

# OOMYCETE GENETICS AND GENOMICS

## Diversity, Interactions, and Research Tools

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Edited by

**Kurt Lamour**

The University of Tennessee  
Knoxville, Tennessee

and

**Sophien Kamoun**

Sainsbury Laboratory  
Norwich, United Kingdom



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# FOREWORD

Francine Govers

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The publication of this book is an important breakthrough; it is the first time that the existing knowledge on Oomycetes has been brought together in one volume. The Oomycetes, also known as water molds, comprise a diverse group of filamentous microorganisms that share many characteristics with Fungi (i.e., members of the taxonomic entity defined as Fungi). They have an absorptive mode of nutrition, grow by polarized hyphal extension, and their reproduction includes the formation of spores. Traditionally the Oomycetes have been presented in textbooks as a phylum in the kingdom Fungi and have been lumped together with other organisms of uncertain affinity as “lower fungi” or “zoosporic fungi.” More recently, the Oomycetes have been placed in another kingdom, in some systems in the kingdom Stramenopila or the stramenopile lineage in the supergroup Chromalveolates; in others in the kingdom Chromista. Phylogenetics has clearly demonstrated that Oomycetes are not Fungi but instead, they are close relatives of heterokont algae. They have lost their plastids and have adopted a fungal-like lifestyle, absorbing their nutrients from the surrounding water or soil or invading the body of another organism to feed. In fact, Oomycetes are “algae in disguise” that qualify as fungi (i.e., organisms sharing the characteristics described above). It is my hope that this book will stimulate scientists, including mycologists, to adopt or rehabilitate Oomycetes as subjects of research.

Like Fungi, Oomycetes have a global distribution and prosper in quite diverse environments. Pathogenic species that live in association with plants, animals, or other microbes can be devastating and completely destroy their hosts. Their victims include natural forests, many crop plants, fish in fish farms, amphibians, and occasionally, humans. In contrast, saprophytic species that

feed on decaying material are beneficial; they play important roles in the decomposition and recycling of biomass. Currently, at least 800 oomycete species are known, but depending on the definition of a species, this number might even reach 1500. Still the species richness is low when compared with the number of species of Fungi known to date (over 100,000), but very likely, there are many more Oomycetes out there to be discovered. In this respect, the genus *Phytophthora* is illustrative. In the last ten years, over 25 new species have been described, expanding the genus to at least 90 members. A few chapters in the first section in this book focus on the phylogeny of Oomycetes and the enormous diversity within the Oomycetes. The diversity at the species level is addressed in the second section.

Oomycete research has a long history. The type species of *Phytophthora*, *P. infestans*, was described 132 years ago by Anton de Bary, the founding father of plant pathology and the founder of modern mycology. This notorious plant pathogen was the cause of the severe late blight epidemic in Europe in the 1840s that resulted in the Irish potato famine and led to a turning point in history, the birth of Irish America. Today, late blight is still a major problem for potato growers worldwide. The same holds for downy mildew on grapes, which is another well-known oomycete disease that emerged in the nineteenth century. Less known is the serious outbreak of a disease in 1877 among the salmon in the rivers Conway and Tweed that spread into most of the rivers of the British Isles within two years. Again a water mold, *Saprolegnia ferax*, was to blame. In the last decade, the rise of industrial fish farming has gone hand-in-hand with the revival of Saprolegniosis as a major disease. The finding that Oomycetes can also cause a disease known as Pythiosis in humans is of a more recent date. The publication of this book is timely; with the (re-)emergence of oomycete diseases in hosts important for the world food economy, such as fish, soybean, potato, and other vegetable crops, or in hosts that shape the landscape (e.g., oak and alder) and inhabit unique ecological niches, interest in oomycete biology and pathology should be challenged, and the research should be intensified and strengthened. This book helps in identifying the challenges. The chapters dealing with sexual and asexual reproduction and interactions with plant hosts and animal hosts provide the necessary background but also point to gaps in our knowledge.

