The Efficacy of the Meiotic Toolkit in Determining the Sexuality and Evolutionary History of Red Algae (Rhodophyta)

by

Saeed Amin

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AUTHOR'S DECLARATION

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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ABSTRACT

Meiosis is a unique mechanism and fundamental process that is shared by sexually reproducing eukaryotic species. Species that undergo meiosis can be determined by identifying the presence of core meiotic genes also known as the "Meiosis Detection Toolkit". These genes include the following: SPO11, HOP1, HOP2, MND1, DMC1, MSH4, MSH5, MER3, and REC. Red algae (Rhodophyta) represent a distinct eukaryotic lineage with a long evolutionary history that dates 1.2 BYA and is represented by a fossil that exhibits characteristics of meiosis. Nonetheless, sexuality has never been observed in unicellular red algal species in the order Cyanidiales (*Galdieria sulphuraria* and *Cyanidioschyzon merolae*) and the Porphyridiales (*Porphyridium purpureum*). To understand the evolution of meiosis in red algae, this study examined the usefulness of the meiotic toolkit to determine the presence of sexuality as well as the utility of these genes as taxonomic markers for the evolutionary history of the red algae. This study used the available red algal genomes of *G. sulphuraria, C. schyzon merolae, C. crispus,* and *Porphyridium purpureum*. As a secondary objective, the genomic DNA of several sexual and asexual red algal taxa were experimentally examined to identify and compare the meiotic genes using a variety of biotechnological methods such as degenerate polymerase chain reaction (PCR) and sequencing analyses. Of the nine core meiotic genes, MND1, which is the essential meiotic gene for nuclear division and new recombination, has been observed in the *Porphyra rediviva* nuclear genome and appear to contain premature stop codons. Moreover, annotated amino acid sequences of six core meiotic genes (DMC1, MND1, SPO11, HOP2, MSH4, and MSH5) were used in the phylogenetic analyses. The data set included animals, fungi, plants, excavates, alveolates, and red algae. The phylogenetic trees indicated that the sexual and unicellular red algae possess the six core meiotic genes and some of these putative genes appear to be associated with horizontal gene transfer (HGT) and gene duplication events followed by subsequent losses in some of eukaryotic lineages. The results of this study suggest one of two possibilities. Either there is cryptic meiosis in the unicellular red algae that

contain these genes, or these genes play alternative roles and are a poor marker for meiotic potential. The phylogenetic tree analyses also suggest that the meiotic toolkit is a not a good source of genes to use for taxonomic classification.

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DEDICATION

To my Wonderful family

And

For those who care about scientific research particularly in Biology

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Chapter 1.0: GENERAL INTRODUCTION

1.1 The Evolution of Sex (Meiosis):

Sexual reproduction is a nearly universal reproductive mechanism among eukaryotes (Bell 1982) and has been defined as a process that combines two haploid gametes and genomes (but not always) from two sources or parents. It also includes species for which the gametes are isogamous (morphologically identical) so that the two sexes (females and males) do not exist (Lehtonen et al. 2012). In sexual reproduction, the occurrence of meiosis (the cellular process that divides parental diploid cells to form haploid gametes) followed by syngamy (gamete fusion) and karyogamy (nuclear fusion) are crucial processes to produce diploid generations that contain unique or novel mixtures of parental genes (Petronczki et al. 2003; Schurko and Logsdon 2008; Ismail et al. 2014). Though meiosis is the mechanism by which genetic variation is attained in eukaryotes; transformation, transduction, and conjugation are also mechanisms that provide genetic variation in prokaryotic organisms (Baron 1996). As sexual reproduction is a ubiquitous mechanism across the eukaryotic tree of life, it suggests that the evolution of sex was a pivotal event in eukaryotic evolution. Furthermore, current theories suggest that sex was present in the last common ancestor of all eukaryotes, but was secondarily lost in some lineages leading to asexual eukaryotes. Additionally, phylogenetic analyses appear to show that asexual lineages are short terminal branches from within sexual clades (Johnson 2006). Hence, sexual reproduction plays a significant role in the manner of long-term survival and the complete loss of this mechanism is rare (Dacks and Roger, 1999; Schurko and Logsdon 2008; Umen and Heitman 2013).

Sexuality (meiosis) does not just produce genetic variation, but provides several useful mechanisms such as the repair of the damage of the double strand DNA (Argueso et al. 2008) and prevents deleterious defects (Lynch 2006) that in turn increase the survival potential for a population that undergoes sexual reproduction. Hence, the offspring of sexual individuals may be better adapted to

a changing environment, and they may have fewer deleterious mutations in regular environmental conditions (Szathmáry and Smith 1995).

Asexual Reproduction & Self-Fertilization (Selfing):

Asexual propagation, on the other hand, is a reproductive process without fertilization or meiosis. Successful asexual reproduction is dependent on mitotic cell divisions in unfertilized egg, which transmits a diploid set of chromosomes to the progeny that generates an identical offspring to each other and their parent. Asexuality includes several features such as short generation time, ability to suppress the meiotic genes (e.g. in *Daphnia*) (Innes and Hebert 1988; Schurko and Logsdon 2008). Even though asexual reproduction is widespread, it is not a successful long-term strategy, and often species that are asexual throughout a season will have a sexual phase as environmental conditions change (Hadany and Comeron 2008; Araujo 2014).

The transition in mating system from cross-fertilization (outcrossing) to self-fertilization (selfing) has been observed as the most frequent evolutionary transition involving reproductive systems in plants (Stebbins 1957; Wright et al. 2008). Self-fertilization, also known as autogamy, is an asexual mechanism to ensure the reproduction for plants that undergo selfing when pollinators and/or mates are rare (Eckert et al. 2006). However, selfing is not a successful long-term strategy because it reduces the potential of adaptation and speciation, and the returning from highly selfing to outcrossing organisms is rarely observed (Takebayashi and Morrell 2001). However, some flowering plants have the ability to undergo both cross-fertilization and self-fertilization, for example, the two members of Brassicaceae: *Arabidopsis thaliana* is known to as a selfing plant while *A. lyrata* is a self-incompatible species (Koch et al. 2001; Charlesworth and Vekemans 2005; Nasrallah et al. 2007). The two forms of uniparental reproduction are genetically and developmentally distinctive in that selfing involves syngamy and recombination whereas asexuality does not (Eckert et al. 2006).

Clonal reproduction is a widespread asexual mode of reproduction among many organisms (Sköld

et al. 2009), and it occurs in up to 80% of plant species (Klimes et al. 1997). Furthermore, most green algae are reproduced by clonal vegetative propagation as well as some red algal species such as *Antithamnionella ternifolia* (Maggs and Stegenga 1998). Clonal reproduction via vegetative propagation is a preferable productive technique to ensure the survival of populations in unfavorable environmental conditions such as environments with limited resources of light and nutrients (Lasker 1990; Svensson et al. 1993). It also allows a small population to grow rapidly, especially for growth in aquaculture (Morgan et al. 2005; Fernandez-Zamudio et al. 2013). By shoot fragmentation, detaching fragments have the ability to form new individuals that functionally are able to photosynthesize and uptake nutrients (Grace 1993). Additionally, because almost all unicellular eukaryotes have the ability to undergo asexual reproduction, they do not require extensive genomic reorganization to achieve an alternation generation (Niklas et al. 2014). Asexual reproduction outperforms sexual reproduction by increasing the dispersal ability of individuals, and reducing the time for the female or male to find a mate (Peck et al. 1998; Lehtonen et al. 2012).

1.2 Origin of Sexual Reproduction:

Successful sexual reproduction in eukaryotes requires a fusion of haploid gametes that would generate a diploid zygote (Szathmáry and Smith 1995). However, the additional sets of chromosomes or double the original DNA content can lead to meiotic failures (Das 1980). Endomitosis is an alternative cell cycle that replicates DNA without cell division that results in polyploid cells (Wolfe 2001; Hollister et al. 2012). Cleveland (1947) proposed that the alternation of the haploid-diploid life history phases might have spontaneously started with a diploidization by endomitosis (Figure 1). In this case, the alternation of phases may have evolved earlier than sexual recombination (Hurst and Nurse 1991). Since sex is consisted of syngamy, nuclear fusion and meiosis, it means that sexual reproduction originated solely after the major transition from prokaryotes to eukaryotes (endosymbiosis) (Cavalier-Smith 2002; Schubert 2011).

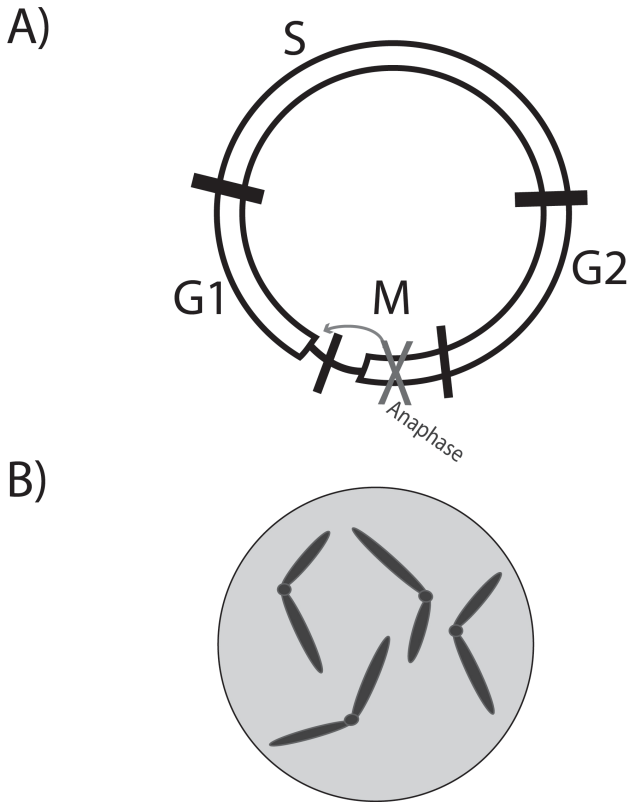


Figure 1 Endomitotic cycle.

a) Endomitosis: cells enter mitosis and chromosomes are being condensed, and then the cells undergo some aspects of mitosis but fail to complete the process, mostly in anaphase (Edgar and Orr-Weaver 2001; Lee et al. 2009).

b) Homologous chromosomes as a result from the second endomitotic round.

Alternation of generations in the Archaeplastida is a switch between multicellular haploid gametophytes and the diploid sporophytes (Raven et al. 2005; Haig 2008). It has been hypothesized that the alternative life history from haploid to diploid might be older than sexual reproduction as the diploid homozygous cell lineage can be produced spontaneously by endomitosis. Additionally, more than two alternating multicellular generations can be occurred with the life history. For instance, the life cycle of Floridophytes "Red Algae" involves alternation of three multicellular generations (gametophyte, carposporophyte, and tetrasporophyte); this is discussed in more detail later (Graham and Wilcox 2000; Graham et al. 2009).

1.3 The Maintenance of Sexual Reproduction and Its Benefits:

Genetic features and environmental conditions can influence the maintenance of sex within a population. Sexual reproduction not only combines the beneficial mutations that are derived from different individuals, but also separates beneficial mutations from detrimental ones in the genome of the offspring (Barton et al. 2007; Gray and Goddard 2012). The combination of both beneficial mutations and new recombination can produce novel genetic variability, which can enable sexual organisms to adapt rapidly to different ecological and environmental conditions (Colegrave 2002; Livnat et al. 2008; Rollo 2014). As a benefit of sexuality, Muller (1964) suggested that over many generations, asexual species might eventually become extinct species due to the accumulation of deleterious mutations in their genome, whereas sexual reproduction is capable of generating ‘mutation-free’ individuals. This is known as Muller's ratchet (Haigh 1978).

In addition, the ‘Red Queen’ hypothesis (RQH) is widely used as a support of the maintenance of sex (Bell 1982). RQH is used to describe two coevolved interaction ideas: 1) a species could become extinct because an evolutionary change have occurred to the other related species which affects the co-evolutionary interactions between these two species, and hence the species which is vulnerable to extinction must evolve in order to survive (Van Valen 1973); 2) co-evolution, particularly between hosts

and parasites, could lead to the maintenance of recombination and segregation, by selecting sexual reproduction (Bell 1982), which generates a progeny with novel genes that could have the ability to reduce the risk of the infection in this progeny (i.e. hosts) (Normark et al. 2003).

1.4 Introduction to Red Algae:

The red algae (Rhodophyta) represent a distinct eukaryotic lineage (Lüning 1990; Freshwater et al. 1994; Baldauf et al. 2000; Yoon et al. 2006a; Müller et al. 2010) that has a long evolutionary history. For instance, *Bangiomorpha pubescens* is the oldest multicellular filamentous red algal fossil that dates 1.2 billion years ago and was obtained from Proterozoic strata on Somerset Island, Canada (Butterfield et al. 1990). This species is similar to the extant species, *Bangia*, in morphology, cell division, and hold fast (Butterfield 2000). The Rhodophyta have primary plastids that originated from a cyanobacterial endosymbiont; a free-living photosynthetic cyanobacterium was taken up by heterotrophic protist and the cyanobacterium (prokaryote) was reduced over time to a plastid enclosed by two membranes (Graham et al. 2009). This pivotal event (or events) led to the rise of three core photosynthetic lineages linked by common ancestry: 1) red algae and those photosynthetic lineages with a plastid derived from red algae through a secondary or tertiary endosymbiotic event, 2) green algae and land plants (and lineages with a secondary plastid derived from a green alga) and 3) glaucophyte algae (Delwiche 1999, 2007; Yoon et al. 2006; Graham et al. 2009; Rodríguez-Ezpeleta et al. 2005; Yoon et al. 2010; Collén et al. 2013). From a cell function perspective, the molecular machinery of nuclear protein import enabled the ancestral plastid over evolutionary time to relinquish functional genes to the nucleus likewise a large number of cyanobacterial endosymbiont genes were transferred to the host's nucleus (Allen 2003; Deusch et al. 2008). These three primary plastids formed Archaeplastida, which comprise the three lineages called Supergroup Plantae derived from a common cyanobacterial ancestor (Adl et al. 2005; Facchinelli 2013). Additionally, genome sequence analyses provides evidence that the primary endosymbiotic event occurred only once in the Archaeplastida (Price et al. 2012). Archaeplastida is a

monophyletic group (Rodríguez-Ezpeleta et al. 2005; Graham et al. 2009; Keeling 2010). Many lines of evidence such as nuclear, plastid, and mitochondrial gene trees support the hypothesis that the red algae are monophyletic group (Freshwater et al. 1994; Yoon et al. 2006; Herron et al. 2013).

1.4.1 Classification of Red Algae

Traditionally, the phylum Rhodophyta has been classified into two subclasses Bangiophycidae and Florideophycidae or two classes Bangiophyceae and Florideophyceae (Gabrielson et al. 1985; Van den Hoek et al. 1995). However, the progress of molecular analyses and organelle ultrastructure studies clarified that this traditional classification did not reflect the diversity of the red algae (Oliveira and Bhattacharya 2000; Müller et al. 2001; Kapraun and Freshwater 2012). Multigene phylogenetic studies have shown that the class Cyanidiophyceae is the earliest diverging of red algae lineages and is often classified as the separate Subphylum Cyanidiophytina with the single order Cyanidiales that includes only three genera: *Cyanidium*, *Cyanidioschyzon*, and *Galdieria*. Cyanidiophyceae is considered to be a sister class of the other red algae (Yoon et al. 2002; Yoon et al. 2004; Müller et al. 2010; Azua-Bustos et al. 2012; Lowell and Castenholz 2013). Saunders and Hommersand (2004) proposed a new taxonomic system that includes an additional phylum to Rhodophyta, the Cyanidiophyta with a single class Cyanidiophyceae. These authors recognized four classes belonging to the phylum Rhodophyta: Rhodellophyceae, Compsopogonophyceae, Bangiophyceae, and Florideophyceae.

The Recent Taxonomic Scheme

Yoon et al. (2006) provided a new classification system of Rhodophyta by using molecular systematic analysis that included a wide range of taxon sampling with multigene analyses. Their classification system includes seven classes under the phylum Rhodophyta. There are six classes under the subphylum Rhodophytina: Bangiophyceae, Florideophyceae, Compsopogonophyceae, Porphyridiophyceae, Rhodellophyceae, and Stylonematophyceae; and one class Cyanidiophyceae under the subphylum Cyanidiophytina. In summary, the current classification of red algae contains one phylum

Rhodophyta and two subphyla - the Rhodophytina with six classes and Cyanidiophytina with one class (Figure 2). However, Bangiophyceae, Stylonematophyceae, and Compsopogonophyceae are early diverging lineages and moreover, these three classes are poorly resolved in the manner of their evolutionary relationships to each other (Yoon et al. 2006; Müller et al. 2010; Verbruggen et al. 2010).

1.4.2 Characteristics of the Red Algae

Cell Biology and Pit Plugs

The cellular features of the red algae are unique (Graham et al. 2009). The cell wall is less rigid and softer than other algae because the extracellular matrix consists of a flaccid net of cellulose microfibrils that is filled with an amorphous gel-like mixture of mucilages and polymers of sulfated galactan. Further, the gelatinous component is more abundant than cellulose in red algal extracellular matrix which makes its consistency softer (Graham et al. 2009). Red algal cell wall is a complex of cellulose, calcium carbonate heteropolysaccharide (agar), and polysaccharide. Biomolecules such as fatty acids and vitamins are observed in the cell wall (Graham et al. 2009; Popper et al. 2011; Gupta et al. 2013). As a storage product, red algae store carbon and produce it as granulated floridean starch in their cytosol rather than in their chloroplast like Chlorophyta (Cole and Sheath 1990; Yoon et al. 2010; Collén et al. 2013).

Pit plugs (pit connections) are another feature that distinguishes red algae. Pit plugs are observed in the sporophytic stage of some members of the Bangiales and in triphasic life cycle of Florideophyceae. The main function of the primary pit plugs is likely to form connections between cells in filamentous genera, and the secondary pit plug is usually formed between the cells of parasitic red algae and their hosts. Primary pit plugs are formed during cytokinesis, and secondary pit plugs are formed at the end of the cell division (Cole and Sheath 1990, Graham et al. 2009).

