

The Molecular Biology of Lichen Symbiosis and Development

by

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Dissertation submitted in partial fulfillment of the requirements for the degree of
Doctor of Philosophy in the Department of Biology
in the Graduate School of Duke University
2009

ABSTRACT
(Biology)

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Abstract

A lichen thallus is an emergent structure formed through the long-lived symbiotic growth of a filamentous fungus and uni-cellular green algae. How the symbionts find and recognize each other, resist potential attack, and proceed to form this unique body remains enigmatic. Based on other well-studied eukaryotic systems, the development of a lichen thallus must rely upon the successful identification and collaboration of these two very different organisms. Identifying the molecular basis of microbe recognition and interactions remains one of the greatest challenges in studying symbiotic systems.

In the development of stratified lichen thalli containing trebouxoid algae, the mycobiont undergoes a change in hyphal morphology when making physical contact with its photobiont. This change is characterized by a switch from apical growth to increased lateral branching, and has been proposed to be a result of thigmotropism (response to shape). This morphological change can be seen when the mycobiont *Cladonia grayi* contacts its symbiotic partner, the trebouxoid photobiont *Asterochloris* sp. Therefore, observations were made using environmental scanning electron microscopy, glass beads, alternative phototrophs, and the development of the lichen symbiosis between *C. grayi* and *Asterochloris* sp. The results of these observations confirmed that the change in growth pattern of *C. grayi* in lichen symbiosis is not a result of thigmotropism or fungal contact with phototrophs, but instead is diagnostic of lichen symbiosis.

This phenotypically defined stage was next used to begin studying the genetic and molecular mechanisms underlying lichen-symbiosis development. Using suppression subtractive hybridization to determine differential gene expression, fungal and algal libraries were made of putatively upregulated genes in the pre-contact and initially post-contact stages of lichen symbiosis. The symbiotic expression levels of select genes were then verified using quantitative PCR. Fungal protein products of genes upregulated in lichen symbiosis show significant matches to proteins putatively involved in fungal self and non-self recognition, lipid metabolism, negative regulation of glucose repressible genes, an oxidoreductase, a dioxygenase, and a conserved hypothetical protein. Algal genes that are upregulated in lichen symbiosis include a chitinase-like protein, an amino acid metabolism protein, a dynamin related protein, and a protein arginine methyltransferase. Furthermore, early stages in lichen development are marked not by a drastic change in transcriptional products, but instead by an overall change in genes that are already expressed.

Finally, one of the candidate fungal genes upregulated in the initial post-contact stage of lichen development, *Lip3*, was cloned from *Cladonia grayi* cDNA, and heterologously expressed in yeast. The protein product of this gene was shown to be a functional secreted extracellular lipase.

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1

Introduction

1.1 Background

Symbiosis, the living together of two unlike organisms (de Bary, 1879), has been proposed to be an important driving force in evolutionary biology (Margulis & Fester, 1992). Because symbiosis can occur between any two different kinds of organisms, and because this can vary temporally, physically, and spacially, investigations into the diversity of symbiotic interactions remain in their infancy. Much remains to be determined about how symbiosis works.

Symbiosis is a successful life strategy for members of the kingdom fungi, with over 30% living symbiotically with plants, bacteria, animals, or other fungi (Kirk *et al.*, 2008). These relationships take place along the symbiotic continuum of parasitism, commensalism, and mutualism. Some of the more notable among these include the

plant pathogen potato blight (*Phytophthora infestans*), the human gut commensalist *Candida albicans*, and mutualists that form mycorrhizal relationships with plant roots such as *Laccaria bicolor* and *Boletus edulis*.

In many cases, fungal symbiosis is accompanied by a shift in morphological growth, specific to the symbiosis. For example, when fungi form ectomycorrhiza with plant roots, their non-specific mycelial growth form changes to highly targeted growth throughout the cortex of plant short roots forming a complex network of fungal hyphae known as the mantle (Martin *et al.*, 2001). The yeast commensalist *Candida albicans*, switches to filamentous growth when assuming a pathogen's role in oral and genital infections (Sánchez-Martínez & Pérez-Martín, 2001).

One of the more successful fungal symbiotic associations is found in lichens, based on fungal species numbers alone, and is formed between a filamentous fungus (the mycobiont) and either a green algae, a cyanobacterium, or both (the photobiont). Based on the transformative properties of the symbiosis to the fungal phenotype, over 13,500 species of lichen-forming fungi have been described including nearly one fifth of all known fungal species (Kirk *et al.*, 2008). Although the emergent properties of symbiotic growth (known as the thallus, or body) were first recognized as the symbiotic growth of different organisms 140 years ago (Schwendener, 1869), the molecular mechanisms of lichenization remain enigmatic. The slow growth and

genetic intractability of lichen symbionts have kept researchers from answering the most basic cellular and molecular based questions of how mycobiont and photobiont partners find and recognize each other, and how they maintain this relationship.

Lichens are often described as a mutualistic association between mycobionts and photobionts, although whether they are the result of a mutualistic or parasitic relationship has yet to be determined. Arguments that mycobionts and photobionts share a parasitic relationship began with Schwendenner, who first described lichen symbiosis in 1869 as mycobionts parasitizing photobionts. Since then, additional researchers have argued that the lichen symbiosis is a form of controlled parasitism, whereby the mycobiont keeps the photobiont alive in order to take nutrients from the alga without giving in return (Ahmadjian & Jacobs, 1981; Kershaw & Millbank, 1970; Peirce, 1900). Evidence in support of this includes: the nature of fungi as obligate heterotrophs and algae as photoautotrophs, the production of autonomously propagating algal flagellated zoospores, as well as the occasional photobiont penetrated by fungal haustoria (Ahmadjian & Jacobs, 1981; Slocum *et al.*, 1980). Additionally, algal cell division changes drastically as the thallus matures (Hill, 1989, 1992). Unlike regions of newly developing thalli, fully differentiated thalline regions house photobiont cells that appear to be cell cycle arrested and twice the size of free-living cells. In general, the description of mycobionts as controlled parasites

of photobionts fits into the larger pathogen subcategory of biotroph. Biotrophic pathogens derive nutrients from living host cells, causing limited damage to the host (Lewis, 1973).

Arguments that mycobionts and photobionts share a mutualistic relationship include: the rare occurrence of thallus-free photobionts in the environment, an increase in the number of available photobiont habitats that are otherwise inhospitable to free-living green algae, and the perennial association between mycobionts and photobionts (Ahmadjian.V, 1982). Whether mycobionts are green algal pathogens, or if the green alga receives benefits from the mycobiont in a mutualistic association, remains to be determined.

Our concept of lichen symbiosis is shaped by a short list of molecules proposed to be involved in mycobiont and photobiont interactions. These molecules include the following: lectins, fungal and plant hormones, fungal secondary compounds, and algal secreted sugars (Ahmadjian, 1992; Bubrick *et al.*, 1985; Honegger, 1986b; Remmer *et al.*, 1986). Additionally, two factors positively correlated with lichen symbiosis are DNA methylation and hydrophobins. The level of mycobiont DNA methylation increases in the symbiotic state, resulting in a change in gene expression (Armaleo & Miao, 1999). A highly hydrophobic fungal-secreted cell wall made of hydrophobin proteins, holds mycobionts and photobionts proximally and mediates

symbiotic interactions (Scherrer *et al.*, 2000, Trembley *et al.*, 2002a,b, Wösten & de Vocht, 2000). The direct function of these factors in lichen symbiosis remain to be tested. The discovery of genes related to lichen symbiosis, and the proteins they encode, remains an open field for investigation.

1.2 Overview of this work

1.2.1 *Phenotypic marker of the Cladonia grayi and Asterochloris sp. symbiosis*

To begin I searched for the best lichen-symbiosis system to use as a model of resynthesis and genetic model system. Genetic model systems are chosen for their ease of use, genetic tractability, and use in answering interesting questions. In general these systems involve short-lived organisms that breed, are fecund and have minimal requirements for physical space and complex nutrition. Regarding lichen symbionts, neither fungal nor algal meiosis has been observed in culture, although many of them readily reproduce vegetatively in the lab, and require minimal amounts of complex media and space. In order to identify a working model lichen genetic system, I chose criteria that could fit within the following parameters. The lichen material had to be locally available, and easy to find. The lichen symbionts had to be easily separated from the symbiotic union in to individual cultures, and easily reproduce vegetatively. Finally, the system had to be amenable to *in vitro* resynthesis, meaning it had to

grow symbiotically under simple laboratory conditions, and produce a recognizable symbiotic specific phenotype within a short period of time. After trying a handful of lichen symbiotic partners that I collected from the greater North Carolina region, I ultimately chose the mycobiont *Cladonia grayi* and its photobiont partner *Asterochloris*. These symbionts were cultures that I had received from Daniele Armaleo, with whom I had been working very closely. Unlike other lichen systems I brought into the lab, *C. grayi* and *Asterochloris* fulfilled all of the lichen model-organism requirements outlined above. In characterizing the initial stages of resynthesis using environmental scanning electron microscopy, I observed a morphological switch in *C. grayi* that had been reported for other stratified lichens containing trebouxoid algae such as *Asterochloris*. Namely, that the fungus undergoes a morphological switch upon contact with its photobiont. To determine if this phenotypic change was specific to lichen symbiosis, and not just a response to shape or photosynthetic organism, I observed the growth of *C. grayi* with glass beads and non-lichen-forming phototrophs. I determined that this shift in fungal morphological growth is specific to early lichen development (Chapter 2). This work has been published in Joneson & Lutzoni (2009).

1.2.2 *Differential gene regulation in early stages of lichen development*

Symbiosis is accompanied by differential gene expression in members of the kingdom Fungi and Chloroplastida (including green algae and land-plants, *fide* Adl *et al.* [2005]). Evidence for differential photobiont and mycobiont gene regulation and expression in lichen symbiosis was reported in Armaleo & Miao (1999) and Trembley *et al.* (2002c). As nothing was known about these genes, I used suppression subtractive hybridization to generate over 2,000 clones of algal and fungal genes potentially upregulated in early lichen development. This timeframe includes the time when the symbionts are close enough to sense one another yet are not physically touching, and when the symbionts have made initial contact.

The mycobiont, Cladonia grayi.

Fungi enter into symbiosis with members of the kingdom Chloroplastida in a number of different ways leading to various symbiotic and phenotypic outcomes. Some pathogenic fungi cause little damage to their host, deriving nutrition from living plant cells through the use of a specialized feeding structure known as a haustorium (Oliver & Ipcho, 2004). Other pathogenic fungi kill the cells of their plant hosts, living off the intracellular contents. In general, these fungi secrete large amounts

of plant cell wall degrading enzymes, and lack any specialized feeding structures (Oliver & Ipcho, 2004). The mutualistic ectomycorrhizal fungi invade the cortical tissue of plant roots, as previously mentioned, developing a complex structure known as the hartig net made of branched hyphae that surround plant cells. Development of the hartig net is concomitant with the coordinated growth of fungal and plant mechanisms for exchanging carbon and minerals (Martin *et al.*, 2001). Endomycorrhizal fungi invade plant root cortical tissue and grow in to cortical cells through a series of dichotomously branched hyphae known as an arbuscule, that penetrates the plant cell walls and invaginate the plasma membrane (Harrison, 2005). It is unknown what signal informs the fungus to grow arbuscules, but upon their development the plant host cell begins to express a mycorrhiza-specific transporter and enzymes.

Based on chapter 2, it is known what *Cladonia grayi* undergoes a morphological change in response to its lichen photobiont. However, it is unknown if any changes in gene transcription or protein production are associated with this symbiotic development. There are two previous global gene expression studies of lichen development. Using 2D-gel analysis, the first study recovered only one gene product, fasciclin, implicated in lichen symbiosis (Paulsrud & Lindblad, 2002). The second study used RNA-fingerprinting to establish that differential gene expression occurs between symbiosis and aposymbiosis, yet this method was unable to isolate specific

genes (Trembley *et al.*, 2002b). In addition, expressed sequence tags (ESTs) and/or cDNA libraries are lacking for any closely related fungi.

Here I present *Cladonia grayi* genes discovered and verified to be upregulated in stage 1 (pre-contact, when the mycobiont and photobiont are not physically touching, yet are close enough to chemically sense one another), and stage 2 (initial contact, after the symbionts have been grown together for at least 21 days) of lichen development. These genes show homology to genes putatively encoding proteins involved in fungal self and non-self recognition, lipid metabolism, negative regulation of glucose repressible genes, a D-arabitol reductase, and two dioxygenases (Chapter 3). Provided herein also is the first evidence that extracellular communication without physical contact can occur between symbionts, and that *C. grayi* gene expression could be influenced by the close proximity (while physically separate) of *Asterochloris*.

The photobiont, Asterochloris sp.

To date, our understanding of symbiosis in Chloroplastida is predominantly from the lineage containing land-plants. In land-plant symbioses with fungi and bacteria, there is a trend that similar sets of plant genes are expressed when presented with either pathogens, commensalists, or mutualists, and that different responses to

symbionts are realized by changes in magnitude of gene expression and their timing (Adomas *et al.*, 2008; Baron & Zambryski, 1995; Mathesius, 2003). This could mean that in examining the differential expression of green algae in lichen symbiosis, one might expect to see the expression of the green algal complement of genes that are expressed in land-plants when presented with microbes. Of the conserved jasmonic acid, salicylic acid, and ethylene signalling pathways that are the major plant hormones induced in plants in recognition of pathogenesis-related-proteins (including those from fungi, bacteria, viruses, and insects)(Bari & Jones, 2009; van Loon *et al.*, 2006), jasmonic acid and ethylene are absent from the available published genomes of the green algae *Chlamydomonas reinhardtii*, *Ostreococcus lucimarinus*, and *O. tauri* (Derelle *et al.*, 2006; Merchant *et al.*, 2007; Palenik *et al.*, 2007). It isn't clear if these algae retain the ability to synthesize salicylic acid or related compounds, or use them in signalling. It therefore is possible to conclude that lichen-forming algae respond to fungal symbionts in a different way from their land-plant counterparts. However, although their genomes haven't been sequenced, lichen-forming green algae and an additional green alga *Ulva intestiformis*, have been shown to produce ethylene in culture (Plettner *et al.*, 2005; Schieleit & Ott, 1996), although jasmonic acid, the synergistic partner of ethylene in land-plant resistance, is so far unknown from lichen-forming algae. It therefore remains possible that lichen-forming green algae

use signalling pathways and mechanisms similar to land-plants, and respond in a way that does not rely upon jasmonic acid. Which cellular machinery and signalling pathways are used by green algae in response to fungal symbiotic partners remains to be determined. Here I present the results of *Asterochloris* sp. genes upregulated in the first two stages of lichen development (Chapter 4). Genes upregulated prior to physical contact between *Asterochloris* and *C. grayi* include a putative chitinase-related gene and an amino acid transporter. Algal genes upregulated during initial contact between the alga and fungus include a protein arginine methyltransferase, and a dynamin related protein.

1.2.3 *Heterologous expression of a lichen-forming fungal gene, in yeast*

Lastly, I wanted to show that the genes I had discovered and verified in stages 1 and 2 of lichen development potentially produced functional gene products. As there is no system for transformation in the present lichen system, I chose to heterologously express a candidate gene of lichen symbiosis in yeast. I used the coding region of the putative extracellular triacylglyceride lipase *Lip3* from *Cladonia grayi* to transform *Saccharomyces cerevisiae*, and express it on olive-oil assay plates under the control of the constitutive GPD promoter (Chapter 5). The *C. grayi* *Lip3* gene encodes a functional and secreted triacylglyceride lipase, whose target in the natural system remains to be determined.

1.3 Future Research

This research is an initial investigation into genes that are involved in lichen symbiont interactions, through the investigation of fungal and algal genes upregulated in early lichen development. Much work remains to be done in the present system to illustrate the extent of genetic mechanisms involved in these interactions, and far more work is needed to elucidate signalling mechanisms in lichens in general. However, these results contribute to the study of lichenology in outlining a model lichen system for future resynthesis assays, and candidate genes of lichen symbiosis for future biochemical, physiological, and phylogenetic testing.

Compatibility and thigmotropism in the lichen symbiosis: A reappraisal.

2.1 Introduction

A differentiated lichen thallus is formed through complex interactions between mycobiont and photobiont cells growing together to form a body unlike either of the two symbionts grown alone (Ahmadjian, 1966; Armaleo, 1991; Galun, 1988; Honegger, 1993). This paper focuses on the early development of stratified thalli that contain trebouxoid photobionts. The sequence of events leading to a stratified thallus can be summarized in four basic stages: stage one, pre-contact, which is prior to physical contact of symbionts but close enough that extracellular interactions are possible; stage two, early-contact, where fungal cells contact algal cells by appressoria and/or subsequently envelop them through an increase in short lateral branches;

stage three, incorporation of growing fungal and algal units into a transitional pre-thallus; and stage four, differentiation of layered tissues (Ahmadjian *et al.*, 1978; Bubrick *et al.*, 1985; Galun, 1988). The molecular mechanisms behind this sequence of events are unknown.

The initial stages of *in situ* and *in vitro* natural lichen development have been the focus of many studies investigating the compatibility and selectivity of symbionts towards their partners (Ahmadjian *et al.*, 1980; Ahmadjian & Jacobs, 1981; Bubrick *et al.*, 1985; Galun & Garty, 1988; Kon *et al.*, 1993; Ott, 1987; Schaper & Ott, 2003). As compatibility and selectivity have been used differently by various authors (Galun, 1988), it is here defined in the context of the present study. Starting with stage one of development, if fungi and phototrophs do not interact to form any of the initial stages of lichen development they are considered non-compatible (which includes neutral and negative interactions). Mycobionts and photobionts are considered compatible when they enter into stage two of lichen development as shown by the envelopment of the alga through increased lateral branching. In some interactions, compatible mycobionts and photobionts form only the early stages of lichen development (stages one and two, sometimes three) but do not proceed to form a stratified thallus. Selection is the process through which appropriate symbionts are distinguished and enter into stage four of development, resulting in the

formation of a stratified thallus. Only when symbionts have entered into stage four of development is selection completely successful. (Specificity, a term describing the relationship between mycobionts and photobionts, is excluded from the discussion as it describes the evolutionary ecology of the selectivity of the symbionts in stage four of development, and the degree to which they are specialists or generalists).

Schwendener first recognized the dual nature of lichens as the symbiosis between mycobionts and photobionts in 1869. Investigations into the compatibility of the symbionts began shortly thereafter. From the detailed drawings of Bornet (1873) and Bonnier (1889b) it is clear that the scientists of the late 19th century were familiar with the hyphal branching of mycobionts and the envelopment of photobiont cells. Bonnier (1889b) was the first to microscopically inspect the growth of mycobionts with inanimate objects and heterologous phototrophs (i.e. all phototrophs other than the known photobiont(s) of a given mycobiont species). Bonnier grew mycobionts with sand, glass wool, and thinly cut straw, and concluded that because the fungi grew over these inanimate items in a manner similar to their photobionts, the mycobiont does not exhibit a distinct growth response to compatible photobionts. Further confirmation came after growing mycobionts with heterologous algae: the golden alga *Vaucheria sessilis*, and two species of the filamentous green alga *Trentepohlia*. However, according to his drawings it appears that the mycobionts failed to

envelop animate and inanimate objects in the same manner as their natural photobionts.

Nearly 100 years later, similar experiments reinvestigated the thigmotropic response using either *Cladonia cristatella* or *Xanthoria parietina* and algal sized glass beads (Ahmadjian & Jacobs, 1981; Bubrick *et al.*, 1985). Members of both *Cladonia* and *Xanthoria* form lichen thalli with trebouxoid algae, and in the presence of compatible photobionts envelop them with the stage 2 specific response (Galun, 1988). In further experiments using *C. cristatella* (Ahmadjian & Jacobs, 1981) reported that the mycobiont grew over glass beads in a manner similar to its compatible photobiont. However, in experiments with *X. parietina*, Bubrick *et al.* (1985) observed that the mycobiont did not interact with the glass beads when simultaneously grown with compatible photobionts. In neither case were illustrations provided. These results have been cited widely in the literature to suggest that a mycobiont does not change its growth habit in response to compatible trebouxoid photobionts; instead that mycobionts respond to thigmotropic stimuli (Galun, 1988; Honegger, 1993; Lawrey, 1984; Trembley *et al.*, 2002c).

As part of an on-going study of the molecular mechanisms underlying the development of mycobionts and photobionts into a stratified thallus, the thigmotropic growth response of mycobionts towards photobionts and inanimate objects was re-

visited. The growth of the lichen-forming fungus *Cladonia grayi* in compatible and non-compatible pairings. The growth of *C. grayi* with its known photobiont *Asterochloris* sp. (a trebouxoid alga) was observed and compared to the growth of this fungus with green algae never found associated with *C. grayi* in nature (*Chlorella vulgaris* and *Trentepohlia* sp.), with moss protonema (*Funaria hygrometrica*), and with glass beads.

2.2 Methods

2.2.1 Cultures and media

All cultures were maintained and used at room temperature, ca. 23-25°. Grinding, pipetting and plating took place in a Nuair Class II, Type A/B3, Biological Safety Cabinet.

Axenic cultures of *Cladonia grayi* and *Asterochloris* sp. were provided by D. Armaleo (Department of Biology, Duke University). These cultures were originally created from the soredia of material deposited at DUKE (Accession No. 0038794). The *C. grayi* culture was maintained in 50-200 mL liquid cultures of Malt Yeast (MY, 20 g malt extract and 2 g yeast extract per liter), in Erlenmeyer flasks on a rotating shaker (100 rev/min), and regularly ground in a commercial Waring blender (every 6-8 weeks).

Asterochloris sp. was maintained on plates of one-quarter strength *Trebouxia* Medium (Ahmadjian, 1967). Cultures of *Chlorella vulgaris* (UTEX 30) and *Trentepohlia* sp. (UTEX 1227), two green algae never found with *Cladonia grayi* in stage four of lichen development, were maintained on plates of MY. All algae were grown under ca. 40 $\mu\text{mol m}^{-2}\text{s}^{-1}$ illuminations for 12 hours a day and subsequently removed for experimental set up by pipetting sterile water onto the plates and pipetting up and down until algal cells were visibly resuspended in the solution.

Spores of *Funaria hygrometrica* were provided by J. Shaw (Department of Biology, Duke University). Spores were washed in bleach for 30 s, next in distilled water for 30 s, and then allowed to germinate on cellophane discs over a reduced nutrient medium made from a 99:1 mix of Bolds Basal Medium (Deason & Bold, 1960), MY medium, and solidified with 2% agar (hereafter referred to as 99:1 medium). Spores were germinated and protonema grown for 20 days under the same growth conditions as the green algae, before placing freshly ground *Cladonia grayi* onto the growing filaments. Although mosses never form lichen thalli with mycobionts, the protonemal stage was chosen based on previous observations that lichen-forming fungi can grow symbiotically with protonemal filaments (Bonnier, 1888, 1889a).

2.2.2 Glass Bead Preparation

Glass beads 3-10 μ m and 10-30 μ m in diameter (PolySciences, Inc., Warrington PA) were washed with acid and thoroughly washed in water before mixing them individually or together with the mycobiont. In brief, enough 5.8 M HCl was used to cover the beads in an Eppendorf tube, and incubated at room temperature for 1 hour. The solution was spun down at maximum speed in a tabletop centrifuge for 2 min. The acid was poured off, and the beads were flooded with autoclaved Ultrapure water (Hydro Picotech[®] 2, Durham NC, USA) before being vortexed and spun down at maximum speed for 2 min, removing the supernate by pipetting. Beads were washed in this manner until the pH of the supernate was increased to 6.3. The beads were subsequently dried in a 37° C incubator overnight, and stored at room temperature.

Each experiment was conducted one to several times from 2004-2007. *Cladonia grayi* cultures were ground one to two days prior to the beginning of each set of experiments and maintained as above, until the experiments began. Experimental plates of *C. grayi*, *C. grayi* with green algae, and *C. grayi* with glass beads were set up by first pipetting a small amount of freshly ground *C. grayi* such that the mycelia could spread out and individual clumps were visible by eye. *Cladonia grayi* was pipetted on to autoclaved hand-cut circular cellophane discs approximately 47

mm in diameter over 2% agarized 99:1 medium, and allowed to dry until the fungus and cellophane disc were secured upon the agar surface (0-2 hours). Next, 100-200 μ L of water mixed with either green algae or glass beads were pipetted on to the fungal material and allowed to dry until all supplemental liquid had evaporated. A minimum of algal cells and glass beads were placed on the plates to allow for even spacing of the cultures or glass beads. Experimental plates of *C. grayi* with moss protonema started with plates of germinated *Funaria hygrometrica* spores (above) onto which the liquid culture of *C. grayi* was pipetted. Plates were parafilm and cultures allowed to grow under the same environmental conditions as the original algal and moss cultures.

2.2.3 *Environmental Scanning Electron Microscopy (ESEM)*

For each experimental setup, small sections of the cultures on cellophane were sampled under sterile conditions for up to seven weeks. Pieces of cellophane were attached to SEM stubs and visualized using a Philips XL30 ESEM microscope (FEI Company). Resulting micrographs were adjusted for improved contrast when needed. Days after initiation of cultured symbiosis were noted on micrographs. This number represents the maximum length of time for possible interaction between symbionts. Micrographs may actually represent younger symbiotic interactions, as these interactions are initiated as the mycobiont grows to established green algae, moss

protonema, or glass beads.

2.3 Results

Cladonia grayi grew in all conditions when grown on cellophane over the reduced nutrient medium 99:1 (alone, with green algae, with moss protonema, or with glass beads), as indicated by an increase in colony sizes after three weeks (data not shown). Growth of *C. grayi* alone is marked by strong apical growth and reduced lateral branching at the edge of a colony (Figure 2.1). This growth pattern did not change over the course of 43 days. Aerial hyphae regularly grew from marginal hyphae, as well as from mycelial clumps.

When grown with *Asterochloris* sp., *Cladonia grayi* grew around algal cells individually (Figure 2.2a), and around algal cells in clumps (Figure 2.2b). Around individual cells of *Asterochloris* sp. the fungus exhibited increased lateral branching (as shown by highly branched hyphae surrounding round algal cells), when compared to growth alone (Figure 2.2a), and hyphae not in contact with algae in Figure 2.2b). This pattern was regularly observed in the mixed cultures, although *C. grayi* did not grow around every algal cell (lower right corner of Figure 2.2a). The envelopment of algal cells through increased lateral hyphal branching was visible by ESEM as early as 21 days after resynthesis, although this response probably occurs earlier. Within 10

days of resynthesis, symbiotic interactions were visible under a dissecting microscope at 16X as aerial hyphae of *C. grayi*, can be observed growing out of *Asterochloris* colonies (data not shown). Cells of *Asterochloris* sp. engulfed by *C. grayi* cannot be dislodged by a gentle stream of water, or mechanical force (squashing material between a cover slip and glass slide).

When grown with glass beads, *Cladonia grayi* never developed the second stage growth response found with compatible algae (Figures 2.3a and 2.3b). *Cladonia grayi* grew over all sizes of glass beads in a non-compatible manner, including those of comparable size to *Asterochloris* sp. cells (15-20 μm in diameter). Glass beads at the edge of *C. grayi* colonies are in general not overgrown with hyphae (Figure 2.3b). Many glass beads remained attached to *C. grayi* hyphae after gentle washing in water, and attempts to dislodge with mechanical force. Aerial hyphae were formed regularly on older clumps of mycelia, as well as from hyphae growing from amongst glass beads.

Cladonia grayi grew amongst proliferating cells of *Chlorella vulgaris*, but was never observed to grow around individual cells, and virtually never formed aerial hyphae within colonies of this green alga (Figures 2.3c and 2.3d). *Chlorella vulgaris* grew quickly on the reduced nutrient medium 99:1, regardless of whether *C. grayi* was present or not. Colonies of *C. vulgaris* rapidly increased and covered both kinds

of plates over a four week period. Aerial hyphae were visible on clumps of *C. grayi* mycelia growing alone.

Cladonia grayi grew over individual filaments and throughout clumps of *Trentepohlia* sp. (Figures 2.3e and 2.3f). *C. grayi* also formed aerial hyphae readily on mycelia mats, as well as on clumps of *Trentepohlia* sp. Although *C. grayi* grew throughout clumps of *Trentepohlia* sp. forming aerial hyphae, the mycobiont never exhibited the symbiotic phenotypic response of the second stage of lichen resynthesis as found with *Asterochloris* sp.

When grown with the protonema of *Funaria hygrometrica*, *Cladonia grayi* grew over the moss filaments, but never entered into stage 2 of thallus development (Figures 2.3g and 2.3h). *Cladonia grayi* never grew on lateral aerial protonemal filaments, instead only over horizontal filaments (Figure 2.3h). Aerial hyphae were present on individual hyphae growing away from mycelial clumps, on mycelial clumps, and within moss protonemal mats.

2.4 Discussion and Conclusion

The results of this study show that *Cladonia grayi* has a distinctive phenotypic response to its compatible photobiont, *Asterochloris* sp., during stage two of lichen development. Consistent with previous studies, the mycobiont *C. grayi* can grow

over green algae, moss protonema, and glass beads. However, *C. grayi* expresses the stage two distinctive envelopment, through increased lateral hyphal branching, exclusively towards its compatible photobiont. This symbiotically induced change in branching pattern of *C. grayi* in response to *Asterochloris* sp. is most apparent when comparing the fungus grown with glass beads and heterologous phototrophs with that of the fungus on its own.

The possibility that *Cladonia grayi* is interacting or communicating in some way with the heterologous phototrophic organisms presented, or even with the glass beads has not been ruled out. The photosynthetic organisms that *C. grayi* was grown with did not appear on a macroscopic level to be inhibited by the growth of the fungus. Colonies of algae and protonema growing amongst the fungi remained green and proliferated in a manner similar to the photosynthesizers grown on their own. No obviously dead colonies of photosynthesizers were found amongst fungi on the resynthesis plates. Therefore it is assumed that *C. grayi* cannot kill these photosynthesizers under the given conditions (i.e., on cellophane discs over a reduced nutrient medium). If, however, *C. grayi* is recognizing or communicating with any of the above photosynthesizers other than *Asterochloris* sp., it does so without forming the diagnostic morphological response of the second stage of thallus development.

Previous authors have noted that in in vitro resynthesis, mycobiont growth is

not directed toward compatible photobionts, instead it is a random growth pattern that brings the symbionts together (Ahmadjian, 1959; Clayden, 1998). This is consistent with the nutrition search strategy found in non-lichen-forming, filamentous ascomycetes (Gaßmann & Ott, 2000), in which these fungi grow over substrates and away from regions in which they have exhausted the resources. This hypothesis is consistent with the results showing that *Cladonia grayi* will grow over, and adhere to, glass beads in its vicinity. This hypothesis can also explain why more glass beads are grown over by *C. grayi* at the center of mycelial mats, than at the edge of fungal colonies. As the density of *C. grayi* hyphae increase, there is a higher chance that a glass bead will be contacted by a roaming hypha. It is interesting that *C. grayi* never adheres to *Chlorella vulgaris*, *Trentepohlia* sp., or *Funaria hygrometrica*, suggesting that a mycobiont will not adhere to everything with which it comes into contact. Perhaps the heterologous phototrophs resist attachment through a defense mechanism. Whether or not a roaming mycobiont will adhere to substrates it encounters cannot to date be predicted and remains to be investigated.

The foraging mycobiont hypothesis differs from the initial developmental stage of arbuscular mycorrhizae (AM). This stage is marked by an increase in hyphal growth and branching, which is thought to bring the fungus closer to plant roots (Paszkowski, 2006). This form of directional growth is induced specifically by host

plant root exudates. The present experimental design did not allow for investigating *Cladonia grayi* response to algal exudates across a concentration gradient. However, attempts in finding directed growth of *C. grayi* towards *Asterochloris* sp. have so far been unsuccessful (the growth of either symbiont grown on strips of filter paper placed approximately 2 mm apart over 99:1 medium plates failed to show directional growth, Joneson unpublished). These results suggest that mycobionts do not grow in a directed manner towards photobionts, although a more sensitive assay with synchronized cultures may still uncover this phenomenon.

The present results are, however, similar to the second stage of AM development. The arbuscular mycorrhiza *Glomus mosseae* forms a phenotypic response exclusively in response to compatible hosts (Giovannetti *et al.*, 1993). The second stage of development begins when the fungus physically contacts a compatible host root and forms an appressorium (Paszkowski, 2006). This response does not occur when *G. mosseae* is grown with simulated roots, or with simulated roots in the presence of host roots (Giovannetti *et al.*, 1993). However, thigmotropic responses have been found to play a role in the initial stages of contact between the ectomycorrhiza *Boletus variegatus* with conifer roots (Read & Armstrong, 1972), and in the biotrophic fungi *Colletotrichum gloeosporioides*, *Magnaporthe grisea*, and *Uromyces appendiculatus* (Jelitto *et al.*, 1994; Kim *et al.*, 1998; Lee & Dean, 1994; Wynn, 1976). Although

fungi in general can show a contour guidance response to objects they encounter in their environs (Perera *et al.*, 1997), thigmotropism does not play an obvious role in the early contact stage of lichen thalli containing trebouxoid algae.

Although *Cladonia grayi* responds to cells of *Asterochloris* sp. in a stage two specific manner, the mycobiont does not respond in the same manner towards every photobiont cell (Figure 2.2a), suggesting that the early stages of thallus development are more complicated than just compatible symbionts finding one another. The inability of every cell within a compatible photobiont species to be enveloped by a compatible mycobiont has also been reported from a similar stratified lichen thallus system formed by the mycobiont *Usnea strigosa* and the photobiont *Trebouxia usneae* (Ahmadjian & Jacobs, 1981). Newly divided algal cells appear to be the most suitable for the development of the primary stage of thallus between mycobionts and photobionts (Honegger, 1986a). In the present experimental design, it was not possible to determine the age of the algae entering stage two of thallus development. Furthermore, it is still not clear at which point *C. grayi* recognizes compatible or selective photobionts. Mycobionts that form stratified lichen thalli in situ have been shown to proceed through stage three of development in in vitro resynthesis with compatible heterologous photobionts (Ahmadjian *et al.*, 1980; Ahmadjian & Jacobs, 1981; Bubrick *et al.*, 1985; Kon *et al.*, 1993; Schaper & Ott, 2003), suggesting that

there are shared molecular mechanisms in the early stages of lichen development between selective and non-selective (yet compatible) symbionts. The recognition of lichen symbionts towards one another has been suggested to be a multi-step process (Bubrick *et al.*, 1985), and warrants further investigation.

The mycobiont *Cladonia grayi* displays a compatible symbiotic phenotype in response to *Asterochloris* sp. in the early contact stage between the two symbionts. This is in contrast to the mycobionts non-compatible response to glass beads and heterologous green symbionts. Using this earliest stage exclusive to lichen symbiosis, it is possible to begin studying the genetic and molecular mechanisms underlying the development of a stratified lichen thallus.

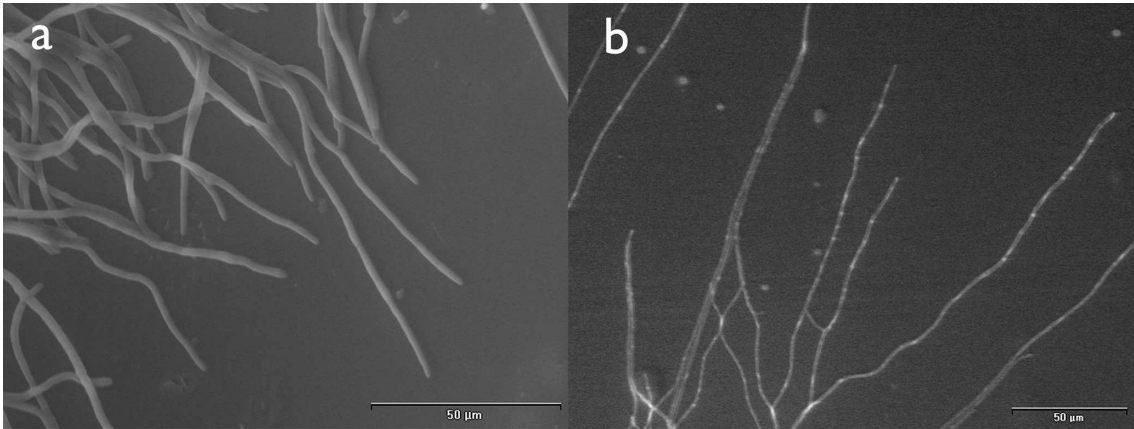


FIGURE 2.1: *Cladonia grayi* growing alone on cellophane over 99:1 medium. 2.1a. Edge of colony, day 5. Bar 50 μ m. 2.1b. Edge of colony, day 43. Bumps between hyphae are abnormalities in the cellophane. Bar 50 μ m.

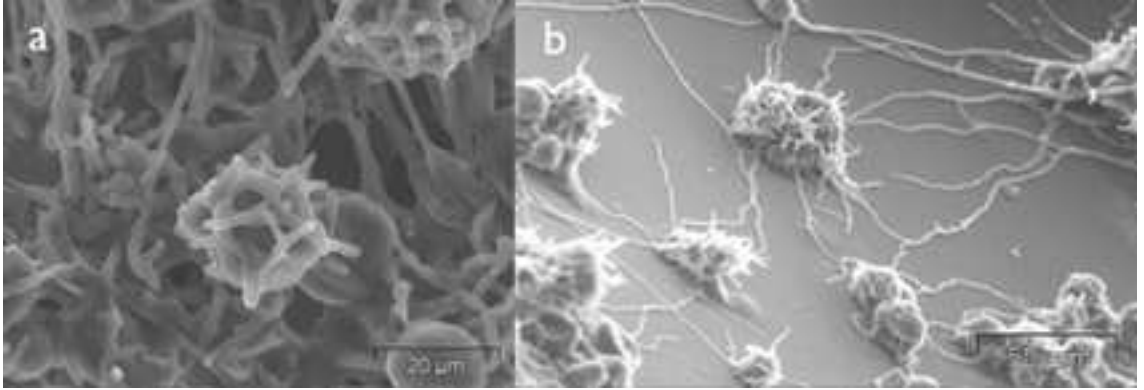


FIGURE 2.2: *Cladonia grayi* growing with *Asterochloris* sp., and surrounding algal cells through increased fungal branching. 2.2a. A single algal cell enveloped by fungal hyphae can be seen in the middle of the micrograph. At least one alga, lower right hand corner, is not engulfed by fungal hyphae, day 49. Bar 20 μ m. 2.2b. Clumps of algal cells and highly branched hyphae can be seen scattered throughout, connected by exploring hyphae, day 49. Note increased branching of *Cladonia* in clumps of algae, when compared to hyphae between clumps and not in contact with algae. Bar 50 μ m.

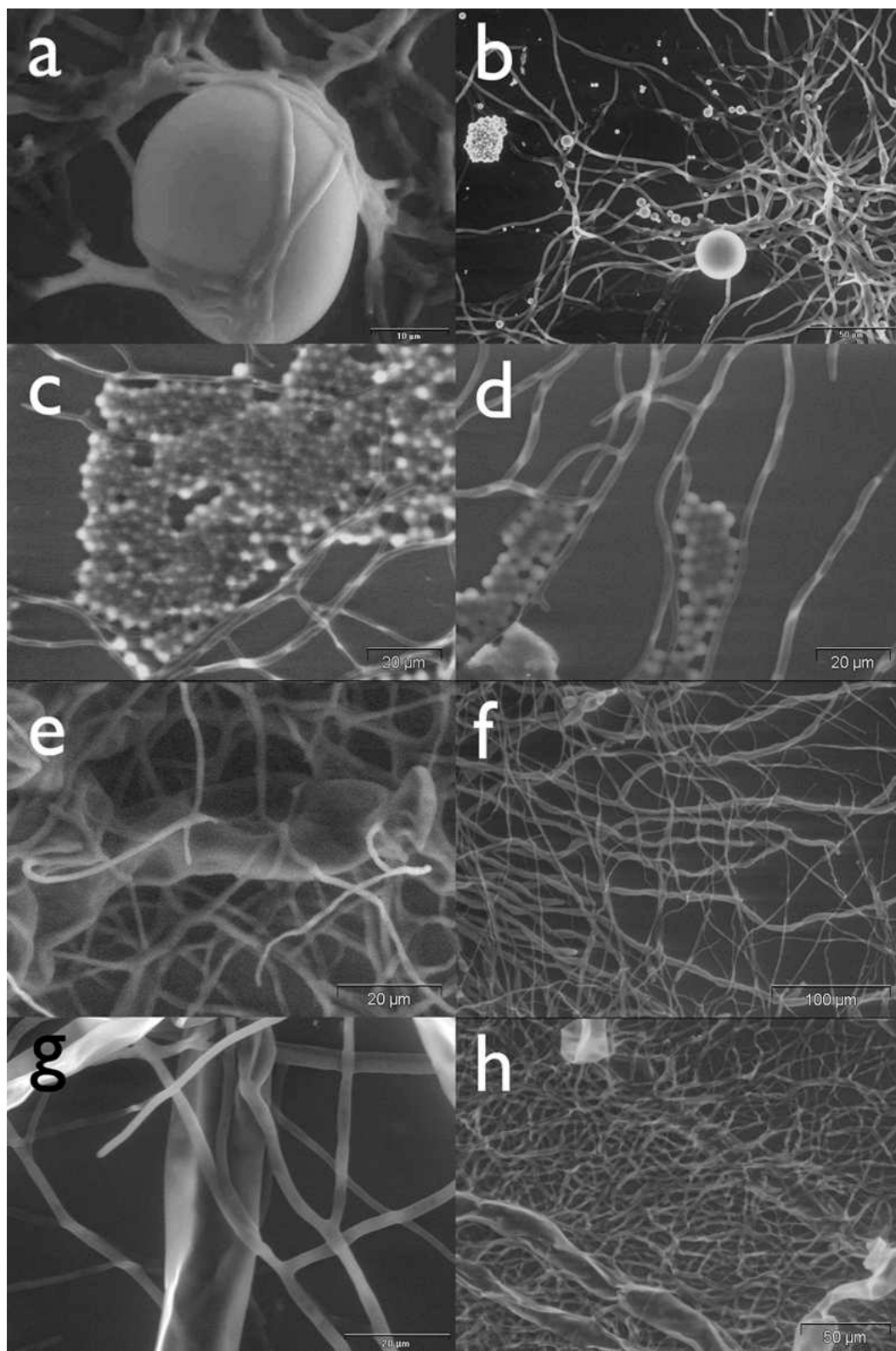


FIGURE 2.3

Figure 2.3 *Cladonia grayi* growing with glass beads and heterologous phototrophs.

2.3a. *Cladonia grayi* growing over a glass bead, towards the center of a mycelial mat. Increased hyphal branching with short lateral hyphae absent, day 29. Bar $10\mu\text{m}$.

2.3b. *Cladonia grayi* growing with glass beads at the edge of a mycelial colony, day 8. Bar $50\mu\text{m}$.

2.3c. *Cladonia grayi* growing with spherical cells of *Chlorella vulgaris*, at the edge of a mycelial colony, day 9. Bar $20\mu\text{m}$.

2.3d. *Cladonia grayi* growing with *C. vulgaris*, day 38. Bar $20\mu\text{m}$.

2.3e. *Cladonia grayi* growing with *Trentepohlia* sp., day 9. At least one filament of *Trentepohlia* sp. can be seen growing horizontally across the center of the micrograph. Bar $20\mu\text{m}$.

2.3f. *Cladonia grayi* growing over the edge of a *Trentepohlia* sp. colony, day 38. Bar $100\mu\text{m}$. Thick filaments of *Trentepohlia* are growing horizontally (mostly) across the micrograph.

2.3g. *Cladonia grayi* growing with protonema of *Funaria hygrometrica*, day 5. One filament of *F. hygrometrica* is shown growing vertically through the center of the micrograph. Bar $20\mu\text{m}$.

2.3h. *Cladonia grayi* growing with several protonemal filaments of *F. hygrometrica*, day 24. *Cladonia grayi* has grown over part of one protonemal filament in the upper right hand third of the micrograph. Portions of the same filament remain free both above and below where *C. grayi* has overgrown it. Growth contacts between fungus and moss lack the phenotypic lichen-symbiotic response of *C. grayi*. Bar $50\mu\text{m}$.

Fungal gene expression in early lichen symbiosis.

3.1 Introduction

Fungi have developed a range of symbiotic interactions with plants (from single celled green algae to multicellular vascular plants) that have allowed them to diversify into an array of terrestrial niches (Selosse & Le Tacon, 1998; Taylor, 1990). The most successful symbioses, based on fungal species numbers alone, is the lichen symbiosis between a filamentous fungus (the mycobiont), and either a green alga, a cyanobacterium, or both (the photobiont). Over 13,500 species of lichen-forming fungi have been described (Kirk *et al.*, 2008) based on the transformative properties of the symbiosis to the fungal phenotype, comprising nearly one fifth of all known fungal species. Because extant lichen-forming fungal species diversity is highly concentrated in one main clade of deep origin within the fungal tree of life, it is possible

that lichens represent the oldest mutualistic symbiosis between fungi and land plants (Arnold *et al.*, 2009; James *et al.*, 2006). Although the emergent properties of lichen thallus development were first recognized as the symbiotic growth of different organisms almost 140 years ago (Schwendener, 1869), the molecular mechanisms of lichenization remain enigmatic. The slow growth and genetic intractability of lichen symbionts have prevented researchers from answering the most basic cellular and molecular based questions of how mycobiont and photobiont partners find and recognize each other, and how they maintain this relationship.

The initial stages in the development of lichen symbiosis can be routinely examined *in vitro* through the axenic culturing of symbionts. In experimental resynthesis, aposymbiotic cultures of mycobionts and photobionts are mixed and allowed to grow together on nitrocellulose filters over reduced nutrients (Armaleo, 1991; Backor & Fahselt, 2003; Oliver *et al.*, 1989). The development of a stratified lichen thallus follows four general developmental stages consisting of: pre-contact, contact and growth together, growth into a transitional pre-thallus, and growth into a stratified thallus (Armaleo, 1991; Ahmadjian *et al.*, 1978; Galun, 1988). These stages are part of a dynamic continuum that originates anywhere novel fungal – algal contacts are made (Armaleo, 1991). The synchronized growth of the latest stage in lichen development cannot be regularly achieved in the lab, and for this reason the present

study focuses on only the first two stages. For the lichen model system formed by the symbiotic growth of *Cladonia grayi* and *Asterochloris* sp., the first two stages of development (pre-contact, and contact) can be observed *in vitro* within 21 days, and the latter step is accompanied by a distinctive phenotypic change in the fungus (Joneson & Lutzoni, 2009). The phenotypic change in *C. grayi* consists of a switch from apical growth with long internodes, to reduced apical growth and internode length with increased lateral branching (Joneson & Lutzoni, 2009; Honegger, 1997). Entry into lichen symbiosis is accompanied by changes in gene regulation and expression in the photobiont and mycobiont (Armaleo & Miao, 1999; Trembley *et al.*, 2002c), although it is not yet known at which step this genetic modification begins.

The *Cladonia grayi* – *Asterochloris* sp. model system of symbiosis was used to explore the combined upregulated transcriptome of early lichen development using. At this time, expressed sequence tags (ESTs) and/or cDNA libraries are lacking for any closely related fungi or green algae to the lichen symbionts, and the annotation of any gene products relies on homology based searches. Suppressive Subtractive Hybridization (SSH) (Diatchenko *et al.*, 1996) was used to determine symbiotically upregulated genes, and quantitative PCR (qPCR) to verify the expression level of candidate genes. Extracellular communication between symbionts prior to contact has generally been disregarded, as chemotropic signals do not appear to be exchanged

(Ahmadjian, 1959; Clayden, 1998). I present here the first evidence that fungal gene expression changes not only after symbiont contact (stage 2) but is influenced by the close, yet physically separate, proximity of its phototrophic partner (stage 1). While the algal portion of this work will be presented elsewhere, I describe here novel fungal genes differentially expressed in the early stages of the development of lichen symbiosis.

3.2 Materials

3.2.1 *Experimental setup*

Axenic cultures of *Cladonia grayi* and *Asterochloris* sp. described in Armaleo & May (2009) were used. Cultures of *Cladonia* were maintained in 50-200 mL liquid cultures of Malt Yeast (MY, 20 g malt extract and 2 g yeast extract per liter), in Ehrlemeyer flasks on a rotating shaker (100 rev/min), and regularly ground in a commercial Waring blender (every 6-8 weeks). *Asterochloris* was grown on nitrocellulose membranes (47 mm in diameter, 0.22 μm pore size, Millipore Cat. #GSWP04700), over one-quarter strength *Trebouxia* Medium (Ahmadjian, 1967), under approximately $40\mu\text{mol m}^{-2} \text{s}^{-1}$ illumination for 12 hours a day.

Day 1 denotes the beginning of the experiment, on which aposymbiotic and symbiotic cultures growing on nitrocellulose filters over 2% agarized plates made from a

99:1 mix of Bold's Basal Medium (Deason & Bold, 1960) and MY (hereafter referred to as 99:1 medium) were created. *Cladonia* cultures were ground on Days -1 or -2, prior to the beginning of the experiment. On Day 1, liquid *Cladonia* cultures were concentrated in a Sorvall floor centrifuge at 7 K for 10 min. Decanted mycelia were resuspended in a minimum of liquid 99:1 medium, filtered through a 74 μ m pore size nylon filter, and the filtrate concentrate determined using a spectrophotometer at 600 nm and a pre-determined concentration of 1 OD = 0.75 mg, dry weight. On Day 1, cultures of *Asterochloris* were scraped and resuspended in a minimum of liquid 99:1 medium using a hand-held glass homogenizer. The algae were subsequently filtered through a 74 μ m nylon filter, and the concentration of the filtrate determined by spectrophotometer at 750 nm, and using a pre-determined concentration of 1 OD = 0.8 mg, dry weight.

An ideal ratio of 7:3 fungus to alga dry weight determined the volume of fungus and/or alga to deposit onto filters by vacuum-filtration, and liquid filtrates were adjusted appropriately with additional liquid 99:1 medium. Approximately 5 mg dry weight of *Cladonia* and 2.14 mg dry weight of *Asterochloris* were vacuum filtered separately onto the membranes either for aposymbiotic growth (each filter culture grown in isolation) or for stage 1 symbiotic growth (an *Asterochloris* filter placed on top of a *C. grayi* filter), or vacuum filtered together onto membranes for stage 2 of

symbiosis.

Stage one aposymbiotic and symbiotic filters were scraped on day 9 and the materials lyophilized in a Freezemobile (Spectrofuge Corporation) at 0°C until dry. RNA extraction proceeded immediately, followed by first strand cDNA synthesis. Stage 2 materials were processed in the same manner, starting on day 21.

3.2.2 RNA extraction

Lyophilized material was ground on a Mini Bead Beater 8 (BioSpec Products, Inc.), using 700 μm zirconia beads. Material and beads were initially frozen in liquid nitrogen, ground for 10 s at full speed, and placed back in liquid nitrogen. 400 μL of guanidine-phenol buffer (Solution A according to Xie & Rothblum (1991), except that the following molarities were substituted: 25 mM sodium citrate and 4 M guanidinium thiocyanate), was added to each tube and subsequently ground for 30 s at half speed. Liquid was removed with a pipette, combining to this an additional 100 μL of buffer that washed the beads. Each sample was heated thoroughly on a 65° C heat block filled with water before grinding. To grind, pestles inserted into an electric screwdriver were used to alternately grind and mix. To the ground material, 1/5 volume of chloroform was added, and vortexed for 1 m. Samples were spun at 8,000 rpm for 5 m. To the upper phase, a 0.11 volume of 10% CTAB (10% (w/v) hexadecyltrimethylammonium bromide, Sigma) was added, mixed until homogenous

and vortexed for 1 m. A chloroform extraction was performed twice by adding equal volumes of chloroform, spinning at maximum speed (max) for 5 m, and collecting the upper phase. Next, equal volumes of isopropanol was added, vortexed, and stored at room temperature (RT) for 3 m (up to 1 h). Samples were spun at max for 5 minutes, the supernatant removed by pipette, and the pellet air-dried. Samples were resuspended in 45 μ L of water and treated with DNase (Promega USA, Madison WI), and the enzyme reaction stopped, all following the manufacturer's guidelines. Next, an equal volume of chloroform was added, vortexed for 1 m, and spun at maximum speed for 5 m, collecting the upper phase in a new tube. A 10% volume of sodium acetate (3 M, pH 5.2) was added, with a 250% volume of 95% ethanol, vortexed for 1 m, and let sit at RT for 3 m. Samples were spun at max for 10 m, discarding the supernatant. Samples were washed with 1 mL of 70% ethanol, spun at max for 2 m, and the supernatant removed by pipette before drying. Pellets were resuspended in 30-50 μ L of water using heat and pipetting.

3.2.3 ss-cDNA synthesis

ss-cDNA for qPCR reactions was carried out using an oligo d(T)₁₆ and SuperScript II Reverse Transcriptase (Invitrogen USA, Carlsbad CA) according to the manufacturer's guidelines, and altered in the following way: initial heating was carried out at 80° C, dNTPs were added during the second step, RNaseOUT was omit-

ted, the final incubation at 42° C was for 2 h, and the reaction was chemically degraded by adding to each 20 μ L reaction volume, 3.5 μ L of a mixture of 0.5 M sodium acetate and 50 mM EDTA (pH 10), followed by an incubation at 65° C for 10 m and adding 5 μ L Tris HCl (1 M, pH 7.5). ss-cDNA was stored at -80° C until used.

3.2.4 Suppression subtractive hybridization and cDNA library construction

First and second strand cDNA was synthesized for SSH using the BD SMARTTM PCR cDNA Synthesis Kit following the manufacturer's guidelines for cDNA Subtraction, except for the following alterations (BD Biosciences Clontech, Palo Alto, CA). *Rsa*I digested double-stranded cDNA (ds-cDNA) was cleaned with QIAquick PCR Purification columns after reduction with n-butanol (Qiagen USA, Valencia, CA), eluted into a final volume of 100 μ L, and precipitated on ice for 30 m with a final wash of 80% ethanol.

SSH was carried out using the PCR-SelectTM cDNA Subtraction kit according to the manufacturer's directions and starting with adaptor ligation, (BD-Biosciences-Clontech, Palo Alto, CA). A truncated (20bp) version of Nested PCR 1 was used to later increase cloning efficiency, 5'CGAGCGGCCGCCCCGGGCAGG3'. For stage 1, SSH was performed using aposymbiotic fungal cDNA as the driver, and symbiotic cDNA as the tester. For stage 2, a mixture of algal and fungal aposymbiotic cDNA as

the driver, and symbiotic cDNA as the tester. Relative proportions of aposymbiotic cDNAs in the stage 2 driver were determined by qPCR. In short, algal and fungal specific LSU primers (Table 4.1) were run separately using symbiotic single-stranded cDNA (ss-cDNA), and compared to known mixtures of varying proportions of algal and fungal ss-cDNA. The ds-cDNA created through SSH was cloned into the pCR2.1-TOPO[®] plasmid, and transformed into TOP-10 *Escherichia coli* cells (Invitrogen USA, Carlsbad, CA), using 7.5 ng of cDNA/reaction.

3.2.5 Clone sequencing and analysis

Double-stranded sequences of clone inserts were cleaned of poor reads and contaminating vector sequences, assembled into contigs using default parameters, and exported as fasta files for local batch blast searches (Altschul *et al.*, 1990) using Sequencher 4.5 (Gene Code Corporation, Ann Arbor, MI). Sequences shorter than 100bp were not analyzed.

A local batch blastx algorithmic search was carried out on the set of non-redundant (nr) proteins from the National Center for Biotechnology and Information (www.ncbi.nlm.nih.gov, accessed on 21 May 2008). Default blastx settings were used, with a threshold e-value $< 10^{-5}$. Sequences were considered significant if they produced a translated sequence of 30 amino acids or longer that matched an entry in the nr database with a percentage identity equal to or greater than 35%. Pro-

tein coding sequences in the proper reading frame were subsequently parsed from the blastx output and used to search the UniProt database (www.ebi.uniprot.org) of protein sequence and function using blastp, and to assign Gene Ontology (GO) annotations (Ashburner *et al.*, 2000) using Blast2GO (Conesa *et al.*, 2005) with an e-value $< 10^{-5}$. Unless otherwise noted, datasets of blastx and Blast2GO results were managed using customized PERL and BioPERL scripts (Stajich *et al.*, 2002).

Genome of origin (fungal, algal, or unknown) was determined for genetic regions by manually classifying up to the top five blastx hits (if available). Sequences were labeled of fungal origin if the top hits were to other fungal sequences. Algal sequences were identified if the top hits matched either all Chloroplastida, or Chloroplastida and cyanobacteria. The remainder of sequences was classified as “either”.

3.2.6 Subtraction efficacy

Subtraction effectiveness was determined by comparing *Cladonia grayi* SSH-cDNA sequences to the top expressed genes of other fungi under normal growth conditions. The rationale is that with low SSH efficiency, the likely set of *C. grayi* genes recovered from SSH would consist of the most highly expressed fungal genes under normal growth conditions. This comparison was carried out by using the tblastx algorithm to compare the list of top expressed genes of *Aspergillus nidulans*, *Neurospora crassa*, and *Saccharomyces cerevisiae* to the complete proteome of

each, and the list of *C. grayi* SSH generated cDNAs to the above 3 proteomes. (For complete proteomes, *A. nidulans* and *N. crassa* were downloaded from the Broad Institute, www.broad.mit.edu, and *S. cerevisiae* from the *Saccharomyces* Genome Database, www.yeastgenome.org, all on 23 October 2008. For the list of top expressed genes, *A. nidulans* [GSM102371] and *N. crassa* [GKI953-S2, 12 hours] expression datasets were downloaded from the NCBI Gene Expression Omnibus. The list of top *S. cerevisiae* expressed genes were extracted from the supplementary data of (Holstege *et al.*, 1998)). It was then asked how many of the top expressed genes of the subject were amongst the top expressed genes of the query. For example, after doing a similarity search of highly expressed *A. nidulans* genes within the complete gene set of *N. crassa*, it was then determined if any of the highly expressed *N. crassa* genes were amongst the list of top tblastx matches to *A. nidulans*. For all non-*Cladonia* gene sets, all reciprocal similarity searches were made. Using a permutations test, the SSH subtraction was considered effective if amongst the sets of reciprocal searches, the number of *C. grayi* SSH-cDNAs that matched another fungus' top expressed genes was lowest within the set.

3.2.7 qPCR and cellular location prediction

qPCR was performed on two experimental replicates per treatment to confirm differential expression of select genes. (Experimental replicates are considered as

different filters set up at the same time, and in each case a single culture was used to set up the aposymbiotic or symbiotic growth of the fungus and the alga). The complete list of qPCR primers can be found in Table 4.1. qPCR in 20 reactions were run using the following conditions: 0.4 units Apex Taq DNA Polymerase (Genesee Scientific, San Diego CA) , 1X Apex PCR Buffer, 3 mM MgCl₂, 0.16 mM dNTPs each, 2% (v/v) ROX passive reference (Eurogentec, San Diego CA), 2.5×10^{-5} % Sybr Green (Invitrogen USA, Carlsbad CA) and an amount of cDNA representative of a standard dilution curve of 1, 10E-01, 10E-02, and 10E-03. qPCR in 20 μ L reactions were conducted in a Chromo4 thermocycler (Bio-Rad, Hercules, CA), with the following parameters: 95° C for 10 m, followed by 44 cycles of 95° C for 30 s, 65° C for 30 s, and 72° C for 30 s. The fluorescence was measured at the end of the 72 °C extension step. Three qPCR reactions were performed for each gene-dilution series, and NRT negative controls were run for each gene, in each experimental replicate. Each sample was normalized against two fungal controls, the efficiency of each primer pair determined using the standard curve method (Rasmussen, 2001), and the final normalized relative expression levels of the symbiotic fold-change in expression level was calculated according to (Pfaffl, 2001). Individual primer efficiencies were averaged within treatments between biological replicates. The subcellular localization of putative homologues of lipid processing proteins (based on the top blastx matches

from other fungi, and an unpublished draft genomic sequence of *Cladonia grayi*) were determined using the N-terminal signal sequence prediction algorithms, TargetP 1.1 (Emanuelsson *et al.*, 2000), and SignalP3.0 (Bendtsen *et al.*, 2004).

3.2.8 Searching the genome for gene duplication of qPCR selected genetic regions.

To determine if SSH generated cDNAs used in qPCR have more than one copy in the fungal genome, an unpublished *Cladonia grayi* genome (approximately 10X coverage, accessed 20 April 2009) was searched using the nucleotide sequences of qPCR primers and SSH-cDNAs. Primer sequences were searched against the genome using blastn. When either the forward or reverse primers for a given pair matched the genome identically over 75% of their nucleotide sequence (arbitrarily chosen as the cut-off point for which mis-matched primers will unsuccessfully anneal at the annealing temperature of 65° C, and therefore will not be able to successfully extend or amplify DNA sequences), the genome was searched using the the tblastx algorithm and the entire corresponding SSH-cDNA nucleotide sequence. Regions of genomic contigs corresponding to the second best match to any given SSH-cDNA were subsequently extracted, and the associated primers were attempted to be aligned to the genomic sequence using Sequencher and low assembly parameters (at least 15 nucleotides, with 65% identity).

3.3 Results

3.3.1 Library sequencing and gene annotation

A stage 1 specific SSH-cDNA library for *Cladonia grayi* containing 576 randomly chosen clones was sequenced. Only clones with non-vector sequence over 100bp in length were considered. Five hundred and twenty two clones (91%) up to 1510bp in length, included in 432 contigs and 90 individual reads, were considered further. A blastx comparison of these sequences against the GenBank nr database of protein sequences resulted in 216 (41.4%) with a significant similarity (e-value $< 10^{-5}$) to protein coding genes.

A stage 2 specific SSH-cDNA library containing 960 clones was constructed. Eight hundred and seventy four clones (91%), containing 806 contigs and 68 individual reads, contained non-vector inserts over 100bp and up to 1515bp in length, and were considered further. A blastx comparison of these sequences against the GenBank nr database of sequences resulted in 475 (54.2%) with a significant similarity (e-value $< 10^{-5}$) to protein encoding genes. Of these, 316 (67%) were of fungal origin, 134 (28%) were of algal origin, and 25 (5%) were classified as “either”. All sequences will be deposited in NCBI’s Trace Archives database (www.ncbi.nlm.nih.gov/Traces/home/).

The top matching GenBank and UniProt entries for each contig or individual read in stages 1 and 2 can be found in Tables A.1 and A.2. GO terms were orga-

nized by organizing principle (biological process, cellular compartment and molecular function), and the first three levels of GO terms for stages 1 and 2 can be seen in Tables A.3 and A.4. A key tying GO terms to Clone IDs can be found in Table A.6.

3.3.2 Subtraction efficacy

To ensure that the SSH in stages 1 and 2 did not enrich for highly expressed house-keeping genes, we carried out a bioinformatic comparison of SSH-cDNA libraries to additional fungal expression libraries. The non-redundant libraries of stages 1 and 2 contained 196 and 240 unique gene regions with a blastx match, respectively. Based on the number of *Cladonia grayi* unique gene regions in each SSH-cDNA library, an equal number of ordered top expressed genes for *Aspergillus nidulans*, *Neurospora crassa*, and *Saccharomyces cerevisiae* were used as the query. The number of *C. grayi* gene sequences that matched the top expressed genes of the 3 alternative fungi was always the smallest amongst the reciprocal and one-way searches of the 3 alternative fungi and *C. grayi* (Table 3.4, $p=0.05$), and the two subtractions were considered effective.

The subset of SSH genes tested by quantitative qPCR gave a more direct estimate of SSH efficiency. When looking at all genes whose symbiotic/aposymbiotic expression ratio is higher than 1, 45% and 50% are upregulated in stages 1 and 2, respectively (Figures 3.1 and 3.2). However, the discussion is limited to genes whose

qPCR expression ratio is at least 2, 14% and 25% in stages 1 and 2, respectively. Previous studies have found that 25-38% of SSH generated candidates show more than a two-fold induction when tested using qPCR (Caturla *et al.*, 2002; Herrero *et al.*, 2007; Nowak *et al.*, 2004).

3.3.3 qPCR results of select genes

qPCR was performed to confirm differential expression of 22 and 20 fungal genes in the first and second stages of lichen resynthesis, respectively. A list of these genes, their putative function, and their top GenBank hit can be found in Tables 3.2 and 3.3. Genes were chosen based on their putative function and a homologue's previous implication in plant and fungal interactions, or general stress and defense responses, as well as genes of unknown function. The no reverse-transcriptase (NRT) controls of all genes showed at least a 32-fold lower background expression level than in the same treatment using reverse-transcriptase.

Relative expression levels of three putative control genes were inspected, as there was no prior knowledge of the expression levels of these genes, actin (*act*), beta-tubulin (β tub), and translation elongation factor 1-alpha (*ef1 α*). Each of the three fungal control genes was normalized to each other to look for consistencies in magnitude of expression level. Fungal *act* and β tub behaved consistently relative to each other across replicates and treatments. On average, the β tub/*act* expression ratio

(\pm standard error) was 1.16 ± 0.37 in the aposymbiotic, and 1.44 ± 0.45 in the symbiotic state. The expression level of *ef1 α* varied within biological replicates across multiple runs, and therefore was dismissed as a control gene.

3.3.4 Stage 1 qPCR

Of 22 stage 1 genes screened (Figure 3.1), three genes (conserved hypothetical protein 2 [CHP2], a D-arabitol dehydrogenase [oxido], and a triacylglyceride lipase [TGL1]) show at least a two-fold increase in expression level in the symbiotic state. Of the remaining genes, 18 (82%) did not change in overall expression level (expression differential between 0.5 and 2), and 1 (4%) decreased. Five additional genes identified by SSH as induced in stage 2 were tested by qPCR in stage 1 (Figure 3.1), but did not show more than a 2 fold increase. In stage 1 (Table 4.4), a gene of unknown function (unkn) has the highest expression relative to controls (26.6 to 37.8), oxido the lowest (0.05 to 0.23).

The putative protein encoded in CHP2 shares sequence similarity with the *Podospora anserina* small S protein, a known self and non-self determinant (Saupe, 2000). The amino acid sequence encoded in CHP2 and the *P. anserina* small S protein match with 31% identical residues over a 103 amino acid stretch, and an e-value = $2e-06$. A second, unnamed *P. anserina* locus matches CHP2 slightly better (over 116 amino acids, 31% identical residues, e-value = $4e-10$). Both this

unnamed locus and the CHP2 gene region have the highest similarity to Guk1p in *Saccharomyces cerevisiae*. Guk1p is a kinase involved in cell wall biosynthesis, whose possible role in self and non-self determination remains to be determined .

3.3.5 Stage 2 qPCR

Of 20 fungal genes screened (Figure 3.2), a negative regulator of glucose repressible genes (HEX2), a heterokaryon incompatibility locus (Het6), lipase 3 (Lip3) a phospholipase (PLA2), and a dioxygenase (YQE1) all show at least a two-fold increase in symbiotic expression levels. Of the remaining genes, 14 (70%) did not change in expression, and one, a carotene synthesis subunit [carRA], decreased in expression. Three additional genes identified by SSH as induced in stage 1 were tested by qPCR in stage 2 (Figure 3.2), but did not show more than an average 2 fold increase. In stage 2, (Table 4.5), cutinase (Cut) has the highest expression (15.5 to 33.9), Het6 the lowest (1.39E-02 to 5.04E-02).

Based on N-terminal signal sequence prediction, the lipid metabolism proteins Lip3 and TGL1 are secreted, and the secretion state of PLA2 can not be determined.

3.3.6 SSH-cDNAs used in qPCR are preliminarily from single copy genes.

Of the 42 fungal primer pairs used in qPCR, 20 pairs contained forward and reverse primers whose second best hits in the genome matched less than 60% of

the original primer sequence identically. Eight pairs matched less than 65%, 7 pairs matched less than 70%, 3 pairs matched less than 75% (the primer pairs for *chit*, *Hsp90*, and *MepB*), 3 pairs matched less than 80% (the primer pairs for *carRA*, *FUI1*, and *GCN1*), and 1 pair matched less than 85% (the primer pair for *H3*). In the last four cases, it was impossible to align the primer pairs with the corresponding genomic region (extracted from the second best *tblastx* hit of the corresponding SSH-cDNA). In addition, each primer pair amplified only one band of uniform size from genomic DNA (as opposed to more than one band, which is presumed to take place if the primer pair is amplifying from both target and non-target regions of the genome). Therefore, the primer pairs used in qPCR were all considered to be amplifying only one gene region, and the genes corresponding to the qPCR SSH-cDNA regions are preliminarily considered as single copy genes.

3.4 Discussion

This study takes the first detailed look into the differential expression of an obligately mutualistic lichen-forming fungus. The upregulation of genes in stages 1 and 2 of lichen development were investigated and identified using SSH. Both up and downregulated genes were discovered in further testing with qPCR. Genes that were upregulated in symbiosis showed significant matches to genes putatively encoding

proteins involved in fungal self and non-self recognition, lipid metabolism, negative regulation of glucose repressible genes, a D-arabitol oxidoreductase, a dioxygenase, and a conserved hypothetical protein. These results include the first evidence that mycobionts and photobionts are able to communicate extracellularly without physical contact.

The Het-6 gene region was chosen for qPCR testing based on the role of Het-6 homologues in self and non-self recognition in fungi (Glass & Kaneko, 2003). The proteins of filamentous ascomycetes containing het domains have also been suggested to play a role in an adaptive fungal response to varying stimuli including development and resistance to pathogens (Fedorova *et al.*, 2005). For example, a different het domain-containing locus, *het-c*, is upregulated in the biotroph *Trichoderma harzianum* in early colonization of tomato plants although the specific role *het-c* plays in this process remains to be determined (Chacón *et al.*, 2007). The approximate 15x swing in expression (from 5-fold repression in stage 1 to 3-fold induction in stage 2 (Figures 3.1 and 3.2) suggests the exciting possibility that the fungal Het system is part of the recognition process involved in lichen thallus formation, a hypothesis that can now be verified with direct experiments.

CHP2 was also chosen as a candidate gene for its possible role in fungal self and non-self recognition due to its high similarity to the *Podospira anserina* small S pro-

tein, also involved in self and non-self recognition (Balguerie *et al.*, 2003). However, a closer look at its most similar homologue in *P. anserina*, and in *Saccharomyces cerevisiae*, revealed it may have more to do with building cell walls. *GUK1*, the closest match to CHP2 in yeast, is a guanylate kinase involved in growth and elongation of cell wall N-linked mannoproteins. This suggests that the close proximity of *Cladonia grayi* to *Asterochloris* sp. stimulates the fungus to increase cell wall biosynthesis, which is consistent with the phenotypic growth changes observed during the *in vitro* interaction of the symbionts (Honegger, 1997; Joneson & Lutzoni, 2009).