With the head title of this book, *Oomycete Genetics and Genomics*, the editors cross a frontier. Mentioning “Oomycete” and “Genetics” in one breath seems odd, and the search for an Oomycete in a genetics textbook is in vain. The genetics timeline begins with Gregor Mendel’s discovery of the basic laws of genetics in 1865 and marks major milestones like the description of the double helix structure of DNA, the unraveling of the genetic code, and the first recombinant DNA experiments. Then, in the 1990s, genomics milestones start to appear: the first whole genome sequence of a prokaryote and a eukaryote, culminating in the human genome sequence in 2001, an event marked by the former U.S. President Bill Clinton as one that will change the history of mankind. Prominent organisms on the genetic timeline are the well-known

models like *Escherichia coli*, yeast, *Caenorhabditis elegans*, or *Arabidopsis* but no Oomycete. David Shaw once called *Phytophthora* a “geneticist’s nightmare.” As exemplified in this book we can now look beyond this nightmare. In recent years, many oomycete researchers have experienced that genomics gives rise to a bright morning with many new milestones at the horizon. To date, five oomycete genomes have been sequenced. The availability of genomics resources and technologies has changed the way we can address various long-standing biological questions and has certainly stimulated researchers to use genomics as an instrument to tackle Oomycetes. The chapters in the tools section of this book describe recent advances in technology aimed at either the functional analysis of individual genes or at overall genome-wide analyses.

This book will serve as an excellent introduction and a valuable resource for students and researchers at all levels. It echoes the enthusiasm of the oomycete research community. I advise the newcomers in this field to take part in this community and to join the oomycete molecular genetics network (OMGN; <http://pmgn.vbi.vt.edu/>). Last, but not least, I commend Kurt Lamour and Sophien Kamoun for taking the initiative to publish this book as well as the authors for their efforts in writing the chapters.



# PREFACE

A bittersweet truth in our fast-paced genetic world is that an organism-specific book on genetics and genomics is outdated by the time it goes to print. This is especially true for the burgeoning field of Oomycete genetics and genomics where the foundations for genetic discovery have only recently been laid. Ten years ago who would have thought there would be genome sequences for multiple *Phytophthora* species, a *Hyaloperonospora*, and soon, a *Pythium*? Strangely enough, these reference genomes may themselves soon be viewed as archaic. Although only touched on in the chapter on genome sequencing, the ongoing quest to develop faster and less costly genetic sequencing has led to sequencing platforms that make it feasible to discover *all* of the changes between multiple whole genomes or transcriptomes — without the need for a reference genome. With so much forward momentum, there is *never* a good time to stop and take stock of where we've been. Our goal is to provide a useful overview of this fascinating group: a resource that can be handed to an incoming graduate student, a new colleague or a potential collaborator.

The book begins by presenting an overview of the evolutionary relationships within the Oomycetes. The diversity of life forms is astounding, and it is clear that additional taxa and sequences will continue to clarify this important area of research. The white blister rusts provide a good example of how genetic data can resolve relationships among morphologically similar yet evolutionarily distinct taxa — an important challenge in an age of costly quarantines and worldwide movement of pathogens. Interesting possibilities spring from the phylogenetic perspective, including the idea that terrestrial plant pathogens may have hitched an evolutionary ride from the open sea via nematodes and switched hosts to plant roots — on more than one occasion. For those

interested in discovering whole new worlds of diversity, a plethora of lower oomycetes are waiting, particularly in the Tropics and Polar regions. A question lingering throughout is whether the parasitic lifestyle is derived from a saprophytic lifestyle or vice versa.

After the introductory chapters is an overview of asexual and sexual reproduction. This provides a useful framework for the next sections, which explore the population structure of representative species in natural populations, and the interactions with plant and animal hosts. These chapters range from overviews of the entrenched pathogen of potato, *Phytophthora infestans*, a staple crop and staple research area since the dawn of micro-organism research, to newly emerging invasive species such as the Sudden Oak Death pathogen, *Phytophthora ramorum*. For investigators familiar with the impact of Oomycetes on sessile organisms, the chapters on Oomycetes that attack fish, crustaceans, and humans should be particularly interesting. And finally, there are specific chapters describing the application and development of molecular tools to better understand these notoriously intractable organisms.

The response by the authors to contribute their work and perspective was overwhelmingly positive, and we are hopeful that this snapshot will stimulate new relationships and research.



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## THE EVOLUTIONARY PHYLOGENY OF OOMYCETES — INSIGHTS GAINED FROM STUDIES OF HOLOCARPIC PARASITES OF ALGAE AND INVERTEBRATES

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... phylogenetic speculations, valueless though these are considered to be....may stimulate studies in the life-history, cytology, morphology etc.... and clear the way for laying the foundations of a more logical system of classification.