Pigments

Since the red algae are a photosynthetic group, chlorophyll *a* is a primary pigment, but they lack

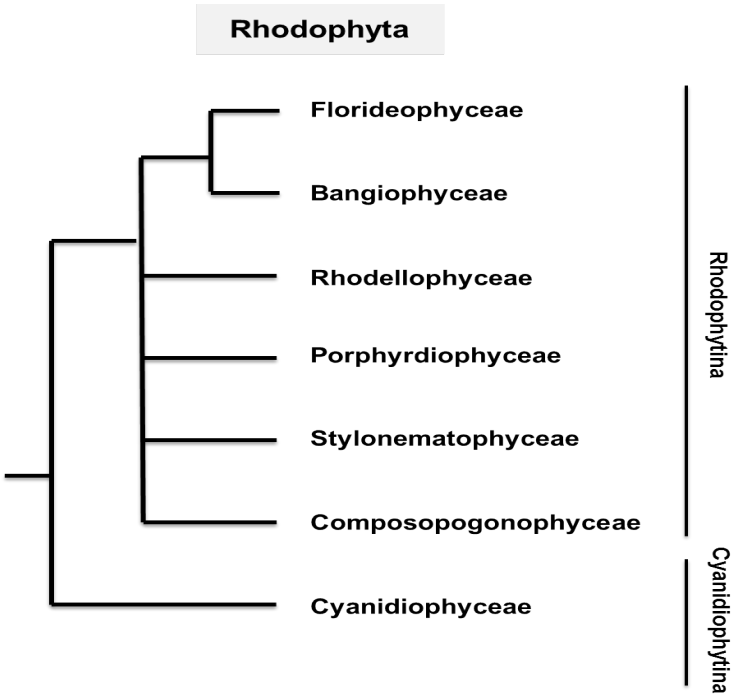


Figure 2 Recent red algal taxonomic system based on the combined plastid protein sequences of psaA and rbcL "Adapted from Yoon et al. 2006; Müller et al. 2010".

of chlorophyll b and c. Red algae contain three important accessory pigments, which are phycoerythrin (PE), phycocyanin (PC), and allophycocyanin (APC). These pigments are light-harvesting protein complexes called phycobiliproteins which assist red algal plastids to harvest light energy that is inaccessible to chlorophyll (Glazer 1977; Beer and Eshel 1985; Liu et al. 1999; Graham et al. 2009).

Additionally, the large amount of red accessory pigment (phycoerythrin) gives red algae a pink to dark red colouration (Graham et al. 2009). However, a small group of the red algae, which are parasitic, appear white or yellowish in colour because their plastids lack photosynthetic pigments (Graham et al. 2009). Freshwater algae are often blue-green coloured due to the preponderance of phycocyanin. In addition, photoprotective carotenoids like antherixanthin, β-carotene, and zeaxanthin may give a yellow, dark violet, brown, or black color to the red algae (Schagerl and Donabaum 2003). These pigments are formed of phycobilisomes on unstacked thylakoids (Graham et al. 2009).

Flagella and Centrioles

In addition to these features, flagella and centrioles are lacking in all life history stages of members of the Rhodophyta and hence this is the most distinctive characteristic of red algae. The reproductive evolution of red alga has affected via the loss of flagella, leading to the widespread occurrence of sexual life histories having two or three multicellular phases (Figure 3) while the maximum life histories of other multicellular algae and land plants is two phases (Young 1977; Searles 1980; Garbary and Gabrielson 1990; Yoon et al. 2006; Graham et al. 2009; Collén et al. 2013). However, a type of motility has been observed in some unicellular species (e.g. *Porphyridium*) and reproductive spores (e.g. *Batrachospermum*) via polysaccharide mucilage secretion and amoeboid movement (Pickett-Heaps et al. 2001).

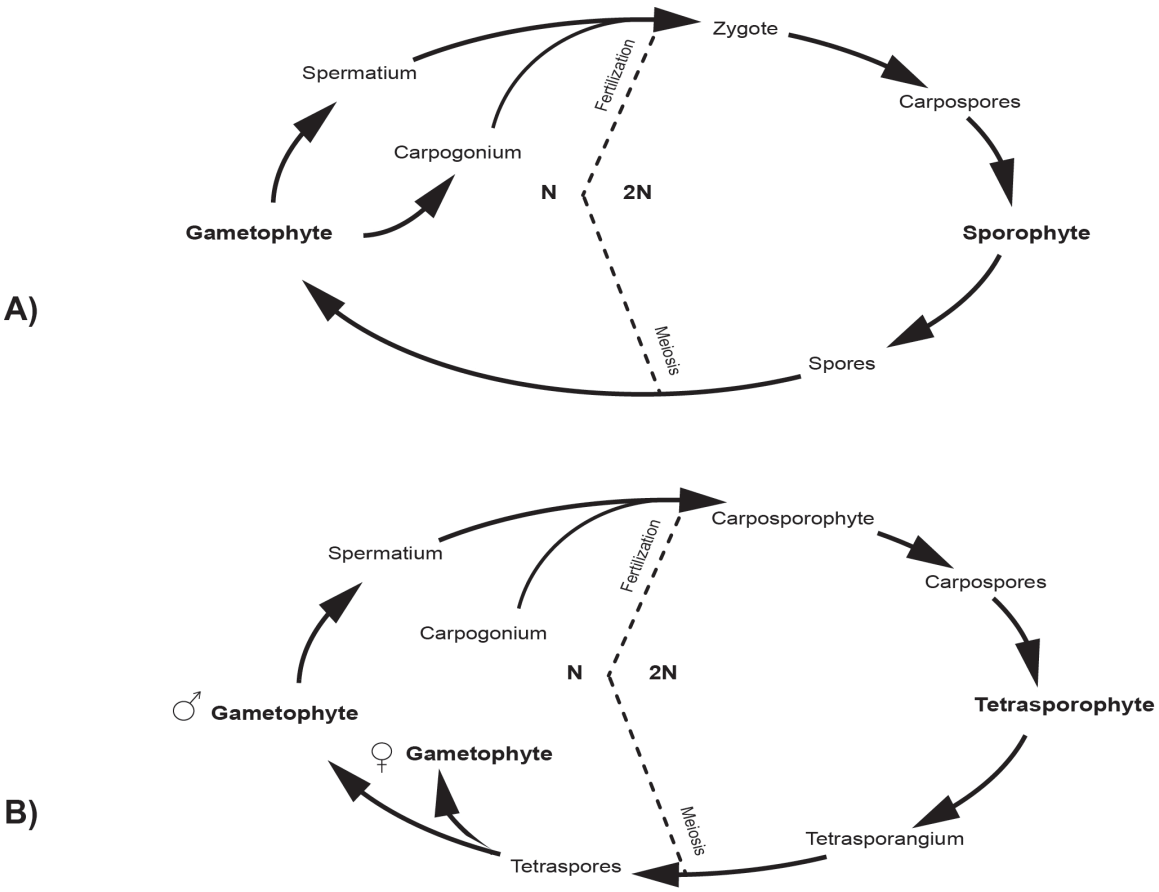


Figure 3 Life history of red algae "Adapted from Graham et al. 2009"

A) Biphasic life history of early-divergent red algae.

B) Triphasic life history later-divergent red algae.

1.4.3 Reproduction and Life History of Red Algae

Red algae are known to produce sexually and asexually, with some species only being described as asexual such as Bangia atropurpurea (Nelson 2007; Spitale et al. 2012), Compsopogon, and Chroodactylon ornatum (Necchi and Dip, 1992; Wehr and Sheath 2003). Sexual reproduction is characterized by gamete fusion and meiosis whereas asexual reproduction can occur whether by spores and mitosis or vegetative fragmentation (Hawkes 1990; Graham et al. 2009). Generally, asexual reproduction occurring by spores is common in early-diverging red algal groups such as Porphyridiales and Cyanidiales (primarily unicellular taxa) (Müller et al. 2001a; Ciniglia et al. 2004, Graham et al. 2009). However, sexual reproduction has also been observed in some early-diverging multicellular red algal taxa including *Rhodochaete, Erythrotrichia, Bangia, Smithora,* and *Porphyra* (Garbary and Gabrielson 1990; Hawkes 1990; Ciniglia et al. 2004; Graham et al. 2009; Müller et al. 2010; De Clerck et al. 2012).

Sexual Reproduction in Red Algae

The majority of Rhodophyta members undergo sexual reproduction, which is typically oogamous, involving fusion of relatively small non-flagellated (non-motile) spermatium (the male gamete) with a large non-flagellated carpogonium (female gamete) (Searles 1980; Graham et al. 2009). Red algal spermatia are formed singly as subapical protrusions in the male sex organs (Hommersand and Fredericq 1990). The female gametes (carpogonia) are also produced separately from carpogonial branches. The carpogonia consist of extended and enlarged cell contacting the nucleus (Hommersand and Fredericq 1990; Graham et al. 2009). In the triphasic life history, fertilization in red algae begins with the adhesion of a spermatium to carpogonium; then, carpogonial extracellular matrix secretes enzymes to enable spermatial nucleus to enter the carpogonium. The cytoplasm then closes the carpogonium to prevent other spermatia from entering (Broadwater and Scott 1982; Graham et al. 2009). Then, the fertilized carpogonia becomes the second phase of the life history, the carposporophyte

(cystocarp), which develops on the female gametophyte while the zygote undergoes repeated mitosis (Kamiya and Kawai 2002; Graham et al. 2009). The mitotic nuclear division of each zygote is supplied by nutritional resources from the female gametophyte to generate diploid cells of filaments called gonimoblast (Turner and Evans 1978; Kamiya and Kawai 2002; Guillemin et al. 2014). A single gonimoblast is equivalent to a carposporophyte, but the first one uses additional nutritional resources from the female gametophyte to generate carposporangia that release diploid carpospores which develop to tetrasporophyte to complete the life cycle (Graham et al. 2009; Guillemin et al. 2014).

Life History

The typical life history of red algae occurs in three generations or phases: haploid (gametophyte) and diploid (sporophyte). The diploid sporophyte develops into female thallus (carposporophyte) and free-living tetrasporophyte which is can be seen in the Florideophyceae and not in the Bangiophyceae. All three phases - gametophyte, carposporophyte, and tetrasporophyte - are obligatory for sexual reproduction; additionally, haploid gametophyte and diploid sporophyte alternate in the life history. Hence, the life cycle of red algae is known as biphasic or triphasic alternation of generations (Figure 3). Furthermore, meiosis occurrence has been observed during the production of spores by sporophytic stage (Searles 1980; Hommersand and Fredericq 1990; Graham et al. 2009; Yao et al. 2009) (Refer to Figure 3).

Asexual Reproduction in Red Algae

Many red algae also reproduce asexually via releasing unicellular spores such as monospores, archeospores, endospores, or neutral spores. The releasing of spores depends on the production and swelling of mucilage mechanism (Graham et al. 2009). To form new red algal plants, spores need to attach to a suitable substrate, and the attachment requires several hours or days to become completed. When spores attach to a substrate, they become resistant to water movement, and the spores grow by repeated mitosis to form new members that are similar to the parents. The other type of asexual

propagation occurs by vegetative fragmentation (Hawkes 1990; Wehr and Sheath 2003; Blouin et al. 2007; Graham et al. 2009).

1.5 Diversity of Red Algae

The Rhodophyta are a diverse group; it has been postulated that there are more than 600 genera and about 20,000 species that are distributed worldwide (Norton et al. 1996; Graham et al. 2009). To address questions of the genes that are in involved meiosis in early eukaryotes (i.e. Rhodophyta), the meiotic genes (meiotic detection toolkit) have to be detected in wide range of red algal species that differ in their reproductive mechanism (sexual or asexual) and habitats. In the next section, several classes and species, will be discussed that exhibit a range in their reproductive mechanisms. These species were used whether directly (e.g. through DNA extraction and sequencing) or indirectly (e.g. using their available complete genome sequences).

1.5.1 Bangiophyceae

Bangiophyceae is a red algal class that contains simple unbranched filaments or a leaf-shaped thallus (Yoon et al. 2010). Molecular phylogenetic studies have supported the monophyly of this class (Oliveira and Bhattacharya 2000; Müller et al. 2001, 2005; Nelson et al. 2005, 2006; Yoon et al. 2006a, 2010). Bangiophyceae is a sister to the Florideophyceae, which are supported both morphologically and molecularly as monophyletic groups (Müller et al. 2001; Yoon 2006a, 2010; Chan et al. 2012). The Bangiophyceae was traditionally classified into one order Bangiales and two genera *Bangia* (e.g. filament) and *Porphyra* (e.g. blade); these genera are molecularly supported as non-monophyletic groups (Oliveira et al. 1995; Müller et al. 2001a; Milstein and De Oliveira 2005). Sutherland et al. (2011) have recognized 15 genera, 7 filamentous and 8 foliose.

Members of Bangiophyceae are typically marine with only one known freshwater species, *Bangia atropurpurea* (Müller et al. 2003). Most species grow in the intertidal zone of marine areas while

others are subtidal. They are typically observed on rocks, shells, or other algae, although some are epiphytic (Sutherland et al. 2011). Cells typically can contain a single plastid with central pyrenoid or several per cell (Graham et al. 2009). The pit plugs are a distinctive feature in the sporophytic phase (conchocelis) of Bangiales, but they are rarely observed in the gametophyte (Müller et al. 2010). The marine members of the Bangiophyceae are typically able to undergo sexual reproduction (Magne 1960; Garbary et al. 1980). In addition, asexuality can occur among members of this class, which results in the production of numerous asexual spores (archeospores), germinating into a thallus, which is identical to the parent (Müller et al. 1998; Wang et al. 2008; Sutherland et al. 2011).

1. *Bangia atropurpurea*

Bangia atropurpurea is a simple multicellular filamentous red algae with little cellular structural complexity (Spitale et al. 2012). Müller et al. (2003) determined that *Bangia atropurpurea*, which is the single freshwater species in the order Bangiales, is clearly distinct based on molecular, cytological, ecological, and reproductive preferences from marine *Bangia* species such *as Bangia fuscopurpurea*. Sexual reproduction has never been observed in *B. atropurpurea* in nature, and it typically reproduces asexually through monosporangia (Wehr and Sheath 2003; Nelson 2007). However, under laboratory conditions, sexual reproduction has been observed by spores development into gametophytic thalli, and the sexuality was confirmed by counting the chromosomes n = 3 and 2n = 6 in the gametophytes and sporophytes (Gargiulo et al. 2001). This species is distributed worldwide attached to rocks or other solid substrates in rivers, streams, and lakes. It has been observed in the British Isles, Japan, New Zealand, and the North American Great Lakes (John et al. 2002; Notoya and Iijima 2003; Müller et al. 2010).

2. *Bangia maxima*

Bangia maxima is a monophyletic species and is morphologically distinguishable among members of the genus *Bangia* due to its large size (up to 35 cm long × 6 mm in diameter), and it has a distally multiseriate hollow filament. It has been reported that *B. maxima* species are having a

monecious form, which helps to increase the proportion of fertilization between male and female reproductive organs (Lynch at al. 2008; Müller et al. 2010). *Bangia maxima* occurs in marine habitats, and it has been found abundantly in littoral boulders in Bolinas Bay, California, USA (Yoon et al. 2006; Lynch at al. 2008). *B. maxima* has been observed to reproduced sexually (Lynch at al. 2008).

3. Porphyra sp.

Porphyra in the narrow sense is a monophyletic genus, which is strongly supported by phylogenetic analyses (Sutherland et al. 2011). Morphologically, *Porphyra* vary from linear (e.g. *P. linearis*) to foliose species (e.g. *P. umbilicalis*) (Müller et al. 2010). This foliose thallus consists of either one (monostromatic) or two (distromatic) cells thick. The gametophyte generation is the blade or foliose phases, and the sporophyte generation is a branched filamentous or conchocelis phase. The conchocelis produces spores to regenerate the blade-forming phase (Graham et al. 2009). Sexual reproduction is the predominant mode among *Porphyra* species. However, in the northwestern Atlantic, asexual reproduction has been noticed on *P. umbilicalis* by releasing spores from the blades (Blouin et al. 2007). *Porphyra* species are distributed worldwide (Blouin et al. 2011).

1.5.2 Florideophyceae

The monophyly of this class and Bangiophyceae has been supported via molecular analyses (Müller et al. 2001a). Among the red algal classes, Florideophyceae is the most diverse group, including more than 20 orders and around 5800 species (Yoon et al. 2004; Le Gall and Saunders 2006). There are five florideophycean subclasses Hildenbrandiophycidae, Nemaliophycidae, Ahnfeltiophycidae, Rhodymeniophycidae (Saunders and Hommersand 2004), and Corallinophycidae (Le Gall and Saunders 2006). Florideophyceans occur in marine habitats with triphasic life cycle: gametophyte, carposporophyte, and tetrasporophyte phases (Graham et al. 2009).

Chondrus crispus

This edible species is known as "Irish moss" having a distinguishable morphology; their bodies are

multiaxial, bushy, and dichotomously branched shape (Graham et al. 2009). *C. crispus* occurs in the lower intertidal and subtidal zones on the both sides of the North Atlantic (Graham et al. 2009). The complete genome of *C. crispus* is available with 105 Mbp genome containing 9,606 of annotated genes (Collén et al. 2013).

1.5.3 Stylonematophyceae

Red algae (Rhodophyta) consist of several early diverging classes, including the Stylonematophyceae (Yoon et al. 2006; Zuccarello et al. 2009). Based on multigene phylogenetic analyses, Yoon et al. (2006) proposed the class Stylonematophyceae has eight genera. It has been reported that this class is a monophyletic group (Yoon et al. 2006; Müller et al. 2010). Recently, Zuccarello et al. (2008) established an additional order that is the Rufusiales with only one genus *Rufusia* (Wujek and Timpano 1986). Fourteen genera under the order Stylonematales have been reported (Müller et al. 2010; Yoon et al. 2010). Thus, Stylonematophyceae includes two orders with 15 genera. Morphologically, this class contains unicellular and pseudo-filamentous forms (Yoon et al. 2006, 2010; Zuccarello et al. 2009; Müller et al. 2010). Their cells consist of thick mucilaginous walls and lack pit plugs; additionally, most genera possess a single stellate plastid with a pyrenoid within their cells (Broadwater and Scott 1994). Stylonematophyceae members are epiphytic and mostly occur in marine rather than freshwater habitats (Wehr and Sheath 2003; Müller et al. 2010; Yoon et al. 2010). Sexual reproduction is rare within this class; however, it has been documented in genus *Kyliniella* (Wehr and Sheath 2003; Zuccarello et al. 2009). In addition, it is worthy to mention that *Stylonema cornu-cervi* and *Stylonema alsidii* are morphologically similar. *S. cornu-cervi*, however, has less branched with more multiseriate thallus (Zuccarello et al. 2009).