Three of the upregulated fungal genes appear to be involved in lipid metabolism, TGL1, Lip3 (both are putative triacylglyceride lipases and predicted to be secreted) and PLA2 (a putative phospholipase). The increase in expression and presumed secretion levels of Lip3 and TGL1 suggests that extracellular lipases play a role in developmental interactions between the symbionts. Upregulation of TGL1 is limited to stage 1 when the fungus and alga are close enough to sense each other through diffusible signals yet are not in cell to cell contact. TGL1 expression is later decreased in stage 2 suggesting that this lipase must be suppressed for normal lichen development to proceed. Conversely, Lip3 and PLA2 are strongly induced in stage 2 (Figure 3.2), and PLA2 is symbiotically induced at least 1.5 times the aposymbiotic level in stage 1 (Figure 3.1). Unlike most other tested genes, the

apobiotic expression of both Lip3 and PLA2 increases between days 9 and 21 (Tables 4.4 and 4.5). However, their symbiotic induction surpasses this apobiotic baseline increase (Figures 3.1 and 3.2). Lipid metabolism plays a role in carbon allocation in growing arbuscular mycorrhizae (Bago *et al.*, 2002), and secreted lipases are essential for infection in some fungal pathogens (Berto *et al.*, 1999; Comm  nil *et al.*, 1998; Voigt *et al.*, 2005). The role of lipases in lichen development remains to be determined.

Oxido, a putative D-arabitol dehydrogenase, was chosen based on its possible role in fungal carbohydrate metabolism. One general characteristic of lichen symbiosis is that symbiotic (and not apobiotic) lichen algae leak carbohydrates into the extracellular matrix (apoplast) shared with mycobionts (Green & Smith, 1974; Richardson & Smith, 1968; Richardson *et al.*, 1968). In trebouxoid algae such as *Asterochloris* sp., this carbohydrate mainly comes in the form of the sugar-alcohol ribitol. Once taken up by the mycobiont, the polyol is converted in to arabitol and mannitol through the pentose phosphate pathway (Lines *et al.*, 1989). The expression of oxido increases 1.95-5.25x in stages 1 and 2, suggesting that ribitol secretion by the alga, and/or the corresponding metabolic changes in the fungus, begin even before direct cell to cell contact between the symbionts and persist after contact. Although the nature of carbohydrate transfer has been confirmed by removing algae

from the lichen thallus (Richardson *et al.*, 1968), this is the first evidence suggesting that carbohydrate leakage starts upon the reintroduction of algae back to symbiosis. In general, the stage 1 induction of oxido and additional fungal genes in this *in vitro* system can be used to search for the extracellular signals mediating pre-contact recognition between fungus and alga.

A significant increase in stage 2 expression of HEX2 was observed. The HEX2 homologue in *Saccharomyces cerevisiae*, *REG1*, acts as a negative regulator of glucose repressible genes (Niederacher & Entian, 1987). In general, Reg1p is necessary to repress genes involved in alternative carbohydrate metabolism when glucose, the preferred carbohydrate of *S. cerevisiae*, is present. That *Cladonia grayi* might use a similar pathway in turning off general sugar metabolism genes used in foraging when polyols from the alga become available is consistent with the upregulation of HEX2. In this manner, *C. grayi* represses alternative carbohydrate metabolic genes through the upregulation of HEX2 after *Asterochloris* sp. starts releasing the carbohydrate currency of symbiosis. For this reason it is curious that HEX2 is not upregulated in stage 1. Although an increase in stage 1 oxido expression was observed (and presumably *Asterochloris* sp. has already started secreting ribitol), perhaps the ribitol level in *C. grayi* is below the critical level needed to induce stage 1 HEX2 induction, or perhaps these pathways remain distinct. Sugar metabolism genes

that are downregulated by HEX2 might possibly be found amongst the many genes hypothesized to be downregulated in lichen symbiosis in Armaleo & Miao (1999), and anonymously selected in Trembley *et al.* (2002c).

Two additional genes, both oxidoreductases, are induced in early lichen development. Using a less conservative cutoff of 1.5, the partial aflatoxin B1 aldehyde reductase (AfB1) recovered in stage 1 contains a conserved domain belonging to the aldo-keto reductase superfamily of NAD(P)(H) oxidoreductases. Aldo-keto reductases are found in prokaryotes and eukaryotes. In eukaryotes AfB1 is best known for its role in reducing the toxicity of aflatoxin in rats (Ellis *et al.*, 2003). It is unclear what this gene is doing in fungi in general, as fungal protein sequence homology searches of GenBank recover only putative or conserved hypothetical proteins. YQE1 is induced in stage 2 and shares homology with alpha-ketoglutarate-dependent dioxygenases. These proteins are found widely in prokaryotes and eukaryotes, and the specific role of this putative oxygenase in early lichen development remains to be determined.

Although not expected from the original screen, at least 4 genes were found to be significantly downregulated in either stages 1 or 2. TUP1 was significantly downregulated in stage 1 of lichen development. Homologues in yeast and filamentous ascomycetes act as negative transcriptional regulators, and are thought to play a role in

the suppression of asexual development (Todd *et al.*, 2003). The down-regulation of this gene in *Cladonia grayi* could therefore be involved in de-repressing transcription of the developmental machinery needed for creating a lichen thallus. In addition to TUP1, Het-6 is downregulated in stage 1, and TGL1 and carRA are downregulated in stage 2. carRA encodes a phytoene synthase/lycopene cyclase that in other fungi is involved in cellular processes such as light reception, protection against oxidation, cell signalling and pigmentation (Linnemannstöns *et al.*, 2002). Light has previously been implicated as necessary for the successful interactions of symbionts in lichen development (Ahmadjian, 1992), however the role of carRA in this process remains to be investigated.

A notable result of the current qPCR data is that all genes are always expressed, and that relatively limited changes in gene expression characterize both the transition from aposymbiotic to early symbiotic growth, as well as the transition from stage 1 to stage 2. Although the expression of symbiosis specific genes was not tested for (which could be amongst the SSH-cDNAs that lacked GenBank matches), it appears that lichen symbiosis development is reliant upon, at least in part, a set of genes that are common to many fungi and to varying metabolic pathways (Tables 3.2 and 3.3). This result is similar to those seen in other mutualistic symbiotic interactions between mycorrhizal fungi and their hosts (Duplessis *et al.*, 2005;

Johansson *et al.*, 2004; Le Quéré *et al.*, 2005; Peter *et al.*, 2003; Polidori *et al.*, 2002; Voiblet *et al.*, 2001). In both mycorrhizal and lichen symbiosis, development appears to be determined by, or concomitant with, epistatic interactions between genes showing slight quantitative variation.

The results described in this paper show that *in vitro* lichen resynthesis is amenable to the molecular investigation of symbiosis. The differential gene expression observed provides evidence that mycobionts and photobionts can communicate. Up- and down- regulated *Cladonia grayi* genes, and their putative roles in additional fungi, provide compelling models upon which to build future testable hypotheses in lichen development.

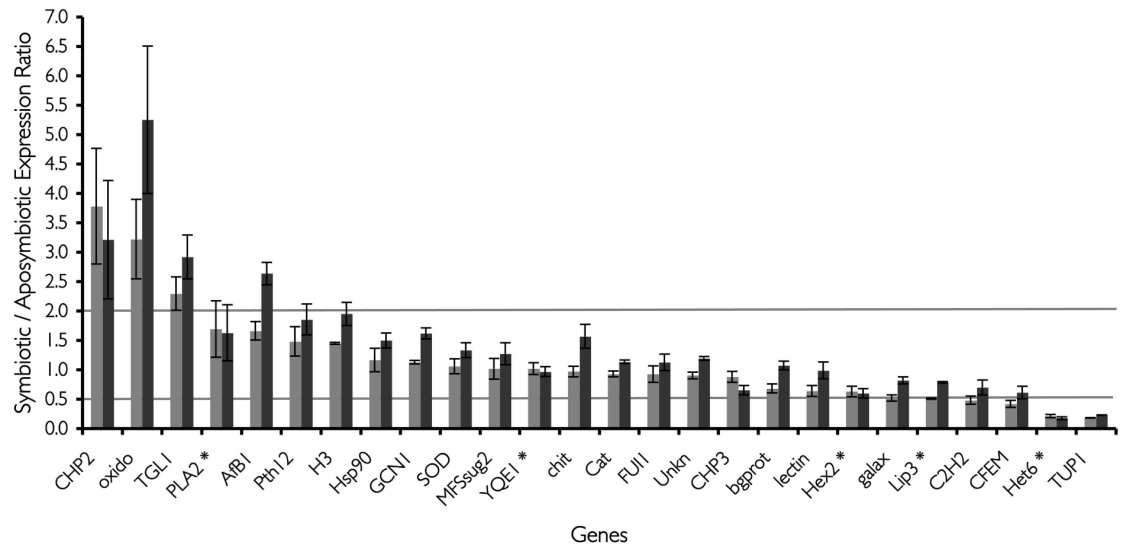


FIGURE 3.1: qPCR expression of 26 SSH-selected *Cladonia grayi* genes in stage 1 of lichen development (assayed after 9 days of symbiotic but segregated growth). Light grey bars correspond to expression levels normalized to actin, and dark grey bars to beta-tubulin. * denotes genes upregulated in stage 2 (and confirmed by qPCR), and tested by qPCR in stage 1. Standard error shown.

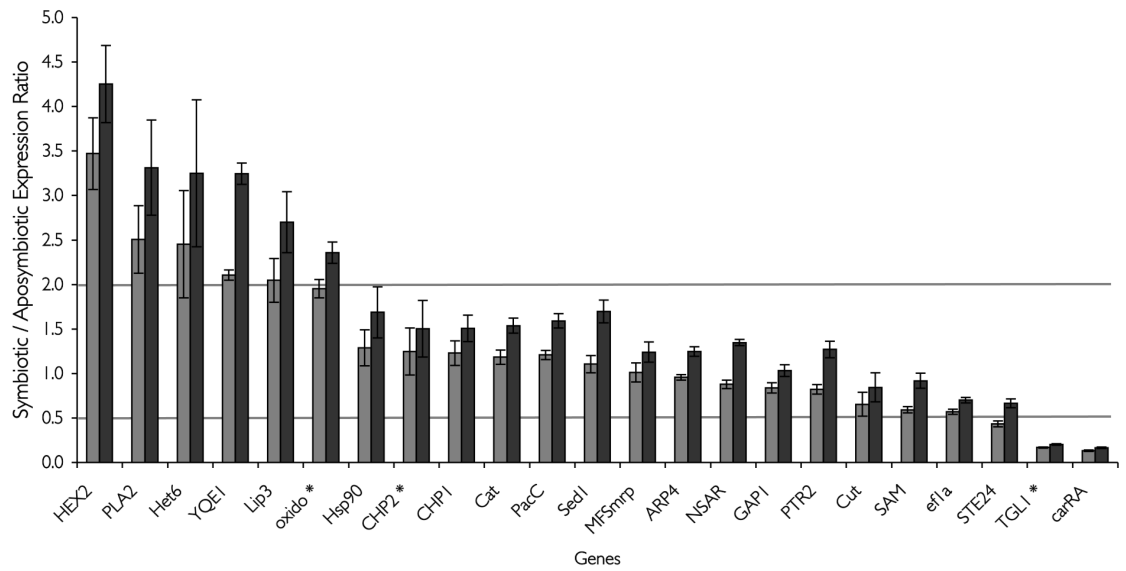


FIGURE 3.2: qPCR expression of 23 SSH-selected *Cladonia grayi* genes in stage 2 of lichen development (assayed after 21 days of symbiotic contact growth). Light grey bars correspond to expression levels normalized to actin, and dark grey bars to beta-tubulin. * denotes genes upregulated in stage 1 (and confirmed by qPCR), and tested by qPCR in stage 2. Standard error shown.

Table 3.1: Primers used in qPCR to determine relative proportions of mycobiont and photobiont in stage 2, and differential expression of select genes in stages 1 and 2.

Name	Primer	Sequence 5'-3'
Act	Clgr_Act_0218F	GAGAGCGAAACCCTCGTAGATTGGT
	Clgr_Act_0344R	CCCCGCTTTCTACGTCTCTATCCAG
AfB1	Clgr_AfB1_0129F	GAAAAGTAATCTGGCGGATCTGGAGA
	Clgr_AfB1_0228R	TAATTGGCCGTGGTAGGCTTGGTTA
ARP4	Clgr_ARP4_0035F	CGAACACAGAACCCGACTCTTCCTTC
	Clgr_ARP4_0117R	GACGGCTACGACGCTTTCTTTGAAT
β tub	Clgr_01_0105F	AGTGTGTGATCTGGAAACCTTGGAG
	Clgr_01_0210R	AAAGGTCATTACACAGAGGGTGCAG
bgprot	Clgr_bgprot_0284F	CGAATCCAAGCATCTATACTCCCTGA
	Clgr_bgprot_0428R	CAGCTCGTCCACTTTGCTCTTCTTT
C2H2	Clgr_C2H2_0068F	CTAAGACCTTTGTCTGCACCCTGTG
	Clgr_C2H2_0172R	TACACTCAAAGGGCTTGTCTGTGAGT
carRA	Clgr_carRA_0009F	CTCGACCGCTTTACGACCTGCTT
	Clgr_carRA_0105R	GCCGAAGGGCAGCTTCATCTTTGATA
Cat	Clgr_Cat_0011F	ATGACAGCCAAGGAGGCCGAAGAG
	Clgr_Cat_0119R	ATTCGCCAATCTTACGGAGAGGGAA
CFEM	Clgr_CFEM_0074F	CCAACAACAACATCACCTGTCTCTG
	Clgr_CFEM_0174R	TTGGCTGGTGTCTACGTCCTTC
chit	Clgr_chit_0222F	CCGTGAACCAGATCAGCGATGGACA
	Clgr_chit_0340R	GCTGGTGACAATAACTTGCTTGGTGCT
CHP1	Clgr_CHP1_0319F	GCCTAGTCATACCTCTGGTGGAGAT
	Clgr_CHP1_0435R	CCGAAATCACTGGAAGGAGGTGTGTT
CHP2	Clgr_CHP2_0295F	GTCGCTCCTGGTCCGAAGTCTAA
	Clgr_CHP2_0434R	CTTGGAAGCTAGGAAGGTCTCGAAT
CHP3	Clgr_CHP3_0102F	GCTGGTCGAGGTTATGCAAGACATAT
	Clgr_CHP3_0244R	TTCGATGAGGAATGCAACGGAGATT
Cut	Clgr_Cut_0191F	CAGCTATCACTTGGCTTATGCAGTCG
	Clgr_Cut_0291R	GCGAGCCGAAGTAGAAATGGAGTAA
efl α	Clgr_efl α _0333F	ATTCGAAGCTGGTATCTCCAAGGAC
	Clgr_efl α _0471R	AATCTCTTGATAACGCTCGCCAGAC
FUI1	Clgr_FUI1_0027F	GTCACCTCCGCACATAGCGTCA
	Clgr_FUI1_0138R	GCATGAACGAGAAAGCCACGAAGAA
galox	Clgr_galox_0446F	GATGCCATTCAGAAAGGAAGGTCT
	Clgr_galox_0585R	CAGAAGGTGTTGGTGTTGCCATC
GAP1	Clgr_GAP1_0250F	CATTCGATCTGGACCCATTGGAACA
	Clgr_GAP1_0349R	CAACTCCACACGCTTAATGCAATTTCAT
GCN1	Clgr_GCN1_0139F	GGCTGGAGGAGAGATTGATTACAAC
	Clgr_GCN1_0254R	ACGTGCCAGTCTTGTACCCGTATT
H3	Clgr_H3_0305F	GGGAATTCTAGCAACATACCATCTCG
	Clgr_H3_0431R	AGGCCGCCTAACTACATGATGTCTC
Het6	Clgr_Het6_0152F	CATGAGTTTCAAGGCTTCTCCATC
	Clgr_Het6_0262R	CCGAGCGGAATTACCAGGTGTCAATA
HEX2	Clgr_HEX2_0029F	CGTTAGACGTGCAATGTGGTCAAGAAGA
	Clgr_HEX2_0176R	CAC TAGGTGACCGAATCCCATTGTCT

Table 3.1 – Continued

Name	Primer	Sequence 5'–3'
Hsp90	Clgr_Hsp90_0141F	CAATCTTGGCCTTCTTCTCCTCATCC
	Clgr_Hsp90_0261R	TTCATTTCTTACCCCATCTACCTCCAT
lectin	Clgr_lectin_0011F	CAACCCAATGTGGTATCAGCGTATCTT
	Clgr_lectin_0124R	CTGGGAGGTGGCATCGGAGAA
Lip3	Clgr_Lip3_0086F	CGATGTGACTACCGTTTATGAGTTCGAG
	Clgr_Lip3_0232R	GGAAAGTCGGCATCTGCTAGGAAGTT
LSU – <i>Asterochloris</i>	AsspLSU250F	CCCTAAGTAGGTGGTAAATCC
	AsspLSU647R	CACCAGTCAGGTTACTTGCGT
LSU – <i>Cladonia</i>	ClgrLSU356F	TGTGACCTGCGATCAAGCCC
	ClgrLSU938R	CGATGATGCGCCCTTAAGGG
MepB	Clgr_MepB_0069F	GATGTGGGCTACTACGGCTACCTCT
	Clgr_MepB_0218R	CTCCATCTCATCCTGGCTTCCAC
MFS1mrp	Clgr_MFS1mrp_0013F	GGGATAGATGTTTCGAGATCGTTTCGACA
	Clgr_MFS1mrp_0160R	CGATACTAGCGATGTTGACCGTAGC
MFSsug2	Clgr_MFSsug2_0146F	GGATCGCACCCCTCGAAGATATTGA
	Clgr_MFSsug2_0297R	CAC TGATAATGGAGCTGTTGCGTCT
NSAR	Clgr_NSAR_0161F	CAGCGAGAGGAGATGAAGGCTAGAG
	Clgr_NSAR_300R	GCGCAACTGATGTGATAGCGGTGTTCT
oxido	Clgr_oxido_0322F	CGTGTCAACAGTATCTCGCCTGGTA
	Clgr_oxido_0472R	CAC TACAGAGTAAGATGACCGCTCCTG
pacC	Clgr_pacC_0119F	GCCATGCAGACA ACTATCTACGACA
	Clgr_pacC_0267R	GCATTGCTATAGTGAGCCGATGGTA
PLA2	Clgr_PLA2_0038F	GTTGTGATAGCACCTGAGCATCGAG
	Clgr_PLA2_0187R	ATTCCTACTGTCTGCGACGTTCTGA
Pth12	Clgr_Pth12_0241F	AGATGCTCTGTAGACGCACTGGACT
	Clgr_Pth12_0363R	TCTCACGTAGTCGAGTCTCAGCTTG
PTR2	Clgr_PTR2_0823F	CATCACTGCTGGCGTCTTCTGGATTT
	Clgr_PTR2_0912R	CTGCCTTCTCACCTTTGTCCTCAAG
SAM	Clgr_SAM_0239F	CCATGTGCAATACTCCAATGACAGCGAA
	Clgr_SAM_0370R	CGTCCAAGGCGATACGGTTATTGACT
Sed1	Clgr_Sed1_0039F	GCCCTTGCACTCTGACTCACAAGA
	Clgr_Sed1_0145R	GACAATGGTGGTAGCGAGAGGACA
SOD	Clgr_SOD_0500F	GGTGGGCATGAACAGAGTAAGGTGAC
	Clgr_SOD_0642R	TCACCTCATCATCCCTCATCACACTAA
STE24	Clgr_STE24_0121F	CCATCATCATTTGGGTTCTGTTGTTT
	Clgr_STE24_0225R	CAGCTTGGA ACTCAAATGTTCTGCTCA
TGL1	Clgr_TGL1_0428F	CACCGCTCCTTCTCAGTCATACATACC
	Clgr_TGL1_0549R	GGAGAAGAATCGCTGCGAGTTAATGC
Tup1	Clgr_Tup1_0070F	GACAGCGTCTATTCTGTTGCCTTTG
	Clgr_Tup1_0175R	TTCTTCGCGTATCCAGAGATAGTTG
Unkn	Clgr_Unkn_0097F	TCACATCCACAGATGGTGAGTGAGTA
	Clgr_Unkn_0233R	GGCAGGGCTACGAGTAATGTATGT
YQE1	Clgr_YQE1_0029F	CAACACCAGTAAGCTCAGAACCAGCAA
	Clgr_YQE1_0170R	CGGTCATGTCTTCGATGTTGGCATTGT

Table 3.2: Gene regions selected from stage 1 of lichen resynthesis, chosen for qPCR analysis. Putative Function determined by GenBank accession description and/or Uniprot description.

Acronym	Putative Function	GenBank Accession No.	Top GenBank blastx hit accession number and description
AfB1	Aflatoxin B1 aldehyde reductase	–	gi 119181595 ref XP_001242002.1 hypothetical protein CIMG_05898 [Coccidioides immitis RS]
bgprot	g-protein, beta-subunit	–	gi 150855716 gb EDN30908.1 guanine nucleotide-binding protein beta subunit [Botryotinia fuckeliana B05.10]
C2H2	C2H2 Transcription factor	–	gi 111434061 gb ABH09883.1 MrgA [Penicillium marneffei]
Cat	Catalase	–	gi 46122635 ref XP_385871.1 hypothetical protein FG05695.1 [Gibberella zeae PH-1]
CFEM	CFEM domain	–	gi 150858408 gb EDN33600.1 predicted protein [Botryotinia fuckeliana B05.10]
Chit	Chitinase	–	No significant match, cysteine rich
CHP2	Conserved hypothetical protein	–	gi 116197513 ref XP_001224568.1 hypothetical protein CHGG_06912 [Chaetomium globosum CBS 148.51]
CHP3	Integral membrane protein	–	gi 150852002 gb EDN27194.1 predicted protein [Botryotinia fuckeliana B05.10]
FUI1	Uracil Transporter	–	gi 111063482 gb EAT84602.1 hypothetical protein SNOG_08326 [Phaeosphaeria nodorum SN15]
galox	Galactose oxidase	–	gi 482303 pir A38084 galactose oxidase (EC 1.1.3.9) precursor [validated] - fungus (Cladobotryum dendroides)
GCN1	GPI-anchored membrane protein	–	gi 67525191 ref XP_660657.1 hypothetical protein AN3053.2 [Aspergillus nidulans FGSC A4]
H3	Histone 3	–	gi 150414169 gb EDN09534.1 histone H3 [Ajellomyces capsulatus Nam1]
hsp90	Heat Shock Protein 90	–	gi 150412789 gb EDN08176.1 ATP-dependent molecular chaperone HSC82 [Ajellomyces capsulatus Nam1]
lectin	Lectin	–	gi 111056115 gb EAT77235.1 hypothetical protein SNOG_15302 [Phaeosphaeria nodorum SN15]
Lip3	Lipase 3	–	gi 111065213 gb EAT86333.1 hypothetical protein SNOG_06502 [Phaeosphaeria nodorum SN15]
MFSsug2	Sugar transporter	–	gi 150853025 gb EDN28217.1 hypothetical protein BC1G_08140 [Botryotinia fuckeliana B05.10]
oxido	D-arabinitol dehydrogenase	–	gi 50552368 ref XP_503594.1 hypothetical protein [Yarrowia lipolytica] unnamed protein product [Yarrowia lipolytica CLIB122]
Pth12	Transcription factor	–	gi 67005921 gb AAY62596.1 Pth12p [Magnaporthe grisea]
SOD	Superoxide Dismutase	–	gi 51702016 sp Q8J0N3 SODC_PAETN Superoxide dismutase [Cu-Zn] copper-zinc superoxide dismutase [Paecilomyces tenuipes]
TGL1	Triacylglyceride lipase	–	gi 119183624 ref XP_001242824.1 hypothetical protein CIMG_06720 [Coccidioides immitis RS]
Tup1	WD repeat transcription factor	–	gi 119467836 ref XP_001257724.1 wd-repeat protein [Neosartorya fischeri NRRL 181]
unkn	Unknown	–	No significant match

Table 3.3: Gene regions selected from stage 2 of lichen resynthesis, chosen for qPCR analysis. Putative Function determined by GenBank accession description and/or Uniprot description.

Acronym	Putative Function	GenBank Accession No.	Top GenBank blastx hit accession number and description	
ARP4	Nuclear actin-related protein 4	–	gi 169769500 ref XP_001819220.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]
carRA	Carotene synthesis subunit	–	gi 46114448 ref XP_383242.1	hypothetical protein FG03066.1 [Gibberella zeae PH-1]
Cat	Catalase	–	gi 46122635 ref XP_385871.1	hypothetical protein FG05695.1 [Gibberella zeae PH-1]
CHP1	Conserved hypothetical protein	–	gi 156059736 ref XP_001595791.1	hypothetical protein SS1G_03881 [Sclerotinia sclerotiorum 1980]
Cut	Cutinase	–	gi 169772161 ref XP_001820550.1	hypothetical protein [Aspergillus oryzae RIB40] triacylglycerol lipase
ef1 α	Elongation Factor 1- α	–	gi 110666917 gb ABG81871.1	translation elongation factor-1 α [Canoparmelia caroliniana]
GAP1	Amino acid permease	–	gi 156035819 ref XP_001586021.1	hypothetical protein SS1G_13114 [Sclerotinia sclerotiorum 1980]
Het6	Heterokaryon Incompatibility 6	–	gi 171689038 ref XP_001909459.1	unnamed protein product [Podospora anserina] unnamed protein product [Podospora anserina]
HEX2	Negative regulator glucose repressible genes	–	gi 154309002 ref XP_001553836.1	hypothetical protein BC1G_08029 [Botryotinia fuckeliana B05.10]
Hsp90	Heat Shock Protein 90	–	gi 154280066 ref XP_001540846.1	ATP-dependent molecular chaperone HSC82 [Ajellomyces capsulatus NAM1]
Lip3	Lipase 3	–	gi 169606904 ref XP_001796872.1	hypothetical protein SNOG_06502 [Phaeosphaeria nodorum SN15]
MFS1mrp	MFS1 transporter	–	gi 154297213 ref XP_001549034.1	hypothetical protein BC1G_12442 [Botryotinia fuckeliana B05.10]
NSAR	Norsolorinic acid reductase	–	gi 164423792 ref XP_961973.2	norsolorinic acid reductase [Neurospora crassa OR74A]
pacC	pacC, transcription factor	–	gi 145233099 ref XP_001399922.1	transcription factor pacC-Aspergillus niger transcription factor pacC-Aspergillus niger
PLA2	Phospholipase 2	–	gi 187981059 gb EDU47685.1	platelet-activating factor acetylhydrolase precursor [Pyrenophora tritici-repentis Pt-1C-BFP]
PTR2	MFS Peptide transporter	–	gi 119193000 ref XP_001247106.1	hypothetical protein CIMG_00877 [Coccidioides immitis RS]
SAM	Sterile alpha motif protein	–	gi 156051186 ref XP_001591554.1	hypothetical protein SS1G_07000 [Sclerotinia sclerotiorum 1980]
Sed1	Cell wall glycoprotein	–	gi 51572467 gb AAU07726.1	Sed1-1p [Saccharomyces pastorianus]
STE24	Sterile24	–	gi 121706852 ref XP_001271650.1	CaaX prenyl protease Ste24 [Aspergillus clavatus NRRL 1] CaaX prenyl protease Ste24 [Aspergillus clavatus NRRL 1]
YQE1	Dioxygenase	–	gi 145231184 ref XP_001389856.1	hypothetical protein An01g14680 [Aspergillus niger] unnamed protein product [Aspergillus niger]

Table 3.4: SSH efficacy as determined by reciprocal tblastx queries of the top expressed genes to proteomes, using three alternative fungi. Percentage of subject top expressed genes found amongst the query's top tblastx hits listed, with numbers in parentheses. A. Stage 1. B. Stage 2.

A Query (Top 196 expressed genes)		Subject (proteome)		
		1	2	3
1	<i>Aspergillus nidulans</i>	100% (196)	33% (64)	45% (89)
2	<i>Neurospora crassa</i>	30% (58)	100% (196)	22% (44)
3	<i>Saccharomyces cerevisiae</i>	69% (136)	35% (68)	100%(196)
4	<i>Cladonia grayi</i>	12% (24)	7% (14)	10% (19)
B Query (Top 240 expressed genes)		Subject (proteome)		
		1	2	3
1	<i>Aspergillus nidulans</i>	100%(240)	33% (80)	45% (109)
2	<i>Neurospora crassa</i>	33%(78)	100%(240)	25% (61)
3	<i>Saccharomyces cerevisiae</i>	66% (158)	38% (92)	100%(240)
4	<i>Cladonia grayi</i>	9% (21)	5% (13)	8% (19)

Table 3.5: Average aposymbiotic and symbiotic qPCR expression levels of 26 *Cladonia grayi* genes after 9 days (stage 1 of lichen development) relative to actin and beta-tubulin. Genes marked with asterisk were tested because of their upregulation in stage 2. Standard error shown.

Gene		actin			beta-tubulin		
Afb1	apo	0.29	±	0.05	0.20	±	0.02
	sym	0.47	±	0.02	0.52	±	0.02
bgprot	apo	13.16	±	0.85	9.06	±	0.19
	sym	8.94	±	1.66	9.73	±	1.14
C2H2	apo	10.02	±	2.06	10.11	±	1.58
	sym	4.61	±	0.66	6.90	±	1.85
Cat	apo	0.86	±	0.08	0.87	±	0.04
	sym	0.79	±	4.14E-03	0.99	±	0.01
CFEM	apo	1.80	±	0.38	1.81	±	0.29
	sym	0.72	±	0.1	1.08	±	0.28
chit	apo	1.03	±	0.16	0.72	±	0.14
	sym	0.98	±	0.03	1.08	±	0.11
CHP2	apo	0.05	±	0.01	0.09	±	0.02
	sym	0.17	±	0.07	0.27	±	0.13
CHP3	apo	0.50	±	0.36	0.74	±	0.53
	sym	0.43	±	0.05	0.48	±	0.08
FUI1	apo	2.34	±	0.61	2.35	±	0.50
	sym	2.03	±	0.01	2.54	±	0.03
galax	apo	13.40	±	0.11	9.25	±	0.47
	sym	6.97	±	1.30	7.58	±	0.9
GCN1	apo	1.12	±	0.02	1.14	±	0.03
	sym	1.26	±	0.05	1.84	±	0.17
H3	apo	7.30	±	0.03	7.86	±	0.33
	sym	10.58	±	0.19	15.31	±	2.58
Het6 *	apo	0.02	±	0.01	0.04	±	8.95E-03
	sym	5.00E-03	±	1.43E-04	0.01	±	2.30E-04
HEX2 *	apo	0.15	±	7.39E-04	0.23	±	7.73E-03
	sym	0.10	±	0.02	0.14	±	0.03
Hsp90	apo	1.92	±	0.28	2.05	±	0.21
	sym	2.19	±	0.55	3.04	±	0.32
lectin	apo	13.65	±	0.40	14.68	±	0.24
	sym	8.75	±	2.18	14.52	±	3.62
Lip3 *	apo	0.11	±	3.54E-03	0.12	±	1.55E-03
	sym	0.06	±	1.19E-03	0.09	±	1.96E-03
MFSsug2	apo	4.51	±	0.25	6.22	±	0.19
	sym	4.57	±	1.35	7.91	±	2.02
oxido	apo	0.07	±	0.03	0.05	±	0.02
	sym	0.21	±	5.74E-03	0.23	±	0.02
PLA2 *	apo	0.05	±	0.02	0.08	±	0.03
	sym	0.08	±	0.02	0.11	±	0.03
Pth12	apo	0.25	±	0.02	0.35	±	0.02
	sym	0.37	±	0.11	0.65	±	0.16
SOD	apo	4.80	±	0.19	6.63	±	0.09
	sym	5.08	±	1.05	8.83	±	1.45
TGL1	apo	0.12	±	0.02	0.16	±	0.04
	sym	0.26	±	0.02	0.45	±	0.02
TUP1	apo	0.96	±	1.22E-03	1.33	±	0.04
	sym	0.18	±	0.01	0.31	±	1.26E-03
unkn	apo	29.45	±	0.61	31.68	±	0.80
	sym	26.61	±	2.80	37.80	±	1.75
YQE1 *	apo	0.05	±	3.05E-03	0.08	±	2.30E-03
	sym	0.05	±	8.19E-03	0.07	±	0.01

Table 3.6: Average aposymbiotic and symbiotic qPCR expression levels of 27 *Cladonia grayi* genes after 21 days (stage 2 of lichen development), relative to actin and beta-tubulin. Genes marked with asterisk were tested because of their upregulation in stage 1. Standard error shown.

Gene		actin			beta-tubulin		
ARP4	apo	0.11	±	0.01	0.16	±	0.01
	sym	0.11	±	4.44E-03	0.20	±	0.02
carRA	apo	0.58	±	0.04	0.77	±	0.04
	sym	0.08	±	6.25E-03	0.13	±	9.37E-03
Cat	apo	1.44	±	0.1	2.05	±	0.14
	sym	1.70	±	0.26	3.15	±	0.36
CHP1	apo	2.67	±	0.51	3.50	±	0.82
	sym	3.17	±	0.12	5.13	±	0.23
CHP2 *	apo	0.05	±	4.83E-03	0.08	±	0.01
	sym	0.07	±	0.02	0.12	±	0.04
Cut	apo	23.83	±	2.35	33.99	±	3.29
	sym	15.51	±	7.64	28.55	±	13.0
ef1 α	apo	6.00	±	0.27	7.89	±	0.16
	sym	3.42	±	0.27	5.54	±	0.40
GAP1	apo	1.05	±	0.03	1.38	±	1.18E-03
	sym	0.88	±	0.10	1.42	±	0.16
Het6	apo	0.01	±	5.89E-03	0.02	±	0.01
	sym	0.03	±	7.19E-04	0.05	±	9.67E-04
HEX2	apo	0.07	±	0.01	0.09	±	0.01
	sym	0.24	±	0.02	0.38	±	0.03
Hsp90	apo	1.52	±	0.28	2.17	±	0.4
	sym	1.93	±	0.65	3.61	±	1.34
Lip3	apo	0.31	±	0.05	0.42	±	0.08
	sym	0.61	±	0.07	1.10	±	0.12
MFSmrp	apo	0.05	±	9.14E-03	0.07	±	0.01
	sym	0.05	±	3.00E-03	0.08	±	0.00
NSAR	apo	0.30	±	0.02	0.36	±	0.02
	sym	0.26	±	0.01	0.49	±	2.96E-03
oxido *	apo	0.03	±	1.7E-03	0.05	±	1.30E-03
	sym	0.06	±	5.23E-03	0.12	±	9.71E-03
pacC	apo	0.62	±	0.04	0.86	±	0.07
	sym	0.75	±	0.02	1.35	±	0.02
PLA2	apo	0.25	±	0.07	0.35	±	0.1
	sym	0.58	±	2.33E-03	1.06	±	0.01
PTR2	apo	0.60	±	0.05	0.74	±	0.09
	sym	0.49	±	0.03	0.93	±	0.02
SAM	apo	0.75	±	0.07	0.92	±	0.11
	sym	0.44	±	0.02	0.83	±	0.08
Sed1	apo	11.98	±	0.51	14.73	±	1.16
	sym	13.21	±	1.96	24.83	±	2.58
STE24	apo	1.35	±	0.11	1.66	±	0.19
	sym	0.58	±	0.06	1.09	±	0.06
TGL1 *	apo	0.16	±	6.39E-03	0.25	±	0.01
	sym	0.03	±	1.04E-03	0.05	±	1.98E-03
YQE1	apo	0.07	±	1.98E-03	0.09	±	0.00
	sym	0.15	±	5.86E-03	0.29	±	1.98E-03

Gene expression of *Asterochloris* sp. in early developmental stages of lichen-symbiosis.

4.1 Introduction

The kingdom Chloroplastida is ubiquitous in terrestrial, aquatic, and oceanic habitats, and encompasses the diversity of all green algal lineages and land plants (Adl *et al.*, 2005; Lewis & McCourt, 2004). It has been proposed that land plants were able to evolve through fungal symbioses with green algae (Pirozynski & Malloch, 1975; Selosse & Le Tacon, 1998). However a basic understanding of green algal – fungal symbiotic relationships is missing. A number of plant and fungal symbioses are dynamic and can be found adopting a pathogenic or mutualistic state, depending on the environmental conditions or combination of partners. Therefore, understanding one form of these symbioses contributes to our understanding of all plant and fungal

symbioses in general. One basic question that remains to be addressed is the evolution of symbiosis related signaling pathways. Do green algae mount conserved or specific signalling pathway responses to symbiotic fungi they encounter?

The nature of the lichen symbiosis has been debated ever since the discovery that lichens are made of mycobionts (filamentous fungi) and photobionts (green algae, cyanobacteria, or both) (Ahmadjian & Jacobs, 1981; Peirce, 1900; Schwendener, 1869). The extent to which mycobionts and photobionts are mutualists, or that mycobionts are controlled parasites of photobiont hosts, remains to be determined. Because of this ambiguity, it is not clear where to search for candidate genes of green algal symbiosis, whether amongst mutualistic or pathogenic Chloroplastida – fungal symbioses. A concurrent study investigating *Cladonia grayi* differential gene expression in early lichen development recovered genes putatively involved in fungal self and non-self recognition, lipid metabolism, negative regulation of glucose repressible genes, a D-arabitol reductase, and two dioxygenases (Joneson *et al.*, 2009). These genes do not readily offer an obvious answer to the question on the nature of the lichen symbiosis, or which kinds of algal genes might be expected to play a role in lichen symbiosis.

In addition to the lack of green algal molecular research, there are very few green algal genomes to survey. Taking a broader perspective, there are only four

non-vascular plant Chloroplastida genomes currently published: the “green-yeast” *Chlamydomonas reinhardtii*, two closely related marine green algal species chosen for their unusually small genomes, *Ostreococcus lucimarinus* and *O. tauri*, and the moss *Physcomitrella patens* (Derelle *et al.*, 2006; Merchant *et al.*, 2007; Palenik *et al.*, 2007; Rensing *et al.*, 2008). Based on the genes that land-plants express in response to pathogens or mutualists, and based on the absence of the homologues of these genes in their genomes, it appears that green algae may respond to pathogens and mutualists in novel ways. Most pathogenesis-related proteins expressed in land-plants are induced through the cell signaling compounds salicylic acid (SA), jasmonic acid (JA), or ethylene (van Loon *et al.*, 2006). Furthermore, a reduction in the signalling of the plant hormone auxin is known to induce resistance to bacterial and fungal pathogens (Domingo *et al.*, 2009; Navarro *et al.*, 2006). JA, ethylene and auxin pathway components are conserved in *P. patens*, although not necessarily to the extent known in vascular plants, and their expression and roles in moss remain to be determined. More importantly, these pathways are absent from any of the above non-vascular Chloroplastida genomes. It is not clear if green algae retain the ability to synthesize salicylic acid or related compounds or use them in signalling. Curiously, although the genomes of lichen-forming green algae (and an additional green alga *Ulva intestiformis*) haven’t been sequenced, these organisms have

been shown to produce ethylene in culture (Plettner *et al.*, 2005; Schieleit & Ott, 1996). Jasmonic acid, the synergistic partner of ethylene in land-plant resistance, has not been reported from lichen-forming algae. Auxin has been reported from an intact lichen (Epstein *et al.*, 1986), although the source of this hormone could be of either algal or fungal origin, or both, as fungi are known to produce auxins (Gruen, 1959; Strzelczyk & Pokojakaburdziej, 1984). It therefore remains possible that lichen-forming green algae use signalling pathways and mechanisms similar to land-plants, and respond in a way that does not rely upon jasmonic acid.

What genes, therefore, are instrumental in regulating symbioses between green algae and fungi? The first two stages of lichen development were surveyed for algal and fungal genes upregulated in early symbiosis, and the fungal portion has been presented elsewhere (Joneson *et al.*, 2009). The development of a stratified lichen thallus can be broadly divided into four stages: pre-contact (when organisms are close enough to potentially sense each other, but do not physically touch), contact and growth together, growth into an undifferentiated mass, and growth and differentiation of a stratified thallus (Ahmadjian *et al.*, 1978; Armaleo, 1991; Galun, 1988). Using the *Asterochloris* sp. (Chlorophyta, *fide* [Adl *et al.*, 2005])–*Cladonia grayi* (Ascomycota) symbiosis, suppression subtractive hybridization (SSH) was employed to survey for algal genes upregulated early in stages 1 and 2 of lichen development,

and quantitative PCR (qPCR) to verify differential expression in a subset of these genes.

4.2 Materials and Methods

Asterochloris sp. and *Cladonia grayi* were grown separately, and together, on nitrocellulose membranes (pore-size = $0.74\mu\text{m}$) over a solid and reduced-nutrient medium. Stage 1 was simulated by growing *Asterochloris* sp. and *C. grayi* separated by a nitrocellulose membrane for nine days under conditions of 12 hours light and 12 hours dark. Stage 2 was simulated by growing a homogenous mixture of *Asterochloris* sp. and *C. grayi* under the same light conditions for 21 days. This time frame was determined by Joneson & Lutzoni (2009). For the complete experimental setup, RNA extraction, suppression subtractive hybridization (SSH), library construction, clone sequencing and analysis, and quantitative PCR (qPCR) see Joneson *et al.* (2009). Primers used in qPCR can be found in Table 4.1. Actin (act) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were both used as qPCR control genes.

4.2.1 Database inquiries

GenBank and UniProt (Bairoch *et al.*, 2008) were used for the initial annotation of sequences, and were accessed on March 4th, 2009. Protein coding sequences in

the proper reading frame were subsequently parsed from the blastx output and used to search the UniProt database (www.ebi.uniprot.org) of protein sequence and function using blastp, and to assign Gene Ontology (GO) annotations (Ashburner *et al.*, 2000) using Blast2GO (Conesa *et al.*, 2005) with an e-value $< 10^{-5}$. Unless otherwise noted, datasets of blastx and Blast2GO results were managed using customized PERL and BioPERL scripts (Stajich *et al.*, 2002). For a subset of gene fragments InterProScan was used to determine the presence of conserved protein domains (Hunter *et al.*, 2009). SignalP3.0 (Bendtsen *et al.*, 2004; Nielsen *et al.*, 1997) and TargetP1.1 (Emanuelsson *et al.*, 2000) were used to determine if signal peptide domains were found in gene sequences containing the 5' coding region of the gene.

4.2.2 Determining stage 2 organism-of-origin for gene fragments

The genome of origin (fungal, algal, or “either”) in stage 2 was determined for gene sequences by manually classifying the top five blastx hits (if available). Sequences were labelled as being of algal origin if the top hits matched either all Chloroplastida, or Chloroplastida and cyanobacteria, or if the gene sequence was present in the exclusively algal stage 1 library. Sequences were identified as fungal if the top hits were to other fungal sequences. The remainder of sequences was classified as “either”. See also Joneson *et al.* (2009)

4.2.3 Searching the genome for possible duplicate genes of qPCR selected genetic regions.

To determine if the SSH generated cDNAs used in qPCR were part of gene families or of duplicated origin in the genome, an unpublished genome of *Asterochlo-
ris* sp. (approximately 2X coverage, accessed 21 April 2009) was searched using qPCR primer and SSH-cDNA nucleotide sequences. Primer sequences were searched against the genome using blastn. When either the forward or reverse primers for a given pair matched the genome identically over 75% of their nucleotide sequence (arbitrarily chosen as the cut-off point for which mis-matched primers will unsuccessfully anneal at the annealing temperature of 65° C, and therefore will not be able to successfully extend or amplify DNA sequences), the genome was searched using the the tblastx algorithm and the entire corresponding SSH-cDNA nucleotide sequence. Regions of genomic contigs corresponding to the second best match to any given SSH-cDNA were subsequently extracted, and the associated primers were attempted to be aligned to the genomic sequence using Sequencher and low assembly parameters (at least 15 nucleotides, with 65% identity).

4.3 Results

4.3.1 Library construction and gene annotation

A stage 1 specific SSH-cDNA library for *Asterochloris* sp. containing 480 randomly chosen clones was constructed. Four hundred and thirty five total sequences, composed of 267 contigs and 168 individual reads, contained non-vector inserts that ranged from 100bp to 1173bp in length. A blastx comparison of these sequences against the GenBank nr database of protein sequences resulted in 134 (31%) with a significant similarity (e-value $< 10^{-5}$) to protein encoding genes.

A stage 2 SSH-cDNA library was constructed containing 960 clones. These results have been partially reported in Joneson *et al.* (2009), and a brief summary follows. Eight hundred and seventy four clones, containing 806 contigs and 68 individual reads, contained non-vector inserts ranging in length from 100bp to 1515bp. Only clones with non-vector inserts over 100bp in length were considered further, using blastx to compare these sequences against the GenBank nr protein database. Four-hundred and seventy-five stage 2 sequences had a significant match (e-value $< 10^{-5}$) to protein encoding genes. Of these, 134 were of algal origin, 316 were of fungal origin, and 25 were classified as “Either”. Sequences will be deposited in NCBI’s Trace Archives database.

A summary of the top GenBank and UniProt matching entries for all Clone IDs

from stages 1 and 2 can be found in Tables B.1 and B.2, respectively. GO terms were organized by organizing principle (biological process, cellular compartment and molecular function), and the first three levels of GO terminology for stages 1 and 2 can be seen in Tables B.3 and B.4. A key linking GO terms to Clone IDs can be found in Tables B.5 and B.6.

4.3.2 qPCR results of select genes

qPCR was performed to confirm differential expression of 18 and 17 algal genes in the first and second stages of lichen synthesis, respectively. A list of these genes, their putative function, and their top GenBank hit can be found in Tables 4.2 and 4.3.

qPCR candidate genes were chosen based on their GenBank and UniProt definitions extracted from the two libraries, on their putative function in plant and fungal interactions or general stress and defense responses, as well as genes of unknown function. Background amplification of no reverse-transcriptase (NRT) controls of all genes was at least 32-fold lower than with reverse-transcriptase.

Relative expression levels of four putative control genes were inspected, as there was no prior knowledge of the expression levels of these genes: actin (act), cytochrome B (CytB) the rubisco small subunit (RbcS), and glyceraldehyde-3-phosphate (GAPDH). Each of the four algal control genes were normalized to each other to look for consistencies in magnitude of expression level. As RbcS is expressed at

levels over 20 times the level of actin (which in itself is much higher than most of the genes being tested herein), RbcS was not further considered as a control gene. Algal act and GAPDH behaved in a relatively similar manner across both experimental replicates in both treatments when compared to each other. On average, GAPDH is expressed 1.14 ± 0.08 times the expression level of act in the aposymbiotic state, and 1.09 ± 0.20 in the symbiotic state (standard error reported).

Stage 1 qPCR

Of 18 genes tested in stage 1 (Figure 4.1), two genes (chit, a chitinase related gene, and HPPD, a putative 4-hydroxyphenylpyruvate dioxygenase) show at least a two-fold increase in expression level in the symbiotic state. Of the remaining genes, 13 (71%) did not change in overall expression level (expression differential between 0.5 and 2), and 3 (18%; acet, amy, per) decreased. Two genes from the stage 2 SSH library were tested as part of this set (anm and DRP), but did not show more than a twofold increase (Figure 4.1). Relative expression levels of stage 1 genes are shown in Table 4.4. The highest expressed gene, a putative cell wall associated hydrolase (hydro), is expressed 29.47 to 83.28 times higher than both control genes. The lowest expressed gene, amylase (amy), is expressed 0.001 to 0.01 times the expression level of both controls.

Stage 2 qPCR

Of 17 algal genes tested in stage 2 (Figure 4.2), a putative arginine methyltransferase (anm), and a putative dynamin related protein (DRP) show at least a twofold increase in expression level. Of the remaining genes, 13 (71%) did not change in expression, and two decreased in expression (HPPD, NFU). Two genes from the stage 1 SSH library were tested as part of this set (chit and HPPD), but did not show more than an average 2 fold increase in expression (Figure 4.2). Relative expression levels of stage 2 genes are shown in Table 4.5. RbcS is expressed at levels 23.84 to 46.54 times that of the both controls. HPPD is the lowest tested expressed gene in stage 2 at 6.16E-04 to 2.62E-02 times the level of actin and GAPDH.

4.3.3 Conserved protein domains.

The gene sequence chit contains the 5' end of the gene, and belongs to the glycoside hydrolase family of proteins (IPR017853) containing a glycoside hydrolase core sequence (SSF51445), two unintegrated InterProScan chitinase domains (PTHR11177 and PTHR11177), and a signal peptide. SignalP3.0 predicts a signal peptide with a cleavage site between nucleotides 21 and 22, with probability 0.99. TargetP1.1 predicts the chit gene product to be extracellularly secreted, with a reliability score of 2 (on a scale of 1 to 5, with 1 indicating the strongest pre-

diction). The gene sequence HPPD contains a 4-hydroxyphenylpyruvate dioxygenase domain (PTHR11959), and two InterProScan unintegrated domains of, 1) no description (G3DSA:3.10.180.10), and 2) a glyoxalase/bleomycin resistance protein/Dihydroxybiphenyl dioxygenase (SSF54593). The DRP gene region belongs to the Ras-like-GTPase superfamily of proteins, and contains the following dynamin related annotations: DYNAMIN (PR00195), *Dynamain_N* (PF00350), DYNc (SM00053), and DYNAMIN (PS00410). An additional, unintegrated InterProScan domain is the P-loop containing nucleoside triphosphate hydrolases (SSF52540). The gene sequence *anm* belongs to the NADB-Rossman superfamily of proteins, and contains only the InterProScan non-integrated domains of : no description (G3DSA:2.70.160.11 and G3DSA:3.40.50.150), the protein arginine N-methyltransferase (PTHR11006), and the S-adenosyl-L-methionine-dependent methyltransferases (SSF53335). The *hydro* gene region contains the following InterProScan unintegrated sequences: CW-hydrolase (PRODOM, PD293281 and PFAM PF10695), and a signal peptidase. SignalP3.0 predicts a signal peptide in *hydro* with a most likely cleavage site between positions 16 and 17, and in TargetP1.1 this protein is expected to be targeted to the mitochondrial membrane with a reliability class of 3.

4.3.4 SSH-cDNAs used in qPCR are preliminarily from single copy genes.

Of the 32 algal primer pairs used in qPCR, 28 pairs contained forward and reverse primers that were less than 75% identical to any sequence in the genome. Four primer pairs contained one primer with less than an 85% match (the primer pairs for acet, anm, bglu, and PII). In each of these four latter cases, it was impossible to align the primer pairs with the corresponding genomic region (extracted from the second best tblastx hit of the corresponding SSH-cDNA). In addition, each primer pair amplified only one band of uniform size from genomic DNA (as opposed to more than one band, which is presumed to take place if the primer pair were amplifying from both a target and non-target regions of the genome). Therefore, the primer pairs used in qPCR were all considered to be amplifying only one gene region, and the genes corresponding to the qPCR SSH-cDNA regions are preliminarily considered as single copy genes.

4.4 Discussion

The genetic programs that regulate Chloroplastida responses to fungal presence have been exclusively investigated using land-plants. This study takes the first foray outside of the land-plant lineage to investigate differential expression in the green alga *Asterochloris* sp. in response to the filamentous ascomycete, *Cladonia grayi*.

SSH was used to find symbiosis upregulated genes in the first two stages of lichen development, and qPCR to subsequently verify the expression level of symbiosis-related-candidate genes. Genes upregulated in *Asterochloris* sp. prior to physical contact with *C. grayi* include a putative chitinase-related gene and an amino acid transporter. *Asterochloris* sp. genes upregulated once the alga and fungus have established contact include a protein arginine methyltransferase, and a dynamin related protein.

Chitinases (EC 3.2.1.14) hydrolyze the β -1,4-glycoside bond found in chitin, and chitin-like molecules found in fungal cell walls and bacterial Nod factors (Kasprzewska, 2003). The *Asterochloris* chit gene sequence is upregulated in stage 1 of lichen development, and belongs to the GH18 chitinase-like superfamily of glycosyl hydrolases. Although it is not known if chit in *Asterochloris* is acting as a chitinase, it raises the exciting possibility that chitin signalling may play a role in lichen development. In symbiotic interactions with some fungi and bacteria, the hydrolyzed targets of secreted Chloroplastida chitinases can act as signals in a receptor mediated pathway that a symbiont is present (Kasprzewska, 2003). The putative chitinase in *Asterochloris* sp. is upregulated in stage 1, pre-contact, and not differentially expressed in symbiosis stage 2, initial contact, suggesting that chitin signaling is important only in the very initial interaction between symbionts. This pattern

is similar to that seen in *Lotus japonicus* after it has been grown with either of its mutualistic partners –the fungus *Glomus mossae*, or the bacterium *Rhizobium* (Deguchi *et al.*, 2007). The possibility that *Asterochloris* can use chitin signalling can be tested through the application of purified chitooligosaccharides to the green alga in culture, and assaying the expression of either putative downstream genes or stage 1 differentially expressed genes.

The HPPD gene sequence from *Asterochloris* sp. is upregulated in stage 1 (Figure 4.1), and contains the InterPro domain 4 - hydroxyphenylpyruvate dioxygenase (IPR005956), which is a dioxygenase involved in tyrosine and phenylalanine metabolism (Moran, 2005). In GenBank this conserved domain is further annotated as an amino acid transport component, based on general function prediction only. It is not immediately clear what the function of HPPD is in *Asterochloris* sp. or lichen development. Homologues of this gene product in *Chlamydomonas reinhardtii* and *Arabidopsis thaliana* are not predicted to be secreted or targeted to an organellar membrane, and therefore this enzyme presumably functions in the cytoplasm. Therefore it can be assumed that the up-regulation of *Asterochloris* sp. HPPD in stage 1 is in response to extracellular signals it is receiving from *Cladonia grayi*, and that the results of this signalling have ended by stage 2, when HPPD is downregulated.

The DRP gene is upregulated in stage 2 of lichen development (Figure 4.2), and

contains a GTPase domain. Dynamin-related-proteins are involved in mitochondrial division, and dynamins in general function in receptor-mediated endocytosis (Praefcke & McMahon, 2004; Sever *et al.*, 1999). The partial *Asterochloris* DRP cDNA encodes the first 170 amino acids of the protein product, but is not enough sequence to allow us to distinguish between the dynamin-related, and the classic dynamin protein products. The participation of this gene's product in the regulation of mitochondrial division in symbiosis, or in the uptake of receptors and ligands involved in symbiotic signalling, remains to be tested.

The *anm* gene sequence in *Asterochloris* sp. is upregulated in stage 2 (Figure 4.2), and shows high similarity with other protein arginine methyltransferases in GenBank, such as with *Chlamydomonas reinhardtii* (XP_001702822.1) and *Arabidopsis thaliana* (NP_563720.1). The function of arginine methylation has mainly been investigated in histone modifications and epigenetics, although it has also been shown to be involved in RNA processing and export, translation, signal transduction, DNA repair, and protein – protein interactions (Pahlich *et al.*, 2006). The upregulation of *anm* in stage 2 of lichen development, and its possible role in either down-regulation of algal genes as observed in Trembley *et al.* (2002c), or as a cell-signaling response of the alga to its fungal envelopment in stage 2, are two hypotheses that can be tested in the future. Discovering the role of *anm* in *Asterochloris* sp. lichen symbiosis will

start with determining the protein targets of anm in *Asterochloris* sp.

A total of six genes are downregulated in either stage 1 or 2: acet, amox, amy, hydro, NFU, and per. The expression of hydro stood out from amongst the rest because of its overall high expression level (Table 4.4). The gene fragment hydro was chosen as a candidate gene based on its UniProt annotation as a cell wall associated hydrolase, and its frequent similarity to GenBank entries of the same annotation. Upon further inspection this is a dubious annotation that cannot be confirmed, as noted in the corresponding Pfam10695 entry. Based on additional mRNA sequencing and an unpublished sequence of the *Asterochloris* genome, the hydro gene sequence is a pseudogene of mitochondrial origin (data not shown). Many TAA and TGA stop codons can be found throughout hydro and other mitochondrially encoded genes that have no eukaryotic homologues, while mitochondrially encoded homologues of COX1 and ATP synthase F0 subunit 8 both contain complete open reading frames. Whether the hydro down-regulation is a result of differential gene regulation, or a reduced mitochondrial to nuclear genome ratio as a result of symbiosis, cannot be determined at this time. It is not immediately apparent what the role of the remaining 5 downregulated genes could play in the development of the lichen symbiosis.

Experiments in this paper were designed to determine *Asterochloris* sp. genes upregulated in early lichen symbiosis, and in learning if any of the genes or signalling

pathways conserved in land-plant – fungal symbioses were relevant to green algal – fungal symbiosis. However, in addition to the low number of true positives recovered through SSH and verified by qPCR, our ability to select candidate genes was limited by the low number of *Asterochloris* sp. gene sequences with homology to proteins of known function. (For example, only 31% of the gene sequences recovered from stage 1 had significant homology [expectation value $< 1 \times 10^{-5}$] to the non-redundant set of proteins in GenBank. This is in contrast to a similar study [Joneson *et al.* 2009], where 50% of *Cladonia grayi* SSH generated cDNAs had a significant match in GenBank.) This highlights an overall shortage of green algal gene information available in public databases, and warrants further green algal based genetic studies and genome sequencing.

In addition to the results of this study, the results of a concurrent investigation in to the upregulated fungal genes in the *Asterochloris* sp. – *Cladonia grayi* symbiosis (Joneson *et al.*, 2009) do not provide obvious comparisons with land-plant – fungal pathogen symbiosis. The previous study recovered fungal genes putatively involved in self and non-self recognition, lipid metabolism, negative regulation of glucose repressible genes, a D-arabitol reductase, and two dioxygenases. Although extracellular lipases such as that found in Joneson *et al.* (2009) have been implicated in the pathogenicity of fungal phytopathogens, their putative plant derived cuticular target

is absent from terrestrial green algae (Raven, 1977). If appropriate target lipids for extracellular fungal lipases exist in green algal cell walls or membranes, remains to be determined. Furthermore, there is no significant source of extracellular glucose in *Asterochloris* sp. in present or past investigations that might act as a source for glucose repressible genes in *C. grayi*. Ribitol is the known molecule of carbohydrate currency in the lichen symbiosis (Green & Smith, 1974; Richardson & Smith, 1968; Richardson *et al.*, 1968), and so far glucose-repression of alternative carbohydrate metabolism genes has not been linked to ribitol signalling. There are no obvious roles for the upregulated fungal derived dioxygenases, nor the algal derived *anm*, *drp* or *HPPD* genes in early lichen symbiosis development. In both algal and fungal SSH-cDNA libraries for stages 1 and 2, relatively low numbers of genes were upregulated when verified with qPCR (11% - 28%), although the number of upregulated fungal genes tended to be higher than in the alga (16% and 28% in *C. grayi*, as opposed to 11% and 12% in *Asterochloris*). These results suggest that an alternative method of transcriptome sequencing, such as in 454-sequencing, would be more effective in future gene-expression studies.

To our knowledge, none of the *Asterochloris* genes upregulated in this study, except for *chit*, have been shown to play a role in land-plant and fungal interactions. This, combined with the observation that the published genomes of non-vascular

Chloroplastida lack the conserved signaling mechanisms that define our current understanding of the Chloroplastida response to fungi, suggests that green algae use original mechanisms in response to fungi. The results of this study show that *Asterochloris* sp. is able to respond to *C. grayi* prior to physical contact, and that green algal upregulated genes are specific to a developmental stage. These sequences will be useful for future studies investigating gene function, and as stage specific markers in lichen developmental assays.

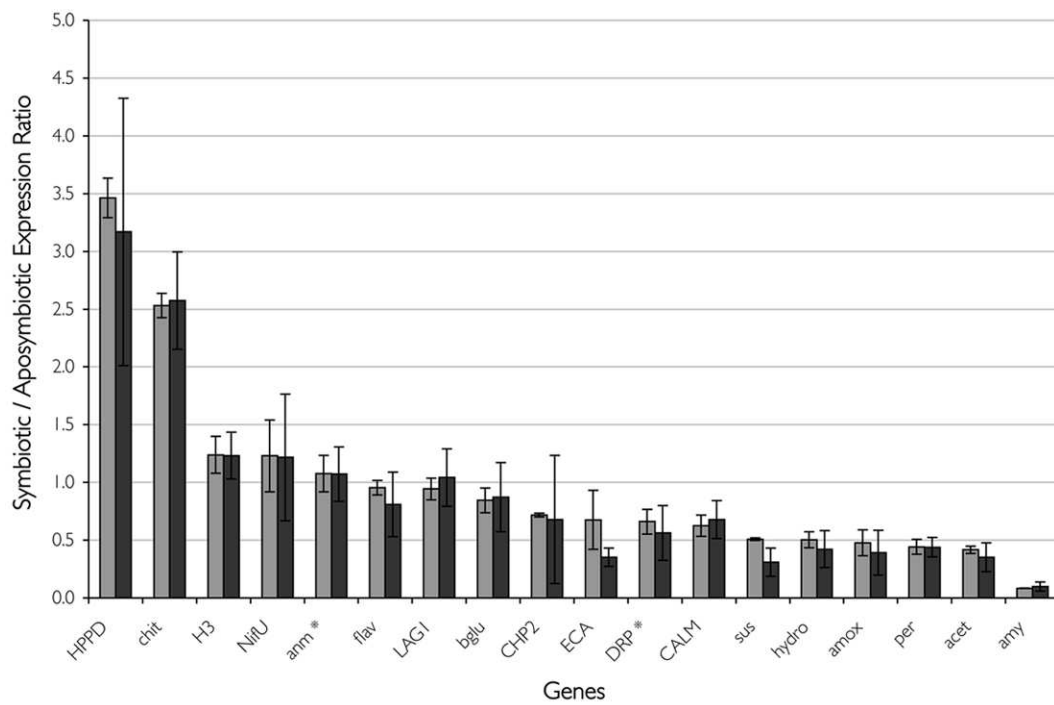


FIGURE 4.1: qPCR expression of 18 SSH-selected *Asterochloris* sp. genes in stage 1 of lichen development (assayed after 9 days of symbiotic but segregated growth). Light grey bars correspond to expression levels normalized to actin, and dark grey bars to GAPDH. * denotes genes upregulated in stage 2 (and confirmed by qPCR), and tested by qPCR in stage 1. Standard error shown.

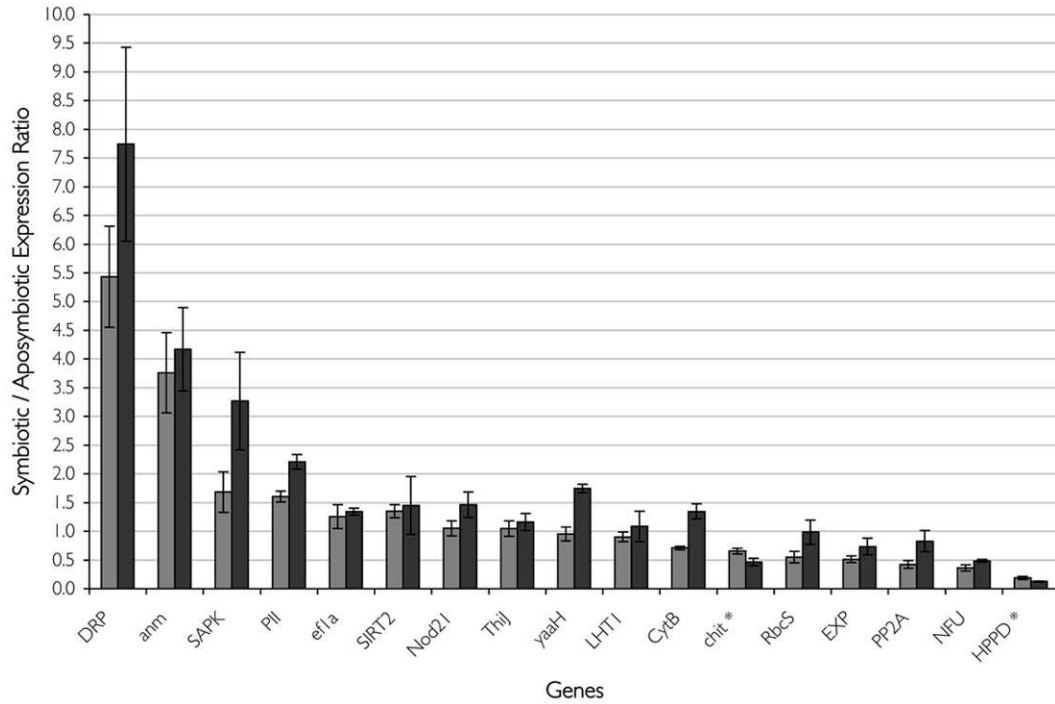


FIGURE 4.2: qPCR expression of 17 SSH-selected *Asterochloris* sp. genes in stage 2 of lichen development (assayed after 21 days of symbiotic contact growth). Light grey bars correspond to expression levels normalized to actin, and dark grey bars to GAPDH. * denotes genes upregulated in stage 1 (and confirmed by qPCR), and tested by qPCR in stage 2. Standard error shown.

Table 4.1: Primers used in qPCR to determine differential expression of select *Asterochloris* sp. genes in stages 1 and 2

Name	Primer	Sequence 5'-3'
act	Assp_act_0042F	GCGAGACCTGACAGATTACATGATGA
	Assp_act_0187R	CCTGCTCATAGTCCAGAGCCACAT
acet	Assp_acet_0353F	CTACAGAGGCCAGTGACAAACTGC
	Assp_acet_0458R	CTTTCTGAAGGTCTCGGCGGTGTT
amy	Assp_amy_0002F	CAATCTGGACAGCCACTACGGCAAT
	Assp_amy_0105R	GGTGGTTGATCACAATGTCGGCTAC
amox	Assp_amox_0050F	GGCGTCTGGGCTCATTTGTGCAA
	Assp_amox_0148R	AGCACAGCTGGCCTGGCACCTCA
anm	Assp_anm_0207F	GATGGGTGGCTGGGCAGAGTTT
	Assp_anm_0307R	AGTATTCCTGCTGCTCCTCCCTGAA
bglu	Assp_bglu_0176F	GCCCTGATGGACAACCTTGAATGGAC
	Assp_bglu_0289R	GAGCCAGGAGAGGGACTGCTTA
CALM	Assp_CALM_0514F	CGTCAAGATGATGATGGCAAAGTGAC
	Assp_CALM_0622R	GGCCCAGCAGGAAGCTATACAAATA
chit	Assp_chit_0035F	GGCTGAGGCTTGATACACCTTGATTC
	Assp_chit_0144R	GCTGAAAGAGATCCTGACCAGCAACA
CHP2	Assp_CHP2_0023F	ACGGCTGACTCCAAGCCTGAGAT
	Assp_CHP2_0165R	GGGCCATACAGGTCCAGCTTCAAT
CytB	Assp_CytB_0278F	TTGCCTTTCATCAACACAGCACAAACT
	Assp_CytB_0393R	GGTTTCAGCCCTATCCAAACCAGAAC
DRP	Assp_DRP_0315F	CAGAGTTCGGGCAAGAGTTC
	Assp_DRP_0429R	CATCCAGATGCGTCAGTTGT
ECA	Assp_ECA_0055F	GTAGGCTCACCAGCTCTCTTGCACT
	Assp_ECA_0181R	AAATAGGAGCACCAGGCACCACTCT
ef1 α	Assp_ef1 α _0233F	CACACTGGGCAGTCACTTCTTCTTG
	Assp_ef1 α _0340R	GACATACGTCAGACTGTGGCTGTTG
EXP	Assp_EXP_0168F	CGGCATCATTCACAATTCTCATGTC
	Assp_EXP_0249R	ATGACCAACACCTTTGGAGCCATCT
flav	Assp_flav_0014F	CTTTGGCGCCCACTTCTGCACT
	Assp_flav_0163R	TCGTAGTCCATGTCCTTGGTCCTCT
GAPDH	Assp_GAPDH_0330F	GCTGCCAACATCATTCCCTCCTC
	Assp_GAPDH_0455R	ATCCACCACTGACACATCCACTGT
H3	Assp_H3_0239F	CGTGCTTAGTTGGCGGACAGAATCAAT
	Assp_H3_0340R	ACACACCACAACCTTCGTGAGAGC
HPPD	Assp_HPPD_0086F	GGCTCACGACATAGCAGTCAGGAA
	Assp_HPPD_0194R	ACCATACAGCTCAATCTCCGACACA
hydro	Assp_hydro_0025F	TTTACACCTTTTCGCCTATTGCGTGGT
	Assp_hydro_0134R	GCTACGTTTAGAGCAAATAACTGCTGA
LAG1	Assp_LAG1_0129F	ATACGGTGTCTCGCACCTTCTTCGT
	Assp_LAG1_0250R	CACTCTGGGCAACCTTGAGGCATT
LHT1	Assp_LHT1_0286F	GCATGTCCTACTCGACCATTGCATTT
	Assp_LHT1_0415R	CCTAGAGCATTGAACACGCCAAAGAG
NifU	Assp_NifU_0165F	GAAGGTATCAAGGCAGCCATCAAAGA
	Assp_NifU_0293R	CCACTCTGCGTTCAGAAACCTCAAGA
NFU	Assp_NFU_0302F	GCATCGAGAACATGCTGAAGCACTA
	Assp_NFU_0431R	AATGATCAATTGGACAGGTGCTGCT

Table 4.1 – Continued

Name	Primer	Sequence 5'–3'
PII	Assp_PII_0025F	ACAACCTGGTGGAGAAGGCCAAG
	Assp_PII_0164R	GACATCGGCTACAGGGACAATGAAA
per	Assp_per_0140F	GGACCGCCTGATATACTGCGACTT
	Assp_per_230R	CAAATCCACATCATCGTCCAGCTTGCT
PP2A	Assp_PP2A_0124F	TGCGACAAGAAGGGTCCAGTGTTAT
	Assp_PP2A_0267R	TGCGATAGATGACTGTCTCCGCATA
RbcS	Assp_RbcS_0121F	CAGGTCAGCTCTTTCCTGGTCCACAG
	Assp_RbcS_0243R	CTGAGCGTTCAAACTGCCTGCTCT
SAPK	Assp_SAPK_0085F	TCAAATACATTGCTCGTGTGGTTGG
	Assp_SAPK_0220R	ATAGCCAGGTGGTCATCGGTGTAGA
SIRT2	Assp_SIRT2_0319F	AGAGAGTCAATGTTCTGAGTGAAGCA
	Assp_SIRT2_0426R	GGAGCTGTTCCCTGGAACTTCAA
sus	Assp_sus_0024F	CGCCAAGGAGAGACACTACTGGAAG
	Assp_sus_0168R	AGTCTGGCCCGTGACAAATTTCC
ThiJ	Assp_ThiJ_0005F	CTCGGGCTTGAGCGATGAGACTG
	Assp_ThiJ_0109R	GCCAGCATGGAGGGTGATTTCTTTACA
yaaH	Assp_yaaH_0312F	TGGCTGGGATACGGAATCTA
	Assp_yaaH_0729R	GAAGATGCCCCAGAGAGACA

Table 4.2: Algal gene regions selected from stage 1 of lichen resynthesis and chosen for qPCR analysis, putative function, and top GenBank hit and description.