—E. A. Bessey (1935), *A Textbook of Mycology*

### 1.1 INTRODUCTION

The unraveling of the evolutionary phylogeny of organisms has been given a tremendous impetus by the application of molecular techniques that have enabled biologists to, in effect, delve for phylogenetic clues in the DNA of organisms in a manner analogous to fossil hunters searching for physical evidence a century earlier. As pointed out by Bessey, a sound phylogenetic framework will hopefully inform and direct future exploration as well as provide a sound basis for classification. This is particularly pertinent in the era of bioinformatics, because this knowledge should help in choosing organisms that might be targeted for genome sequencing. The oomycetes are fungus-like heterotrophs that are saprophytes or parasites of diverse hosts in marine,

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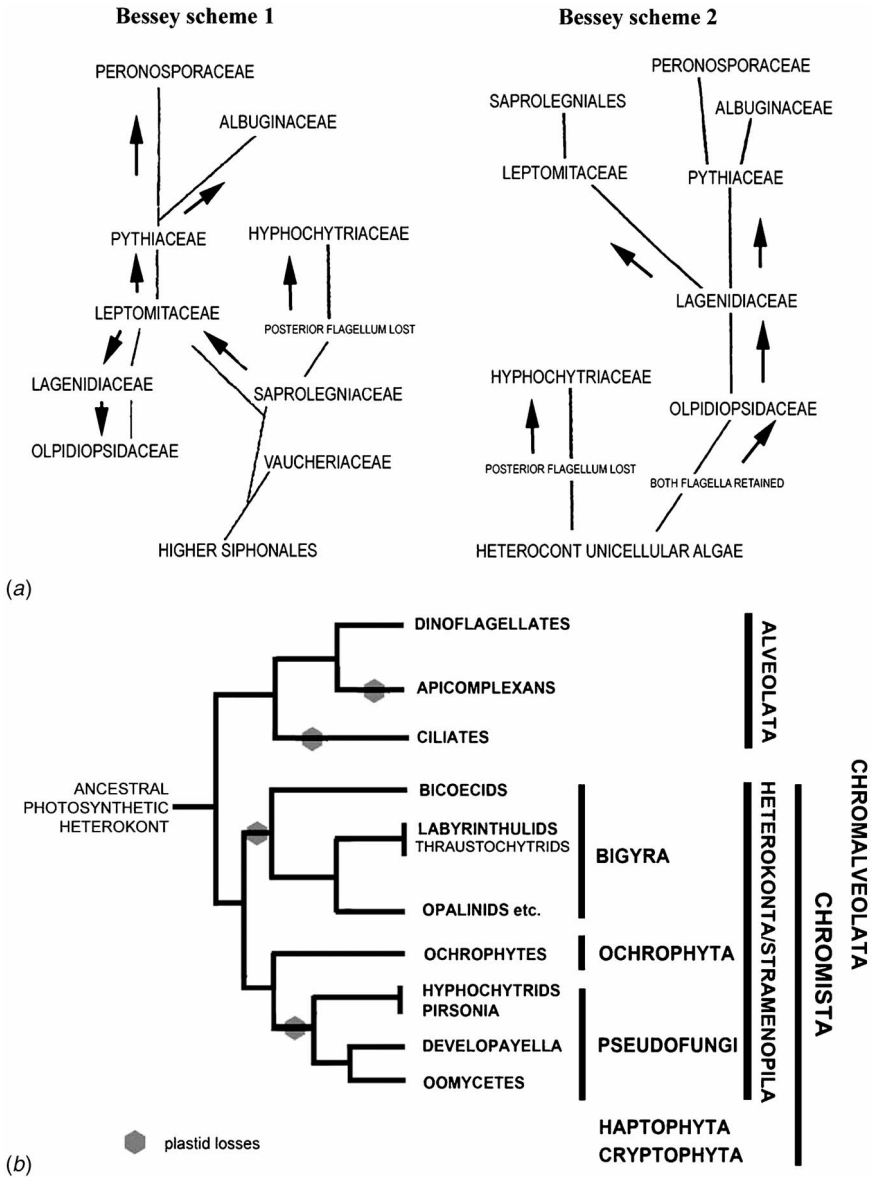
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freshwater, and terrestrial environments (Sparrow, 1960; Karling, 1981; Dick, 2001; Johnson et al., 2002). However, as a group, they are best known as devastating pathogens of plants.