Kyliniella latvica

Kyliniella latvica is a monophyletic group (Yoon et al. 2006) formed of unbranched pseudo-filaments with a discoid pseudoparenchymatous base occurring in freshwater habitats (Wehr and Sheath

2003). *Kyliniella latvica* is epiphytic and has been observed in water streams in Rhode Island in U.S.A (Vis and Sheath 1993); additionally, this species has been observed in Alhárabe River in Spain (Fernández et al. 2012). *Kyliniella latvica* is the only member of the class Stylonematophyceae that has been reported to be sexually reproductive (Wehr and Sheath 2003).

1.5.4 Cyanidiophyceae

This class is well-supported monophyletic group (Yoon et al. 2006). The cyanidiophytes are unicellular members, and they are acidophilic (pH 0.5-3.0) and thermophilic (up to 57°C) taxa, and thrive around hot springs or acidic sulfur fumes (Graham et al. 2009; Ciniglia et al. 2014). Asexuality has been observed in cyanidiophytes both in natural populations and in laboratory conditions (Ciniglia et al. 2004; 2014). The class Cyanidiophyceae is supported phylogenetically as an early diverging lineage of the red algae, likely dating to more than 1300 MYA (Müller et al. 2001b; Yoon et al. 2006). There are currently three genera and seven species: *Cyanidium* (1 sp.), *Cyanidioschyzon* (1 sp.), and *Galdieria* (5 spp.) (Yoon et al. 2006b; Pinto et al. 2007).

1. Cyanidioschyzon merolae

This small (2 μm diameter) unicellular red algal species inhabits photoautotrophically sulphate-rich hot springs (Matsuzaki et al. 2004). *Cyanidioscyzon merolae* consists of non-rigid cell wall and one nucleus, mitochondrion and plastid which is considered such an excellent model systems for the study of plant cell biology and metabolism; for example, the study of mitochondrial and chloroplast divisions (Kuroiwa 1998). *Cyanidioscyzon merolae* has one of the smallest genomes among photosynthetic eukaryotic organisms with 20 chromosomes in total size of ≈ 16.6Mbp (Matsuzaki et al. 2004; Nozaki et al. 2007).

2. Galdieria sulphuraria

Galderia sulphuraria is unicellular and grows either photoautotrophically or heterotrophically in harsher environmental conditions; for instance, it thrives naturally on volcanic hot sulfur springs,

solfatara soils, and anthropogenic hostile environments (Gross and Schnarrenberger 1995). It has relatively a smaller genome than *C. merolae* with 13.7Mbp (Schönknecht et al. 2013).

1.5.5 Porphyridiophyceae

The Porphyridiophyceae is morphologically and molecularly supported as non- monophyletic group (Müller et al. 2001a,b; Yoon et al. 2006). Porphyridiophyceae contains three unicellular genera: *Porphyridium, Erythrolobus*, and *Flintiella* (Yoon et al. 2006). The members of this group, particularly, *Porphyridium* and *Erythrolobus* can be found on wet soils (Sheath and Sherwood 2002).

Porphyridium sp.

The red coloured mesophilic *Porphyridium purpureum* and the olive-green *Porphyridium sordidum* are unicellular red algal species and inhabit areas of high salt content (Ott 1987; Broadwater and Scott 1994). Their cells consist of mucilaginous matrix that enables them to move slowly via mucilage excretion (Graham et al. 2009). The complete genome of *P. purpureum* is available with 19.7 Mbp containing 8,355 of predicted genes (Bhattacharya et al. 2013). Despite this *P. purpureum* is a unicellular species, 8 of 9 core meiotic genes (DMC1, MND1, SPO11, HOP2, HOP1, MSH4, MSH5, and MER3) were identified in its genome (Bhattacharya et al. 2013).

1.6 Pseudogenes and Gene Duplication

Pseudogenes

Generally, pseudogenes are copies of functional genes that are no longer able to encode functional proteins (Harrison and Gerstein 2002). Pseudogenes have been observed in all eukaryotes (Wang et al. 2012; Jackson et al. 2009; Eschenlauer et al. 2006; Zheng et al. 2007) and can be divided into three groups. The first group includes unprocessed pseudogenes (duplicated pseudogenes), which are the result of the duplication of a functional gene (Salmena 2014). The second group consists of processed pseudogenes, which derived by the reverse transcription of an mRNA back into DNA generating cDNA

and randomly inserted into the genome (Salmena 2014). These pseudogenes usually are lacking in regulatory sequences and introns due to the occurrence of a mutation during the copying process or are missing crucial regions of the original gene (Gerstein and Zheng 2006; Wang et al. 2006). It is possible that this second group of pseudogenes was initially functional but over time and due to the accumulation of deleterious mutations, the nucleotide sequence has premature stop codons (or other elements), which prevent of the successful expression. The last group is the unitary pseudogenes, which possibly arose through spontaneous mutations in protein-coding genes associated with losses (Mitchell and Graur 2005).

Gene duplication

Gene duplication is an event from which another copy of the same gene is duplicated and can be operationally is similar to the original gene. These duplicated genes form paralogs as they remain in the same genome and orthologs when they are transferred into a different genome (Magadum et al. 2013). The occurrence of duplicated genes has been observed in the all three domains of life and thus it is hypothesized that gene duplication has contributed to gene evolution by adding new functions (Zhang 2003). Hence, gene duplication is considered as a source of paralogs forming gene families (Li et al. 2001; Martin and Holland 2014), many protein-coding genes can be classified into genes families that gene duplication event has contributed their history (Dayhoff 1976; Vasiliou et al. 2000; Pascual-Anaya et al. 2013). For example, the hemoglobin β chain is a paralog of the α chain hemoglobin and of myoglobin which they evolved from the same ancestral globin gene through gene multiple duplication events (Gogarten and Olendzenski 1999).

There are four different mechanisms by which genes can be duplicated: 1) unequal crossing-over, 2) retrotransposition 3), duplicated DNA transposition, and 4) whole-genome duplication (Magadum et al. 2013; Dharia et al. 2014).

1. **Unequal crossing over:** This mechanism provides tandem repeated DNA sequences. The

tandem duplication depends on where the cross over occurs whether in portion of a gene, a complete gene, or a segment encoding a number of different genes (Zhang 2003). These sequences can be duplicated with their introns if they were presented in the original genes (Zhang 2003).

2. **Retrotransposition:** genes can be duplicated when a messenger RNA (mRNA) is reverse transcribed to complementary DNA (cDNA) forming retrogenes followed inserted into the genome (Long 2001). The retrogenes usually are not similar to the origin genes -unlike the unequal crossing over- because the retrogenes are lack of introns (Long et al. 2003).
3. **Duplicated DNA transposition:** it is also called duplicative transposition, which occurs during the stage of DNA repair, can produce more new duplications due to the recombination of non-allelic homologous (Samonte and Eichler 2002). It is believed that duplicative transposition contributes the rise of gene families (Dharia et al. 2014).
4. **Whole genome duplication (WGD):** it is also called polyploidization or polyploidy. This mechanism is the fourth type of gene duplication, and it is common in plants more than in animals (Masterson 1994). Whole genome duplication is a process that hybridizationally combines two or more genomes in one nucleus (Magadum et al. 2013); however, polyploidization is also associated with gene loss (Kellis et al. 2004; Dehal and Boore 2005).

1.7 Horizontal Gene Transfer

Horizontal, or lateral, gene transfer is the process of genes transfer between organisms and was recognized first in prokaryotes due to the abundant amount of available genomic sequence compared to eukaryotes (Ochman et al. 2000; Boucher et al. 2003). However, despite the well-recognized importance of horizontal gene transfer (HGT) in prokaryotes, it has likely played an important role in eukaryotes as well (Andersson 2005). For example, in eukaryotes, lateral genetic transfer can occur by different mechanisms such as DNA being transferred to the eukaryotic nucleus via endosymbiotic gene transfer

(from mitochondrion or plastid to the nucleus) (Lill 2009; Hirt et al. 2015). Furthermore, it is possible that eukaryotes can horizontally obtain genes from virus transmission that occurs between eukaryotic or prokaryotic hosts (Liu et al. 2010; Gilbert and Cordaux 2013). For example, seven of (sphingolipid) genes were horizontally transferred between the eukaryotic organism *Emiliania huxleyi* and the virus *EhV* (Monier et al. 2009). Hence, many eukaryotes possess encoded genes from mitochondria, and HGT from parasite viruses, and free-living organisms have contributed to the evolution of the eukaryotic genome (Huang 2013; Schönknecht et al. 2014).

1.8 The Modes of Evolution

Divergent, convergent, and parallel are the different modes (patterns) of evolution (Pagel et al. 2006; Conte et al. 2012; Soria-Carrasco et al. 2014). Divergent evolution can be described as a short period of evolution occurs after long period of stability within a population of organisms associated with speciation (Ricklefs 2006; Pagel et al. 2006). The result of this mode of evolution is to form new species that are genetically isolated and phenotypically distinct (Buckner and Krienen 2013). Isolation can accelerate genes frequency changes and mutant genes adaptation (Buckner and Krienen 2013). Duplicated genes in an isolated population are vulnerable to the evolutionary of divergence that may affect their original genetic background; for example, divergent evolution may change the primary functions or genomic locations of such genes (Bikard et al. 2009; Ouyang et al. 2010).

Speciation that is associated with parallel evolution may occur in closely related linages, and the descendants of this speciation would have some properties that are missing in the common ancestor (Schluter and Nagel 1995). For example, the descendants from such speciation may do not have the same reproductive traits that they were existed in the common ancestor; the descendants do not have the same ability of mating that their common ancestor does (Schluter and Nagel 1995; Conte et al. 2012). In another words, the descendants are not reproductively isolated from one another, but they may not have the same traits of the common ancestor. Additionally, geographic or environmental changes may not be

able to prevent the generation of the new traits that are generated by the parallel evolution; for instance, reproductive isolation may happen in closely related (similar) populations even if they live in different geographic or environmental areas (Schluter and Nagel 1995; Soria-Carrasco et al. 2014).

However, convergent evolution is the process of adapting similar environmental or biological properties by different species that are distantly related (non-monophyletic); as a result from this event, these organisms, which are not closely related, possess similar traits (Conte et al. 2012). It is possible to say that the opposite of the parallel is convergent evolution. The parallel evolution occurs to populations that are closely related and ends up in speciation that having traits that were not observed on the ancestor while the convergent evolution occurs to populations that are distantly related which results in speciation that evolved similar traits (Conte et al. 2012; Schluter and Nagel 1995; Soria-Carrasco et al. 2014).

1.9 Core Meiotic Genes

Meiosis is the most essential process for sexual reproduction, which is ubiquitous among all eukaryotes. Meiosis has several unique processes, for instance, synapsis, high levels of recombination, and no S phase prior to Meiosis II (White 1978; Page and Hawley 2003). Meiosis I reduces the chromosomes sets from two to one set in each nucleus and the second meiosis division is still equational (does not reduce the chromosomes numbers) but the separated chromatids are not identical due to the new recombination (Marcon and Moens 2005). In most eukaryotic species, the complex meiotic prophase I involves leptotene (to align the homolog chromosomes), zygotene (to imitate synapsis between the two homologs via synaptonemal complex proteins (SC), pachytene (to ensure the homologs are completely synapsed), and diplotene (to recognize the sites that do not synapse and allows the crossing over occur) (Marcon and Moens 2005; Hamant et al. 2006).

Detecting meiotic genes (Table1) in a given genome is a suitable solution for sexuality determination because the presence of most or all these genes provides a significant mark that the

organism has the ability to undergo meiosis, whereas the absence of some or all of these meiotic genes would be associated with asexual reproduction (Schurko and Logsdon 2008). The presence of only one or two of meiotic genes would not provide strong proof for meiosis while several genes are necessary to be present in order to prove the sexuality of the organism. These genes signify the best candidate indicators for detecting the presence of meiosis and sexual reproduction across all eukaryotes; additionally, almost all meiotic genes have been identified in animals, plants, fungi, protists, and red algae (e.g. *P. purpureum*) (Ramesh et al. 2005; Malik et al. 2008; Schurko and Logsdon 2008; Carr et al. 2010; Hanson et al. 2013; Bhattacharya et al. 2013).

The Concept of Meiosis Detection Toolkit

The dramatic development of genome sequencing technology increases the methods that can be used to search genomes for genes required for sexual reproduction. In the flowering plant Silene latifolia, tracing the evolution of sex chromosomes, and comparing the closely related species with and without sex chromosomes can be determined by finding a set of genes in the S. latifolia genome (Filatov 2005). Likewise the present of the complementary mating-type (MAT) gene in the fungus Aspergillus fumigatus is associated with obligate sexual outbreeding and mating partner (Pöggeler 2002; Paoletti et al. 2005). To make a comparative genomics approach applicable to all eukaryotes, it is necessary to define a set of genes that are universally required for sexual reproduction. This is precisely the aim of the ‘‘meiosis detection toolkit’’ approach (Schurko and Logsdon 2008).

This meiosis detection toolkit is a powerful approach to detect sexual reproduction in eukaryotes for several reasons: First, applicable to all eukaryotes. Second, since the meiotic genes are in the genomic DAN (Schurko and Logsdon 2008; Bhattacharya et al. 2013), small sample size is sufficient. Third, as sexual reproduction is a ubiquitous mechanism across the eukaryotes, it suggests that the evolution of sex (meiosis) was a pivotal event in eukaryotic evolution (Bell 1982), so the meiotic toolkit

may have an importance to evolutionary biology. On the other hand, the difficulty of determining a true negative result and direct assessing gene expression are the pitfall points of the meiosis detection toolkit.

In the case of the red algae, asexuality is common in early-diverging groups, and it occurs in different red algal members (Graham et al. 2009); therefore, it would be difficult to determine whether asexual species undergo sexual reproduction via inducing sex under laboratory conditions. To overcome this challenge, using the meiosis detection toolkit to detect the meiosis genes in the genomes of different red algal species may an effective alternative solution for inferring the presence of sexuality.

Table 1 Brief description and key functions of the core meiotic genes that are considered to be components of the meiosis detection toolkit.

Core Meiotic Genes	Description and Some Key Functions
SPO11	• SPO11 proteins are homologs of an archaebacterial topoisomerase VI subunit (Bergerat et al. 1997). • Meiosis-specific protein that stimulates the formation of double-strand breaks (DSBs) in DNA, particularly, in meiosis in order to initiate meiotic recombination (Keeney 2008). • **Homologs:** SPO11-1, SPO11-2 and SPO11-3 (Hartung and Puchta 2000); SPO11-1 and SPO11-2 are the essential for meiotic recombination (Hartung et al. 2007; Hörandl and Hadacek 2013).
HOP1	• Important protein for chromosome pairing and DSBs binding during meiosis (Hollingsworth et al. 1990). • This protein also contributes in the formation of axial and lateral elements of synaptonemal complex (SC) between homologous chromosomes (Anuradha and Muniyappa 2004).
HOP2	• Prevents synapsis between non-homologous chromosomes and ensures synapsis between homologous chromosomes (Halary et al. 2011). • HOP2 and Mnd1 form a heterodimeric complex that promotes the accurate homology pairing and meiotic DSBs repair during pachytene stage of meiotic prophase (Pezza et al. 2006).
MND1	• Meiotic nuclear division and recombination requires MND1; • Mnd1 forms a heterodimeric complex with Hop2 to ensure accurate meiotic DSBs formation (Riley and Corradi 2013). • MND1, HOP2, and RAD51 form essential heterodimer complex to ensure synapsis between homologous chromosomes during

Core Meiotic Genes	Description and Some Key Functions
	meiosis (Cho et al. 2014).
DMC1	• DMC1 is homolog of RAD51, which both are eukaryotic proteins; DMC1 is homolog of the eubacterial RECA (Stassen et al. 1997). • DMC1 and RAD51 are essential meiotic proteins for normal SC formation, meiotic recombination, and DNA damage repair (Masson and West 2001).
MSH4 and MSH5	• The eukaryotic MSH4 and MSH5 genes are homologous of eubacterial mismatch repair MutS genes (Halary et al. 2011). • MSH4 and MSH5 form a heterodimer complex that facilitates and controls the crossovers between homologs "Holliday Junction"* and meiotic recombination (Borts et al. 2000; Rakshambikai et al. 2013).
MER3	• Meiosis-specific protein which encourages DEAD-box helicase* to work in order to disengage Holliday Junction (HJ)* and insure crossover interference quality (Nakagawa and Ogawa 1999; Chen et al. 2005). * DEAD-box proteins are the largest family of DNA and RNA helicases. The origin of DEAD-box's name is came from the motif II includes the sequence D-E-A-D (Asp-Glu-Ala-Asp) (Linder et al. 1989). * Holliday junctions (HJs) are 4-stranded DNA crossover structures (X-shaped). HJ occurs during genetic recombination and repair (Holliday 1964; Boddy et al. 2001).

Core Meiotic Genes	Description and Some Key Functions
RECA	• REC8 is paralog of RAD21 (Urban et al. 2014). • Sister chromatid cohesion is regulated via RAD21; then, RAD21 is replaced via its meiosis-specific paralog REC8 which bounds sister chromatids between the centromeres during meiosis I until the beginning of anaphase II (Haering and Hasmyth 2003; Urban et al. 2014).

1.10 Objectives of the Thesis

Objective 1: To examine the usefulness of six meiotic toolkit genes (DMC1, MND1, SPO11, HOP2, MSH4, and **MSH5) in the determination of sexuality using available red algal genomes: *Galdieria sulphuraria, Cyanidioschyzon merolae, Chondrus crispus,* and *Porphyridium purpureum*).**

Sexual reproduction has not been observed in unicellular red algal species (*C. merolae*, and *P. purpureum*). However, it appears that meiotic genes have been reported (Schurko and Logsdon 2008; Bhattacharya et al. 2013).

Hypothesis: Meiotic genes are present in the unicellular red algae.

i. *Possible outcome 1:* Genomes of the unicellular red algal species contain the core meiotic genes.

   Explanation: These red algal species may have all or some of the meiotic toolkit genes, and hence it provides support for the common ancestor having the ability to undergo meiosis, and the unicellular red algae may undergo cryptic sexual reproduction.

   Hence: The meiosis detection toolkit would be useful markers for sexual reproduction determination.

ii. *Possible outcome 2:* Genomes of the unicellular red algal species contain non-functional meiotic genes (i.e. pseudogenes) that are no longer under selection.

   Explanation: These genes are may not longer under selective constraint and may no longer be functional; hence those genes will exhibit characteristics of pseudogenes in coding regions (Ka=Ks) and behaving differently (Schurko and Logsdon 2008; Pei et al. 2012).

Hence: The meiosis detection toolkit would not be a good source to determine the sexuality of these red algal species (*G. sulphuraria*, *C. merolae*, and *P. purpureum*).