Acronym	Putative Function	GenBank Accession No.	Top GenBank blastx hit accession number and description	
acet	Acetamidase/formamidase	–	ref XP_002181627.1	formamidase-like protein [Phaeodactylum tri-cornutum CCAP 1055/1] gb EEC46841.1
amox	Amine oxidase	–	ref YP_001866143.1	amine oxidase [Nostoc punctiforme PCC 73102] gb ACC81200.1
amy	Alpha amylase	–	emb CAL58037.1	alpha amylase 1 (IC) [Ostreococcus tauri]
bglu	Beta-glucosidase	–	gb AAF03675.1 AF149311.1	raucaffricine-O-beta-D-glucosidase [Rauvolfia serpentina]
CALM	Calmodulin	–	emb CAL50295.1	calmodulin mutant SYNCAM9 (ISS) [Ostreococcus tauri]
chit	Chitinase-like protein	–	ref NP_192013.1	glycosyl hydrolase family 18 protein [Arabidopsis thaliana] emb CAB80913.1 hypothetical protein
CHP2	Conserved Hypothetical Protein	–	gb AAG49030.1 AF204783.1	ripening regulated protein DDTFR8 [Lycopersicon esculentum]
ECA	Calcium-transporting ATPase	–	gb EAY96338.1	hypothetical protein OsL18241 [Oryza sativa Indica Group]
flav	Flavoprotein	–	ref XP_001756079.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ78945.1 predicted protein
H3	Histone 3	–	ref XP_537880.2	PREDICTED: similar to histone 1, H2ai (predicted) isoform 1 [Canis familiaris]
HPPD	4-hydroxyphenylpyruvate dioxygenase	–	emb CAR47957.1	p-hydroxyphenylpyruvate dioxygenase [Chlamydomonas reinhardtii]
hydro	Bacterial cell wall associated hydrolase	–	ref ZP_01689674.1	cell wall-associated hydrolase [Microscilla marina ATCC 23134] gb EAY29055.1
LAG1	Longevity assurance gene	–	ref XP_001697632.1	hypothetical protein CHLREDRAFT_41962 [Chlamydomonas reinhardtii] gb EDP00026.1
NifU	Nitrogen fixation protein nifU	–	gb EEF47225.1	Nitrogen fixation protein nifU, putative [Ricinus communis]
per	Peroxisome biogenesis factor	–	gb EEF44734.1	peroxisome biogenesis factor, putative [Ricinus communis]
sus	Sucrose synthase	–	gb ACL36485.1	starch synthase VI precursor [Picea glauca]

Table 4.3: Algal gene regions selected from stage 2 of lichen resynthesis and chosen for qPCR analysis, putative function, and top GenBank hit and description.

Acronym	Putative Function	GenBank Accession No.	Top GenBank blastx hit accession number and description
anm	Protein arginine methyltransferase	—	ref XP_001702822.1 protein arginine N-methyltransferase [Chlamydomonas reinhardtii] gb EDO96867.1
CytB	Cytochrome B	—	ref NP_042262.1 cytochrome b [Prototheca wickerhamii] gb AAD12650.1
DRP	Dynamin-related protein	—	ref XP_001700931.1 dynamin-related GTPase [Chlamydomonas reinhardtii] gb EDP07185.1
ef1 α	Translation elongation factor 1-alpha	—	gb ACL97363.1 elongation factor-1 alpha [Ignatius tetrasporus]
EXP	Expansin	—	ref XP_001767753.1 predicted protein [Physcomitrella patens subsp. patens] gb EDQ67504.1
LHT1	Amino acid transporter (lysine-histidine)	—	emb CAD89802.1 histidine amino acid transporter [Oryza sativa (indica cultivar-group)]
NFU	Iron-sulphur cluster assembly	—	ref XP_001690805.1 iron-sulfur cluster assembly protein [Chlamydomonas reinhardtii] gb EDP05251.1
Nod21	Nodulin 21	—	ref XP_001700022.1 hypothetical protein CHLREDRAFT_112030 [Chlamydomonas reinhardtii] gb—EDP07718.1
PII	Nitrogen regulatory protein	—	ref XP_001703658.1 nitrogen regulatory protein PII [Chlamydomonas reinhardtii] gb EDO96407.1
PP2A	Protein Phosphatase 2A	—	ref XP_001752983.1 predicted protein [Physcomitrella patens subsp. patens] gb EDQ82024.1
RbcS	RuBisCO small subunit	—	gb ACF16408.1 chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit [Myrmecia incisa]
SAPK	Serine / threonine protein kinase	—	gb ACD44937.1 osmotic stress/ABA-activated protein kinase [Zea mays]
SIRT2	NAD-dependent deacetylase, sirtuin 2	—	ref XP_001419772.1 predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO98065.1
ThiJ	ThiJ/PfpI domain protein	—	ref XP_001759218.1 predicted protein [Physcomitrella patens subsp. patens] gb EDQ75902.1
yaaH	GPR1/FUN34/yaaH family of multi-pass cell membrane proteins	—	ref XP_001835110.1 predicted protein [Coprinopsis cinerea okayama7#130]

Table 4.4: Average aposymbiotic and symbiotic qPCR expression levels of 18 *Asterochloris* sp. genes after 9 days (stage 1 of lichen development) relative to actin and GAPDH. Genes marked with asterisk were tested because of their upregulation in stage 2. Standard error shown.

Gene		actin			GAPDH		
acet	apo	0.09	±	4.21E-03	0.13	±	0.02
	sym	0.04	±	4.62E-03	0.04	±	0.01
amox	apo	0.09	±	0.02	0.12	±	0.03
	sym	0.04	±	7.90E-03	0.04	±	0.02
amy	apo	0.01	±	4.48E-04	9.92E-03	±	4.39E-04
	sym	9.87E-04	±	8.87E-06	9.68E-04	±	3.36E-04
anm *	apo	0.12	±	0.02	0.17	±	0.03
	sym	0.12	±	0.02	0.17	±	0.01
bglu	apo	0.13	±	5.90E-03	0.19	±	4.58E-03
	sym	0.11	±	0.02	0.16	±	0.05
CALM	apo	2.05	±	0.31	1.71	±	0.25
	sym	1.25	±	0.25	1.13	±	0.16
chit	apo	0.02	±	1.91E-03	0.04	±	3.63E-03
	sym	0.07	±	1.46E-03	0.10	±	0.01
CHP2	apo	0.53	±	8.24E-03	0.44	±	4.10E-03
	sym	0.39	±	6.70E-03	0.37	±	0.13
DRP *	apo	0.08	±	0.02	0.13	±	0.01
	sym	0.05	±	4.82E-03	0.07	±	0.03
ECA	apo	0.02	±	4.37E-03	0.01	±	3.54E-03
	sym	0.01	±	4.62E-03	0.00	±	1.12E-03
flav	apo	0.49	±	0.03	0.69	±	0.12
	sym	0.46	±	0.04	0.54	±	0.12
H3	apo	4.58	±	0.14	6.67	±	0.34
	sym	5.66	±	1.23	8.20	±	1.08
HPPD	apo	0.01	±	2.87E-04	0.01	±	1.80E-03
	sym	0.02	±	1.40E-03	0.02	±	5.03E-03
hydro	apo	59.8	±	8.76	83.2	±	2.81
	sym	29.4	±	5.54	35.1	±	11.4
LAG1	apo	0.39	±	0.02	0.32	±	0.02
	sym	0.36	±	0.05	0.34	±	0.06
NifU	apo	0.66	±	0.17	0.97	±	0.27
	sym	0.76	±	0.25	1.09	±	0.27
per	apo	0.07	±	4.66E-03	0.11	±	4.42E-03
	sym	0.03	±	8.52E-03	0.04	±	8.05E-03
sus	apo	0.61	±	0.01	1.28	±	0.38
	sym	0.31	±	6.57E-03	0.36	±	0.06

Table 4.5: Average aposymbiotic and symbiotic qPCR expression levels of 17 *Asterochloris* sp. genes after 21 days (stage 2 of lichen development) relative to actin and GAPDH. Genes marked with asterisk were tested because of their upregulation in stage 1. Standard error shown.

Gene		actin			GAPDH		
anm	apo	0.03	±	4.90E-03	0.03	±	9.44E-03
	sym	0.12	±	0.03	0.12	±	7.75E-03
chit *	apo	0.07	±	0.00	0.14	±	0.01
	sym	0.05	±	0.00	0.06	±	0.01
CytB	apo	1.45	±	0.10	0.98	±	0.02
	sym	1.02	±	0.01	1.33	±	0.23
DRP	apo	0.02	±	5.93E-03	0.02	±	6.86E-03
	sym	0.11	±	6.02E-03	0.12	±	0.01
ef1 α	apo	0.17	±	0.02	0.17	±	9.32E-03
	sym	0.22	±	0.06	0.23	±	0.01
EXP	apo	0.59	±	0.10	0.50	±	0.14
	sym	0.29	±	0.02	0.34	±	0.07
HPPD *	apo	2.62E-03	±	1.43E-04	4.86E-03	±	9.91E-05
	sym	4.84E-04	±	1.17E-04	6.16E-04	±	8.99E-05
LHT1	apo	0.55	±	0.03	0.55	±	0.12
	sym	0.50	±	0.07	0.57	±	0.20
NFU	apo	0.95	±	0.15	0.78	±	0.04
	sym	0.34	±	0.07	0.38	±	0.02
Nod21	apo	1.27	±	0.21	1.08	±	0.28
	sym	1.30	±	0.18	1.47	±	2.72E-03
PII	apo	0.34	±	0.03	0.28	±	3.16E-03
	sym	0.54	±	0.02	0.62	±	0.06
PP2A	apo	0.05	±	9.76E-03	0.04	±	4.8E-03
	sym	0.02	±	4.07E-03	0.03	±	0.01
RbcS	apo	46.5	±	12.3	31.3	±	6.76
	sym	23.8	±	4.33	29.4	±	8.71
SAPK	apo	0.09	±	0.03	0.06	±	0.02
	sym	0.13	±	1.59E-03	0.16	±	0.03
SIRT2	apo	0.14	±	0.01	0.13	±	0.04
	sym	0.19	±		0.17	±	–
ThiJ	apo	0.54	±	0.04	0.53	±	0.12
	sym	0.56	±	0.12	0.59	±	4.51E-03
yaaH	apo	1.89	±	0.02	1.29	±	0.08
	sym	1.80	±	0.40	2.25	±	0.09

Heterologous expression of *Cladonia grayi* *Lip3* in yeast.

5.1 Introduction

Cladonia grayi is a filamentous lichen-forming ascomycete that lives symbiotically with the green alga *Asterochloris* sp. One result of this symbiosis is the physical manifestation of a new body made of both organisms, the thallus, unlike either symbiont grown on its own. Lichens are ubiquitous, and can be found in all but the purely aquatic ecosystems. Although lichen thalli grow relatively slowly, and the *in vitro* resynthesis of thalli remains difficult, their success in the wild is thought to be due to a closely regulated and coordinated symbiotic growth (Honegger, 1998).

Many fungi communicate with their symbiotic partners through extracellular cell-membrane/wall protein signalling, and the products of extracellular enzymes can be used to inform an organism of its neighbors (Dean, 1997; Eckhardt, 2008;

Kolattukudy *et al.*, 1995). Secreted lipases have been suggested to play a role in the invasion of plant tissue by fungal plant pathogens by breaking down the plant's waxy cuticular coating (Comm  nil *et al.*, 1999; Nasser Eddine *et al.*, 2001; Voigt *et al.*, 2005), although they have not been reported from mutualistic symbioses between mycorrhizal fungi and plants.

A putative extracellular triacylglycerol lipase, *Lip3* was predicted to be secreted from *Cladonia grayi* in an early stage of lichen development when the fungus first makes contact with its symbiotic green alga, *Asterochloris* sp. (Joneson *et al.*, 2009). At this time, no functional data exists to support this, and there is no transformable or classical genetics system available in lichen-forming-fungi. To test candidate genes in fungal-lichen symbiosis, it is therefore necessary to use a heterologous expression (Sinnemann *et al.*, 2000).

The genomic coding region and full *Lip3* mRNA coding region from *Cladonia grayi* was identified, heterologously expressed it in yeast, and assayed its function using olive oil agar plates. Triacylglycerol lipase activity must be verified with tri-oleoylglycerol (18:1-18:1-18:1), as different length triacylglycerols can be hydrolyzed by esterases other than lipase, and these esterases are present in yeast. Olive oil is an inexpensive substitute, as in its purified state it contains more than 70% 18:1 (Jensen, 1983). Lipase enzymes (EC:3.1.1) hydrolyze the ester linkages of triacyl-

glycerols into diacyl- and monoacylglycerols and free fatty acids and glycerol. The extent of triacylglycerols in *Asterochloris* or other lichen-forming green algae is unknown, and the target of *Lip3* from *C. grayi* remains to be determined. This study investigates for the first time the heterologous expression of a lichen-forming fungal gene involved in lichen symbiosis.

5.2 Methods

5.2.1 Strains and Cultivation

Cultivation of *Cladonia grayi* DA1 (Armaleo & May, 2009) was performed as described in Joneson & Lutzoni (2009).

Saccharomyces cerevisiae strain W3031A was grown in synthetic complete (SC) medium -ura with 2% glucose as the carbon source in transformant selection (Burke *et al.*, 2000).

5.2.2 Nucleotide Sequences

Isolation of RNA was performed according to Joneson *et al.* (2009). Total genomic DNA was isolated using a modified protocol of Zolan & Pukkila (1986), using 2% sodium dodecyl sulphate as the extraction buffer. Partial 5' and 3' ends of the coding mRNA were sequenced using a 5'-and-3'-RACE library provided by Tami McDonald (Duke University).

Sequencing was carried out on an Applied Biosystems 3730xl DNA automated sequencer (Duke IGSP sequencing facility) using Big-Dye Terminator Cycle Sequencing v3.1 (PE Applied Biosystems, Foster City, CA, U.S.A.). Sequences were analysed using Sequencher 4.8 (Gene Code Corporation, Ann Arbor, MI).

5.2.3 Plasmid Construction

Saccharomyces cerevisiae strain W3031A (MATa ade2-1 can1-100 his3-11,15 leu2-3112 trp1-1 ura3-1) and yeast expression vector p416GPD-2xHA were gifts from Dr. Chandra Tucker (Duke University, NC, USA).

The *Lip3* coding region from *Cladonia grayi* was cloned by homologous recombination into the plasmid p416GPD (Mumberg *et al.*, 1995) in which a 2xHA tag (GMRYPYDVPDYAGYPYDVPDYASGR) had been added (Hopper *et al.*, 2008). In this plasmid, *Lip3* is under the control of the glyceraldehyde-3phosphate dehydrogenase promoter (GAPD). Forward and reverse PCR primers were designed to complement the p416GPD vector at the insert site, and to ensure that the 2x HA tag was joined to the *Lip3* C-terminus, using Phusion (New England Biolabs). High efficiency transformation was carried out according to the Clontech user manual PT3529-1. All clones were verified by sequencing.

The p416GPD-2xHA plasmid without *Lip3* was also transformed in to W3031A for use as a negative control.

5.2.4 Searching the yeast genome with Lip3

To determine if secreted triacylglycerol lipase homologues are present in the *Saccharomyces cerevisiae* genome, the blastp algorithm (Altschul *et al.*, 1990) was used to query the *Lip3* amino acid sequence against the yeast genome database of proteins (www.yeastgenome.org). A word based search was also used, to query ‘extracellular’ and ‘lipase’.

5.2.5 Enzyme Assay

Olive oil plates were created using a SC -ura base, with an SC -ura olive oil (OO) overlay. Nine mls of SC -ura were poured in to 55mm diameter plates and allowed to solidify before pouring 3 mLs of SC-OO overlay on top. The SC-OO medium was prepared by adding 7.5mL-OO/L to SC -ura medium and homogenizing in a Waring blender on high speed for one minute, and then stirring on a heated stir plate at 60°C for five minutes to reduce foaming.

Before plating yeast for olive oil assays, yeast were grown in liquid SC -ura medium containing 2% glucose, at 200 RPM and 37°C for three days. Culture density was measured using a Hauser Scientific Improved Neubauer haemocytometer and a light microscope. Spots of 50,000 cells in 10 μ L were pipetted onto olive oil assay plates. Yeast cells were grown on SC-OO plates in an 37°C incubator for

19 days. Plates were photographed, colonies were washed from the plates with 95% ethanol, and the plates photographed again to observe lipase activity.

5.2.6 Protein Sequence

Protein molecular weight was determined using the protein molecular weight module available at Stothard's sequence manipulation suite (Stothard, 2000). The signal peptide sequence was determined using the N-terminal signal sequence prediction algorithms, TargetP 1.1 (Emanuelsson *et al.*, 2000), and SignalP3.0 (Bendtsen *et al.*, 2004). Predicted protein secondary and tertiary structure was determined using the SCRATCH protein predictor (Cheng *et al.*, 2005).

5.3 Results

5.3.1 Nucleotide and Protein Sequences.

The *Lip3* gene of *C. grayi* consists of an open reading frame of 1,066 bp (GenBank accession no. XXXXXXXX), interrupted by two introns of 57 and 80 nucleotides in length at positions 71 and 303, respectively. The encoded protein consists of 309 amino acids with a predicted molecular weight of 32.86 kDa (Figure. 5.1). The N-terminal region contains a deduced signal peptide, composed of the first 17 amino acid residues, and the putative cleavage site for the signal peptidase is predicted to be between residues 17 and 18. Cleavage of the signal peptide results in a peptide

292 amino acids in length with a molecular weight of 31.05kDa.

Lip3 contains conserved domains of the lipase 3 superfamily (Figure 5.1). The catalytic triad Ser-Asp-His (Derewenda *et al.*, 1992b) are found at the pre-peptide residues Ser170, Asp225, His284. *Lip3* contains an active site lid (Derewenda *et al.*, 1992a) encoded in amino acids Val111 - Asp117 which forms a short alpha helix that covers the active site in an aqueous environment. The conserved nucleophilic elbow motif GXSXG (Kohno *et al.*, 1996) is encoded in amino acids Gly168 - His169 - Ser170 - Leu171 - Gly172.

5.3.2 Searching the yeast genome with *Lip3*

When searching the yeast genome through the *Saccharomyces* Genome Database (SGD; www.yeastgenome.org) with *Lip3*, the best match is to YJR107W, an uncharacterized protein with a class 3 lipase active site. This putative protein shares 27% identical residues over a 206 amino acid stretch with *Lip3*, but is predicted to be intracellular. No extracellular triacylglyceride lipases could be found in the yeast genome.

5.3.3 Heterologous Expression

Cladonia grayi currently lacks a transformation system. Functional expression of *Lip3* was therefore carried out by heterologous expression of *Lip3* in the yeast

Saccharomyces cerevisiae. To determine whether *Lip3* from *C. grayi* is secreted and lipolytically active *in vitro*, the yeast heterologous expression system was used with an olive-oil plate assay. Extracellular lipolytic activity is detectable by substrate hydrolysis, which causes a clearing in olive-oil supplemented agar (which when photographed appears as a halo). Yeast transformed with *Lip3* secreted a functional lipase as shown by a halo on assay plates, as compared to yeast transformed with an expression vector without *Lip3* that lacked this halo (Figure 5.2).

5.4 Discussion

Through heterologous expression in yeast, it was determined that *Lip3* from *Cladonia grayi* is a functional and secreted extracellular lipase. The previously determined upregulation of *Lip3* in early lichen development (Joneson *et al.*, 2009), and the functional expressivity of *Lip3* in yeast, strongly suggests that the activity of *C. grayi* is acting upon extracellular lipid-containing compounds during early lichen development.

It is not possible to verify at this time if a lipase inhibitor such as ebalactone would inhibit *Lip3* activity and role in establishing the lichen symbiosis. It takes at least 21 days to establish the 2nd stage of lichen development, the stage in which *Lip3* was previously found to be upregulated. At this time it is not clear at which point

in development to add a lipase inhibitor, or how often, or for how long. Furthermore a lichen development specific assay in which to look for its effect is currently lacking.

The putative roles of extracellular fungal lipases include nutritional lipid digestion, development, adhesion to host cells and tissues, and penetration of host cells and tissue (Chu *et al.*, 2008; Gácsér *et al.*, 2007). In phytopathogenic fungi in particular, some extracellular lipases have been important in pathogenicity determination (Berto *et al.*, 1999; Gácsér *et al.*, 2007; Nasser Eddine *et al.*, 2001; Voigt *et al.*, 2005). In other symbiotic interactions, even though lipase activity may be heavily induced, it is not necessary for causing disease (Feng *et al.*, 2005; Reis *et al.*, 2005). In phytopathogenic fungi, secreted extracellular lipases are thought to aid in host penetration through destruction of the secreted waxy outer layer of plants known as the cuticle (Berto *et al.*, 1999; Voigt *et al.*, 2005), however the exact mechanism of how this works remains to be shown. A possible algal substrate of *Cladonia grayi* *Lip3* also remains to be determined.

Aquatic and terrestrial green algae are not known to produce a cuticular layer (Raven, 1977), the presumed target of extracellular lipases from phytopathogenic fungi. The cell walls of closely related green algae are thought to be made of non-lipid derived cellulose, glycoproteins, and polysaccharides (Konig & Peveling, 1984; Domozych *et al.*, 1980). Although lichen-forming green algae related to *Asterochlo-*

ris are known to produce large amounts of triacylglycerols, these are presumably storage products (Guschina *et al.*, 2003), and not in their cell-membrane. One possible source for *Lip3* targets could be in degrading mother cells (aplanosporangia, or zoosporangia), that burst open to give rise to *Asterochloris* daughter spores. Alternatively, the cell wall of lichen-forming fungi has been proposed to serve as a protective cuticular layer for trebouxoid algae such as *Asterochloris* sp. upon initial contact, such as in stage 2 of lichen development (Honegger, 1990). In this manner, the algal cell wall becomes entirely covered by a fungal secreted mixture of lipids and phenolic secondary metabolites (Honegger, 1990). It remains an intriguing possibility that the target of *Lip3* may be a source of fungal derived lipids, modified to help coat the lichen-forming *Asterochloris* sp.

In this study, the isolation and preliminary characterization of *Lip3* in *Cladonia grayi* is reported. This extracellular lipase encodes a peptide that is similar to other class 3 lipases. The further investigation of *Lip3* and its entire spectrum of target lipids will be useful in determining the function of this extracellular lipase in the *C. grayi* and *Asterochloris* sp. symbiosis.


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1      MFKIAAAITF  FLSSTLVAGPV  QSRSIDSTTF  ANLQLFEQFA  AAAYCPGNNN
51     ITKGGTKLSC  PLSNNCPLVE  ADDVTTVYEF  ENSLLTDVTG  YVAVDNTRSL
101    TVLAFRGSES  VRNFLADADF  PTVPTDICSG  CEADQGFYNS  WLEARTDVTS
151    ALHSAAAANP  SYKVVVVGHS  LGGAIAAIAA  AEIRDQGTDA  DLYTYGQPRI
201    GGSTISSYIT  NQNKGGNFRV  THYDDPVPRL  PPLLGGFVHI  SPEYYIDTGN
251    YVPVTAADIK  ELTGSINLLG  NAGNIGDGVD  LDAHGWYFNN  ISSCDTSDSL
301    EFKRAVISG.

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FIGURE 5.1: Pre-peptide amino acid sequence of *Lip3* in *Cladonia grayi*. Signal cut site between residues 17 and 18, marked with a v. Catalytic triad Ser170, Asp225, His284 in bold and underlined. Active site flap/lid , Val111 - Asp117, in bold and boxed. Nucleophilic elbow motif Gly168 - His169 - Ser170 - Leu171 - Gly172, highlighted in grey.

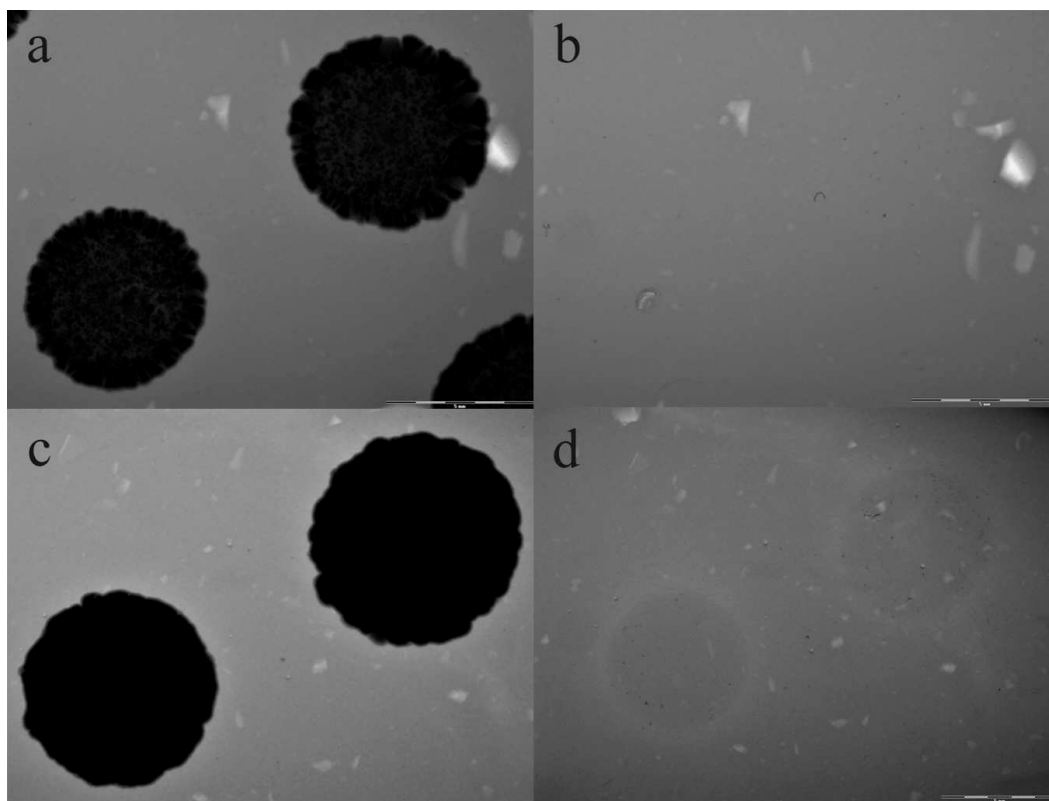


FIGURE 5.2: Heterologous expression of *Lip3* in yeast. a) Yeast transformed with expression vector without *Lip3*. b) Plate washed of yeast without *Lip3*. c) Yeast transformed with *Lip3*. d) Plate washed of yeast with *Lip3*, zone of hydrolysis visible as halo. In b) and d), compare abnormalities in agar with a) and c) to determine previous location of yeast.

Appendices

A

GenBank, UniProt, and Gene Ontology summary information for *Cladonia grayi* in stages 1 and 2 of lichen development.

A.1 Combined GenBank and UniProt summary information for stages 1 and 2 *Cladonia grayi* gene fragments.

A local batch blastx algorithmic search was carried out for stages 1 and 2 SSH-cDNAs using the non-redundant (nr) set of proteins from the National Center for Biotechnology and Information (www.ncbi.nlm.nih.gov, accessed on 4 March 2009). Default blastx settings were used with a lower threshold e-value $< 10^{-5}$. Sequences were considered significant if they produced a translated sequence of 30 amino acids or longer that matched an nr database entry with a percentage identity equal to or greater than 35%. Protein coding sequences in the proper reading frame were subsequently parsed from the blastx output, and used to search the UniProt database (www.ebi.uniprot.org) of protein sequence and function using the blastp algorithm.

Genome of origin (fungal, algal, or “either”) was determined for genetic regions by manually classifying the top five blastx hits. Sequences were labeled of fungal origin if the top hits were to all fungal sequences. Algal sequences were identified if the top hits matched either all Chloroplastida, or Chloroplastida and cyanobacteria. The remainder of sequences was classified as ‘either’. Blastx and UniProt datasets were managed using customized PERL and BioPERL scripts (Stajich *et al.*, 2002).

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments.

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0003]	gi 111062632 gb EAT83752.1	hypothetical protein SNOG_08584 [Phaeosphaeria nodorum SN15]	P51401 RL9B_YEAST	60S ribosomal protein L9-B – Saccharomyces cerevisiae (Baker's yeast)
Contig[0007]	gi 70998522 ref XP_753983.1	acyl-CoA dehydrogenase family protein [Aspergillus fumigatus Af293]	Q5ZHT1 ACD11_CHICK	Acyl-CoA dehydrogenase family member 11 – Gallus gallus (Chicken)
Contig[0012]	gi 23270399 ref NP_690845.1	Mitochondrial protein of unknown function, overexpression suppresses an rpo41 mutation affecting mitochondrial RNA polymerase; encoded within the 25S rRNA gene on the opposite strand [Saccharomyces cerevisiae] Protein TAR1 (Transcript antisense to ribosomal RNA protein 1) unknown [Saccharomyces cerevisiae]	Q8TGM6 TAR1_YEAST	Protein TAR1 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0016]	gi 150853025 gb EDN28217.1	hypothetical protein BC1G_08140 [Botryotinia fuckeliana B05.10]	P39932 STL1_YEAST	Sugar transporter STL1 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0021]	gi 111062505 gb EAT83625.1	hypothetical protein SNOG_09433 [Phaeosphaeria nodorum SN15]	O59948 ERF1_PODAN	Eukaryotic peptide chain release factor subunit 1 – Podospira anserina
Contig[0023]	gi 83772686 dbj BAE62814.1	unnamed protein product [Aspergillus oryzae]	Q5QA93 HSV2_PICAN	SVP1-like protein 2 – Pichia angusta (Yeast) (Hansenula polymorpha)
Contig[0024]	gi 119193787 ref XP_001247497.1	hypothetical protein CIMG_01268 [Coccidioides immitis RS] hypothetical protein CIMG_01268 [Coccidioides immitis RS]	P25346 GIT1_YEAST	Probable metabolite transport protein GIT1 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0031]	gi 145250085 ref XP_001396556.1	hypothetical protein An15g00170 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q10452 GSK3_SCHPO	Protein kinase gsk3 – Schizosaccharomyces pombe (Fission yeast)
Contig[0036]	gi 119184318 ref XP_001243083.1	hypothetical protein CIMG_06979 [Coccidioides immitis RS] hypothetical protein CIMG_06979 [Coccidioides immitis RS]	P15703 BGL2_YEAST	Glucan 1,3-beta-glucosidase precursor – Saccharomyces cerevisiae (Baker's yeast)
Contig[0040]	gi 150414583 gb EDN09945.1	conserved hypothetical protein [Ajellomyces capsulatus NAm1]	P53981 YNB0_YEAST	Uncharacterized protein YNL010W – Saccharomyces cerevisiae (Baker's yeast)
Contig[0046]	gi 150414940 gb EDN10302.1	conserved hypothetical protein [Ajellomyces capsulatus NAm1]	O74846 SEC6_SCHPO	Exocyst complex component sec6 – Schizosaccharomyces pombe (Fission yeast)
Contig[0047]	gi 119188903 ref XP_001245058.1	hypothetical protein CIMG_04499 [Coccidioides immitis RS] hypothetical protein CIMG_04499 [Coccidioides immitis RS]	Q4WI01 RS21_ASPFU	40S ribosomal protein S21 – Aspergillus fumigatus (Sartorya fumigata)
Contig[0071]	gi 67525191 ref XP_660657.1	hypothetical protein AN3053.2 [Aspergillus nidulans FGSC A4]	P53301 CRH1_YEAST	Probable glycosidase CRH1 precursor – Saccharomyces cerevisiae (Baker's yeast)
Contig[0077]	gi 111606543 gb ABH10636.1	elongation factor 2 [Coccidioides posadasii]	Q96X45 EF2_NEUCR	Elongation factor 2 – Neurospora crassa
Contig[0087]	gi 116179676 ref XP_001219687.1	histone H2B [Chaetomium globosum CBS 148.51] Histone H2B histone H2B [Chaetomium globosum CBS 148.51]	Q2HH38 H2B_CHAGB	Histone H2B – Chaetomium globosum (Soil fungus)

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0088]	gi 119178093 ref XP_001240751.1	hypothetical protein CIMG_07914 [Coccidioides immitis RS] hypothetical protein CIMG_07914 [Coccidioides immitis RS]	Q02336 ADA2_YEAST	Transcriptional adapter 2 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0089]	gi 145239495 ref XP_001392394.1	hypothetical protein An08g02770 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q9U5N0 VATH_MANSE	Vacuolar ATP synthase subunit H – <i>Manduca sexta</i> (Tobacco hawkmoth) (Tobacco hornworm)
Contig[0095]	gi 150410195 gb EDN05583.1	40S ribosomal protein S25 [Ajelomyces capsulatus NAM1]	Q7SC06 RS25_NEUCR	40S ribosomal protein S25 – <i>Neurospora crassa</i>
Contig[0101]	gi 50552368 ref XP_503594.1	hypothetical protein [Yarrowia lipolytica] unnamed protein product [Yarrowia lipolytica CLIB122]	P50166 ARDH_CANTR	D-arabinitol 2-dehydrogenase [ribulose-forming] – <i>Candida tropicalis</i> (Yeast)
Contig[0105]	gi 83772625 dbj BAE62753.1	unnamed protein product [Aspergillus oryzae]	P35128 UBCD3_DROME	Ubiquitin-conjugating enzyme E2-17 kDa – <i>Drosophila melanogaster</i> (Fruit fly)
Contig[0106]	gi 150850158 gb EDN25351.1	hypothetical protein BC1G_06195 [Botryotinia fuckeliana B05.10]	Q10475 IF4G_SCHPO	Eukaryotic translation initiation factor 4 gamma – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0111]	gi 119480931 ref XP_001260494.1	60S acidic ribosomal protein P2/allergen Asp F 8 [Neosartorya fischeri NRRL 181] 60S acidic ribosomal protein P2/allergen Asp F 8 [Neosartorya fischeri NRRL 181]	Q9UUZ6 RLA2_ASPFU	60S acidic ribosomal protein P2 – <i>Aspergillus fumigatus</i> (Sartorya fumigata)
Contig[0114]	gi 115491589 ref XP_001210422.1	hypothetical protein ATEG_00336 [Aspergillus terreus NIH2624] hypothetical protein ATEG_00336 [Aspergillus terreus NIH2624]	O13990 BGL2_SCHPO	Glucan 1,3-beta-glucosidase precursor – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0122]	gi 121705634 ref XP_001271080.1	D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase, putative [Aspergillus clavatus NRRL 1] D-xylulose 5-phosphate/D-fructose 6-phosphate phosphoketolase, putative [Aspergillus clavatus NRRL 1]	O74770 PHK_SCHPO	Probable phosphoketolase – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0134]	gi 111063775 gb EAT84895.1	hypothetical protein SNOG_07429 [Phaeosphaeria nodorum SN15]	O14359 YB4E_SCHPO	Uncharacterized protein C30D10.14 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0137]	gi 71000788 ref XP_755075.1	mitochondrial carrier protein, putative [Aspergillus fumigatus Af293]	Q12251 YP011_YEAST	Probable mitochondrial carrier YPR011C – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0144]	gi 121715530 ref XP_001275374.1	nuclear protein export protein Yrb2, putative [Aspergillus clavatus NRRL 1] nuclear protein export protein Yrb2, putative [Aspergillus clavatus NRRL 1]	Q9P6P8 YIP4_SCHPO	Protein YIP4 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0146]	gi 46125549 ref XP_387328.1	hypothetical protein FG07152.1 [Gibberella zeae PH-1]	P22151 GRG1_NEUCR	Glucose-repressible gene protein – <i>Neurospora crassa</i>
Contig[0156]	gi 119189363 ref XP_001245288.1	heat shock protein hsp1 [Coccidioides immitis RS] heat shock protein hsp1 [Coccidioides immitis RS]	Q90474 HS90A_DANRE	Heat shock protein HSP 90-alpha – <i>Danio rerio</i> (Zebrafish) (Brachydanio rerio)
Contig[0159]	gi 67537086 ref XP_662317.1	hypothetical protein AN4713.2 [Aspergillus nidulans FGSC A4]	P40152 YNV7_YEAST	Putative metallophosphoesterase YNL217W precursor – <i>Saccharomyces cerevisiae</i> (Baker's yeast)

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0163]	gi 145235874 ref XP_001390585.1	unnamed protein product [Aspergillus niger] unnamed protein product [Aspergillus niger]	P53722 YN8A_YEAST	Uncharacterized protein YNR020C – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0164]	gi 46115270 ref XP_383653.1	hypothetical protein FG03477.1 [Gibberella zeae PH-1]	P53099 TPN1_YEAST	Vitamin B6 transporter TPN1 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0166]	gi 67517173 ref XP_658469.1	hypothetical protein AN0865.2 [Aspergillus nidulans FGSC A4]	Q80UY1 CI041_MOUSE	Uncharacterized protein C9orf41 homolog – <i>Mus musculus</i> (Mouse)
Contig[0171]	gi 46122635 ref XP_385871.1	hypothetical protein FG05695.1 [Gibberella zeae PH-1]	P30263 CATA_PICAN	Peroxisomal catalase – <i>Pichia angusta</i> (Yeast) (<i>Hansenula polymorpha</i>)
Contig[0172]	gi 47824822 emb CAG30552.1	TipA protein [Emericella nidulans]	Q12199 TIP41_YEAST	Type 2A phosphatase activator TIP41 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0175]	gi 111056115 gb EAT77235.1	hypothetical protein SNOG_15302 [Phaeosphaeria nodorum SN15]	Q01745 GAOA_GIBZE	Galactose oxidase precursor – <i>Gibberella zeae</i> (<i>Fusarium graminearum</i>)
Contig[0181]	gi 150846582 gb EDN21775.1	hypothetical protein BC1G_14974 [Botryotinia fuckeliana B05.10]	Q6C0Z6 CCPR_YARLI	Cytochrome c peroxidase, mitochondrial precursor – <i>Yarrowia lipolytica</i> (<i>Candida lipolytica</i>)
Contig[0182]	gi 150842366 gb EDN17559.1	hypothetical protein BC1G_00137 [Botryotinia fuckeliana B05.10]	P12611 WHI2_YEAST	Growth regulation protein – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0183]	gi 83771637 dbj BAE61767.1	unnamed protein product [Aspergillus oryzae]	Q3T099 SYWM_BOVIN	Tryptophanyl-tRNA synthetase, mitochondrial precursor – <i>Bos taurus</i> (Bovine)
Contig[0186]	gi 145229241 ref XP_001388929.1	hypothetical protein An01g05030 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q6JQN1 ACD10_HUMAN	Acyl-CoA dehydrogenase family member 10 – <i>Homo sapiens</i> (Human)
Contig[0187]	gi 70989741 ref XP_749720.1	hexokinase Kxk, putative [Aspergillus fumigatus Af293]	P33284 HXX_KLULA	Hexokinase – <i>Kluyveromyces lactis</i> (Yeast) (<i>Candida sphaerica</i>)
Contig[0190]	gi 150858231 gb EDN33423.1	hypothetical protein BC1G_12045 [Botryotinia fuckeliana B05.10]	O94321 MPR1_SCHPO	Multistep phosphorelay regulator 1 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0191]	gi 145242140 ref XP_001393716.1	hypothetical protein An09g04000 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q6C9W0 UBC12_YARLI	NEDD8-conjugating enzyme UBC12 – <i>Yarrowia lipolytica</i> (<i>Candida lipolytica</i>)
Contig[0201]	gi 150856386 gb EDN31578.1	hypothetical protein BC1G_10638 [Botryotinia fuckeliana B05.10]	Q06567 YL253_YEAST	ABC1 family protein YLR253W – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0207]	gi 83767336 dbj BAE57475.1	unnamed protein product [Aspergillus oryzae]	Q9P5L0 CYB5_NEUCR	Probable cytochrome b5 – <i>Neurospora crassa</i>
Contig[0213]	gi 117840 sp P00161 CYB_EMENI	Cytochrome b apocytochrome b	P00161 CYB_EMENI	Cytochrome b – <i>Emericella nidulans</i> (<i>Aspergillus nidulans</i>)
Contig[0222]	gi 150414583 gb EDN09945.1	conserved hypothetical protein [Ajellomyces capsulatus NAml]	P53981 YNB0_YEAST	Uncharacterized protein YNL010W – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0225]	gi 150857094 gb EDN32286.1	hypothetical protein BC1G_10814 [Botryotinia fuckeliana B05.10]	O74933 UAP1_CANAL	UDP-N-acetylglucosamine pyrophosphorylase – <i>Candida albicans</i> (Yeast)

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0227]	gi 119501338 ref XP_001267426.1	mitochondrial protein sorting (Msf1), putative [Neosartorya fischeri NRRL 181] mitochondrial protein sorting (Msf1), putative [Neosartorya fischeri NRRL 181]	Q04006 YD185_YEAST	PRELI/MSF1 domain-containing protein YDR185C – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0234]	gi 150414745 gb EDN10107.1	proteasome component PRE2 precursor [Ajellomyces capsulatus NAml]	P30656 PSB5_YEAST	Proteasome component PRE2 precursor – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0235]	gi 145233295 ref XP_001400020.1	hypothetical protein An02g08850 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q5R5I4 UB2L3_PONPY	Ubiquitin-conjugating enzyme E2 L3 – <i>Pongo pygmaeus</i> (Orangutan)
Contig[0237]	gi 150856640 gb EDN31832.1	histone H2B [Botryotinia fuckeliana B05.10]	Q8J1K2 H2B_ROSNE	Histone H2B – <i>Rosellinia necatrix</i> (White root-rot fungus)
Contig[0240]	gi 115433342 ref XP_001216808.1	conserved hypothetical protein [Aspergillus terreus NIH2624] conserved hypothetical protein [Aspergillus terreus NIH2624]	P22151 GRG1_NEUCR	Glucose-repressible gene protein – <i>Neurospora crassa</i>
Contig[0242]	gi 150852731 gb EDN27923.1	hypothetical protein BC1G_07780 [Botryotinia fuckeliana B05.10]	P37211 ATPA_NEUCR	ATP synthase subunit alpha, mitochondrial precursor – <i>Neurospora crassa</i>
Contig[0246]	gi 145235505 ref XP_001390401.1	hypothetical protein An03g05200 [Aspergillus niger] unnamed protein product [Aspergillus niger]	P34946 CPS1_PENJA	Carboxypeptidase S1 – <i>Penicillium janthinellum</i> (<i>Penicillium vitale</i>)
Contig[0251]	gi 150849017 gb EDN24210.1	predicted protein [Botryotinia fuckeliana B05.10]	P52553 PFD6_YEAST	Prefoldin subunit 6 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0252]	gi 150411355 gb EDN06743.1	cytochrome c1 [Ajellomyces capsulatus NAml]	P07142 CY1_NEUCR	Cytochrome c1 heme protein, mitochondrial precursor – <i>Neurospora crassa</i>
Contig[0253]	gi 111058828 gb EAT79948.1	predicted protein [Phaeosphaeria nodorum SN15]	O74226 KRE9_CANAL	Cell wall synthesis protein KRE9 precursor – <i>Candida albicans</i> (Yeast)
Contig[0255]	gi 150848019 gb EDN23212.1	hypothetical protein BC1G_00685 [Botryotinia fuckeliana B05.10]	Q08548 YO175_YEAST	Putative membrane-bound O-acyltransferase YOR175C – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0259]	gi 115390164 ref XP_001212587.1	hypothetical protein ATEG_03409 [Aspergillus terreus NIH2624] hypothetical protein ATEG_03409 [Aspergillus terreus NIH2624]	P33313 CNS1_YEAST	Cyclophilin seven suppressor 1 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0260]	gi 111066850 gb EAT87970.1	hypothetical protein SNOG_04210 [Phaeosphaeria nodorum SN15]	P25338 YGB0_YEAST	Uncharacterized endoplasmic reticulum membrane protein YGL010W – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0262]	gi 70999125 ref XP_754284.1	ribose-phosphate pyrophosphokinase [Aspergillus fumigatus Af293]	Q12265 KPR5_YEAST	Probable ribose-phosphate pyrophosphokinase 5 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0266]	gi 150413169 gb EDN08552.1	hypothetical protein HCAG_05051 [Ajellomyces capsulatus NAml]	Q0ULD0 NACB_PHANO	Nascent polypeptide-associated complex subunit beta – <i>Phaeosphaeria nodorum</i> (<i>Sep-toria nodorum</i>)
Contig[0268]	gi 482303 pir A38084	galactose oxidase (EC 1.1.3.9) precursor [validated] – fungus (<i>Cladobotryum dendroides</i>)	Q01745 GAOA_GIBZE	Galactose oxidase precursor – <i>Gibberella zeae</i> (<i>Fusarium graminearum</i>)
Contig[0274]	gi 51702016 sp Q8J0N3 SODC_PAETN	Superoxide dismutase [Cu-Zn] copper-zinc superoxide dismutase [Paecilomyces tenuipes]	Q8J0N3 SODC_PAETN	Superoxide dismutase [Cu-Zn] – <i>Paecilomyces tenuipes</i>

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0278]	gi 85104930 ref XP_961835.1	40S RIBOSOMAL PROTEIN S17 (CRP3) [Neurospora crassa OR74A] 40S ribosomal protein S17 (CRP3) ribosomal protein [Neurospora crassa] 40S RIBOSOMAL PROTEIN S17 (CRP3) [Neurospora crassa]	P27770 RS17_NEUCR	40S ribosomal protein S17 – Neurospora crassa
Contig[0285]	gi 85105087 ref XP_961885.1	hypothetical protein ((AJ236906) 64 kDa mitochondrial NADH dehydrogenase [Neurospora crassa OR74A]) hypothetical protein ((AJ236906) 64 kDa mitochondrial NADH dehydrogenase [Neurospora crassa])	P32340 NDI1_YEAST	Rotenone-insensitive NADH-ubiquinone oxidoreductase, mitochondrial precursor – Saccharomyces cerevisiae (Baker's yeast)
Contig[0299]	gi 119467836 ref XP_001257724.1	wd-repeat protein [Neosartorya fischeri NRRL 181] wd-repeat protein [Neosartorya fischeri NRRL 181]	P78706 RCO1_NEUCR	Transcriptional repressor rco-1 – Neurospora crassa
Contig[0303]	gi 145238840 ref XP_001392067.1	hypothetical protein An07g09920 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q9C102 GLT1_SCHPO	Putative glutamate synthase [NADPH] – Schizosaccharomyces pombe (Fission yeast)
Contig[0305]	gi 150847800 gb EDN22993.1	prohibitin [Botryotinia fuckeliana B05.10]	P50085 PHB2_YEAST	Prohibitin-2 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0306]	gi 121706746 ref XP_001271617.1	37S ribosomal protein Rsm25 [Aspergillus clavatus NRRL 1] 37S ribosomal protein Rsm25 [Aspergillus clavatus NRRL 1]	P40496 RT25_YEAST	Mitochondrial 37S ribosomal protein S25 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0307]	gi 119467490 ref XP_001257551.1	glutathione-s-transferase theta, gst [Neosartorya fischeri NRRL 181] glutathione-s-transferase theta, gst [Neosartorya fischeri NRRL 181]	O59827 GST2_SCHPO	Glutathione S-transferase II – Schizosaccharomyces pombe (Fission yeast)
Contig[0310]	gi 83772625 dbj BAE62753.1	unnamed protein product [Aspergillus oryzae]	P35128 UBCD3_DROME	Ubiquitin-conjugating enzyme E2-17 kDa – Drosophila melanogaster (Fruit fly)
Contig[0313]	gi 119184891 ref XP_001243300.1	hypothetical protein CIMG_07196 [Coccidioides immitis RS] hypothetical protein CIMG_07196 [Coccidioides immitis RS]	Q09116 SPN2_SCHPO	Septin homolog spn2 – Schizosaccharomyces pombe (Fission yeast)
Contig[0318]	gi 119493011 ref XP_001263761.1	protein translocase, putative [Neosartorya fischeri NRRL 181] protein translocase, putative [Neosartorya fischeri NRRL 181]	Q9C2D4 SC61G_NEUCR	Probable protein transport protein SEC61 subunit gamma – Neurospora crassa
Contig[0320]	gi 4572458 gb AAD23831.1 AF123482_1	NAD-dependent formate dehydrogenase [Mycosphaerella graminicola]	Q03134 FDH_EMENI	Probable formate dehydrogenase – Emericella nidulans (Aspergillus nidulans)
Contig[0322]	gi 150846588 gb EDN21781.1	NADH-ubiquinone oxidoreductase 29.9 kDa subunit [Botryotinia fuckeliana B05.10]	P24919 NDUA5_NEUCR	NADH-ubiquinone oxidoreductase 29.9 kDa subunit, mitochondrial precursor – Neurospora crassa
Contig[0323]	gi 150412789 gb EDN08176.1	ATP-dependent molecular chaperone HSC82 [Ajellomyces capsulatus NAm1]	O43109 HSP90_PODAN	Heat shock protein 90 homolog – Podospira anserina
Contig[0328]	gi 145253883 ref XP_001398454.1	hypothetical protein An17g02250 [Aspergillus niger] unnamed protein product [Aspergillus niger]	P35682 BD31C_ORYSJ	Protein BUD31 homolog 3 – Oryza sativa subsp. japonica (Rice)
Contig[0329]	gi 39972029 ref XP_367405.1	hypothetical protein MGG_07330 [Magnaporthe grisea 70-15] hypothetical protein MGG_07330 [Magnaporthe grisea 70-15]	P36088 YKG9_YEAST	UPF0067 protein YKL069W – Saccharomyces cerevisiae (Baker's yeast)

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0342]	gi 150843705 gb EDN18898.1	hypothetical protein BC1G_03047 [Botryotinia fuckeliana B05.10]	Q12164 YL023_YEAST	Uncharacterized membrane protein YLL023C – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0346]	gi 23270399 ref NP_690845.1	Mitochondrial protein of unknown function, overexpression suppresses an rpo41 mutation affecting mitochondrial RNA polymerase; encoded within the 25S rRNA gene on the opposite strand [Saccharomyces cerevisiae] Protein TAR1 (Transcript antisense to ribosomal RNA protein 1) unknown [Saccharomyces cerevisiae]	Q8TGM6 TAR1_YEAST	Protein TAR1 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0349]	gi 150414169 gb EDN09534.1	histone H3 [Ajellomyces capsulatus NAml]	P61834 H3_PENFN	Histone H3 – <i>Penicillium funiculosum</i>
Contig[0357]	gi 145236130 ref XP_001390713.1	unnamed protein product [Aspergillus niger] unnamed protein product [Aspergillus niger]	P50198 LINX_PSEPA	2,5-dichloro-2,5-cyclohexadiene-1,4-diol dehydrogenase – <i>Pseudomonas paucimobilis</i> (<i>Sphingomonas paucimobilis</i>)
Contig[0366]	gi 150415209 gb EDN10562.1	conserved hypothetical protein [Ajellomyces capsulatus NAml]	Q9Y7K7 YGI5_SCHPO	Uncharacterized FAM18-like protein C2A9.05c – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0373]	gi 119480027 ref XP_001260042.1	GPR/FUN34 family protein [Neosartorya fischeri NRRL 181] GPR/FUN34 family protein [Neosartorya fischeri NRRL 181]	P25613 YQCQ0_YEAST	Uncharacterized protein YCR010C – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0374]	gi 150408416 gb EDN03957.1	conserved hypothetical protein [Ajellomyces capsulatus NAml]	P24276 SSD1_YEAST	Protein SSD1 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0377]	gi 150858408 gb EDN33600.1	predicted protein [Botryotinia fuckeliana B05.10]	Q4WL B9 YA090_ASPFU	Uncharacterized protein AFUA_6G14090 precursor – <i>Aspergillus fumigatus</i> (<i>Sartorya fumigata</i>)
Contig[0384]	gi 115384126 ref XP_001208610.1	conserved hypothetical protein [Aspergillus terreus NIH2624] conserved hypothetical protein [Aspergillus terreus NIH2624]	Q9P6A5 SEC17_NEUCR	Probable vesicular-fusion protein sec17 homolog – <i>Neurospora crassa</i>
Contig[0386]	gi 150855716 gb EDN30908.1	guanine nucleotide-binding protein beta subunit [Botryotinia fuckeliana B05.10]	Q01369 GBLP_NEUCR	Guanine nucleotide-binding protein subunit beta-like protein – <i>Neurospora crassa</i>
Contig[0388]	gi 85093899 ref XP_959782.1	hypothetical protein [Neurospora crassa OR74A] hypothetical protein [Neurospora crassa]	Q50228 FMDA_METME	Formamidase – <i>Methylophilus methylotrophus</i> (<i>Bacterium W3A1</i>)
Contig[0389]	gi 111065213 gb EAT86333.1	hypothetical protein SNOG_06502 [Phaeosphaeria nodorum SN15]	P61869 MDLA_PENCY	Mono- and diacylglycerol lipase precursor – <i>Penicillium cyclopium</i>
Contig[0397]	gi 119181595 ref XP_001242002.1	hypothetical protein CIMG_05898 [Coccidioides immitis RS] hypothetical protein CIMG_05898 [Coccidioides immitis RS]	P38918 ARK73_RAT	Aflatoxin B1 aldehyde reductase member 3 – <i>Rattus norvegicus</i> (Rat)
Contig[0398]	gi 150848818 gb EDN24011.1	conserved hypothetical protein [Botryotinia fuckeliana B05.10]	Q50228 FMDA_METME	Formamidase – <i>Methylophilus methylotrophus</i> (<i>Bacterium W3A1</i>)
Contig[0399]	gi 150413349 gb EDN08732.1	40S ribosomal protein S10-A [Ajellomyces capsulatus NAml]	O13614 RS10B_SCHPO	40S ribosomal protein S10-B – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0401]	gi 111434061 gb ABH09883.1	MrgA [<i>Penicillium marneffei</i>]	P39959 YEW0_YEAST	Zinc finger protein YER130C – <i>Saccharomyces cerevisiae</i> (Baker's yeast)

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0407]	gi 116499720 gb EAU82615.1	hypothetical protein CC1G_07897 [Coprinopsis cinerea okayama7#130]	Q12612 TRI4_FUSSP	Trichodiene oxygenase – Fusarium sporotrichioides
Contig[0409]	gi 111063370 gb EAT84490.1	predicted protein [Phaeosphaeria nodorum SN15]	Q95V31 RS16_SPOFR	40S ribosomal protein S16 – Spodoptera frugiperda (Fall armyworm)
Contig[0410]	gi 150854172 gb EDN29364.1	hypothetical protein BC1G_08370 [Botryotinia fuckeliana B05.10]	P15703 BGL2_YEAST	Glucan 1,3-beta-glucosidase precursor – Saccharomyces cerevisiae (Baker's yeast)
Contig[0411]	gi 150857626 gb EDN32818.1	40S ribosomal protein S22 [Botryotinia fuckeliana B05.10]	Q7RV75 RS22_NEUCR	40S ribosomal protein S22 – Neurospora crassa
Contig[0415]	gi 119472804 ref XP_001258421.1	NADH-ubiquinone oxidoreductase 39 kDa subunit, putative [Neosartorya fischeri NRRL 181] NADH-ubiquinone oxidoreductase 39 kDa subunit, putative [Neosartorya fischeri NRRL 181]	P25284 NDUA9_NEUCR	NADH-ubiquinone oxidoreductase 40 kDa subunit, mitochondrial precursor – Neurospora crassa
Contig[0418]	gi 119178708 ref XP_001240995.1	acyl-CoA desaturase [Coccidioides immitis RS] acyl-CoA desaturase [Coccidioides immitis RS]	Q12618 ACO1_AJECA	Acyl-CoA desaturase – Ajellomyces capsulata (Histoplasma capsulatum)
Contig[0422]	gi 150408899 gb EDN04355.1	predicted protein [Ajellomyces capsulatus NAM1]	Q6CF41 FKBP_YARLI	FK506-binding protein 1 – Yarrowia lipolytica (Candida lipolytica)
Contig[0424]	gi 119194621 ref XP_001247914.1	hypothetical protein CIMG_01685 [Coccidioides immitis RS] hypothetical protein CIMG_01685 [Coccidioides immitis RS]	P29453 RL8B_YEAST	60S ribosomal protein L8-B – Saccharomyces cerevisiae (Baker's yeast)
Contig[0430]	gi 111062996 gb EAT84116.1	hypothetical protein SNOG_08948 [Phaeosphaeria nodorum SN15]	P22217 TRX1_YEAST	Thioredoxin I – Saccharomyces cerevisiae (Baker's yeast)
Contig[0431]	gi 150409611 gb EDN05055.1	hypothetical protein HCAG_08709 [Ajellomyces capsulatus NAM1]	Q1DP69 DBP2_COCIM	ATP-dependent RNA helicase DBP2 – Coccidioides immitis
Contig[0438]	gi 111063599 gb EAT84719.1	hypothetical protein SNOG_08443 [Phaeosphaeria nodorum SN15]	Q9P3R1 ERG6_NEUCR	Sterol 24-C-methyltransferase – Neurospora crassa
Contig[0441]	gi 67005921 gb AAY62596.1	Pth12p [Magnaporthe grisea]	O70477 PKNX1_MOUSE	Homeobox protein PKNOX1 – Mus musculus (Mouse)
Contig[0443]	gi 119178714 ref XP_001240997.1	hypothetical protein CIMG_08160 [Coccidioides immitis RS] hypothetical protein CIMG_08160 [Coccidioides immitis RS]	P25375 PRTD_YEAST	Saccharolysin – Saccharomyces cerevisiae (Baker's yeast)
Contig[0445]	gi 150412301 gb EDN07688.1	proteasome component Pre1 [Ajellomyces capsulatus NAM1]	Q9P6U7 PSB2_NEUCR	Probable proteasome subunit beta type 2 – Neurospora crassa
Contig[0446]	gi 111064907 gb EAT86027.1	hypothetical protein SNOG_06196 [Phaeosphaeria nodorum SN15]	Q63060 GLPK_RAT	Glycerol kinase – Rattus norvegicus (Rat)
Contig[0461]	gi 146324608 ref XP_746718.2	37S ribosomal protein S24 [Aspergillus fumigatus Af293]	P26782 RS24_YEAST	40S ribosomal protein S24 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0462]	gi 23270399 ref NP_690845.1	Mitochondrial protein of unknown function, overexpression suppresses an rpo41 mutation affecting mitochondrial RNA polymerase; encoded within the 25S rRNA gene on the opposite strand [Saccharomyces cerevisiae] Protein TAR1 (Transcript antisense to ribosomal RNA protein 1) unknown [Saccharomyces cerevisiae]	Q8TGM6 TAR1_YEAST	Protein TAR1 – Saccharomyces cerevisiae (Baker's yeast)

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0475]	gi 83772686 dbj BAE62814.1	unnamed protein product [Aspergillus oryzae]	Q5QA93 HSV2_PICAN	SVP1-like protein 2 – Pichia angusta (Yeast) (Hansenula polymorpha)
Contig[0478]	gi 150414583 gb EDN09945.1	conserved hypothetical protein [Ajellomyces capsulatus NAml]	P53981 YNB0_YEAST	Uncharacterized protein YNL010W – Saccharomyces cerevisiae (Baker's yeast)
Contig[0482]	gi 50552368 ref XP_503594.1	hypothetical protein [Yarrowia lipolytica] unnamed protein product [Yarrowia lipolytica CLIB122]	P50166 ARDH_CANTR	D-arabinitol 2-dehydrogenase [ribulose-forming] – Candida tropicalis (Yeast)
Contig[0484]	gi 46122635 ref XP_385871.1	hypothetical protein FG05695.1 [Gibberella zeae PH-1]	P30263 CATA_PICAN	Peroxisomal catalase – Pichia angusta (Yeast) (Hansenula polymorpha)
Contig[0486]	gi 150856640 gb EDN31832.1	histone H2B [Botryotinia fuckeliana B05.10]	Q8J1K2 H2B_ROSNE	Histone H2B – Rosellinia necatrix (White root-rot fungus)
Contig[0491]	gi 121706746 ref XP_001271617.1	37S ribosomal protein Rsm25 [Aspergillus clavatus NRRL 1] 37S ribosomal protein Rsm25 [Aspergillus clavatus NRRL 1]	P40496 RT25_YEAST	Mitochondrial 37S ribosomal protein S25 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0494]	gi 150414169 gb EDN09534.1	histone H3 [Ajellomyces capsulatus NAml]	P61834 H3_PENFN	Histone H3 – Penicillium funiculosum
Contig[0496]	gi 150846081 gb EDN21274.1	hypothetical protein BC1G_14884 [Botryotinia fuckeliana B05.10]	Q01447 ERG24_FUSSO	Delta(14)-sterol reductase – Fusarium solani subsp. pisi (Nectria haematococca)
SLJClgrIII01M13RF05_F05	gi 91212581 ref YP_542567.1	Cold-shock DEAD-box protein A [Escherichia coli UTI89] Cold-shock DEAD-box protein A [Escherichia coli UTI89]	P0A9P8 DEAD_SHIFL	Cold-shock DEAD box protein A – Shigella flexneri
SLJClgrIII01M13RF08_F08	gi 111064038 gb EAT85158.1	predicted protein [Phaeosphaeria nodorum SN15]	Q12087 RS30_YEAST	40S ribosomal protein S30 – Saccharomyces cerevisiae (Baker's yeast)
SLJClgrIII01T7B04_B04	gi 150409611 gb EDN05055.1	hypothetical protein HCAG_08709 [Ajellomyces capsulatus NAml]	Q1DP69 DBP2_COCIM	ATP-dependent RNA helicase DBP2 – Coccidioides immitis
SLJClgrIII01T7F05_F05	gi 91212581 ref YP_542567.1	Cold-shock DEAD-box protein A [Escherichia coli UTI89] Cold-shock DEAD-box protein A [Escherichia coli UTI89]	P0A9P8 DEAD_SHIFL	Cold-shock DEAD box protein A – Shigella flexneri
SLJClgrIII01T7F12_F12	gi 119188389 ref XP_001244801.1	hypothetical protein CIMG_04242 [Coccidioides immitis RS] hypothetical protein CIMG_04242 [Coccidioides immitis RS]	Q9UT44 PROA_SCHPO	Probable gamma-glutamyl phosphate reductase – Schizosaccharomyces pombe (Fission yeast)
SLJClgrIII01T7H10_H10	gi 150855850 gb EDN31042.1	40S ribosomal protein S13 [Botryotinia fuckeliana B05.10]	P33192 RS13_CANMA	40S ribosomal protein S13 – Candida maltosa (Yeast)
SLJClgrIII03M13RB08_B08	gi 150854172 gb EDN29364.1	hypothetical protein BC1G_08370 [Botryotinia fuckeliana B05.10]	P43070 BGL2_CANAL	Glucan 1,3-beta-glucosidase precursor – Candida albicans (Yeast)
SLJClgrIII03M13RF01_F01	gi 150414094 gb EDN09459.1	mitochondrial chaperone BCS1 [Ajellomyces capsulatus NAml]	P32839 BCS1_YEAST	Mitochondrial chaperone BCS1 – Saccharomyces cerevisiae (Baker's yeast)
SLJClgrIII03M13RF05_F05	gi 150857802 gb EDN32994.1	clathrin coat assembly protein [Botryotinia fuckeliana B05.10]	Q7SAQ1 AP2S_NEUCR	AP-2 complex subunit sigma – Neurospora crassa
SLJClgrIII03M13RF06_F06	gi 111063599 gb EAT84719.1	hypothetical protein SNOG_08443 [Phaeosphaeria nodorum SN15]	Q9P3R1 ERG6_NEUCR	Sterol 24-C-methyltransferase – Neurospora crassa
SLJClgrIII03M13RG01_G01	gi 111060388 gb EAT81508.1	hypothetical protein SNOG_11009 [Phaeosphaeria nodorum SN15]	P22394 LIP2_GEOCN	Lipase 2 precursor – Geotrichum candidum (Oospora lactis) (Dipodascus geotrichum)

Table A.1: Combined GenBank and UniProt summary information for stage 1 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
SLJClgrIII03T7FH11_H11	gi 150842351 gb EDN17544.1	tubulin beta chain [Botryotinia fuckeliana B05.10]	P41388 TBB_VENIN	Tubulin beta chain – Venturia inaequalis (Apple scab fungus)
SLJClgrIII05M13RE04_E04	gi 4572458 gb AAD23831.1	NAD-dependent formate dehydrogenase [Mycosphaerella graminicola]	Q03134 FDH_EMENI	Probable formate dehydrogenase – Emericella nidulans (Aspergillus nidulans)
SLJClgrIII06M13RA01_A01	gi 70999197 ref XP_754320.1	Zn-dependent hydro-lase/oxidoreductase family protein, putative [Aspergillus fumigatus Af293]	P64760 Y930_MYCBO	Uncharacterized protein Mb0930 precursor – Mycobacterium bovis
SLJClgrIII06T7E03_E03	gi 111057936 gb EAT79056.1	hypothetical protein SNOG_13609 [Phaeosphaeria nodorum SN15]	Q9P7J8 GAD8_SCHPO	Serine/threonine-protein kinase gad8 – Schizosaccharomyces pombe (Fission yeast)
SLJClgrIII06T7F02_F02	gi 150408808 gb EDN04264.1	conserved hypothetical protein [Ajellomyces capsulatus NAm1]	Q8AYC2 MRTFA_XENLA	Myocardin-related transcription factor A – Xenopus laevis (African clawed frog)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0001]	gi 164422140 ref YP_001648754.1	cytochrome oxidase subunit 2 [Mycosphaerella graminicola] cytochrome oxidase subunit 2 [Mycosphaerella graminicola]	P20682 COX2_PODAN	Cytochrome c oxidase subunit 2 – Podospira anserina
Contig[0006]	gi 187973019 gb EDU40518.1	CDP–diacylglycerol– glycerol–3–phosphate 3– phosphatidyltransferase [Pyrenophora tritici–repentis Pt–1C–BFP]	P25578 PGPS1_YEAST	CDP–diacylglycerol– glycerol–3–phosphate 3– phosphatidyltransferase – Sac- charomyces cerevisiae (Baker's yeast)
Contig[0007]	gi 154323079 ref XP_001560854.1	hypothetical protein BC1G_00882 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_00882 [Botryotinia fuckeliana B05.10]	Q03178 PIR1_YEAST	Protein PIR1 precursor – Saccha- romyces cerevisiae (Baker's yeast)
Contig[0009]	gi 187973918 gb EDU41417.1	glutaryl–CoA dehydrogenase, mi- tochondrial precursor [Pyrenophora tritici–repentis Pt–1C–BFP]	Q60759 GCDH_MOUSE	Glutaryl–CoA dehydrogenase, mi- tochondrial precursor – Mus muscu- lus (Mouse)
Contig[0011]	gi 116204595 ref XP_001228108.1	hypothetical protein CHGG_10181 [Chaetomium globosum CBS 148.51] hypothetical protein CHGG_10181 [Chaetomium globo- sum CBS 148.51]	Q4U3E8 MPG1_ASPFU	Mannose–1–phosphate guanyltrans- ferase – Aspergillus fumigatus (Sar- torya fumigata)
Contig[0013]	gi 187973428 gb EDU40927.1	mannose–1–phosphate guanyltrans- ferase [Pyrenophora tritici–repentis Pt–1C–BFP]		
Contig[0015]	gi 70992189 ref XP_750943.1	arrestin domain protein [As- pergillus fumigatus Af293] arrestin domain protein [Aspergillus fumi- gatus A1163]		
Contig[0016]	gi 67521664 ref XP_658893.1	hypothetical protein AN1289.2 [As- pergillus nidulans FGSC A4] Pro- tein rmd11 precursor	Q5BDU1 RMD11_EMENI	Protein rmd11 precursor – Emericella nidulans (Aspergillus nidulans)
Contig[0019]	gi 169609703 ref XP_001798270.1	hypothetical protein SNOG_07944 [Phaeosphaeria nodorum SN15]		
Contig[0022]	gi 169770325 ref XP_001819632.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]	O60177 YG42_SCHPO	Uncharacterized ATP–dependent helicase C23E6.02 – Schizosaccha- romyces pombe (Fission yeast)
Contig[0024]	gi 115399884 ref XP_001215531.1	hypothetical protein ATEG_06353 [Aspergillus terreus NIH2624] hypo- thetical protein ATEG_06353 [As- pergillus terreus NIH2624]		
Contig[0025]	gi 169606530 ref XP_001796685.1	hypothetical protein SNOG_06308 [Phaeosphaeria nodorum SN15]	P36062 AVT3_YEAST	Vacuolar amino acid transporter 3 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0027]	gi 145603699 ref XP_369584.2	hypothetical protein MGG_05880 [Magnaporthe grisea 70–15] hypo- thetical protein MGG_05880 [Mag- naporthe grisea 70–15]		
Contig[0028]	gi 50555151 ref XP_504984.1	YITSR1 [Yarrowia lipolytica] YITSR1 [Yarrowia lipolytica CLIB122]		
Contig[0031]	gi 119192652 ref XP_001246932.1	hypothetical protein CIMG_00703 [Coccidioides immitis RS] hypo- thetical protein CIMG_00703 [Coc- cidioides immitis RS]		

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0034]	gi 156059736 ref XP_001595791.1	hypothetical protein SS1G_03881 [Sclerotinia sclerotiorum 1980]		
Contig[0043]	gi 119178466 ref XP_001240906.1	hypothetical protein SS1G_03881 [Sclerotinia sclerotiorum 1980]		
Contig[0044]	gi 156044714 ref XP_001588913.1	hypothetical protein CIMG_08069 [Coccidioides immitis RS] hypo- thetical protein CIMG_08069 [Coc- cidioides immitis RS]	P34054 INDA1_TRIAT	Amino-acid permease inda1 – Trichoderma atroviride (Hypocrea atroviridis)
Contig[0049]	gi 171685918 ref XP_001907900.1	hypothetical protein SS1G_10461 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_10461 [Sclerotinia sclerotiorum 1980]	P38279 YBZ7_YEAST	Uncharacterized membrane protein YBR147W – Saccharomyces cere- visiae (Baker's yeast)
Contig[0050]	gi 50551127 ref XP_503037.1	unnamed protein product [Po- dospora anserina] unnamed protein product [Podospora anserina]		
Contig[0051]	gi 187972522 gb EDU40021.1	hypothetical protein [Yarrowia lipolytica] unnamed protein prod- uct [Yarrowia lipolytica CLIB122]	O42626 NRC2_NEUCR	Serine/threonine-protein kinase nrc-2 – Neurospora crassa
Contig[0052]	gi 154292281 ref XP_001546716.1	serine/threonine-protein kinase ppk14 [Pyrenophora tritici-repentis Pt-1C-BFP]	Q60759 GCDH_MOUSE	Glutaryl-CoA dehydrogenase, mi- tochondrial precursor – Mus muscu- lus (Mouse)
Contig[0054]	gi 156044714 ref XP_001588913.1	conserved hypothetical protein [Botryotinia fuckeliana B05.10] conserved hypothetical protein [Botryotinia fuckeliana B05.10]	Q9P768 YI26_SCHPO	Uncharacterized amino-acid perme- ase P7G5.06 – Schizosaccharomyces pombe (Fission yeast)
Contig[0058]	gi 154311873 ref XP_001555265.1	hypothetical protein SS1G_10461 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_10461 [Sclerotinia sclerotiorum 1980]		
Contig[0062]	gi 482303 pir A38084	hypothetical protein BC1G_05970 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_05970 [Botryotinia fuckeliana B05.10]	Q01745 GAOA_GIBZE	Galactose oxidase precursor – Gibberella zeae (Fusarium graminearum)
Contig[0065]	gi 154282291 ref XP_001541941.1	galactose oxidase (EC 1.1.3.9) precursor [validated] – fungus (Cladobotryum dendroides)	Q6CA53 APT_YARLI	Adenine phosphoribosyltransferase – Yarrowia lipolytica (Candida lipolytica)
Contig[0067]	gi 187983150 gb EDU48638.1	adenine phosphoribosyltransferase 1 [Ajellomyces capsulatus NAm1] adenine phosphoribosyltransferase 1 [Ajellomyces capsulatus NAm1]		
Contig[0068]	gi 187985079 gb EDU50567.1	RING finger domain containing protein [Pyrenophora tritici- repentis Pt-1C-BFP]		
Contig[0070]	gi 46122635 ref XP_385871.1	conserved hypothetical protein [Pyrenophora tritici-repentis Pt-1C-BFP]	Q96VB8 CATA_CANBO	Peroxisomal catalase – Candida boidinii (Yeast)
Contig[0073]	gi 169597099 ref XP_001791973.1	hypothetical protein FG05695.1 [Gibberella zeae PH-1]		
Contig[0079]	gi 145240599 ref XP_001392946.1	hypothetical protein SNOG_01331 [Phaeosphaeria nodorum SN15] hypothetical protein An08g08330 [Aspergillus niger] unnamed protein product [Aspergillus niger]		
Contig[0081]	gi 187978014 gb EDU44640.1	hypothetical protein PTRG_11590 [Pyrenophora tritici-repentis Pt- 1C-BFP]		