Oomycetes are similar to the true fungi in that they produce complex branching, tip-growing, hyphal systems (forming mycelia) and have similar modes of nutrition and ecological roles (Richards et al., 2006). Summaries of the early speculations as to the likely evolutionary relationships of oomycetes to other organisms have been reviewed by Karling (1942), Dick (2001), and Johnson et al. (2002). Candidates cited as their likely ancestors have included amoebas, heterotrophic flagellates, diverse algal groups, and even chytrid fungi. However, most opinions tended to divide sharply between those, such as Scherffel, who considered oomycetes to have evolved from heterotrophic flagellates (Karling, 1942), and those like Bessey, who thought that photosynthetic algae were the more likely ancestors. In a seminal analysis, Bessey (1942) outlined two possible alternative evolutionary pathways within the oomycete lineage (Fig. 1.1a). In the first, it was suggested that oomycetes evolved from siphonaceous (coenocytic) algae and that they shared a common ancestor with the xanthophyte alga *Vaucheria*. The saprotrophic Saprolegniales were considered to be the most primitive order, which in turn gave rise to the Leptomitales, after which the lineage split and created the plant pathogenic Peronosporales along one branch and the holocarpic Lagenidiales along the other. The other scheme postulated that the most likely ancestor was an unknown “heterocont unicellular algae,” which was ancestral to both the uniflagellate hyphochytrids and the biflagellate oomycetes. In this pathway, the holocarpic Olpidiopsidales were thought to be the most likely basal family and yielded the Lagenidiales. From these, the plant pathogenic Peronosporales diverged on one branch and the water moulds (Saprolegniales via the Leptomitales) on the other. In this review, we will summarize current views on the likely phylogeny and taxonomy of these organisms in the light of recent work that we have carried out on some of the less widely studied parasites of seaweeds, crustacea and nematodes.

## 1.2 ANIMAL OR VEGETABLE—WHERE DO OOMYCETES BELONG ON THE TREE OF LIFE?

The sequencing of conserved genes over the past two decades has led to a firm phylogenetic placement for most groups of living organisms. These studies have shown that the oomycetes are heterokonts (see Fig. 1.1b based on Cavalier-Smith and Chao, 2006; Tsui et al., 2008) within the chromalveolate “super kingdom” (Baldauf et al., 2000). The chromist section contains three, wholly or partially, photosynthetic lineages: the cryptomonads, haptophytes, and heterokonts, although the evidence for the inclusion of the former pair with the heterokonts is still not particularly strong (discussed by Harper et al., 2005). The alveolate section contains the parasitic apicomplexa, phagotrophic ciliates, and mixotrophic dinoflagellates (Fig. 1.1b). The heterokonts/stramenopiles



**FIG. 1.1** Schematic summaries of the likely phylogenetic relationships of oomycetes and their relatives. (a) Schematic summary of two possible phylogenetic schemes showing the likely origins and family relationships within the oomycetes outlined by Bessey (1942). (b) Summary of the likely relationships between main classes and phyla within the Chromalveolata Superkingdom based on the terminology and information presented in Cavalier-Smith and Chao (2006) and Tsui et al. (2008).

(Fig. 1.1b) are an extraordinarily diverse assemblage (Cavalier-Smith and Choa, 2006) that encompasses both autotrophic and heterotrophic organisms, including the chlorophyll c-containing algae (diatoms, chrysophytes, xanthophytes, phaeophytes, etc.), free-living bacteriotrophic flagellates (bicoecids, etc.), a group of absorptive gut commensals/parasites (opalanids, proteromonads, and *Blastocystis*), as well as the fungal-like osmotrophic representatives (labyrinthulids, hyphochytrids, oomycetes, etc.). Recent multigene analyses have indicated that the Rhizaria (a very diverse group, including filose amoeboid organisms and flagellates) are the sister group to the “Stramenopiles,” which has led to this lineage being renamed as the SAR (Stramenopile/Alveolate/Rhizaria) clade (Burki et al., 2007).

The first published phylogenetic trees, which are mostly based on nuclear-encoded ribosomal gene (SSU rDNA) sequences, showed that all the early branching heterokonts were nonphotosynthetic organisms, which suggested the late acquisition of plastids in the line (Leipe et al., 1996). Most recent evidence points to the whole chromalveolate lineage having developed from a common biflagellate (mastigonate) ancestor, which had acquired photosynthetic capabilities as a result of a single unique red algal enslavement (Patron et al., 2004; Harper et al., 2005; Cavalier-Smith and Chao, 2006). It is now thought that chloroplast loss has occurred many times within the lineage, including at least twice in the heterokont line (Fig. 1.1b; Cavalier-Smith and Chao, 2006; Tsui et al., 2008). Genomic data have also provided direct evidence for the photosynthetic ancestry of oomycetes with the discovery of vestigial plastid genes within the nuclear genome of *Phytophthora* (Lamour et al., 2007).