Objective 2: Determine whether the meiotic toolkit is a useful tool for evolutionary history.

Hypothesis: Since meiosis detection toolkit is considered to be a universal set of genes, it may be useful as a molecular tool to determine the evolutionary history of many organisms.

Possible outcome: It may correspond to the evolutionary history and ordinal taxonomic level of red algae as determined by standard gene phylogenies using18S rRNA and rbcL genes (Yoon et al. 2006a; Müller et al. 2010).

Chapter 2.0: METHODS

2.1 DNA Extraction and Samples

Most samples were already DNA extracted and stored in a –20 °C freezer, and DNA extraction was applied as needed (Table. 2). Frozen samples (100 - 400 mg) were disrupted in liquid nitrogen using mortar and pestle. DNA extracted using the QIAgen ® DNeasy Plant Mini Kit (Qiagen, Mississauga, Ontario, Canada) and DNA was eluted in 50 µl of either 50 mM Tris-HCl (pH 8.0) or DNA/RNA free water.

Table 2 List of samples

A list of algal species, the sampling sites, reproductive types, and preferable habitats.

* The highlighted species was partly sequenced.

Taxon	Species	Sexuality	Locality	Habitat
Bangiophyceae	*Bangia atropurpurea*	Asexual Sexual (Gargiulo et al. 2001)	Lake Ontario Lake Michigan	Freshwater
	Bangia maxima	Sexual	Bolinas Bay, CA	Marine
	Porphyra rediviva	Sexual	HBI	Marine
	Porphyra umbilicalis	Sexual Asexual (Blouin and Brawley 2012)	SCO. 12 AC	Marine
	Porphyra rediviva	Sexual	ce 73	Marine
Stylonematophyceae	*Stylonema cornu-cervi*	Asexual Sexual?		Marine
	Stylonema alsidii	Sexual	CA - MLVC	Marine
	Kyliniella latvica	Asexual Sexual	EB	Freshwater
Porphyridiophyceae	*Porphyridium sordidum*	Asexual?	EB	

2.2 Primer Design

Primers for targeted meiotic genes (SPO11, MND1, and DMC1) were designed based on Expressed Sequence Tags (ESTs) from *Porphyra* and *Pyropia* species and the available red algal genomes (*Cyanidioschyzon merolae, Chondrus crispus*, *Galdieria sulphuraria* and *Porphyridium purpureum*) using NCBI Primer Designing Tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Primers were designed to be suitable to amplify the meiotic genes in the genome of more than one red algal genus. Phylogenetic analyses from previous studies (Yoon et al. 2006a; Müller et al. 2010) were used to determine the most closely related red algal species so that each designed primer can amplify several related red algal species (Table 3).

Table 3 Sequences of the primers used for PCR amplification and sequencing.

Tm: annealing temperature.

*The results of the highlighted primers were successful.

Gene	Primer name	Primer sequence 5' ——— 3'	Length (bp)	Tm	Genera
DMC1	DMC1_P.umb-F	AAGCCGAGGAGGAACGCA	18	52 °C	*Porphyra* and *Bangia*
	DMC1_P.umb-R	CAGCGTTGGGGAGTCGT	17		
MND1	F-MND1-A	GGTGGAGCTGCTGCAAG	17	55 °C	*Porphyra* and *Bangia*
	R-MND1-A	CCAAACTTGTCACTCACG	18		
DMC1	DMC1-Pdium-F	GACTCAGCTGTCTCACACCC	20	52 °C	*Porphyridium* and *Stylonema*
	DMC1-Pdium-R	TGGTACTTGCGTGAGCCATT	20		
SPO11	SPO11 - Pdium-F	GGACCTGTCGGCTGTGCGCG	20	50 °C	*Porphyridium* and *Stylonema*
	SPO11 - Pdium-R	CCGAGCCACTTGATGTCCGG	20		
SPO11	SPO11 – Purpra - F	AAAATCGTGCCAACGCAGAG	20	47 °C	*Porphyra* and *Bangia*
	SPO11- Purpra - R	GGTCTTTACGCGCCTCTCAG	20		

2.3 PCR Amplification of Meiotic Genes

To obtain a sufficient amount of PCR product, 95 µl as a final volume of reaction mixtures were prepared containing 10 µl 10X taq buffer with KCl (100 mM Tris-HCl (pH 8.8 at 25 °C), 500 mM KCl, 0.8% (v/v) Nonidet P-40), 8 µl 25 mM $MgCl_2$, 2mM each of dATP, dCTP, dGTP, and dTTP, 4 µl 0.01 mM of each specific meiotic primers (Table 3), 0.4 µl taq DNA polymerase (5 U/µL), and 2 µl of extracted genomic DNA from the red algal species listed in (Table 2). Eppendorf Mastercycler® Gradient 5331 (Eppendorf, C.A., U.S.A.) and MultiGene II™ Personal Thermal Cycler (Labnet International, N.J., U.S.A.) were used for PCR amplifications. 35 cycles of 2 min of pre-denaturation at 95 °C followed by 1 min of denaturation at 93 °C, 1 min of annealing (Tm are listed in table.3), and 4 min of extension at 72 °C; then a final extension of 6 min at 72 °C.

Gradient PCR was used to increase primers specificity (Hecker and Roux 1996; Schneider et al. 2012). It was performed using Eppendorf Mastercycler® Gradient 5331 (Eppendorf, C.A., U.S.A.) with similar reagent concentrations as the previous PCR reaction but with the following changes: 5 µl 10X taq buffer with KCl (100 mM Tris-HCl (pH 8.8 at 25°C), 500 mM KCl, 0.8% (v/v) Nonidet P-40), 4 µl 25 mM $MgCl_2$, 2 µl 0.01 mM of each specific meiotic primers (Table.3), 0.4 µl Taq DNA Polymerase (5 U/µL), and 1 µl of extracted genomic DNA in 50 µl as final volume. The protocol was 35 cycles of 2 min of pre-denaturation at 95 °C, 1 min of denaturation at 93°C, 1 min of annealing with gradient temperature from 48.2°C to 63°C, 4 min of extension at 72 °C and a final extension of 6 min at 72°C.

Each amplification product was electrophoresed through a 1.0% agarose gel in 1x TBE buffer at 120 V for 45 min. Fluorescent nucleic acid dye "GelRed" (GelRed Nucleic Acid Gel Stain, 10,000X in water) was added to the gel (3 µl of GelRed for each 50 ml of agarose gel) to make DNA visible under UV lights. Products were visualized using the Syngene Bioimaging System (Synoptics Ltd., United Kingdom). PCR product size and concentrations were estimated using DNA ruler (GeneRuler 100 bp

Plus DNA Ladder 50µg, Thermo Fisher Scientific Inc.) that was electrophoresed alongside the PCR products.

2.4 Gel and PCR Purification

Forty µl of PCR products were purified using QIAquick® PCR Purification (Qiagen, Mississauga, Ontario, Canada) followed by elution in 50 µl of either 50 mM Tris-HCl (pH 8.0) or DNA/RNA free water. For the PCR products that showing multiple bands, gel purification was applied. Agarose gels were prepared from 1.0% agarose in 1x TBE buffer and stained by GelRed. Combs were used to form 10mm wide and 3 mm depth sample wells. 30 µl of PCR product were loaded in each well. Electrophoresis was carried out in 1x TBE buffer at 100 V for 70 min. DNA was visualized using the Syngene Bioimaging System (Synoptics Ltd., United Kingdom). Each targeted band were extracted from the gel by cutting it into a slice using a clean razor blade, and the slices were placed individually into a clean 1.5 ml microcentrifuge tube. The slices were purified using QIAquick ® Gel Extraction Kit (Qiagen, Mississauga, Ontario, Canada), and DNA was eluted in 50 µl of either 50 mM Tris-HCl (pH 8.0) or DNA/RNA free water.

2.5 Sequencing of DNA from PCR products

The samples were prepared and sent to the Centre for Applied Genomics at the Hospital for Sick Children in Toronto. Genetic Analysis Facility at the hospital provides high-quality capillary-based fluorescent sequencing on dual ABI 3730XL instruments for DNA sequencing. Each sample was prepared as follow: 14 µl purified template DNA was divided equally into two 0.5 ml microcentrifuge tubes (7 µl and 7 µl), and 0.7 µl – 1 µl (≈50 ng) of forward primer was added to the first tube and similar volume of reverse primer to the second tube; the total volume in each tube was 7.7 µl – 8.0 µl. The concentration of PCR products was required as follow: (<1kb) - ≈ 7ng/ 1 µl, (1-2kb) – 7 to 14ng /1 µl, and (2-4kb) – 14 to 21 ng/ 1 µl. Primers and PCR products were quantified using a NanoDrop ND2000

spectrophotometer (Thermo Scientific, Wilmington, DE) before being sequenced. Nuclease-free water was added and Savant Speed Vac (Sc110) dryer was used to adjust the concentration.

2.6 Sequence Alignment and Phylogenetic Analyses

Due to DNA mutational bias at the third codon positions (Savolainen et al. 2002; Magallón 2010), annotated amino acid sequences of six core meiotic genes "Meiosis Detection Toolkit" were used in the phylogenetic analysis. The data set included the amino acid sequences of animals, fungi, plants, excavate, alveolates, and red algae, and the sequences of 6 core meiotic genes (DMC1, MND1, SPO11, HOP2, MSH4, and MSH5) were obtained from GenBank (Appendix 1). The protein sequences were aligned using Clustal Omega (ClustalO) (Sievers et al. 2011). The Gblocks program was used to select blocks of conserved sites for pylogenetic analyses (Castresana 2000; Talavera and Castresana 2007). A maximum-likelihood (ML) tree was constructed using PhyML 3.1 (Guindon et al. 2010) with a WAG model using the default settings for amino acids. Clade support was evaluated by applying approximate likelihood ratio test (aLRT) statistics (Anisimova and Gascuel 2006). Trees were visualized with FigTree v**1.4**.**2** (http://tree.bio.ed.ac.uk/software/figtree/). Trees were edited and finalized via Adobe Illustrator CC. MEGA 6.06 was used to determined sequence divergence (p-distance model) and the number of amino acids differences (No. of differences model) (Tamura et al. 2013).

BLASTx (http://blast.ncbi.nlm.nih.gov/Blast.cgi), which searches protein database using a translated nucleotide query, was applied to identify open reading frames homologous to known proteins. Then, using ExPASy Translate Tool (http://web.expasy.org/translate/) was used to obtain amino acid sequences.

Chapter 3.0: RESULTS

3.1 Sequencing Results

The 5' portion of the meiotic gene MND1 was successfully obtained from the red algal species *Porphyra rediviva* (Bangiales) after purifying the PCR product. The amplified MND1 sequence is 1138 nucleotides in length (Figure 4) and encodes a 375 amino acid partial MND1 fragment (Figure 5). The obtained MND1 sequence was blasted against the NCBI non-redundant (nr) database using BLASTx http://blast.ncbi.nlm.nih.gov/Blast.cgi, which searches the protein database using a translated nucleotide query. This revealed a partial hit between the MND1 sequence from *P. rediviva* and full length MND1 reference sequences. The first three detected hits were the sequences of *Cyanidioschyzon merolae* (GenBank Accession number XP_005535845.1, *E*-value 6e-09), *Chondrus crispus* (GenBank Accession number XP_005717880.1, *E*-value 3e-08), and *Galdieria sulphuraria* (GenBank Accession number XP_005703331.1, *E*-value 6e-08) from the first 5'3' translated frame (Figure 6). The obtained sequence from *P. rediviva* also appears to have premature stop codons in the translated sequence (Figure 5). The alignment of MND1 was then generated from amino acids sequences of 24 eukaryotic organisms including the protein sequence of *P. rediviva*, other red algal species, plants, fungi, excavata, alveolate, and animals.

Figure 4 Nucleotide sequence of the MND1 gene in *Porphyra rediviva.*

Figure 5 Sequence of the open reading frame of the MND1 gene from *P. rediviva.* Premature stop codons are noted with red stars.

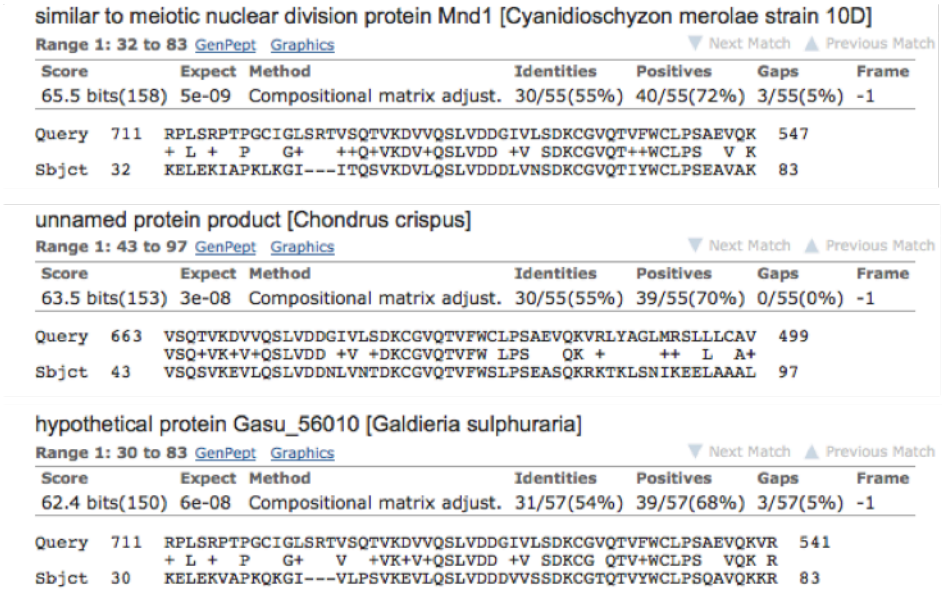


Figure 6 BLASTx search including the first three hits of the MND1 sequence from *Porphyra rediviva* indicating the matches of obtained protein sequence of MND1 from P. rediviva and the amino acids sequences of C. merolae, C. crispus, and G. sulphuraria.

3.2 Phylogenetic Analyses

Sequences of previously published meiotic genes (DMC1, MND1, SOP11, HOP2, MSH4, and MSH5) were obtained from GenBank and are included in Appendix 1. Due to DNA mutational bias at the third codon position, as noted in chapter 2, phylogenetic analyses were based on amino acid alignments. Branch support was determined using the approximate likelihood ratio test (aLRT).

3.2.1 DMC1

Figure 7 depicts a likelihood tree of the DMC1 protein family including three additional paralogs (RAD51, RAD57, and RECA). From a phylogenetic perspective, this phylogenetic tree appears to have some monophyletic groups. For example, the fungi, animals, green plant lineage, and red algae were well-supported as separate but monophyletic groups (0.96, 1.0, 0.79, and 1.0 aLRT, respectively). However, *Trichomonas vaginalis* formed a weakly supported paraphyletic group with the alveolates (*Trypanosoma cruzi* and *Oxytricha trifallax*), and *Trichomonas vaginalis* appears to be basal to the alveolates (Figure 7). Interestingly, two copies of the DMC1 gene were identified in *Galdieria sulphuraria* (Cyanidiales) (GenBank Accession numbers EME32481 and EME32482), which represent two isoforms.

The RAD51 cluster did not reflect the taxonomy and evolutionary history of these groups as was noted in the DMC1 clades (Figure 7). For instance, sequences from the red algae form a paraphyletic group with *Trichomonas vaginalis* (0.71 aLRT), however the alveolates did form a monophyletic group (0.72 aLRT). Moreover, the two members of the order Cyanidiales, *G. sulphuraria* and *Cyanidioschyzon merolae,* also do not form a monophyletic group. In addition, the florideophyte *Chondrus crispus* appears to be weakly supported as grouping with *C. merolae* (Figure 7).

The green plant lineage appears to be a non monophyletic group because the green algae are not clustering with this group and *Chlamydomonas reinhardtii* was basal within RAD51cluster; however,

Arabidopsis thaliana and *Medicago truncatula* are well-supported as being monophyletic group (1.0 aLRT). Fungi and animals formed well-supported separate monophyletic groups (0.99 and 1.0 aLRT, respectively) and are included in a larger supported (0.79 aLRT) clade (Figure 7). Furthermore, the red algal species *C. merolae* (BAM81186), *G. sulphuraria* (EME29355), and *C. crispus* (CDF39004) form a well-supported monophyletic group (0.97 aLRT) for RAD57. In addition, the RACA gene sequence of *C. merolae* (BAM80454) appears to be basal to the entire tree (Figure 7).

3.2.2 MND1

The likelihood tree of MND1 shows some groups that were incongruent with taxonomic and evolutionary history. For example, the green plant lineage appears as non-monophyletic group, and *Arabidopsis thaliana* and *Cryptococcus neoformans* cluster together to form sister taxa with a weak aLRT support (Figure 8). The alveolates were not supported as a monophyletic group. *Trichomonas vaginalis*, however, formed a weak supported paraphyletic group (0.68 aLRT) with animals while this latter group consisting of *Homo sapiens, Mus musculus*, and *Danio rerio* form a well-supported monophyletic group (0.98 aLRT) (Figure 8).