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0082]	gi 145609319 ref XP_367597.2	hypothetical protein MGG_13410 [Magnaporthe grisea 70–15] hypothetical protein MGG_13410 [Magnaporthe grisea 70–15]		
Contig[0083]	gi 169613897 ref XP_001800365.1	hypothetical protein SNOG_10083 [Phaeosphaeria nodorum SN15]	Q9URM2 TKT_SCHPO	Probable transketolase – Schizosaccharomyces pombe (Fission yeast)
Contig[0085]	gi 157061845 gb ABV03820.1	extracellular lipase [Aureobasidium pullulans]	Q9P979 FAEA_ASPAW	Feruloyl esterase A precursor – Aspergillus awamori
Contig[0091]	gi 187978504 gb EDU45130.1	benomyl/methotrexate resistance protein [Pyrenophora tritici-repentis Pt-1C-BFP]	Q12256 TPO4_YEAST	Polyamine transporter 4 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0094]	gi 187973918 gb EDU41417.1	glutaryl-CoA dehydrogenase, mitochondrial precursor [Pyrenophora tritici-repentis Pt-1C-BFP]	Q60759 GCDH_MOUSE	Glutaryl-CoA dehydrogenase, mitochondrial precursor – Mus musculus (Mouse)
Contig[0099]	gi 156059188 ref XP_001595517.1	glucan 1,3- β -glucosidase [Sclerotinia sclerotiorum 1980] glucan 1,3- β -glucosidase [Sclerotinia sclerotiorum 1980]	O13990 BGL2_SCHPO	Glucan 1,3- β -glucosidase precursor – Schizosaccharomyces pombe (Fission yeast)
Contig[0100]	gi 156048420 ref XP_001590177.1	hypothetical protein SS1G_08941 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_08941 [Sclerotinia sclerotiorum 1980]		
Contig[0101]	gi 169603183 ref XP_001795013.1	hypothetical protein SNOG_04600 [Phaeosphaeria nodorum SN15]		
Contig[0104]	gi 154305938 ref XP_001553370.1	hypothetical protein BC1G_08200 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_08200 [Botryotinia fuckeliana B05.10]	Q2U9E2 FES1_ASPOR	Hsp70 nucleotide exchange factor fes1 – Aspergillus oryzae
Contig[0111]	gi 159128420 gb EDP53535.1	aspartic-type endopeptidase, putative [Aspergillus fumigatus A1163]		
Contig[0116]	gi 71019727 ref XP_760094.1	hypothetical protein UM03947.1 [Ustilago maydis 521] hypothetical protein UM03947.1 [Ustilago maydis 521]	P34946 CPS1_PENJA	Carboxypeptidase S1 – Penicillium janthinellum (Penicillium vitale)
Contig[0117]	gi 154284576 ref XP_001543083.1	predicted protein [Ajellomyces capsulatus NAM1] predicted protein [Ajellomyces capsulatus NAM1]		
Contig[0123]	gi 169772161 ref XP_001820550.1	hypothetical protein [Aspergillus oryzae RIB40] triacylglycerol lipase [Aspergillus oryzae] unnamed protein product [Aspergillus oryzae]	Q8TGB8 CUTI_MONFR	Cutinase precursor – Monilinia fructicola
Contig[0124]	gi 46114448 ref XP_383242.1	hypothetical protein FG03066.1 [Gibberella zeae PH-1]	P37295 PSY_NEUCR	Phytoene synthase – Neurospora crassa
Contig[0125]	gi 159128013 gb EDP53128.1	histone H1 [Aspergillus fumigatus A1163]	Q9P8F8 H1_EMENI	Histone H1 – Emericella nidulans (Aspergillus nidulans)
Contig[0126]	gi 46109376 ref XP_381746.1	hypothetical protein FG01570.1 [Gibberella zeae PH-1]	Q00298 CUTI_BOTFU	Cutinase precursor – Botryotinia fuckeliana (Noble rot fungus) (Botrytis cinerea)
Contig[0127]	gi 50549899 ref XP_502421.1	hypothetical protein [Yarrowia lipolytica] unnamed protein product [Yarrowia lipolytica CLIB122]	P38929 ATC2_YEAST	Calcium-transporting ATPase 2 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0130]	gi 145238876 ref XP_001392085.1	hypothetical protein An07g10100 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q9HE76 KAD_NEUCR	Probable adenylate kinase – Neurospora crassa
Contig[0139]	gi 45356863 gb AAS58470.1	heat shock protein 70 [Aspergillus fumigatus]	Q92260 HSP70_PENCI	Heat shock 70 kDa protein – Penicillium citrinum

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0141]	gi 154303659 ref XP_001552236.1	hypothetical protein BC1G_08714 [Botryotinia fuckeliana B05.10]		
Contig[0148]	gi 156065107 ref XP_001598475.1	hypothetical protein BC1G_08714 [Botryotinia fuckeliana B05.10]		
Contig[0153]	gi 169599390 ref XP_001793118.1	predicted protein [Sclerotinia sclerotiorum 1980] predicted protein [Sclerotinia sclerotiorum 1980]		
Contig[0156]	gi 187981103 gb EDU47729.1	hypothetical protein SNOG_02514 [Phaeosphaeria nodorum SN15]	Q39172 P1_ARATH	Probable NADP-dependent oxidoreductase P1 – Arabidopsis thaliana (Mouse-ear cress)
Contig[0161]	gi 119192624 ref XP_001246918.1	NADP-dependent leukotriene B4 12-hydroxydehydrogenase [Pyrenophora tritici-repentis Pt-1C-BFP]	Q12252 PHM7_YEAST	Phosphate metabolism protein 7 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0163]	gi 156060529 ref XP_001596187.1	hypothetical protein CIMG_00689 [Coccidioides immitis RS]		
Contig[0166]	gi 119194599 ref XP_001247903.1	hypothetical protein CIMG_00689 [Coccidioides immitis RS]		
Contig[0171]	gi 154295099 ref XP_001547987.1	hypothetical protein SS1G_02403 [Sclerotinia sclerotiorum 1980]		
Contig[0172]	gi 156494375 ref YP_001427401.1	hypothetical protein CIMG_01674 [Coccidioides immitis RS]	Q9Y7M1 YGK4_SCHPO	Putative 2-hydroxyacyl-CoA lyase – Schizosaccharomyces pombe (Fission yeast)
Contig[0174]	gi 154304085 ref XP_001552448.1	hypothetical protein BC1G_13678 [Botryotinia fuckeliana B05.10]	P05510 NU5M_NEUCR	NADH-ubiquinone oxidoreductase chain 5 – Neurospora crassa
Contig[0175]	gi 169622210 ref XP_001804514.1	NADH dehydrogenase subunit 5 [Phaeosphaeria nodorum SN15]	P53716 YBJ5_CANAL	Uncharacterized protein in WHS11 5' region – Candida albicans (Yeast)
Contig[0177]	gi 154283583 ref XP_001542587.1	hypothetical protein BC1G_09678 [Botryotinia fuckeliana B05.10]	O93866 HSP70_TRIRU	Heat shock 70 kDa protein – Trichophyton rubrum
Contig[0179]	gi 67516039 ref XP_657905.1	hypothetical protein BC1G_09678 [Botryotinia fuckeliana B05.10]		
Contig[0181]	gi 156051982 ref XP_001591952.1	hypothetical protein SNOG_14322 [Phaeosphaeria nodorum SN15]		
Contig[0184]	gi 187984106 gb EDU49594.1	predicted protein [Ajellomyces capsulatus NAM1] predicted protein [Ajellomyces capsulatus NAM1]		
Contig[0185]	gi 119480027 ref XP_001260042.1	hypothetical protein AN0301.2 [Aspergillus nidulans FGSC A4]	O14201 MUG86_SCHPO	Meiotically up-regulated gene 86 protein – Schizosaccharomyces pombe (Fission yeast)
Contig[0190]	gi 60600824 gb AA26869.1	hypothetical protein SS1G_07398 [Sclerotinia sclerotiorum 1980]		
Contig[0192]	gi 156060529 ref XP_001596187.1	hypothetical protein SS1G_07398 [Sclerotinia sclerotiorum 1980]		
		predicted protein [Pyrenophora tritici-repentis Pt-1C-BFP]		
		GPR/FUN34 family protein [Neosartorya fischeri NRRL 181]		
		GPR/FUN34 family protein [Neosartorya fischeri NRRL 181]		
		unknown [Schistosoma japonicum]		
		hypothetical protein SS1G_02403 [Sclerotinia sclerotiorum 1980]		
		hypothetical protein SS1G_02403 [Sclerotinia sclerotiorum 1980]		

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0193]	gi 169597897 ref XP_001792372.1	hypothetical protein SNOG_01740 [Phaeosphaeria nodorum SN15]	P47044 YJF5_YEAST	Uncharacterized protein YJL055W – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0198]	gi 169615879 ref XP_001801355.1	hypothetical protein SNOG_11105 [Phaeosphaeria nodorum SN15]		
Contig[0202]	gi 154323079 ref XP_001560854.1	hypothetical protein BC1G_00882 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_00882 [Botryotinia fuckeliana B05.10]	Q03178 PIR1_YEAST	Protein PIR1 precursor – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0203]	gi 169601846 ref XP_001794345.1	hypothetical protein SNOG_03799 [Phaeosphaeria nodorum SN15]	P38329 YB85_YEAST	Uncharacterized membrane protein YBR235W – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0205]	gi 169781470 ref XP_001825198.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]		
Contig[0209]	gi 46109376 ref XP_381746.1	hypothetical protein FG01570.1 [Gibberella zeae PH-1]	Q00298 CUTI_BOTFU	Cutinase precursor – <i>Botryotinia fuckeliana</i> (Noble rot fungus) (<i>Botrytis cinerea</i>)
Contig[0215]	gi 154274856 ref XP_001538279.1	hypothetical protein HCAG_05884 [Ajellomyces capsulatus NAm1] hypothetical protein HCAG_05884 [Ajellomyces capsulatus NAm1]	P53319 6PGD2_YEAST	6-phosphogluconate dehydrogenase, decarboxylating 2 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0216]	gi 171687345 ref XP_001908613.1	unnamed protein product [Podospora anserina] unnamed protein product [Podospora anserina]	P43083 CP52V_CANAP	Cytochrome P450 52E1 – <i>Candida apicola</i> (Yeast)
Contig[0220]	gi 169769500 ref XP_001819220.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]	Q4WHA3 ARP4_AS PFU	Actin-related protein 4 – <i>Aspergillus fumigatus</i> (<i>Sartorya fumigata</i>)
Contig[0222]	gi 29839372 sp Q8TGB8 CUTI_MONFR	Cutinase precursor (Cutin hydrolase) cutinase [Monilinia fructicola] cutinase [Monilinia fructicola]	Q8TGB8 CUTI_MONFR	Cutinase precursor – <i>Monilinia fructicola</i>
Contig[0226]	gi 154314752 ref XP_001556700.1	conserved hypothetical protein [Botryotinia fuckeliana B05.10] conserved hypothetical protein [Botryotinia fuckeliana B05.10]	Q9URX7 ANM1_SCHPO	Probable protein arginine N-methyltransferase – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0227]	gi 154311552 ref XP_001555105.1	hypothetical protein BC1G_06235 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_06235 [Botryotinia fuckeliana B05.10]		
Contig[0228]	gi 154285676 ref XP_001543633.1	predicted protein [Ajellomyces capsulatus NAm1] predicted protein [Ajellomyces capsulatus NAm1]		
Contig[0230]	gi 169598610 ref XP_001792728.1	hypothetical protein SNOG_02111 [Phaeosphaeria nodorum SN15]		
Contig[0234]	gi 154301173 ref XP_001551000.1	hypothetical protein BC1G_10559 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_10559 [Botryotinia fuckeliana B05.10]		
Contig[0235]	gi 169603183 ref XP_001795013.1	hypothetical protein SNOG_04600 [Phaeosphaeria nodorum SN15]		
Contig[0237]	gi 171690566 ref XP_001910208.1	unnamed protein product [Podospora anserina] unnamed protein product [Podospora anserina]	O74885 YQE1_SCHPO	Putative dioxygenase C576.01c – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0241]	gi 187985079 gb EDU50567.1	conserved hypothetical protein [Pyrenophora tritici-repentis Pt-1C-BFP]		

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0242]	gi 156494375 ref YP_001427401.1	NADH dehydrogenase subunit 5 [Phaeosphaeria nodorum SN15]	Q6QU67 NU5M_ASPNG	NADH-ubiquinone oxidoreductase chain 5 – <i>Aspergillus niger</i>
Contig[0247]	gi 169763212 ref XP_001727506.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]	Q9Y815 RSV2_SCHPO	Zinc finger protein rsv2 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0249]	gi 156059188 ref XP_001595517.1	glucan 1,3-beta-glucosidase [Sclerotinia sclerotiorum 1980] glucan 1,3-beta-glucosidase [Sclerotinia sclerotiorum 1980]	O13990 BGL2_SCHPO	Glucan 1,3-beta-glucosidase precursor – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0260]	gi 46109376 ref XP_381746.1	hypothetical protein FG01570.1 [Gibberella zeae PH-1]	Q00298 CUTI_BOTFU	Cutinase precursor – <i>Botryotinia fuckeliana</i> (Noble rot fungus) (<i>Botrytis cinerea</i>)
Contig[0267]	gi 67522168 ref XP_659145.1	hypothetical protein AN1541.2 [Aspergillus nidulans FGSC A4]	Q4L966 ROCA_STAHJ	1-pyrroline-5-carboxylate dehydrogenase – <i>Staphylococcus haemolyticus</i> (strain JCSC1435)
Contig[0270]	gi 154314752 ref XP_001556700.1	conserved hypothetical protein [Botryotinia fuckeliana B05.10] conserved hypothetical protein [Botryotinia fuckeliana B05.10]	Q9URX7 ANM1_SCHPO	Probable protein arginine N-methyltransferase – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0272]	gi 119488145 ref XP_001262616.1	folylpolyglutamate synthase [Neosartorya fischeri NRRL 181] folylpolyglutamate synthase [Neosartorya fischeri NRRL 181]	O13492 FOLE_NEUCR	Folylpolyglutamate synthase – <i>Neurospora crassa</i>
Contig[0274]	gi 70997299 ref XP_753399.1	arrestin (or S-antigen), N-terminal domain protein [Aspergillus fumigatus Af293] conserved hypothetical protein [Aspergillus fumigatus A1163]	Q10347 YDA5_SCHPO	Uncharacterized protein C1F12.05 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0276]	gi 187983566 gb EDU49054.1	hypothetical protein PTRG_06134 [Pyrenophora tritici-repentis Pt-1C-BFP]	Q10347 YDA5_SCHPO	Uncharacterized protein C1F12.05 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0278]	gi 159130791 gb EDP55904.1	hypothetical protein AFUB_006050 [Aspergillus fumigatus A1163]		
Contig[0280]	gi 156049113 ref XP_001590523.1	hypothetical protein SS1G_08263 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_08263 [Sclerotinia sclerotiorum 1980]		
Contig[0283]	gi 67539734 ref XP_663641.1	G6PI_ASPOR Glucose-6-phosphate isomerase (GPI) (Phosphoglucose isomerase) (PGI) (Phosphohexose isomerase) (PHI) [Aspergillus nidulans FGSC A4]	Q9HGZ2 G6PI_ASPOR	Glucose-6-phosphate isomerase – <i>Aspergillus oryzae</i>
Contig[0285]	gi 116191875 ref XP_001221750.1	hypothetical protein CHGG_05655 [Chaetomium globosum CBS 148.51] hypothetical protein CHGG_05655 [Chaetomium globosum CBS 148.51]	Q7WLE5 NPD_BORBR	NAD-dependent deacetylase – <i>Bordetella bronchiseptica</i> (<i>Alcaligenes bronchisepticus</i>)
Contig[0288]	gi 169856913 ref XP_001835110.1	predicted protein [Coprinosin cinerea okayama7#130] predicted protein [Coprinosin cinerea okayama7#130]	P0ACA0 YAAH_SHIFL	Inner membrane protein yaaH – <i>Shigella flexneri</i>
Contig[0290]	gi 119196353 ref XP_001248780.1	hypothetical protein CIMG_02551 [Coccidioides immitis RS] hypothetical protein CIMG_02551 [Coccidioides immitis RS]	P49374 HGT1_KLULA	High-affinity glucose transporter – <i>Kluyveromyces lactis</i> (Yeast) (<i>Candida sphaerica</i>)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0293]	gi 154276240 ref XP_001538965.1	conserved hypothetical protein [Ajellomyces capsulatus NAM1]		
Contig[0296]	gi 187975477 gb EDU42103.1	conserved hypothetical protein [Ajellomyces capsulatus NAM1] plasma membrane calcium-transporting ATPase 3 [Pyrenophora tritici-repentis Pt-1C-BFP]	P38929 ATC2_YEAST	Calcium-transporting ATPase 2 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0297]	gi 154273338 ref XP_001537521.1	rab GDP-dissociation inhibitor [Ajellomyces capsulatus NAM1] rab GDP-dissociation inhibitor [Ajellomyces capsulatus NAM1]	P50396 GDIA_MOUSE	Rab GDP dissociation inhibitor alpha – <i>Mus musculus</i> (Mouse)
Contig[0298]	gi 169609116 ref XP_001797977.1	hypothetical protein SNOG_07644 [Phaeosphaeria nodorum SN15]	Q05567 SGPL_YEAST	Sphingosine-1-phosphate lyase – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0305]	gi 67516039 ref XP_657905.1	hypothetical protein AN0301.2 [Aspergillus nidulans FGSC A4]		
Contig[0306]	gi 156040491 ref XP_001587232.1	hypothetical protein SS1G_12262 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_12262 [Sclerotinia sclerotiorum 1980]		
Contig[0307]	gi 145248800 ref XP_001400739.1	hypothetical protein An14g01110 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q9Y757 CP52L_DEBHA	Cytochrome P450 52A12 – <i>Debaryomyces hansenii</i> (Yeast) (<i>Torulaspora hansenii</i>)
Contig[0308]	gi 154282291 ref XP_001541941.1	adenine phosphoribosyltransferase 1 [Ajellomyces capsulatus NAM1] adenine phosphoribosyltransferase 1 [Ajellomyces capsulatus NAM1]	Q6BZF9 APT_DEBHA	Adenine phosphoribosyltransferase – <i>Debaryomyces hansenii</i> (Yeast) (<i>Torulaspora hansenii</i>)
Contig[0312]	gi 115442904 ref XP_001218259.1	60S ribosomal protein L19 [Aspergillus terreus NIH2624] 60S ribosomal protein L19 [Aspergillus terreus NIH2624]	P05734 RL19_SCHPO	60S ribosomal protein L19 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0313]	gi 145233099 ref XP_001399922.1	transcription factor pacC–Aspergillus niger transcription factor pacC–Aspergillus niger	Q00203 PACC_ASPNG	pH-response transcription factor pacC/RIM101 – <i>Aspergillus niger</i>
Contig[0320]	gi 145603699 ref XP_369584.2	hypothetical protein MGG_05880 [Magnaporthe grisea 70–15] hypothetical protein MGG_05880 [Magnaporthe grisea 70–15]		
Contig[0326]	gi 156065107 ref XP_001598475.1	predicted protein [Sclerotinia sclerotiorum 1980] predicted protein [Sclerotinia sclerotiorum 1980]		
Contig[0330]	gi 156060463 ref XP_001596154.1	hypothetical protein SS1G_02370 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_02370 [Sclerotinia sclerotiorum 1980]	Q9P8U4 GEL2_ASPFU	1,3-beta-glucanosyltransferase gel2 precursor – <i>Aspergillus fumigatus</i> (<i>Sartorya fumigata</i>)
Contig[0331]	gi 156032403 ref XP_001585039.1	hypothetical protein SS1G_13899 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_13899 [Sclerotinia sclerotiorum 1980]	O14201 MUG86_SCHPO	Meiotically up-regulated gene 86 protein – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0334]	gi 146324608 ref XP_746718.2	37S ribosomal protein S24 [Aspergillus fumigatus Af293] 37S ribosomal protein S24 [Aspergillus fumigatus Af293]	P26782 RS24_YEAST	40S ribosomal protein S24 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0334]	gi 146324608 ref XP_746718.2	37S ribosomal protein S24 [Aspergillus fumigatus Af293] 37S ribosomal protein S24 [Aspergillus fumigatus Af293]	P26782 RS24_YEAST	40S ribosomal protein S24 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0335]	gi 154308532 ref XP_001553602.1	hypothetical protein BC1G_08326 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_08326 [Botryotinia fuckeliana B05.10]	P19752 HSP30_NEUCR	30 kDa heat shock protein – <i>Neurospora crassa</i>
Contig[0338]	gi 187978561 gb EDU45187.1	heat shock 70 kDa protein [Pyrenophora tritici-repentis Pt-1C-BFP]	P87047 HSP70_PARBR	Heat shock 70 kDa protein – <i>Paracoccidioides brasiliensis</i>
Contig[0339]	gi 169763212 ref XP_001727506.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]		
Contig[0340]	gi 154311736 ref XP_001555197.1	hypothetical protein BC1G_06327 [Botryotinia fuckeliana B05.10] fructose transporter 1 [Botryotinia fuckeliana] hypothetical protein BC1G_06327 [Botryotinia fuckeliana B05.10]	Q8VZ80 PLT5_ARATH	Polyol transporter 5 – <i>Arabidopsis thaliana</i> (Mouse-ear cress)
Contig[0348]	gi 156054316 ref XP_001593084.1	hypothetical protein SS1G_06006 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_06006 [Sclerotinia sclerotiorum 1980]	P17064 FCY2_YEAST	Purine-cytosine permease FCY2 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0349]	gi 70989381 ref XP_749540.1	GPR/FUN34 family protein [Aspergillus fumigatus Af293] GPR/FUN34 family protein [Aspergillus fumigatus A1163]	P25613 YCQ0_YEAST	Uncharacterized protein YCR010C – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0353]	gi 156056458 ref XP_001594153.1	malate synthase [Sclerotinia sclerotiorum 1980] malate synthase [Sclerotinia sclerotiorum 1980]	P28344 MASY_EMENI	Malate synthase, glyoxysomal – <i>Emmericella nidulans</i> (<i>Aspergillus nidulans</i>)
Contig[0354]	gi 119487126 ref XP_001262418.1	ELL complex subunit Eap30, putative [Neosartorya fischeri NRRL 181] ELL complex subunit Eap30, putative [Neosartorya fischeri NRRL 181]	Q5RK19 SNF8_RAT	Vacuolar-sorting protein SNF8 – <i>Rattus norvegicus</i> (Rat)
Contig[0357]	gi 169772161 ref XP_001820550.1	hypothetical protein [Aspergillus oryzae RIB40] triacylglycerol lipase [Aspergillus oryzae] unnamed protein product [Aspergillus oryzae]	Q8TGB8 CUTI_MONFR	Cutinase precursor – <i>Monilinia fructicola</i>
Contig[0359]	gi 70997299 ref XP_753399.1	arrestin (or S-antigen), N-terminal domain protein [Aspergillus fumigatus Af293] conserved hypothetical protein [Aspergillus fumigatus A1163]	Q10347 YDA5_SCHPO	Uncharacterized protein C1F12.05 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0366]	gi 85107476 ref XP_962380.1	hypothetical protein NCU06346 [<i>Neurospora crassa</i> OR74A] hypothetical protein NCU06346 [<i>Neurospora crassa</i> OR74A]		
Contig[0368]	gi 154282291 ref XP_001541941.1	adenine phosphoribosyltransferase 1 [Ajellomyces capsulatus NAm1] adenine phosphoribosyltransferase 1 [Ajellomyces capsulatus NAm1]	Q6BZF9 APT_DEBHA	Adenine phosphoribosyltransferase – <i>Debaryomyces hansenii</i> (Yeast) (<i>Torulaspora hansenii</i>)
Contig[0369]	gi 154280066 ref XP_001540846.1	ATP-dependent molecular chaperone HSC82 [Ajellomyces capsulatus NAm1] ATP-dependent molecular chaperone HSC82 [Ajellomyces capsulatus NAm1]	O43109 HSP90_PODAN	Heat shock protein 90 homolog – <i>Podospora anserina</i>
Contig[0370]	gi 187983566 gb EDU49054.1	hypothetical protein PTRG_06134 [Pyrenophora tritici-repentis Pt-1C-BFP]	Q10347 YDA5_SCHPO	Uncharacterized protein C1F12.05 – <i>Schizosaccharomyces pombe</i> (Fission yeast)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0373]	gi 156054316 ref XP_001593084.1	hypothetical protein SS1G_06006 [Sclerotinia sclerotiorum 1980]	P17064 FCY2_YEAST	Purine-cytosine permease FCY2 – <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0374]	gi 46109376 ref XP_381746.1	hypothetical protein SS1G_06006 [Sclerotinia sclerotiorum 1980]	Q00298 CUTI_BOTFU	Cutinase precursor – <i>Botryotinia fuckeliana</i> (Noble rot fungus) (<i>Botrytis cinerea</i>)
Contig[0378]	gi 116179518 ref XP_001219608.1	hypothetical protein FG01570.1 [Gibberella zeae PH-1]	Q9URY7 FMDA_SCHPO	Putative formamidase C869.04 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0380]	gi 187975924 gb EDU42550.1	conserved hypothetical protein [Chaetomium globosum CBS 148.51]	Q7XZP5 APX5_ARATH	L-ascorbate peroxidase 5, peroxisomal precursor – <i>Arabidopsis thaliana</i> (Mouse-ear cress)
Contig[0381]	gi 171689038 ref XP_001909459.1	conserved hypothetical protein [Pyrenophora tritici-repentis Pt-1C-BFP]	Q9UV10 HET6_NEUCR	Heterokaryon incompatibility protein 6, OR allele – <i>Neurospora crassa</i>
Contig[0382]	gi 169600787 ref XP_001793816.1	unnamed protein product [Podospira anserina]	Q54P77 4CL1_DICDI	Probable 4-coumarate-CoA ligase 1 – <i>Dictyostelium discoideum</i> (Slime mold)
Contig[0385]	gi 169600787 ref XP_001793816.1	unnamed protein product [Podospira anserina]	Q54P77 4CL1_DICDI	Probable 4-coumarate-CoA ligase 1 – <i>Dictyostelium discoideum</i> (Slime mold)
Contig[0385]	gi 156064057 ref XP_001597950.1	hypothetical protein SNOG_03246 [Phaeosphaeria nodorum SN15]	P22151 GRG1_NEUCR	Glucose-repressible gene protein – <i>Neurospora crassa</i>
Contig[0386]	gi 154297049 ref XP_001548953.1	Grg1 protein [Sclerotinia sclerotiorum 1980]	P22151 GRG1_NEUCR	Glucose-repressible gene protein – <i>Neurospora crassa</i>
Contig[0388]	gi 187983047 gb EDU48535.1	Grg1 protein [Sclerotinia sclerotiorum 1980]	P22151 GRG1_NEUCR	Glucose-repressible gene protein – <i>Neurospora crassa</i>
Contig[0394]	gi 70992559 ref XP_751128.1	hypothetical protein BC1G_12613 [Botryotinia fuckeliana B05.10]	Q9P6P9 PDK_SCHPO	[Pyruvate dehydrogenase [lipoamide]] kinase, mitochondrial precursor – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0399]	gi 154305426 ref XP_001553115.1	kinase isozyme 4, mitochondrial precursor [Pyrenophora tritici-repentis Pt-1C-BFP]	Q9P6P9 PDK_SCHPO	[Pyruvate dehydrogenase [lipoamide]] kinase, mitochondrial precursor – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0404]	gi 169625664 ref XP_001806235.1	AhpC/TSA family protein [Aspergillus fumigatus Af293]	O14313 PMP20_SCHPO	Putative peroxiredoxin pmp20 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0405]	gi 46110262 ref XP_382189.1	AhpC/TSA family protein [Aspergillus fumigatus A1163]	O14313 PMP20_SCHPO	Putative peroxiredoxin pmp20 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0406]	gi 156065107 ref XP_001598475.1	hypothetical protein BC1G_08482 [Botryotinia fuckeliana B05.10]	Q6BRD5 ACOX_DEBHA	Acyl-coenzyme A oxidase – <i>Debaryomyces hansenii</i> (Yeast) (<i>Torulasporea hansenii</i>)
Contig[0408]	gi 145603699 ref XP_369584.2	hypothetical protein BC1G_08482 [Botryotinia fuckeliana B05.10]	Q6BRD5 ACOX_DEBHA	Acyl-coenzyme A oxidase – <i>Debaryomyces hansenii</i> (Yeast) (<i>Torulasporea hansenii</i>)
Contig[0412]	gi 169600135 ref XP_001793490.1	hypothetical protein SNOG_16108 [Phaeosphaeria nodorum SN15]	Q6BRD5 ACOX_DEBHA	Acyl-coenzyme A oxidase – <i>Debaryomyces hansenii</i> (Yeast) (<i>Torulasporea hansenii</i>)
Contig[0413]	gi 169618527 ref XP_001802677.1	hypothetical protein FG02013.1 [Gibberella zeae PH-1]	Q6BRD5 ACOX_DEBHA	Acyl-coenzyme A oxidase – <i>Debaryomyces hansenii</i> (Yeast) (<i>Torulasporea hansenii</i>)
		predicted protein [Sclerotinia sclerotiorum 1980]		
		predicted protein [Sclerotinia sclerotiorum 1980]		
		hypothetical protein MGG_05880 [Magnaporthe grisea 70-15]		
		hypothetical protein MGG_05880 [Magnaporthe grisea 70-15]		
		hypothetical protein SNOG_02896 [Phaeosphaeria nodorum SN15]		
		hypothetical protein SNOG_12454 [Phaeosphaeria nodorum SN15]		

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0414]	gi 154297213 ref XP_001549034.1	hypothetical protein BC1G_12442 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_12442 [Botryotinia fuckeliana B05.10]	P28873 BMRP_CANAL	Benomyl/methotrexate resistance protein – <i>Candida albicans</i> (Yeast)
Contig[0415]	gi 154308532 ref XP_001553602.1	hypothetical protein BC1G_08326 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_08326 [Botryotinia fuckeliana B05.10]	P19752 HSP30_NEUCR	30 kDa heat shock protein – <i>Neurospora crassa</i>
Contig[0417]	gi 187985079 gb EDU50567.1	conserved hypothetical protein [Pyrenophora tritici-repentis Pt-1C-BFP]		
Contig[0423]	gi 156056218 ref XP_001594033.1	hypothetical protein SS1G_05461 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_05461 [Sclerotinia sclerotiorum 1980]		
Contig[0425]	gi 119173175 ref XP_001239084.1	hypothetical protein CIMG_10106 [Coccidioides immitis RS] hypothetical protein CIMG_10106 [Coccidioides immitis RS]	P28344 MASY_EMENI	Malate synthase, glyoxysomal – <i>Emericella nidulans</i> (<i>Aspergillus nidulans</i>)
Contig[0429]	gi 6687541 emb CAB65007.1	transmembrane protein [Erysiphe pisi]		
Contig[0430]	gi 187979077 gb EDU45703.1	conserved hypothetical protein [Pyrenophora tritici-repentis Pt-1C-BFP]		
Contig[0433]	gi 119195217 ref XP_001248212.1	hypothetical protein CIMG_01983 [Coccidioides immitis RS] hypothetical protein CIMG_01983 [Coccidioides immitis RS]	P54422 GGT_BACSU	Gamma-glutamyltranspeptidase precursor – <i>Bacillus subtilis</i>
Contig[0437]	gi 121701967 ref XP_001269248.1	integral membrane protein [Aspergillus clavatus NRRL 1] integral membrane protein [Aspergillus clavatus NRRL 1]		
Contig[0439]	gi 154301173 ref XP_001551000.1	hypothetical protein BC1G_10559 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_10559 [Botryotinia fuckeliana B05.10]		
Contig[0444]	gi 156055914 ref XP_001593881.1	hypothetical protein SS1G_05309 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_05309 [Sclerotinia sclerotiorum 1980]	O14359 YB4E_SCHPO	Uncharacterized protein C30D10.14 – <i>Schizosaccharomyces pombe</i> (Fission yeast)
Contig[0445]	gi 156051296 ref XP_001591609.1	ATP synthase delta chain, mitochondrial precursor [Sclerotinia sclerotiorum 1980] ATP synthase delta chain, mitochondrial precursor [Sclerotinia sclerotiorum 1980]	P56525 ATPD_NEUCR	ATP synthase subunit delta, mitochondrial precursor – <i>Neurospora crassa</i>
Contig[0446]	gi 119194517 ref XP_001247862.1	hypothetical protein CIMG_01633 [Coccidioides immitis RS] hypothetical protein CIMG_01633 [Coccidioides immitis RS]		
Contig[0448]	gi 46123023 ref XP_386065.1	hypothetical protein FG05889.1 [Gibberella zeae PH-1]		
Contig[0450]	gi 156039433 ref XP_001586824.1	hypothetical protein SS1G_11853 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_11853 [Sclerotinia sclerotiorum 1980]		
Contig[0454]	gi 187972489 gb EDU39988.1	elongation factor 1-beta [Pyrenophora tritici-repentis Pt-1C-BFP]	P32471 EF1B_YEAST	Elongation factor 1-beta – <i>Saccharomyces cerevisiae</i> (Baker's yeast)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0455]	gi 169613647 ref XP_001800240.1	hypothetical protein SNOG_09955 [Phaeosphaeria nodorum SN15]		
Contig[0456]	gi 145603699 ref XP_369584.2	hypothetical protein MGG_05880 [Magnaporthe grisea 70–15] hypothetical protein MGG_05880 [Magnaporthe grisea 70–15]		
Contig[0459]	gi 119495238 ref XP_001264408.1	transketolase TktA [Neosartorya fischeri NRRL 181] transketolase TktA [Neosartorya fischeri NRRL 181]	Q9URM2 TKT_SCHPO	Probable transketolase – Schizosaccharomyces pombe (Fission yeast)
Contig[0463]	gi 169622210 ref XP_001804514.1	hypothetical protein SNOG_14322 [Phaeosphaeria nodorum SN15]	O93866 HSP70_TRIRU	Heat shock 70 kDa protein – Trichophyton rubrum
Contig[0466]	gi 169597897 ref XP_001792372.1	hypothetical protein SNOG_01740 [Phaeosphaeria nodorum SN15]	P47044 YJF5_YEAST	Uncharacterized protein YJL055W – Saccharomyces cerevisiae (Baker's yeast)
Contig[0467]	gi 169609703 ref XP_001798270.1	hypothetical protein SNOG_07944 [Phaeosphaeria nodorum SN15]		
Contig[0472]	gi 187974056 gb EDU41555.1	3-alpha-(or 20-beta)-hydroxysteroid dehydrogenase [Pyrenophora tritici-repentis Pt-1C-BFP]	Q9LBG2 LVR_LEIAQ	Levodione reductase – Leifsonia aquatica (Corynebacterium aquaticum)
Contig[0473]	gi 156494375 ref YP_001427401.1	NADH dehydrogenase subunit 5 [Phaeosphaeria nodorum SN15]	P05510 NU5M_NEUCR	NADH-ubiquinone oxidoreductase chain 5 – Neurospora crassa
Contig[0475]	gi 154310457 ref XP_001554560.1	hypothetical protein BC1G_07149 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_07149 [Botryotinia fuckeliana B05.10]	P52718 PEPF_ASPNG	Serine-type carboxypeptidase F precursor – Aspergillus niger
Contig[0479]	gi 119189291 ref XP_001245252.1	hypothetical protein CIMG_04693 [Coccidioides immitis RS] hypothetical protein CIMG_04693 [Coccidioides immitis RS]	P33192 RS13_CANMA	40S ribosomal protein S13 – Candida maltosa (Yeast)
Contig[0481]	gi 187981059 gb EDU47685.1	platelet-activating factor acetylhydrolase precursor [Pyrenophora tritici-repentis Pt-1C-BFP]	Q28017 PAFA_BOVIN	Platelet-activating factor acetylhydrolase precursor – Bos taurus (Bovine)
Contig[0483]	gi 119183624 ref XP_001242824.1	hypothetical protein CIMG_06720 [Coccidioides immitis RS] hypothetical protein CIMG_06720 [Coccidioides immitis RS]	O74430 TGCE1_SCHPO	Probable lipase C1672.09 – Schizosaccharomyces pombe (Fission yeast)
Contig[0484]	gi 169599190 ref XP_001793018.1	hypothetical protein SNOG_02412 [Phaeosphaeria nodorum SN15]	P36008 EF1G2_YEAST	Elongation factor 1-gamma 2 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0486]	gi 169615879 ref XP_001801355.1	hypothetical protein SNOG_11105 [Phaeosphaeria nodorum SN15]		
Contig[0488]	gi 170109536 ref XP_001885975.1	predicted protein [Laccaria bicolor S238N-H82] predicted protein [Laccaria bicolor S238N-H82]	O67610 FABG_AQUAE	3-oxoacyl-[acyl-carrier-protein] reductase – Aquifex aeolicus
Contig[0491]	gi 121710780 ref XP_001273006.1	peptidyl-prolyl cis-trans isomerase Cpr7, putative [Aspergillus clavatus NRRL 1] peptidyl-prolyl cis-trans isomerase Cpr7, putative [Aspergillus clavatus NRRL 1]	Q4HXF6 PPID_GIBZE	Peptidyl-prolyl cis-trans isomerase D – Gibberella zeae (Fusarium graminearum)
Contig[0493]	gi 119188149 ref XP_001244681.1	conserved hypothetical protein [Coccidioides immitis RS] conserved hypothetical protein [Coccidioides immitis RS] porin [Coccidioides posadasii]	P07144 VDAC_NEUCR	Outer mitochondrial membrane protein porin – Neurospora crassa

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0494]	gi 145231184 ref XP_001389856.1	hypothetical protein An01g14680 [Aspergillus niger] unnamed protein product [Aspergillus niger]	O74885 YQE1_SCHPO	Putative dioxygenase C576.01c – Schizosaccharomyces pombe (Fission yeast)
Contig[0495]	gi 121701909 ref XP_001269219.1	conserved hypothetical protein [Aspergillus clavatus NRRL 1] conserved hypothetical protein [Aspergillus clavatus NRRL 1]		
Contig[0497]	gi 154299019 ref XP_001549930.1	hypothetical protein BC1G_11822 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_11822 [Botryotinia fuckeliana B05.10]	Q27514 C13A5_CAEEL	Putative cytochrome P450 CYP13A5 – Caenorhabditis elegans
Contig[0499]	gi 67537110 ref XP_662329.1	hypothetical protein AN4725.2 [Aspergillus nidulans FGSC A4]	O94728 YG0G_SCHPO	Uncharacterized protein C1604.16c – Schizosaccharomyces pombe (Fission yeast)
Contig[0502]	gi 121703337 ref XP_001269933.1	quinone oxidoreductase, putative [Aspergillus clavatus NRRL 1] quinone oxidoreductase, putative [Aspergillus clavatus NRRL 1]	Q53FA7 QORX_HUMAN	Putative quinone oxidoreductase – Homo sapiens (Human)
Contig[0509]	gi 119193000 ref XP_001247106.1	hypothetical protein CIMG_00877 [Coccidioides immitis RS] hypothetical protein CIMG_00877 [Coccidioides immitis RS]	P32901 PTR2_YEAST	Peptide transporter PTR2 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0511]	gi 154310104 ref XP_001554384.1	hypothetical protein BC1G_06972 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_06972 [Botryotinia fuckeliana B05.10]		
Contig[0525]	gi 60600824 gb AAX26869.1	unknown [Schistosoma japonicum]		
Contig[0528]	gi 119471791 ref XP_001258221.1	NADPH cytochrome P450 reductase (CprA), putative [Neosartorya fischeri NRRL 181] NADPH cytochrome P450 reductase (CprA), putative [Neosartorya fischeri NRRL 181]	P36587 NCPR_SCHPO	NADPH—cytochrome P450 reductase – Schizosaccharomyces pombe (Fission yeast)
Contig[0529]	gi 156044714 ref XP_001588913.1	hypothetical protein SS1G_10461 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_10461 [Sclerotinia sclerotiorum 1980]	Q9URZ4 YI0B_SCHPO	Uncharacterized amino-acid permease C869.11 – Schizosaccharomyces pombe (Fission yeast)
Contig[0530]	gi 119182668 ref XP_001242456.1	hypothetical protein CIMG_06352 [Coccidioides immitis RS] hypothetical protein CIMG_06352 [Coccidioides immitis RS]	Q9HGT6 SYSC_CANAL	Seryl-tRNA synthetase, cytoplasmic – Candida albicans (Yeast)
Contig[0536]	gi 119179426 ref XP_001241302.1	hypothetical protein CIMG_08465 [Coccidioides immitis RS] hypothetical protein CIMG_08465 [Coccidioides immitis RS]	P78617 TREA_EMENI	Acid trehalase precursor – Emericella nidulans (Aspergillus nidulans)
Contig[0538]	gi 159124226 gb EDP49344.1	MFS phospholipid transporter (Git1), putative [Aspergillus fumigatus A1163]	O94342 YHM9_SCHPO	Probable metabolite transport protein C1271.09 – Schizosaccharomyces pombe (Fission yeast)
Contig[0539]	gi 169763654 ref XP_001727727.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]	Q54TE3 GTAJ_DICDI	GATA zinc finger domain-containing protein 10 – Dictyostelium discoideum (Slime mold)
Contig[0543]	gi 145247084 ref XP_001395791.1	hypothetical protein An12g07400 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q9USN4 YJA1_SCHPO	Uncharacterized transporter C1529.01 – Schizosaccharomyces pombe (Fission yeast)
Contig[0544]	gi 169614604 ref XP_001800718.1	hypothetical protein SNOG_10448 [Phaeosphaeria nodorum SN15]		

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0552]	gi 145231184 ref XP_001389856.1	hypothetical protein An01g14680 [Aspergillus niger] unnamed protein product [Aspergillus niger]	O74885 YQE1_SCHPO	Putative dioxygenase C576.01c – Schizosaccharomyces pombe (Fission yeast)
Contig[0554]	gi 115390192 ref XP_001212601.1	conserved hypothetical protein [Aspergillus terreus NIH2624] conserved hypothetical protein [Aspergillus terreus NIH2624]	Q96CN4 EVI5L_HUMAN	EV15-like protein – Homo sapiens (Human)
Contig[0559]	gi 187977500 gb EDU44126.1	phospholipase/Carboxylesterase superfamily protein [Pyrenophora tritici-repentis Pt-1C-BFP]	O14304 YE88_SCHPO	Uncharacterized hydrolase C9G1.08c – Schizosaccharomyces pombe (Fission yeast)
Contig[0560]	gi 187983502 gb EDU48990.1	conserved hypothetical protein [Pyrenophora tritici-repentis Pt-1C-BFP]		
Contig[0561]	gi 164423792 ref XP_961973.2	norsolorinic acid reductase [Neurospora crassa OR74A] norsolorinic acid reductase [Neurospora crassa OR74A]	Q00258 NORA_ASPPA	Norsolorinic acid reductase – Aspergillus parasiticus
Contig[0562]	gi 790973 emb CAA56190.1	purine permease [Emericella nidulans]	P48777 UAPC_EMENI	Purine permease – Emericella nidulans (Aspergillus nidulans)
Contig[0569]	gi 119184640 ref XP_001243201.1	hypothetical protein CIMG_07097 [Coccidioides immitis RS] hypothetical protein CIMG_07097 [Coccidioides immitis RS]	P07872 ACOX1_RAT	Peroxisomal acyl-coenzyme A oxidase 1 – Rattus norvegicus (Rat)
Contig[0571]	gi 119177733 ref XP_001240606.1	hypothetical protein CIMG_07769 [Coccidioides immitis RS] hypothetical protein CIMG_07769 [Coccidioides immitis RS]		
Contig[0574]	gi 164422145 ref YP_001648759.1	ATP synthase subunit 8 [Mycosphaerella graminicola] ATP synthase subunit 8 [Mycosphaerella graminicola]	Q08656 ATP8_NEUCR	ATP synthase protein 8 – Neurospora crassa
Contig[0575]	gi 154311873 ref XP_001555265.1	hypothetical protein BC1G_05970 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_05970 [Botryotinia fuckeliana B05.10]		
Contig[0576]	gi 156035819 ref XP_001586021.1	hypothetical protein SS1G_13114 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_13114 [Sclerotinia sclerotiorum 1980]	P34054 INDA1_TRIAT	Amino-acid permease inda1 – Trichoderma atroviride (Hypocrea atroviridis)
Contig[0577]	gi 145616440 ref XP_366389.2	enolase [Magnaporthe grisea 70–15] enolase [Magnaporthe grisea 70–15]	Q9HDT3 ENO_ALTAL	Enolase – Alternaria alternata (Alternaria rot fungus)
Contig[0585]	gi 11345300 gb AAG34660.1 AF295432.1	prepropenicillopepsin-JT3 [Penicillium janthinellum]	P41748 PEPF_AS PFU	Aspergillopepsin-F precursor – Aspergillus fumigatus (Sartorya fumigata)
Contig[0588]	gi 51572467 gb AAU07726.1	Sed1-1p [Saccharomyces pastorianus]	Q01589 SED1_YEAST	Cell wall protein SED1 precursor – Saccharomyces cerevisiae (Baker's yeast)
Contig[0589]	gi 39977569 ref XP_370172.1	hypothetical protein MGG_06669 [Magnaporthe grisea 70–15] hypothetical protein MGG_06669 [Magnaporthe grisea 70–15]	Q11118 WOS2_SCHPO	Protein wos2 – Schizosaccharomyces pombe (Fission yeast)
Contig[0593]	gi 145235437 ref XP_001390367.1	hypothetical protein An03g04860 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q12207 NCE2_YEAST	Non-classical export protein 2 – Saccharomyces cerevisiae (Baker's yeast)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0601]	gi 119194517 ref XP_001247862.1	hypothetical protein CIMG_01633 [Coccidioides immitis RS] hypothetical protein CIMG_01633 [Coccidioides immitis RS]	Q10242 GNTK_SCHPO	Probable gluconokinase – Schizosaccharomyces pombe (Fission yeast)
Contig[0606]	gi 145603699 ref XP_369584.2	hypothetical protein MGG_05880 [Magnaporthe grisea 70–15] hypothetical protein MGG_05880 [Magnaporthe grisea 70–15]		
Contig[0607]	gi 156049361 ref XP_001590647.1	hypothetical protein SS1G_08387 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_08387 [Sclerotinia sclerotiorum 1980]		
Contig[0611]	gi 154288304 ref XP_001544947.1	conserved hypothetical protein [Ajellomyces capsulatus NAM1] conserved hypothetical protein [Ajellomyces capsulatus NAM1]		
Contig[0613]	gi 119193356 ref XP_001247284.1	hypothetical protein CIMG_01055 [Coccidioides immitis RS] hypothetical protein CIMG_01055 [Coccidioides immitis RS]		
Contig[0614]	gi 169599515 ref XP_001793180.1	hypothetical protein SNOG_02577 [Phaeosphaeria nodorum SN15]		
Contig[0616]	gi 154301968 ref XP_001551395.1	hypothetical protein BC1G_10221 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_10221 [Botryotinia fuckeliana B05.10]	P48825 BGL1_ASPAC	Beta-glucosidase 1 precursor – Aspergillus aculeatus
Contig[0620]	gi 156065107 ref XP_001598475.1	predicted protein [Sclerotinia sclerotiorum 1980] predicted protein [Sclerotinia sclerotiorum 1980]		
Contig[0621]	gi 169769194 ref XP_001819067.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]	Q9UUZ3 MANBA_ASPNG	Beta-mannosidase precursor – Aspergillus niger
Contig[0626]	gi 119181817 ref XP_001242091.1	hypothetical protein CIMG_05987 [Coccidioides immitis RS] hypothetical protein CIMG_05987 [Coccidioides immitis RS]	Q09912 PSI1_SCHPO	Protein psi1 – Schizosaccharomyces pombe (Fission yeast)
Contig[0630]	gi 27728593 gb AAO18690.1	glyceraldehyde 3-phosphate dehydrogenase [Cladonia arbuscula]	Q8WZN0 G3P_SORMA	Glyceraldehyde-3-phosphate dehydrogenase – Sordaria macrospora
Contig[0633]	gi 187979783 gb EDU46409.1	zinc finger transcription factor 1 [Pyrenophora tritici-repentis Pt-1C-BFP]	O59746 YN2B_SCHPO	Uncharacterized transcriptional regulatory protein C530.11c – Schizosaccharomyces pombe (Fission yeast)
Contig[0634]	gi 169606904 ref XP_001796872.1	hypothetical protein SNOG_06502 [Phaeosphaeria nodorum SN15]	P61869 MDLA_PENCY	Mono- and diacylglycerol lipase precursor – Penicillium cyclopium
Contig[0636]	gi 169621183 ref XP_001804002.1	hypothetical protein SNOG_13799 [Phaeosphaeria nodorum SN15]		
Contig[0640]	gi 46128001 ref XP_388554.1	hypothetical protein FG08378.1 [Gibberella zeae PH-1]	P48397 CHSA_IPOPU	Chalcone synthase A – Ipomoea purpurea (Common morning glory) (Pharbitis purpurea)
Contig[0644]	gi 115398880 ref XP_001215029.1	6-phosphogluconate dehydrogenase [Aspergillus terreus NIH2624] 6-phosphogluconate dehydrogenase [Aspergillus terreus NIH2624]	O13287 6PGD_CANAL	6-phosphogluconate dehydrogenase, decarboxylating – Candida albicans (Yeast)
Contig[0649]	gi 121706852 ref XP_001271650.1	CaaX prenyl protease Ste24 [Aspergillus clavatus NRRL 1] CaaX prenyl protease Ste24 [Aspergillus clavatus NRRL 1]	P47154 STE24_YEAST	CAAX prenyl protease 1 – Saccharomyces cerevisiae (Baker's yeast)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0650]	gi 156060529 ref XP_001596187.1	hypothetical protein SS1G_02403 [Sclerotinia sclerotiorum 1980]		
Contig[0651]	gi 85102240 ref XP_961298.1	hypothetical protein SS1G_02403 [Sclerotinia sclerotiorum 1980] heat shock protein 90 [Neurospora crassa OR74A] heat shock protein 80 [Neurospora crassa] heat shock protein 90 [Neurospora crassa OR74A]	O43109 HSP90_PODAN	Heat shock protein 90 homolog – Podospora anserina
Contig[0653]	gi 154311873 ref XP_001555265.1	hypothetical protein BC1G_05970 [Botryotinia fuckeliana B05.10]		
Contig[0654]	gi 119194517 ref XP_001247862.1	hypothetical protein BC1G_05970 [Botryotinia fuckeliana B05.10] hypothetical protein CIMG_01633 [Coccidioides immitis RS] hypothetical protein CIMG_01633 [Coccidioides immitis RS]	Q10242 GNTK_SCHPO	Probable gluconokinase – Schizosaccharomyces pombe (Fission yeast)
Contig[0660]	gi 121709383 ref XP_001272405.1	isoleucyl-tRNA synthetase, putative [Aspergillus clavatus NRRL 1]	Q8YXY3 SYLANASP	Isoleucyl-tRNA synthetase – Anabaena sp. (strain PCC 7120)
Contig[0668]	gi 67539518 ref XP_663533.1	isoleucyl-tRNA synthetase, putative [Aspergillus clavatus NRRL 1] hypothetical protein AN5929.2 [Aspergillus nidulans FGSC A4]		
Contig[0670]	gi 154323350 ref XP_001560989.1	hypothetical protein BC1G_00074 [Botryotinia fuckeliana B05.10]		
Contig[0671]	gi 70989381 ref XP_749540.1	hypothetical protein BC1G_00074 [Botryotinia fuckeliana B05.10] GPR/FUN34 family protein [Aspergillus fumigatus Af293]	P25613 YCQ0_YEAST	Uncharacterized protein YCR010C – Saccharomyces cerevisiae (Baker's yeast)
Contig[0672]	gi 187979091 gb EDU45717.1	GPR/FUN34 family protein [Aspergillus fumigatus A1163] proline iminopeptidase [Pyrenophora tritici-repentis Pt-1C-BFP]	P46547 PIP_AERSO	Proline iminopeptidase – Aeromonas sobria
Contig[0673]	gi 145231184 ref XP_001389856.1	hypothetical protein An01g14680 [Aspergillus niger] unnamed protein product [Aspergillus niger]	O74885 YQE1_SCHPO	Putative dioxygenase C576.01c – Schizosaccharomyces pombe (Fission yeast)
Contig[0676]	gi 169615278 ref XP_001801055.1	hypothetical protein SNOG_10796 [Phaeosphaeria nodorum SN15]	Q5R8Q7 GTPB1_PONPY	GTP-binding protein 1 – Pongo pygmaeus (Bornean orangutan)
Contig[0677]	gi 119195801 ref XP_001248504.1	hypothetical protein CIMG_02275 [Coccidioides immitis RS] hypothetical protein CIMG_02275 [Coccidioides immitis RS]		
Contig[0681]	gi 154309002 ref XP_001553836.1	hypothetical protein BC1G_08029 [Botryotinia fuckeliana B05.10]	Q00816 HEX2_YEAST	Protein HEX2 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0684]	gi 27728593 gb AAO18690.1	hypothetical protein BC1G_08029 [Botryotinia fuckeliana B05.10] glyceraldehyde 3-phosphate dehydrogenase [Cladonia arbuscula]	Q8WZN0 G3P_SORMA	Glyceraldehyde-3-phosphate dehydrogenase – Sordaria macrospora
Contig[0685]	gi 156040491 ref XP_001587232.1	hypothetical protein SS1G_12262 [Sclerotinia sclerotiorum 1980]		
Contig[0691]	gi 119481919 ref XP_001260988.1	hypothetical protein SS1G_12262 [Sclerotinia sclerotiorum 1980] small oligopeptide transporter, OPT family [Neosartorya fischeri NRRL 181] small oligopeptide transporter, OPT family [Neosartorya fischeri NRRL 181]	P40900 ISP4_SCHPO	Sexual differentiation process protein isp4 – Schizosaccharomyces pombe (Fission yeast)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0692]	gi 156051390 ref XP_001591656.1	hypothetical protein SS1G_07102 [Sclerotinia sclerotiorum 1980]	Q6TWC4 LEU3_SORMA	3-isopropylmalate dehydrogenase – Sordaria macrospora
Contig[0697]	gi 2772940 gb AAB96651.1	hypothetical protein SS1G_07102 [Sclerotinia sclerotiorum 1980]		
Contig[0698]	gi 187977056 gb EDU43682.1	putative cruciform DNA binding protein [Glomus versiforme]	P0A390 NAHF_PSEU8	Salicylaldehyde dehydrogenase – Pseudomonas sp. (strain C18)
Contig[0699]	gi 156065107 ref XP_001598475.1	salicylaldehyde dehydrogenase [Pyrenophora tritici-repentis Pt-1C-BFP]		
Contig[0707]	gi 169597099 ref XP_001791973.1	predicted protein [Sclerotinia sclerotiorum 1980] predicted protein [Sclerotinia sclerotiorum 1980]		
Contig[0708]	gi 67903440 ref XP_681976.1	hypothetical protein SNOG_01331 [Phaeosphaeria nodorum SN15]	P55250 FUMH_RHIOH	Fumarate hydratase, mitochondrial precursor – Rhizopus oryzae (Rhizopus delemar)
Contig[0709]	gi 154274858 ref XP_001538280.1	hypothetical protein AN8707.2 [Aspergillus nidulans FGSC A4]		
Contig[0710]	gi 154274858 ref XP_001538280.1	conserved hypothetical protein [Ajellomyces capsulatus NAM1]	Q7SFL7 ERFB_NEUCR	Palmitoyltransferase ERF2 – Neurospora crassa
Contig[0715]	gi 171684967 ref XP_001907425.1	conserved hypothetical protein [Ajellomyces capsulatus NAM1]		
Contig[0718]	gi 108935817 sp P40108 ALDH_CLAHE	unnamed protein product [Podospira anserina] unnamed protein product [Podospira anserina]	P40108 ALDH_CLAHE	Aldehyde dehydrogenase – Cladosporium herbarum (Davidiella tassiana)
Contig[0724]	gi 156059736 ref XP_001595791.1	Aldehyde dehydrogenase (ALDDH) (Allergen Cla h 10) (Cla h 3) (Cla h III) aldehyde dehydrogenase (NAD+) [Davidiella tassiana]		
Contig[0725]	gi 187985561 gb EDU51049.1	hypothetical protein SS1G_03881 [Sclerotinia sclerotiorum 1980]		
Contig[0726]	gi 119193000 ref XP_001247106.1	hypothetical protein SS1G_03881 [Sclerotinia sclerotiorum 1980]		
Contig[0727]	gi 154298154 ref XP_001549501.1	membrane transporter [Pyrenophora tritici-repentis Pt-1C-BFP]	P32901 PTR2_YEAST	Peptide transporter PTR2 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0729]	gi 9082283 gb AAF82789.1 AF275347.1	hypothetical protein CIMG_00877 [Coccidioides immitis RS] hypothetical protein CIMG_00877 [Coccidioides immitis RS]		
Contig[0730]	gi 156052034 ref XP_001591978.1	hypothetical protein BC1G_12042 [Botryotinia fuckeliana B05.10]	O59827 GST2_SCHPO	Glutathione S-transferase 2 – Schizosaccharomyces pombe (Fission yeast)
Contig[0733]	gi 145238412 ref XP_001391853.1	hypothetical protein BC1G_12042 [Botryotinia fuckeliana B05.10]		
		glutathione-s-transferase theta, gst [Neosartorya fischeri NRRL 181]	P40108 ALDH_CLAHE	Aldehyde dehydrogenase – Cladosporium herbarum (Davidiella tassiana)
		glutathione-s-transferase theta, gst [Neosartorya fischeri NRRL 181]		
		aldehyde dehydrogenase; ALDH [Cladosporium fulvum]		
		hypothetical protein SS1G_07425 [Sclerotinia sclerotiorum 1980]		
		hypothetical protein SS1G_07425 [Sclerotinia sclerotiorum 1980]		
		hypothetical protein An07g07780 [Aspergillus niger] unnamed protein product [Aspergillus niger]	P09940 ATIF_PICJA	ATPase inhibitor, mitochondrial – Pichia jadinii (Yeast) (Candida utilis)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0737]	gi 119173175 ref XP_001239084.1	hypothetical protein CIMG_10106 [Coccidioides immitis RS] hypothetical protein CIMG_10106 [Coccidioides immitis RS]	P28344 MASY_EMENI	Malate synthase, glyoxysomal – Emericella nidulans (Aspergillus nidulans)
Contig[0738]	gi 121701967 ref XP_001269248.1	integral membrane protein [Aspergillus clavatus NRRL 1] integral membrane protein [Aspergillus clavatus NRRL 1]		
Contig[0740]	gi 154312332 ref XP_001555494.1	uricase [Botryotinia fuckeliana B05.10] uricase [Botryotinia fuckeliana B05.10]	P33282 URIC_EMENI	Uricase – Emericella nidulans (Aspergillus nidulans)
Contig[0746]	gi 154293128 ref XP_001547114.1	hypothetical protein BC1G_14516 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_14516 [Botryotinia fuckeliana B05.10]	Q00614 CACP_CANTR	Carnitine O-acetyltransferase, mitochondrial precursor – Candida tropicalis (Yeast)
Contig[0747]	gi 121710780 ref XP_001273006.1	peptidyl-prolyl cis-trans isomerase Cpr7, putative [Aspergillus clavatus NRRL 1] peptidyl-prolyl cis-trans isomerase Cpr7, putative [Aspergillus clavatus NRRL 1]	Q4HXF6 PPID_GIBZE	Peptidyl-prolyl cis-trans isomerase D – Gibberella zeae (Fusarium graminearum)
Contig[0751]	gi 67516039 ref XP_657905.1	hypothetical protein AN0301.2 [Aspergillus nidulans FGSC A4]		
Contig[0754]	gi 187973918 gb EDU41417.1	glutaryl-CoA dehydrogenase, mitochondrial precursor [Pyrenophora tritici-repentis Pt-1C-BFP]	Q60759 GCDH_MOUSE	Glutaryl-CoA dehydrogenase, mitochondrial precursor – Mus musculus (Mouse)
Contig[0756]	gi 187983047 gb EDU48535.1	kinase isozyme 4, mitochondrial precursor [Pyrenophora tritici-repentis Pt-1C-BFP]	Q9P6P9 PDK_SCHPO	[Pyruvate dehydrogenase [lipoamide]] kinase, mitochondrial precursor – Schizosaccharomyces pombe (Fission yeast)
Contig[0760]	gi 154291945 ref XP_001546551.1	hypothetical protein BC1G_14275 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_14275 [Botryotinia fuckeliana B05.10]	Q12256 TPO4_YEAST	Polyamine transporter 4 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0765]	gi 154303659 ref XP_001552236.1	hypothetical protein BC1G_08714 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_08714 [Botryotinia fuckeliana B05.10]		
Contig[0770]	gi 70989381 ref XP_749540.1	GPR/FUN34 family protein [Aspergillus fumigatus Af293] GPR/FUN34 family protein [Aspergillus fumigatus A1163]	P25613 YCQ0_YEAST	Uncharacterized protein YCR010C – Saccharomyces cerevisiae (Baker's yeast)
Contig[0771]	gi 119186131 ref XP_001243672.1	40S ribosomal protein S22 [Coccidioides immitis RS] 40S ribosomal protein S22 [Coccidioides immitis RS]	Q7RV75 RS22_NEUCR	40S ribosomal protein S22 – Neurospora crassa
Contig[0773]	gi 156060315 ref XP_001596080.1	cell division control protein 2 [Sclerotinia sclerotiorum 1980] cell division control protein 2 [Sclerotinia sclerotiorum 1980]	P54119 CDC2_AJECA	Cell division control protein 2 – Ajellomyces capsulata (Histoplasma capsulatum)
Contig[0776]	gi 156062614 ref XP_001597229.1	hypothetical protein SS1G_01423 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_01423 [Sclerotinia sclerotiorum 1980]	O22212 PRP4_ARATH	U4/U6 small nuclear ribonucleoprotein PRP4-like protein – Arabidopsis thaliana (Mouse-ear cress)
Contig[0778]	gi 156062842 ref XP_001597343.1	hypothetical protein SS1G_01537 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_01537 [Sclerotinia sclerotiorum 1980]		

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0779]	gi 85102240 ref XP_961298.1	heat shock protein 90 [Neurospora crassa OR74A] heat shock protein 80 [Neurospora crassa] heat shock protein 90 [Neurospora crassa OR74A]	O43109 HSP90_PODAN	Heat shock protein 90 homolog – Podospora anserina
Contig[0781]	gi 156040808 ref XP_001587390.1	hypothetical protein SS1G_11382 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_11382 [Sclerotinia sclerotiorum 1980]	P34946 CPS1_PENJA	Carboxypeptidase S1 – Penicillium janthinellum (Penicillium vitale)
Contig[0782]	gi 169780962 ref XP_001824945.1	hypothetical protein [Aspergillus oryzae RIB40] unnamed protein product [Aspergillus oryzae]		
Contig[0783]	gi 159123649 gb EDP48768.1	mitochondrial ATPase subunit ATP4, putative [Aspergillus fumigatus A1163]	Q870C4 ATPF_PARBR	ATP synthase subunit 4, mitochondrial precursor – Paracoccidioides brasiliensis
Contig[0792]	gi 121699693 ref XP_001268112.1	transcription factor TFIID complex 145 kDa subunit, putative [Aspergillus clavatus NRRL 1] transcription factor TFIID complex 145 kDa subunit, putative [Aspergillus clavatus NRRL 1]	Q09813 T111_SCHPO	Putative transcription initiation factor TFIID 111 kDa subunit – Schizosaccharomyces pombe (Fission yeast)
Contig[0795]	gi 145247120 ref XP_001395809.1	hypothetical protein An12g07600 [Aspergillus niger] unnamed protein product [Aspergillus niger]	P61167 Y1785_RHOPA	UPF0311 protein RPA1785 – Rhodopseudomonas palustris
Contig[0800]	gi 154308532 ref XP_001553602.1	hypothetical protein BC1G_08326 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_08326 [Botryotinia fuckeliana B05.10]	P19752 HSP30_NEUCR	30 kDa heat shock protein – Neurospora crassa
Contig[0801]	gi 38567290 emb CAE76579.1	related to cell division control protein CDC91 [Neurospora crassa]	P41733 CDC91_YEAST	GPI transamidase component GAB1 – Saccharomyces cerevisiae (Baker's yeast)
Contig[0806]	gi 145231244 ref XP_001389886.1	hypothetical protein An01g14980 [Aspergillus niger] unnamed protein product [Aspergillus niger]		
Contig[0809]	gi 119196239 ref XP_001248723.1	heat shock 70 kDa protein [Coccidioides immitis RS] heat shock 70 kDa protein [Coccidioides immitis RS]	Q00043 HSP70_AJECA	Heat shock 70 kDa protein – Ajelomyces capsulata (Histoplasma capsulatum)
Contig[0810]	gi 119502054 ref XP_001267666.1	hypothetical protein NFIA_061330 [Neosartorya fischeri NRRL 181] hypothetical protein NFIA_061330 [Neosartorya fischeri NRRL 181]		
Contig[0811]	gi 156051186 ref XP_001591554.1	hypothetical protein SS1G_07000 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_07000 [Sclerotinia sclerotiorum 1980]	Q4WJS2 VTS1_ASPFU	Protein vts1 – Aspergillus fumigatus (Sartorya fumigata)
Contig[0813]	gi 119178330 ref XP_001240846.1	hypothetical protein CIMG_08009 [Coccidioides immitis RS] hypothetical protein CIMG_08009 [Coccidioides immitis RS]		
Contig[0817]	gi 46139263 ref XP_391322.1	hypothetical protein FG11146.1 [Gibberella zeae PH-1]		
Contig[0827]	gi 156056458 ref XP_001594153.1	malate synthase [Sclerotinia sclerotiorum 1980] malate synthase [Sclerotinia sclerotiorum 1980]	P28344 MASY_EMENI	Malate synthase, glyoxysomal – Emericella nidulans (Aspergillus nidulans)

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0829]	gi 46109376 ref XP_381746.1	hypothetical protein FG01570.1 [Gibberella zeae PH-1]	Q00298 CUTL_BOTFU	Cutinase precursor – Botryotinia fuckeliana (Noble rot fungus) (Botrytis cinerea)
Contig[0838]	gi 156044714 ref XP_001588913.1	hypothetical protein SS1G_10461 [Sclerotinia sclerotiorum 1980] hypothetical protein SS1G_10461 [Sclerotinia sclerotiorum 1980]	Q9P768 YI26_SCHPO	Uncharacterized amino-acid permease P7G5.06 – Schizosaccharomyces pombe (Fission yeast)
Contig[0840]	gi 187973918 gb EDU41417.1	glutaryl-CoA dehydrogenase, mitochondrial precursor [Pyrenophora tritici-repentis Pt-1C-BFP]	Q60759 GCDH_MOUSE	Glutaryl-CoA dehydrogenase, mitochondrial precursor – Mus musculus (Mouse)
Contig[0842]	gi 169622210 ref XP_001804514.1	hypothetical protein SNOG_14322 [Phaeosphaeria nodorum SN15]	O93866 HSP70_TRIRU	Heat shock 70 kDa protein – Trichophyton rubrum
Contig[0843]	gi 156065107 ref XP_001598475.1	predicted protein [Sclerotinia sclerotiorum 1980] predicted protein [Sclerotinia sclerotiorum 1980]		
Contig[0844]	gi 67516039 ref XP_657905.1	hypothetical protein AN0301.2 [Aspergillus nidulans FGSC A4]		
Contig[0847]	gi 39968601 ref XP_365691.1	hypothetical protein MGG_02393 [Magnaporthe grisea 70-15] hypothetical protein MGG_02393 [Magnaporthe grisea 70-15]	Q00298 CUTL_BOTFU	Cutinase precursor – Botryotinia fuckeliana (Noble rot fungus) (Botrytis cinerea)
Contig[0851]	gi 145603699 ref XP_369584.2	hypothetical protein MGG_05880 [Magnaporthe grisea 70-15] hypothetical protein MGG_05880 [Magnaporthe grisea 70-15]		
Contig[0852]	gi 156065107 ref XP_001598475.1	predicted protein [Sclerotinia sclerotiorum 1980] predicted protein [Sclerotinia sclerotiorum 1980]		
Contig[0854]	gi 156056458 ref XP_001594153.1	malate synthase [Sclerotinia sclerotiorum 1980] malate synthase [Sclerotinia sclerotiorum 1980]	P28344 MASY_EMENI	Malate synthase, glyoxysomal – Emericella nidulans (Aspergillus nidulans)
Contig[0857]	gi 46109376 ref XP_381746.1	hypothetical protein FG01570.1 [Gibberella zeae PH-1]	Q00298 CUTL_BOTFU	Cutinase precursor – Botryotinia fuckeliana (Noble rot fungus) (Botrytis cinerea)
Contig[0865]	gi 70989381 ref XP_749540.1	GPR/FUN34 family protein [Aspergillus fumigatus Af293] GPR/FUN34 family protein [Aspergillus fumigatus A1163]	P25613 YCQ0_YEAST	Uncharacterized protein YCR010C – Saccharomyces cerevisiae (Baker's yeast)
Contig[0866]	gi 145231184 ref XP_001389856.1	hypothetical protein An01g14680 [Aspergillus niger] unnamed protein product [Aspergillus niger]	O74885 YQE1_SCHPO	Putative dioxygenase C576.01c – Schizosaccharomyces pombe (Fission yeast)
Contig[0867]	gi 156065107 ref XP_001598475.1	predicted protein [Sclerotinia sclerotiorum 1980] predicted protein [Sclerotinia sclerotiorum 1980]		
Contig[0869]	gi 121716178 ref XP_001275698.1	dioxygenase, putative [Aspergillus clavatus NRRL 1] dioxygenase, putative [Aspergillus clavatus NRRL 1]		
Contig[0870]	gi 169597099 ref XP_001791973.1	hypothetical protein SNOG_01331 [Phaeosphaeria nodorum SN15]		
SLJC grI101M13RC02	gi 121699500 ref XP_001268041.1	conserved hypothetical protein [Aspergillus clavatus NRRL 1] conserved hypothetical protein [Aspergillus clavatus NRRL 1]		

Table A.2: Combined GenBank and UniProt summary information for stage 2 *Cladonia grayi* gene fragments – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
SLJC grII01M13RG11	gi 154310767 ref XP_001554714.1	hypothetical protein BC1G_06362 [Botryotinia fuckeliana B05.10] hypothetical protein BC1G_06362 [Botryotinia fuckeliana B05.10]	P40108 ALDH_CLAHE	Aldehyde dehydrogenase – Cladosporium herbarum (Davidiella tassiana)
SLJC grII03M13RD03	gi 145232573 ref XP_001399728.1	hypothetical protein An02g05920 [Aspergillus niger] unnamed protein product [Aspergillus niger]	Q09887 YC9D_SCHPO	Uncharacterized amino-acid permease C584.13 – Schizosaccharomyces pombe (Fission yeast)
SLJC grII10M13RC04	gi 46109376 ref XP_381746.1	hypothetical protein FG01570.1 [Gibberella zeae PH-1]	Q00298 CUTL_BOTFU	Cutinase precursor – Botryotinia fuckeliana (Noble rot fungus) (Botrytis cinerea)
SLJC grII10M13RG01	gi 119182793 ref XP_001242508.1	hypothetical protein CIMG_06404 [Coccidioides immitis RS] Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) hypothetical protein CIMG_06404 [Coccidioides immitis RS]	Q1DTF9 G3P_COCIM	Glyceraldehyde-3-phosphate dehydrogenase – Coccidioides immitis
SLJC grII10T7F11	gi 67516039 ref XP_657905.1	hypothetical protein AN0301.2 [Aspergillus nidulans FGSC A4]		

A.2 Summary of Gene Ontology (GO) organizing principles for *Cladonia grayi* in stages 1 and 2 of lichen development

Functional classification of ESTs. To group SSH-cDNA sequences into functional categories, Blast2GO (Conesa *et al.*, 2005) was used to compare each sequence to the GenBank nr protein database, and subsequently Gene Ontology (GO; <http://www.geneontology.org>) terms were extracted from associated annotated blast hits. GO terms were organized by organizing principle (biological process, cellular compartment and molecular function), and the first three levels of GO terms for the non-normalized gene sequences from stages 1 and 2 can be seen in Tables A.3 and A.4. For stage 1, 215 gene fragments had a significant blastx match, and of these 166 had at least one associated GO term (GO database accessed 24 June, 2007). In stage 2, of 316 fungal gene fragments with a significant blastx match, 253 had at least one associated GO term (GO database accessed 19 May, 2008).