### 1.3 KINGDOM WARS AND FAMILY TIES—A CASE OF CONFLICTING NOMENCLATURE

There is still debate as to the correct (and taxonomically legal) kingdom/phylum/class names to be used for the lineage that contains the oomycetes. Dick (2001) formally proposed (and diagnosed) the kingdom Straminipila for the heterokont lineage, pointing out the incorrect etymological derivation of the by then widely used informal term “Stramenopile,” which was first introduced by Patterson (1989) in reference to the “straw-like” flagellum hairs (mastigonemes) possessed by most members of this group. However, in their attempt to bring order and consistency to the naming of protists, algae, and fungi, Adl et al. (2005) forcefully argued for the continued use of the name Stramenopile for this lineage, although they side stepped the issue of assigning hierarchical taxonomic ranks. Cavalier-Smith and Chao (2006) in their review of the phylogeny of phagotrophic heterokonts considered Dick’s kingdom Straminipila to be synonymous with the kingdom Chromista erected by Cavalier-Smith (1981); this is the name that is used in many current nomenclatural databases.

Which phylum the oomycetes should be placed in has been no less controversial. The name Heterokonta has been used, respectively, to define



both a “phylum” (Dick, 2001) and an “infrakingdom” (Cavalier-Smith and Chao, 2006). The Heterokonta infrakingdom was split into three phyla (see Fig. 1.1b), the Ochrophyta (encompassing all photosynthetic heterokonts), Bygyra (thraustochytrids, labyrinthulids, opalinids, etc.) and Pseudofungi (Cavalier-Smith and Chao, 2006). This includes, in addition to the oomycetes, the anteriorly unflagellate hyphochytrids and associated sister clade, the flagellate parasitoid *Pirsonia* (Kühn et al., 2004), and the free-living bacteriotrophic marine zooflagellate *Developayella*. The latter species usually forms the sister clade to the oomycetes in small ribosomal subunit phylogenetic trees (Figs. 1b, 1.2a; Leipe et al., 1996). Patterson (1999) introduced yet another name, Sloomycetes, for a clade that contains all the osmotrophic fungal-like heterokonts. Perhaps because of the plethora of conflicting higher level taxonomic schemes, it is not surprising that many review volumes and textbooks continue to afford the oomycetes/oomycota their own phylum status.

The separation of the photosynthetic ochrophyte and heterotrophic oomycete lineages into two parallel clades derived from a common ancestor (Fig. 1.1b) is supported in the most recent phylogenetic trees (e.g., Cavalier-Smith and Chao, 2006; Tsui et al., 2008). This makes evolutionary sense as it explains the often reciprocal host–pathogen relationships observed between members of these two groups. For instance, both the hyphochytrid *Anisopidium ectocarpus* and the oomycete *Eurychasma dicksonii* are parasites of ectocarpalean phaeophyte algae (Küpper and Müller, 1999) and *Pirsonia*, *Ectrogella*, and *Lagenisma* all infect centric marine diatoms (Kühn et al., 2004; Schnepf et al., 1977, 1978; Raghu Kumar, 1980), which suggests the coevolution of parasitism between these two heterokont lineages (Cavalier-Smith and Chao, 2006). Environmental SSU rDNA sequences derived from small nanoplanktonic organisms sampled from diverse marine locations and ecosystems have shown that many of these lineages not only cluster within existing stramenopile clades, such as the hyphochytrids and oomycetes, but also form many “novel stramenopile” clades whose identities largely remain a mystery (Massana et al., 2004, 2006). The inclusion of such environmental sequence data in phylogenetic analyses significantly alters the topography of the heterokont tree and suggests that the *Pirsonia*/hyphochytrid clade may not be related as closely to the oomycetes as shown in Fig. 1.1b, although they undoubtedly share a common ancestor (Massana et al., 2004, 2006). It is to be expected that a systematic multigene approach to determining phylogeny in this lineage, as well as a significantly increased taxon sampling, will result in a much better understanding of the precise branching relationships of these various groups.