The MND1 genes within the red algae formed two separate groups: a well-supported monophyletic clade (0.96 aLRT) and a polyphyletic group. The monophyletic group consists of four sequences from the red algal order, Bangiales: *Porphyra purpurea*, *Porphyra umbilicalis*, *Porphyra rediviva, Porphyridium purpurea*; one from the Floridieophyceae, *Chondrus crispus*; and two from the order Cyandiales, *Cyanidioschyzon merolae*, and *Galdieria sulphuraria* (Figure 8). However, the other red algal group contains three other sequences for *C. merolae* (GenBank Accession numbers 544210302, 544216417, and BAM81906) and an extra sequence for *G. sulphuraria* (GenBank Accession No EME27752). In other words, there are four sequences from *C. merolae* that appear to be MND1, but

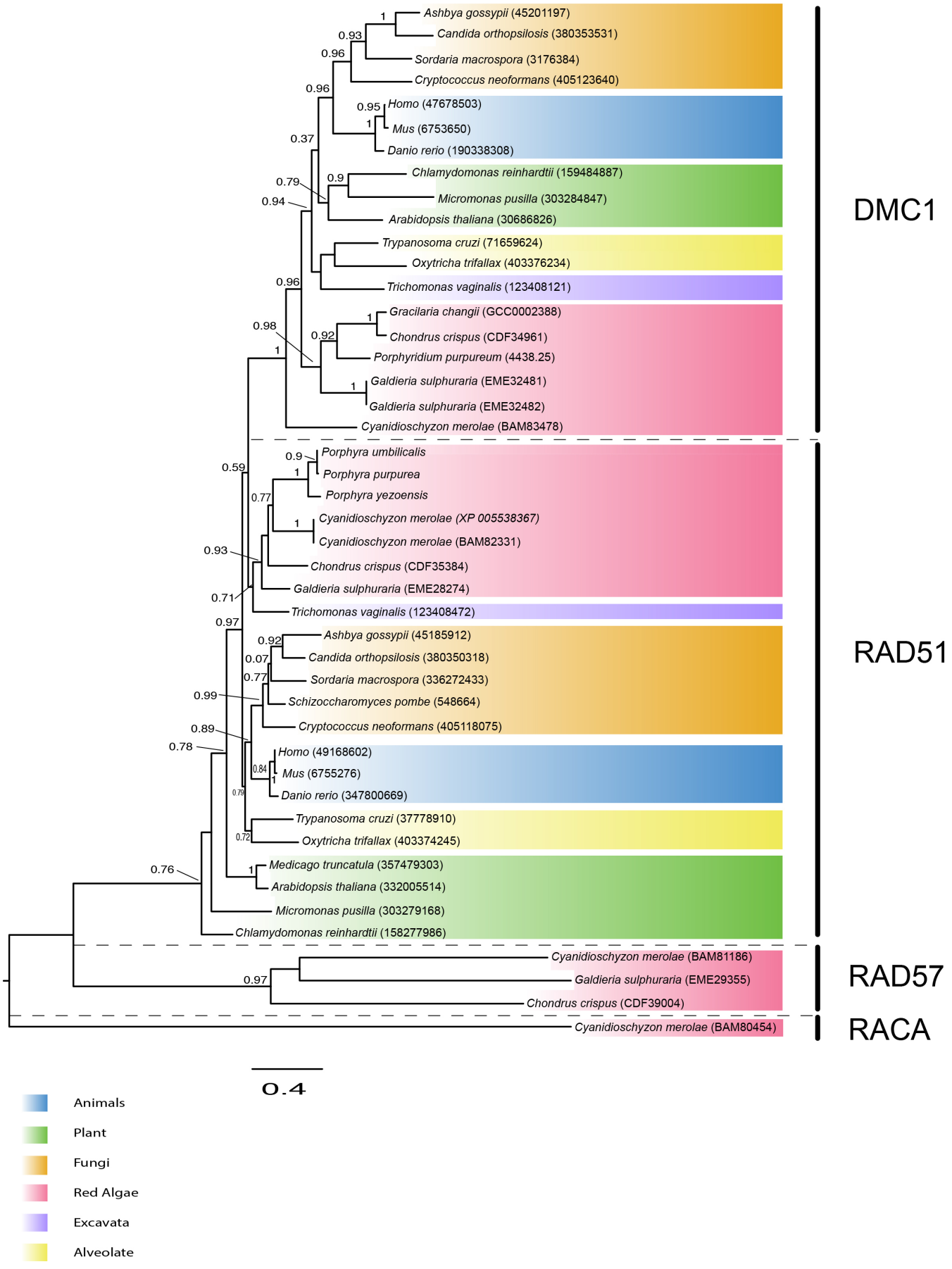

Ashbya gossypii (45201197)
Candida orthopsilosis (380353531)
Sordaria macrospora (3176384)
Cryptococcus neoformans (405123640)
Homo (47678503)
Mus (6753650)
Danio rerio (190338308)
Chlamydomonas reinhardtii (159484887)
Micromonas pusilla (303284847)
Arabidopsis thaliana (30686826)
Trypanosoma cruzi (71659624)
Oxytricha trifallax (403376234)
Trichomonas vaginalis (123408121)
Gracilaria changii (GCC0002388)
Chondrus crispus (CDF34961)
Porphyridium purpureum (4438.25)
Galdieria sulphuraria (EME32481)
Galdieria sulphuraria (EME32482)
Cyanidioschyzon merolae (BAM83478)
Porphyra umbilicalis
Porphyra purpurea
Porphyra yezoensis
Cyanidioschyzon merolae (XP 005538367)
Cyanidioschyzon merolae (BAM82331)
Chondrus crispus (CDF35384)
Galdieria sulphuraria (EME28274)
Trichomonas vaginalis (123408472)
Ashbya gossypii (45185912)
Candida orthopsilosis (380350318)
Sordaria macrospora (336272433)
Schizoccharomyces pombe (548664)
Cryptococcus neoformans (405118075)
Homo (49168602)
Mus (6755276)
Danio rerio (347800669)
Trypanosoma cruzi (37778910)
Oxytricha trifallax (403374245)
Medicago truncatula (357479303)
Arabidopsis thaliana (332005514)
Micromonas pusilla (303279168)
Chlamydomonas reinhardtii (158277986)
Cyanidioschyzon merolae (BAM81186)
Galdieria sulphuraria (EME29355)
Chondrus crispus (CDF39004)
Cyanidioschyzon merolae (BAM80454)
DMC1
RAD51
RAD57
RACA
0.4
Animals
Plant
Fungi
Red Algae
Excavata
Alveolate

Figure 7 Phylogenetic tree for the DMC1 gene and its homologs RAD51, RAD57, and RECA.

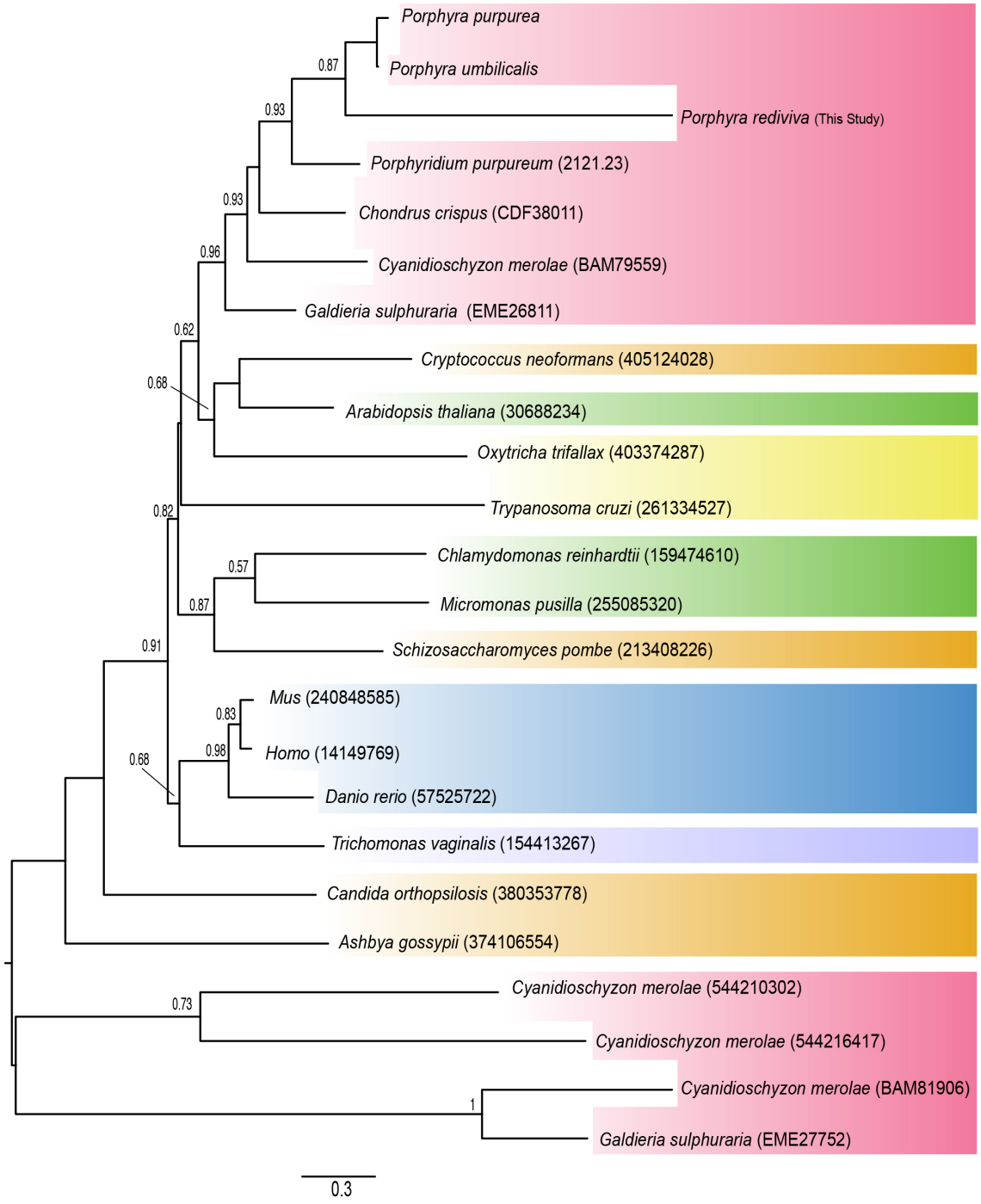
This phylogenetic tree was generated from amino acids sequences of 45 species and 602 sites as total alignment. The amino acid subsets were selected manually by Gblocks. Support for branches with (aLRT) values less than 0.50 are not shown.

they do not form a monophyletic cluster. It is worth noting that the members of the Bangiales clustered together and were moderately supported as such (0.87 aLRT); however, *P. rediviva*, which was sequenced in this study, is positioned on a much longer branch than *P. purpurea*, *P. umbilicalis* (Figure 8). This is not surprising, as noted previously, this sequence had premature stop codons (Figure 5).

3.2.3 SPO11

The likelihood tree depicts the three homologs (SPO11-1, SPO11-3, and SPO11-2) of the SPO11 protein in which the SPO11-1 and SPO11-3 sequences formed a large clade and the SPO11-2 sequences in smaller clade (Figure 9). This phylogenetic analysis of SPO11-1 depicts fungi, alveolates and animals as well-supported and as monophyletic groups (0.95, 0.91 and 1.0 aLRT support, respectively). Furthermore, the two SPO11-1 gene sequences of the red algal species *Galdieria sulphuraria* (GenBank Accession numbers EME30795 and EME30796) formed a well-supported monophyletic group (1.0 aLRT). However, *Trichomonas vaginalis* and *Medicago truncatula* formed a paraphyletic clade with SPO11 sequences from animals (Figure 9). For SPO11-3, the monophyly of the red algae taxa, *Cyanidioschyzon merolae* (Cyanidiales) (BAM82023), *Porphyridium purpureum* (Porphyridiales) (Contig number 2255.5), and *Chondrus crispus* (Florideophyceae) (CDF39919) was well-supported (0.93 aLRT) and the green algae, *Chlamydomonas reinhardtii* and *Micromonas pusilla,* also formed a monophyletic group (0.63 aLRT) (Figure 9).

The SPO11-2 did not appear as a monophyletic clade. Interestingly, the sequence from *Arabidopsis thaliana* was clustered with red algae forming paraphyletic group (0.96 aLRT). In this group, the red algal clade does not appear to correspond to the taxonomy and evolutionary history of this phylum (Figure 9). For example, the florideophyte, *C. crispus* (CDF33912), is closely related to the *C. merolae* (BAM82494) (Cyanidiales) rather than being associated with the other copy of the SPO11-2 in *C. crispus* (CDF36452). Unexpectedly, the gene sequence of *G. sulphuraria* (Cyanidiales) (EME31699)


Porphyra purpurea
Porphyra umbilicalis
Porphyra rediviva (This Study)
Porphyridium purpureum (2121.23)
Chondrus crispus (CDF38011)
Cyanidioschyzon merolae (BAM79559)
Galdieria sulphuraria (EME26811)
Cryptococcus neoformans (405124028)
Arabidopsis thaliana (30688234)
Oxytricha trifallax (403374287)
Trypanosoma cruzi (261334527)
Chlamydomonas reinhardtii (159474610)
Micromonas pusilla (255085320)
Schizosaccharomyces pombe (213408226)
Mus (240848585)
Homo (14149769)
Danio rerio (57525722)
Trichomonas vaginalis (154413267)
Candida orthopsilosis (380353778)
Ashbya gossypii (374106554)
Cyanidioschyzon merolae (544210302)
Cyanidioschyzon merolae (544216417)
Cyanidioschyzon merolae (BAM81906)
Galdieria sulphuraria (EME27752)
0.87
0.93
0.93
0.96
0.62
0.68
0.82
0.57
0.87
0.91
0.83
0.98
0.68
0.73
1
0.3
Animals
Plant
Fungi
Red Algae
Excavata
Alveolate

MND1

Figure 8 MND1 likelihood tree.

The MND1 phylogeny was generated from amino acids sequences of 24 species and 676 sites as total alignment. The amino acid subsets were selected manually by Gblocks. Support for branches with (aLRT) values less than 0.50 are not shown.

was well-supported as being associated with a SPO11-2 gene from *Porphyridium purpureum* (Porphyridiales) (Contig number 3410.8) (0.83 aLRT) instead of being associated with *C. merolae* (BAM82494) (Figure 9).

Pairwise sequence divergence between SPO11 homologs was different. The sequence divergence between the species of SPO11-1 clade ranged from 0.000 to 0.800 (0 to 24 amino acids) (Appendix 2), and *Galdieria sulphuraria* EME30796 with *G. sulphuraria* EME30795 formed the lowest sequence divergence while *Candida orthopsilosis* with *Trichomonas vaginalis* and *C. orthopsilosis* with *Medicago truncatula* formed the highest sequence divergence in the SPO11-1 clade. For SPO11-3 clade, the sequence divergence between species ranged from 0.067 to 0.200 (2 to 6 amino acids) (Appendix 2). The lowest sequence divergence for SPO11-3 was formed by CDF39919 with *Cyanidioschyzon merolae* BAM82023 and *Porphyridium purpureum* 2255.5 with *C. merolae* BAM82023 while the highest sequence divergence formed by *P. purpureum* 2255.5 with *Micromonas pusilla* and *C. crispus* CDF39919 with *M. pusilla*. In SPO11-2 clade, the range of the sequence divergence was 0.567 to 0.867 (17 to 26 amino acids) (Appendix 2). *Arabidopsis thaliana* with *G. sulphuraria* EME31699 formed the lowest sequence divergence and *C. crispus* CDF33912 with *A. thaliana* formed the highest sequence divergence of the SPO11-2 clade.

3.2.4 HOP2

The HOP2 phylogenetic tree appears to have some groups that correspond to taxonomic and evolutionary history that they can be seen in two separate large clades. For example, red algae formed well-supported monophyletic group (0.95 aLRT), and Cyanidiales *Galdieria sulphuraria* and *Cyanidioschyzon merolae* formed well-supported cluster (0.82 aLRT). Additionally, the florideophyte *Chondrus crispus* and the bangiophyte *Porphyra purpurea* also formed a well-supported cluster (0.89 aLRT) (Figure 10). The green plant lineage, however, was weakly supported as being monophyletic with aLRT value less than 0.50 (not shown). However, the excavata *Trichomonas vaginalis* and alveolate

Oxytricha trifallax formed a non-monophyletic clade. The second clade contains two well-supported monophyletic groups: the fungi (0.83 aLRT) and animals (0.99 aLRT) (Figure 10).

3.2.5 MSH4

The maximum likelihood analysis of the MSH4 gene depicts the animals, Fungi, and green plant lineage as well-supported (1.0, 1.0 and 0.89 aLRT, respectively) as a separate group in a larger clade that is a sister clade to the sequences from the red algae (Figure 11). This red algal clade was well-supported as being monophyletic (0.96 aLRT). Surprisingly, the sequences of the MSH4 from *Oxytricha fallax* and *Trichomonas vaginalis* also formed a well-supported cluster (0.95 aLRT) that is sister to the larger clade containing the animals, Fungi, plants and red algae (Figure 11).

However, the cluster of the red algae does not reflect the evolutionary history of this group. Two members of the order Cyanidiales, *G. sulphuraria* and *C. merolae*, do not form a monophyletic group, and *C. merolae* was basal to the remaining red algae (Figure 11). Two copies of the MSH4 gene were identified in *G. sulphuraria* (GenBank Accession numbers EME26709 and EME26710), which may represent two isoforms. Interestingly, *C. crispus* (Florideophyceae) appears to be more closely related to *G. sulphuraria* with a supported value of 0.9 aLRT. In addition, the two sequences of taxa from the Bangiales, *Porphyra purpurea* and *Porphyra umbilicalis* formed well-supported (1.0 aLRT) cluster (Figure 11).

3.2.6 MSH5

The MSH5 likelihood tree appears to have some groups that do not correspond to taxonomic and evolutionary history, but were well-supported as being monophyletic. For instance, the animals, green plant lineage, and red algae are well-supported as separate monophyletic groups (1.0, 0.87 and 0.98 aLRT, respectively) and are included in a larger well-supported (0.92 aLRT) clade (Figure 12). The three sequences from the Fungi, however, formed a polyphyletic group. In addition, *Oxytricha trifallax* and *Trichomonas vaginalis* were supported as a paraphyletic group (0.88 aLRT) (Figure 12).