Table A.3: GO categorization of a non-normalized SSH library of 166 gene products for *Cladonia grayi* in stage 1 of lichen development. Individual gene products may be represented by more than one GO term, and subordinate terms can represent multiple higher-level terms. First 3 levels of GO terms annotated through increasing indentation.

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
BIOLOGICAL PROCESS	GO:0008150	91	100%
biological regulation	GO:0065007	10	11%
cellular process	GO:0009987	77	85%
developmental process	GO:0032502	5	5%
establishment of localization	GO:0051234	23	25%
localization	GO:0051179	23	25%
metabolic process	GO:0008152	75	82%
multicellular organismal process	GO:0032501	2	2%
response to stimulus	GO:0050896	8	9%
aging	GO:0007568	2	2%
anatomical structure development	GO:0048856	2	2%
anatomical structure formation	GO:0048646	1	1%
anatomical structure morphogenesis	GO:0009653	1	1%

Table A.3 Stage 1 *Cladonia grayi* Gene Ontology summary – Continued

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
biosynthetic process	GO:0009058	25	27%
blood circulation	GO:0008015	1	1%
catabolic process	GO:0009056	7	8%
cell communication	GO:0007154	2	2%
cell cycle	GO:0007049	2	2%
cell development	GO:0048468	1	1%
cell division	GO:0051301	3	3%
cellular component organization and biogenesis	GO:0016043	19	21%
cellular developmental process	GO:0048869	2	2%
cellular homeostasis	GO:0019725	1	1%
cellular localization	GO:0051641	5	5%
cellular metabolic process	GO:0044237	66	73%
cellular response to stimulus	GO:0051716	1	1%
embryonic development	GO:0009790	1	1%
establishment of cellular localization	GO:0051649	5	5%
establishment of protein localization	GO:0045184	4	4%
generation of precursor metabolites and energy	GO:0006091	6	7%
macromolecule localization	GO:0033036	4	4%
macromolecule metabolic process	GO:0043170	48	53%
multicellular organismal development	GO:0007275	2	2%
nitrogen compound metabolic process	GO:0006807	3	3%
pattern specification process	GO:0007389	1	1%
primary metabolic process	GO:0044238	61	67%
regulation of biological process	GO:0050789	10	11%
regulation of biological quality	GO:0065008	2	2%
regulation of cellular process	GO:0050794	9	10%
regulation of metabolic process	GO:0019222	7	8%
response to biotic stimulus	GO:0009607	2	2%
response to chemical stimulus	GO:0042221	6	7%
response to endogenous stimulus	GO:0009719	2	2%
response to external stimulus	GO:0009605	1	1%
response to stress	GO:0006950	7	8%
secondary metabolic process	GO:0019748	1	1%
secretion	GO:0046903	2	2%
transport	GO:0006810	23	25%
CELLULAR COMPONENT	GO:0005575	68	100%
cell	GO:0005623	67	99%
cell part	GO:0044464	67	99%
envelope	GO:0031975	6	9%
macromolecular complex	GO:0032991	25	37%
organelle	GO:0043226	40	59%
organelle part	GO:0044422	15	22%
cell fraction	GO:0000267	2	3%
intracellular	GO:0005622	57	84%
intracellular organelle	GO:0043229	40	59%
intracellular organelle part	GO:0044446	15	22%
intracellular part	GO:0044424	52	76%
membrane	GO:0016020	26	38%
membrane part	GO:0044425	15	22%
membrane-bounded organelle	GO:0043227	28	41%
non-membrane-bounded organelle	GO:0043228	17	25%
organelle envelope	GO:0031967	6	9%
organelle membrane	GO:0031090	9	13%
protein complex	GO:0043234	13	19%
ribonucleoprotein complex	GO:0030529	12	18%
vesicle	GO:0031982	2	3%
MOLECULAR FUNCTION	GO:0003674	116	100%
antioxidant activity	GO:0016209	3	3%
binding	GO:0005488	61	53%
catalytic activity	GO:0003824	69	59%
enzyme regulator activity	GO:0030234	1	1%
structural molecule activity	GO:0005198	10	9%
transcription regulator activity	GO:0030528	2	2%
translation regulator activity	GO:0045182	2	2%
transporter activity	GO:0005215	9	8%
cofactor binding	GO:0048037	3	3%
enzyme activator activity	GO:0008047	1	1%
helicase activity	GO:0004386	4	3%
hydrolase activity	GO:0016787	28	24%
ion binding	GO:0043167	17	15%
isomerase activity	GO:0016853	1	1%
ligase activity	GO:0016874	6	5%
lipid binding	GO:0008289	2	2%
lyase activity	GO:0016829	2	2%
metal cluster binding	GO:0051540	1	1%
nucleic acid binding	GO:0003676	20	17%
nucleotide binding	GO:0000166	20	17%
oxidoreductase activity	GO:0016491	21	18%
peroxidase activity	GO:0004601	2	2%
protein binding	GO:0005515	9	8%
structural constituent of ribosome	GO:0003735	10	9%
substrate-specific transporter activity	GO:0022892	6	5%
tetrapyrrole binding	GO:0046906	4	3%

Table A.3 Stage 1 *Cladonia grayi* Gene Ontology summary – Continued

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
transcription activator activity	GO:0016563	1	1%
transcription factor activity	GO:0003700	1	1%
transferase activity	GO:0016740	12	10%
translation factor activity, nucleic acid binding	GO:0008135	2	2%
transmembrane transporter activity	GO:0022857	6	5%

Table A.4: GO categorization of a non-normalized SSH library of 253 gene products for *Cladonia grayi* in stage 2 of lichen development. Individual gene products may be represented by more than one GO term, and subordinate terms can represent multiple higher-level terms. First 3 levels of GO terms annotated through increasing indentation.

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
BIOLOGICAL PROCESS	GO:0008150	153	100%
biological regulation	GO:0065007	7	5%
cellular process	GO:0009987	69	45%
developmental process	GO:0032502	41	27%
establishment of localization	GO:0051234	27	18%
localization	GO:0051179	27	18%
metabolic process	GO:0008152	101	66%
multicellular organismal process	GO:0032501	33	22%
multi-organism process	GO:0051704	33	22%
reproduction	GO:0000003	2	1%
response to stimulus	GO:0050896	20	13%
anatomical structure development	GO:0048856	33	22%
biosynthetic process	GO:0009058	22	14%
catabolic process	GO:0009056	16	10%
cell communication	GO:0007154	4	3%
cell cycle	GO:0007049	3	2%
cell cycle process	GO:0022402	1	1%
cell division	GO:0051301	2	1%
cellular component organization and biogenesis	GO:0016043	4	3%
cellular localization	GO:0051641	2	1%
cellular metabolic process	GO:0044237	62	41%
cellular response to stimulus	GO:0051716	1	1%
conjugation	GO:0000746	1	1%
developmental maturation	GO:0021700	8	5%
establishment of cellular localization	GO:0051649	2	1%
establishment of protein localization	GO:0045184	5	3%
generation of precursor metabolites and energy	GO:0006091	12	8%
interspecies interaction between organisms	GO:0044419	33	22%
macromolecule localization	GO:0033036	5	3%
macromolecule metabolic process	GO:0043170	31	20%
multicellular organismal development	GO:0007275	33	22%
nitrogen compound metabolic process	GO:0006807	6	4%
primary metabolic process	GO:0044238	68	44%
regulation of biological process	GO:0050789	6	4%
regulation of cellular process	GO:0050794	5	3%
regulation of metabolic process	GO:0019222	3	2%
regulation of molecular function	GO:0065009	2	1%
response to chemical stimulus	GO:0042221	10	7%
response to external stimulus	GO:0009605	1	1%
response to stress	GO:0006950	11	7%
sexual reproduction	GO:0019953	2	1%
transport	GO:0006810	27	18%
CELLULAR COMPONENT	GO:0005575	89	100%
cell	GO:0005623	88	99%
cell part	GO:0044464	88	99%
envelope	GO:0031975	8	9%
extracellular region	GO:0005576	1	1%
macromolecular complex	GO:0032991	15	17%
membrane-enclosed lumen	GO:0031974	1	1%
organelle	GO:0043226	35	39%
organelle part	GO:0044422	14	16%
cell fraction	GO:0000267	1	1%
external encapsulating structure	GO:0030312	3	3%
intracellular	GO:0005622	60	67%
intracellular organelle	GO:0043229	35	39%
intracellular organelle part	GO:0044446	14	16%
intracellular part	GO:0044424	56	63%
membrane	GO:0016020	38	43%
membrane part	GO:0044425	21	24%
membrane-bounded organelle	GO:0043227	30	34%
non-membrane-bounded organelle	GO:0043228	7	8%
organelle envelope	GO:0031967	8	9%
organelle lumen	GO:0043233	1	1%
organelle membrane	GO:0031090	12	13%
protein complex	GO:0043234	9	10%
ribonucleoprotein complex	GO:0030529	7	8%
MOLECULAR FUNCTION	GO:0003674	155	100%
antioxidant activity	GO:0016209	2	1%
binding	GO:0005488	79	51%
catalytic activity	GO:0003824	105	68%
chaperone regulator activity	GO:0030188	1	1%
enzyme regulator activity	GO:0030234	2	1%
molecular transducer activity	GO:0060089	3	2%

Table A.4 Stage 2 *Cladonia grayi* Gene Ontology summary – Continued

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
structural molecule activity	GO:0005198	4	3%
transcription regulator activity	GO:0030528	1	1%
translation regulator activity	GO:0045182	2	1%
transporter activity	GO:0005215	19	12%
carbohydrate binding	GO:0030246	1	1%
chaperone activator activity	GO:0030189	1	1%
cofactor binding	GO:0048037	15	10%
enzyme activator activity	GO:0008047	1	1%
GTPase regulator activity	GO:0030695	2	1%
helicase activity	GO:0004386	1	1%
hydrolase activity	GO:0016787	27	17%
ion binding	GO:0043167	22	14%
isomerase activity	GO:0016853	3	2%
ligase activity	GO:0016874	3	2%
lyase activity	GO:0016829	9	6%
nucleic acid binding	GO:0003676	11	7%
nucleotide binding	GO:0000166	32	21%
oxidoreductase activity	GO:0016491	34	22%
peroxidase activity	GO:0004601	1	1%
protein binding	GO:0005515	9	6%
signal transducer activity	GO:0004871	3	2%
structural constituent of ribosome	GO:0003735	4	3%
substrate-specific transporter activity	GO:0022892	14	9%
tetrapyrrole binding	GO:0046906	2	1%
transcription factor activity	GO:0003700	1	1%
transferase activity	GO:0016740	29	19%
translation factor activity, nucleic acid binding	GO:0008135	2	1%
transmembrane transporter activity	GO:0022857	15	10%
vitamin binding	GO:0019842	1	1%

A.3 Keys for linking GO terms to individual Clone IDs, in *Cladonia grayi*

Stages 1 and 2 GO terms can be linked to Clone IDs using Tables A.5 and A.6 respectively.

Table A.5: Key for linking stage 1 *Cladonia grayi* GO terms and Clone IDs.

Level	GO ID	GO TERM	Clone ID
1	GO:0008150	BIOLOGICAL PROCESS	SLJClgrIII03M13RF01, Contig[0323], Contig[0156], Contig[0187], Contig[0415], Contig[0430], Contig[0023], Contig[0475], SLJClgrIII06T7E03, Contig[0266], Contig[0137], Contig[0016], Contig[0031], Contig[0325], Contig[0418], Contig[0252], Contig[0207], Contig[0213], Contig[0021], Contig[0313], Contig[0014], Contig[0077], Contig[0111], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0274], Contig[0318], SLJClgrIII01T7F12, Contig[0431], SLJClgrIII01T7B04, SLJClgrIII02M13RH11, SLJClgrIII06T7F02, Contig[0438], Contig[0496], Contig[0262], Contig[0362], Contig[0197], Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0401], Contig[0305], Contig[0171], Contig[0484], Contig[0253], Contig[0303], SLJClgrIII03M13RF06, Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0396], Contig[0384], SLJClgrIII03M13RF05, Contig[0441], Contig[0105], Contig[0310], Contig[0235], Contig[0191], Contig[0143], Contig[0234], Contig[0446], Contig[0029], Contig[0443], Contig[0182], Contig[0037], Contig[0225], Contig[0357], Contig[0413], Contig[0398], Contig[0388], Contig[0114], SLJClgrIII06M13RA02, Contig[0259], Contig[0251], Contig[0422], Contig[0245], Contig[0183]
2	GO:0065007	biological regulation	Contig[0187], SLJClgrIII06T7F02, Contig[0197], Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0305], Contig[0441], Contig[0430]
2	GO:0009987	cellular process	SLJClgrIII03M13RF01, Contig[0187], Contig[0415], Contig[0021], Contig[0313], Contig[0014], Contig[0077], Contig[0111], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0274], Contig[0318], SLJClgrIII01T7F12, Contig[0431], SLJClgrIII01T7B04, SLJClgrIII02M13RH11, SLJClgrIII06T7F02, Contig[0438], Contig[0496], Contig[0262], Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0266], Contig[0401], Contig[0305], Contig[0171], Contig[0484], Contig[0253], Contig[0430], Contig[0303], SLJClgrIII03M13RF06, Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0396], Contig[0384], SLJClgrIII03M13RF05, Contig[0441], Contig[0252], Contig[0105], Contig[0310], Contig[0235], Contig[0191], Contig[0143], Contig[0234], Contig[0197], Contig[0362], Contig[0446], Contig[0029], Contig[0443], Contig[0182], Contig[0037], SLJClgrIII06T7E03, Contig[0031], Contig[0259], Contig[0251], Contig[0323], Contig[0156], Contig[0422], Contig[0245], Contig[0418], Contig[0183]
2	GO:0032502	developmental process	SLJClgrIII05T7FA11, Contig[0305], Contig[0187], Contig[0197], Contig[0299]
2	GO:0051234	establishment of localization	Contig[0023], Contig[0475], SLJClgrIII06T7E03, Contig[0266], Contig[0137], Contig[0016], Contig[0031], Contig[0325], Contig[0418], Contig[0252], Contig[0207], Contig[0213], Contig[0187], Contig[0318], Contig[0362], Contig[0430], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0396], Contig[0384], SLJClgrIII03M13RF05
2	GO:0051179	localization	Contig[0023], Contig[0475], SLJClgrIII06T7E03, Contig[0266], Contig[0137], Contig[0016], Contig[0031], Contig[0325], Contig[0418], Contig[0252], Contig[0207], Contig[0213], Contig[0187], Contig[0318], Contig[0362], Contig[0430], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0396], Contig[0384], SLJClgrIII03M13RF05
2	GO:0008152	metabolic process	SLJClgrIII03M13RF01, Contig[0187], Contig[0415], Contig[0021], Contig[0077], Contig[0111], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0274], SLJClgrIII01T7F12, Contig[0431], SLJClgrIII01T7B04, Contig[0438], Contig[0496], Contig[0262], Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, SLJClgrIII06T7F02, Contig[0266], Contig[0401], Contig[0305], Contig[0171], Contig[0484], Contig[0303], SLJClgrIII03M13RF06, Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0441], Contig[0252], Contig[0105], Contig[0310], Contig[0235], Contig[0191], Contig[0143], Contig[0234], Contig[0362], Contig[0253], Contig[0446], Contig[0029], Contig[0443], Contig[0182], Contig[0037], SLJClgrIII06T7E03, Contig[0031], Contig[0225], Contig[0357], Contig[0413], Contig[0398], Contig[0388], Contig[0114], Contig[0259], Contig[0251], Contig[0323], Contig[0156], Contig[0422], Contig[0245], Contig[0418], Contig[0183]
2	GO:0032501	multicellular organismal process	SLJClgrIII05T7FA11, Contig[0197]
2	GO:0050896	response to stimulus	Contig[0323], Contig[0156], Contig[0187], Contig[0430], Contig[0171], Contig[0484], Contig[0349], Contig[0494]
3	GO:0007568	aging	Contig[0305], Contig[0187]
3	GO:0048856	anatomical structure development	Contig[0197], Contig[0299]
3	GO:0048646	anatomical structure formation	Contig[0299]

Table A.5: Key for linking stage 1 *Cladonia grayi* GO terms and Clone ID – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0009653	anatomical structure morphogenesis	Contig[0299]
3	GO:0009058	biosynthetic process	Contig[0415], Contig[0021], Contig[0077], Contig[0111], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], SLJClgrIII01T7F12, Contig[0438], Contig[0496], Contig[0303], SLJClgrIII03M13RF06, Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0262], Contig[0418], Contig[0183]
3	GO:0008015	blood circulation	Contig[0197]
3	GO:0009056	catabolic process	Contig[0171], Contig[0484], Contig[0187], Contig[0143], Contig[0234], Contig[0431], SLJClgrIII01T7B04
3	GO:0007154	cell communication	Contig[0187], Contig[0014]
3	GO:0007049	cell cycle	Contig[0313], Contig[0014]
3	GO:0048468	cell development	Contig[0197]
3	GO:0051301	cell division	Contig[0313], Contig[0014], SLJClgrIII02M13RH11
3	GO:0016043	cellular component organization and biogenesis	SLJClgrIII03M13RF01, Contig[0021], Contig[0318], Contig[0411], Contig[0047], Contig[0431], SLJClgrIII01T7B04, Contig[0253], Contig[0430], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0396], Contig[0384], SLJClgrIII03M13RF05, Contig[0424], Contig[0197]
3	GO:0048869	cellular developmental process	Contig[0197], Contig[0299]
3	GO:0019725	cellular homeostasis	Contig[0430]
3	GO:0051641	cellular localization	Contig[0318], Contig[0430], Contig[0396], Contig[0384], SLJClgrIII03M13RF05
3	GO:0044237	cellular metabolic process	Contig[0187], Contig[0415], Contig[0021], Contig[0077], Contig[0111], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0274], SLJClgrIII01T7F12, Contig[0431], SLJClgrIII01T7B04, Contig[0438], Contig[0496], Contig[0262], Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, SLJClgrIII06T7F02, Contig[0266], Contig[0401], Contig[0305], Contig[0171], Contig[0484], SLJClgrIII03M13RF01, Contig[0430], Contig[0303], SLJClgrIII03M13RF06, Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0441], Contig[0252], Contig[0349], Contig[0494], Contig[0105], Contig[0310], Contig[0235], Contig[0191], Contig[0143], Contig[0234], Contig[0362], Contig[0253], Contig[0446], Contig[0029], Contig[0443], Contig[0182], Contig[0037], SLJClgrIII06T7E03, Contig[0031], Contig[0259], Contig[0251], Contig[0323], Contig[0156], Contig[0422], Contig[0245], Contig[0418], Contig[0183]
3	GO:0051716	cellular response to stimulus	Contig[0187]
3	GO:0009790	embryonic development	SLJClgrIII05T7FA11
3	GO:0051649	establishment of cellular localization	Contig[0318], Contig[0430], Contig[0396], Contig[0384], SLJClgrIII03M13RF05
3	GO:0045184	establishment of protein localization	Contig[0318], Contig[0384], Contig[0396], SLJClgrIII03M13RF05
3	GO:0006091	generation of precursor metabolites and energy	SLJClgrIII03M13RF01, Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0252]
3	GO:0033036	macromolecule localization	Contig[0318], Contig[0384], Contig[0396], SLJClgrIII03M13RF05
3	GO:0043170	macromolecule metabolic process	SLJClgrIII03M13RF01, Contig[0187], Contig[0021], Contig[0077], Contig[0111], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0431], SLJClgrIII01T7B04, Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0305], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0105], Contig[0310], Contig[0235], Contig[0191], Contig[0143], Contig[0234], Contig[0362], Contig[0253], Contig[0029], Contig[0443], Contig[0182], Contig[0037], SLJClgrIII06T7E03, Contig[0031], Contig[0430], Contig[0259], Contig[0251], Contig[0323], Contig[0156], Contig[0422], Contig[0245], Contig[0183]
3	GO:0007275	multicellular organismal development	SLJClgrIII05T7FA11, Contig[0197]
3	GO:0006807	nitrogen compound metabolic process	SLJClgrIII01T7F12, Contig[0303], Contig[0183]
3	GO:0007389	pattern specification process	SLJClgrIII05T7FA11

Table A.5: Key for linking stage 1 *Cladonia grayi* GO terms and Clone ID – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0044238	primary metabolic process	SLJClgrIII03M13RF01, Contig[0187], Contig[0021], Contig[0077], Contig[0111], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], SLJClgrIII01T7F12, Contig[0431], SLJClgrIII01T7B04, Contig[0438], Contig[0496], Contig[0262], Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, SLJClgrIII06T7F02, Contig[0266], Contig[0401], Contig[0305], Contig[0303], SLJClgrIII03M13RF06, Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0441], Contig[0349], Contig[0494], Contig[0105], Contig[0310], Contig[0235], Contig[0191], Contig[0143], Contig[0234], Contig[0362], Contig[0253], Contig[0029], Contig[0443], Contig[0182], Contig[0037], SLJClgrIII06T7E03, Contig[0031], Contig[0430], Contig[0259], Contig[0251], Contig[0323], Contig[0156], Contig[0422], Contig[0245], Contig[0446], Contig[0418], Contig[0183]
3	GO:0050789	regulation of biological process	Contig[0187], SLJClgrIII06T7F02, Contig[0197], Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0305], Contig[0441], Contig[0430]
3	GO:0065008	regulation of biological quality	Contig[0430], Contig[0187]
3	GO:0050794	regulation of cellular process	Contig[0187], SLJClgrIII06T7F02, Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0305], Contig[0441], Contig[0430]
3	GO:0019222	regulation of metabolic process	Contig[0187], Contig[0299], SLJClgrIII05T7FA11, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0305], Contig[0441]
3	GO:0009607	response to biotic stimulus	Contig[0323], Contig[0156]
3	GO:0042221	response to chemical stimulus	Contig[0323], Contig[0156], Contig[0187], Contig[0430], Contig[0171], Contig[0484]
3	GO:0009719	response to endogenous stimulus	Contig[0349], Contig[0494]
3	GO:0009605	response to external stimulus	Contig[0187]
3	GO:0006950	response to stress	Contig[0323], Contig[0156], Contig[0430], Contig[0171], Contig[0484], Contig[0349], Contig[0494]
3	GO:0019748	secondary metabolic process	Contig[0415]
3	GO:0046903	secretion	Contig[0430], Contig[0396]
3	GO:0006810	transport	Contig[0023], Contig[0475], SLJClgrIII06T7E03, Contig[0266], Contig[0137], Contig[0016], Contig[0031], Contig[0325], Contig[0418], Contig[0252], Contig[0207], Contig[0213], Contig[0187], Contig[0318], Contig[0362], Contig[0430], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0396], Contig[0384], SLJClgrIII03M13RF05
1	GO:0005575	CELLULAR COMPONENT	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0415], Contig[0242], Contig[0302], Contig[0473], Contig[0023], Contig[0475], Contig[0182], SLJClgrIII01T7F12, Contig[0329], Contig[0021], Contig[0197], Contig[0095], Contig[0396], Contig[0089], SLJClgrIII03M13RF05, SLJClgrIII06T7F02, Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0143], Contig[0234], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], SLJClgrIII05T7FA11, Contig[0431], SLJClgrIII01T7B04, SLJClgrIII02M13RH11, Contig[0384], Contig[0207], Contig[0430], Contig[0187], Contig[0318], Contig[0362], Contig[0325], Contig[0418], Contig[0496], Contig[0235], Contig[0014], Contig[0092], Contig[0016], Contig[0024], Contig[0373], Contig[0303], Contig[0245], SLJClgrIII06M13RA02, Contig[0401], Contig[0393], Contig[0178], Contig[0171], Contig[0484], Contig[0183]
2	GO:0005623	cell	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0415], Contig[0242], Contig[0302], Contig[0473], Contig[0023], Contig[0475], Contig[0182], SLJClgrIII01T7F12, Contig[0329], Contig[0021], Contig[0197], Contig[0095], Contig[0396], Contig[0089], SLJClgrIII03M13RF05, SLJClgrIII06T7F02, Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0143], Contig[0234], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], SLJClgrIII05T7FA11, Contig[0431], SLJClgrIII01T7B04, SLJClgrIII02M13RH11, Contig[0384], Contig[0207], Contig[0430], Contig[0187], Contig[0318], Contig[0362], Contig[0325], Contig[0418], Contig[0496], Contig[0235], Contig[0014], Contig[0092], Contig[0016], Contig[0024], Contig[0373], Contig[0303], Contig[0245], SLJClgrIII06M13RA02, Contig[0401], Contig[0393], Contig[0178], Contig[0171], Contig[0484]

Table A.5: Key for linking stage 1 *Cladonia grayi* GO terms and Clone ID – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0044464	cell part	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0415], Contig[0242], Contig[0302], Contig[0473], Contig[0023], Contig[0475], Contig[0182], SLJClgrIII01T7F12, Contig[0329], Contig[0021], Contig[0197], Contig[0095], Contig[0396], Contig[0089], SLJClgrIII03M13RF05, SLJClgrIII06T7F02, Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0143], Contig[0234], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], SLJClgrIII05T7FA11, Contig[0431], SLJClgrIII01T7B04, SLJClgrIII02M13RH11, Contig[0384], Contig[0207], Contig[0430], Contig[0187], Contig[0318], Contig[0362], Contig[0325], Contig[0418], Contig[0496], Contig[0235], Contig[0014], Contig[0092], Contig[0016], Contig[0024], Contig[0373], Contig[0303], Contig[0245], SLJClgrIII06M13RA02, Contig[0401], Contig[0393], Contig[0178], Contig[0171], Contig[0484]
2	GO:0031975	envelope	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137]
2	GO:0032991	macromolecular complex	Contig[0252], Contig[0095], Contig[0242], Contig[0396], Contig[0302], Contig[0473], Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0143], Contig[0234], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0089]
2	GO:0043226	organelle	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0415], Contig[0242], Contig[0302], Contig[0473], Contig[0197], Contig[0396], SLJClgrIII03M13RF05, Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], SLJClgrIII05T7FA11, Contig[0431], SLJClgrIII01T7B04, SLJClgrIII02M13RH11, Contig[0384], Contig[0089], Contig[0318], Contig[0418], Contig[0207], Contig[0171], Contig[0484]
2	GO:0044422	organelle part	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0197], Contig[0396], SLJClgrIII03M13RF05, Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0089]
3	GO:0000267	cell fraction	Contig[0089], Contig[0207]
3	GO:0005622	intracellular	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0415], Contig[0242], Contig[0302], Contig[0473], Contig[0023], Contig[0475], Contig[0182], SLJClgrIII01T7F12, Contig[0329], Contig[0021], Contig[0197], Contig[0095], Contig[0396], SLJClgrIII06T7F02, SLJClgrIII03M13RF05, Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0143], Contig[0234], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0384], Contig[0089], Contig[0430], Contig[0187], SLJClgrIII06M13RA02, Contig[0014], Contig[0401], Contig[0393], Contig[0178], Contig[0318], Contig[0418], Contig[0207], Contig[0171], Contig[0484]
3	GO:0043229	intracellular organelle	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0415], Contig[0242], Contig[0302], Contig[0473], Contig[0197], Contig[0396], SLJClgrIII03M13RF05, Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], SLJClgrIII05T7FA11, Contig[0431], SLJClgrIII01T7B04, SLJClgrIII02M13RH11, Contig[0384], Contig[0089], Contig[0318], Contig[0418], Contig[0207], Contig[0171], Contig[0484]
3	GO:0044446	intracellular organelle part	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0197], Contig[0396], SLJClgrIII03M13RF05, Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0089]
3	GO:0044424	intracellular part	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0415], Contig[0242], Contig[0302], Contig[0473], Contig[0023], Contig[0475], Contig[0182], SLJClgrIII01T7F12, Contig[0329], Contig[0021], Contig[0197], Contig[0095], Contig[0396], SLJClgrIII06T7F02, SLJClgrIII03M13RF05, Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0143], Contig[0234], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], SLJClgrIII05T7FA11, Contig[0431], SLJClgrIII01T7B04, SLJClgrIII02M13RH11, Contig[0384], Contig[0089], Contig[0430], Contig[0187], Contig[0318], Contig[0418], Contig[0207], Contig[0171], Contig[0484]

Table A.5: Key for linking stage 1 *Cladonia grayi* GO terms and Clone ID – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0016020	membrane	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0242], Contig[0396], Contig[0302], Contig[0473], SLJClgrIII03M13RF05, Contig[0089], Contig[0318], Contig[0362], Contig[0325], Contig[0418], Contig[0207], Contig[0496], Contig[0235], Contig[0014], Contig[0092], Contig[0016], Contig[0024], Contig[0373], Contig[0303], Contig[0245]
3	GO:0044425	membrane part	Contig[0252], Contig[0213], Contig[0242], Contig[0396], Contig[0302], Contig[0473], SLJClgrIII03M13RF05, Contig[0089], Contig[0318], Contig[0137], Contig[0362], Contig[0325], Contig[0418], Contig[0207], Contig[0496]
3	GO:0043227	membrane-bounded organelle	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0415], Contig[0242], Contig[0302], Contig[0473], Contig[0396], SLJClgrIII03M13RF05, SLJClgrIII05T7FA11, Contig[0431], SLJClgrIII01T7B04, Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], SLJClgrIII02M13RH11, Contig[0384], Contig[0089], Contig[0318], Contig[0418], Contig[0207], Contig[0171], Contig[0484]
3	GO:0043228	non-membrane-bounded organelle	Contig[0197], Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494]
3	GO:0031967	organelle envelope	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137]
3	GO:0031090	organelle membrane	Contig[0252], Contig[0213], Contig[0305], Contig[0322], SLJClgrIII03M13RF01, Contig[0137], Contig[0396], SLJClgrIII03M13RF05, Contig[0089]
3	GO:0043234	protein complex	Contig[0252], Contig[0242], Contig[0396], Contig[0302], Contig[0473], Contig[0143], Contig[0234], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0089]
3	GO:0030529	ribonucleoprotein complex	Contig[0095], Contig[0342], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461]
3	GO:0031982	vesicle	Contig[0396], SLJClgrIII03M13RF05
1	GO:0003674	MOLECULAR FUNCTION	Contig[0252], Contig[0415], Contig[0303], Contig[0007], SLJClgrIII05T7FA11, Contig[0143], Contig[0234], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0171], Contig[0484], Contig[0259], Contig[0362], Contig[0274], Contig[0396], Contig[0443], Contig[0401], Contig[0393], Contig[0178], Contig[0251], Contig[0323], Contig[0156], Contig[0398], Contig[0388], Contig[0021], Contig[0391], Contig[0418], Contig[0207], Contig[0191], SLJClgrIII06T7E03, Contig[0031], Contig[0413], Contig[0214], Contig[0046], Contig[0101], Contig[0482], Contig[0213], Contig[0397], Contig[0091], SLJClgrIII03M13RF01, Contig[0438], Contig[0318], Contig[0313], Contig[0014], Contig[0077], Contig[0183], Contig[0431], SLJClgrIII01T7B04, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0187], Contig[0384], Contig[0088], Contig[0137], Contig[0326], Contig[0114], Contig[0420], Contig[0268], Contig[0389], SLJClgrIII06T7F02, Contig[0197], Contig[0496], Contig[0235], Contig[0105], Contig[0310], Contig[0225], Contig[0186], Contig[0023], Contig[0475], Contig[0285], Contig[0029], Contig[0430], Contig[0322], Contig[0422], Contig[0037], Contig[0410], Contig[0036], Contig[0383], SLJClgrIII03M13RF06, Contig[0316], Contig[0122], Contig[0446], Contig[0440], Contig[0040], Contig[0222], Contig[0478], SLJClgrIII03M13RB08, Contig[0245], SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461], Contig[0374], SLJClgrIII01T7F12, Contig[0262], Contig[0182], Contig[0016], Contig[0325], Contig[0441], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0094], Contig[0159], Contig[0181], Contig[0407]
2	GO:0016209	antioxidant activity	Contig[0171], Contig[0484], Contig[0274]
2	GO:0005488	binding	Contig[0415], Contig[0303], Contig[0007], SLJClgrIII05T7FA11, Contig[0259], Contig[0274], Contig[0396], Contig[0443], Contig[0401], Contig[0393], Contig[0178], Contig[0251], Contig[0323], Contig[0156], Contig[0021], Contig[0391], Contig[0418], Contig[0252], Contig[0207], Contig[0318], Contig[0313], Contig[0014], Contig[0077], Contig[0089], Contig[0183], SLJClgrIII06T7E03, Contig[0431], SLJClgrIII01T7B04, Contig[0242], SLJClgrIII03M13RF01, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0187], Contig[0031], Contig[0384], Contig[0088], Contig[0137], Contig[0326], Contig[0114], Contig[0420], SLJClgrIII06T7F02, Contig[0197], Contig[0046], Contig[0023], Contig[0475], Contig[0285], Contig[0171], Contig[0484], Contig[0213], Contig[0245], Contig[0374], Contig[0091], Contig[0461], Contig[0441], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0094], Contig[0182]

Table A.5: Key for linking stage 1 *Cladonia grayi* GO terms and Clone ID – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0003824	catalytic activity	Contig[0252], Contig[0143], Contig[0234], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0171], Contig[0484], Contig[0398], Contig[0388], Contig[0191], SLJClgrIII06T7E03, Contig[0031], Contig[0413], Contig[0214], Contig[0046], Contig[0101], Contig[0482], Contig[0213], Contig[0397], Contig[0091], SLJClgrIII03M13RF01, Contig[0438], Contig[0007], Contig[0418], Contig[0415], Contig[0268], Contig[0389], Contig[0496], Contig[0235], Contig[0105], Contig[0310], Contig[0391], Contig[0225], Contig[0186], Contig[0029], Contig[0285], Contig[0183], Contig[0431], SLJClgrIII01T7B04, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0430], Contig[0187], Contig[0322], Contig[0422], Contig[0443], Contig[0037], Contig[0410], Contig[0036], Contig[0383], SLJClgrIII03M13RF06, Contig[0316], Contig[0122], Contig[0446], Contig[0440], Contig[0040], Contig[0222], Contig[0478], SLJClgrIII03M13RB08, Contig[0303], Contig[0077], Contig[0114], SLJClgrIII01T7F12, Contig[0262], Contig[0182], Contig[0374], Contig[0362]
2	GO:0030234	enzyme regulator activity	Contig[0089]
2	GO:0005198	structural molecule activity	SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461]
2	GO:0030528	transcription regulator activity	SLJClgrIII05T7FA11, SLJClgrIII06T7F02
2	GO:0045182	translation regulator activity	Contig[0021], Contig[0077]
2	GO:0005215	transporter activity	Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0362], Contig[0318], Contig[0137], Contig[0016], Contig[0325]
3	GO:0048037	cofactor binding	Contig[0415], Contig[0303], Contig[0007]
3	GO:0008047	enzyme activator activity	Contig[0089]
3	GO:0004386	helicase activity	Contig[0431], SLJClgrIII01T7B04, SLJClgrIII01T7F05, SLJClgrIII01M13RF05
3	GO:0016787	hydrolase activity	Contig[0143], Contig[0234], Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0398], Contig[0388], SLJClgrIII03M13RF01, Contig[0235], Contig[0029], Contig[0431], SLJClgrIII01T7B04, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0443], Contig[0037], Contig[0410], Contig[0036], Contig[0440], Contig[0040], Contig[0222], Contig[0478], SLJClgrIII03M13RB08, Contig[0077], Contig[0114], Contig[0182], Contig[0374]
3	GO:0043167	ion binding	Contig[0274], Contig[0396], Contig[0443], Contig[0401], Contig[0393], Contig[0178], Contig[0391], Contig[0046], Contig[0285], Contig[0303], Contig[0418], Contig[0252], Contig[0207], Contig[0171], Contig[0484], Contig[0213], Contig[0242]
3	GO:0016853	isomerase activity	Contig[0422]
3	GO:0016874	ligase activity	Contig[0105], Contig[0310], Contig[0235], Contig[0191], Contig[0183], Contig[0383]
3	GO:0008289	lipid binding	Contig[0023], Contig[0475]
3	GO:0016829	lyase activity	Contig[0415], Contig[0122]
3	GO:0051540	metal cluster binding	Contig[0303]
3	GO:0003676	nucleic acid binding	SLJClgrIII05T7FA11, Contig[0021], Contig[0077], Contig[0431], SLJClgrIII01T7B04, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0374], Contig[0441], Contig[0087], Contig[0237], Contig[0486], Contig[0349], Contig[0494], Contig[0094], Contig[0182], Contig[0401], Contig[0393], Contig[0178], Contig[0091]
3	GO:0000166	nucleotide binding	Contig[0303], Contig[0007], Contig[0313], Contig[0014], Contig[0077], Contig[0089], Contig[0183], SLJClgrIII06T7E03, Contig[0431], SLJClgrIII01T7B04, Contig[0242], SLJClgrIII03M13RF01, SLJClgrIII01T7F05, SLJClgrIII01M13RF05, Contig[0187], Contig[0323], Contig[0156], Contig[0031], Contig[0091], Contig[0461]
3	GO:0016491	oxidoreductase activity	Contig[0252], Contig[0171], Contig[0484], Contig[0413], Contig[0214], Contig[0046], Contig[0101], Contig[0482], Contig[0213], Contig[0397], Contig[0091], Contig[0007], Contig[0418], Contig[0496], Contig[0391], Contig[0285], Contig[0430], Contig[0322], Contig[0303], SLJClgrIII01T7F12, Contig[0415]
3	GO:0004601	peroxidase activity	Contig[0171], Contig[0484]
3	GO:0005515	protein binding	Contig[0259], Contig[0251], Contig[0323], Contig[0156], Contig[0318], SLJClgrIII06T7F02, Contig[0396], Contig[0197], Contig[0245]
3	GO:0003735	structural constituent of ribosome	SLJClgrIII01M13RF08, Contig[0424], Contig[0003], Contig[0111], Contig[0411], Contig[0047], Contig[0278], Contig[0409], SLJClgrIII01T7H10, Contig[0461]
3	GO:0022892	substrate-specific transporter activity	Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0362], Contig[0318]
3	GO:0046906	tetrapyrrole binding	Contig[0391], Contig[0418], Contig[0252], Contig[0207]

Table A.5: Key for linking stage 1 *Cladonia grayi* GO terms and Clone ID – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0016563	transcription activator activity	SLJClgrIII06T7F02
3	GO:0003700	transcription factor activity	SLJClgrIII05T7FA11
3	GO:0016740	transferase activity	Contig[0191], SLJClgrIII06T7E03, Contig[0031], Contig[0438], Contig[0225], Contig[0186], Contig[0187], SLJ- ClgrIII03M13RF06, Contig[0316], Contig[0446], Contig[0262], Contig[0362]
3	GO:0008135	translation factor activity, nucleic acid binding	Contig[0021], Contig[0077]
3	GO:0022857	transmembrane transporter activity	Contig[0089], Contig[0242], Contig[0302], Contig[0473], Contig[0362], Contig[0318]

Table A.6: Key for linking stage 2 *Cladonia grayi* GO terms and Clone IDs.

Level	GO ID	GO TERM	Clone ID
1	GO:0008150	BIOLOGICAL PROCESS	Contig[0483], Contig[0085], Contig[0493], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04, Contig[0543], Contig[0455], Contig[0127], Contig[0296], Contig[0242], Contig[0172], Contig[0473], Contig[0562], Contig[0614], Contig[0529], Contig[0001], Contig[0203], Contig[0576], Contig[0378], Contig[0660], Contig[0013], Contig[0484], Contig[0454], Contig[0007], Contig[0202], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0676], Contig[0312], Contig[0692], Contig[0801], Contig[0773], Contig[0368], Contig[0065], Contig[0308], Contig[0338], Contig[0139], Contig[0651], Contig[0779], Contig[0809], Contig[0369], Contig[0175], Contig[0463], Contig[0842], Contig[0340], Contig[0234], Contig[0439], Contig[0554], Contig[0408], Contig[0488], Contig[0163], Contig[0130], Contig[0058], Contig[0575], Contig[0653], Contig[0481], Contig[0737], Contig[0117], Contig[0425], Contig[0577], Contig[0192], Contig[0650], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0215], Contig[0626], Contig[0724], Contig[0290], Contig[0539], Contig[0730], Contig[0633], Contig[0354], Contig[0760], Contig[0070], Contig[0285], Contig[0740], Contig[0413], Contig[0601], Contig[0654], Contig[0382], Contig[0272], SLJClgrII10M13RG01, Contig[0388], Contig[0756], Contig[0125], Contig[0220], Contig[0691], Contig[0827], Contig[0708], Contig[0353], Contig[0854], Contig[0644], Contig[0630], Contig[0684], Contig[0283], Contig[0811], Contig[0297], Contig[0445], Contig[0783], Contig[0574], Contig[0621], Contig[0589], Contig[0746], Contig[0331], Contig[0715], SLJClgrII01M13RG11, Contig[0330], Contig[0640], Contig[0380], Contig[0025], Contig[0298], Contig[0267], Contig[0433], Contig[0559], Contig[0198], Contig[0174], Contig[0044], SLClgrII03M13RD03, Contig[0585], Contig[0649], Contig[0116], Contig[0502], Contig[0226], Contig[0270], Contig[0486], Contig[0052], Contig[0698], Contig[0009], Contig[0094], Contig[0754], Contig[0840], Contig[0414], Contig[0083], Contig[0459], Contig[0024], Contig[0569], Contig[0670], Contig[0536], Contig[0616], Contig[0124], Contig[0399], Contig[0530]
2	GO:0065007	biological regulation	Contig[0539], Contig[0730], Contig[0285], Contig[0691], Contig[0589], Contig[0297], Contig[0554]
2	GO:0009987	cellular process	Contig[0576], Contig[0378], Contig[0660], Contig[0242], Contig[0172], Contig[0473], Contig[0013], Contig[0484], Contig[0454], Contig[0007], Contig[0202], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0676], Contig[0312], Contig[0692], Contig[0801], Contig[0773], Contig[0368], Contig[0065], Contig[0308], Contig[0539], Contig[0730], Contig[0633], Contig[0234], Contig[0439], Contig[0354], Contig[0070], Contig[0285], Contig[0740], Contig[0382], Contig[0272], SLJClgrII10M13RG01, Contig[0388], Contig[0756], Contig[0125], Contig[0130], Contig[0220], Contig[0691], Contig[0827], Contig[0708], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0644], Contig[0215], Contig[0630], Contig[0684], Contig[0283], Contig[0577], Contig[0445], Contig[0783], Contig[0574], Contig[0589], Contig[0298], Contig[0585], Contig[0649], Contig[0116], Contig[0651], Contig[0779], Contig[0369], Contig[0124], Contig[0554], Contig[0399], Contig[0569], Contig[0530]
2	GO:0032502	developmental process	Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04, Contig[0340], Contig[0234], Contig[0439], Contig[0554], Contig[0408], Contig[0488], Contig[0163], Contig[0455], Contig[0651], Contig[0779], Contig[0334], Contig[0130], Contig[0479], Contig[0058], Contig[0575], Contig[0653], Contig[0481], Contig[0737], Contig[0117], Contig[0676], Contig[0369], Contig[0425], Contig[0577], Contig[0192], Contig[0650], Contig[0312], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0215], Contig[0626]
2	GO:0051234	establishment of localization	Contig[0493], Contig[0455], Contig[0127], Contig[0296], Contig[0242], Contig[0172], Contig[0473], Contig[0562], Contig[0614], Contig[0529], Contig[0001], Contig[0543], Contig[0203], Contig[0724], Contig[0340], Contig[0290], Contig[0760], Contig[0811], Contig[0691], Contig[0297], Contig[0445], Contig[0783], Contig[0574], Contig[0007], Contig[0202], Contig[0044], SLClgrII03M13RD03
2	GO:0051179	localization	Contig[0493], Contig[0455], Contig[0127], Contig[0296], Contig[0242], Contig[0172], Contig[0473], Contig[0562], Contig[0614], Contig[0529], Contig[0001], Contig[0543], Contig[0203], Contig[0724], Contig[0340], Contig[0290], Contig[0760], Contig[0811], Contig[0691], Contig[0297], Contig[0445], Contig[0783], Contig[0574], Contig[0007], Contig[0202], Contig[0044], SLClgrII03M13RD03

Table A.6: Key for linking stage 2 *Cladonia grayi* GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0008152	metabolic process	Contig[0483], Contig[0085], Contig[0576], Contig[0378], Contig[0660], Contig[0242], Contig[0172], Contig[0473], Contig[0484], Contig[0454], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0676], Contig[0312], Contig[0692], Contig[0368], Contig[0065], Contig[0308], Contig[0539], Contig[0730], Contig[0633], Contig[0234], Contig[0439], Contig[0354], Contig[0070], Contig[0285], Contig[0740], Contig[0413], Contig[0601], Contig[0654], Contig[0382], Contig[0272], SLJClgrII10M13RG01, Contig[0125], Contig[0013], Contig[0130], Contig[0220], Contig[0827], Contig[0708], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0644], Contig[0215], Contig[0630], Contig[0684], Contig[0283], Contig[0577], Contig[0445], Contig[0783], Contig[0574], Contig[0621], Contig[0298], Contig[0585], Contig[0649], Contig[0116], Contig[0801], Contig[0773], Contig[0715], SLJClgrII01M13RG11, Contig[0640], Contig[0488], Contig[0502], Contig[0127], Contig[0226], Contig[0270], Contig[0486], Contig[0052], Contig[0698], Contig[0009], Contig[0094], Contig[0754], Contig[0840], Contig[0414], Contig[0083], Contig[0459], Contig[0024], Contig[0198], Contig[0296], Contig[0651], Contig[0779], Contig[0369], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04, Contig[0536], Contig[0616], Contig[0388], Contig[0756], Contig[0124], Contig[0399], Contig[0569], Contig[0530]
2	GO:0032501	multicellular organismal process	Contig[0340], Contig[0234], Contig[0439], Contig[0554], Contig[0408], Contig[0488], Contig[0163], Contig[0455], Contig[0651], Contig[0779], Contig[0334], Contig[0130], Contig[0479], Contig[0058], Contig[0575], Contig[0653], Contig[0481], Contig[0737], Contig[0117], Contig[0676], Contig[0369], Contig[0425], Contig[0577], Contig[0192], Contig[0650], Contig[0312], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0215], Contig[0626]
2	GO:0051704	multi-organism process	Contig[0691], Contig[0746], Contig[0331], Contig[0715], SLJClgrII01M13RG11, Contig[0330], Contig[0640], Contig[0801], Contig[0234], Contig[0439], Contig[0380], Contig[0025], Contig[0562], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], Contig[0298], Contig[0267], Contig[0433], SLJClgrII10M13RC04, Contig[0783], Contig[0771], Contig[0559], Contig[0730], Contig[0378], Contig[0198], Contig[0174], Contig[0670], Contig[0649]
2	GO:0000003	reproduction	Contig[0691], Contig[0569]
2	GO:0050896	response to stimulus	Contig[0543], Contig[0338], Contig[0139], Contig[0651], Contig[0779], Contig[0809], Contig[0369], Contig[0175], Contig[0463], Contig[0842], Contig[0070], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04, Contig[0691]
3	GO:0048856	anatomical structure development	Contig[0340], Contig[0234], Contig[0439], Contig[0554], Contig[0408], Contig[0488], Contig[0163], Contig[0455], Contig[0651], Contig[0779], Contig[0334], Contig[0130], Contig[0479], Contig[0058], Contig[0575], Contig[0653], Contig[0481], Contig[0737], Contig[0117], Contig[0676], Contig[0369], Contig[0425], Contig[0577], Contig[0192], Contig[0650], Contig[0312], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0215], Contig[0626]
3	GO:0009058	biosynthetic process	Contig[0576], Contig[0378], Contig[0660], Contig[0484], Contig[0454], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0676], Contig[0312], Contig[0692], SLJClgrII10M13RG01, Contig[0013], Contig[0272], Contig[0445], Contig[0783], Contig[0574], Contig[0283], Contig[0801], Contig[0124], Contig[0530]
3	GO:0009056	catabolic process	Contig[0070], Contig[0827], Contig[0708], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0644], Contig[0215], Contig[0630], Contig[0684], Contig[0283], Contig[0577], SLJClgrII10M13RG01, Contig[0399], Contig[0569]
3	GO:0007154	cell communication	Contig[0388], Contig[0756], Contig[0691], Contig[0554]
3	GO:0007049	cell cycle	Contig[0013], Contig[0589], Contig[0773]
3	GO:0022402	cell cycle process	Contig[0773]
3	GO:0051301	cell division	Contig[0801], Contig[0773]
3	GO:0016043	cellular component organization and biogenesis	Contig[0007], Contig[0202], Contig[0285], Contig[0125]
3	GO:0051641	cellular localization	Contig[0007], Contig[0202]

Table A.6: Key for linking stage 2 *Cladonia grayi* GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0044237	cellular metabolic process	Contig[0576], Contig[0378], Contig[0660], Contig[0242], Contig[0172], Contig[0473], Contig[0484], Contig[0454], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0676], Contig[0312], Contig[0692], Contig[0368], Contig[0065], Contig[0308], Contig[0539], Contig[0730], Contig[0633], Contig[0234], Contig[0439], Contig[0354], Contig[0070], Contig[0285], Contig[0740], Contig[0382], Contig[0272], SLJClgrII10M13RG01, Contig[0130], Contig[0220], Contig[0827], Contig[0708], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0644], Contig[0215], Contig[0630], Contig[0684], Contig[0283], Contig[0577], Contig[0445], Contig[0783], Contig[0298], Contig[0585], Contig[0649], Contig[0116], Contig[0801], Contig[0773], Contig[0651], Contig[0779], Contig[0369], Contig[0388], Contig[0756], Contig[0124], Contig[0399], Contig[0569], Contig[0530]
3	GO:0051716	cellular response to stimulus	Contig[0691]
3	GO:0000746	conjugation	Contig[0691]
3	GO:0021700	developmental maturation	Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04
3	GO:0051649	establishment of cellular localization	Contig[0007], Contig[0202]
3	GO:0045184	establishment of protein localization	Contig[0811], Contig[0691], Contig[0297], Contig[0007], Contig[0202]
3	GO:0006091	generation of precursor metabolites and energy	Contig[0242], Contig[0172], Contig[0473], Contig[0827], Contig[0708], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0445], Contig[0783], Contig[0574]
3	GO:0044419	interspecies interaction between organisms	Contig[0746], Contig[0331], Contig[0715], SLJClgrII01M13RG11, Contig[0330], Contig[0640], Contig[0801], Contig[0234], Contig[0439], Contig[0691], Contig[0380], Contig[0025], Contig[0562], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], Contig[0298], Contig[0267], Contig[0433], SLJClgrII10M13RC04, Contig[0783], Contig[0771], Contig[0559], Contig[0730], Contig[0378], Contig[0198], Contig[0174], Contig[0670], Contig[0649]
3	GO:0033036	macromolecule localization	Contig[0811], Contig[0691], Contig[0297], Contig[0007], Contig[0202]
3	GO:0043170	macromolecule metabolic process	Contig[0660], Contig[0484], Contig[0454], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0676], Contig[0312], Contig[0539], Contig[0730], Contig[0285], Contig[0125], Contig[0644], Contig[0215], Contig[0630], Contig[0684], Contig[0283], Contig[0577], SLJClgrII10M13RG01, Contig[0585], Contig[0649], Contig[0116], Contig[0801], Contig[0773], Contig[0651], Contig[0779], Contig[0369], Contig[0388], Contig[0756], Contig[0530]
3	GO:0007275	multicellular organismal development	Contig[0340], Contig[0234], Contig[0439], Contig[0554], Contig[0408], Contig[0488], Contig[0163], Contig[0455], Contig[0651], Contig[0779], Contig[0334], Contig[0130], Contig[0479], Contig[0058], Contig[0575], Contig[0653], Contig[0481], Contig[0737], Contig[0117], Contig[0676], Contig[0369], Contig[0425], Contig[0577], Contig[0192], Contig[0650], Contig[0312], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0215], Contig[0626]
3	GO:0006807	nitrogen compound metabolic process	Contig[0576], Contig[0378], Contig[0660], Contig[0692], SLJClgrII10M13RG01, Contig[0530]
3	GO:0044238	primary metabolic process	Contig[0483], Contig[0085], Contig[0660], Contig[0484], Contig[0454], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0676], Contig[0312], Contig[0692], Contig[0368], Contig[0065], Contig[0308], Contig[0539], Contig[0730], Contig[0633], Contig[0234], Contig[0439], Contig[0354], Contig[0285], Contig[0740], Contig[0413], Contig[0601], Contig[0654], SLJClgrII10M13RG01, Contig[0130], Contig[0220], Contig[0644], Contig[0215], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0630], Contig[0684], Contig[0283], Contig[0577], Contig[0445], Contig[0783], Contig[0574], Contig[0621], Contig[0585], Contig[0649], Contig[0116], Contig[0801], Contig[0773], Contig[0651], Contig[0779], Contig[0369], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04, Contig[0536], Contig[0616], Contig[0388], Contig[0756], Contig[0124], Contig[0399], Contig[0569], Contig[0530]
3	GO:0050789	regulation of biological process	Contig[0539], Contig[0730], Contig[0285], Contig[0691], Contig[0589], Contig[0554]
3	GO:0050794	regulation of cellular process	Contig[0539], Contig[0730], Contig[0285], Contig[0589], Contig[0554]
3	GO:0019222	regulation of metabolic process	Contig[0539], Contig[0730], Contig[0285]
3	GO:0065009	regulation of molecular function	Contig[0297], Contig[0554]
3	GO:0042221	response to chemical stimulus	Contig[0543], Contig[0070], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04
3	GO:0009605	response to external stimulus	Contig[0691]

Table A.6: Key for linking stage 2 *Cladonia grayi* GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0006950	response to stress	Contig[0338], Contig[0139], Contig[0651], Contig[0779], Contig[0809], Contig[0369], Contig[0175], Contig[0463], Contig[0842], Contig[0070], Contig[0691]
3	GO:0019953	sexual reproduction	Contig[0691], Contig[0569]
3	GO:0006810	transport	Contig[0493], Contig[0455], Contig[0127], Contig[0296], Contig[0242], Contig[0172], Contig[0473], Contig[0562], Contig[0614], Contig[0529], Contig[0001], Contig[0543], Contig[0203], Contig[0724], Contig[0340], Contig[0290], Contig[0760], Contig[0811], Contig[0691], Contig[0297], Contig[0445], Contig[0783], Contig[0574], Contig[0007], Contig[0202], Contig[0044], SLJClgrII03M13RD03
1	GO:0005575	CELLULAR COMPONENT	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0593], Contig[0082], Contig[0484], Contig[0163], Contig[0811], Contig[0715], SLJClgrII01M13RG11, Contig[0455], Contig[0630], Contig[0684], Contig[0283], Contig[0013], Contig[0692], Contig[0193], Contig[0466], Contig[0530], SLJClgrII10M13RG01, Contig[0049], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0760], Contig[0330], Contig[0007], Contig[0202], Contig[0676], Contig[0285], Contig[0166], Contig[0368], Contig[0065], Contig[0308], Contig[0274], Contig[0359], Contig[0454], Contig[0104], Contig[0708], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125], Contig[0589], Contig[0539], Contig[0801], Contig[0724], Contig[0340], Contig[0691], Contig[0562], Contig[0127], Contig[0614], Contig[0044], Contig[0543], Contig[0290], Contig[0203], Contig[0296], SLJClgrII03M13RD03, Contig[0408], Contig[0216], Contig[0185], Contig[0529], Contig[0058], Contig[0575], Contig[0653], Contig[0649], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0554], Contig[0414], Contig[0499], Contig[0730], Contig[0577], Contig[0569], Contig[0740], Contig[0394], Contig[0399], Contig[0621]
2	GO:0005623	cell	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0593], Contig[0082], Contig[0484], Contig[0163], Contig[0811], Contig[0715], SLJClgrII01M13RG11, Contig[0455], Contig[0630], Contig[0684], Contig[0283], Contig[0013], Contig[0692], Contig[0193], Contig[0466], Contig[0530], SLJClgrII10M13RG01, Contig[0049], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0760], Contig[0330], Contig[0007], Contig[0202], Contig[0676], Contig[0285], Contig[0166], Contig[0368], Contig[0065], Contig[0308], Contig[0274], Contig[0359], Contig[0454], Contig[0104], Contig[0708], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125], Contig[0589], Contig[0539], Contig[0801], Contig[0724], Contig[0340], Contig[0691], Contig[0562], Contig[0127], Contig[0614], Contig[0044], Contig[0543], Contig[0290], Contig[0203], Contig[0296], SLJClgrII03M13RD03, Contig[0408], Contig[0216], Contig[0185], Contig[0529], Contig[0058], Contig[0575], Contig[0653], Contig[0649], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0554], Contig[0414], Contig[0499], Contig[0730], Contig[0577], Contig[0569], Contig[0740], Contig[0394], Contig[0399]
2	GO:0044464	cell part	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0593], Contig[0082], Contig[0484], Contig[0163], Contig[0811], Contig[0715], SLJClgrII01M13RG11, Contig[0455], Contig[0630], Contig[0684], Contig[0283], Contig[0013], Contig[0692], Contig[0193], Contig[0466], Contig[0530], SLJClgrII10M13RG01, Contig[0049], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0760], Contig[0330], Contig[0007], Contig[0202], Contig[0676], Contig[0285], Contig[0166], Contig[0368], Contig[0065], Contig[0308], Contig[0274], Contig[0359], Contig[0454], Contig[0104], Contig[0708], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125], Contig[0589], Contig[0539], Contig[0801], Contig[0724], Contig[0340], Contig[0691], Contig[0562], Contig[0127], Contig[0614], Contig[0044], Contig[0543], Contig[0290], Contig[0203], Contig[0296], SLJClgrII03M13RD03, Contig[0408], Contig[0216], Contig[0185], Contig[0529], Contig[0058], Contig[0575], Contig[0653], Contig[0649], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0554], Contig[0414], Contig[0499], Contig[0730], Contig[0577], Contig[0569], Contig[0740], Contig[0394], Contig[0399]
2	GO:0031975	envelope	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493]
2	GO:0005576	extracellular region	Contig[0621]
2	GO:0032991	macromolecular complex	Contig[0676], Contig[0783], Contig[0574], Contig[0285], Contig[0445], Contig[0484], Contig[0454], Contig[0104], Contig[0708], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125], Contig[0577]
2	GO:0031974	membrane-enclosed lumen	Contig[0285]

Table A.6: Key for linking stage 2 *Cladonia grayi* GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0043226	organelle	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0082], Contig[0484], Contig[0163], Contig[0049], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0285], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125], Contig[0589], Contig[0539], Contig[0193], Contig[0466], Contig[0801], Contig[0691], Contig[0569], Contig[0740], Contig[0394], Contig[0399], Contig[0760]
2	GO:0044422	organelle part	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0049], Contig[0285], Contig[0125], Contig[0801], Contig[0569], Contig[0760]
3	GO:0000267	cell fraction	Contig[0330]
3	GO:0030312	external encapsulating structure	Contig[0330], Contig[0007], Contig[0202]
3	GO:0005622	intracellular	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0082], Contig[0484], Contig[0163], Contig[0811], Contig[0715], SLJClgrII01M13RG11, Contig[0455], Contig[0630], Contig[0684], Contig[0283], Contig[0013], Contig[0692], Contig[0193], Contig[0466], Contig[0530], SLJClgrII10M13RG01, Contig[0049], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0676], Contig[0285], Contig[0166], Contig[0368], Contig[0065], Contig[0308], Contig[0274], Contig[0359], Contig[0454], Contig[0104], Contig[0708], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125], Contig[0589], Contig[0539], Contig[0801], Contig[0554], Contig[0414], Contig[0499], Contig[0730], Contig[0691], Contig[0577], Contig[0569], Contig[0740], Contig[0394], Contig[0399], Contig[0760]
3	GO:0043229	intracellular organelle	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0082], Contig[0484], Contig[0163], Contig[0049], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0455], Contig[0285], Contig[0104], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125], Contig[0589], Contig[0539], Contig[0193], Contig[0466], Contig[0801], Contig[0691], Contig[0569], Contig[0740], Contig[0394], Contig[0399], Contig[0760]
3	GO:0044446	intracellular organelle part	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0049], Contig[0285], Contig[0125], Contig[0801], Contig[0569], Contig[0760]
3	GO:0044424	intracellular part	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0082], Contig[0484], Contig[0163], Contig[0811], Contig[0715], SLJClgrII01M13RG11, Contig[0455], Contig[0630], Contig[0684], Contig[0283], Contig[0013], Contig[0692], Contig[0193], Contig[0466], Contig[0530], SLJClgrII10M13RG01, Contig[0049], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0676], Contig[0285], Contig[0166], Contig[0368], Contig[0065], Contig[0308], Contig[0274], Contig[0359], Contig[0454], Contig[0104], Contig[0708], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125], Contig[0589], Contig[0539], Contig[0801], Contig[0691], Contig[0577], Contig[0569], Contig[0740], Contig[0394], Contig[0399], Contig[0760]
3	GO:0016020	membrane	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0049], Contig[0760], Contig[0801], Contig[0724], Contig[0340], Contig[0691], Contig[0562], Contig[0127], Contig[0614], Contig[0044], Contig[0614], Contig[0044], Contig[0543], Contig[0290], Contig[0203], Contig[0296], SLClgrII03M13RD03, Contig[0330], Contig[0408], Contig[0216], Contig[0185], Contig[0529], Contig[0058], Contig[0575], Contig[0653], Contig[0649], Contig[0606], Contig[0851], Contig[0027], Contig[0320], Contig[0456], Contig[0569]
3	GO:0044425	membrane part	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0493], Contig[0783], Contig[0574], Contig[0445], Contig[0801], Contig[0724], Contig[0340], Contig[0691], Contig[0562], Contig[0127], Contig[0614], Contig[0044], Contig[0543], Contig[0290], Contig[0203], Contig[0296], SLClgrII03M13RD03
3	GO:0043227	membrane-bounded organelle	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0082], Contig[0484], Contig[0163], Contig[0049], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0285], Contig[0589], Contig[0539], Contig[0193], Contig[0466], Contig[0125], Contig[0801], Contig[0691], Contig[0569], Contig[0740], Contig[0394], Contig[0399], Contig[0760]
3	GO:0043228	non-membrane-bounded organelle	Contig[0104], Contig[0484], Contig[0334], Contig[0771], Contig[0479], Contig[0312], Contig[0125]
3	GO:0031967	organelle envelope	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493]
3	GO:0043233	organelle lumen	Contig[0285]
3	GO:0031090	organelle membrane	Contig[0242], Contig[0172], Contig[0473], Contig[0001], Contig[0445], Contig[0783], Contig[0574], Contig[0493], Contig[0049], Contig[0801], Contig[0569], Contig[0760]
3	GO:0043234	protein complex	Contig[0783], Contig[0574], Contig[0285], Contig[0445], Contig[0484], Contig[0454], Contig[0708], Contig[0125], Contig[0577]

Table A.6: Key for linking stage 2 *Cladonia grayi* GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0030529	ribonucleoprotein complex	Contig[0676], Contig[0104], Contig[0484], Contig[0334], Contig[0771], Contig[0479], Contig[0312]
1	GO:0003674	MOLECULAR FUNCTION	Contig[0171], Contig[0644], Contig[0215], Contig[0052], Contig[0009], Contig[0094], Contig[0754], Contig[0840], Contig[0399], Contig[0569], Contig[0388], Contig[0756], Contig[0773], Contig[0298], Contig[0692], Contig[0539], Contig[0708], Contig[0445], Contig[0783], Contig[0640], Contig[0070], Contig[0285], Contig[0630], Contig[0684], SLJClgrII10M13RG01, Contig[0792], Contig[0124], Contig[0006], Contig[0446], Contig[0746], Contig[0483], Contig[0724], Contig[0340], Contig[0290], Contig[0554], Contig[0502], Contig[0022], Contig[0677], Contig[0730], Contig[0067], Contig[0649], Contig[0651], Contig[0779], Contig[0369], Contig[0378], Contig[0001], Contig[0216], Contig[0493], Contig[0574], Contig[0203], Contig[0034], Contig[0760], Contig[0486], Contig[0198], Contig[0013], Contig[0267], Contig[0494], Contig[0552], Contig[0673], Contig[0866], Contig[0698], Contig[0394], Contig[0237], Contig[0433], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0127], Contig[0577], Contig[0368], Contig[0065], Contig[0308], Contig[0297], Contig[0676], Contig[0338], Contig[0139], Contig[0809], Contig[0130], Contig[0530], Contig[0175], Contig[0463], Contig[0842], Contig[0296], Contig[0272], Contig[0247], Contig[0373], Contig[0156], Contig[0348], Contig[0488], Contig[0491], Contig[0497], Contig[0747], Contig[0313], Contig[0024], Contig[0312], Contig[0413], Contig[0062], Contig[0382], Contig[0249], Contig[0099], Contig[0634], Contig[0589], Contig[0626], Contig[0740], Contig[0475], Contig[0529], Contig[0601], Contig[0654], Contig[0528], Contig[0633], Contig[0691], Contig[0585], Contig[0330], Contig[0715], SLJClgrII01M13RG11, Contig[0116], Contig[0536], Contig[0660], Contig[0226], Contig[0270], Contig[0044], SLClgrII03M13RD03, Contig[0484], Contig[0454], Contig[0672], Contig[0085], Contig[0083], Contig[0459], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04, Contig[0621], Contig[0334], Contig[0771], Contig[0479], Contig[0104], Contig[0616], Contig[0811], Contig[0242], Contig[0172], Contig[0473], Contig[0562], Contig[0614], Contig[0543], Contig[0448], Contig[0283], Contig[0125], Contig[0499]
2	GO:0016209	antioxidant activity	Contig[0070], Contig[0394]
2	GO:0005488	binding	Contig[0644], Contig[0215], Contig[0052], Contig[0009], Contig[0094], Contig[0754], Contig[0840], Contig[0399], Contig[0569], Contig[0298], Contig[0539], Contig[0285], Contig[0630], Contig[0684], SLJClgrII10M13RG01, Contig[0554], Contig[0502], Contig[0022], Contig[0677], Contig[0730], Contig[0067], Contig[0649], Contig[0651], Contig[0779], Contig[0369], Contig[0216], Contig[0001], Contig[0692], Contig[0127], Contig[0577], Contig[0676], Contig[0338], Contig[0388], Contig[0756], Contig[0139], Contig[0773], Contig[0809], Contig[0130], Contig[0530], Contig[0175], Contig[0463], Contig[0842], Contig[0296], Contig[0247], Contig[0373], Contig[0156], Contig[0348], Contig[0488], Contig[0491], Contig[0497], Contig[0747], Contig[0494], Contig[0552], Contig[0673], Contig[0866], Contig[0313], Contig[0171], Contig[0024], Contig[0312], Contig[0589], Contig[0626], Contig[0070], Contig[0528], Contig[0633], Contig[0445], Contig[0368], Contig[0065], Contig[0308], Contig[0484], Contig[0454], Contig[0536], Contig[0104], Contig[0811], Contig[0660], Contig[0448], Contig[0334], Contig[0272], Contig[0125], Contig[0499]
2	GO:0003824	catalytic activity	Contig[0171], Contig[0388], Contig[0756], Contig[0773], Contig[0692], Contig[0708], Contig[0445], Contig[0783], Contig[0640], Contig[0070], Contig[0298], Contig[0792], Contig[0124], Contig[0006], Contig[0446], Contig[0746], Contig[0483], Contig[0378], Contig[0001], Contig[0486], Contig[0198], Contig[0013], Contig[0502], Contig[0267], Contig[0494], Contig[0552], Contig[0673], Contig[0866], Contig[0698], Contig[0394], Contig[0237], Contig[0433], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0368], Contig[0065], Contig[0308], Contig[0399], Contig[0569], Contig[0272], Contig[0216], Contig[0413], Contig[0062], Contig[0156], Contig[0382], Contig[0249], Contig[0099], Contig[0634], Contig[0740], Contig[0475], Contig[0285], Contig[0601], Contig[0654], Contig[0491], Contig[0747], Contig[0577], SLJClgrII10M13RG01, Contig[0530], Contig[0585], Contig[0330], Contig[0528], Contig[0022], Contig[0649], Contig[0715], SLJClgrII01M13RG11, Contig[0116], Contig[0536], Contig[0660], Contig[0644], Contig[0215], Contig[0226], Contig[0270], Contig[0130], Contig[0672], Contig[0085], Contig[0574], Contig[0083], Contig[0459], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0260], Contig[0857], SLJClgrII10M13RC04, Contig[0621], Contig[0630], Contig[0684], Contig[0052], Contig[0009], Contig[0094], Contig[0754], Contig[0840], Contig[0127], Contig[0296], Contig[0676], Contig[0616], Contig[0242], Contig[0172], Contig[0473], Contig[0283]
2	GO:0030188	chaperone regulator activity	Contig[0589]
2	GO:0030234	enzyme regulator activity	Contig[0554], Contig[0297]
2	GO:0060089	molecular transducer activity	Contig[0388], Contig[0756], Contig[0034]
2	GO:0005198	structural molecule activity	Contig[0334], Contig[0771], Contig[0479], Contig[0312]
2	GO:0030528	transcription regulator activity	Contig[0539]

Table A.6: Key for linking stage 2 *Cladonia grayi* GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0045182	translation regulator activity	Contig[0484], Contig[0454]
2	GO:0005215	transporter activity	Contig[0445], Contig[0783], Contig[0724], Contig[0340], Contig[0290], Contig[0001], Contig[0493], Contig[0574], Contig[0203], Contig[0760], Contig[0529], Contig[0691], Contig[0044], SLJClgrII03M13RD03, Contig[0127], Contig[0296], Contig[0562], Contig[0614], Contig[0543]
3	GO:0030246	carbohydrate binding	Contig[0536]
3	GO:0030189	chaperone activator activity	Contig[0589]
3	GO:0048037	cofactor binding	Contig[0644], Contig[0215], Contig[0052], Contig[0009], Contig[0094], Contig[0754], Contig[0840], Contig[0399], Contig[0569], Contig[0298], Contig[0285], Contig[0630], Contig[0684], SLJClgrII10M13RG01, Contig[0528]
3	GO:0008047	enzyme activator activity	Contig[0554]
3	GO:0030695	GTPase regulator activity	Contig[0554], Contig[0297]
3	GO:0004386	helicase activity	Contig[0022]
3	GO:0016787	hydrolase activity	Contig[0445], Contig[0783], Contig[0483], Contig[0378], Contig[0001], Contig[0475], Contig[0285], Contig[0585], Contig[0649], Contig[0116], Contig[0536], Contig[0672], Contig[0085], Contig[0574], Contig[0829], Contig[0374], Contig[0847], Contig[0126], Contig[0209], Contig[0260], Contig[0857], SLJClgrII10M13RC04, Contig[0621], Contig[0127], Contig[0296], Contig[0676], Contig[0616]
3	GO:0043167	ion binding	Contig[0554], Contig[0539], Contig[0502], Contig[0285], Contig[0022], Contig[0677], Contig[0730], Contig[0067], Contig[0649], Contig[0692], Contig[0127], Contig[0577], Contig[0296], Contig[0001], Contig[0216], Contig[0070], Contig[0528], Contig[0633], Contig[0445], Contig[0368], Contig[0065], Contig[0308]
3	GO:0016853	isomerase activity	Contig[0491], Contig[0747], Contig[0283]
3	GO:0016874	ligase activity	Contig[0272], Contig[0530], Contig[0660]
3	GO:0016829	lyase activity	Contig[0171], Contig[0708], Contig[0298], Contig[0577], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425]
3	GO:0003676	nucleic acid binding	Contig[0539], Contig[0484], Contig[0454], Contig[0811], Contig[0633], Contig[0285], Contig[0022], Contig[0125], Contig[0554], Contig[0499], Contig[0730]
3	GO:0000166	nucleotide binding	Contig[0052], Contig[0009], Contig[0094], Contig[0754], Contig[0840], Contig[0399], Contig[0569], Contig[0676], Contig[0338], Contig[0388], Contig[0756], Contig[0139], Contig[0773], Contig[0127], Contig[0651], Contig[0779], Contig[0809], Contig[0130], Contig[0022], Contig[0369], Contig[0530], Contig[0175], Contig[0463], Contig[0842], Contig[0296], Contig[0528], Contig[0104], Contig[0811], Contig[0660], Contig[0448], Contig[0334], Contig[0272]
3	GO:0016491	oxidoreductase activity	Contig[0692], Contig[0070], Contig[0001], Contig[0486], Contig[0198], Contig[0502], Contig[0267], Contig[0494], Contig[0552], Contig[0673], Contig[0866], Contig[0698], Contig[0394], Contig[0237], Contig[0399], Contig[0569], Contig[0216], Contig[0740], SLJClgrII10M13RG01, Contig[0528], Contig[0715], SLJClgrII01M13RG11, Contig[0644], Contig[0215], Contig[0630], Contig[0684], Contig[0052], Contig[0009], Contig[0094], Contig[0754], Contig[0840], Contig[0242], Contig[0172], Contig[0473]
3	GO:0004601	peroxidase activity	Contig[0070]
3	GO:0005515	protein binding	Contig[0651], Contig[0779], Contig[0369], Contig[0127], Contig[0589], Contig[0022], Contig[0677], Contig[0067], Contig[0626]
3	GO:0004871	signal transducer activity	Contig[0388], Contig[0756], Contig[0034]
3	GO:0003735	structural constituent of ribosome	Contig[0334], Contig[0771], Contig[0479], Contig[0312]
3	GO:0022892	substrate-specific transporter activity	Contig[0445], Contig[0783], Contig[0724], Contig[0340], Contig[0290], Contig[0001], Contig[0493], Contig[0574], Contig[0203], Contig[0691], Contig[0044], SLJClgrII03M13RD03, Contig[0127], Contig[0296]
3	GO:0046906	tetrapyrrole binding	Contig[0216], Contig[0001]
3	GO:0003700	transcription factor activity	Contig[0539]
3	GO:0016740	transferase activity	Contig[0388], Contig[0756], Contig[0773], Contig[0640], Contig[0792], Contig[0124], Contig[0006], Contig[0446], Contig[0746], Contig[0486], Contig[0198], Contig[0013], Contig[0433], Contig[0827], Contig[0353], Contig[0854], Contig[0737], Contig[0425], Contig[0368], Contig[0065], Contig[0308], Contig[0601], Contig[0654], Contig[0330], Contig[0226], Contig[0270], Contig[0130], Contig[0083], Contig[0459]
3	GO:0008135	translation factor activity, nucleic acid binding	Contig[0484], Contig[0454]

Table A.6: Key for linking stage 2 *Cladonia grayi* GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0022857	transmembrane transporter activity	Contig[0445], Contig[0783], Contig[0724], Contig[0340], Contig[0290], Contig[0001], Contig[0493], Contig[0574], Contig[0203], Contig[0760], Contig[0529], Contig[0044], SLClgrII03M13RD03, Contig[0127], Contig[0296]
3	GO:0019842	vitamin binding	Contig[0298]

B

GenBank, UniProt, and Gene Ontology summary information for *Asterochloris* sp. in stages 1 and 2 of lichen development.