#### 1.4 THE NAME GAME—THE TAXONOMY OF “CROWN” OOMYCETES

The current taxonomic organization of the oomycetes has largely been forged by two eminent scholars of zoosporic fungi, Frederick Sparrow (Sparrow, 1960,

1976) and Michael Dick (Dick et al., 1984; Dick, 2001). In his encyclopedic treatise on aquatic fungi, Sparrow (1960) split the oomycetes into four orders, the Lagenidiales, Leptomitales, Peronosporales, and Saprolegniales. In his final synthesis, Sparrow (1976) suggested that all oomycetes could be assigned to one of two groups, which he informally termed “galaxies.” Within the “saprolegnian galaxy,” he placed the order Saprolegniales (in which he included the Leptomitaceae as a family) and introduced a new order the Eurychasmatales, in which he placed many marine oomycete families. Within the “peronosporalean galaxy,” he placed the Peronosporales (in which the Peronosporaceae, Pythiaceae, and Rhipidiaceae were included as families) and the holocarpic Lagenidiales.

Dick continued to refine oomycete classification culminating in his final synthesis, which he outlined in his *magnum opus* Straminipilous Fungi, in which he expanded the number of orders to around 12 (Dick, 2001). Sparrow (1976) had pointed out the inappropriateness of the name oomycete, which had been first introduced in 1879, and this was acted on by Dick (1998, 2001) who formally renamed the class the Peronosporomycetes. However, there has been a general reluctance to abandon the traditional name, and its retention does not apparently contravene the International Code of Nomenclature. Dick’s major revision was substantially carried out before the advent of wide-ranging molecular studies and was based mostly on a scholarly reinterpretation of the available morphological and ecological data. The application of molecular methodologies has revolutionized understanding of the likely phylogenetic relationships throughout biology, and it has become increasingly apparent that many of the more radical changes introduced by Dick (2001) are not supported by molecular data and will require revision.

For oomycetes, most molecular studies have used the sequences of either the nuclear-encoded SSU (Dick et al., 1999; Spencer et al., 2002), large ribosomal subunit (LSU) genes (Riethmüller et al., 1999, 2002; Petersen and Rosendahl, 2000; Leclerc et al., 2000) or associated internal spacer region (ITS) sequences (Cooke et al., 2000), or the mitochondrial-encoded cytochrome c oxidase subunit II (*cox2*) gene (Hudspeth et al., 2000; Cook et al., 2001; Thines et al., 2008). Phylogenetic sequence data for the oomycetes is still far from complete, and the current analyses should be viewed as work in progress. It is not possible, for instance, to assemble all species for which molecular data are available into a single all-encompassing tree. There are also significant gaps in data, particularly for many of the less economically important taxa and, particularly, for those holocarpic species that cannot be brought into laboratory culture.