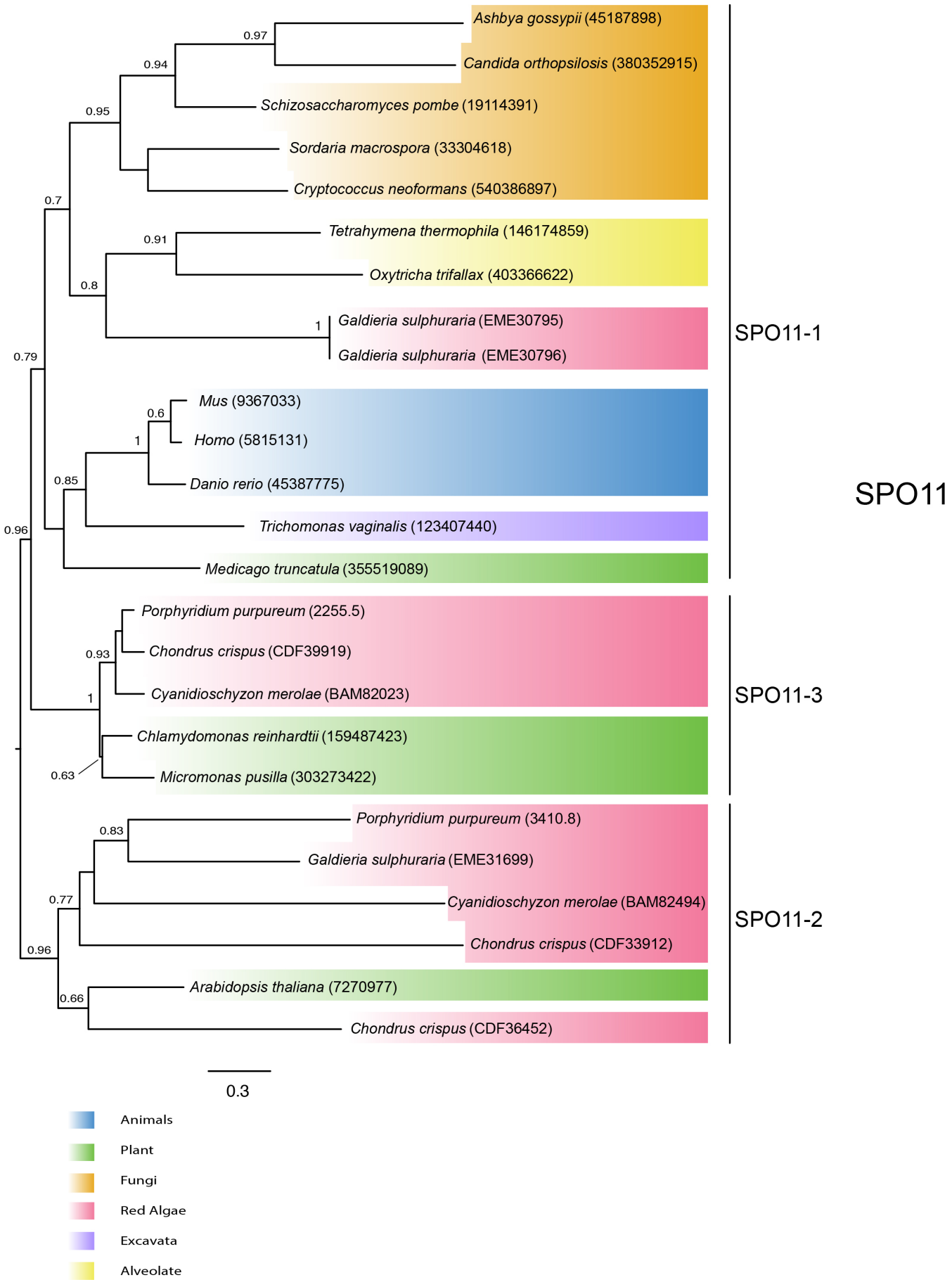
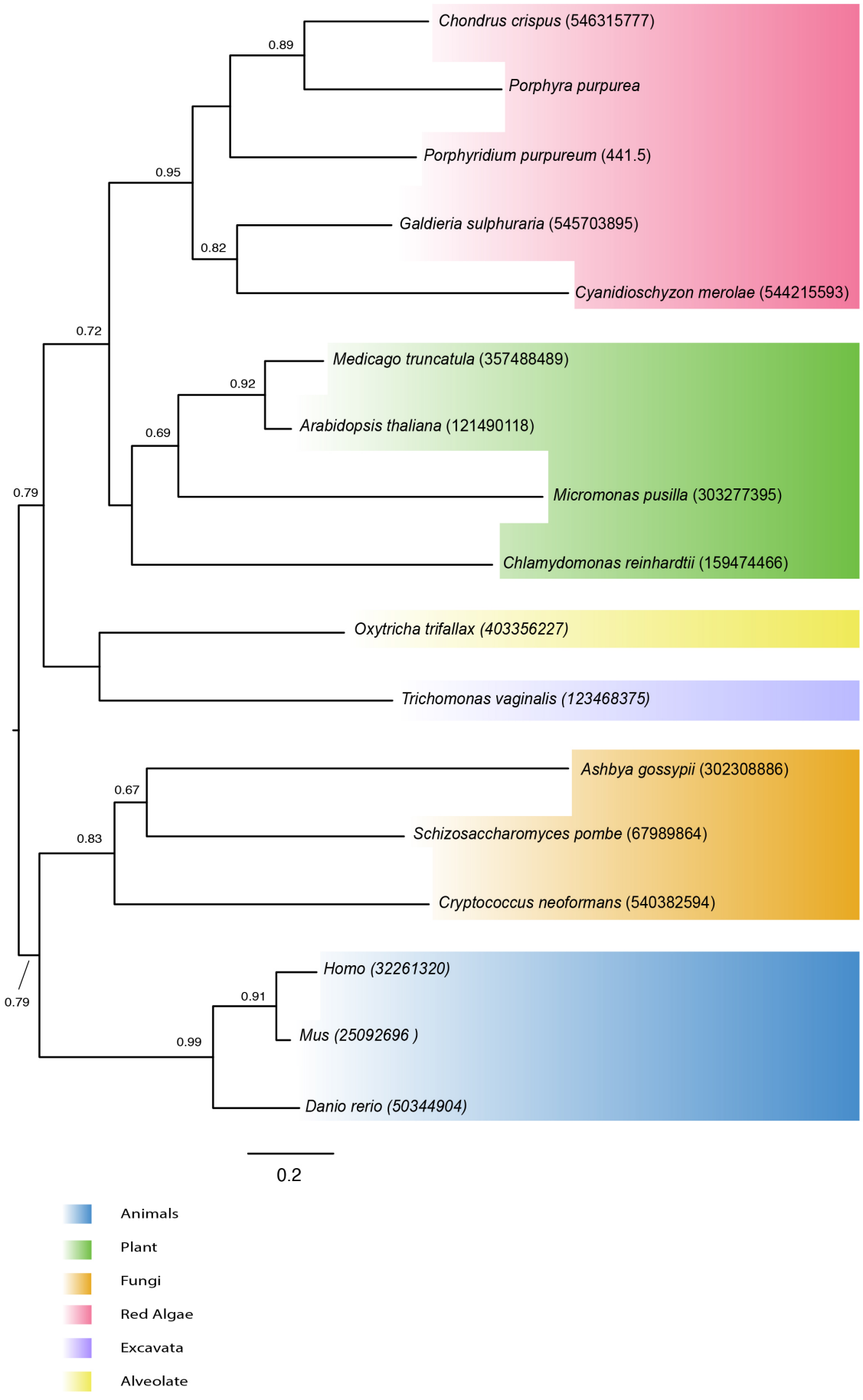

Ashbya gossypii (45187898)
Candida orthopsilosis (380352915)
Schizosaccharomyces pombe (19114391)
Sordaria macrospora (33304618)
Cryptococcus neoformans (540386897)
Tetrahymena thermophila (146174859)
Oxytricha trifallax (403366622)
Galdieria sulphuraria (EME30795)
Galdieria sulphuraria (EME30796)
Mus (9367033)
Homo (5815131)
Danio rerio (45387775)
Trichomonas vaginalis (123407440)
Medicago truncatula (355519089)
Porphyridium purpureum (2255.5)
Chondrus crispus (CDF39919)
Cyanidioschyzon merolae (BAM82023)
Chlamydomonas reinhardtii (159487423)
Micromonas pusilla (303273422)
Porphyridium purpureum (3410.8)
Galdieria sulphuraria (EME31699)
Cyanidioschyzon merolae (BAM82494)
Chondrus crispus (CDF33912)
Arabidopsis thaliana (7270977)
Chondrus crispus (CDF36452)
0.97
0.94
0.95
0.7
0.91
0.8
1
0.79
0.6
1
0.85
0.96
0.93
1
0.63
0.83
0.77
0.96
0.66
SPO11-1
SPO11-3
SPO11-2
SPO11
0.3
Animals
Plant
Fungi
Red Algae
Excavata
Alveolate

Figure 9 Maximum likelihood phylogeny of the SPO11 gene and its homologs SPO11-1, SPO11-2, and SPO11-3.

The SPO11 phylogeny was generated from amino acids sequences of 25 species and 877 sites as total alignment. The amino acid subsets were selected manually by Gblocks. Support for branches with (aLRT) values less than 0.50 are not shown.

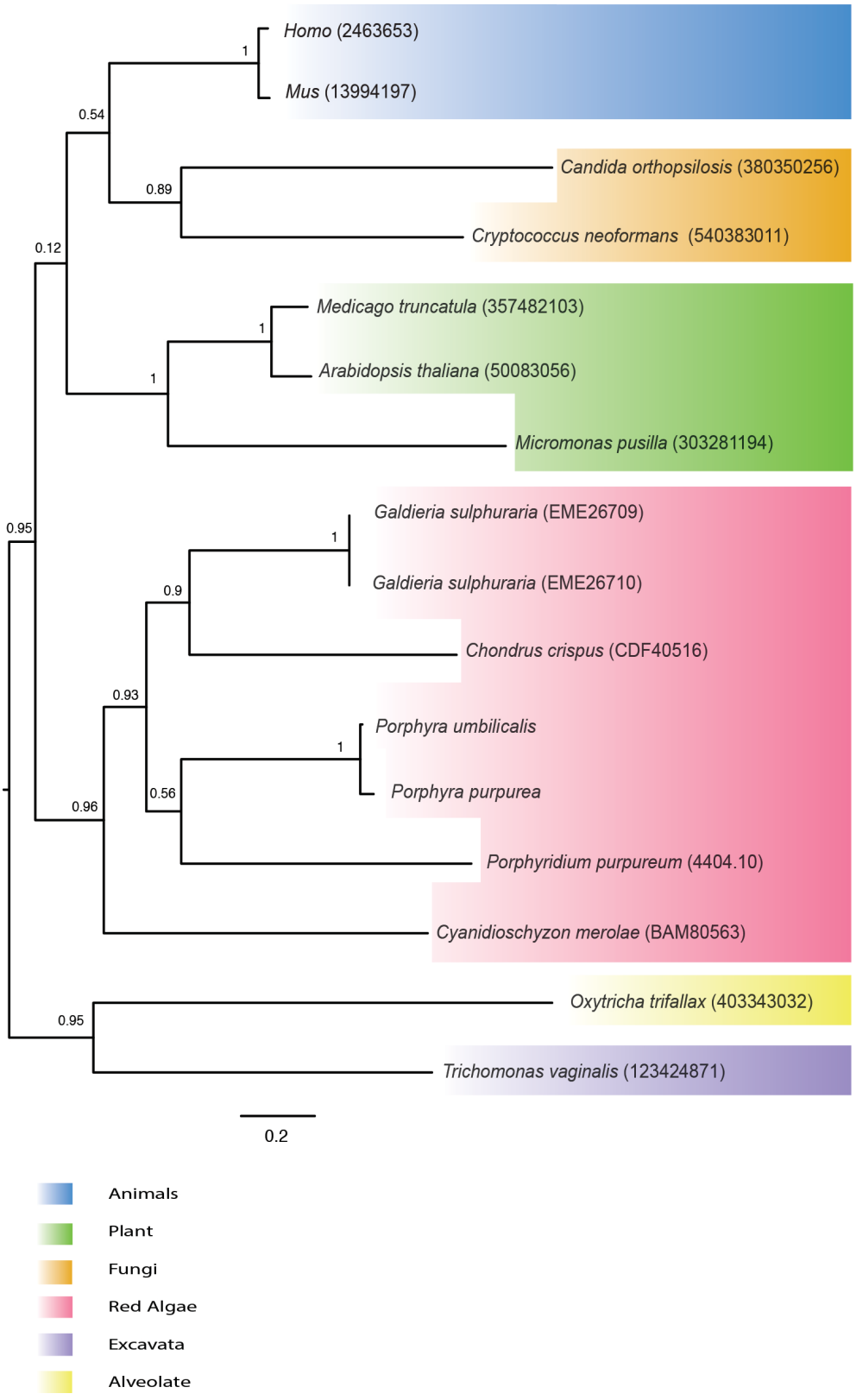
As noted previously, the MSH5 genes in the red algae were well-supported as being monophyletic (0.98 aLRT). However, the clustering of the MSH5 gene in the red algae does not reflect the evolutionary history of this group. For example, the two members of the order Cyanidiales, *Galdieria sulphuraria* and *Cyanidioschyzon merolae*, do not form a monophyletic group and *G. sulphuraria* appears to be basal to the remaining red algae. Furthermore, the MSH5 gene sequence from the order Cyanidiales *C. merolae* appears to be closely related to the order Porphyridiales *Porphyridium purpureum* though not well-supported by aLRT (Figure 12). Interestingly, two copies of the MSH5 gene were identified in *Chondrus crispus* (GenBank Accession numbers CDF39323 and CDF39324), which may indicate a gene duplication event.


Chondrus crispus (546315777)
Porphyra purpurea
Porphyridium purpureum (441.5)
Galdieria sulphuraria (545703895)
Cyanidioschyzon merolae (544215593)
Medicago truncatula (357488489)
Arabidopsis thaliana (121490118)
Micromonas pusilla (303277395)
Chlamydomonas reinhardtii (159474466)
Oxytricha trifallax (403356227)
Trichomonas vaginalis (123468375)
Ashbya gossypii (302308886)
Schizosaccharomyces pombe (67989864)
Cryptococcus neoformans (540382594)
Homo (32261320)
Mus (25092696)
Danio rerio (50344904)
0.89
0.95
0.82
0.72
0.92
0.69
0.79
0.67
0.83
0.79
0.91
0.99
0.2
Animals
Plant
Fungi
Red Algae
Excavata
Alveolate

HOP2

Figure 10 HOP2 Phylogenetic tree.

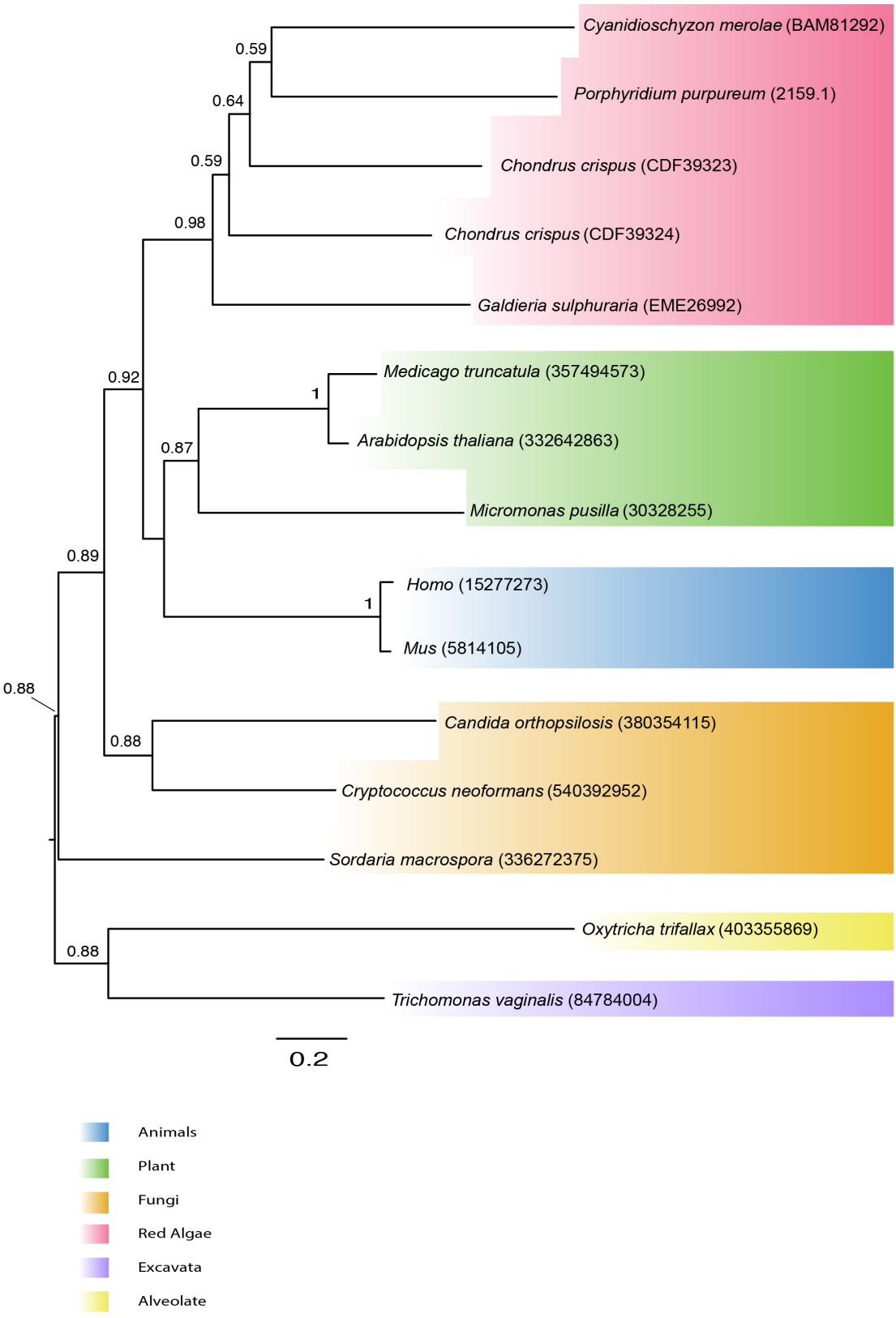
The maximum likelihood analysis of HOP2 was generated from amino acids sequences of 17 species and 623 sites as total alignment. The amino acid subsets were selected manually by Gblocks. Support for branches with (aLRT) values less than 0.50 are not shown.


Homo (2463653)
Mus (13994197)
Candida orthopsilosis (380350256)
Cryptococcus neoformans (540383011)
Medicago truncatula (357482103)
Arabidopsis thaliana (50083056)
Micromonas pusilla (303281194)
Galdieria sulphuraria (EME26709)
Galdieria sulphuraria (EME26710)
Chondrus crispus (CDF40516)
Porphyra umbilicalis
Porphyra purpurea
Porphyridium purpureum (4404.10)
Cyanidioschyzon merolae (BAM80563)
Oxytricha trifallax (403343032)
Trichomonas vaginalis (123424871)
1
0.54
0.89
0.12
1
1
0.95
1
0.9
0.93
1
0.56
0.96
0.95
0.2
Animals
Plant
Fungi
Red Algae
Excavata
Alveolate

MSH4

Figure 11 Phylogenetic tree of MSH4.

The MSH4 phylogenetic tree was generated from amino acids sequences of 16 species and 1962 sites as total alignment. The amino acid subsets were selected manually by Gblocks. Support for branches with (aLRT) values less than 0.50 are not shown.


