....-- .G........ .......... ..........
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........

                                  421         431         441         451         461         471
                                  |           |           |           |           |           |
'MPN6171 (from C. pocillum)'      GGCACTTGGC CGAAATTCAG TGATACTGCA GGGACCGTTA ATCGGACTCC AGCTTGGTAG
'RK1088 (from C. pocillum)'       ...G...... .......... .......... ........C. .......... ..........
'RK885 (from C. pocillum)'        ...G...... .......... .......... ........C. .......... ..........
'MPN6232 (from C. pyxidata 1)'    .......... .......... .......... ...G.....G .......... ..........
'MPN6776 (from C. pyxidata 1)'    .......... T......... ...C...... .......... .......... ..........
'RK969 (from C. pyxidata 1)'      ...G...... .......... .......... ........C. .......... ..........
'RK862 (from C. pyxidata 1)'      .......... .......... ...C...... .......... .......... ..........
'MPN7072 (from C. pyxidata 2)'    .......... .......... ...C...... .......... .......... ..........
'AA51297 (from C. monomorpha)'    .......... T......... ...C...... .......... .......... ..........
'MS4553 (from C. magyarica)'      .......... .......... .......... .......... .......... ..........
'RK1064 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........

                                  481         491         501         511         521         531
                                  |           |           |           |           |           |
'MPN6171 (from C. pocillum)'      GCTTTTCCCT TGTGGAATTA TGCATGCCGC TGTTGGCCGT GGACCACTGC AGCTGTCAAG
'RK1088 (from C. pocillum)'       .......... C......... .......... .......... .......... T...A.....
'RK885 (from C. pocillum)'        .......... C......... .......... .......... .......... T...A.....
'MPN6232 (from C. pyxidata 1)'    .......... .......... .......... .......... .......... .........T
'MPN6776 (from C. pyxidata 1)'    .......... .......G.. .......... .......... .......... ..........
'RK969 (from C. pyxidata 1)'      .......... C......... .......... .........- ---------- ----------
'RK862 (from C. pyxidata 1)'      .......... .......G.. .......... .......... .......... ..........
'MPN7072 (from C. pyxidata 2)'    .......... .......G.. .......... .......... .......... ..........
'AA51297 (from C. monomorpha)'    .......... .......G.. .......... .......... .......... ..........
'MS4553 (from C. magyarica)'      .......... .......... .......... .......... .......... ..........
'RK1064 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........
'RK1091 (from C. chlorophaea)'    .......... .......... .......... .......... .......... ..........
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                                 541         551
                                 !           |
'MPN6171 (from C. pocillum)'     CAGGGAAACC TTTCAAATT
'RK1088 (from C. pocillum)'      .......... .........
'RK885 (from C. pocillum)'       .......... .........
'MPN6232 (from C. pyxidata 1)'   .......... .........
'MPN6776 (from C. pyxidata 1)'   .......... .........
'RK969 (from C. pyxidata 1)'     ---------- ---------
'RK862 (from C. pyxidata 1)'     .......... .........
'MPN7072 (from C. pyxidata 2)'   .......... .........
'AA51297 (from C. monomorpha)'   .......... ....G....
'MS4553 (from C. magyarica)'     .......... .........
'RK1064  (from C. chlorophaea)'  .......... .........
'RK1091 (from C. chlorophaea)'   .......... .........
```