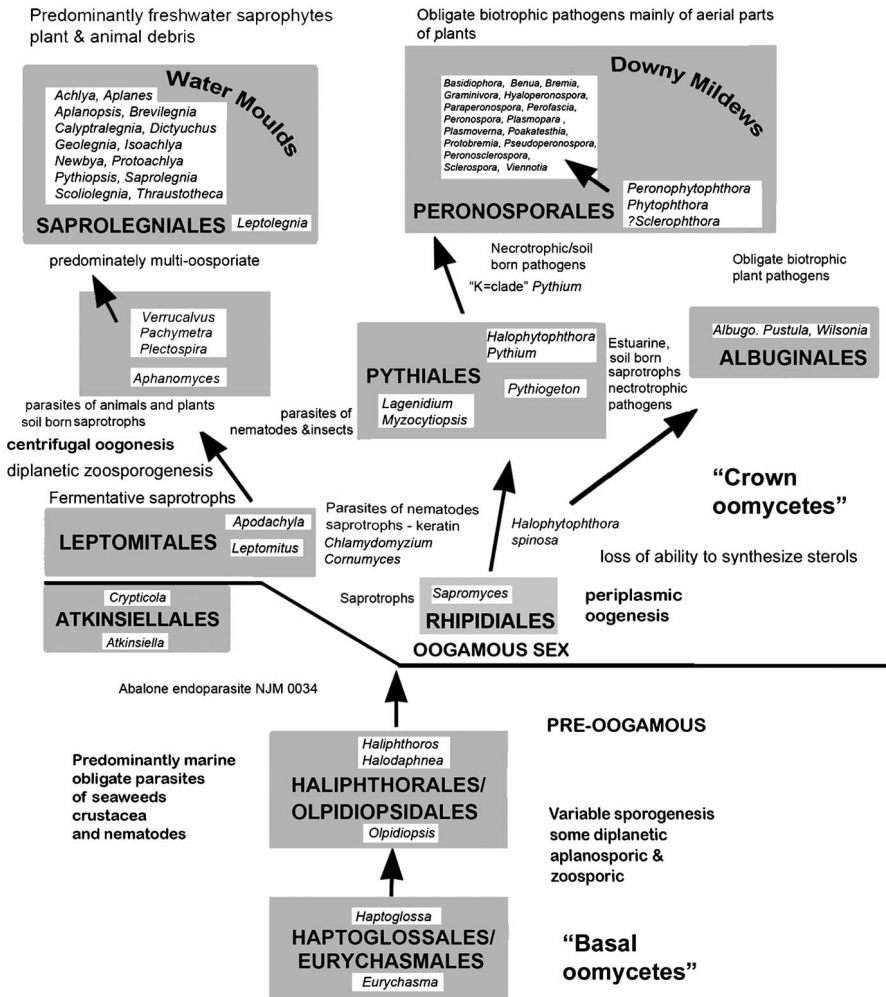
The early molecular studies all supported both the monophyletic origins of the oomycetes (Riethmüller et al., 1999; Hudspeth et al., 2000; Petersen and Rosendahl, 2000) and the broad “galaxy split” proposed by Sparrow (1976), which were assigned formal subclass rank (Saprolegniomycetidae and Peronosporomycetidae) by Dick et al. (1999). However, it seems likely that these higher taxonomic ranks will also require major revision, particularly if the

oomycetes are considered to be a phylum in their own right. The two main plant pathogenic orders, the Pythiales and Peronosporales, were also fairly well supported by sequence data (Cooke et al., 2000; Riethmüller et al., 2002; Hudspeth et al., 2003). Most analyses revealed the genus *Phytophthora* to be part of the Peronosporales rather than the Pythiales where it had traditionally been placed (Cooke et al., 2000; Riethmüller et al., 2002). Some larger genera of plant pathogenic oomycetes, such as *Phytophthora* (Cooke et al., 2000; Blair et al., 2008) and *Pythium* (Lévesque and de Cock, 2004), have been split into several clades, which ultimately may warrant at least genus-level separation. The K-clade of *Pythium* is phylogenetically interesting because it seems to form a clade that is intermediate between the Pythiales and Peronosporales orders as currently constituted (Lévesque and de Cock, 2004).

Another major surprise was the early divergence within this line of the white blister rusts (*Albugo*) and their clear separation from all other members of the Peronosporales (Fig. 1.2b; Petersen and Rosendahl, 2000; Hudspeth et al., 2003). They have now been placed in their own order, the Albuginales (Fig. 1.2b; Riethmüller et al., 2002; Voglmayr and Riethmüller, 2006). On the basis of their unusually long and unique COII amino acid sequence (derived from the *cox2* gene analysis), Hudspeth et al. (2003) considered them to be the earliest diverging clade in the Peronosporomycetidae, and they have been assigned their own subclass rank, which is called Albugomycetidae in some analyses (Thines et al., 2008).

The Rhipidiales are a small group of saprotrophic species associated with submerged twigs and fruit, most of which show restricted thallus development, consisting of a basal cell, holdfasts, and constricted (jointed) hyphal branches (Sparrow, 1960). They are a phylogenetically significant group that sits at the cusp of the saprolegnian-peronosporalean clade divergence (Figs. 1.2 and 1.3). Dick (2001) proposed that they be given their own order and subclass status (Rhipidiales, Rhipidiomycetidae), although he acknowledged the limited data on which this was based. Unfortunately, *Sapromyces elongatus* is still the only representative of this clade to have been sequenced and is a species whose placement has proven problematic (compare Fig. 1.2a and b). It has been reported as the basal clade to the Peronosporomycetidae in *cox2* trees (Hudspeth et al., 2000) and the basal clade to the Saprolegniomycetidae in LSU rDNA trees (Riethmüller et al., 1999; Petersen and Rosendahl, 2000). In our SSU rDNA trees (Fig. 1.2a), it forms part of a clade together with the holocarpic nematode parasite *Chlamydomyrium*, which diverges before both the major subclasses. However, the derived COII amino acid sequence showed that *Sapromyces* has the same signature amino acid insertion-deletion (indel) sequence (LEF/T) as that found in members of the Pythiales in contrast to the YTD indel sequence found in members of the Leptomitaceae (Hudspeth et al., 2000, 2003; Cook et al., 2001). Other members of the genus, such as *C. oviparasiticum* (Glockling and Beakes, 2006a), are diplanetic and have K-bodies in their zoospores (saprolegnian characteristics) but release their zoospores into a transient vesicle (a peronosporalean characteristic). Nakagiri