B.1 Combined GenBank and UniProt summary information for stages 1 and 2 *Asterochloris* sp. gene fragments

A local batch blastx algorithmic search was carried out for stages 1 and 2 *Asterochloris* sp. SSH-cDNAs, using the non-redundant set of proteins from the National Center for Biotechnology and Information (www.ncbi.nlm.nih.gov, accessed on 4 March 2009). An outline of how nucleotide and amino acid sequences were summarized, and how stage 2 SSH-cDNAs were assigned to organism, can be found in Appendix A.1.

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments.

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0003]	ref YP_001277992.1	carbonic anhydrase [Roseiflexus sp. RS-1] gb ABQ92042.1 carbonic anhydrase [Roseiflexus sp. RS-1]	P64798 Y1315_MYCBO	Uncharacterized protein Mb1315 - Mycobacterium bovis
Contig[0008]	ref XP_001417190.1	Mn-Superoxide dismutase [Ostreococcus lucimarinus CCE9901] gb ABO95483.1 Mn-Superoxide dismutase [Ostreococcus lucimarinus CCE9901]	P28762 SODM_EPTST	Superoxide dismutase [Mn], mitochondrial - Eptatretus stoutii (Pacific hagfish)
Contig[0013]	ref XP_001699186.1	glycosyl transferase, type ALG6, ALG8 [Chlamydomonas reinhardtii] gb EDO98826.1 glycosyl transferase, type ALG6, ALG8 [Chlamydomonas reinhardtii]	Q9FF17 ALG6_ARATH	Probable dolichyl pyrophosphate Man9GlcNAc2 alpha-1,3-glucosyltransferase - Arabidopsis thaliana (Mouse-ear cress)
Contig[0014]	ref XP_001702638.1	20S proteasome alpha subunit C [Chlamydomonas reinhardtii] gb EDP06417.1 20S proteasome alpha subunit C [Chlamydomonas reinhardtii]	P52427 PSA4_SPIOL	Proteasome subunit alpha type 4 - Spinacia oleracea (Spinach)
Contig[0015]	ref XP_001703643.1	type-II calcium-dependent NADH dehydrogenase [Chlamydomonas reinhardtii] gb EDO96450.1 type-II calcium-dependent NADH dehydrogenase [Chlamydomonas reinhardtii]	P40215 NDH1_YEAST	External NADH-ubiquinone oxidoreductase 1, mitochondrial precursor - Saccharomyces cerevisiae (Baker's yeast)
Contig[0020]	gb AAF03675.1 AF149311.1	raucacaffricine-O-beta-D-glucosidase [Rauvolfia serpentina]	Q03506 BGLA_BACCI	Beta-glucosidase - Bacillus circulans
Contig[0026]	gb ACL53533.1	unknown [Zea mays]	O08795 GLU2B_MOUSE	Glucosidase 2 subunit beta precursor - Mus musculus (Mouse)
Contig[0029]	ref XP_001695978.1	early light-inducible protein [Chlamydomonas reinhardtii] gb EDP01315.1 early light-inducible protein [Chlamydomonas reinhardtii]	P27516 CBR_DUNBA	Carotene biosynthesis-related protein CBR, chloroplast precursor - Dunaliella bardawil
Contig[0038]	gb ACF16408.1	chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit [Myrmecia incisa]	Q38692 RBS6_ACECL	Ribulose bisphosphate carboxylase small chain 6, chloroplast precursor - Acetabularia cliftonii (Green alga)
Contig[0042]	ref XP_001767800.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ67314.1 predicted protein [Physcomitrella patens subsp. patens]	P13798 ACPH_HUMAN	Acylamino-acid-releasing enzyme - Homo sapiens (Human)
Contig[0046]	ref XP_001695456.1	coiled-coil domain 6-like protein [Chlamydomonas reinhardtii] gb EDP01714.1 coiled-coil domain 6-like protein [Chlamydomonas reinhardtii]		
Contig[0048]	gb AAB96651.1	putative cruciform DNA binding protein [Glomus versiforme]		
Contig[0052]	gb ABK25112.1	unknown [Picea sitchensis]		
Contig[0054]	gb ACI31237.1	cytochrome f [Volvox carteri]	Q1KVS1 CYF_SCEOB	Apocytochrome f precursor - Scenedesmus obliquus

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0060]	ref XP_001754044.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ80945.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0084]	ref YP_001660751.1	30S ribosomal protein S3 [Microcystis aeruginosa NIES-843] sp B0JHZ7 RS3_MICAN RecName: Full=30S ribosomal protein S3 dbj BAG05559.1 30S ribosomal protein S3 [Microcystis aeruginosa NIES-843]	P73314 RS3_SYNY3	30S ribosomal protein S3 - Synechocystis sp. (strain PCC 6803)
Contig[0087]	ref XP_001703311.1	SEC61-alpha subunit of ER-translocon [Chlamydomonas reinhardtii] gb EDP05993.1 SEC61-alpha subunit of ER-translocon [Chlamydomonas reinhardtii]	P38379 SC61A_PYRSA	Protein transport protein Sec61 subunit alpha - Pyrenomonas salina
Contig[0094]	gb ACF16408.1	chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit [Myrmecia incisa]	Q38692 RBS6_ACECL	Ribulose biphosphate carboxylase small chain 6, chloroplast precursor - Acetabularia cliftonii (Green alga)
Contig[0097]	ref XP_002167513.1	PREDICTED: similar to Tubulin Tyrosine Ligase Like family member (ttll-15) [Hydra magnipapillata]		
Contig[0099]	ref XP_002115957.1	hypothetical protein TRIADDRAFT_29967 [Trichoplax adhaerens] gb EDV21809.1 hypothetical protein TRIADDRAFT_29967 [Trichoplax adhaerens]	Q304B9 NCASE_ARATH	Neutral ceramidase precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0100]	ref XP_537880.2	PREDICTED: similar to histone 1, H2ai (predicted) isoform 1 [Canis familiaris] ref XP_859248.1 PREDICTED: similar to histone 1, H2ai (predicted) isoform 3 [Canis familiaris]	P22843 H3_ACRFO	Histone H3 - Acropora formosa (Staghorn coral)
Contig[0101]	gb ABM74386.1	major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina] gb ABM74387.1 major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina]	P27517 CB2_DUNTE	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor - Dunaliella tertiolecta
Contig[0111]	gb EEF30171.1	Protein ABC1, mitochondrial precursor, putative [Ricinus communis]	P73627 Y1770_SYNY3	Uncharacterized protein sll1770 - Synechocystis sp. (strain PCC 6803)
Contig[0116]	ref XP_001697670.1	Qc-SNARE protein, SFT1 family [Chlamydomonas reinhardtii] gb EDP00064.1 Qc-SNARE protein, SFT1 family [Chlamydomonas reinhardtii]	Q8VXX9 BETL1_ARATH	Bet1-like protein At4g14600 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0118]	ref YP_615223.1	alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen [Sphingopyxis alaskensis RB2256] gb ABF51890.1 alkyl hydroperoxide reductase/ Thiol specific antioxidant/ Mal allergen [Sphingopyxis alaskensis RB2256]	Q7F8S5 PR2E2_ORYSJ	Peroxioredoxin-2E-2, chloroplast precursor - Oryza sativa subsp. japonica (Rice)

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0121]	ref XP_001703187.1	acetyl-coa carboxylase beta-carboxyltransferase subunit of plastidic multimeric ACCase [Chlamydomonas reinhardtii] gb EDO96563.1 acetyl-coa carboxylase beta-carboxyltransferase subunit of plastidic multimeric AC-Case [Chlamydomonas reinhardtii]	P56293 ACCD_CHLVU	Acetyl-coenzyme A carboxylase carboxyl transferase subunit beta - Chlorella vulgaris (Green alga)
Contig[0126]	ref XP_001790911.1	hypothetical protein SNOG_00220 [Phaeosphaeria nodorum SN15] gb EAT91715.2 hypothetical protein SNOG_00220 [Phaeosphaeria nodorum SN15]	Q46845 YGHU_ECOLI	Uncharacterized GST-like protein yghU - Escherichia coli
Contig[0127]	gb EEC82025.1	hypothetical protein OsI_25990 [Oryza sativa Indica Group]	O82660 P2SAF_ARATH	Photosystem II stability/assembly factor HCF136, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0138]	ref XP_001696194.1	photosystem II stability/assembly factor HCF136 [Chlamydomonas reinhardtii] gb EDP08171.1 photosystem II stability/assembly factor HCF136 [Chlamydomonas reinhardtii]		
Contig[0140]	ref NP_191188.1	unknown protein [Arabidopsis thaliana] emb CAB87428.1 putative protein [Arabidopsis thaliana] gb AAL32581.1 putative protein [Arabidopsis thaliana] gb AAM10237.1 putative protein [Arabidopsis thaliana] dbj BAF00005.1 hypothetical protein [Arabidopsis thaliana]		
Contig[0141]	ref XP_001777570.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ57613.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0142]	gb AAG49030.1 AF204783_1	ripening regulated protein DDTRF8 [Lycopersicon esculentum]	Q11118 WOS2_SCHPO	Protein wos2 - Schizosaccharomyces pombe (Fission yeast)
Contig[0143]	ref XP_001422007.1	predicted protein [Ostreococcus lucimarinus CCE9901] gb ABP00301.1 predicted protein [Ostreococcus lucimarinus CCE9901]		
Contig[0148]	emb CAJ30047.1	conserved hypothetical protein [Magnetospirillum gryphiswaldense]		
Contig[0150]	ref XP_001609713.1	Histone H3 [Babesia bovis T2Bo] gb EDO06145.1 Histone H3, putative [Babesia bovis]	P22843 H3_ACRFO	Histone H3 - Acropora formosa (Staghorn coral)
Contig[0151]	gb ABM74386.1	major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina] gb ABM74387.1 major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina]	P27517 CB2_DUNTE	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor - Dunaliella tertiolecta
Contig[0152]	gb AAB70556.1	chlorophyll a/b binding protein [Tetraselmis sp. RG-15]	P27517 CB2_DUNTE	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor - Dunaliella tertiolecta

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0153]	gb ABM74386.1	major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina]	P27517 CB2_DUNTE	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor - Dunaliella tertiolecta
Contig[0154]	ref XP_001697670.1	gb ABM74387.1 major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina]	Q8VXX9 BETL1_ARATH	Bet1-like protein At4g14600 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0155]	gb ACL36485.1	Qc-SNARE protein, SFT1 family [Chlamydomonas reinhardtii]	Q43846 SSY3_SOLTU	starch synthase VI precursor [Picea glauca]
Contig[0161]	ref XP_001697632.1	Qc-SNARE protein, SFT1 family [Chlamydomonas reinhardtii]	Q6EUN0 ASCL1_ORYSJ	Soluble starch synthase 3, chloroplast precursor - Solanum tuberosum (Potato)
Contig[0169]	gb EED14557.1	hypothetical protein TSTA_040370 [Talaromyces stipitatus ATCC 10500]	Q9SID3 GLO2N_ARATH	ASC1-like protein 1 - Oryza sativa subsp. japonica (Rice)
Contig[0181]	ref XP_001765155.1	predicted protein [Physcomitrella patens subsp. patens]	Q9XES1 ECA4_ARATH	Putative hydroxyacylglutathione hydrolase 2, mitochondrial precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0182]	gb EDQ69883.1	predicted protein [Physcomitrella patens subsp. patens]	P34313 BUD31_CAEEL	Protein BUD31 homolog - Caenorhabditis elegans
Contig[0185]	ref XP_001690520.1	hypothetical protein CHLREDRAFT_114566 [Chlamydomonas reinhardtii]	Q9XES1 ECA4_ARATH	Calcium-transporting ATPase 4, endoplasmic reticulum-type - Arabidopsis thaliana (Mouse-ear cress)
Contig[0189]	gb EAY96338.1	hypothetical protein OsI_18241 [Oryza sativa Indica Group]	Q8DJY2 DFA1_SYNEL	Putative diflavin flavoprotein A 1 - Synechococcus elongatus (Thermosynechococcus elongatus)
Contig[0190]	ref XP_001756079.1	predicted protein [Physcomitrella patens subsp. patens]	P49598 PP2C4_ARATH	Protein phosphatase 2C - Arabidopsis thaliana (Mouse-ear cress)
Contig[0193]	gb ACM67554.1	ADP-ribosylation factor-like protein [Musa acuminata AAA Group]	Q9XF89 CB26_ARATH	Chlorophyll a-b binding protein CP26, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0201]	ref XP_001770874.1	predicted protein [Physcomitrella patens subsp. patens]	Q9CQU8 IMP1L_MOUSE	Mitochondrial inner membrane protease subunit 1 - Mus musculus (Mouse)
Contig[0202]	gb ABD37916.1	light-harvesting chlorophyll-a/b binding protein Lhcb5 [Chlamydomonas incerta]		
Contig[0217]	ref XP_001769005.1	predicted protein [Physcomitrella patens subsp. patens]		
	gb EEF42900.1	conserved hypothetical protein [Ricinus communis]		

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0226]	ref XP_001692287.1	elongation factor EF-3 [Chlamydomonas reinhardtii] gb EDP04237.1 elongation factor EF-3 [Chlamydomonas reinhardtii]	Q08972 NEW1_YEAST	[NU+] prion formation protein 1 - Saccharomyces cerevisiae (Baker's yeast)
Contig[0230]	gb EAY99194.1	hypothetical protein OsI_21150 [Oryza sativa Indica Group]		
Contig[0233]	emb CAL58037.1	alpha amylase 1 (IC) [Ostreococcus tauri]		
Contig[0235]	ref XP_001702022.1	ornithine transaminase [Chlamydomonas reinhardtii] gb EDO97297.1 ornithine transaminase [Chlamydomonas reinhardtii]	P29758 OAT_MOUSE	Ornithine aminotransferase, mito- chondrial precursor - Mus musculus (Mouse)
Contig[0237]	ref XP_001776615.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ58603.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0238]	gb ABJ98607.1	40S ribosomal protein S14 [Scoph- thalmus maximus]	P62264 RS14_MOUSE	40S ribosomal protein S14 - Mus musculus (Mouse)
Contig[0243]	ref XP_001765745.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ69336.1 predicted protein [Physcomitrella patens subsp. patens]	P90727 U2AF2_CAEBR	Splicing factor U2AF 65 kDa sub- unit - Caenorhabditis briggsae
Contig[0245]	ref XP_001692870.1	2-oxoglutarate dehydrogenase, E1 subunit [Chlamydomonas reinhardtii] gb EDP03439.1 2- oxoglutarate dehydrogenase, E1 subunit [Chlamydomonas rein- hardtii]	Q60597 ODO1_MOUSE	2-oxoglutarate dehydrogenase E1 component, mitochondrial precu- sor - Mus musculus (Mouse)
Contig[0246]	emb CAR47957.1	p-hydroxyphenylpyruvate dioxyge- nase [Chlamydomonas reinhardtii]	O23920 HPPD_DAUCA	4-hydroxyphenylpyruvate dioxyge- nase - Daucus carota (Carrot)
Contig[0250]	ref XP_001701464.1	ribosomal protein S30, component of cytosolic 80S ribosome and 40S small subunit [Chlamydomonas reinhardtii] gb EDO97461.1 ribo- somal protein S30 [Chlamydomonas reinhardtii]		
Contig[0251]	ref YP_002300088.1	hypothetical protein RC1_3934 [Rhodospirillum centenum SW] gb ACJ01276.1 conserved hypo- thetical protein [Rhodospirillum centenum SW]		
Contig[0252]	ref NP_958369.1	ribosomal protein L2 [Chlamydomonas reinhardtii] sp Q8HTL2 RK2_CHLRE Rec- Name: Full=50S riboso- mal protein L2, chloroplastic gb AAN60082.1 ribosomal protein L2 [Chlamydomonas reinhardtii] tpg DAA00915.1 TPA_inf: riboso- mal protein L2 [Chlamydomonas reinhardtii] gb ACJ50102.1 ribo- somal protein L2 [Chlamydomonas reinhardtii]	Q8HTL2 RK2_CHLRE	Chloroplast 50S ribosomal protein L2 - Chlamydomonas reinhardtii

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0255]	ref XP_001782634.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ52537.1 predicted protein [Physcomitrella patens subsp. patens]	Q8CST1 PNP_STAES	Polyribonucleotide nucleotidyl-transferase - Staphylococcus epidermidis (strain ATCC 12228)
Contig[0256]	ref XP_001756647.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ78601.1 predicted protein [Physcomitrella patens subsp. patens]	P07591 TRXM_SPIOL	Thioredoxin M-type, chloroplast precursor - Spinacia oleracea (Spinach)
Contig[0273]	ref XP_001698718.1	hypothetical protein CHLREDRAFT_205904 [Chlamydomonas reinhardtii] gb EDO99400.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0284]	emb CAO66084.1	unnamed protein product [Vitis vinifera]		
Contig[0286]	ref XP_001618200.1	hypothetical protein NEMVEDRAFT_v1g155353 [Nematostella vectensis] ref XP_001619109.1 hypothetical protein NEMVEDRAFT_v1g152336 [Nematostella vectensis] gb EDO26100.1 predicted protein [Nematostella vectensis] gb EDO27009.1 predicted protein [Nematostella vectensis]	Q6CQE5 TAR1_KLULA	Protein TAR1 - Kluyveromyces lactis (Yeast) (Candida sphaerica)
Contig[0287]	ref NP_181407.1	peptidyl-prolyl cis-trans isomerase, putative / cyclophilin, putative / rotamase, putative [Arabidopsis thaliana] gb AAC67345.1 putative peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana] gb AAN72042.1 putative peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana] gb AAP21373.1 At2g38730 [Arabidopsis thaliana] gb AAS75305.1 single domain cyclophilin type peptidyl-prolyl cis-trans isomerase [Arabidopsis thaliana]	O43447 PPIH_HUMAN	Peptidyl-prolyl cis-trans isomerase H - Homo sapiens (Human)
Contig[0291]	gb AAB01561.1	heat shock protein 17.0 [Picea glauca]	P46516 HSP21_HELAN	17.9 kDa class II heat shock protein - Helianthus annuus (Common sunflower)
Contig[0294]	ref XP_001699299.1	hypothetical protein CHLREDRAFT_205579 [Chlamydomonas reinhardtii] gb EDO98729.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0295]	gb ACN25606.1	unknown [Zea mays]	Q0IUB5 VATL_ORYSJ	Vacuolar ATP synthase 16 kDa proteolipid subunit - Oryza sativa subsp. japonica (Rice)
Contig[0300]	ref XP_001692287.1	elongation factor EF-3 [Chlamydomonas reinhardtii] gb EDP04237.1 elongation factor EF-3 [Chlamydomonas reinhardtii]	Q08972 NEW1_YEAST	[NU+] prion formation protein 1 - Saccharomyces cerevisiae (Baker's yeast)

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0301]	ref XP_001692870.1	2-oxoglutarate dehydrogenase, E1 subunit [Chlamydomonas reinhardtii] gb EDP03439.1 2-oxoglutarate dehydrogenase, E1 subunit [Chlamydomonas reinhardtii]	Q60597 ODO1_MOUSE	2-oxoglutarate dehydrogenase E1 component, mitochondrial precursor - Mus musculus (Mouse)
Contig[0302]	ref ZP_01550605.1	hypothetical protein SIAM614_17184 [Stappia aggregata IAM 12614] gb EAV40767.1 hypothetical protein SIAM614_17184 [Stappia aggregata IAM 12614]		
Contig[0304]	gb AAG12204.1 AF287482.5	Orf122 [Chlorobium tepidum]		
Contig[0306]	gb AAK49897.1 AF295670.1	plastidic 6-phosphogluconate dehydrogenase [Spinacia oleracea]	P21577 6PGD_SYNP7	6-phosphogluconate dehydrogenase, decarboxylating - Synechococcus sp. (strain PCC 7942) (Anacystis nidulans R2)
Contig[0314]	ref XP_001693430.1	solanesyl diphosphate synthase [Chlamydomonas reinhardtii] gb EDP08684.1 solanesyl diphosphate synthase [Chlamydomonas reinhardtii]	Q1XDL8 PREA_PORYE	Prenyl transferase - Porphyra yezoensis
Contig[0316]	ref XP_001697173.1	26S proteasome regulatory subunit [Chlamydomonas reinhardtii] gb EDP00428.1 26S proteasome regulatory subunit [Chlamydomonas reinhardtii]		
Contig[0330]	ref XP_001756079.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ78945.1 predicted protein [Physcomitrella patens subsp. patens]	Q8DJY2 DFA1_SYNEL	Putative diflavin flavoprotein A 1 - Synechococcus elongatus (Thermosynechococcus elongatus)
Contig[0331]	ref XP_001778887.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ56337.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0332]	ref XP_001694372.1	predicted protein [Chlamydomonas reinhardtii] gb EDP02805.1 predicted protein [Chlamydomonas reinhardtii]	Q9T096 YIPL6_ARATH	Protein yippee-like At4g27745 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0334]	ref ZP_01689674.1	cell wall-associated hydrolase [Microscilla marina ATCC 23134] gb EAY29055.1 cell wall-associated hydrolase [Microscilla marina ATCC 23134]	Q9PLI5 Y114_CHLMU	Uncharacterized protein TC_0114 - Chlamydia muridarum
Contig[0344]	ref XP_001698342.1	mitochondrial inner membrane translocase [Chlamydomonas reinhardtii] gb EDP07835.1 mitochondrial inner membrane translocase [Chlamydomonas reinhardtii]	Q9SP35 TIM17_ARATH	Mitochondrial import inner membrane translocase subunit Tim17 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0345]	ref XP_002181627.1	formamidase-like protein [Phaeodactylum tricornutum CCAP 1055/1] gb EEC46841.1 formamidase-like protein [Phaeodactylum tricornutum CCAP 1055/1]		

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0346]	gb ACN25606.1	unknown [Zea mays]	Q0IUB5 VATL_ORYSJ	Vacuolar ATP synthase 16 kDa proteolipid subunit - <i>Oryza sativa</i> subsp. japonica (Rice)
Contig[0350]	ref YP_172643.1	hypothetical protein syc1933_c [Synechococcus elongatus PCC 6301] ref YP_401178.1 hypothetical protein Synpcc7942_2161 [Synechococcus elongatus PCC 7942] dbj BAD80123.1 unknown protein [Synechococcus elongatus PCC 6301] gb ABB58191.1 conserved hypothetical protein [Synechococcus elongatus PCC 7942]		
Contig[0355]	gb AAB49030.1	DnaJ homolog [Arabidopsis thaliana]	Q94AW8 DNAJ3_ARATH	Chaperone protein dnaJ 3 - <i>Arabidopsis thaliana</i> (Mouse-ear cress)
Contig[0367]	ref NP_192013.1	glycosyl hydrolase family 18 protein [Arabidopsis thaliana] emb CAB80913.1 hypothetical protein [Arabidopsis thaliana]	Q922Q9 CHID1_MOUSE	Chitinase domain-containing protein 1 precursor - <i>Mus musculus</i> (Mouse)
Contig[0368]	ref XP_002292159.1	predicted protein [Thalassiosira pseudonana CCMP1335] gb EED91010.1 predicted protein [Thalassiosira pseudonana CCMP1335]	P48375 FKB12_DROME	12 kDa FK506-binding protein - <i>Drosophila melanogaster</i> (Fruit fly)
Contig[0371]	ref XP_001691093.1	predicted protein [Chlamydomonas reinhardtii] gb EDP05539.1 predicted protein [Chlamydomonas reinhardtii]		
SLJClgrIV01M13RC09_C09	emb CAL52060.2	PsaL photosystem I subunit XI precursor (IC) [Ostreococcus tauri]	P23993 PSAL_HORVU	Photosystem I reaction center subunit XI, chloroplast precursor - <i>Hordeum vulgare</i> (Barley)
SLJClgrIV01M13RE08_E08	ref XP_001697526.1	chlorophyll a-b binding protein of LHCII [Chlamydomonas reinhardtii] gb AAD03732.2 light harvesting complex II protein precursor [Chlamydomonas reinhardtii] gb EDP00188.1 chlorophyll a-b binding protein of LHCII [Chlamydomonas reinhardtii]	P14273 CB2_CHLRE	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor - <i>Chlamydomonas reinhardtii</i>
SLJClgrIV01M13RG06_G06	ref XP_001417190.1	Mn-Superoxide dismutase [Ostreococcus lucimarinus CCE9901] gb ABO95483.1 Mn-Superoxide dismutase [Ostreococcus lucimarinus CCE9901]	P28762 SODM_EPTST	Superoxide dismutase [Mn], mitochondrial - <i>Eptatretus stoutii</i> (Pacific hagfish)
SLJClgrIV01T7A06_A06	ref XP_001695238.1	predicted protein [Chlamydomonas reinhardtii] gb EDP01946.1 predicted protein [Chlamydomonas reinhardtii]	Q9BSH5 HDHD3_HUMAN	Haloacid dehalogenase-like hydrolase domain-containing protein 3 - <i>Homo sapiens</i> (Human)
SLJClgrIV01T7F03_F03	ref XP_001417654.1	predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO95947.1 predicted protein [Ostreococcus lucimarinus CCE9901]		

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
SLJClgrIV02M13RA01_A01	ref XP_001767409.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ67733.1 predicted protein [Physcomitrella patens subsp. patens]	O24301 SUS2_PEA	Sucrose synthase 2 - Pisum sativum (Garden pea)
SLJClgrIV02M13RB07_B07	gb ACF16408.1	chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit [Myrmecia incisa]	Q38692 RBS6_ACECL	Ribulose biphosphate carboxylase small chain 6, chloroplast precursor - Acetabularia cliftonii (Green alga)
SLJClgrIV02M13RC08_C08	gb EDM11640.1	rCG30465, isoform CRA_b [Rattus norvegicus]	O35242 FAN_MOUSE	Protein FAN - Mus musculus (Mouse)
SLJClgrIV02M13RD06_D06	ref YP_001866143.1	amine oxidase [Nostoc punctiforme PCC 73102] gb ACC81200.1 amine oxidase [Nostoc punctiforme PCC 73102]		
SLJClgrIV02M13RF09_F09	ref XP_001697715.1	hypothetical protein CHLREDRAFT_105431 [Chlamydomonas reinhardtii] gb EDO99867.1 predicted protein [Chlamydomonas reinhardtii]	Q6FF50 RLMB_ACIAD	23S rRNA - Acinetobacter sp. (strain ADP1)
SLJClgrIV02M13RG10_G10	dbj BAB96757.1	glucose-6-phosphate dehydrogenase 1 [Chlorella vulgaris]	Q9LK23 G6PD5_ARATH	Glucose-6-phosphate 1-dehydrogenase, cytoplasmic isoform 1 - Arabidopsis thaliana (Mouse-ear cress)
SLJClgrIV02M13RH06_H06	gb AAF37876.1 AF234163.1	starch synthase I [Hordeum vulgare]	Q43654 SSY1_WHEAT	Starch synthase 1, chloroplast precursor - Triticum aestivum (Wheat)
SLJClgrIV02T7A01_A01	ref XP_001767409.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ67733.1 predicted protein [Physcomitrella patens subsp. patens]		
SLJClgrIV02T7C04_C04	gb EEF47225.1	Nitrogen fixation protein nifU, putative [Ricinus communis]	Q93W77 NIFU1_ARATH	NifU-like protein 1, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
SLJClgrIV02T7F06_F06	ref XP_001690968.1	hypothetical protein CHLREDRAFT_182999 [Chlamydomonas reinhardtii] gb EDP05414.1 predicted protein [Chlamydomonas reinhardtii]		
SLJClgrIV02T7F07_F07	ref ZP_02079996.1	hypothetical protein CLOLEP_01448 [Clostridium leptum DSM 753] gb EDO61937.1 hypothetical protein CLOLEP_01448 [Clostridium leptum DSM 753]		
SLJClgrIV02T7G01_G01	ref XP_001751134.1	hypothetical protein [Monosiga brevicollis MX1] gb EDQ84054.1 predicted protein [Monosiga brevicollis MX1]	Q9VNV3 DDX1_DROME	ATP-dependent RNA helicase Ddx1 - Drosophila melanogaster (Fruit fly)
SLJClgrIV02T7H04_H04	ref XP_001701753.1	prohibitin [Chlamydomonas reinhardtii] gb EDP06728.1 prohibitin [Chlamydomonas reinhardtii]	P50085 PHB2_YEAST	Prohibitin-2 - Saccharomyces cerevisiae (Baker's yeast)
SLJClgrIV03M13RA01_A01	gb ACL36485.1	starch synthase VI precursor [Picea glauca]	Q43846 SSY3_SOLTU	Soluble starch synthase 3, chloroplast precursor - Solanum tuberosum (Potato)

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
SLJClgrIV03M13RD02_D02	ref XP_001689471.1	splicing factor, component of the U5 snRNP and of the spliceosome [Chlamydomonas reinhardtii] gb EDP09209.1 splicing factor, component of the U5 snRNP and of the spliceosome [Chlamydomonas reinhardtii]	Q99PV0 PRP8_MOUSE	Pre-mRNA-processing-splicing factor 8 - Mus musculus (Mouse)
SLJClgrIV03M13RF01_F01	gb EEF44734.1	peroxisome biogenesis factor, putative [Ricinus communis]	O43933 PEX1_HUMAN	Peroxisome biogenesis factor 1 - Homo sapiens (Human)
SLJClgrIV03M13RG01_G01	emb CAL50295.1	calmodulin mutant SYNCAM9 (ISS) [Ostreococcus tauri]	P62155 CALM_XENLA	Calmodulin - Xenopus laevis (African clawed frog)
SLJClgrIV03T7D04_D04	emb CAL58543.1	ferredoxin I (ISS) [Ostreococcus tauri]	P00233 FER_GLEJA	Ferredoxin - Gleichenia japonica (Urajiro) (Fern)
SLJClgrIV03T7D07_D07	ref ZP_00630616.1	conserved hypothetical protein [Paracoccus denitrificans PD1222]		
SLJClgrIV04M13RD09_D09	gb ACN12926.1	myo-inositol phosphate synthase [Phaseolus vulgaris]	Q9LW96 INO1_TOBAC	Inositol-3-phosphate synthase - Nicotiana tabacum (Common tobacco)
SLJClgrIV04T7A08_A08	emb CAP96575.1	Pc21g16780 [Penicillium chrysogenum Wisconsin 54-1255]		
SLJClgrIV04T7B02_B02	ref XP_001703188.1	ribosomal protein L36, component of cytosolic 80S ribosome and 60S large subunit [Chlamydomonas reinhardtii] gb EDO96564.1 ribosomal protein L36, component of cytosolic 80S ribosome and 60S large subunit [Chlamydomonas reinhardtii]	O80929 RL361_ARATH	60S ribosomal protein L36-1 - Arabidopsis thaliana (Mouse-ear cress)
SLJClgrIV04T7B11_B11	ref XP_001699466.1	short-chain dehydrogenase/reductase SDR [Chlamydomonas reinhardtii] gb EDO98712.1 short-chain dehydrogenase/reductase SDR [Chlamydomonas reinhardtii]	P28643 FABG_CUPLA	3-oxoacyl-[acyl-carrier-protein] reductase, chloroplast precursor - Cuphea lanceolata
SLJClgrIV04T7C01_C01	ref XP_453845.1	unnamed protein product [Kluyveromyces lactis]	Q8TGM5 ART3_YEAST	Uncharacterized protein ART3 - Saccharomyces cerevisiae (Baker's yeast)
SLJClgrIV04T7D07_D07	ref XP_001696180.1	hypothetical protein CHLREDRAFT_142043 [Chlamydomonas reinhardtii] gb EDP08157.1 predicted protein [Chlamydomonas reinhardtii]		
SLJClgrIV04T7D11_D11	ref XP_001417569.1	predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO95862.1 predicted protein [Ostreococcus lucimarinus CCE9901]	Q55158 RIBD_SYNY3	Riboflavin biosynthesis protein ribD [Includes: Diaminohydroxyphosphoribosylaminopyrimidine deaminase - Synechocystis sp. (strain PCC 6803)]
SLJClgrIV04T7F12_F12	ref XP_001419772.1	predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO98065.1 predicted protein [Ostreococcus lucimarinus CCE9901]	Q5RBF1 SIRT2_PONPY	NAD-dependent deacetylase sirtuin-2 - Pongo pygmaeus (Orangutan)
SLJClgrIV04T7G10_G10	sp P56408 FER_CHLFU	RecName: Full=Ferredoxin pdb 1AWD A Chain A, Ferredoxin [2Fe-2S] Oxidized Form From Chlorella Fusca	P56408 FER_CHLFU	Ferredoxin - Chlorella fusca

Table B.1: Combined GenBank and UniProt summary information for stage 1 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
SLJClgrIV05M13RA06_A06	ref[NP_866427.1]	hypothetical protein RB4971 [Rhodopirellula baltica SH 1] emb[CAD78208.1] conserved hypothetical protein [Rhodopirellula baltica SH 1]		
SLJClgrIV05M13RF03_F03	gb AAC32131.1]	heat shock protein [Picea mariana]	P51819 HSP83_IPONI	Heat shock protein 83 - Ipomoea nil (Japanese morning glory) (Pharbitis nil)
SLJClgrIV05M13RF05_F05	dbj BAA78583.1]	60S ribosomal protein L31 [Chlamydomonas sp. HS-5]	P45841 RL31_CHLRE	60S ribosomal protein L31 - Chlamydomonas reinhardtii
SLJClgrIV05M13RG03_G03	ref XP_001419128.1]	predicted protein [Ostreococcus lucimarinus CCE9901] ref XP_001420602.1] predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO97421.1] predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO98895.1] predicted protein [Ostreococcus lucimarinus CCE9901]	P16868 H2B4_VOLCA	Histone H2B.4 - Volvox carteri
SLJClgrIV05T7C07_C07	ref XP_001699466.1]	short-chain dehydrogenase/reductase SDR [Chlamydomonas reinhardtii] gb EDO98712.1] short-chain dehydrogenase/reductase SDR [Chlamydomonas reinhardtii]	P28643 FABG_CUPLA	3-oxoacyl-[acyl-carrier-protein] reductase, chloroplast precursor - Cuphea lanceolata
SLJClgrIV05T7D10_D10	ref ZP_01689674.1]	cell wall-associated hydrolase [Microscilla marina ATCC 23134] gb EAY29055.1] cell wall-associated hydrolase [Microscilla marina ATCC 23134]		
SLJClgrIV05T7E07_E07	ref XP_001761641.1]	predicted protein [Physcomitrella patens subsp. patens] gb EDQ73709.1] predicted protein [Physcomitrella patens subsp. patens]	Q96558 UGDH_SOYBN	UDP-glucose 6-dehydrogenase - Glycine max (Soybean)
SLJClgrIV05T7F11_F11	sp Q8GTZ9 CCS1_CHLRE	RecName: Full=Cytochrome c biogenesis protein CCS1, chloroplastic; AltName: Full=C-type cytochrome synthesis protein 1; Flags: Precursor gb AAB95195.1] CCS1 [Chlamydomonas reinhardtii] gb AAB95196.1] CCS1 [Chlamydomonas reinhardtii] gb AAM23259.1] c-type cytochrome synthesis 1 [Chlamydomonas reinhardtii]	P51363 YCF44_PORPU	Uncharacterized protein ycf44 - Porphyra purpurea
SLJClgrIV05T7G06_G06	ref XP_001757529.1]	predicted protein [Physcomitrella patens subsp. patens] gb EDQ77586.1] predicted protein [Physcomitrella patens subsp. patens]	O80832 YU87_ARATH	UPF0187 protein At2g45870, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments.

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0003]	ref XP_001697614.1	signal peptidase, catalytic subunit [Chlamydomonas reinhardtii] gb EDP00008.1 signal peptidase, catalytic subunit [Chlamydomonas reinhardtii]	P42667 SC11A_RAT	Signal peptidase complex catalytic subunit SEC11A - Rattus norvegicus (Rat)
Contig[0008]	gb ACF16408.1	chloroplast ribulose 1,5-bisphosphate carboxylase/oxygenase small subunit [Myrmecia incisa]	Q38692 RBS6_ACECL	Ribulose biphosphate carboxylase small chain 6, chloroplast precursor - Acetabularia cliftonii (Green alga)
Contig[0012]	emb CAL54647.1	enoyl- (ISS) [Ostreococcus tauri]	P80030 FABI_BRANA	Enoyl-[acyl-carrier-protein] reductase [NADH], chloroplast precursor - Brassica napus (Rape)
Contig[0021]	ref XP_001694167.1	ER retention protein [Chlamydomonas reinhardtii] gb EDP03103.1 ER retention protein [Chlamydomonas reinhardtii]	O48671 RER1B_ARATH	Protein RER1B - Arabidopsis thaliana (Mouse-ear cress)
Contig[0032]	ref XP_001752983.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ82024.1 predicted protein [Physcomitrella patens subsp. patens]	Q06190 P2R3A_HUMAN	Serine/threonine-protein phosphatase 2A regulatory subunit B" subunit alpha - Homo sapiens (Human)
Contig[0033]	ref XP_001694100.1	predicted protein [Chlamydomonas reinhardtii] gb EDP03036.1 predicted protein [Chlamydomonas reinhardtii]	Q42541 UBC13_ARATH	Ubiquitin-conjugating enzyme E2 13 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0035]	gb ABK23267.1	unknown [Picea sitchensis] gb ABK26898.1 unknown [Picea sitchensis]	Q02960 MIF_CHICK	Macrophage migration inhibitory factor - Gallus gallus (Chicken)
Contig[0041]	ref XP_001702034.1	predicted protein [Chlamydomonas reinhardtii] gb EDO97309.1 predicted protein [Chlamydomonas reinhardtii]	Q9M2D2 YU88_ARATH	UPF0187 protein At3g61320, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0045]	gb AAM61009.1	UDP-glucose dehydrogenase, putative [Arabidopsis thaliana]	Q96558 UGDH_SOYBN	UDP-glucose 6-dehydrogenase - Glycine max (Soybean)
Contig[0046]	gb AAC36344.1	AJH1 [Arabidopsis thaliana]	Q8LAZ7 CSN5B_ARATH	COP9 signalosome complex subunit 5b - Arabidopsis thaliana (Mouse-ear cress)
Contig[0047]	ref XP_001696940.1	prohibitin [Chlamydomonas reinhardtii] gb EDP00632.1 prohibitin [Chlamydomonas reinhardtii]	Q9P7H3 PHB1_SCHPO	Prohibitin-1 - Schizosaccharomyces pombe (Fission yeast)
Contig[0055]	ref XP_001698863.1	Mu2-Adaptin [Chlamydomonas reinhardtii] gb EDO99145.1 Mu2-Adaptin [Chlamydomonas reinhardtii]	Q32Q06 AP1M1_RAT	AP-1 complex subunit mu-1 - Rattus norvegicus (Rat)
Contig[0059]	gb EEF43733.1	aldo-keto reductase, putative [Ricinus communis]	P80276 ALDR_PIG	Aldose reductase - Sus scrofa (Pig)
Contig[0072]	gb ACG32861.1	mitochondrial ribosomal protein L43 [Zea mays]		
Contig[0075]	ref XP_001701693.1	predicted protein [Chlamydomonas reinhardtii] gb EDP06668.1 predicted protein [Chlamydomonas reinhardtii]	Q54Y03 KAD6_DICDI	Probable adenylate kinase isoenzyme 6 - Dictyostelium discoideum (Slime mold)

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0090]	ref XP_001700022.1	hypothetical protein CHLREDRAFT_112030 [Chlamydomonas reinhardtii]		
Contig[0097]	ref XP_001694100.1	gb EDP07718.1 predicted protein [Chlamydomonas reinhardtii]	Q42541 UBC13_ARATH	Ubiquitin-conjugating enzyme E2 13 - Arabidopsis thaliana (Mouse- ear cress)
Contig[0098]	gb ACD44937.1	predicted protein [Chlamydomonas reinhardtii] gb EDP03036.1 pre- dicted protein [Chlamydomonas reinhardtii]	Q75V57 SAPK9_ORYSJ	Serine/threonine-protein kinase SAPK9 - Oryza sativa subsp. japonica (Rice)
Contig[0105]	ref XP_001691328.1	osmotic stress/ABA-activated protein kinase [Zea mays] gb ACG36261.1 serine/threonine- protein kinase SAPK8 [Zea mays]	Q93XM7 MCAT_ARATH	Mitochondrial carnitine carrier-like protein - Arabidopsis thaliana (Mouse-ear cress)
Contig[0115]	gb ACL52455.1	mitochondrial carrier protein [Chlamydomonas reinhardtii] gb EDP05061.1 mitochondrial carrier protein [Chlamydomonas reinhardtii]	O24573 RLA0_MAIZE	60S acidic ribosomal protein P0 - Zea mays (Maize)
Contig[0134]	ref XP_001700659.1	unknown [Zea mays]	Q9SJU4 ALFC1_ARATH	Probable fructose-bisphosphate al- dolase 1, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0138]	ref XP_001702107.1	fructose-1,6-bisphosphate aldolase [Chlamydomonas reinhardtii] gb EDO97897.1 fructose-1,6- bisphosphate aldolase [Chlamy- domonas reinhardtii]	Q56YA5 SGAT_ARATH	Serine-glyoxylate aminotransferase - Arabidopsis thaliana (Mouse-ear cress)
Contig[0140]	ref XP_001760433.1	serine glyoxylate aminotransferase [Chlamydomonas reinhardtii] gb EDO97196.1 serine glyoxylate aminotransferase [Chlamydomonas reinhardtii]	Q0JNK5 COPB2_ORYSJ	Coatomer subunit beta-2 - Oryza sativa subsp. japonica (Rice)
Contig[0142]	ref XP_002311676.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ74824.1 predicted pro- tein [Physcomitrella patens subsp. patens]	P58728 SF3BB_ARATH	Uncharacterized protein At4g14342 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0144]	ref XP_001691305.1	predicted protein [Populus tri- chocarpa] gb ABK93878.1 un- known [Populus trichocarpa] gb EEE89043.1 predicted protein [Populus trichocarpa]	Q54FR4 PX24D_DICDI	PXMP2/4 family protein 4 - Dic- tyostelium discoideum (Slime mold)
Contig[0146]	ref XP_001776952.1	predicted protein [Chlamydomonas reinhardtii] gb EDP05038.1 pre- dicted protein [Chlamydomonas reinhardtii]	Q03965 L181_CHLEU	Chlorophyll a-b binding protein L1818, chloroplast precursor - Chlamydomonas eugametos
Contig[0149]	ref XP_001693654.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ58201.1 predicted pro- tein [Physcomitrella patens subsp. patens]	O44017 ERD2_ENTHI	ER lumen protein retaining recep- tor - Entamoeba histolytica
Contig[0158]	emb CAC80388.1	KDEL receptor B [Chlamydomonas reinhardtii] gb EDP08908.1 KDEL receptor B [Chlamydomonas rein- hardtii]	P12859 G3PB_PEA	Glyceraldehyde-3-phosphate dehy- drogenase B, chloroplast precursor - Pisum sativum (Garden pea)

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0170]	ref XP_001770321.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ64811.1 predicted protein [Physcomitrella patens subsp. patens]	P21343 PFPB_SOLTU	Pyrophosphate-fructose 6-phosphate 1-phosphotransferase subunit beta - Solanum tuberosum (Potato)
Contig[0180]	ref XP_001692548.1	light-harvesting protein of photosystem I [Chlamydomonas reinhardtii] gb EDP04026.1 light-harvesting protein of photosystem I [Chlamydomonas reinhardtii]	Q9XF89 CB26_ARATH	Chlorophyll a-b binding protein CP26, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0199]	gb EEF47836.1	ribulose-5-phosphate-3-epimerase, putative [Ricinus communis]	Q43157 RPE_SPIOL	Ribulose-phosphate 3-epimerase, chloroplast precursor - Spinacia oleracea (Spinach)
Contig[0200]	gb ABV02033.1	glyceraldehyde 3-phosphate dehydrogenase [Nicotiana langsdorffii x Nicotiana sanderae]	P25861 G3PC_ANTMA	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic - Antirrhinum majus (Garden snapdragon)
Contig[0204]	ref XP_001697636.1	iron-sulfur cluster assembly protein [Chlamydomonas reinhardtii] gb AAL29443.1 AF428268.1 iron-sulfur cluster assembly protein IscA [Chlamydomonas reinhardtii] gb EDP00030.1 iron-sulfur cluster assembly protein [Chlamydomonas reinhardtii]	Q8LBM4 ISAM1_ARATH	Iron-sulfur assembly protein IscA-like 1, mitochondrial precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0211]	gb EAY81264.1	hypothetical protein OsI_36442 [Oryza sativa Indica Group]	O97860 PPA5_RABIT	Tartrate-resistant acid phosphatase type 5 precursor - Oryctolagus cuniculus (Rabbit)
Contig[0212]	gb EEF43789.1	Brain protein, putative [Ricinus communis]	O01578 YXX3_CAEEL	UPF0041 protein F53F10.3 - Caenorhabditis elegans
Contig[0223]	ref XP_001700659.1	fructose-1,6-bisphosphate aldolase [Chlamydomonas reinhardtii] gb EDO97897.1 fructose-1,6-bisphosphate aldolase [Chlamydomonas reinhardtii]	Q9SJU4 ALFC1_ARATH	Probable fructose-bisphosphate aldolase 1, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0224]	ref XP_001702822.1	protein arginine N-methyltransferase [Chlamydomonas reinhardtii] gb EDO96867.1 protein arginine N-methyltransferase [Chlamydomonas reinhardtii]	Q9MAT5 ANM42_ARATH	Probable protein arginine N-methyltransferase 4.2 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0229]	ref XP_001759218.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ75902.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0244]	ref XP_001695426.1	methionine aminopeptidase [Chlamydomonas reinhardtii] gb EDP01684.1 methionine aminopeptidase [Chlamydomonas reinhardtii]		
Contig[0251]	gb ABD37909.1	light-harvesting chlorophyll-a/b binding protein LhcbM1 [Chlamydomonas incerta]	P14277 CB2F_SOLLC	Chlorophyll a-b binding protein 3B, chloroplast precursor - Solanum lycopersicum (Tomato) (Lycopersicon esculentum)
Contig[0269]	gb AAL88456.1 AF479777.1	major light-harvesting complex II protein m10 [Chlamydomonas reinhardtii]	P14273 CB2_CHLRE	Chlorophyll a-b binding protein of LHCI type I, chloroplast precursor - Chlamydomonas reinhardtii

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0271]	ref XP_001697230.1	photosystem I reaction center subunit psaK [Chlamydomonas reinhardtii] sp P14225 PSAK_CHLRE RecName: Full=Photosystem I reaction center subunit psaK, chloroplastic; AltName: Full=Photosystem I subunit X; AltName: Full=PSI-K; AltName: Full=Light-harvesting complex I 8.4 kDa protein; AltName: Full=P37 protein; Flags: Precursor emb CAA33258.1 polypeptide 37 precursor [Chlamydomonas reinhardtii] gb EDP00485.1 photosystem I reaction center subunit psaK [Chlamydomonas reinhardtii] prf 1613444C photosystem I P37 protein	P14225 PSAK_CHLRE	Photosystem I reaction center subunit psaK, chloroplast precursor - Chlamydomonas reinhardtii
Contig[0277]	ref XP_001696569.1	predicted protein [Chlamydomonas reinhardtii] gb EDP08546.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0281]	ref XP_001691084.1	photosystem I reaction center subunit XI [Chlamydomonas reinhardtii] gb EDP05530.1 photosystem I reaction center subunit XI [Chlamydomonas reinhardtii]	Q9SUI4 PSAL_ARATH	Photosystem I reaction center subunit XI, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0284]	ref XP_001703658.1	nitrogen regulatory protein PII [Chlamydomonas reinhardtii] gb EDO96407.1 nitrogen regulatory protein PII [Chlamydomonas reinhardtii]	O66513 GLNB_AQUAE	Nitrogen regulatory protein P-II - Aquifex aeolicus
Contig[0287]	ref XP_001757403.1	MM-ALDH [Physcomitrella patens subsp. patens] gb EDQ77888.1 MM-ALDH [Physcomitrella patens subsp. patens]	Q0WM29 MMSA_ARATH	Methylmalonate-semialdehyde dehydrogenase [acylating], mitochondrial precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0288]	ref XP_001835110.1	predicted protein [Coprinosia cinerea okayama7#130]		
Contig[0292]	emb CAJ30047.1	conserved hypothetical protein [Magnetospirillum gryphiswaldense]		
Contig[0302]	gb ACA30301.1	putative senescence-associated protein [Cupressus sempervirens]		
Contig[0304]	ref XP_001699664.1	naphthoate synthase [Chlamydomonas reinhardtii] gb EDP07360.1 naphthoate synthase [Chlamydomonas reinhardtii]	Q9CLV5 MENB_PASMU	Naphthoate synthase - Pasteurella multocida
Contig[0317]	ref XP_001416526.1	predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO94819.1 predicted protein [Ostreococcus lucimarinus CCE9901]	Q9SN86 MDHP_ARATH	Malate dehydrogenase, chloroplast precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0318]	emb CAA06481.1	replication factor C/activator 1 subunit [Cicer arietinum]	Q05B83 RFC2_BOVIN	Replication factor C subunit 2 - Bos taurus (Bovine)

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0332]	ref YP_636252.1	envelope membrane protein [Pseudendoclonium akinetum] sp Q3ZJ15 CEMA_PSEAK Rec-Name: Full=Chloroplast envelope membrane protein gb AAV80674.1 chloroplast envelope membrane protein [Pseudendoclonium akinetum]	Q3ZJ15 CEMA_PSEAK	Chloroplast envelope membrane protein - Pseudendoclonium akinetum (Green alga)
Contig[0333]	gb ACG32861.1	mitochondrial ribosomal protein L43 [Zea mays]		
Contig[0336]	ref XP_001692728.1	predicted protein [Chlamydomonas reinhardtii] gb EDP03747.1 predicted protein [Chlamydomonas reinhardtii]	P27250 YJGB_ECOLI	Uncharacterized zinc-type alcohol dehydrogenase-like protein yjgB - Escherichia coli (strain K12)
Contig[0341]	gb EAZ02969.1	hypothetical protein OsI_25109 [Oryza sativa Indica Group]	P27516 CBR_DUNBA	Carotene biosynthesis-related protein CBR, chloroplast precursor - Dunaliella bardawil
Contig[0344]	gb EAZ02969.1	hypothetical protein OsI_25109 [Oryza sativa Indica Group]	P27516 CBR_DUNBA	Carotene biosynthesis-related protein CBR, chloroplast precursor - Dunaliella bardawil
Contig[0345]	ref XP_002302753.1	predicted protein [Populus trichocarpa] gb ABK94325.1 unknown [Populus trichocarpa] gb EEE82026.1 predicted protein [Populus trichocarpa]	Q32L10 TM205_BOVIN	Transmembrane protein 205 - Bos taurus (Bovine)
Contig[0350]	emb CAR78980.1	phosphoenolpyruvate carboxykinase [Setaria viridis]	Q9SLZ0 PEPCK_MAIZE	Phosphoenolpyruvate carboxykinase [ATP] - Zea mays (Maize)
Contig[0361]	ref XP_001699672.1	hypothetical protein CHLREDRAFT_182131 [Chlamydomonas reinhardtii] gb EDP07368.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0362]	ref XP_001784831.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ50341.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0372]	gb ABM74386.1	major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina] gb ABM74387.1 major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina]	P27517 CB2_DUNTE	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor - Dunaliella tertiolecta
Contig[0383]	ref XP_001766081.1	Rab8/RabE-family small GTPase [Physcomitrella patens subsp. patens] gb EDQ69140.1 Rab8/RabE-family small GTPase [Physcomitrella patens subsp. patens]	Q39433 RB1BV_BETVU	Ras-related protein RAB1BV - Beta vulgaris (Sugar beet)
Contig[0384]	ref XP_001775449.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ59778.1 predicted protein [Physcomitrella patens subsp. patens]	Q8LAZ7 CSN5B_ARATH	COP9 signalosome complex subunit 5b - Arabidopsis thaliana (Mouse-ear cress)

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0390]	ref XP_001691218.1	ribosomal protein S4, component of cytosolic 80S ribosome and 40S small subunit [Chlamydomonas reinhardtii] gb EDP04951.1 ribosomal protein S4, component of cytosolic 80S ribosome and 40S small subunit [Chlamydomonas reinhardtii]	P87158 RS4A_SCHPO	40S ribosomal protein S4-A - Schizosaccharomyces pombe (Fission yeast)
Contig[0391]	ref XP_001691452.1	eukaryotic initiation factor 4A-like protein [Chlamydomonas reinhardtii] gb EDP05185.1 eukaryotic initiation factor 4A-like protein [Chlamydomonas reinhardtii]	P41376 IF4A1_ARATH	Eukaryotic initiation factor 4A-1 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0392]	ref XP_001761261.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ73983.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0401]	ref XP_001701674.1	predicted protein [Chlamydomonas reinhardtii] gb EDP06649.1 predicted protein [Chlamydomonas reinhardtii]	Q99M01 SYFM_MOUSE	Phenylalanyl-tRNA synthetase, mitochondrial precursor - Mus musculus (Mouse)
Contig[0420]	ref XP_001695317.1	Qb-SNARE protein, Gos1/GS28-family [Chlamydomonas reinhardtii] gb EDP01575.1 Qb-SNARE protein, Gos1/GS28-family [Chlamydomonas reinhardtii]	O22151 GOS12_ARATH	Golgi SNARE 12 protein - Arabidopsis thaliana (Mouse-ear cress)
Contig[0426]	ref XP_001418138.1	psaN, PSI-N, photosystem I polypeptide [Ostreococcus lucimarinus CCE9901] gb ABO96431.1 psaN, PSI-N, photosystem I polypeptide [Ostreococcus lucimarinus CCE9901]	Q9SBN5 PSAN_VOLCA	Photosystem I reaction center subunit N, chloroplast precursor - Volvox carteri
Contig[0435]	sp Q9SBN4 PGKH_VOLCA	RecName: Full=Phosphoglycerate kinase, chloroplastic; Flags: Precursor gb AAD55564.1 AF110782.1 phosphoglycerate kinase precursor [Volvox carteri f. nagariensis]	Q9SBN4 PGKH_VOLCA	Phosphoglycerate kinase, chloroplast precursor - Volvox carteri
Contig[0449]	gb ACL97363.1	elongation factor-1 alpha [Ignatius tetrasporus]	Q40034 EF1A2_HORVU	Elongation factor 1-alpha - Hordeum vulgare (Barley)
Contig[0461]	ref XP_001767753.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ67504.1 predicted protein [Physcomitrella patens subsp. patens]	Q9LN94 EXPA7_ARATH	Expansin-A7 precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0474]	ref ZP_02296571.1	mutator MutT protein [Rhizobium leguminosarum bv. trifolii WSM1325] gb EDR75048.1 mutator MutT protein [Rhizobium leguminosarum bv. trifolii WSM1325]	P32090 MUTT_PROVU	Mutator mutT protein - Proteus vulgaris
Contig[0476]	ref XP_001698645.1	oxygenase-like protein [Chlamydomonas reinhardtii] gb EDP08138.1 oxygenase-like protein [Chlamydomonas reinhardtii]	Q9M547 FLS_EUSGR	Flavonol synthase/flavanone 3-hydroxylase - Eustoma grandiflorum (Bluebells) (Lisianthus russellianus)
Contig[0485]	gb ACG32861.1	mitochondrial ribosomal protein L43 [Zea mays]		

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0498]	ref XP_001766967.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ68133.1 predicted protein [Physcomitrella patens subsp. patens]	P22734 COMT_RAT	Catechol O-methyltransferase - Rattus norvegicus (Rat)
Contig[0505]	ref NP_817269.1	ORF64c [Pinus koraiensis]		
Contig[0515]	emb CAD89802.1	histidine amino acid transporter [Oryza sativa (indica cultivar-group)]		
Contig[0516]	ref XP_001698451.1	hypothetical protein CHLREDRAFT_205676 [Chlamydomonas reinhardtii] gb EDP07944.1 hypothetical protein CHLREDRAFT_205676 [Chlamydomonas reinhardtii]		
Contig[0518]	ref XP_001695861.1	hypothetical protein CHLREDRAFT_149200 [Chlamydomonas reinhardtii] gb EDP01198.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0535]	ref XP_001771532.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ63732.1 predicted protein [Physcomitrella patens subsp. patens]	Q8GY23 UPL1_ARATH	E3 ubiquitin-protein ligase UPL1 - Arabidopsis thaliana (Mouse-ear cress)
Contig[0542]	gb AAZ30377.1	PHB2 [Nicotiana benthamiana]	Q54Q31 PHB2_DICDI	Prohibitin-2 - Dictyostelium discoideum (Slime mold)
Contig[0545]	gb AAF03432.1 AC010797.8	putative amino acid permease [Arabidopsis thaliana]		
Contig[0547]	ref XP_001754691.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ80661.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0550]	gb ABM74386.1	major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina] gb ABM74387.1 major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina]	P27517 CB2_DUNTE	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor - Dunaliella tertiolecta
Contig[0551]	ref XP_001694445.1	O-acetyltransferase-related protein [Chlamydomonas reinhardtii] gb EDP02440.1 O-acetyltransferase-related protein [Chlamydomonas reinhardtii]	Q7TN73 CASD1_MOUSE	CAS1 domain-containing protein 1 precursor - Mus musculus (Mouse)
Contig[0555]	ref XP_001766967.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ68133.1 predicted protein [Physcomitrella patens subsp. patens]	P22734 COMT_RAT	Catechol O-methyltransferase - Rattus norvegicus (Rat)
Contig[0556]	ref XP_001701227.1	hypothetical protein CHLREDRAFT_139920 [Chlamydomonas reinhardtii] gb EDO97737.1 predicted protein [Chlamydomonas reinhardtii]	O94183 NPC2_ASPOR	Phosphatidylglycerol/phosphatidylinositol transfer protein precursor - Aspergillus oryzae

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0566]	ref XP_001692870.1	2-oxoglutarate dehydrogenase, E1 subunit [Chlamydomonas reinhardtii] gb EDP03439.1 2-oxoglutarate dehydrogenase, E1 subunit [Chlamydomonas reinhardtii]	Q9ULD0 OGDHL_HUMAN	2-oxoglutarate dehydrogenase E1 component-like, mitochondrial precursor - Homo sapiens (Human)
Contig[0584]	ref XP_001417012.1	predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO95305.1 predicted protein [Ostreococcus lucimarinus CCE9901]	Q5R7H4 LBR_PONPY	Lamin-B receptor - Pongo pygmaeus (Bornean orangutan)
Contig[0590]	ref XP_001695426.1	methionine aminopeptidase [Chlamydomonas reinhardtii] gb EDP01684.1 methionine aminopeptidase [Chlamydomonas reinhardtii]	Q9SLN5 AMP1A_ARATH	Methionine aminopeptidase 1A - Arabidopsis thaliana (Mouse-ear cress)
Contig[0600]	ref XP_001697636.1	iron-sulfur cluster assembly protein [Chlamydomonas reinhardtii] gb AAL29443.1 AF428268.1 iron-sulfur cluster assembly protein IscA [Chlamydomonas reinhardtii] gb EDP00030.1 iron-sulfur cluster assembly protein [Chlamydomonas reinhardtii]	Q8LBM4 ISAM1_ARATH	Iron-sulfur assembly protein IscA-like 1, mitochondrial precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0609]	emb CAA98166.1	RAB5A [Lotus japonicus]	P31583 RHN1_NICPL	Ras-related protein RHN1 - Nicotiana plumbaginifolia (Leadwort-leaved tobacco)
Contig[0618]	ref XP_001694630.1	predicted protein [Chlamydomonas reinhardtii] gb EDP02625.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0619]	ref XP_001695112.1	predicted protein [Chlamydomonas reinhardtii] gb EDP01820.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0622]	gb ACG34836.1	NADH-cytochrome b5 reductase-like protein [Zea mays]	P83291 NCB5R_ARATH	NADH-cytochrome b5 reductase-like protein - Arabidopsis thaliana (Mouse-ear cress)
Contig[0624]	ref XP_001690805.1	iron-sulfur cluster assembly protein [Chlamydomonas reinhardtii] gb EDP05251.1 iron-sulfur cluster assembly protein [Chlamydomonas reinhardtii]	Q9LIG6 NIFU4_ARATH	NifU-like protein 4, mitochondrial precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0631]	ref XP_001753651.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ81403.1 predicted protein [Physcomitrella patens subsp. patens]	P49729 UCRI1_TOBAC	Cytochrome b-c1 complex subunit Rieske-1, mitochondrial precursor - Nicotiana tabacum (Common tobacco)
Contig[0638]	ref XP_001419772.1	predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO98065.1 predicted protein [Ostreococcus lucimarinus CCE9901]	Q7ZVK3 SIRT2_DANRE	NAD-dependent deacetylase sirtuin-2 - Danio rerio (Zebrafish) (Brachydanio rerio)
Contig[0642]	emb CAL54647.1	enoyl- (ISS) [Ostreococcus tauri]	P80030 FABI_BRANA	Enoyl-[acyl-carrier-protein] reductase [NADH], chloroplast precursor - Brassica napus (Rape)

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0648]	tpg[DAA05901.1]	TPA_inf: chloroplast light-harvesting complex I protein precursor Lhca6 [Acetabularia acetabulum]	P10708 CB12_SOLLC	Chlorophyll a-b binding protein 7, chloroplast precursor - Solanum lycopersicum (Tomato) (Lycopersicon esculentum)
Contig[0655]	ref[XP_001702343.1]	hypothetical protein CHLREDRAFT_108071 [Chlamydomonas reinhardtii] gb EDO96963.1 predicted protein [Chlamydomonas reinhardtii]	Q9R1T3 CATZ_RAT	Cathepsin Z precursor - Rattus norvegicus (Rat)
Contig[0656]	ref[XP_001691053.1]	predicted protein [Chlamydomonas reinhardtii] gb EDP05499.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0658]	gb ACF82208.1]	unknown [Zea mays] gb ACG24605.1 hydrogen-transporting ATP synthase, rotational mechanism [Zea mays] gb ACG31641.1 hydrogen-transporting ATP synthase, rotational mechanism [Zea mays] gb ACG36080.1 hydrogen-transporting ATP synthase, rotational mechanism [Zea mays] gb ACG38190.1 hydrogen-transporting ATP synthase, rotational mechanism [Zea mays] gb ACG41671.1 hydrogen-transporting ATP synthase, rotational mechanism [Zea mays]		
Contig[0659]	gb ABV02033.1]	glyceraldehyde 3-phosphate dehydrogenase [Nicotiana glauca x Nicotiana glauca]	P25861 G3PC_ANTMA	Glyceraldehyde-3-phosphate dehydrogenase, cytosolic - Antirrhinum majus (Garden snapdragon)
Contig[0662]	ref[NP_001118500.1]	ARP (apurinic endonuclease-redox protein) [Arabidopsis thaliana]	P45951 ARP_ARATH	Apurinic endonuclease-redox protein - Arabidopsis thaliana (Mouse-ear cress)
Contig[0663]	ref[XP_001784363.1]	predicted protein [Physcomitrella patens subsp. patens] gb EDQ50832.1 predicted protein [Physcomitrella patens subsp. patens]		

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0664]	ref NP_001049096.1	Os03g0169100 [Oryza sativa (japonica cultivar-group)] sp Q9ZTP5 RPE_ORYSJ Rec-Name: Full=Ribulose-phosphate 3-epimerase, chloroplastic; Alt-Name: Full=Pentose-5-phosphate 3-epimerase; Short=PPE; AltName: Full=R5P3E; Short=RPE; Flags: Precursor gb AAL84303.1 AC073556.20 ribulose-5-phosphate-3-epimerase [Oryza sativa (japonica cultivar-group)] gb ABF94188.1 Ribulose-phosphate 3-epimerase, chloroplast precursor, putative, expressed [Oryza sativa (japonica cultivar-group)] dbj BAF11010.1 Os03g0169100 [Oryza sativa (japonica cultivar-group)] dbj BAG88103.1 unnamed protein product [Oryza sativa Japonica Group] dbj BAG89902.1 unnamed protein product [Oryza sativa Japonica Group] dbj BAG94000.1 unnamed protein product [Oryza sativa Japonica Group] gb EEC74595.1 hypothetical protein OsL10180 [Oryza sativa Indica Group] gb EEE58398.1 hypothetical protein OsJ_09567 [Oryza sativa Japonica Group]	Q9ZTP5 RPE_ORYSJ	Ribulose-phosphate 3-epimerase, chloroplast precursor - Oryza sativa subsp. japonica (Rice)
Contig[0665]	ref YP_001382145.1	apocytochrome f of cytochrome b6/f complex [Leptosira terrestris] sp A6YG68 CYF_LEPTE Rec-Name: Full=Apocytochrome f; Flags: Precursor gb ABO69289.1 apocytochrome f of cytochrome b6/f complex [Leptosira terrestris]	Q1KVS1 CYF_SCEOB	Apocytochrome f precursor - Scenedesmus obliquus
Contig[0675]	ref XP_001762079.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ73183.1 predicted protein [Physcomitrella patens subsp. patens]	Q9ZU75 UB12L_ARATH	Probable NEDD8-conjugating enzyme Ubc12-like - Arabidopsis thaliana (Mouse-ear cress)
Contig[0687]	ref XP_001782243.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ52915.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0689]	ref XP_001698932.1	hypothetical protein CHLREDRAFT_185240 [Chlamydomonas reinhardtii] gb EDO99214.1 predicted protein [Chlamydomonas reinhardtii]	Q60700 M3K12_MOUSE	Mitogen-activated protein kinase kinase 12 - Mus musculus (Mouse)
Contig[0696]	gb ABR18369.1	unknown [Picea sitchensis]	O15270 SPTC2_HUMAN	Serine palmitoyltransferase 2 - Homo sapiens (Human)

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0716]	ref[NP_045829.1]	photosystem II protein V [Chlorella vulgaris] sp P56309 PSBE_CHLVU RecName: Full=Cytochrome b559 subunit alpha; AltName: Full=PSII reaction center subunit V dbj BAA57904.1 cytochrome b559 a subunit [Chlorella vulgaris]	P56309 PSBE_CHLVU	Cytochrome b559 subunit alpha - Chlorella vulgaris (Green alga)
Contig[0717]	ref[ZP_02296571.1]	mutator MutT protein [Rhizobium leguminosarum bv. trifolii WSM1325] gb EDR75048.1 mutator MutT protein [Rhizobium leguminosarum bv. trifolii WSM1325]		
Contig[0720]	ref[XP_001693625.1]	predicted protein [Chlamydomonas reinhardtii] gb EDP08879.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0739]	ref[XP_001700931.1]	dynammin-related GTPase [Chlamydomonas reinhardtii] gb EDP07185.1 dynammin-related GTPase [Chlamydomonas reinhardtii]	Q39821 SDLCA_SOYBN	Dynammin-related protein 12A - Glycine max (Soybean)
Contig[0741]	ref[XP_001417563.1]	predicted protein [Ostreococcus lucimarinus CCE9901] gb ABO95856.1 predicted protein [Ostreococcus lucimarinus CCE9901]	P16148 PLZ12_LUPPO	Protein PPLZ12 - Lupinus polyphyllus (Large-leaved lupin)
Contig[0752]	ref[XP_001778721.1]	predicted protein [Physcomitrella patens subsp. patens] gb EDQ56419.1 predicted protein [Physcomitrella patens subsp. patens]	Q8L8Q8 Y5486_ARATH	Uncharacterized protein At5g64816 precursor - Arabidopsis thaliana (Mouse-ear cress)
Contig[0757]	ref[XP_001703170.1]	flagellar inner arm dynein 1 heavy chain alpha [Chlamydomonas reinhardtii] gb EDO96546.1 flagellar inner arm dynein 1 heavy chain alpha [Chlamydomonas reinhardtii]	Q9SMH3 DYH1A_CHLRE	Dynein-1-alpha heavy chain, flagellar inner arm II complex - Chlamydomonas reinhardtii
Contig[0763]	ref[XP_001702343.1]	hypothetical protein CHLREDRAFT_108071 [Chlamydomonas reinhardtii] gb EDO96963.1 predicted protein [Chlamydomonas reinhardtii]	Q9R1T3 CATZ_RAT	Cathepsin Z precursor - Rattus norvegicus (Rat)
Contig[0768]	ref[XP_001697425.1]	predicted protein [Chlamydomonas reinhardtii] gb EDP00087.1 predicted protein [Chlamydomonas reinhardtii]		
Contig[0772]	gb ABM74386.1]	major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina] gb ABM74387.1 major light-harvesting chlorophyll a/b protein 3 [Dunaliella salina]	P27517 CB2_DUNTE	Chlorophyll a-b binding protein of LHCII type I, chloroplast precursor - Dunaliella tertiolecta
Contig[0785]	ref[ZP_01999045.1]	ADP-ribosylation/Crystallin J1 [Beggiatoa sp. PS] gb EDN70960.1 ADP-ribosylation/Crystallin J1 [Beggiatoa sp. PS]		
Contig[0793]	ref[XP_001689944.1]	predicted protein [Chlamydomonas reinhardtii] gb EDP09682.1 predicted protein [Chlamydomonas reinhardtii]	Q59516 DHGY_METEX	Glycerate dehydrogenase - Methylobacterium extorquens (Protomonas extorquens)

Table B.2: Combined GenBank and UniProt summary information for stage 2 *Asterochloris* sp. gene fragments. – Continued

Clone ID	Top GenBank BLASTx hit	Top GenBank BLASTx description	Uniprot top hit	Uniprot top hit description
Contig[0797]	ref XP_001778574.1	predicted protein [Physcomitrella patens subsp. patens] gb EDQ56604.1 predicted protein [Physcomitrella patens subsp. patens]		
Contig[0807]	gb ACG31637.1	60S ribosomal protein L11-1 [Zea mays]	P46287 RL11_MEDSA	60S ribosomal protein L11 - Medicago sativa (Alfalfa)
Contig[0815]	ref XP_001701487.1	sugar phosphate/phosphate translocator-like protein [Chlamydomonas reinhardtii] gb EDO97484.1 sugar phosphate/phosphate translocator-like protein [Chlamydomonas reinhardtii]	P49131 TPT_FLAPR	Triose phosphate/phosphate translocator, chloroplast precursor - Flaveria pringlei
SLJClgrII02T7A09_016	ref XP_001695643.1	hypothetical protein CHLREDRAFT_130814 [Chlamydomonas reinhardtii] gb EDP01430.1 predicted protein [Chlamydomonas reinhardtii]		
SLJClgrII02T7B04_002	ref XP_001697940.1	ribosomal protein L34, component of cytosolic 80S ribosome and 60S large subunit [Chlamydomonas reinhardtii] gb EDO99823.1 ribosomal protein L34 [Chlamydomonas reinhardtii]	Q9FE65 RL342_ARATH	60S ribosomal protein L34-2 - Arabidopsis thaliana (Mouse-ear cress)
SLJClgrII10M13RA09_A09	ref NP_042262.1	cytochrome b [Prototheca wickerhamii] gb AAD12650.1 cytochrome b [Prototheca wickerhamii]	P26852 CYB_MARPO	Cytochrome b - Marchantia polymorpha (Liverwort)
SLJClgrII10M13RC06_C06	ref XP_001703001.1	ferredoxin-dependent glutamate synthase [Chlamydomonas reinhardtii] gb EDO96739.1 ferredoxin-dependent glutamate synthase [Chlamydomonas reinhardtii]	P55038 GLTS_SYNY3	Ferredoxin-dependent glutamate synthase 2 - Synechocystis sp. (strain PCC 6803)
SLJClgrII10T7F07_F07	ref XP_001697887.1	cyclic nucleotide dependent protein kinase [Chlamydomonas reinhardtii] gb EDO99770.1 cyclic nucleotide dependent protein kinase [Chlamydomonas reinhardtii]	P67999 KS6B1_RAT	Ribosomal protein S6 kinase beta-1 - Rattus norvegicus (Rat)

B.2 Summary of Gene Ontology (GO) organizing principles for *Asterochloris* sp. in stages 1 and 2 of lichen development

Functional classification of ESTs. To group SSH-cDNA sequences into functional categories, Blast2GO (Conesa *et al.*, 2005) was used to compare each sequence to the GenBank nr protein database, and subsequently Gene Ontology (GO; <http://www.geneontology.org>) terms were extracted from associated annotated blast hits. GO terms were organized by organizing principle (biological process, cellular compartment and molecular function), and the first three levels of GO terms for stages 1 and 2 can be seen in Tables B.3 and B.4. One hundred and thirty-three stage 1 gene fragments had a significant blastx match, and of these 108 had at least one associated GO term (GO database accessed 24 June, 2007). In stage 2, of 134 algal gene fragments with a significant blastx match, 116 had at least one associated GO term (GO database accessed 19 May, 2008).