Cyanidioschyzon merolae (BAM81292)
Porphyridium purpureum (2159.1)
Chondrus crispus (CDF39323)
Chondrus crispus (CDF39324)
Galdieria sulphuraria (EME26992)
Medicago truncatula (357494573)
Arabidopsis thaliana (332642863)
Micromonas pusilla (30328255)
Homo (15277273)
Mus (5814105)
Candida orthopsilosis (380354115)
Cryptococcus neoformans (540392952)
Sordaria macrospora (336272375)
Oxytricha trifallax (403355869)
Trichomonas vaginalis (84784004)
0.59
0.64
0.59
0.98
0.92
1
0.87
0.89
1
0.88
0.88
0.88
0.2
Animals
Plant
Fungi
Red Algae
Excavata
Alveolate

MSH5

Figure 12 Phylogenetic tree of MSH5.

The likelihood tree analysis of the MSH5 was generated from amino acids sequences of 15 species and 1328 sites as total alignment. The amino acid subsets were selected manually by Gblocks. Support for branches with (aLRT) values less than 0.50 are not shown.

Chapter 4.0: Discussion

The research performed in this thesis represents the first time the meiotic toolkit (core meiotic genes) has been tested to determine the sexuality and the evolutionary history of a group of organisms and in this case the red algae (Rhodophyta). Red algae are a distinct eukaryotic lineage and characterized by the lack of flagella and centrioles; however, there are sexual species and other species that have never been reported as sexual (Nelson 2007; Spitale et al. 2012). Due to the small number of available red algal genomes, the phylogenetic analyses in this study are confined to the genomes of the cyanidiophytes (*Galdieria sulphuraria* and *Cyanidioschyzon merolae*), the florideophyte *Chondrus crispus*, the porphyridiophyte *Porphyridium purpureum*, and the bangiophytes (*Porphyra purpurea*, *Porphyra umbilicalis*, and *Porphyra rediviva*); moreover, animals, plants, fungi, alveolates, and excavate species were added to the analyses to examine trends over the eukaryotic tree of life. In this study, six out of nine core meiotic genes (DMC1, MND1, SPO11, HOP2, MSH4, and MSH5) were examined. These meiotic genes are more universal in the genomes of eukaryotes (Schurko and Logsdon 2008; Malik et al. 2008; Chi et al. 2013). The results from the present study suggested that both unicellular and multicellular red algal species possess these meiotic genes and this may indicate cryptic meiosis in some lineages. However, it appears that the meiotic toolkit genes are not useful for determining the taxonomy and evolutionary history of the red algae when compared to more commonly used genes, such as the 18S rRNA and *rbc*L genes (Saunders and Hommersand 2004; Yoon et al. 2006). Some of the meiotic genes examined appear to show complex evolutionary histories that reflect gene duplication and possible horizontal gene transfer.

4.1 DMC1, MND1, and SPO11 Phylogenetic Tree analyses

In this study, three out of six core meiotic genes, DMC1, MND1, and SPO11, have complex evolutionary histories that show examples of both horizontal gene transfer and gene duplication events as seen in the various phylogenies presented in this study (Figures 7, 8, 9). On the other hand, the

meiotic genes, HOP2, MSH4, and MSH5, do not exhibit such complexity (Figures 10, 11, 12) and depict an evolutionary history that reflects the current taxonomy of those groups (Yoon et al. 2006a).

4.1.1 DMC1

The DMC1 gene is a homolog of the RAD51 gene. Both are eukaryotic proteins and are also homologs of the eubacterial RECA gene (Stassen et al. 1997). Both DMC1 and RAD51 are essential meiotic proteins for normal synaptonemal complex formation (SC), meiotic recombination, and DNA damage repair (Masson and West 2001). The DMC1 phylogenetic tree indicates that either a lateral gene transfer or an ancient gene duplication led to the homologs DMC1 and RAD51, and followed by subsequent RAD57 and RECA gene losses. The branching pattern similarities in animals, plants and green algae, red algae, fungi, excavata, and alveolates in the DMC1 and RAD51 are more suggestive of an ancient gene duplication or horizontal transfer in the common ancestor of these eukaryotic groups because they both are presented in all eukaryotic lineages. Interestingly, gene loss has likely happened in the closely related members of the red algal group, Cyanidiales, where the species *Galdieria sulphuraria* has two isoforms of the DMC1 gene (EME32481 and EME32482) and a single copy of RAD51 gene (EME28274); whereas the other species, *Cyanidioschyzon merolae* has a single copy of the RAD51 gene with different assemblies (XP005538367 and BAM82331) and a single copy of the DMC1 gene (BAM83478) (Figure 7). In the same phylogenetic tree, only the red algae *C. merolae* BAM81186, *G. sulphuraria* EME29355, and *Chondrus crispus* CDF39004 have the RAD57 gene, but they are positioned on longer branches suggesting a recent gene dulication. For the eubacterial RECA gene, Lin et al. (2006) suggested that a secondary endosymbiotic gene transfer derived the RECA to the nuclear genomes of ancestral eukaryotes. However, the current phylogenetic analyses showed that the RECA is only present in *C. merolae* and missing in other eukaryotic lineages and that is more suggestive of recent gene duplication.

4.1.2 SPO11

The SPO11 proteins are homologs of an archaebacterial topoisomerase VI subunit, which functions to separate replicated chromosomes in archaebacteria (Bergerat et al. 1997; Corbett et al. 2007). SPO11 is a meiosis-specific protein that stimulates the formation of double-strand breaks (DSBs) in DNA, particularly, in meiosis in order to initiate meiotic recombination (Keeney 2008). The absence of SPO11 in eukaryotic species may lead to meiosis failure (Keeney 2001). SPO11-1, SPO11-2 and SPO11-3 are homologs of SPO11 (Hartung and Puchta 2000), and SPO11-1 and SPO11-2 are essential for meiotic recombination (Hartung et al. 2007; Hörandl and Hadacek 2013).

The phylogenetic tree of SPO11 appears to have three orthologs (SPO11-1, SPO11-2, and SPO11-3) and only red algae have the three prologs of the SPO11. However, the clustering of green plants and red algae are suggestive of either HGT or an ancient gene duplication event in the common ancestor of these groups. Subsequent gene loss can explain why some closely related species do not contain a second copy. For example, the genome of *Galdieria sulphuraria* (Cyanidiales) contains three copies of SPO11 (two similar SPO11-1 gene copies and a single SPO11-2 gene) while the other member of the Cyanidiales, *Cyanidioschyzon merolae* does not have the SPO11-1 copy but does have a copy of the SPO11-2 and SPO11-3 genes (Figure 9), which is consistent with the finding by Malik et al. (2007). The one member of the Porphyridiales, *Porphyridium purpureum,* appears to have two paralogs of SPO11 (SPO11-2 and SPO11-3) while the SPO11-1 gene is lost as was shown by Bhattacharya et al. (2013). Similarly, in the green plant linage, there are multiple gene losses in this linage and that is inconsistent with Malik et al. (2007) finding which indicated that the SPO11-1 is present in *Arabidopsis.*

These recent findings are in agreement with the suggestion that the SPO11-2 gene is not observed in animals, fungi and *Trichomonas* (Malik et al. 2007) while the SPO11-1 gene is present in animals, fungi, and some alveolates (Ramesh et al. 2005). It has been proposed that the red algae and green plant lineages have acquired the SPO11-2 and SPO11-3 genes from archaebacteria via horizontal

gene transfer and this would explain why the homolog copies of SPO11 (SPO11-2 and SPO11-3) are absent in other eukaryotes (Yin et al. 2002; Jain et al. 2006). However, the phylogenetic analyses in the current study indicate that the eukaryotic SPO11 gene homologs evolved via gene duplication events that led to arise the SPO11 orthologs SPO11-3 and SPO11-2 (Figure 9). Moreover, the absence of the SPO11-1 and SPO11-3 genes in *Chlamydomonas reinhardtii* and *Micromonas pusilla* suggest that the SPO11 orthologs may not be derived via secondary endosymbiotic gene transfer because these orthologs are absent in other eukaryotic lineages (Figure 9). Similarly, not all red algal species have SPO11 orthologs (Figure 9), which may indicate that SPO11-2 and SPO11-3 orthologs were lost from several eukaryotic lineages. In addition, the red algal species that clustered in SPO11-2 gene clade exhibit long branches compared to the remaining red algae. These differences in branches lengths may be due to being associated with different protein structural environments and function (Thorne 2000).

4.1.3 MND1

Meiotic nuclear division and recombination requires the MND1 gene, and the protein forms a heterodimeric complex with HOP2 to ensure accurate meiotic DNA double-strand break (DSB) formation (Riley and Corradi 2013). The MND1-HOP2 heterodimeric also contributes in telomeres maintenance (Cho et al. 2014). The phylogenetic tree of the MND1gene exhibits gene loss and multi gene duplication events in the red algal species (Figure 8). In the MND1 gene phylogeny the red algae form a paraphyletic group, and the cyanidiophytes *Galdieria sulphuraria* and *Cyanidioschyzon merolae* have two paralogs of MND1; the MND1 paralog observed only in the cyanidiophytes has not yet been annotated in the genome sequences (Nozaki et al. 2007; Bhattacharya et al. 2013; Schönknecht et al. 2013). Since the other copy of the MND1 gene is missing in other eukaryotic lineages, it is possible that there is a recent MND1 gene duplication in the Cyanidiales or an ancient horizontal transfer event in a common ancestor of red algae. Additionally, the duplicated MND1 gene copies are only distantly related in the tree and positioned on longer branches. *Porphyra rediviva* (the sequence obtained in this study) is

clustered with two other members of the genus *Porphyra*, *P. purpurea* and *P. umbilicalis*, but it is positioned on a much longer branch (Figure 8). In addition, this sequence contains premature stop codons (Figure 5) and may be a pseudogene and is likely no longer under selection.

4.2 Core Meiotic Genes

The presence of most or all of these genes, in a given genome, provides an indication that the organism has the ability to undergo meiosis; on the other hand, the absence of some or all of these meiotic genes may not associated with the absence of meiosis (Schurko and Logsdon 2008). For example, 5 out of 9 core meiotic genes (DMC1, HOP2, MND1, MSH4 and MSH5) are absent in the genome of *Drosophila melanogaster*, which is well known as a sexual organism (Jensen et al. 2015).

Sexual reproduction is well documented in multicellular members of Rhodophyta. Triphasic alternation of generations occur in the multicellular Florideophyceae and a biphasic life history occurs in other multicellular taxa, as in order Bangiales including *Porphyra* and *B. maxima* (Lynch at al. 2008; Müller et al. 2010). Nonetheless, very little is known about sexual reproduction in the other taxa, particularly for the unicellular red algal species such as *Galdieria sulphuraria, Cyanidioschyzon merolae,* and *Porphyridium purpureum*, and it has been suggested that they are asexual (Bhattacharya et al. 2013; Ciniglia et al. 2004; 2014). To address the possibility of cryptic meiosis, detecting meiotic genes in such organisms would be an effective solution for determining the presence of sex. The detection meiotic toolkit was widely used to determine the sexuality, for example, excavates, fungi, and animals (Malik et al. 2008; Schurko et al. 2009; Riley and Corradi 2013). The presence of most or all these meiosis-specific proteins (DMC1, MND1, SOP11, HOP1, HOP2, MSH4, MSH5, RECA, and MER3) provide a significant sign of the ability to undergo meiosis, whereas the absence of some or all of these meiotic genes may be associated with the inability to undergo sexual reproduction (Schurko and Logsdon 2008).

In this study, six out of nine core meiotic genes (DMC1, MND1, SPO11, HOP2, MSH4, and MSH5) were examined. The results showed that the all well-known sexual eukaryotic species that were clustered in the phylogenetic trees possess at least on copy of the meiotic genes (Figures 7, 8, 9, 10, 11, 12). However, the unicellular red algal species *G. sulphuraria, C. merolae,* and *P. purpureum*, which are have never been observed as a sexual genera in nature (Bhattacharya et al. 2013; Ciniglia et al. 2004; 2014), do not just possess a copy of these meiotic genes but two or more of (DMC1 and its homolog RAD51, MND1, SPO11, and MSH4) (Figures 7, 8, 9, 11) and that is consistent with the maintenance of sexuality but does not prove the occurrence of sexual reproduction in these unicellular species.

The multiple copies of the meiotic genes, as discussed earlier, were either derived from HGT or gene duplication. Since these duplicate genes remain in the genome of the unicellular red algae, the duplicate genes may become duplicated pseudogenes. However, keeping pseudogenes in a genome may be disadvantageous; for example, the organism may pay the energy cost of nonfunctional pseudogenes (Tutar 2012). Hence, why does an organism keep these pseudogenes rather than getting rid of them? Gene duplication, however, may generate new genes associated with different function (Magadum et al. 2013), and some pseudogenes may have operational functions, or they maintain the functionality of the original genes (Pink and Carter 2013), and that may explain why the duplicated pseudogenes are still present in the unicellular genomes of red algae. Some pseudogenes that accumulate the deleterious mutations and will eventually will be lost while the non-deleterious may generate other copies and contribute to genetic diversity overtime (Singh and Cole 2011).

On the other hand, the six core meiotic genes, which appear to be ubiquitous among all eukaryotes, are essential to the meiotic process including synapsis, high levels of recombination, and no S phase prior to Meiosis II (White 1978; Page and Hawley 2003). These genes were present in all sexual eukaryotic lineages (Figures 7, 8, 9, 10, 11, 12). This suggests that the unicellular red algae have the original meiotic genes and the other duplicated copies may maintain the functionality of the original

genes, which provides support for the common ancestor having the ability to undergo meiosis, and that suggests the unicellular red algae may undergo cryptic sex. Hence, in this case, the detection meiotic toolkit appears to be useful in determination of sexuality in unicellular red algae.

In summary, as it has been reported that the detection meiotic toolkit was widely used to determine the sexuality, and the presence of most or all these core meiotic genes provide a significant sign of the ability to undergo sexuality, whereas the absence of some or all of these meiotic genes may be associated with asexual reproduction (Schurko and Logsdon 2008; Malik et al. 2008; Schurko et al. 2009; Riley and Corradi 2013).

Sexuality has been documented in the multicellular red algal species *Chondrus crispus*, so the presence of the 6 core meiotic genes in its genome is not being surprising. However, the presence of the same meiotic genes in the genome of the unicellular red algal species *Galdieria sulphuraria*, *Cyanidioschyzon merolae,* and *Porphyra umbilicalis*, which have never been observed as sexual, is considered as an interesting finding. In the case of the red algae, the determination of sexuality of the unicellular red algal species would be challenging if we only rely on the detection meiotic toolkit. Further laboratory experiments have to be carried to ensure these unicellular red algae have the ability to undergo sexual reproduction. In other words, the presence of the core meiotic genes (meiotic toolkit) in the genomes of the unicellular red algae may suggest the maintenance of sexuality but does not prove the occurrence of sexual reproduction in these unicellular species.

4.3 Core Meiotic Genes in Taxonomy

The red algae (Rhodophyta) are a distinct lineage characterized by the lack of flagella and centrioles. In traditional taxonomic scheme, the phylum Rhodophyta contained two classes Bangiophyceae and Florideophyceae (Hoek et al. 1995). Then, Saunders and Hommersand (2004) recognized four classes Bangiophyceae, Florideophyceae Rhodellophyceae and Compsopogonophyceae.

The recent taxonomic scheme proposed by Yoon et al. (2006a) contains one phylum Rhodophyta and two subphyla: 1) the Rhodophytina with six classes Bangiophyceae, Florideophyceae, Rhodellophyceae, Porphyridiophyceae, Compsopogonophyceae, and Stylonematophyceae; 2) Cyanidiophytina with one class Cyanidiophyceae. This taxonomic scheme was determined using nuclear and plastid small subunit ribosomal (SSU) genes and also included several morphological characteristics.

The phylogenetic tree analyses of this study suggest that the detection meiotic toolkit is a not a good source of genes to use for ordinal classification and in general only the animals appeared monophyletic in which *Homo sapiens*, *Mus musculus*, and *Danio rerio* were clustering together in all phylogenetic trees (Figures 7, 8, 9, 10, 11, 12). However, this is a very small sampling of the animal group.

In the RAD51 gene phylogeny, the red algae did not form a monophyletic group and the florideophyte, *Chondrus crispus*, appears to be closely related to the order Cyanidiales. This differs from 18S rRNA and *rbc*L gene phylogenies in which the Florideophyceae is typically associated with the order Bangiales (Yoon et al. 2006a; Müller et al. 2011). The phylogenetic tree of the MND1 gene also did not reflect the evolutionary history. For example, the green plants and fungi did not appear as separate monophyletic groups, and within the red algae, the Porphyridiales appears to be more closely related to the Bangiales. The green plants did not form a monophyletic cluster in the meiotic gene SPO11 phylogeny, and the red alga, *C. crispus*, was a sister taxa to the plant, *A. thaliana*. In the MSH4 gene phylogeny, the cyanidiophytes appeared to be closely related to the Florideophyceae, which again does not agree with current taxonomy (Yoon et al. 2006a). Similarly, in the MSH5 gene phylogeny the cyanidiophytes, *G. sulphuraria* and *C. merolae* did not cluster together as expected. However, the HOP2 gene phylogeny appears to correspond with evolutionary history as determined by 18S rRNA and *rbc*L gene phylogenies (Yoon et al. 2006a; Müller et al. 2011). For example, all monophyletic groups appear to correspond to the ordinal classification in the HOP2 phylogeny (Figure 10).

The lack of correspondence among the meiotic gene phylogenies and current accepted taxonomy of these lineages may be due to compositional biases (CB). Compositional biases are known as local shifts in amino acids or nucleotides sequences, and it may occur in genomes, proteomes or even in a part of genes determining certain proteins features (Antonets and Nizhnikov 2013). As mentioned previously, the phylogenetic analyses, were generated from the amino acids sequence alignment; hence, proteins sequences may be affected by the GCs and ATs content that can end up forcing the protein to look different which in turn provide unreliable phylogeny (Foster and Hickey 1999; Antonets and Nizhnikov 2013). In summary and based on this study, the meiotic genes (MND1, RAD51, SPO11, MSH4, MSH5) are not good source of genes to determine the evolutionary history and taxonomical system (Figure 7, 8, 9, 11, 12) because of the CB and the GCs and ATs content while the meiotic genes (DMC1 and HOP2) are consider as useful taxonomic markers for the red algae or other eukaryotic organisms (Figure 7 and 10).