**FIG. 1.3** Schematic summary of the likely phylogenetic relationships between the main orders within the oomycetes, based on molecular sequence data. The species listed are those for which sequence data are available. Some main ecological and morphological characteristics are also mapped onto this scheme. See the text for sources.

(2002) has also reported that *Halophytophthora spinosa* is not closely related to other members of the genus and apparently clusters close to *Sapromyces*.

The sequence data that support the early divergence of the Leptomitales clade in Saprolegniomycetidae comes from two taxa *Apodachlya* and *Leptomitius*, which are both members of the family Leptomitaceae (Riethmüller et al., 1999; Dick et al., 1999; Petersen and Rosendahl, 2000). This order, however, also includes the Leptolegnielliaceae, which contains many holocarpic genera,

such as *Aphanomycoopsis*, *Brevilegniella*, *Leptolegniella*, and the nematode parasite *Nematophthora*. *Cornumyces* was also tentatively included in this family by Dick (2001). *Cornumyces* isolates form a clade close to the Leptomitales at the base of the saprolegnian line (Inaba and Harayama, 2006) and also close to *Chlamydomyzium* when this species is included in the analyses (Inaba unpublished data). Unfortunately, no sequence data are available for any other of the genera in the Leptolegniellaceae. From the current, scant, molecular data, it seems that the clades located close to the point where the two main subclasses diverge (encompassing the Rhipidiales, Leptomitales, Atkinsiellales etc. Figs. 1.2 and 1.3) cannot be properly resolved until there has been far greater taxon and gene sampling.

### 1.5 ALL AT SEA—THE EARLIEST DIVERGING OOMYCETE CLADES

The first indication that some genera might fall outside the two main “crown” subclasses came from the study of Cook et al. (2001) who sequenced the *cox2* gene for several parasites of marine crustaceans. Two genera, *Haliphthoros* (Fig. 1.4p) and *Halocrusticida* (Fig. 1.4n and o), which has been reclassified as *Halodaphnea* by Dick, 1998, 2001), formed a well-supported clade that diverged before the main crown subclasses (Cook et al., 2001). However, another enigmatic marine crustacean parasite, *Atkinsiella*, formed a deeply branched clade basal to the Saprolegniomycetidae. This study indicated that these obscure marine genera might hold the key to understanding the evolutionary origins of the oomycetes as a whole. This conclusion was reinforced when it was reported that *E. dicksonii*, which is a holocarpic parasite of brown seaweeds (Fig. 1.4a and b), was found to be the earliest diverging member of the oomycete lineage (Küpper et al., 2006).

A range of marine parasites of seaweeds and invertebrates was selected for an integrated study into their molecular phylogeny, morphological development, and ultrastructural characteristics (Sekimoto, 2008; Sekimoto et al., 2007, 2008a–c). Phylogenetic trees based on the SSU rDNA (Fig. 1.2a) and *cox2* genes (Fig. 1.2b) revealed that most of these marine holocarpic species fell into one of two deeply branched early diverging clades, which we have termed “basal oomycetes” (Fig. 1.3). The first clade in both SSU rDNA (Fig. 1.2a) and *cox2* gene (Fig. 1.2b) trees encompassed two genera, *Eurychasma* and *Haptoglossa* (Beakes et al., 2006; Hakariya et al., 2007; Sekimoto et al., 2008b). These two genera have few apparent morphological and structural features in common (cf. Fig. 1.4a,b, f–l) and would never have been linked without molecular data. These two genera may merit their own order status, the Eurychasmales and Haptoglossales, although they do seem to form a distinct clade, albeit showing long branch separation (Fig. 1.2a and b). *Eurychasma* is an obligate parasite of filamentous brown seaweeds, mostly in the Ectocarpales (Fig. 1.4a and b), but it has a broad host range (Küpper and Müller, 1999).