Table B.3: GO categorization of a non-normalized SSH library of 108 gene products with at least one GO term for *Asterochloris* sp. in stage 1 of lichen development. Individual gene products may be represented by more than one GO term, and subordinate terms can represent multiple higher-level terms. First 3 levels of GO terms annotated through increasing indentation.

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
BIOLOGICAL PROCESS	GO:0008150	73	100%
cellular process	GO:0009987	66	90%
metabolic process	GO:0008152	58	79%
localization	GO:0051179	12	16%
response to stimulus	GO:0050896	6	8%
biological regulation	GO:0065007	6	8%
developmental process	GO:0032502	4	5%
multicellular organismal process	GO:0032501	4	5%
homeostatic process	GO:0042592	2	3%
reproduction	GO:0000003	2	3%
reproductive process	GO:0022414	1	1%
growth	GO:0040007	1	1%
cellular metabolic process	GO:0044237	52	71%
primary metabolic process	GO:0044238	43	59%

Table B.3 Stage 1 *Asterochloris* sp. Gene Ontology summary – Continued

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
macromolecule metabolic process	GO:0043170	36	49%
biosynthetic process	GO:0009058	22	30%
cell organization and biogenesis	GO:0016043	14	19%
establishment of localization	GO:0051234	12	16%
photosynthesis	GO:0015979	10	14%
regulation of biological process	GO:0050789	6	8%
regulation of cellular process	GO:0050794	6	8%
regulation of metabolic process	GO:0019222	5	7%
response to chemical stimulus	GO:0042221	5	7%
protein localization	GO:0008104	4	5%
catabolic process	GO:0009056	4	5%
response to stress	GO:0006950	4	5%
cellular localization	GO:0051641	3	4%
carbon utilization	GO:0015976	3	4%
multicellular organismal development	GO:0007275	3	4%
cell homeostasis	GO:0019725	2	3%
nitrogen compound metabolic process	GO:0006807	2	3%
anatomical structure development	GO:0048856	2	3%
reproductive developmental process	GO:0003006	1	1%
cell development	GO:0048468	1	1%
response to biotic stimulus	GO:0009607	1	1%
cell cycle process	GO:0022402	1	1%
post-embryonic development	GO:0009791	1	1%
cellular developmental process	GO:0048869	1	1%
embryonic development	GO:0009790	1	1%
neurological process	GO:0050877	1	1%
sexual reproduction	GO:0019953	1	1%
urea cycle intermediate metabolic process	GO:0000051	1	1%
chemical homeostasis	GO:0048878	1	1%
secondary metabolic process	GO:0019748	1	1%
cell cycle	GO:0007049	1	1%
death	GO:0016265	1	1%
CELLULAR COMPONENT	GO:0005575	59	100%
cell	GO:0005623	59	100%
cell part	GO:0044464	59	100%
organelle	GO:0043226	54	92%
protein complex	GO:0043234	24	41%
organelle part	GO:0044422	24	41%
membrane-enclosed lumen	GO:0031974	2	3%
envelope	GO:0031975	1	2%
intracellular	GO:0005622	57	97%
intracellular part	GO:0044424	57	97%
intracellular organelle	GO:0043229	54	92%
membrane-bound organelle	GO:0043227	53	90%
intracellular organelle part	GO:0044446	24	41%
membrane	GO:0016020	19	32%
membrane part	GO:0044425	12	20%
non-membrane-bound organelle	GO:0043228	8	14%
vesicle	GO:0031982	6	10%
photosystem II	GO:0009523	6	10%
photosystem I	GO:0009522	5	8%
ribonucleoprotein complex	GO:0030529	5	8%
nucleosome	GO:0000786	3	5%
ribulose biphosphate carboxylase complex	GO:0048492	3	5%
small ribosomal subunit	GO:0015935	2	3%
organelle lumen	GO:0043233	2	3%
proton-transporting two-sector ATPase complex	GO:0016469	2	3%
eukaryotic 43S preinitiation complex	GO:0016282	1	2%
organelle envelope	GO:0031967	1	2%
eukaryotic 48S initiation complex	GO:0016283	1	2%
mitochondrial inner membrane presequence translocase complex	GO:0005744	1	2%
proteasome complex (sensu Eukaryota)	GO:0000502	1	2%
proteasome core complex (sensu Eukaryota)	GO:0005839	1	2%
photosystem II antenna complex	GO:0009783	1	2%
alpha-glucosidase II complex	GO:0017177	1	2%
chromatin remodeling complex	GO:0016585	1	2%
ESCRT III complex	GO:0000815	1	2%
MOLECULAR FUNCTION	GO:0003674	81	100%
catalytic activity	GO:0003824	56	69%
binding	GO:0005488	40	49%
structural molecule activity	GO:0005198	5	6%
transporter activity	GO:0005215	5	6%
enzyme regulator activity	GO:0030234	1	1%
hydrolase activity	GO:0016787	22	27%
ion binding	GO:0043167	17	21%
oxidoreductase activity	GO:0016491	16	20%
transferase activity	GO:0016740	12	15%
nucleic acid binding	GO:0003676	10	12%
protein binding	GO:0005515	7	9%
nucleotide binding	GO:0000166	6	7%
isomerase activity	GO:0016853	4	5%
carrier activity	GO:0005386	4	5%
structural constituent of ribosome	GO:0003735	4	5%

Table B.3 Stage 1 *Asterochloris* sp. Gene Ontology summary – Continued

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
ion transporter activity	GO:0015075	3	4%
ATPase activity, coupled to movement of substances	GO:0043492	3	4%
lyase activity	GO:0016829	3	4%
cofactor binding	GO:0048037	2	2%
drug binding	GO:0008144	2	2%
protein transporter activity	GO:0008565	2	2%
ligase activity	GO:0016874	1	1%
enzyme activator activity	GO:0008047	1	1%
helicase activity	GO:0004386	1	1%
metal cluster binding	GO:0051540	1	1%
peptide binding	GO:0042277	1	1%
tetrapyrrole binding	GO:0046906	1	1%
deaminase activity	GO:0019239	1	1%
vitamin binding	GO:0019842	1	1%

Table B.4: GO categorization of a non-normalized SSH library of 116 gene products with at least one GO term for *Asterochloris* sp. in stage 2 of lichen development. Individual gene products may be represented by more than one GO term, and subordinate terms can represent multiple higher-level terms. First 3 levels of GO terms annotated through increasing indentation.

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
BIOLOGICAL PROCESS	GO:0008150	75	100%
biological regulation	GO:0065007	11	15%
cellular process	GO:0009987	62	83%
developmental process	GO:0032502	5	7%
establishment of localization	GO:0051234	14	19%
growth	GO:0040007	1	1%
localization	GO:0051179	15	20%
metabolic process	GO:0008152	61	81%
multicellular organismal process	GO:0032501	4	5%
multi-organism process	GO:0051704	2	3%
reproduction	GO:0000003	1	1%
response to stimulus	GO:0050896	6	8%
aging	GO:0007568	1	1%
anatomical structure development	GO:0048856	4	5%
anatomical structure morphogenesis	GO:0009653	2	3%
behavior	GO:0007610	1	1%
biosynthetic process	GO:0009058	17	23%
carbon utilization	GO:0015976	4	5%
catabolic process	GO:0009056	11	15%
cell communication	GO:0007154	3	4%
cell development	GO:0048468	2	3%
cell motility	GO:0006928	1	1%
cell proliferation	GO:0008283	1	1%
cellular component organization and biogenesis	GO:0016043	9	12%
cellular developmental process	GO:0048869	2	3%
cellular homeostasis	GO:0019725	1	1%
cellular localization	GO:0051641	4	5%
cellular metabolic process	GO:0044237	56	75%
cellular response to stimulus	GO:0051716	1	1%
defense response	GO:0006952	1	1%
developmental growth	GO:0048589	1	1%
embryonic development	GO:0009790	1	1%
establishment of cellular localization	GO:0051649	4	5%
establishment of protein localization	GO:0045184	4	5%
generation of precursor metabolites and energy	GO:0006091	9	12%
localization of cell	GO:0051674	1	1%
macromolecule localization	GO:0033036	4	5%
macromolecule metabolic process	GO:0043170	37	49%
multicellular organismal development	GO:0007275	4	5%
muscle adaptation	GO:0043500	1	1%
nitrogen compound metabolic process	GO:0006807	6	8%
photosynthesis	GO:0015979	15	20%
primary metabolic process	GO:0044238	46	61%
regulation of biological process	GO:0050789	10	13%
regulation of biological quality	GO:0065008	1	1%
regulation of cellular process	GO:0050794	7	9%
regulation of developmental process	GO:0050793	1	1%
regulation of growth	GO:0040008	1	1%
regulation of metabolic process	GO:0019222	8	11%
regulation of response to stimulus	GO:0048583	1	1%
response to abiotic stimulus	GO:0009628	2	3%
response to biotic stimulus	GO:0009607	2	3%
response to chemical stimulus	GO:0042221	4	5%
response to endogenous stimulus	GO:0009719	3	4%
response to external stimulus	GO:0009605	1	1%
response to other organism	GO:0051707	2	3%
response to stress	GO:0006950	3	4%
secondary metabolic process	GO:0019748	2	3%
secretion	GO:0046903	1	1%
sexual reproduction	GO:0019953	1	1%
stomatal movement	GO:0010118	1	1%
transport	GO:0006810	14	19%
CELLULAR COMPONENT	GO:0005575	77	100%
cell	GO:0005623	77	100%
cell part	GO:0044464	77	100%
envelope	GO:0031975	4	5%
macromolecular complex	GO:0032991	24	31%
membrane-enclosed lumen	GO:0031974	2	3%
organelle	GO:0043226	62	81%
organelle part	GO:0044422	27	35%
cell fraction	GO:0000267	1	1%
cell projection	GO:0042995	1	1%
cell surface	GO:0009986	1	1%
external encapsulating structure	GO:0030312	1	1%
intracellular	GO:0005622	70	91%

Table B.4 Stage 2 *Asterochloris* sp. Gene Ontology summary – Continued

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
intracellular organelle	GO:0043229	62	81%
intracellular organelle part	GO:0044446	27	35%
intracellular part	GO:0044424	69	90%
membrane	GO:0016020	31	40%
membrane part	GO:0044425	15	19%
membrane-bounded organelle	GO:0043227	57	74%
non-membrane-bounded organelle	GO:0043228	6	8%
organelle envelope	GO:0031967	4	5%
organelle lumen	GO:0043233	2	3%
organelle membrane	GO:0031090	11	14%
protein complex	GO:0043234	20	26%
ribonucleoprotein complex	GO:0030529	4	5%
vesicle	GO:0031982	4	5%
MOLECULAR FUNCTION	GO:0003674	80	100%
binding	GO:0005488	47	59%
catalytic activity	GO:0003824	57	71%
enzyme regulator activity	GO:0030234	2	3%
motor activity	GO:0003774	1	1%
structural molecule activity	GO:0005198	6	8%
transcription regulator activity	GO:0030528	1	1%
translation regulator activity	GO:0045182	2	3%
transporter activity	GO:0005215	8	10%
acetylglutamate kinase regulator activity	GO:0010307	1	1%
cofactor binding	GO:0048037	3	4%
helicase activity	GO:0004386	1	1%
hydrolase activity	GO:0016787	14	18%
ion binding	GO:0043167	13	16%
isomerase activity	GO:0016853	2	3%
kinase regulator activity	GO:0019207	1	1%
ligase activity	GO:0016874	5	6%
lyase activity	GO:0016829	6	8%
metal cluster binding	GO:0051540	3	4%
microtubule motor activity	GO:0003777	1	1%
nucleic acid binding	GO:0003676	6	8%
nucleotide binding	GO:0000166	12	15%
oxidoreductase activity	GO:0016491	18	23%
peptide binding	GO:0042277	1	1%
protein binding	GO:0005515	15	19%
structural constituent of ribosome	GO:0003735	3	4%
substrate-specific transporter activity	GO:0022892	8	10%
tetrapyrrole binding	GO:0046906	6	8%
transcription activator activity	GO:0016563	1	1%
transcription cofactor activity	GO:0003712	1	1%
transferase activity	GO:0016740	14	18%
translation factor activity, nucleic acid binding	GO:0008135	2	3%
transmembrane transporter activity	GO:0022857	6	8%

B.3 Keys for linking GO terms to individual Clone IDs, in *Asterochloris* sp.

Stages 1 and 2 GO terms and Clone IDs can be found in Tables B.5 and B.6 respectively.

Table B.5: Key for linking stage 1 *Asterochloris* sp. GO terms and Clone IDs.

Level	GO ID	GO TERM	Clone ID
1	GO:0008150	BIOLOGICAL PROCESS	SLJClgrIV05M13RF03, SLJClgrIV04T7F12, SLJClgrIV04M13RD09, Contig[0185], SLJClgrIV04T7G10, Contig[0256], Contig[0054], Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, SLJClgrIV05T7F11, Contig[0287], SLJClgrIV01M13RG06, Contig[0087], SLJClgrIV02M13RB07, Contig[0094], Contig[0052], Contig[0291], Contig[0181], Contig[0246], Contig[0226], Contig[0235], Contig[0038], SLJClgrIV02M13RD06, SLJClgrIV02T7C04, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01, Contig[0161], SLJClgrIV02M13RG10, Contig[0306], Contig[0127], Contig[0295], Contig[0346], Contig[0344], Contig[0121], Contig[0013], Contig[0182], SLJClgrIV02T7H04, SLJClgrIV02M13RC08, SLJClgrIV02T7G01, Contig[0014], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], SLJClgrIV04T7D11, SLJClgrIV05T7E07, Contig[0026], SLJClgrIV03T7D04, Contig[0169], Contig[0193], Contig[0189], Contig[0330], Contig[0202], Contig[0138], SLJClgrIV04T7B11, Contig[0245], Contig[0301], Contig[0233], SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0355], Contig[0368], Contig[0201], Contig[0314], Contig[0300], SLJClgrIV02M13RF09
2	GO:0009987	cellular process	SLJClgrIV04T7F12, SLJClgrIV04M13RD09, Contig[0185], SLJClgrIV04T7G10, Contig[0256], Contig[0054], Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, SLJClgrIV05T7F11, Contig[0287], SLJClgrIV01M13RG06, Contig[0087], SLJClgrIV02M13RB07, Contig[0094], Contig[0052], Contig[0181], Contig[0246], Contig[0226], Contig[0038], SLJClgrIV02M13RD06, SLJClgrIV02T7C04, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01, Contig[0161], SLJClgrIV02M13RG10, Contig[0306], Contig[0127], Contig[0295], Contig[0346], Contig[0344], Contig[0121], Contig[0013], SLJClgrIV02T7H04, SLJClgrIV02M13RC08, SLJClgrIV02T7G01, Contig[0014], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], SLJClgrIV04T7D11, SLJClgrIV05T7E07, Contig[0026], SLJClgrIV03T7D04, Contig[0169], Contig[0193], Contig[0189], Contig[0330], Contig[0202], SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0355], Contig[0368], SLJClgrIV05M13RF03, Contig[0201], Contig[0314], Contig[0300], Contig[0235], SLJClgrIV02M13RF09
2	GO:0008152	metabolic process	SLJClgrIV04T7F12, SLJClgrIV04M13RD09, Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, SLJClgrIV05T7F11, SLJClgrIV01M13RG06, SLJClgrIV02M13RB07, Contig[0094], Contig[0181], Contig[0246], Contig[0226], Contig[0038], SLJClgrIV02M13RD06, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01, Contig[0161], SLJClgrIV02M13RG10, Contig[0306], Contig[0295], Contig[0346], Contig[0287], Contig[0121], Contig[0013], Contig[0054], SLJClgrIV02T7G01, Contig[0014], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], SLJClgrIV04T7D11, SLJClgrIV05T7E07, Contig[0026], SLJClgrIV04T7B11, SLJClgrIV02M13RC08, Contig[0245], Contig[0301], Contig[0233], SLJClgrIV02T7C04, SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0355], Contig[0368], SLJClgrIV05M13RF03, Contig[0201], Contig[0314], Contig[0235], SLJClgrIV02M13RF09
2	GO:0051179	localization	Contig[0185], SLJClgrIV04T7G10, Contig[0256], Contig[0054], Contig[0287], Contig[0087], Contig[0127], Contig[0295], Contig[0346], Contig[0344], Contig[0306], SLJClgrIV02T7H04
2	GO:0050896	response to stimulus	SLJClgrIV05M13RF03, SLJClgrIV04T7F12, Contig[0291], Contig[0181], Contig[0185], SLJClgrIV01M13RG06
2	GO:0065007	biological regulation	Contig[0238], SLJClgrIV01M13RG06, SLJClgrIV04T7F12, Contig[0161], SLJClgrIV02M13RC08, SLJClgrIV02T7G01
2	GO:0032502	developmental process	SLJClgrIV04T7F12, Contig[0182], SLJClgrIV02M13RC08, SLJClgrIV02T7G01
2	GO:0032501	multicellular organismal process	Contig[0235], SLJClgrIV04T7F12, Contig[0182], SLJClgrIV02T7G01
2	GO:0042592	homeostatic process	Contig[0256], Contig[0185]
2	GO:0000003	reproduction	Contig[0182], SLJClgrIV02T7G01
2	GO:0022414	reproductive process	Contig[0182]
2	GO:0040007	growth	Contig[0182]

Table B.5: Key for linking stage 1 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0044237	cellular metabolic process	SLJClgrIV04T7F12, SLJClgrIV04M13RD09, Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, SLJClgrIV05T7F11, SLJClgrIV01M13RG06, SLJClgrIV02M13RB07, Contig[0094], Contig[0181], Contig[0246], Contig[0226], Contig[0038], SLJClgrIV02M13RD06, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01, Contig[0161], SLJClgrIV02M13RG10, Contig[0306], Contig[0295], Contig[0346], Contig[0287], Contig[0121], Contig[0013], SLJClgrIV02T7G01, Contig[0014], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], SLJClgrIV04T7D11, SLJClgrIV05T7E07, SLJClgrIV04T7G10, Contig[0256], Contig[0054], Contig[0202], SLJClgrIV02T7C04, SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0355], Contig[0368], SLJClgrIV05M13RF03, Contig[0201], Contig[0314], Contig[0235], SLJClgrIV02M13RF09
3	GO:0044238	primary metabolic process	SLJClgrIV04M13RD09, Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, SLJClgrIV05T7F11, SLJClgrIV02M13RB07, Contig[0094], SLJClgrIV01M13RG06, Contig[0246], SLJClgrIV04T7F12, Contig[0226], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01, Contig[0161], SLJClgrIV02M13RG10, Contig[0306], Contig[0295], Contig[0346], Contig[0287], Contig[0121], Contig[0013], SLJClgrIV02T7G01, Contig[0014], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0202], Contig[0138], SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0355], Contig[0368], SLJClgrIV05M13RF03, Contig[0314], Contig[0235], SLJClgrIV02M13RF09
3	GO:0043170	macromolecule metabolic process	SLJClgrIV04M13RD09, Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, SLJClgrIV05T7F11, SLJClgrIV02M13RB07, Contig[0094], SLJClgrIV01M13RG06, SLJClgrIV04T7F12, Contig[0226], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV02M13RG10, Contig[0306], Contig[0287], Contig[0013], SLJClgrIV02T7G01, Contig[0014], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0202], Contig[0138], SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0355], Contig[0368], SLJClgrIV05M13RF03, SLJClgrIV02M13RF09
3	GO:0009058	biosynthetic process	SLJClgrIV04M13RD09, Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, SLJClgrIV02M13RB07, Contig[0094], SLJClgrIV05T7F11, Contig[0246], SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01, Contig[0161], Contig[0295], Contig[0346], Contig[0121], Contig[0013], SLJClgrIV04T7D11, SLJClgrIV02M13RA01, SLJClgrIV02T7A01, SLJClgrIV02T7G01, Contig[0314]
3	GO:0016043	cell organization and biogenesis	Contig[0287], Contig[0087], Contig[0052], Contig[0238], SLJClgrIV04T7F12, SLJClgrIV02T7C04, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0344], SLJClgrIV02T7G01, Contig[0226], SLJClgrIV05M13RF05, Contig[0300]
3	GO:0051234	establishment of localization	Contig[0185], SLJClgrIV04T7G10, Contig[0256], Contig[0054], Contig[0287], Contig[0087], Contig[0127], Contig[0295], Contig[0346], Contig[0344], Contig[0306], SLJClgrIV02T7H04
3	GO:0015979	photosynthesis	SLJClgrIV02M13RB07, Contig[0094], Contig[0038], Contig[0054], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0201]
3	GO:0050789	regulation of biological process	Contig[0238], SLJClgrIV01M13RG06, SLJClgrIV04T7F12, Contig[0161], SLJClgrIV02M13RC08, SLJClgrIV02T7G01
3	GO:0050794	regulation of cellular process	Contig[0238], SLJClgrIV01M13RG06, SLJClgrIV04T7F12, Contig[0161], SLJClgrIV02M13RC08, SLJClgrIV02T7G01
3	GO:0019222	regulation of metabolic process	Contig[0238], SLJClgrIV01M13RG06, SLJClgrIV04T7F12, Contig[0161], SLJClgrIV02T7G01
3	GO:0042221	response to chemical stimulus	SLJClgrIV05M13RF03, SLJClgrIV04T7F12, Contig[0181], Contig[0185], SLJClgrIV01M13RG06
3	GO:0008104	protein localization	Contig[0287], Contig[0087], Contig[0127], Contig[0344]
3	GO:0009056	catabolic process	Contig[0181], SLJClgrIV02M13RG10, Contig[0306], Contig[0014]
3	GO:0006950	response to stress	SLJClgrIV05M13RF03, SLJClgrIV04T7F12, Contig[0291], SLJClgrIV01M13RG06
3	GO:0051641	cellular localization	Contig[0287], Contig[0087], Contig[0344]
3	GO:0015976	carbon utilization	SLJClgrIV02M13RB07, Contig[0094], Contig[0038]
3	GO:0007275	multicellular organismal development	SLJClgrIV04T7F12, Contig[0182], SLJClgrIV02T7G01
3	GO:0019725	cell homeostasis	Contig[0256], Contig[0185]
3	GO:0006807	nitrogen compound metabolic process	Contig[0246], Contig[0235]
3	GO:0048856	anatomical structure development	SLJClgrIV04T7F12, Contig[0182]

Table B.5: Key for linking stage 1 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0003006	reproductive developmental process	Contig[0182]
3	GO:0048468	cell development	SLJClgrIV02M13RC08
3	GO:0009607	response to biotic stimulus	SLJClgrIV05M13RF03
3	GO:0022402	cell cycle process	SLJClgrIV04T7F12
3	GO:0009791	post-embryonic development	Contig[0182]
3	GO:0048869	cellular developmental process	SLJClgrIV02M13RC08
3	GO:0009790	embryonic development	Contig[0182]
3	GO:0050877	neurological process	Contig[0235]
3	GO:0019953	sexual reproduction	SLJClgrIV02T7G01
3	GO:0000051	urea cycle intermediate metabolic process	Contig[0235]
3	GO:0048878	chemical homeostasis	Contig[0185]
3	GO:0019748	secondary metabolic process	SLJClgrIV05T7F11
3	GO:0007049	cell cycle	SLJClgrIV04T7F12
3	GO:0016265	death	SLJClgrIV02M13RC08
1	GO:0005575	CELLULAR COMPONENT	Contig[0235], Contig[0026], Contig[0287], SLJClgrIV02M13RH06, Contig[0118], Contig[0238], Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV04T7B02, Contig[0138], Contig[0201], Contig[0344], Contig[0029], SLJClgrIV02M13RC08, Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0226], Contig[0355], Contig[0202], SLJClgrIV01T7A06, Contig[0013], Contig[0300], Contig[0127], SLJClgrIV01M13RG06, Contig[0014], Contig[0087], Contig[0042], SLJClgrIV02T7H04, Contig[0314], SLJClgrIV04T7F12, Contig[0368], Contig[0193], Contig[0054], Contig[0121], SLJClgrIV05T7F11, SLJClgrIV04T7G10, Contig[0246], Contig[0084], Contig[0304], Contig[0295], Contig[0346], Contig[0306], SLJClgrIV05M13RF05, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0332], SLJClgrIV01M13RC09, SLJClgrIV02M13RG10, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0185], Contig[0052], SLJClgrIV05T7E07, Contig[0161]
2	GO:0005623	cell	Contig[0235], Contig[0026], Contig[0287], SLJClgrIV02M13RH06, Contig[0118], Contig[0238], Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV04T7B02, Contig[0138], Contig[0201], Contig[0344], Contig[0029], SLJClgrIV02M13RC08, Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0226], Contig[0355], Contig[0202], SLJClgrIV01T7A06, Contig[0013], Contig[0300], Contig[0127], SLJClgrIV01M13RG06, Contig[0014], Contig[0087], Contig[0042], SLJClgrIV02T7H04, Contig[0314], SLJClgrIV04T7F12, Contig[0368], Contig[0193], Contig[0054], Contig[0121], SLJClgrIV05T7F11, SLJClgrIV04T7G10, Contig[0246], Contig[0084], Contig[0304], Contig[0295], Contig[0346], Contig[0306], SLJClgrIV05M13RF05, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0332], SLJClgrIV01M13RC09, SLJClgrIV02M13RG10, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0185], Contig[0052], SLJClgrIV05T7E07, Contig[0161]
2	GO:0044464	cell part	Contig[0235], Contig[0026], Contig[0287], SLJClgrIV02M13RH06, Contig[0118], Contig[0238], Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV04T7B02, Contig[0138], Contig[0201], Contig[0344], Contig[0029], SLJClgrIV02M13RC08, Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0226], Contig[0355], Contig[0202], SLJClgrIV01T7A06, Contig[0013], Contig[0300], Contig[0127], SLJClgrIV01M13RG06, Contig[0014], Contig[0087], Contig[0042], SLJClgrIV02T7H04, Contig[0314], SLJClgrIV04T7F12, Contig[0368], Contig[0193], Contig[0054], Contig[0121], SLJClgrIV05T7F11, SLJClgrIV04T7G10, Contig[0246], Contig[0084], Contig[0304], Contig[0295], Contig[0346], Contig[0306], SLJClgrIV05M13RF05, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0332], SLJClgrIV01M13RC09, SLJClgrIV02M13RG10, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0185], Contig[0052], SLJClgrIV05T7E07, Contig[0161]
2	GO:0043226	organelle	Contig[0235], Contig[0026], Contig[0287], SLJClgrIV02M13RH06, Contig[0118], Contig[0238], Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV04T7B02, Contig[0138], Contig[0201], Contig[0344], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0226], Contig[0355], Contig[0202], SLJClgrIV01T7A06, Contig[0013], Contig[0300], Contig[0127], SLJClgrIV01M13RG06, Contig[0014], Contig[0087], Contig[0042], SLJClgrIV02T7H04, Contig[0314], Contig[0193], Contig[0054], SLJClgrIV05T7F11, SLJClgrIV04T7G10, Contig[0246], Contig[0084], Contig[0304], SLJClgrIV04T7F12, SLJClgrIV05M13RF05, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0332], SLJClgrIV01M13RC09, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0185], Contig[0052], Contig[0295], SLJClgrIV05T7E07, Contig[0306], Contig[0346], Contig[0161]

Table B.5: Key for linking stage 1 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0043234	protein complex	Contig[0026], Contig[0344], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0287], SLJClgrIV04T7F12, Contig[0295], Contig[0346], Contig[0238], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, Contig[0201], Contig[0014], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0127], Contig[0084]
2	GO:0044422	organelle part	Contig[0235], Contig[0026], Contig[0138], Contig[0201], Contig[0344], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0054], Contig[0287], SLJClgrIV04T7F12, Contig[0238], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0127], Contig[0185], Contig[0052], Contig[0084]
2	GO:0031974	membrane-enclosed lumen	Contig[0235], SLJClgrIV04T7F12
2	GO:0031975	envelope	Contig[0344]
3	GO:0005622	intracellular	Contig[0235], Contig[0026], Contig[0287], SLJClgrIV02M13RH06, Contig[0118], Contig[0238], Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV04T7B02, Contig[0138], Contig[0201], Contig[0344], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0226], Contig[0355], Contig[0202], SLJClgrIV01T7A06, Contig[0013], Contig[0300], Contig[0127], SLJClgrIV01M13RG06, Contig[0014], Contig[0087], Contig[0042], SLJClgrIV02T7H04, Contig[0314], SLJClgrIV04T7F12, Contig[0368], Contig[0193], Contig[0054], Contig[0121], SLJClgrIV05T7F11, SLJClgrIV04T7G10, Contig[0246], Contig[0084], Contig[0304], SLJClgrIV05M13RF05, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0332], SLJClgrIV01M13RC09, SLJClgrIV02M13RG10, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0185], Contig[0052], Contig[0295], SLJClgrIV05T7E07, Contig[0306], Contig[0346], Contig[0161]
3	GO:0044424	intracellular part	Contig[0235], Contig[0026], Contig[0287], SLJClgrIV02M13RH06, Contig[0118], Contig[0238], Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV04T7B02, Contig[0138], Contig[0201], Contig[0344], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0226], Contig[0355], Contig[0202], SLJClgrIV01T7A06, Contig[0013], Contig[0300], Contig[0127], SLJClgrIV01M13RG06, Contig[0014], Contig[0087], Contig[0042], SLJClgrIV02T7H04, Contig[0314], SLJClgrIV04T7F12, Contig[0368], Contig[0193], Contig[0054], Contig[0121], SLJClgrIV05T7F11, SLJClgrIV04T7G10, Contig[0246], Contig[0084], Contig[0304], SLJClgrIV05M13RF05, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0332], SLJClgrIV01M13RC09, SLJClgrIV02M13RG10, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0185], Contig[0052], Contig[0295], SLJClgrIV05T7E07, Contig[0306], Contig[0346], Contig[0161]
3	GO:0043229	intracellular organelle	Contig[0235], Contig[0026], Contig[0287], SLJClgrIV02M13RH06, Contig[0118], Contig[0238], Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV04T7B02, Contig[0138], Contig[0201], Contig[0344], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0226], Contig[0355], Contig[0202], SLJClgrIV01T7A06, Contig[0013], Contig[0300], Contig[0127], SLJClgrIV01M13RG06, Contig[0014], Contig[0087], Contig[0042], SLJClgrIV02T7H04, Contig[0314], Contig[0193], Contig[0054], SLJClgrIV05T7F11, SLJClgrIV04T7G10, Contig[0246], Contig[0084], Contig[0304], SLJClgrIV04T7F12, SLJClgrIV05M13RF05, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0332], SLJClgrIV01M13RC09, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0185], Contig[0052], Contig[0295], SLJClgrIV05T7E07, Contig[0306], Contig[0346], Contig[0161]
3	GO:0043227	membrane-bound organelle	Contig[0235], Contig[0026], Contig[0287], SLJClgrIV02M13RH06, Contig[0118], Contig[0238], Contig[0155], SLJClgrIV03M13RA01, SLJClgrIV04T7B02, Contig[0138], Contig[0201], Contig[0344], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0226], Contig[0355], Contig[0202], SLJClgrIV01T7A06, Contig[0013], Contig[0300], Contig[0127], SLJClgrIV01M13RG06, Contig[0014], Contig[0087], Contig[0042], SLJClgrIV02T7H04, Contig[0314], Contig[0193], Contig[0054], SLJClgrIV05T7F11, SLJClgrIV04T7G10, Contig[0246], Contig[0084], Contig[0304], SLJClgrIV04T7F12, Contig[0332], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, SLJClgrIV01M13RC09, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0185], Contig[0052], Contig[0295], SLJClgrIV05T7E07, Contig[0306], Contig[0346], Contig[0161]
3	GO:0044446	intracellular organelle part	Contig[0235], Contig[0026], Contig[0138], Contig[0201], Contig[0344], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0054], Contig[0287], SLJClgrIV04T7F12, Contig[0238], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0038], SLJClgrIV02M13RB07, Contig[0094], Contig[0127], Contig[0185], Contig[0052], Contig[0084]
3	GO:0016020	membrane	Contig[0344], Contig[0054], Contig[0295], Contig[0346], Contig[0306], Contig[0127], Contig[0185], Contig[0052], Contig[0152], Contig[0151], Contig[0153], Contig[0101], Contig[0087], Contig[0161], SLJClgrIV05T7F11, Contig[0202], Contig[0013], SLJClgrIV01M13RE08, Contig[0201]
3	GO:0044425	membrane part	Contig[0344], Contig[0054], Contig[0295], Contig[0346], Contig[0127], Contig[0185], Contig[0052], Contig[0152], Contig[0151], Contig[0153], Contig[0101], Contig[0087]

Table B.5: Key for linking stage 1 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0043228	non-membrane-bound organelle	SLJClgrIV04T7F12, Contig[0238], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0084]
3	GO:0031982	vesicle	Contig[0295], SLJClgrIV05T7E07, Contig[0306], Contig[0346], Contig[0185], SLJClgrIV02T7H04
3	GO:0009523	photosystem II	Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0201]
3	GO:0009522	photosystem I	Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101]
3	GO:0030529	ribonucleoprotein complex	Contig[0287], Contig[0238], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, Contig[0084]
3	GO:0000786	nucleosome	Contig[0100], Contig[0150], SLJClgrIV05M13RG03
3	GO:0048492	ribulose biphosphate carboxylase complex	Contig[0038], SLJClgrIV02M13RB07, Contig[0094]
3	GO:0015935	small ribosomal subunit	Contig[0238], Contig[0084]
3	GO:0043233	organelle lumen	Contig[0235], SLJClgrIV04T7F12
3	GO:0016469	proton-transporting two-sector ATPase complex	Contig[0295], Contig[0346]
3	GO:0016282	eukaryotic 43S preinitiation complex	Contig[0238]
3	GO:0031967	organelle envelope	Contig[0344]
3	GO:0016283	eukaryotic 48S initiation complex	Contig[0238]
3	GO:0005744	mitochondrial inner membrane pre-sequence translocase complex	Contig[0344]
3	GO:0000502	proteasome complex (sensu Eukaryota)	Contig[0014]
3	GO:0005839	proteasome core complex (sensu Eukaryota)	Contig[0014]
3	GO:0009783	photosystem II antenna complex	Contig[0201]
3	GO:0017177	alpha-glucosidase II complex	Contig[0026]
3	GO:0016585	chromatin remodeling complex	SLJClgrIV04T7F12
3	GO:0000815	ESCRT III complex	Contig[0127]
1	GO:0003674	MOLECULAR FUNCTION	Contig[0306], SLJClgrIV04T7F12, Contig[0246], Contig[0235], Contig[0295], SLJClgrIV02M13RB07, Contig[0094], SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0014], Contig[0346], Contig[0126], Contig[0255], Contig[0331], SLJClgrIV04T7D11, SLJClgrIV05M13RF03, Contig[0054], Contig[0193], SLJClgrIV04M13RD09, Contig[0008], Contig[0038], SLJClgrIV02M13RD06, Contig[0084], Contig[0226], Contig[0060], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0368], Contig[0087], SLJClgrIV03M13RF01, Contig[0185], SLJClgrIV02T7G01, Contig[0121], SLJClgrIV03T7D04, Contig[0169], SLJClgrIV05T7C07, Contig[0189], Contig[0330], Contig[0042], Contig[0138], Contig[0355], Contig[0026], Contig[0256], Contig[0013], Contig[0202], SLJClgrIV04T7B11, SLJClgrIV05T7E07, Contig[0233], SLJClgrIV04T7G10, SLJClgrIV01M13RG06, Contig[0245], Contig[0301], Contig[0300], SLJClgrIV02M13RF09, Contig[0287], Contig[0344], Contig[0181], SLJClgrIV02M13RC08, SLJClgrIV01T7A06, SLJClgrIV02T7C04, Contig[0238], SLJClgrIV05M13RF05, SLJClgrIV04T7B02, Contig[0314], Contig[0367], Contig[0020], Contig[0099], SLJClgrIV02M13RG10, Contig[0161], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0243], Contig[0127], Contig[0345], SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01
2	GO:0003824	catalytic activity	Contig[0246], Contig[0295], SLJClgrIV02M13RB07, Contig[0094], SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0014], Contig[0346], Contig[0126], Contig[0255], Contig[0331], SLJClgrIV04T7D11, Contig[0193], SLJClgrIV04M13RD09, Contig[0008], Contig[0038], SLJClgrIV02M13RD06, Contig[0226], Contig[0060], Contig[0121], SLJClgrIV05T7C07, Contig[0189], Contig[0330], Contig[0042], Contig[0013], Contig[0202], SLJClgrIV04T7B11, SLJClgrIV04T7F12, SLJClgrIV05T7E07, SLJClgrIV04T7G10, Contig[0256], Contig[0054], SLJClgrIV01M13RG06, SLJClgrIV02T7G01, Contig[0245], Contig[0301], SLJClgrIV03M13RF01, Contig[0300], SLJClgrIV02M13RF09, Contig[0235], Contig[0287], Contig[0368], Contig[0233], Contig[0306], Contig[0181], SLJClgrIV01T7A06, Contig[0314], Contig[0185], Contig[0026], Contig[0367], Contig[0020], Contig[0099], SLJClgrIV02M13RG10, SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01

Table B.5: Key for linking stage 1 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0005488	binding	Contig[0306], SLJClgrIV04T7F12, Contig[0235], SLJClgrIV04T7D11, SLJClgrIV05M13RF03, Contig[0054], Contig[0084], Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0368], Contig[0226], SLJClgrIV03M13RF01, Contig[0185], SLJClgrIV02T7G01, SLJClgrIV03T7D04, Contig[0169], Contig[0138], Contig[0355], Contig[0026], Contig[0256], Contig[0233], SLJClgrIV04T7G10, Contig[0295], Contig[0008], Contig[0346], Contig[0189], Contig[0330], SLJClgrIV02M13RF09, Contig[0238], Contig[0287], Contig[0300], Contig[0161], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0255], Contig[0243]
2	GO:0005198	structural molecule activity	SLJClgrIV02T7C04, Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02
2	GO:0005215	transporter activity	Contig[0295], Contig[0346], Contig[0087], Contig[0344], Contig[0185]
2	GO:0030234	enzyme regulator activity	SLJClgrIV02M13RC08
3	GO:0016787	hydrolase activity	Contig[0295], Contig[0014], Contig[0346], Contig[0193], Contig[0226], Contig[0060], SLJClgrIV04T7D11, Contig[0042], Contig[0202], SLJClgrIV04T7B11, SLJClgrIV04T7F12, SLJClgrIV02T7G01, SLJClgrIV03M13RF01, Contig[0300], Contig[0233], Contig[0181], SLJClgrIV01T7A06, Contig[0185], Contig[0026], Contig[0367], Contig[0020], Contig[0099]
3	GO:0043167	ion binding	SLJClgrIV04T7D11, SLJClgrIV04T7F12, Contig[0152], SLJClgrIV01M13RE08, Contig[0151], Contig[0153], Contig[0101], Contig[0233], SLJClgrIV04T7G10, Contig[0054], Contig[0295], Contig[0008], Contig[0355], Contig[0346], Contig[0185], Contig[0189], Contig[0330]
3	GO:0016491	oxidoreductase activity	Contig[0246], SLJClgrIV02M13RB07, Contig[0094], SLJClgrIV04T7D11, Contig[0008], Contig[0038], SLJClgrIV02M13RD06, SLJClgrIV05T7E07, SLJClgrIV04T7G10, Contig[0256], Contig[0054], SLJClgrIV01M13RG06, Contig[0245], Contig[0301], Contig[0306], SLJClgrIV02M13RG10
3	GO:0016740	transferase activity	SLJClgrIV02M13RA01, SLJClgrIV02T7A01, Contig[0126], Contig[0255], Contig[0331], Contig[0013], SLJClgrIV02M13RF09, Contig[0235], Contig[0314], SLJClgrIV02M13RH06, Contig[0155], SLJClgrIV03M13RA01
3	GO:0003676	nucleic acid binding	Contig[0084], SLJClgrIV02M13RF09, Contig[0238], SLJClgrIV02T7G01, Contig[0161], Contig[0100], Contig[0150], SLJClgrIV05M13RG03, Contig[0255], Contig[0243]
3	GO:0005515	protein binding	SLJClgrIV04T7F12, SLJClgrIV05M13RF03, Contig[0138], Contig[0355], Contig[0026], Contig[0256], Contig[0084]
3	GO:0000166	nucleotide binding	Contig[0226], SLJClgrIV03M13RF01, Contig[0185], SLJClgrIV02T7G01, SLJClgrIV05M13RF03, Contig[0300]
3	GO:0016853	isomerase activity	SLJClgrIV04M13RD09, Contig[0256], Contig[0287], Contig[0368]
3	GO:0005386	carrier activity	Contig[0295], Contig[0346], Contig[0087], Contig[0185]
3	GO:0003735	structural constituent of ribosome	Contig[0238], Contig[0084], SLJClgrIV05M13RF05, SLJClgrIV04T7B02
3	GO:0015075	ion transporter activity	Contig[0295], Contig[0346], Contig[0185]
3	GO:0043492	ATPase activity, coupled to movement of substances	Contig[0295], Contig[0346], Contig[0185]
3	GO:0016829	lyase activity	Contig[0038], SLJClgrIV02M13RB07, Contig[0094]
3	GO:0048037	cofactor binding	Contig[0306], Contig[0235]
3	GO:0008144	drug binding	Contig[0368], Contig[0287]
3	GO:0008565	protein transporter activity	Contig[0087], Contig[0344]
3	GO:0016874	ligase activity	Contig[0121]
3	GO:0008047	enzyme activator activity	SLJClgrIV02M13RC08
3	GO:0004386	helicase activity	SLJClgrIV02T7G01
3	GO:0051540	metal cluster binding	SLJClgrIV04T7G10
3	GO:0042277	peptide binding	Contig[0287]
3	GO:0046906	tetrapyrrole binding	Contig[0054]
3	GO:0019239	deaminase activity	SLJClgrIV04T7D11
3	GO:0019842	vitamin binding	Contig[0235]

Table B.6: Key for linking stage 2 *Asterochloris* sp. GO terms and Clone IDs.

Level	GO ID	GO TERM	Clone ID
1	GO:0008150	BIOLOGICAL PROCESS	SLJClgrII10T7F07, Contig[0284], Contig[0098], SLJClgrII10M13RA09, Contig[0420], Contig[0631], Contig[0609], Contig[0716], Contig[0665], Contig[0815], Contig[0244], Contig[0590], Contig[0449], Contig[0115], Contig[0391], Contig[0390], SLJClgrII02T7B04, Contig[0807], Contig[0287], Contig[0140], Contig[0435], Contig[0664], Contig[0199], Contig[0008], SLJClgrII10M13RC06, Contig[0075], Contig[0638], Contig[0383], Contig[0768], Contig[0757], Contig[0211], Contig[0033], Contig[0097], Contig[0158], Contig[0332], Contig[0021], Contig[0696], Contig[0317], Contig[0134], Contig[0223], Contig[0200], Contig[0659], Contig[0170], Contig[0149], Contig[0350], Contig[0055], Contig[0584], Contig[0271], Contig[0251], Contig[0675], Contig[0304], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0142], Contig[0655], Contig[0763], Contig[0003], Contig[0476], SLJClgrII02T7A09, Contig[0035], Contig[0318], Contig[0426], Contig[0045], Contig[0566], Contig[0624], Contig[0204], Contig[0600], Contig[0146], Contig[0281], Contig[0535], Contig[0642], Contig[0012], Contig[0401]
2	GO:0065007	biological regulation	SLJClgrII10T7F07, Contig[0098], Contig[0244], Contig[0590], Contig[0449], Contig[0115], Contig[0391], Contig[0033], Contig[0097], Contig[0284], Contig[0211]
2	GO:0009987	cellular process	SLJClgrII10T7F07, Contig[0098], Contig[0244], Contig[0590], Contig[0449], Contig[0115], Contig[0391], Contig[0390], SLJClgrII02T7B04, Contig[0807], Contig[0287], Contig[0140], Contig[0435], Contig[0664], Contig[0199], Contig[0008], SLJClgrII10M13RC06, Contig[0075], Contig[0284], Contig[0638], Contig[0383], Contig[0768], Contig[0757], Contig[0158], Contig[0021], Contig[0696], Contig[0317], Contig[0134], Contig[0223], Contig[0200], Contig[0659], Contig[0170], Contig[0350], Contig[0055], Contig[0271], Contig[0665], Contig[0251], Contig[0033], Contig[0097], Contig[0304], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0142], Contig[0655], Contig[0763], Contig[0003], Contig[0211], Contig[0318], Contig[0675], Contig[0426], Contig[0624], Contig[0204], Contig[0600], Contig[0716], Contig[0146], Contig[0281], Contig[0535], Contig[0642], Contig[0012], Contig[0401]
2	GO:0032502	developmental process	SLJClgrII10T7F07, Contig[0390], Contig[0638], Contig[0675], Contig[0757]
2	GO:0051234	establishment of localization	SLJClgrII10M13RA09, Contig[0420], Contig[0631], Contig[0609], Contig[0716], Contig[0665], Contig[0815], Contig[0140], Contig[0757], Contig[0332], Contig[0021], Contig[0149], Contig[0383], Contig[0055]
2	GO:0040007	growth	SLJClgrII10T7F07
2	GO:0051179	localization	SLJClgrII10M13RA09, Contig[0420], Contig[0631], Contig[0609], Contig[0716], Contig[0665], Contig[0815], Contig[0140], Contig[0757], Contig[0332], Contig[0021], Contig[0149], Contig[0383], Contig[0055], SLJClgrII10T7F07
2	GO:0008152	metabolic process	Contig[0244], Contig[0590], Contig[0449], Contig[0115], Contig[0391], Contig[0390], SLJClgrII02T7B04, Contig[0807], Contig[0287], Contig[0284], Contig[0435], Contig[0664], Contig[0199], Contig[0008], SLJClgrII10M13RC06, Contig[0075], Contig[0638], Contig[0383], Contig[0768], Contig[0033], Contig[0097], Contig[0158], Contig[0696], Contig[0317], Contig[0134], Contig[0223], Contig[0200], Contig[0659], Contig[0170], Contig[0350], Contig[0584], Contig[0271], Contig[0665], Contig[0251], Contig[0304], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0142], Contig[0655], Contig[0763], Contig[0003], SLJClgrII10T7F07, Contig[0476], Contig[0055], Contig[0318], Contig[0675], Contig[0426], Contig[0045], Contig[0566], Contig[0624], Contig[0204], Contig[0600], Contig[0716], Contig[0146], Contig[0281], Contig[0535], Contig[0642], Contig[0012], Contig[0401]
2	GO:0032501	multicellular organismal process	SLJClgrII10T7F07, Contig[0390], Contig[0638], Contig[0675]
2	GO:0051704	multi-organism process	Contig[0098], SLJClgrII10T7F07
2	GO:0000003	reproduction	SLJClgrII10T7F07
2	GO:0050896	response to stimulus	SLJClgrII10T7F07, Contig[0284], Contig[0098], Contig[0211], SLJClgrII02T7A09, Contig[0035]
3	GO:0007568	aging	SLJClgrII10T7F07
3	GO:0048856	anatomical structure development	SLJClgrII10T7F07, Contig[0390], Contig[0638], Contig[0757]
3	GO:0009653	anatomical structure morphogenesis	Contig[0638], Contig[0757]
3	GO:0007610	behavior	SLJClgrII10T7F07
3	GO:0009058	biosynthetic process	Contig[0244], Contig[0590], Contig[0449], Contig[0115], Contig[0391], Contig[0390], SLJClgrII02T7B04, Contig[0807], SLJClgrII10M13RC06, Contig[0284], Contig[0696], Contig[0350], Contig[0584], Contig[0304], Contig[0642], Contig[0012], Contig[0401]
3	GO:0015976	carbon utilization	Contig[0435], Contig[0664], Contig[0199], Contig[0008]
3	GO:0009056	catabolic process	Contig[0317], Contig[0664], Contig[0134], Contig[0223], Contig[0199], Contig[0435], Contig[0200], Contig[0659], Contig[0170], Contig[0033], Contig[0097]

Table B.6: Key for linking stage 2 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0007154	cell communication	Contig[0098], SLJClgrII10T7F07, Contig[0383]
3	GO:0048468	cell development	Contig[0638], SLJClgrII10T7F07
3	GO:0006928	cell motility	SLJClgrII10T7F07
3	GO:0008283	cell proliferation	SLJClgrII10T7F07
3	GO:0016043	cellular component organization and biogenesis	Contig[0391], Contig[0140], Contig[0075], Contig[0757], Contig[0638], Contig[0021], Contig[0055], SLJClgrII02T7B04, Contig[0115]
3	GO:0048869	cellular developmental process	Contig[0638], SLJClgrII10T7F07
3	GO:0019725	cellular homeostasis	Contig[0211]
3	GO:0051641	cellular localization	Contig[0140], Contig[0757], Contig[0021], Contig[0055]
3	GO:0044237	cellular metabolic process	Contig[0244], Contig[0590], Contig[0449], Contig[0115], Contig[0391], Contig[0390], SLJClgrII02T7B04, Contig[0807], Contig[0287], Contig[0435], Contig[0664], Contig[0199], Contig[0008], SLJClgrII10M13RC06, Contig[0075], Contig[0284], Contig[0638], Contig[0383], Contig[0768], Contig[0158], Contig[0696], Contig[0317], Contig[0134], Contig[0223], Contig[0200], Contig[0659], Contig[0170], Contig[0350], Contig[0271], Contig[0665], Contig[0251], Contig[0033], Contig[0097], Contig[0304], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0142], Contig[0655], Contig[0763], Contig[0003], SLJClgrII10T7F07, Contig[0318], Contig[0675], Contig[0426], Contig[0624], Contig[0204], Contig[0600], Contig[0716], Contig[0146], Contig[0281], Contig[0535], Contig[0642], Contig[0012], Contig[0401]
3	GO:0051716	cellular response to stimulus	SLJClgrII10T7F07
3	GO:0006952	defense response	Contig[0098]
3	GO:0048589	developmental growth	SLJClgrII10T7F07
3	GO:0009790	embryonic development	Contig[0675]
3	GO:0051649	establishment of cellular localization	Contig[0140], Contig[0757], Contig[0021], Contig[0055]
3	GO:0045184	establishment of protein localization	Contig[0140], Contig[0149], Contig[0383], Contig[0055]
3	GO:0006091	generation of precursor metabolites and energy	Contig[0317], Contig[0426], Contig[0716], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0146], Contig[0281]
3	GO:0051674	localization of cell	SLJClgrII10T7F07
3	GO:0033036	macromolecule localization	Contig[0140], Contig[0149], Contig[0383], Contig[0055]
3	GO:0043170	macromolecule metabolic process	Contig[0244], Contig[0590], Contig[0449], Contig[0115], Contig[0391], Contig[0390], SLJClgrII02T7B04, Contig[0807], Contig[0075], Contig[0638], Contig[0383], Contig[0768], Contig[0033], Contig[0097], Contig[0664], Contig[0134], Contig[0223], Contig[0199], Contig[0435], Contig[0317], Contig[0200], Contig[0659], Contig[0170], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0142], Contig[0655], Contig[0763], Contig[0003], SLJClgrII10T7F07, Contig[0055], Contig[0318], Contig[0675], Contig[0535], Contig[0401]
3	GO:0007275	multicellular organismal development	SLJClgrII10T7F07, Contig[0390], Contig[0638], Contig[0675]
3	GO:0043500	muscle adaptation	SLJClgrII10T7F07
3	GO:0006807	nitrogen compound metabolic process	Contig[0287], SLJClgrII10M13RC06, Contig[0768], Contig[0696], Contig[0401]
3	GO:0015979	photosynthesis	Contig[0435], Contig[0664], Contig[0199], Contig[0008], Contig[0271], Contig[0665], Contig[0251], Contig[0426], Contig[0716], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0146], Contig[0281]
3	GO:0044238	primary metabolic process	Contig[0244], Contig[0590], Contig[0449], Contig[0115], Contig[0391], Contig[0390], SLJClgrII02T7B04, Contig[0807], Contig[0287], Contig[0435], Contig[0664], Contig[0199], Contig[0008], SLJClgrII10M13RC06, Contig[0075], Contig[0284], Contig[0638], Contig[0383], Contig[0768], Contig[0033], Contig[0097], Contig[0696], Contig[0134], Contig[0223], Contig[0317], Contig[0200], Contig[0659], Contig[0170], Contig[0350], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0142], Contig[0655], Contig[0763], Contig[0003], SLJClgrII10T7F07, Contig[0055], Contig[0318], Contig[0675], Contig[0158], Contig[0535], Contig[0642], Contig[0012], Contig[0401]
3	GO:0050789	regulation of biological process	SLJClgrII10T7F07, Contig[0098], Contig[0244], Contig[0590], Contig[0075], Contig[0638], Contig[0383], Contig[0033], Contig[0097], Contig[0284]
3	GO:0065008	regulation of biological quality	Contig[0211]

Table B.6: Key for linking stage 2 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0050794	regulation of cellular process	SLJClgrII10T7F07, Contig[0098], Contig[0244], Contig[0590], Contig[0075], Contig[0638], Contig[0383]
3	GO:0050793	regulation of developmental process	SLJClgrII10T7F07
3	GO:0040008	regulation of growth	SLJClgrII10T7F07
3	GO:0019222	regulation of metabolic process	Contig[0244], Contig[0590], Contig[0075], Contig[0638], Contig[0383], Contig[0033], Contig[0097], Contig[0284]
3	GO:0048583	regulation of response to stimulus	Contig[0098]
3	GO:0009628	response to abiotic stimulus	Contig[0098], SLJClgrII10T7F07
3	GO:0009607	response to biotic stimulus	Contig[0098], SLJClgrII10T7F07
3	GO:0042221	response to chemical stimulus	SLJClgrII10T7F07, Contig[0284], Contig[0211], Contig[0098]
3	GO:0009719	response to endogenous stimulus	SLJClgrII10T7F07, Contig[0284], Contig[0098]
3	GO:0009605	response to external stimulus	SLJClgrII10T7F07
3	GO:0051707	response to other organism	Contig[0098], SLJClgrII10T7F07
3	GO:0006950	response to stress	Contig[0211], Contig[0098], SLJClgrII10T7F07
3	GO:0019748	secondary metabolic process	Contig[0284], Contig[0476]
3	GO:0046903	secretion	Contig[0021]
3	GO:0019953	sexual reproduction	SLJClgrII10T7F07
3	GO:0010118	stomatal movement	Contig[0098]
3	GO:0006810	transport	SLJClgrII10M13RA09, Contig[0420], Contig[0631], Contig[0609], Contig[0716], Contig[0665], Contig[0815], Contig[0140], Contig[0757], Contig[0332], Contig[0021], Contig[0149], Contig[0383], Contig[0055]
1	GO:0005575	CELLULAR COMPONENT	Contig[0170], SLJClgrII10T7F07, Contig[0622], Contig[0757], Contig[0716], Contig[0281], Contig[0687], Contig[0515], Contig[0551], Contig[0642], Contig[0815], Contig[0012], Contig[0545], Contig[0271], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0648], Contig[0211], Contig[0180], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0251], Contig[0149], Contig[0333], Contig[0072], Contig[0624], Contig[0696], Contig[0485], Contig[0401], Contig[0204], Contig[0600], Contig[0318], Contig[0287], Contig[0244], Contig[0590], Contig[0449], Contig[0200], Contig[0659], Contig[0350], Contig[0662], Contig[0341], Contig[0344], Contig[0665], Contig[0435], Contig[0146], Contig[0505], Contig[0332], Contig[0284], Contig[0055], Contig[0140], Contig[0075], Contig[0584], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115], Contig[0032], Contig[0046], Contig[0768], Contig[0426], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0609], Contig[0542], Contig[0345], Contig[0047], Contig[0021], Contig[0383], Contig[0655], Contig[0763]
2	GO:0005623	cell	SLJClgrII10T7F07, Contig[0622], Contig[0757], Contig[0716], Contig[0281], Contig[0687], Contig[0515], Contig[0551], Contig[0642], Contig[0815], Contig[0012], Contig[0545], Contig[0271], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0648], Contig[0211], Contig[0180], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0251], Contig[0149], Contig[0333], Contig[0072], Contig[0624], Contig[0696], Contig[0485], Contig[0401], Contig[0204], Contig[0600], Contig[0318], Contig[0287], Contig[0244], Contig[0590], Contig[0449], Contig[0200], Contig[0659], Contig[0350], Contig[0662], Contig[0341], Contig[0344], Contig[0665], Contig[0170], Contig[0435], Contig[0146], Contig[0505], Contig[0332], Contig[0284], Contig[0055], Contig[0140], Contig[0075], Contig[0584], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115], Contig[0032], Contig[0046], Contig[0768], Contig[0426], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0609], Contig[0542], Contig[0345], Contig[0047], Contig[0021], Contig[0383], Contig[0655], Contig[0763]
2	GO:0044464	cell part	SLJClgrII10T7F07, Contig[0622], Contig[0757], Contig[0716], Contig[0281], Contig[0687], Contig[0515], Contig[0551], Contig[0642], Contig[0815], Contig[0012], Contig[0545], Contig[0271], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0648], Contig[0211], Contig[0180], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0251], Contig[0149], Contig[0333], Contig[0072], Contig[0624], Contig[0696], Contig[0485], Contig[0401], Contig[0204], Contig[0600], Contig[0318], Contig[0287], Contig[0244], Contig[0590], Contig[0449], Contig[0200], Contig[0659], Contig[0350], Contig[0662], Contig[0341], Contig[0344], Contig[0665], Contig[0170], Contig[0435], Contig[0146], Contig[0505], Contig[0332], Contig[0284], Contig[0055], Contig[0140], Contig[0075], Contig[0584], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115], Contig[0032], Contig[0046], Contig[0768], Contig[0426], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0609], Contig[0542], Contig[0345], Contig[0047], Contig[0021], Contig[0383], Contig[0655], Contig[0763]

Table B.6: Key for linking stage 2 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0031975	envelope	Contig[0622], SLJClgrII10M13RA09, Contig[0631], Contig[0584]
2	GO:0032991	macromolecular complex	Contig[0170], SLJClgrII10T7F07, Contig[0716], Contig[0281], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0271], Contig[0251], Contig[0055], Contig[0140], Contig[0757], Contig[0075], Contig[0318], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115], Contig[0584], Contig[0642], Contig[0012], Contig[0008], Contig[0003]
2	GO:0031974	membrane-enclosed lumen	Contig[0622], Contig[0075]
2	GO:0043226	organelle	Contig[0622], Contig[0716], Contig[0281], Contig[0687], Contig[0515], Contig[0551], Contig[0642], Contig[0815], Contig[0012], Contig[0545], Contig[0271], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0251], Contig[0149], Contig[0333], Contig[0072], Contig[0624], Contig[0696], Contig[0485], Contig[0401], Contig[0204], Contig[0600], Contig[0318], Contig[0287], Contig[0662], Contig[0341], Contig[0344], Contig[0665], Contig[0435], Contig[0146], Contig[0505], Contig[0332], Contig[0284], Contig[0757], Contig[0140], Contig[0055], Contig[0075], Contig[0584], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115], Contig[0032], Contig[0046], SLJClgrII10T7F07, Contig[0768], Contig[0426], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0655], Contig[0763], Contig[0021]
2	GO:0044422	organelle part	Contig[0622], Contig[0716], Contig[0281], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0271], Contig[0251], Contig[0665], Contig[0757], Contig[0140], Contig[0055], Contig[0075], Contig[0318], Contig[0584], Contig[0807], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0021]
3	GO:0000267	cell fraction	SLJClgrII10T7F07
3	GO:0042995	cell projection	Contig[0757]
3	GO:0009986	cell surface	SLJClgrII10T7F07
3	GO:0030312	external encapsulating structure	Contig[0622]
3	GO:0005622	intracellular	SLJClgrII10T7F07, Contig[0622], Contig[0716], Contig[0281], Contig[0687], Contig[0515], Contig[0551], Contig[0642], Contig[0815], Contig[0012], Contig[0545], Contig[0271], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0251], Contig[0149], Contig[0333], Contig[0072], Contig[0624], Contig[0696], Contig[0485], Contig[0401], Contig[0204], Contig[0600], Contig[0318], Contig[0287], Contig[0244], Contig[0590], Contig[0449], Contig[0200], Contig[0659], Contig[0350], Contig[0662], Contig[0341], Contig[0344], Contig[0665], Contig[0170], Contig[0435], Contig[0146], Contig[0505], Contig[0332], Contig[0284], Contig[0055], Contig[0757], Contig[0140], Contig[0075], Contig[0584], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115], Contig[0032], Contig[0046], Contig[0768], Contig[0426], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0383], Contig[0655], Contig[0763], Contig[0021]
3	GO:0043229	intracellular organelle	Contig[0622], Contig[0716], Contig[0281], Contig[0687], Contig[0515], Contig[0551], Contig[0642], Contig[0815], Contig[0012], Contig[0545], Contig[0271], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0251], Contig[0149], Contig[0333], Contig[0072], Contig[0624], Contig[0696], Contig[0485], Contig[0401], Contig[0204], Contig[0600], Contig[0318], Contig[0287], Contig[0244], Contig[0590], Contig[0449], Contig[0200], Contig[0659], Contig[0350], Contig[0662], Contig[0341], Contig[0344], Contig[0665], Contig[0170], Contig[0435], Contig[0146], Contig[0505], Contig[0332], Contig[0284], Contig[0055], Contig[0757], Contig[0140], Contig[0075], Contig[0584], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115], Contig[0032], Contig[0046], Contig[0768], Contig[0426], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0383], Contig[0655], Contig[0763], Contig[0021]
3	GO:0044446	intracellular organelle part	Contig[0622], Contig[0716], Contig[0281], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0271], Contig[0251], Contig[0665], Contig[0757], Contig[0140], Contig[0055], Contig[0075], Contig[0318], Contig[0584], Contig[0807], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0021]

Table B.6: Key for linking stage 2 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0044424	intracellular part	SLJClgrII10T7F07, Contig[0622], Contig[0716], Contig[0281], Contig[0687], Contig[0515], Contig[0551], Contig[0642], Contig[0815], Contig[0012], Contig[0545], Contig[0271], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0251], Contig[0149], Contig[0333], Contig[0072], Contig[0624], Contig[0696], Contig[0485], Contig[0401], Contig[0204], Contig[0600], Contig[0318], Contig[0287], Contig[0244], Contig[0590], Contig[0449], Contig[0200], Contig[0659], Contig[0350], Contig[0662], Contig[0341], Contig[0344], Contig[0665], Contig[0170], Contig[0435], Contig[0146], Contig[0505], Contig[0332], Contig[0284], Contig[0055], Contig[0757], Contig[0140], Contig[0075], Contig[0584], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115], Contig[0032], Contig[0046], Contig[0768], Contig[0426], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0655], Contig[0763], Contig[0021]
3	GO:0016020	membrane	Contig[0664], Contig[0281], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0665], Contig[0055], Contig[0140], Contig[0584], Contig[0149], Contig[0372], Contig[0550], Contig[0772], Contig[0815], Contig[0332], Contig[0716], Contig[0003], Contig[0269], Contig[0515], Contig[0696], Contig[0551], Contig[0609], Contig[0545], Contig[0271], Contig[0542], Contig[0146], Contig[0345], Contig[0047], Contig[0021], Contig[0251]
3	GO:0044425	membrane part	SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0665], Contig[0055], Contig[0140], Contig[0584], Contig[0149], Contig[0372], Contig[0550], Contig[0772], Contig[0815], Contig[0332], Contig[0716], Contig[0003]
3	GO:0043227	membrane-bounded organelle	Contig[0622], Contig[0716], Contig[0281], Contig[0687], Contig[0515], Contig[0551], Contig[0642], Contig[0815], Contig[0012], Contig[0545], Contig[0271], Contig[0664], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0251], Contig[0149], Contig[0333], Contig[0072], Contig[0624], Contig[0696], Contig[0485], Contig[0401], Contig[0204], Contig[0600], Contig[0318], Contig[0287], Contig[0662], Contig[0341], Contig[0344], Contig[0665], Contig[0435], Contig[0146], Contig[0505], Contig[0332], Contig[0284], Contig[0140], Contig[0055], Contig[0075], Contig[0584], Contig[0032], Contig[0046], SLJClgrII10T7F07, Contig[0768], Contig[0426], Contig[0008], Contig[0134], Contig[0223], Contig[0003], Contig[0655], Contig[0763], Contig[0021]
3	GO:0043228	non-membrane-bounded organelle	Contig[0757], Contig[0318], Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115]
3	GO:0031967	organelle envelope	Contig[0622], SLJClgrII10M13RA09, Contig[0631], Contig[0584]
3	GO:0043233	organelle lumen	Contig[0622], Contig[0075]
3	GO:0031090	organelle membrane	Contig[0664], Contig[0281], Contig[0199], SLJClgrII10M13RA09, Contig[0631], Contig[0420], Contig[0665], Contig[0140], Contig[0055], Contig[0584], Contig[0003]
3	GO:0043234	protein complex	Contig[0170], SLJClgrII10T7F07, Contig[0716], Contig[0281], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0271], Contig[0251], Contig[0055], Contig[0140], Contig[0757], Contig[0075], Contig[0318], Contig[0584], Contig[0642], Contig[0012], Contig[0008], Contig[0003]
3	GO:0030529	ribonucleoprotein complex	Contig[0807], Contig[0390], SLJClgrII02T7B04, Contig[0115]
3	GO:0031982	vesicle	Contig[0140], Contig[0055], Contig[0696], Contig[0551]
1	GO:0003674	MOLECULAR FUNCTION	Contig[0696], SLJClgrII10M13RA09, Contig[0631], SLJClgrII02T7A09, Contig[0008], Contig[0134], Contig[0223], Contig[0287], Contig[0200], Contig[0659], Contig[0045], Contig[0098], Contig[0170], Contig[0138], Contig[0498], Contig[0555], Contig[0815], Contig[0284], Contig[0716], Contig[0665], Contig[0622], Contig[0332], SLJClgrII10T7F07, Contig[0662], Contig[0642], Contig[0012], Contig[0476], Contig[0390], Contig[0807], Contig[0757], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0739], Contig[0449], Contig[0383], Contig[0435], Contig[0350], Contig[0075], Contig[0401], Contig[0318], Contig[0391], SLJClgrII10T7F07, Contig[0317], Contig[0624], Contig[0662], Contig[0642], Contig[0012], Contig[0146], Contig[0244], Contig[0590], Contig[0664], SLJClgrII10M13RC06, Contig[0046], Contig[0675], Contig[0199], Contig[0055], Contig[0003], Contig[0655], Contig[0763], Contig[0535], Contig[0033], Contig[0097], Contig[0032], Contig[0638], Contig[0251], Contig[0566], Contig[0515], Contig[0545], Contig[0224], Contig[0211], Contig[0158], Contig[0204], Contig[0600], Contig[0115], Contig[0551], Contig[0304], Contig[0609], Contig[0584]
2	GO:0005488	binding	Contig[0200], Contig[0659], Contig[0045], Contig[0716], Contig[0665], Contig[0390], Contig[0807], Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0739], Contig[0449], Contig[0383], Contig[0435], Contig[0350], Contig[0075], Contig[0401], Contig[0318], Contig[0757], Contig[0391], SLJClgrII10T7F07, Contig[0317], Contig[0624], Contig[0662], Contig[0642], Contig[0012], Contig[0146], Contig[0244], Contig[0590], Contig[0664], Contig[0098], SLJClgrII10M13RC06, Contig[0046], Contig[0384], Contig[0675], Contig[0199], Contig[0055], Contig[0032], SLJClgrII10M13RA09, Contig[0631], Contig[0638], Contig[0251], Contig[0609], Contig[0204], Contig[0600], Contig[0584]

Table B.6: Key for linking stage 2 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
2	GO:0003824	catalytic activity	Contig[0696], SLJClgrII10M13RA09, Contig[0631], Contig[0008], Contig[0134], Contig[0223], Contig[0287], Contig[0098], Contig[0170], Contig[0138], Contig[0498], Contig[0555], Contig[0622], SLJClgrII10T7F07, Contig[0662], Contig[0642], Contig[0012], Contig[0476], Contig[0757], Contig[0717], Contig[0474], Contig[0384], Contig[0768], Contig[0003], Contig[0655], Contig[0763], Contig[0046], Contig[0535], Contig[0033], Contig[0097], Contig[0675], Contig[0045], Contig[0350], Contig[0716], Contig[0665], Contig[0391], Contig[0244], Contig[0590], Contig[0566], Contig[0401], Contig[0075], Contig[0317], Contig[0435], Contig[0224], Contig[0211], Contig[0638], Contig[0158], Contig[0200], Contig[0659], Contig[0551], Contig[0739], Contig[0449], Contig[0304], Contig[0664], Contig[0199], Contig[0318], SLJClgrII10M13RC06
2	GO:0030234	enzyme regulator activity	Contig[0284], SLJClgrII10T7F07
2	GO:0003774	motor activity	Contig[0757]
2	GO:0005198	structural molecule activity	Contig[0624], Contig[0204], Contig[0600], Contig[0390], Contig[0115], Contig[0807]
2	GO:0030528	transcription regulator activity	Contig[0075]
2	GO:0045182	translation regulator activity	Contig[0449], Contig[0391]
2	GO:0005215	transporter activity	SLJClgrII10M13RA09, Contig[0631], SLJClgrII02T7A09, Contig[0815], Contig[0332], Contig[0055], Contig[0515], Contig[0545]
3	GO:0010307	acetylglutamate kinase regulator activity	Contig[0284]
3	GO:0048037	cofactor binding	Contig[0200], Contig[0659], Contig[0045]
3	GO:0004386	helicase activity	Contig[0391]
3	GO:0016787	hydrolase activity	Contig[0662], Contig[0757], Contig[0003], Contig[0655], Contig[0763], Contig[0046], Contig[0391], Contig[0244], Contig[0590], Contig[0211], Contig[0638], Contig[0739], Contig[0449], Contig[0318]
3	GO:0043167	ion binding	Contig[0269], Contig[0372], Contig[0550], Contig[0772], Contig[0032], SLJClgrII10M13RA09, Contig[0631], Contig[0716], Contig[0665], Contig[0244], Contig[0590], Contig[0638], Contig[0251]
3	GO:0016853	isomerase activity	Contig[0664], Contig[0199]
3	GO:0019207	kinase regulator activity	SLJClgrII10T7F07
3	GO:0016874	ligase activity	Contig[0535], Contig[0033], Contig[0097], Contig[0675], Contig[0401]
3	GO:0016829	lyase activity	Contig[0134], Contig[0223], Contig[0350], Contig[0662], Contig[0008], Contig[0304]
3	GO:0051540	metal cluster binding	Contig[0631], Contig[0204], Contig[0600]
3	GO:0003777	microtubule motor activity	Contig[0757]
3	GO:0003676	nucleic acid binding	Contig[0390], Contig[0807], Contig[0449], Contig[0391], Contig[0318], Contig[0584]
3	GO:0000166	nucleotide binding	Contig[0739], Contig[0449], Contig[0383], Contig[0435], Contig[0350], Contig[0075], Contig[0401], Contig[0318], Contig[0757], Contig[0391], SLJClgrII10T7F07, Contig[0609]
3	GO:0016491	oxidoreductase activity	SLJClgrII10M13RA09, Contig[0631], Contig[0008], Contig[0287], Contig[0622], Contig[0642], Contig[0012], Contig[0476], Contig[0768], Contig[0045], Contig[0716], Contig[0665], Contig[0566], Contig[0317], Contig[0158], Contig[0200], Contig[0659], SLJClgrII10M13RC06
3	GO:0042277	peptide binding	SLJClgrII10T7F07
3	GO:0005515	protein binding	Contig[0244], Contig[0590], Contig[0664], Contig[0098], Contig[0350], SLJClgrII10M13RC06, Contig[0046], Contig[0384], Contig[0318], Contig[0675], Contig[0391], Contig[0199], Contig[0055], Contig[0075], Contig[0383]
3	GO:0003735	structural constituent of ribosome	Contig[0390], Contig[0115], Contig[0807]
3	GO:0022892	substrate-specific transporter activity	SLJClgrII10M13RA09, Contig[0631], SLJClgrII02T7A09, Contig[0815], Contig[0332], Contig[0055], Contig[0515], Contig[0545]
3	GO:0046906	tetrapyrrole binding	Contig[0716], Contig[0665], Contig[0269], Contig[0372], Contig[0550], Contig[0772]
3	GO:0016563	transcription activator activity	Contig[0075]
3	GO:0003712	transcription cofactor activity	Contig[0075]
3	GO:0016740	transferase activity	Contig[0696], Contig[0098], Contig[0170], Contig[0138], Contig[0498], Contig[0555], SLJClgrII10T7F07, Contig[0642], Contig[0012], Contig[0350], Contig[0075], Contig[0435], Contig[0224], Contig[0551]

Table B.6: Key for linking stage 2 *Asterochloris* sp. GO terms and Clone IDs – Continued

Level	GO ID	GO TERM	Clone ID
3	GO:0008135	translation factor activity, nucleic acid binding	Contig[0449], Contig[0391]
3	GO:0022857	transmembrane transporter activity	SLJCIgrII10M13RA09, Contig[0631], Contig[0815], Contig[0332], Contig[0515], Contig[0545]

C

GenBank, UniProt, and Gene Ontology summary information for “either” in stages 1 and 2 of lichen development.