Chapter 5.0: Conclusion and Future Studies

Meiosis, which is the most essential process for sexual reproduction in eukaryotes, requires several nuclear genes to perform properly. Schurko and Logsdon (2008) suggested that there are nine core meiotic genes "Detection meiotic toolkit", and the presence of all or some of these genes would be likely be associated with sexual reproduction. Despite having an important role in meiosis process, not much is known about the evolutionary history of these core meiotic genes. Red algae are a distinct eukaryotic lineage having a long evolutionary history that dates 1.2 billion years ago represented by a fossil that exhibits characteristics of meiosis (Butterfield et al. 1990). However, sexual reproduction has never been observed in many of the unicellular red algal species including *Galdieria sulphuraria*, *Cyanidioschyzon merolae*, and *Porphyridium purpureum* Bhattacharya et al. 2013; Ciniglia et al. 2004; 2014). Detecting core meiotic genes in the genome of red algal species would provide significant indication that cryptic meiosis is occurring in the putative asexual lineages. The present study was initiated to facilitate a better understanding of the evolutionary history of meiotic genes by:

1. Examining the presence of six core meiotic genes (DMC1, MND1, SPO11, HOP2, MSH4, and MSH5) in the available red algal genomes: *G. sulphuraria*, *C. merolae*, *P. purpureum*, and *C. crispus*) to determine the sexuality of the unicellular red algal species and the usefulness of the meiotic toolkit in the determination of sexuality.
2. Determine whether the meiotic toolkit is a useful tool to determine the evolutionary history of eukaryotes, particularly the red algae.

In this study, amino acids alignments were used to generate the phylogenetic trees. The amino acids sequences of the six core meiotic genes were retrieved from all completely sequenced eukaryotic genomes stored in the GenBank database using BLASTx. The results indicated that the core meiotic genes DMC1, MND1, and SPO11 have been associated with subsequent gene loss (Figures 7, 8, 9) and

that may explain why some eukaryotic species do not contain a second copy of these three genes. HOP2, MSH4, and MSH5 genes, however, do not exhibit such complexity (Figures 10, 11, 12).

Overall, the phylogenetic analyses indicate that the six core meiotic genes examined are present in sexual multicellular (*Porphyra* spp. and *C. crispus*) and unicellular red algae (*G. sulphuraria*, *C. merolae*, and *P. purpureum*). The latter of which sexual reproduction has never been reported (Bhattacharya et al. 2013; Ciniglia et al. 2004; 2014) (Figures 7, 8, 9, 10, 11, 12). Hence:

I. This suggests support for the common ancestor having the ability to undergo meiosis as expected.

II. The validity of using the meiotic toolkit for sexuality determination may rely on the following:

A. The meiotic genes may have an alternative function to their role in meiosis (Pink and Carter 2013).

B. The meiotic genes may be pseudogenes without deleterious mutations: These pseudogenes may generate other copies and contribute to genetic diversity overtime (Singh and Cole 2011).

In these two cases, the meiotic toolkit is not a good source of genes to determine sexual reproduction.

C. The meiotic gene homologs may function as true meiotic genes: The unicellular red algae may still use these genes and undergo cryptic meiosis.

In the third case in which the genes in the meiotic toolkit are still functioning for meiosis, it may be possible that these are maintaining the ability for unicellular red algae to undergo meiosis, possibly in a cryptic manner that has not been observed.

Taxonomically, the phylogenetic analyses of this study indicate that the meiotic genes HOP2 and DMC1 may consider as useful genes for classification and taxonomic system while the other genes

within the meiotic toolkit are not good taxonomic markers for the red algae or other eukaryotic organisms due to the gene duplication and potential horizontal transfer (Figures 7, 8, 9, 10, 11, 12).

Future studies

In this study, the core meiotic genes are present in the unicellular red algae suggesting that they may have the ability to undergo cryptic meiosis. Further work could be performed to provide more support by examining the presence of the core meiotic genes in other asexual eukaryotic organisms such as the haptophyte *Emiliania huxleyi*, the green algae *Trebouxia* and the red algae *B*. *atropurpurea* (Wehr and Sheath 2003; Graham et al. 2009). Moreover, to ensure the usefulness of the meiotic toolkit, more studies need to be conducted on the genes expression of the meiotic too kit of the unicellular red algae, and compare the expressed gene result with well-known sexual species. Finally, it may be possible that the unicellular red algal species are alternative life phases of another currently unknown red algal species. The conchocelis (sporophytic) stage of *Porphyra* was initially identified as a separate species (Drew 1949; Nelson et al. 1999; Gantt et al. 2010). Moreover, some species of the red algae, *Audouinella,* have been determined to be the asexually produced alternate life history stages (also called chantransia) of members of the genus *Batrachospermum* (Necchi & Zucchi 1997; Chiasson et al. 2005). Hence, the possibility of some taxa being alternate life histories of unknown red algae is still a possibility.

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APPENDIXES

Appendix 1

The meiotic genes and their amino acids accession numbers in GenBank for the organisms that were used in this study.

Gene/protein	Organism	Species	Accession Number
DMC1			
	Animals	Homo sapiens	47678503 9168602
		Mus musculus (House mouse)	6753650 6755276
		Danio rerio (zebrafish)	190338308 347800669
	Plants and Green algae	*Arabidopsis thaliana*	30686826 332005514
		Medicago truncatula	357479303
		Chlamydomonas reinhardtii	159484887 158277986
		Micromonas pusilla	303284847 303279168
	Red algae	*Galdieria sulphuraria*	EME32481 EME32482 EME29355 EME28274 XP 005709001
		Gracilaria changii	GCC0002388 (PartiGeneDB)

Gene/protein	Organism	Species	Accession Number
		Chondrus crispus	CDF34961 CDF35384 CDF39004 CDF39532
		Porphyridium purpureum	Contig# 4438.25 (Bhattacharya et al. 2013)
		Cyanidioschyzon merolae	BAM83478 BAM81186 BAM80454 BAM82331 XP 005538367
		Porphyra purpurea *Porphyra umbilicalis* *Porphyra yezoensis*	
	Fungi	*Ashbya gossypii*	45201197 45185912
		Candida orthopsilosis	380353531 380350318
		Sordaria macrospora	3176384 336272433
		Cryptococcus neoformans	405123640 405118075
		Schizoccharomyces pombe	548664
	Excavata	*Trichomonas vaginalis*	123408121 123408472
	Alveolates	*Trypanosoma cruzi*	71659624

Gene/protein	Organism	Species	Accession Number
			37778910
		Oxytricha trifallax	403376234 403374245
MND1			
	Animals	Homo sapiens	14149769
		Mus musculus (House mouse)	240848585
		Danio rerio (zebrafish)	57525722
	Plants and Green algae	*Arabidopsis thaliana*	30688234
		Chlamydomonas reinhardtii	159474610
		Micromonas pusilla	255085320
	Red algae	*Galdieria sulphuraria*	EME26811 EME27752
		Chondrus crispus	CDF38011
		Porphyridium purpureum	Contig# 2121.23 (Bhattacharya et al. 2013)
		Cyanidioschyzon merolae	BAM79559 544210302 544216417 BAM81906
		Porphyra rediviva	**This study**
		Porphyra purpurea *Porphyra umbilicalis*	

Gene/protein	Organism	Species	Accession Number
	Fungi	*Ashbya gossypii*	374106554
		Candida orthopsilosis	380353778
		Sordaria macrospora	3176384 336272433
		Cryptococcus neoformans	405124028
		Schizoccharomyces pombe	213408226
	Excavata	*Trichomonas vaginalis*	154413267
	Alveolates	*Trypanosoma cruzi*	261334527
		Oxytricha trifallax	403374287
SPO11			
	Animals	Homo sapiens	5815131
		Mus musculus (House mouse)	9367033
		Danio rerio (zebrafish)	45387775
	Plants and Green algae	*Arabidopsis thaliana*	7270977
		Medicago truncatula	303273422
		Chlamydomonas reinhardtii	159487423
		Micromonas pusilla	303273422
	Red algae	*Galdieria sulphuraria*	EME31699 EME30796 EME30795
		Chondrus crispus	CDF39919 CDF36452 CDF33912
		Porphyridium purpureum	Contig# 3410.8 (SPO11-2) Contig# 2255.5

Gene/protein	Organism	Species	Accession Number
			(SPO11-3) (Bhattacharya et al. 2013)
		Cyanidioschyzon merolae	BAM82023 BAM82494
	Fungi	*Ashbya gossypii*	45187898
		Candida orthopsilosis	380352915
		Sordaria macrospora	33304618
		Cryptococcus neoformans	540386897
		Schizoccharomyces pombe	19114391
	Excavata	*Trichomonas vaginalis*	123407440
	Alveolates	*Tetrahymena thermophila*	146174859
		Oxytricha trifallax	403366622
HOP2			
	Animals	Homo sapiens	32261320
		Mus musculus (House mouse)	25092696
		Danio rerio (zebrafish)	50344904
	Plants and Green algae	*Arabidopsis thaliana*	121490118
		Medicago truncatula	357488489
		Chlamydomonas reinhardtii	159474466
		Micromonas pusilla	303277395
	Red algae	*Galdieria sulphuraria*	545703895 EME31309 EME31308
		Chondrus crispus	546315777

Gene/protein	Organism	Species	Accession Number
		Porphyridium purpureum	Contig# 441.5 (Bhattacharya et al. 2013)
		Cyanidioschyzon merolae	544215593 BAM82928
	Fungi	*Ashbya gossypii*	302308886
		Cryptococcus neoformans	540382594
		Schizoccharomyces pombe	67989864
	Excavata	*Trichomonas vaginalis*	123468375
	Alveolates	*Oxytricha trifallax*	403356227
MSH4			
	Animals	Homo sapiens	2463653
		Mus musculus (House mouse)	13994197
	Plants and Green algae	*Arabidopsis thaliana*	50083056
		Medicago truncatula	357482103
		Micromonas pusilla	303281194
	Red algae	*Galdieria sulphuraria*	EME26709 EME26710
		Chondrus crispus	CDF40516
		Porphyridium purpureum	Contig# 4404.10 (Bhattacharya et al. 2013)
		Cyanidioschyzon merolae	BAM80563
	Fungi	*Candida orthopsilosis*	380350256
		Cryptococcus neoformans	540383011

Gene/protein	Organism	Species	Accession Number
	Excavata	*Trichomonas vaginalis*	123424871
	Alveolates	*Oxytricha trifallax*	403343032

MSH5			
	Animals	Homo sapiens	15277273
		Mus musculus (House mouse)	5814105
	Plants and Green algae	*Arabidopsis thaliana*	332642863
		Medicago truncatula	357494573
		Micromonas pusilla	30328255
	Red algae	*Galdieria sulphuraria*	EME26992
		Chondrus crispus	CDF39323 CDF39324
		Porphyridium purpureum	Contig# 2159.1 (Bhattacharya et al. 2013)
		Cyanidioschyzon merolae	BAM81292
	Fungi	*Candida orthopsilosis*	380354115
		Cryptococcus neoformans	540392952
		Sordaria macrospora	336272375
	Excavata	*Trichomonas vaginalis*	84784004
	Alveolates	*Oxytricha trifallax*	403355869

Appendix 2

Pairwise distances of sequence variation in aligned amino acids sequences of 877 sites. The first matrix shows the sequence divergence between SPO11 homologs of 25 species, and the second matrix shows the number of differences of amino acids between SPO11 homologs of the 25 species.

[1] *Mus musculus* |9367033

[2] *Homo sapiens* |5815131

[3] *Danio rerio* |45387775

[4] *Medicago truncatula* |355519089

[5] *Chlamydomonas reinhardtii* |159487423

[6] *Micromonas pusilla* |303273422

[7] *Arabidopsis thaliana* |7270977

[8] *Tetrahymena thermophila*|146174859

[9] *Oxytricha trifallax* |403366622

[10] *Trichomonas vaginalis* |123407440

[11] *Ashbya gossypii* |45187898

[12] *Candida orthopsilosis* |380352915

[13] *Shizcosaccharomyces pombe* |19114391

[14] *Sordaria macrospora* |33304618

[15] *Cryptococcus neoformans* |540386897

[16] *Cyanidioschyzon merolae* |BAM82023

[17] *Cyanidioschyzon merolae* |BAM82494

[18] *Galdieria sulphuraria* |EME30795

[19] *Galdieria sulphuraria* |EME30796

[20] *Galdieria sulphuraria* |EME31699

[21] *Chondrus crispus* |CDF33912

[22] *Chondrus crispus* |CDF36452

[23] *Chondrus crispus* |CDF39919

[24] *Porphyridium purpureum* |2255.5

[25] *Porphyridium purpureum* |3410.8

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25

[1]

[2] 0.067

[3] 0.267 0.233

[4] 0.633 0.667 0.633

[5] 0.467 0.500 0.433 0.533

[6] 0.400 0.400 0.400 0.600 0.100

[7] 0.533 0.500 0.567 0.667 0.500 0.533

[8] 0.600 0.567 0.600 0.633 0.567 0.633 0.600

[9] 0.567 0.500 0.533 0.733 0.600 0.533 0.600 0.567

[10] 0.600 0.600 0.700 0.667 0.633 0.600 0.600 0.667 0.733

[11] 0.700 0.633 0.667 0.667 0.700 0.667 0.600 0.600 0.633 0.667

[12] 0.633 0.567 0.667 0.800 0.700 0.700 0.667 0.633 0.600 0.800 0.600

[13] 0.567 0.567 0.567 0.533 0.500 0.467 0.633 0.467 0.500 0.667 0.600 0.567

[14] 0.500 0.500 0.500 0.733 0.633 0.633 0.667 0.533 0.600 0.767 0.600 0.667 0.467

[15] 0.567 0.567 0.633 0.733 0.633 0.600 0.667 0.633 0.633 0.700 0.700 0.733 0.467 0.567

[16] 0.500 0.533 0.467 0.533 0.100 0.167 0.533 0.533 0.600 0.667 0.633 0.700 0.500 0.600 0.633

[17] 0.767 0.733 0.667 0.700 0.700 0.733 0.633 0.633 0.600 0.800 0.667 0.833 0.700 0.700 0.767 0.700

[18] 0.533 0.567 0.633 0.633 0.533 0.567 0.500 0.633 0.667 0.600 0.733 0.733 0.667 0.700 0.733 0.567 0.667

[19] 0.533 0.567 0.633 0.633 0.533 0.567 0.500 0.633 0.667 0.600 0.733 0.733 0.667 0.700 0.733 0.567 0.667 0.000

[20] 0.633 0.600 0.500 0.700 0.667 0.667 0.567 0.733 0.633 0.700 0.767 0.700 0.733 0.767 0.767 0.667 0.700 0.667 0.667

[21] 0.900 0.900 0.833 0.733 0.800 0.800 0.867 0.867 0.833 0.867 0.900 0.967 0.800 0.900 0.900 0.800 0.800 0.800 0.800 0.800

[22] 0.733 0.767 0.700 0.600 0.567 0.633 0.600 0.700 0.800 0.767 0.833 0.767 0.600 0.733 0.800 0.633 0.733 0.667 0.667 0.800 0.833

[23] 0.500 0.533 0.467 0.567 0.167 0.200 0.533 0.567 0.600 0.667 0.633 0.733 0.533 0.633 0.633 0.067 0.700 0.567 0.567 0.667 0.800 0.667

[24] 0.500 0.533 0.467 0.567 0.167 0.200 0.533 0.567 0.600 0.667 0.633 0.733 0.533 0.633 0.633 0.067 0.700 0.567 0.567 0.667 0.800 0.667 0.000

[25] 0.633 0.600 0.600 0.567 0.533 0.600 0.633 0.633 0.667 0.767 0.733 0.667 0.667 0.667 0.800 0.567 0.633 0.567 0.567 0.500 0.800 0.667 0.600 0.600

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
[1]																									
[2]	2.000																								
[3]	8.000	7.000																							
[4]	19.000	20.000	19.000																						
[5]	14.000	15.000	13.000	16.000																					
[6]	12.000	12.000	12.000	18.000	3.000																				
[7]	16.000	15.000	17.000	20.000	15.000	16.000																			
[8]	18.000	17.000	18.000	19.000	17.000	19.000	18.000																		
[9]	17.000	15.000	16.000	22.000	18.000	16.000	18.000	17.000																	
[10]	18.000	18.000	21.000	20.000	19.000	18.000	18.000	20.000	22.000																
[11]	21.000	19.000	20.000	20.000	21.000	20.000	18.000	18.000	19.000	20.000															
[12]	19.000	17.000	20.000	24.000	21.000	21.000	20.000	19.000	18.000	24.000	18.000														
[13]	17.000	17.000	17.000	16.000	15.000	14.000	19.000	14.000	15.000	20.000	18.000	17.000													
[14]	15.000	15.000	15.000	22.000	19.000	19.000	20.000	16.000	18.000	23.000	18.000	20.000	14.000												
[15]	17.000	17.000	19.000	22.000	19.000	18.000	20.000	19.000	19.000	21.000	21.000	22.000	14.000	17.000											
[16]	15.000	16.000	14.000	16.000	3.000	5.000	16.000	16.000	18.000	20.000	19.000	21.000	15.000	18.000	19.000										
[17]	23.000	22.000	20.000	21.000	21.000	22.000	19.000	19.000	18.000	24.000	20.000	25.000	21.000	21.000	23.000	21.000									
[18]	16.000	17.000	19.000	19.000	16.000	17.000	15.000	19.000	20.000	18.000	22.000	22.000	20.000	21.000	22.000	17.000	20.000								
[19]	16.000	17.000	19.000	19.000	16.000	17.000	15.000	19.000	20.000	18.000	22.000	22.000	20.000	21.000	22.000	17.000	20.000	0.000							
[20]	19.000	18.000	15.000	21.000	20.000	20.000	17.000	22.000	19.000	21.000	23.000	21.000	22.000	23.000	23.000	20.000	21.000	20.000	20.000						
[21]	27.000	27.000	25.000	22.000	24.000	24.000	26.000	26.000	25.000	26.000	27.000	29.000	24.000	27.000	27.000	24.000	24.000	24.000	24.000	24.000					
[22]	22.000	23.000	21.000	18.000	17.000	19.000	18.000	21.000	24.000	23.000	25.000	23.000	18.000	22.000	24.000	19.000	22.000	20.000	20.000	24.000	25.000				
[23]	15.000	16.000	14.000	17.000	5.000	6.000	16.000	17.000	18.000	20.000	19.000	22.000	16.000	19.000	19.000	2.000	21.000	17.000	17.000	20.000	24.000	20.000			
[24]	15.000	16.000	14.000	17.000	5.000	6.000	16.000	17.000	18.000	20.000	19.000	22.000	16.000	19.000	19.000	2.000	21.000	17.000	17.000	20.000	24.000	20.000	0.000		
[25]	19.000	18.000	18.000	17.000	16.000	18.000	19.000	19.000	20.000	23.000	22.000	20.000	20.000	20.000	24.000	17.000	19.000	17.000	17.000	15.000	24.000	20.000	18.000	18.000	