C.1 Combined GenBank and UniProt summary information for stage 2 “either” gene fragments

A local batch blastx algorithmic search was carried out for stage 2 “either” SSH-cDNAs, using the non-redundant set of proteins from the National Center for Biotechnology and Information (www.ncbi.nlm.nih.gov, accessed on 4 March 2009). An outline of how nucleotide and amino acid sequences were summarized, and how stage 2 SSH-cDNAs were assigned to organism, can be found in Appendix A.1.

Table C.1: Combined GenBank and UniProt summary information for stage 2 “either” gene fragments.

Clone ID	Top GenBank blastx hit	Top GenBank blastx description	Uniprot top hit	Uniprot top hit description
Contig[0004]	gi 66810195 ref XP_638821.1	hypothetical protein DDBDRAFT_0185766 [Dictyostelium discoideum AX4] hypothetical protein DDBDRAFT_0185766 [Dictyostelium discoideum AX4]	P36151 YK50_YEAST	Uncharacterized protein YKR070W - <i>Saccharomyces cerevisiae</i> (Baker's yeast)
Contig[0040]	gb ABT13580.1	hypothetical protein MT325_M026L [Paramecium bursaria chlorella virus MT325]	P52643 LDHD.ECOLI	D-lactate dehydrogenase - <i>Escherichia coli</i> (strain K12)
Contig[0048]	gi 126665512 ref ZP_01736494.1	hypothetical protein MELB17_23005 [Marinobacter sp. ELB17] hypothetical protein MELB17_23005 [Marinobacter sp. ELB17]		
Contig[0056]	gi 119502998 ref ZP_01625083.1	hypothetical protein MGP2080_06817 [marine gamma proteobacterium HTCC2080] hypothetical protein MGP2080_06817 [marine gamma proteobacterium HTCC2080]		
Contig[0074]	gi 85057541 ref YP_456457.1	hypothetical protein AYWB_261 [Aster yellows witches'-broom phytoplasma AYWB] hypothetical protein AYWB_471 [Aster yellows witches'-broom phytoplasma AYWB] conserved hypothetical protein [Aster yellows witches'-broom phytoplasma AYWB] conserved hypothetical protein [Aster yellows witches'-broom phytoplasma AYWB]		
Contig[0090]	gi 110598319 ref ZP_01386593.1	Protein of unknown function DUF125, transmembrane [Chlorobium ferrooxidans DSM 13031] Protein of unknown function DUF125, transmembrane [Chlorobium ferrooxidans DSM 13031]		
Contig[0102]	gi 118637026 emb CAI77913.1	aldo/keto reductase precursor [Guillardia theta]	P0A9T5 TAS_SHIFL	Protein tas - <i>Shigella flexneri</i>
Contig[0105]	gi 145344249 ref XP_001416649.1	MC family transporter: carnitine/acylcarnitine [Ostreococcus lucimarinus CCE9901] MC family transporter: carnitine/acylcarnitine [Ostreococcus lucimarinus CCE9901]		
Contig[0110]	gi 149726847 ref XP_001502587.1	PREDICTED: similar to C11 protein [Equus caballus]	P62498 ERF1_XENTR	Eukaryotic peptide chain release factor subunit 1 - <i>Xenopus tropicalis</i> (Western clawed frog) (<i>Silurana tropicalis</i>)
Contig[0129]	gi 71279547 ref YP_270885.1	membrane protein [Colwellia psychrerythraea 34H] membrane protein [Colwellia psychrerythraea 34H]		
Contig[0229]	gi 145352312 ref XP_001420494.1	predicted protein [Ostreococcus lucimarinus CCE9901] predicted protein [Ostreococcus lucimarinus CCE9901]		
Contig[0256]	gi 115379856 ref ZP_01466920.1	CsgA [Stigmatella aurantiaca DW4/3-1] CsgA [Stigmatella aurantiaca DW4/3-1]	Q5R6U1 DRS7B_PONPY	Dehydrogenase/reductase SDR family member 7B - <i>Pongo pygmaeus</i> (Bornean orangutan)

Table C.1: Combined GenBank and UniProt summary information for stage 2 “either” gene fragments. – Continued

Clone ID	Top GenBank blastx hit	Top GenBank blastx description	Uniprot top hit	Uniprot top hit description
Contig[0294]	gi 50312719 ref XP_453843.1	unnamed protein product [Kluyveromyces lactis] unnamed protein product [Kluyveromyces lactis NRRL Y-1140]		
Contig[0300]	gi 33637548 gb AAQ23918.1	metallothionein IIIB [Crassostrea vir- ginica]		
Contig[0308]	gi 150410121 gb EDN05509.1	adenine phosphoribosyltransferase 1 [Ajellomyces capsulatus NAM1]		
Contig[0310]	gi 84701662 ref ZP_01016237.1	hypothetical protein PB2503_01852 [Parvularcula bermudensis HTCC2503] hypothetical protein PB2503_01852 [Parvularcula bermudensis HTCC2503]		
Contig[0316]	gi 148550720 ref YP_001260159.1	hypothetical protein Swit_5284 [Sphin- gomonas wittichii RW1] conserved hypo- thetical protein [Sphingomonas wittichii RW1]		
Contig[0336]	gi 87306455 ref ZP_01088602.1	Zinc-containing alcohol dehydrogenase superfamily protein [Blastopirellula ma- rina DSM 3645] Zinc-containing alco- hol dehydrogenase superfamily protein [Blastopirellula marina DSM 3645]		
Contig[0363]	gi 148656395 ref YP_001276600.1	protein tyrosine/serine phosphatase [Roseiflexus sp. RS-1] protein tyro- sine/serine phosphatase [Roseiflexus sp. RS-1]		
Contig[0432]	gi 82541487 ref XP_724982.1	hypothetical protein PY04653 [Plas- modium yoelii yoelii str. 17XNL] hy- pothetical protein [Plasmodium yoelii yoelii]		
Contig[0474]	gi 116254030 ref YP_769868.1	putative NTP pyrophosphohydrolase [Rhizobium leguminosarum bv. viciae 3841]		
Contig[0490]	gi 125841193 ref XP_693754.2	PREDICTED: similar to D-aspartate oxidase [Danio rerio]	A3KCL7 OXDD_PIG	D-aspartate oxidase - Sus scrofa (Pig)
Contig[0498]	gi 120404255 ref YP_954084.1	Catechol O-methyltransferase [My- cobacterium vanbaalenii PYR-1] Catechol O-methyltransferase [My- cobacterium vanbaalenii PYR-1]		
Contig[0505]	gi 29565713 ref NP_817269.1	ORF64c [Pinus koraiensis]		
Contig[0555]	gi 120404255 ref YP_954084.1	Catechol O-methyltransferase [My- cobacterium vanbaalenii PYR-1] Catechol O-methyltransferase [My- cobacterium vanbaalenii PYR-1]		
Contig[0568]	gi 145846039 gb EDK22957.1	hypothetical protein RUMTOR_02877 [Ruminococcus torques ATCC 27756] hypothetical protein RUMTOR_02846 [Ruminococcus torques ATCC 27756] hypothetical protein RUMTOR_02820 [Ruminococcus torques ATCC 27756] hypothetical protein RUMTOR_02777 [Ruminococcus torques ATCC 27756] hypothetical protein RUMTOR_02003 [Ruminococcus torques ATCC 27756] hypothetical protein RUMTOR_01389 [Ruminococcus torques ATCC 27756]	Q9PLI5 Y114_CHLMU	Uncharacterized protein TC_0114 - Chlamydia muridarum
Contig[0655]	gi 945054 gb AAA74445.1	cathepsin B-like protease		

Table C.1: Combined GenBank and UniProt summary information for stage 2 “either” gene fragments. – Continued

Clone ID	Top GenBank blastx hit	Top GenBank blastx description	Uniprot top hit	Uniprot top hit description
Contig[0657]	gi 119502998 ref ZP_01625083.1	hypothetical protein MGP2080_06817 [marine gamma proteobacterium HTCC2080] hypothetical protein MGP2080_06817 [marine gamma proteobacterium HTCC2080]		
Contig[0689]	gi 121914819 gb EAY19614.1	TKL family protein kinase [Trichomonas vaginalis G3]		
Contig[0703]	gi 85057541 ref YP_456457.1	hypothetical protein AYWB_261 [Aster yellows witches'-broom phytoplasma AYWB] hypothetical protein AYWB_471 [Aster yellows witches'-broom phytoplasma AYWB] conserved hypothetical protein [Aster yellows witches'-broom phytoplasma AYWB] conserved hypothetical protein [Aster yellows witches'-broom phytoplasma AYWB]		
Contig[0717]	gi 116254030 ref YP_769868.1	putative NTP pyrophosphohydrolase [Rhizobium leguminosarum bv. viciae 3841] putative NTP pyrophosphohydrolase [Rhizobium leguminosarum bv. viciae 3841]		
Contig[0757]	gi 30580468 sp Q9SMH3	DYH1A_CHLREDynein-1-alpha heavy chain, flagellar inner arm II complex (1-alpha DHC) (Dynein-1, subspecies f) 1-alpha dynein heavy chain [Chlamydomonas reinhardtii]		
Contig[0763]	gi 945054 gb AAA74445.1	cathepsin B-like protease		
Contig[0785]	gi 86156860 ref YP_463645.1	Inositol monophosphatase/ADP-ribosylglycohydrolase [Anaeromyxobacter dehalogenans 2CP-C] Inositol monophosphatase/ADP-ribosylglycohydrolase [Anaeromyxobacter dehalogenans 2CP-C]		
Contig[0793]	gi 118472396 ref YP_890517.1	glyoxylate reductase [Mycobacterium smegmatis str. MC2 155] glyoxylate reductase [Mycobacterium smegmatis str. MC2 155]		
Contig[0798]	gi 82541487 ref XP_724982.1	hypothetical protein PY04653 [Plasmodium yoelii yoelii str. 17XNL] hypothetical protein [Plasmodium yoelii yoelii]		
Contig[0812]	gi 94468884 gb ABF18291.1	multifunctional 14-3-3 family chaperone [Aedes aegypti] 14-3-3 protein sigma, gamma, zeta, beta/alpha [Aedes aegypti]	Q1HR36 1433Z_AEDAE	14-3-3 protein zeta - Aedes aegypti (Yellowfever mosquito)
Contig[0849]	gi 85057541 ref YP_456457.1	hypothetical protein AYWB_261 [Aster yellows witches'-broom phytoplasma AYWB] hypothetical protein AYWB_471 [Aster yellows witches'-broom phytoplasma AYWB] conserved hypothetical protein [Aster yellows witches'-broom phytoplasma AYWB] conserved hypothetical protein [Aster yellows witches'-broom phytoplasma AYWB]		

C.2 Summary of Gene Ontology (GO) organizing principles for “either” in stage 2 of lichen development

Functional classification of ESTs. To group SSH-cDNA sequences into functional categories, Blast2GO (Conesa *et al.*, 2005) was used to compare each sequence to the GenBank nr protein database, and subsequently Gene Ontology (GO; <http://www.geneontology.org>) terms were extracted from associated annotated blast hits. GO terms were organized by organizing principle (biological process, cellular compartment and molecular function), and the first three levels of GO terms for stage 2 can be seen in Table C.2. Thirty-eight stage 2 gene fragments had a significant blastx match, and of these 14 had at least one associated GO term (GO database accessed 18 May, 2008). A key for linking GO terms to individual Clone IDs can be found in Table C.3

Table C.2: GO categorization of a non-normalized SSH library of 14 gene products for “either” in stage 2 of lichen development. Individual gene products may be represented by more than one GO term, and subordinate terms can represent multiple higher-level terms. First 3 levels of GO terms annotated through increasing indentation.

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
BIOLOGICAL PROCESS	GO:0008150	5	100%
biological regulation	GO:0065007	2	40%
cellular process	GO:0009987	5	100%
developmental process	GO:0032502	2	40%
metabolic process	GO:0008152	2	40%
multicellular organismal process	GO:0032501	1	20%
reproduction	GO:0000003	1	20%
response to stimulus	GO:0050896	1	20%
anatomical structure development	GO:0048856	2	40%
anatomical structure formation	GO:0048646	1	20%
anatomical structure morphogenesis	GO:0009653	2	40%
anatomical structure organization	GO:0048532	2	40%
behavior	GO:0007610	1	20%
biosynthetic process	GO:0009058	2	40%
cell communication	GO:0007154	1	20%
cell cycle	GO:0007049	1	20%
cell development	GO:0048468	1	20%
cell fate commitment	GO:0045165	1	20%
cell fate determination	GO:0001709	1	20%

Table C.2 Stage 2 “either” Gene Ontology summary – Continued

Gene Ontology Organizing Principle and Terms	GO ID	Total	Percentage
cell proliferation	GO:0008283	1	20%
cellular component organization	GO:0016043	2	40%
cellular developmental process	GO:0048869	1	20%
cellular metabolic process	GO:0044237	2	40%
chromosome segregation	GO:0007059	1	20%
embryonic development	GO:0009790	1	20%
macromolecule metabolic process	GO:0043170	1	20%
multicellular organismal development	GO:0007275	1	20%
neurological system process	GO:0050877	1	20%
nitrogen compound metabolic process	GO:0006807	1	20%
pattern specification process	GO:0007389	1	20%
primary metabolic process	GO:0044238	2	40%
regulation of biological process	GO:0050789	2	40%
regulation of cellular process	GO:0050794	2	40%
regulation of metabolic process	GO:0019222	1	20%
regulation of molecular function	GO:0065009	1	20%
response to chemical stimulus	GO:0042221	1	20%
sexual reproduction	GO:0019953	1	20%
CELLULAR COMPONENT	GO:0005575	2	100%
biological regulation	GO:0005623	2	100%
biological regulation	GO:0044464	2	100%
biological regulation	GO:0005576	1	50%
biological regulation	GO:0044421	1	50%
biological regulation	GO:0043226	1	50%
intercellular bridge	GO:0045171	1	50%
intracellular	GO:0005622	2	100%
intracellular organelle	GO:0043229	1	50%
intracellular part	GO:0044424	2	100%
membrane-bounded organelle	GO:0043227	1	50%
MOLECULAR FUNCTION	GO:0003674	7	100%
biological regulation	GO:0005488	5	71%
biological regulation	GO:0003824	2	29%
biological regulation	GO:0030234	1	14%
biological regulation	GO:0045182	1	14%
enzyme activator activity	GO:0008047	1	14%
enzyme inhibitor activity	GO:0004857	1	14%
kinase regulator activity	GO:0019207	1	14%
nucleic acid binding	GO:0003676	1	14%
oxidoreductase activity	GO:0016491	1	14%
protein binding	GO:0005515	2	29%
ribonucleoprotein binding	GO:0043021	1	14%
translation factor activity, nucleic acid binding	GO:0008135	1	14%

Table C.3: Key for linking stage 2 “either” GO terms and Clone ID.

Level	GO ID	GO TERM	Clone ID
1	GO:0008150	BIOLOGICAL PROCESS	Contig[0798], Contig[0432], Contig[0294], Contig[0812], Contig[0110]
2	GO:0065007	biological regulation	Contig[0812], Contig[0110]
2	GO:0009987	cellular process	Contig[0798], Contig[0432], Contig[0294], Contig[0812], Contig[0110]
2	GO:0032502	developmental process	Contig[0812], Contig[0110]
2	GO:0008152	metabolic process	Contig[0812], Contig[0110]
2	GO:0032501	multicellular organismal process	Contig[0812]
2	GO:0000003	reproduction	Contig[0812]
2	GO:0050896	response to stimulus	Contig[0812]
3	GO:0048856	anatomical structure development	Contig[0812], Contig[0110]
3	GO:0048646	anatomical structure formation	Contig[0812]
3	GO:0009653	anatomical structure morphogenesis	Contig[0812], Contig[0110]
3	GO:0048532	anatomical structure organization	Contig[0110], Contig[0812]
3	GO:0007610	behavior	Contig[0812]
3	GO:0009058	biosynthetic process	Contig[0812], Contig[0110]
3	GO:0007154	cell communication	Contig[0812]
3	GO:0007049	cell cycle	Contig[0812]
3	GO:0048468	cell development	Contig[0812]
3	GO:0045165	cell fate commitment	Contig[0812]
3	GO:0001709	cell fate determination	Contig[0812]
3	GO:0008283	cell proliferation	Contig[0812]
3	GO:0016043	cellular component organization	Contig[0110], Contig[0812]
3	GO:0048869	cellular developmental process	Contig[0812]
3	GO:0044237	cellular metabolic process	Contig[0812], Contig[0110]
3	GO:0007059	chromosome segregation	Contig[0812]
3	GO:0009790	embryonic development	Contig[0812]
3	GO:0043170	macromolecule metabolic process	Contig[0110]
3	GO:0007275	multicellular organismal development	Contig[0812]
3	GO:0050877	neurological system process	Contig[0812]
3	GO:0006807	nitrogen compound metabolic process	Contig[0812]
3	GO:0007389	pattern specification process	Contig[0812]
3	GO:0044238	primary metabolic process	Contig[0812], Contig[0110]
3	GO:0050789	regulation of biological process	Contig[0812], Contig[0110]
3	GO:0050794	regulation of cellular process	Contig[0812], Contig[0110]
3	GO:0019222	regulation of metabolic process	Contig[0110]
3	GO:0065009	regulation of molecular function	Contig[0812]
3	GO:0042221	response to chemical stimulus	Contig[0812]
3	GO:0019953	sexual reproduction	Contig[0812]

Table C.3: Key for linking stage 2 “either” GO and Clone ID – Continued

Level	GO ID	GO TERM	Clone ID
1	GO:0005575	CELLULAR COMPONENT	Contig[0812], Contig[0110]
2	GO:0005623	cell	Contig[0812], Contig[0110]
2	GO:0044464	cell part	Contig[0812], Contig[0110]
2	GO:0005576	extracellular region	Contig[0812]
2	GO:0044421	extracellular region part	Contig[0812]
2	GO:0043226	organelle	Contig[0812]
3	GO:0045171	intercellular bridge	Contig[0812]
3	GO:0005622	intracellular	Contig[0812], Contig[0110]
3	GO:0043229	intracellular organelle	Contig[0812]
3	GO:0044424	intracellular part	Contig[0812], Contig[0110]
3	GO:0043227	membrane-bounded organelle	Contig[0812]
1	GO:0003674	MOLECULAR FUNCTION	Contig[0812], Contig[0110], Contig[0040], Contig[0798], Contig[0432], Contig[0294], Contig[0490]
2	GO:0005488	binding	Contig[0812], Contig[0110], Contig[0798], Contig[0432], Contig[0294]
2	GO:0003824	catalytic activity	Contig[0040], Contig[0490]
2	GO:0030234	enzyme regulator activity	Contig[0812]
2	GO:0045182	translation regulator activity	Contig[0110]
3	GO:0008047	enzyme activator activity	Contig[0812]
3	GO:0004857	enzyme inhibitor activity	Contig[0812]
3	GO:0019207	kinase regulator activity	Contig[0812]
3	GO:0003676	nucleic acid binding	Contig[0110]
3	GO:0016491	oxidoreductase activity	Contig[0490]
3	GO:0005515	protein binding	Contig[0812], Contig[0110]
3	GO:0043021	ribonucleoprotein binding	Contig[0110]
3	GO:0008135	translation factor activity, nucleic acid binding	Contig[0110]

Bibliography

- Adl SM, Simpson AGB, Farmer MA, Andersen RA, Anderson OR, Barta JR, Bowser SS, Brugerolle G, Fensome RA, Fredericq S, James TY, Karpov S, Kugrens P, Krug J, Lane CE, Lewis LA, Lodge J, Lynn DH, Mann DG, McCourt RM, Mendoza L, Moestrup O, Mozley-Standridge SE, Nerad TA, Shearer CA, Smirnov AV, Spiegel FW, Taylor M (2005) The new higher level classification of eukaryotes with emphasis on the taxonomy of protists. *Journal of Eukaryotic Microbiology* 52(5):399–451.
- Adomas A, Heller G, Olson A, Osborne J, Karlsson M, Nahalkova J, Van Zyl L, Sederoff R, Stenlid J, Finlay R, Asiegbu FO (2008) Comparative analysis of transcript abundance in *Pinus sylvestris* after challenge with a saprotrophic, pathogenic or mutualistic fungus. *Tree Physiology* 28(6):885–897.
- Ahmadjian V (1959) A contribution toward lichen synthesis. *Mycologia* 51(1):56–60.
- Ahmadjian V (1966) Lichens. In Henry, SM, ed., *Symbiosis*. Academic Press, New York, pp. 35–97.
- Ahmadjian V (1967) *The Lichen Symbiosis*. Blaisdell Pub., Waltham, Mass, 1st ed.
- Ahmadjian V (1982) Algal/fungal symbioses. In Round, RE and Chapman, D J, ed., *Progress in Phycological Research*, vol. 1. Elsevier Biomedical Press, Amsterdam, pp. 179–223.
- Ahmadjian V (1992) Basic mechanisms of signal exchange, recognition, and regulation in lichens. In *Algae and Symbioses: Plants, Animals, Fungi, Viruses, Interactions Explored*. Biopress Ltd., Bristol, pp. 675–697.
- Ahmadjian V, Jacobs JB (1981) Relationship between fungus and alga in the lichen *Cladonia cristatella* Tuck. *Nature* 289(5794):169–172.

- Ahmadjian V, Jacobs JB, Russell LA (1978) Scanning electron-microscope study of early lichen synthesis. *Science* 200(4345):1062–1064.
- Ahmadjian V, Russell LA, Hildreth KC (1980) Artificial reestablishment of lichens. I. Morphological interactions between the phycobionts of different lichens and the mycobionts *Cladonia cristatella* and *Lecanora chrysoleuca*. *Mycologia* 72(1):73–89.
- Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of Molecular Biology* 215(3):403–410.
- Armaleo D (1991) Experimental microbiology of lichens - mycelia fragmentation, a novel growth chamber, and the origins of thallus differentiation. *Symbiosis* 11(2-3):163–177.
- Armaleo D, May S (2009) Sizing the fungal and algal genomes of the lichen *Cladonia grayi* through quantitative PCR. *Symbiosis* (*in press*).
- Armaleo D, Miao V (1999) Symbiosis and DNA methylation in the *Cladonia* lichen fungus. *Symbiosis* 26(2):143–163.
- Arnold AE, Miadlikowska J, Higgins KL, Sarvate SD, Gugger P, Way A, Hofstetter V, Kauff F, Lutzoni F (2009) A phylogenetic estimation of trophic transition networks for ascomycetous fungi: Are lichens cradles of symbiotrophic fungal diversification? *Systematic Biology* (*In press*).
- Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM, Davis AP, Dolinski K, Dwight SS, Eppig JT, Harris MA, Hill DP, Issel-Tarver L, Kasarskis A, Lewis S, Matese JC, Richardson JE, Ringwald M, Rubin GM, Sherlock G, Gene Ontology C (2000) Gene Ontology: tool for the unification of biology. *Nature Genetics* 25(1):25–29.
- Backor M, Fahselt D (2003) Cellulose-acetate disks as novel substrate for the resynthesis and culture of lichens. *Bryologist* 106(3):439–442.
- Bago B, Zipfel W, Williams RM, Jun J, Arreola R, Lammers PJ, Pfeffer PE, Shachar-Hill Y (2002) Translocation and utilization of fungal storage lipid in the arbuscular mycorrhizal symbiosis. *Plant Physiology* 128(1):108–124.
- Bairoch A, Bougueleret L, Altairac S, Amendolia V, Auchincloss A, Puy GA, Axelsen K, Baratin D, Blatter MC, Boeckmann B, Bollondi L, Boutet E, Quintaje SB,

- Breuzer L, Bridge A, Saux VBL, deCastro E, Ciampina L, Coral D, Coudert E, Cusin I, David F, Delbard G, Dornevil D, Duek-Roggli P, Duvaud S, Estreicher A, Famiglietti L, Farriol-Mathis N, Ferro S, Feuermann M, Gasteiger E, Gateau A, Gehant S, Gerritsen V, Gos A, Gruaz-Gumowski N, Hinz U, Hulo C, Hulo N, Innocenti A, James J, Jain E, Jimenez S, Jungo F, Junker V, Keller G, Lachaize C, Lane-Guermontprez L, Langendijk-Genevaux P, Lara V, Le Mercier P, Lieberherr D, Lima TD, Mangold V, Martin X, Michoud K, Moinat M, Morgat A, Nicolas M, Paesano S, Pedruzzi I, Perret D, Phan I, Pilbout S, Pillet V, Poux S, Pozzato M, Redaschi N, Reynaud S, Rivoire C, Roehert B, Sapsezian C, Schneider M, Sigrist C, Sonesson K, Staehli S, Stutz A, Sundaram S, Tognolli M, Verbregue L, Veuthey AL, Vitorello C, Yip L, Zuletta LF, Apweiler R, Alam-Faruque Y, Barrell D, Bower L, Browne P, Chan WM, Daugherty L, Donate ES, Eberhardt R, Fedotov A, Foulger R, Frigerio G, Garavelli J, Golin R, Horne A, *et al.* (2008) The Universal Protein Resource (UniProt). *Nucleic Acids Research* 36:D190–D195.
- Balguerie A, Dos Reis S, Ritter C, Chaignepain S, Coullary-Salin B, Forge V, Bathany K, Lascu I, Schmitter JM, Riek R, Saupe SJ (2003) Domain organization and structure-function relationship of the HET-s prion protein of *Podospora anserina*. *Embo Journal* 22(9):2071–2081.
- Bari R, Jones J (2009) Role of plant hormones in plant defence responses. *Plant Molecular Biology* 69(4):473–488.
- Baron C, Zambryski PC (1995) The plant response in pathogenesis, symbiosis, and wounding: Variations on a common theme? *Annual Review of Genetics* 29:107–129.
- de Bary H (1879) *Die Erscheinung der Symbiose*. Privately printed in Strasburg. (Translated from German by Leon Shernoff, 2004, In, *The Origins of Symbiosis*).
- Bendtsen JD, Nielsen H, von Heijne G, Brunak S (2004) Improved prediction of signal peptides: SignalP 3.0. *Journal of Molecular Biology* 340(4):783–795.
- Berto P, Comm  nil P, Belingheri L, Dehorter B (1999) Occurrence of a lipase in spores of *Alternaria brassicicola* with a crucial role in the infection of cauliflower leaves. *Fems Microbiology Letters* 180(2):183–189.
- Bonnier G (1888) Germination des spores de lichens sur les proton  mas des mousses et sur des Algues diff  rant des gonidies du Lichen. *Comptes Rendus Hebdomadaires des S  ances et M  moires de la Soci  t   de Biologie* 40:541–543.

- Bonnier G (1889a) Germination des lichens sur les protonemas des mousses. *Revue Générale de Botanique* 1:165–169.
- Bonnier G (1889b) Recherches sur la synthèse des lichens. In *Annales des Sciences Naturelles, Botanique VII*, vol. 9. s.n., Paris, pp. 1–34.
- Bornet E (1873) Recherches sur les gonidies des lichens. In *Annales des Sciences Naturelles, Botanique V*, vol. 17. s.n., Paris, pp. 45–110.
- Bubrick P, Frensdorff A, Galun M (1985) Selectivity in the lichen symbiosis. In Brown, D H, ed., *Lichen Physiology and Cell Biology*. Plenum, New York, pp. 319–334.
- Burke DT, Dawson D, Stearns T (2000) *Methods in Yeast Genetics: A Cold Spring Harbor Laboratory Course Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Caturla M, Chaparro C, Schroeyers K, Holsters M (2002) Suppression subtractive hybridization to enrich low-abundance and submergence-enhanced transcripts of adventitious root primordia of *Sesbania rostrata*. *Plant Science* 162(6):915–921.
- Chacón MR, Rodríguez-Galán O, Benítez T, Sousa S, Rey M, Llobell A, Delgado-Jarana J (2007) Microscopic and transcriptome analyses of early colonization of tomato roots by *Trichoderma harzianum*. *International Microbiology* 10(1):19–27.
- Cheng J, Randall AZ, Sweredoski MJ, Baldi P (2005) SCRATCH: a protein structure and structural feature prediction server. *Nucleic Acids Research* 33:W72–W76.
- Chu FH, Wang SY, Lee LC, Shaw JF (2008) Identification and characterization of a lipase gene from *Antrodia cinnamomea*. *Mycological Research* 112:1421–1427.
- Clayden SR (1998) Thallus initiation and development in the lichen *Rhizocarpon lecanorinum*. *New Phytologist* 139(4):685–695.
- Comménil P, Belingheri L, Bauw G, Dehorter B (1999) Molecular characterization of a lipase induced in *Botrytis cinerea* by components of grape berry cuticle. *Physiological and Molecular Plant Pathology* 55(1):37–43.

- Comménil P, Belingheri L, Dehorter B (1998) Antilipase antibodies prevent infection of tomato leaves by *Botrytis cinerea*. *Physiological and Molecular Plant Pathology* 52(1):1–14.
- Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M (2005) Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21(18):3674–3676.
- Dean RA (1997) Signal pathways and appressorium morphogenesis. *Annual Review of Phytopathology* 35:211–234.
- Deason T, Bold H (1960) *Exploratory studies of Texas soil algae*, vol. No. 6022. U. Texas Pub.
- Deguchi Y, Banba M, Shimoda Y, Chechetka SA, Suzuri R, Okusako Y, Ooki Y, Toyokura K, Suzuki A, Uchiumi T, Higashi S, Abe M, Kouchi H, Izui K, Hata S (2007) Transcriptome profiling of *Lotus japonicus* roots during arbuscular mycorrhiza development and comparison with that of nodulation. *DNA Research* 14(3):117–133.
- Derelle E, Ferraz C, Rombauts S, Rouzé P, Worden AZ, Robbens S, Partensky F, Degroeve S, Echeynié S, Cooke R, Saeys Y, Wuyts J, Jabbari K, Bowler C, Panaud O, Piégu B, Ball SG, Ral JP, Bouget FY, Piganeau G, De Baets B, Picard A, Delseny M, Demaille J, Van de Peer Y, Moreau H (2006) Genome analysis of the smallest free-living eukaryote *Ostreococcus tauri* unveils many unique features. *PNAS* 103(31):11647–11652.
- Derewenda U, Brzozowski AM, Lawson DM, Derewenda ZS (1992a) Catalysis at the interface - the anatomy of a conformational change in a triglyceride lipase. *Biochemistry* 31(5):1532–1541.
- Derewenda ZS, Derewenda U, Dodson GG (1992b) The crystal and molecular-structure of the *Rhizomucor miehei* triacylglyceride lipase at 1.9-angstrom resolution. *Journal of Molecular Biology* 227(3):818–839.
- Diatchenko L, Lau YFC, Campbell AP, Chenchik A, Moqadam F, Huang B, Lukyanov S, Lukyanov K, Gurskaya N, Sverdlov ED, Siebert PD (1996) Suppression subtractive hybridization: A method for generating differentially regulated or tissue-specific cDNA probes and libraries. *Proceedings of the National Academy of Sciences of the United States of America* 93(12):6025–6030.

- Domingo C, Andrés F, Tharreau D, Iglesias DJ, Talón M (2009) Constitutive expression of OsGH3.1 reduces auxin content and enhances defense response and resistance to a fungal pathogen in rice. *Molecular Plant-Microbe Interactions* 22(2):201–210.
- Domozych DS, Stewart KD, Mattox KR (1980) The comparative aspects of cell-wall chemistry in the green-algae (Chlorophyta). *Journal of Molecular Evolution* 15(1):1–12.
- Duplessis S, Courty PE, Tagu D, Martin F (2005) Transcript patterns associated with ectomycorrhiza development in *Eucalyptus globulus* and *Pisolithus microcarpus*. *New Phytologist* 165(2):599–611.
- Eckhardt NA (2008) Chitin Signaling in Plants: Insights into the Perception of Fungal Pathogens and Rhizobacterial Symbionts. *Plant Cell* 20:241–243.
- Ellis EM, Slattery CM, Hayes JD (2003) Characterization of the rat aflatoxin B-1 aldehyde reductase gene, AKR7A1. Structure and chromosomal localization of AKR7A1 as well as identification of antioxidant response elements in the gene promoter. *Carcinogenesis* 24(4):727–737.
- Emanuelsson O, Nielsen H, Brunak S, von Heijne G (2000) Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. *Journal of Molecular Biology* 300(4):1005–1016.
- Epstein E, Sagee O, Cohen JD, Garty J (1986) Endogenous auxin and ethylene in the lichen *Ramalina duriaei*. *Plant Physiology* 82(4):1122–1125.
- Fedorova ND, Badger JH, Robson GD, Wortman JR, Nierman WC (2005) Comparative analysis of programmed cell death pathways in filamentous fungi. *BMC Genomics* 6:14.
- Feng J, Liu GS, Selvaraj G, Hughes GR, Wei YD (2005) A secreted lipase encoded by LIP1 is necessary for efficient use of saturated triglyceride lipids in *Fusarium graminearum*. *Microbiology-Sgm* 151:3911–3921.
- Gácsér A, Stehr F, Kröger C, Kredics L, Schäfer W, Nosanchuk JD (2007) Lipase 8 affects the pathogenesis of *Candida albicans*. *Infection and Immunity* 75(10):4710–4718.

- Galun M (1988) Recognition in fungus alga interaction in lichens. *Phytoparasitica* 16(2):190–190.
- Galun M, Garty J (1988) Soredia formation of compatible and incompatible lichen symbionts. In Cannerini, SS and Bonfante-Fasolo, P and Gianinazzi-Pearson, V, ed., *Cell to cell signals in plant, animal, and microbial symbiosis*, vol. H17 of *NATO ASI series.; Series H,; Cell biology ;; vol. 17;* . Springer-Verlag, Berlin, pp. 207–217.
- Gaßmann A, Ott S (2000) Growth strategy and the gradual symbiotic interactions of the lichen *Ochrolechia frigida*. *Plant Biology* 2(3):368–378.
- Giovannetti M, Avio L, Sbrana C, Citerinesi AS (1993) Factors affecting appressorium development in the vesicular arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol & Gerd) Gerd and Trappe. *New Phytologist* 123(1):115–122.
- Glass NL, Kaneko I (2003) Fatal attraction: Nonspecific recognition and heterokaryon incompatibility in filamentous fungi. *Eukaryotic Cell* 2(1):1–8.
- Green TGA, Smith D (1974) Lichen Physiology XIV. Differences between lichen algae in symbiosis and in isolation. *The New Phytologist* 73(4):753 – 766.
- Gruen HE (1959) Auxins and fungi. *Annual Review of Plant Physiology and Plant Molecular Biology* 10:405–440.
- Guschina IA, Dobson G, Harwood JL (2003) Lipid metabolism in cultured lichen photobionts with different phosphorus status. *Phytochemistry* 64(1):209–217.
- Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. *Annual Review of Microbiology* 59:19–42.
- Herrero S, Amnuaykanjanasin A, Daub ME (2007) Identification of genes differentially expressed in the phytopathogenic fungus *Cercospora nicotianae* between cercosporin toxin-resistant and -susceptible strains. *FEMS Microbiology Letters* 275(2):326–337.
- Hill DJ (1989) The control of the cell-cycle in microbial symbionts. *New Phytologist* 112(2):175–184.

- Hill DJ (1992) Lobe growth in lichen-thalli. *Symbiosis* 12(1):43–55.
- Holstege FCP, Jennings EG, Wyrick JJ, Lee TI, Hengartner CJ, Green MR, Golub TR, Lander ES, Young RA (1998) Dissecting the regulatory circuitry of a eukaryotic genome. *Cell* 95(5):717–728.
- Honegger R (1986a) Ultrastructural studies in lichens. I. Haustorial types and their frequencies in a range of lichens with trebouxoid photobionts. *New Phytologist* 103(4):785–795.
- Honegger R (1986b) Ultrastructural studies in lichens. II. Mycobiont and photobiont cell-wall surface-layers and adhering crystalline lichen products in 4 Parmeliaceae. *New Phytologist* 103(4):797–808.
- Honegger R (1990) Surface interactions in lichens. In Wiessner, W and Robinson, DG and Starr, RC, ed., *Experimental phycology . Cell walls and surfaces, reproduction, photosynthesis*. Springer, Berlin, pp. 40–54.
- Honegger R (1993) Developmental biology of lichens. *New Phytologist* 125(4):659–677.
- Honegger R (1997) Metabolic interactions at the mycobiont–photobiont interface in lichens. In *The Mycota V, Part A, Plant relationships*, vol. V(A). Springer-Verlag, Berlin-Heidelberg, Germany, pp. 209–221.
- Honegger R (1998) The Lichen symbiosis - What is so spectacular about it? *Lichenologist* 30:193–212.
- Hopper ED, Pittman AMC, Fitzgerald MC, Tucker CL (2008) In vivo and in vitro examination of stability of primary hyperoxaluria-associated human alanine: glyoxylate aminotransferase. *Journal of Biological Chemistry* 283(45):30493–30502.
- Hunter S, Apweiler R, Attwood TK, Bairoch A, Bateman A, Binns D, Bork P, Das U, Daugherty L, Duquenne L, Finn RD, Gough J, Haft D, Hulo N, Kahn D, Kelly E, Laugraud A, Letunic I, Lonsdale D, Lopez R, Madera M, Maslen J, McAnulla C, McDowall J, Mistry J, Mitchell A, Mulder N, Natale D, Orengo C, Quinn AF, Selengut JD, Sigrist CJA, Thimma M, Thomas PD, Valentin F, Wilson D, Wu CH, Yeats C (2009) InterPro: the integrative protein signature database. *Nucleic Acids Research* 37:D211–D215.

- James TY, Kauff F, Schoch CL, Matheny PB, Hofstetter V, Cox CJ, Celio G, Gueidan C, Fraker E, Miadlikowska J, Lumbsch HT, Rauhut A, Reeb V, Arnold AE, Amtoft A, Stajich JE, Hosaka K, Sung GH, Johnson D, O'Rourke B, Crockett M, Binder M, Curtis JM, Slot JC, Wang Z, Wilson AW, Schussler A, Longcore JE, O'Donnell K, Mozley-Standridge S, Porter D, Letcher PM, Powell MJ, Taylor JW, White MM, Griffith GW, Davies DR, Humber RA, Morton JB, Sugiyama J, Rossman AY, Rogers JD, Pfister DH, Hewitt D, Hansen K, Hambleton S, Shoemaker RA, Kohlmeyer J, Volkmann-Kohlmeyer B, Spotts RA, Serdani M, Crous PW, Hughes KW, Matsuura K, Langer E, Langer G, Untereiner WA, Lucking R, Budel B, Geiser DM, Aptroot A, Diederich P, Schmitt I, Schultz M, Yahr R, Hibbett DS, Lutzoni F, McLaughlin DJ, Spatafora JW, Vilgalys R (2006) Reconstructing the early evolution of Fungi using a six-gene phylogeny. *Nature* 443(7113):818–822.
- Jelitto TC, Page HA, Read ND (1994) Role of external signals in regulating the pre-penetration phase of infection by the rice blast fungus, *Magnaporthe grisea*. *Planta* 194(4):471–477.
- Jensen RG (1983) Detection and determination of lipase (acylglycerol hydrolase) activity from various sources. *Lipids* 18(9):650–657.
- Johansson T, Le Quéré A, Ahren D, Söderström B, Erlandsson R, Lundeberg J, Uhlen M, Tunlid A (2004) Transcriptional responses of *Paxillus involutus* and *Betula pendula* during formation of ectomycorrhizal root tissue. *Molecular Plant-Microbe Interactions* 17(2):202–215.
- Joneson S, Armaleo D, Lutzoni F (2009) Fungal gene expression in early symbiotic lichen development. *In Prep.*
- Joneson S, Lutzoni F (2009) Compatibility and thigmotropism in the lichen symbiosis: A reappraisal. *Symbiosis* 47:109–115.
- Kasprzewska A (2003) Plant chitinases - Regulation and function. *Cellular & Molecular Biology Letters* 8(3):809–824.
- Kershaw KA, Millbank JW (1970) Nitrogen metabolism in lichens. 2. Partition of cephalodial-fixed nitrogen between mycobiont and phycobionts of *Peltigera aphthosa*. *New Phytologist* 69(1):75–79.
- Kim YK, Li DX, Kolattukudy PE (1998) Induction of Ca²⁺-calmodulin signaling by hard-surface contact primes *Colletotrichum gloeosporioides* conidia to germinate and form appressoria. *Journal of Bacteriology* 180(19):5144–5150.

- Kirk P, Ainsworth G, Bisby G (2008) *Dictionary of the fungi*. CABI Publishing, Wallingford, UK, 10th ed.
- Kohno M, Funatsu J, Mikami B, Kugimiya W, Matsuo T, Morita Y (1996) The crystal structure of lipase II from *Rhizopus niveus* at 2.2 angstrom resolution. *Journal of Biochemistry* 120(3):505–510.
- Kolattukudy PE, Li DX, Hwang CS, Flaishman MA (1995) Host signals in fungal gene-expression involved in penetration into the host. *Canadian Journal of Botany-Revue Canadienne De Botanique* 73:S1160–S1168.
- Kon Y, Kashiwadani H, Masada M, Tamura G (1993) Artificial syntheses of mycobionts of *Usnea confusa* ssp. *kitamiensis* and *Usnea orientalis* with their natural and non-natural phycobiont. *Journal of Japanese Botany* 68:129–137.
- König J, Peveling E (1984) Cell-walls of the phycobionts *Trebouxia* and *Pseudotrebourxia* - constituents and their localization. *Lichenologist* 16:129–144.
- Lawrey J (1984) *Biology of Lichenized fungi*. Praeger, New York.
- Le Quéré A, Wright DP, Söderström B, Tunlid A, Johansson T (2005) Global patterns of gene regulation associated with the development of ectomycorrhiza between birch *Betula pendula* Roth.) and *Paxillus involutus* (Batsch) Fr. *Molecular Plant-Microbe Interactions* 18(7):659–673.
- Lee YH, Dean RA (1994) Hydrophobicity of contact surface induces appressorium formation in *Magnaporthe grisea*. *FEMS Microbiology Letters* 115(1):71–75.
- Lewis DH (1973) Concepts in fungal nutrition and origin of biotrophy. *Biological Reviews of the Cambridge Philosophical Society* 48(2):261–278.
- Lewis LA, McCourt RM (2004) Green algae and the origin of land plants. *American Journal of Botany* 91(10):1535–1556.
- Lines CEM, Ratcliffe RG, Rees TAV, Southon TE (1989) A C-13 NMR-Study of photosynthate transport and metabolism in the lichen *Xanthoria calcicola* Oxner. *New Phytologist* 111(3):447–456.

- Linnemannstöns P, Prado MM, Fernández-Martín R, Tudzynski B, Avalos J (2002) A carotenoid biosynthesis gene cluster in *Fusarium fujikuroi*: the genes *carB* and *carRA*. *Molecular Genetics and Genomics* 267(5):593–602.
- Margulis L, Fester R (1992) *Symbiosis as a Source of Evolutionary Innovation: Speciation and Morphogenesis*. MIT Press, Cambridge, MA.
- Martin F, Duplessis S, Ditengou F, Lagrange H, Voiblet C, Lapeyrie F (2001) Developmental cross talking in the ectomycorrhizal symbiosis: signals and communication genes. *New Phytologist* 151(1):145–154.
- Mathesius U (2003) Conservation and divergence of signalling pathways between roots and soil microbes - the *Rhizobium*-legume symbiosis compared to the development of lateral roots, mycorrhizal interactions and nematode-induced galls. *Plant and Soil* 255(1):105–119.
- Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB, Terry A, Salamov A, Fritz-Laylin LK, Marechal-Drouard L, Marshall WF, Qu LH, Nelson DR, Sanderfoot AA, Spalding MH, Kapitonov VV, Ren QH, Ferris P, Lindquist E, Shapiro H, Lucas SM, Grimwood J, Schmutz J, Cardol P, Cerutti H, Chanfreau G, Chen CL, Cognat V, Croft MT, Dent R, Dutcher S, Fernandez E, Fukuzawa H, Gonzalez-Balle D, Gonzalez-Halphen D, Hallmann A, Hanikenne M, Hippler M, Inwood W, Jabbari K, Kalanon M, Kuras R, Lefebvre PA, Lemaire SD, Lobanov AV, Lohr M, Manuell A, Meir I, Mets L, Mittag M, Mittelmeier T, Moroney JV, Moseley J, Napoli C, Nedelcu AM, Niyogi K, Novoselov SV, Paulsen IT, Pazour G, Purton S, Ral JP, Riano-Pachon DM, Riekhof W, Rymarquis L, Schroda M, Stern D, Umen J, Willows R, Wilson N, Zimmer SL, Allmer J, Balk J, Bisova K, Chen CJ, Elias M, Gendler K, Hauser C, Lamb MR, Ledford H, Long JC, Minagawa J, Page MD, Pan JM, Pootakham W, Roje S, Rose A, Stahlberg E, Terauchi AM, Yang PF, Ball S, Bowler C, Dieckmann CL, Gladyshev VN, Green P, Jorgensen R, Mayfield S, Mueller-Roeber B, Rajamani S, Sayre RT, Brokstein P, *et al.* (2007) The *Chlamydomonas* genome reveals the evolution of key animal and plant functions. *Science* 318(5848):245–251.
- Moran GR (2005) 4-Hydroxyphenylpyruvate dioxygenase. *Archives of Biochemistry and Biophysics* 433(1):117–128.
- Mumberg D, Muller R, Funk M (1995) Yeast vectors for the controlled expression of heterologous proteins in different genetic backgrounds. *Gene* 156(1):119–122.

- Nasser Eddine A, Hannemann F, Schafer W (2001) Cloning and expression analysis of NhL1, a gene encoding an extracellular lipase from the fungal pea pathogen *Nectria haematococca* MP VI (*Fusarium solani* f. sp *pisi*) that is expressed *in planta*. Molecular Genetics and Genomics 265(2):215–224.
- Navarro L, Dunoyer P, Jay F, Arnold B, Dharmasiri N, Estelle M, Voinnet O, Jones JDG (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science 312(5772):436–439.
- Niederacher D, Entian KD (1987) Isolation and characterization of the regulatory HEX2 gene necessary for glucose repression in yeast. Molecular & General Genetics 206(3):505–509.
- Nielsen H, Engelbrecht J, Brunak S, von Heijne G (1997) Identification of prokaryotic and eukaryotic signal peptides and prediction of their cleavage sites. Protein Engineering 10(1):1–6.
- Nowak TS, Woodards AC, Jung YH, Adema CM, Loker ES (2004) Identification of transcripts generated during the response of resistant *Biomphalaria glabrata* to *Schistosoma mansoni* infection using suppression subtractive hybridization. Journal of Parasitology 90(5):1034–1040.
- Oliver E, Crittenden PD, Beckett A, Brown DH (1989) Growth of lichen-forming fungi on membrane filters. Lichenologist 21:387–392.
- Oliver RP, Ipcho SVS (2004) *Arabidopsis* pathology breathes new life into the necrotrophs-vs.-biotrophs classification of fungal pathogens. Molecular Plant Pathology 5(4):347–352.
- Ott S (1987) Sexual reproduction and developmental adaptations in *Xanthoria parietina*. Nordic Journal of Botany 7(2):219–228.
- Pahlich S, Zakaryan RP, Gehring H (2006) Protein arginine methylation: Cellular functions and methods of analysis. Biochimica Et Biophysica Acta-Proteins and Proteomics 1764(12):1890–1903.
- Paszkowski U (2006) A journey through signaling in arbuscular mycorrhizal symbioses 2006. New Phytologist 172(1):35–46.

- Palenik B, Grimwood J, Aerts A, Rouze P, Salamov A, Putnam N, Dupont C, Jorgensen R, Derelle E, Rombauts S, Zhou KM, Otillar R, Merchant SS, Podell S, Gaasterland T, Napoli C, Gendler K, Manuell A, Tai V, Vallon O, Piganeau G, Jancek S, Heijde M, Jabbari K, Bowler C, Lohr M, Robbens S, Werner G, Dubchak I, Pazour GJ, Ren QH, Paulsen I, Delwiche C, Schmutz J, Rokhsar D, Van de Peer Y, Moreau H, Grigoriev IV (2007) The tiny eukaryote *Ostreococcus* provides genomic insights into the paradox of plankton speciation. *Proceedings of the National Academy of Sciences of the United States of America* 104(18):7705–7710.
- Paulsrud P, Lindblad P (2002) Fasciclin domain proteins are present in *Nostoc* symbionts of lichens. *Applied and Environmental Microbiology* 68(4):2036–2039.
- Peirce GJ (1900) The relation of fungus and alga in lichens. *American Naturalist* 34:245–253.
- Perera THS, Gregory DW, Marshall D, Gow NAR (1997) Contact-sensing by hyphae of dermatophytic and saprophytic fungi. *Journal of Medical and Veterinary Mycology* 35(4):289–293.
- Peter M, Courty PE, Kohler A, Delaruelle C, Martin D, Tagu D, Frey-Klett P, Duplessis S, Chalot M, Podila G, Martin F (2003) Analysis of expressed sequence tags from the ectomycorrhizal basidiomycetes *Laccaria bicolor* and *Pisolithus microcarpus*. *New Phytologist* 159(1):117–129.
- Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Research* 29(9):6.
- Pirozynski KA, Malloch DW (1975) The origin of land plants: a matter of mycotropism. *Biosystems* 6(3):153–164.
- Plettner I, Steinke M, Malin G (2005) Ethene (ethylene) production in the marine macroalga *Ulva (Enteromorpha) intestinalis* L. (Chlorophyta, Ulvophyceae): effect of light-stress and co-production with dimethyl sulphide. *Plant Cell and Environment* 28(9):1136–1145.
- Polidori E, Agostini D, Zeppa S, Potenza L, Palma F, Sisti D, Stocchi V (2002) Identification of differentially expressed cDNA clones in *Tilia platyphyllos* - *Tuber borchii* ectomycorrhizae using a differential screening approach. *Molecular Genetics and Genomics* 266(5):858–864.

- Praefcke GJK, McMahon HT (2004) The dynamin superfamily: Universal membrane tubulation and fission molecules? *Nature Reviews Molecular Cell Biology* 5(2):133–147.
- Rasmussen R (2001) Quantification on the LightCycler. In Meuer, S and Wittwer, C and Nakagawara, K, ed., *Rapid Cycle Real time PCR: Methods and Applications*. Springer, Heidelberg, pp. 21–34.
- Raven J (1977) The evolution of vascular land plants in relation to supracellular transport processes. *Advances in Botanical Research* 5:153–219.
- Read DJ, Armstrong W (1972) Relationship between oxygen transport and formation of ectotrophic mycorrhizal sheath in conifer seedlings. *New Phytologist* 71(1):49–53.
- Reis H, Pfiffi S, Hahn M (2005) Molecular and functional characterization of a secreted lipase from *Botrytis cinerea*. *Molecular Plant Pathology* 6(3):257–267.
- Remmer S, Ahmadjian V, Livdahl T (1986) Effects of IAA (indole-3-acetic acid) and kinetin (6-furfurylamino-purine) on the synthetic lichen *Cladonia cristatella* and its isolated symbionts. *Lichen Physiology and Biochemistry* 1:1–25.
- Rensing SA, Lang D, Zimmer AD, Terry A, Salamov A, Shapiro H, Nishiyama T, Perroud PF, Lindquist EA, Kamisugi Y, Tanahashi T, Sakakibara K, Fujita T, Oishi K, Shin-I T, Kuroki Y, Toyoda A, Suzuki Y, Hashimoto S, Yamaguchi K, Sugano S, Kohara Y, Fujiyama A, Anterola A, Aoki S, Ashton N, Barbazuk WB, Barker E, Bennetzen JL, Blankenship R, Cho SH, Dutcher SK, Estelle M, Fawcett JA, Gundlach H, Hanada K, Heyl A, Hicks KA, Hughes J, Lohr M, Mayer K, Melkozernov A, Murata T, Nelson DR, Pils B, Prigge M, Reiss B, Renner T, Rombauts S, Rushton PJ, Sanderfoot A, Schween G, Shiu SH, Stueber K, Theodoulou FL, Tu H, Van de Peer Y, Verrier PJ, Waters E, Wood A, Yang LX, Cove D, Cuming AC, Hasebe M, Lucas S, Mishler BD, Reski R, Grigoriev IV, Quatrano RS, Boore JL (2008) The *Physcomitrella* genome reveals evolutionary insights into the conquest of land by plants. *Science* 319(5859):64–69.
- Richardson D, Hill DJ, Smith DC (1968) Lichen physiology XI. Role of alga in determining pattern of carbohydrate movement between lichen symbionts. *New Phytologist* 67(3):469–486.
- Richardson D, Smith DC (1968) Lichen physiology. X. Isolated algal and fungal symbionts of *Xanthoria aureola*. *New Phytologist* 67(1):69–77.

- Sánchez-Martínez C, Pérez-Martín J (2001) Dimorphism in fungal pathogens: *Candida albicans* and *Ustilago maydis* - similar inputs, different outputs. *Current Opinion in Microbiology* 4(2):214–221.
- Saupe SJ (2000) Molecular genetics of heterokaryon incompatibility in filamentous ascomycetes. *Microbiology and Molecular Biology Reviews* 64(3):489–502.
- Schaper T, Ott S (2003) Photobiont selectivity and interspecific interactions in lichen communities. I. Culture experiments with the mycobiont *Fulgensia bracteata*. *Plant Biology* 5(4):441–450.
- Scherrer S, De Vries OMH, Dudler R, Wessels JGH, Honegger R (2000) Interfacial self-assembly of fungal hydrophobins of the lichen-forming ascomycetes *Xanthoria parietina* and *X. ectaneoides*. *Fungal Genetics and Biology* 30(1):81–93.
- Schieleit P, Ott S (1996) Ethylene production and 1-aminocyclopropane-1-carboxylic acid content of lichen bionts. *Symbiosis* 21(3):223–231.
- Schwendener S (1869) Die Flechten als Parasiten der Algen. *Verhandlungen der Schweizerischen Naturforschenden Gesellschaft in Basel* (Translated by Leon Shernoff, 2004, In, *The Origins of Symbiosis*). 5:527–550.
- Selosse MA, Le Tacon F (1998) The land flora: a phototroph-fungus partnership? *Trends in Ecology & Evolution* 13(1):15–20.
- Sever S, Muhlberg AB, Schmid SL (1999) Impairment of dynamin's GAP domain stimulates receptor-mediated endocytosis. *Nature* 398(6727):481–486.
- Slocum RD, Ahmadjian V, Hildreth KC (1980) Zoosporogenesis in *Trebouxia gelatinosa* - ultrastructure potential for zoospore release and implications for the lichen association. *Lichenologist* 12:173–187.
- Sinnemann SJ, Andresson OS, Brown DW, Miao VPW (2000) Cloning and heterologous expression of *Solorina crocea* pyrG. *Current Genetics* 37(5):333–338.
- Stajich JE, Block D, Boulez K, Brenner SE, Chervitz SA, Dagdigian C, Fuellen G, Gilbert JGR, Korf I, Lapp H, Lehvaslaiho H, Matsalla C, Mungall CJ, Osborne BI, Pocock MR, Schattner P, Senger M, Stein LD, Stupka E, Wilkinson MD, Birney E (2002) The bioperl toolkit: Perl modules for the life sciences. *Genome Research* 12(10):1611–1618.

- Stothard P (2000) The sequence manipulation suite: JavaScript programs for analyzing and formatting protein and DNA sequences. *Biotechniques* 28(6):1102–1104.
- Strzelczyk E, Pokojakaburdziej A (1984) Production of auxins and gibberellin-like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and the mycorrhizosphere of pine (*Pinus silvestris* L.). *Plant and Soil* 81(2):185–194.
- Taylor TN (1990) Fungal associations in the terrestrial paleoecosystem. *Trends in Ecology & Evolution* 5(1):21–25.
- Todd RB, Greenhalgh JR, Hynes MJ, Andrianopoulos A (2003) *TupA*, the *Penicillium marneffei* *Tup1p* homologue, represses both yeast and spore development. *Molecular Microbiology* 48(1):85–94.
- Trembley ML, Ringli C, Honegger R (2002a) Differential expression of hydrophobins DGH1, DGH2 and DGH3 and immunolocalization of DGH1 in strata of the lichenized basidiocarp of *Dictyonema glabratum*. *New Phytologist* 154(1):185–195.
- Trembley ML, Ringli C, Honegger R (2002b) Hydrophobins DGH1, DGH2, and DGH3 in the lichen-forming basidiomycete *Dictyonema glabratum*. *Fungal Genetics and Biology* 35(3):247–259.
- Trembley ML, Ringli C, Honegger R (2002c) Morphological and molecular analysis of early stages in the resynthesis of the lichen *Baeomyces rufus*. *Mycological Research* 106:768–776.
- van Loon LC, Rep M, Pieterse CMJ (2006) Significance of inducible defense-related proteins in infected plants. *Annual Review of Phytopathology* 44:135–162.
- Voiblet C, Duplessis S, Encelot N, Martin F (2001) Identification of symbiosis-regulated genes in *Eucalyptus globulus*-*Pisolithus tinctorius* ectomycorrhiza by differential hybridization of arrayed cDNAs. *Plant Journal* 25(2):181–191.
- Voigt CA, Schäfer W, Salomon S (2005) A secreted lipase of *Fusarium graminearum* is a virulence factor required for infection of cereals. *Plant Journal* 42(3):364–375.
- Wösten HAB, de Vocht ML (2000) Hydrophobins, the fungal coat unravelled. *Biochimica Et Biophysica Acta-Reviews on Biomembranes* 1469(2):79–86.

- Wynn WK (1976) Appressorium formation over stomates by bean rust fungus - response to a surface contact stimulus. *Phytopathology* 66(2):136–146.
- Xie WQ, Rothblum LI (1991) Rapid, small-scale RNA isolation from tissue-culture cells. *Biotechniques* 11(3):324–327.
- Zolan ME, Pukkila PJ (1986) Inheritance of DNA methylation in *Coprinus cinereus*. *Molecular and Cellular Biology* 6(1):195–200.

Biography

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EDUCATION

Duke University, PhD. Candidate in Biology, August 2003-present
University of Washington, Botany, M.S., 2003
University of Washington, Botany, B.S., 1999
Fairhaven College, Western Washington University, B.A., 1992

CURRENT RESEARCH INTERESTS

The molecular basis for plant and fungal communication, lichen symbiosis.

PUBLICATIONS

- Joneson, S.** and F. Lutzoni. 2009. Compatibility and Thigmotropism in the Lichen Symbiosis: A Reappraisal. *Symbiosis* 47:109-115.
- Gage, S., **S. Joneson**, B.V. Yu, N.A. Eremenko, and H. Takahashi. 2006. A newly compiled checklist of the vascular plants of the Habomais, the little Kurils. In Takahashi, H. & Ohara, M. (eds.): Biodiversity and Biogeography of the Kuril Islands and Sakhalin 2, pp. 67-91. The Hokkaido University Museum, Sapporo.
- Joneson, S.**, T. Kashiwadani, S. Tschabanenko, and S. Gage. 2004. *Ramalina* of the Kuril Islands. *Bryologist* 107(1): 98-106.
- Joneson, S.** & K. Glew. 2003. *Acroscyphus* of North America. *Bryologist* 106(3): 443-446.
- Takahashi H., V.Y. Barkalov, S. Gage, **S. Joneson**, M. Ilyushko, & Y.N. Zhuravlev. 2002. A Floristic Study of the Vascular Plants of Raikoke, Kuril Islands. *Acta Phytotax. Geobot.* 53(1): 17-33.

In Preparation

- Joneson, S., D. Armaleo and F. Lutzoni. *In prep.* Fungal gene expression in early lichen symbiosis. To be submitted to *Fungal Genetics and Biology*.
- Joneson, S., D. Armaleo and F. Lutzoni. *In prep.* Gene expression of *Asterochloris* sp. in early developmental stages of lichen-symbiosis. To be submitted to *The New Phytologist*.
- Joneson, S., D. Armaleo and F. Lutzoni. *In prep.* Heterologous expression of *Cladonia grayi* Lip3 in yeast. To be submitted to *Mycological Research*.

GRANTS AND AWARDS

- 2006 Mycological Society of America - Backus Award
- 2006 Botanical Society of America - Graduate Student Research
- 2006 Sigma Xi - Graduate Student Mini-Grant
- 2004 Biology Internal Grant Program, Duke University
- 2003 Mellon Fellowship, Biology Department, Duke University
- 2003 Trevor Goward Award, best student paper, Northwest Scientific Association Annual Meeting.
- 2001 Northwest Scientific Association, Graduate Student Fellowship
- 2001 Giles Graduate Student Field Research Award, University of Washington
- 2000 Pat Jones and Daniel E. Stuntz Annual Teaching Assistant Award in Botany and Biology, University of Washington

TEACHING EXPERIENCE

- 2003-current Teaching Assistant, Department of Biology, Duke University: Principles of Biology, Genetics and Molecular Biology, Organismal Diversity, Microbiology, and Principles of Evolution
- 1999-2003 Teaching Assistant, Department of Botany, University of Washington: Introductory Biology (Cellular and Molecular, Biodiversity and Botany), Introductory Botany, and Flowering Plant Identification and Classification

SCIENTIFIC MEETINGS, TALKS

- 2008 Mycological Society of America, "Up-regulated Fungal Genes in Early Lichen Symbiosis."
- 2008 *Invited Speaker*, International Association for Lichenology, "Revisiting thigmotropism and the early stages of lichen symbiosis."
- 2008 International Association for Lichenology, "Differentially regulated genes of *Cladonia grayi* and the early stages of lichen symbiosis."
- 2007 *Invited Speaker*, Fungal Genetics Society of America, "Discovering differentially regulated genes in the lichen symbiosis."
- 2007 *Invited Speaker*, Mycological Society of America, "Differentially regulated genes and lichen symbiosis."
- 2003 Northwest Scientific Association annual meeting, Forks WA. "*Acroscyphus* of North America."
- 2001 International Symposium on Kuril Island Biodiversity, Sapporo JAPAN. "The lichenized fungal genus *Ramalina* in the Kuril Islands".

PROFESSIONAL SOCIETIES

- American Bryological and Lichenological Society
- Botanical Society of America
- British Lichen Society
- California Lichen Society
- Genetics Society of America
- International Association for Lichenology
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