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Minireview

Integrating chytrid fungal parasites into plankton ecology: research gaps and needs

Thijs Frenken,[1] Elisabet Alacid,[2] Stella A. Berger,[3] Elizabeth C. Bourne,[4,5] Mélanie Gerphagnon,[5] Hans-Peter Grossart,[3,6] Alena S. Gsell,[1] Bas W. Ibelings,[7] Maiko Kagami,[8] Frithjof C. Küpper,[9] Peter M. Letcher,[10] Adeline Loyau,[11,12,13] Takeshi Miki,[14,15] Jens C. Nejstgaard,[3] Serena Rasconi,[16] Albert Reñé,[2] Thomas Rohrlack,[17] Keilor Rojas-Jimenez,[3,18] Dirk S. Schmeller,[12,13] Bettina Scholz,[19,20] Kensuke Seto,[8,21] Télesphore Sime-Ngando,[22] Assaf Sukenik,[23] Dedmer B. Van de Waal,[1] Silke Van den Wyngaert,[3] Ellen Van Donk,[1,24] Justyna Wolinska,[5,25] Christian Wurzbacher,[26,27] and Ramsy Agha[5]*

[1]*Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Droevendaalsesteeg 10, Wageningen, PB, 6708, The Netherlands.*

[2]*Departament de Biologia Marina i Oceanografia, Institut de Ciències del Mar (CSIC), Pg. Marítim de la Barceloneta, 37-49, Barcelona, 08003, Spain.*

[3]*Department of Experimental Limnology, Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Alte Fischerhuette 2, Stechlin, D-16775, Germany.*

[4]*Berlin Center for Genomics in Biodiversity Research, Königin-Luise-Straβe 6-8, Berlin, D-14195, Germany.*

[5]*Department of Ecosystem Research, Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Müggelseedamm 301, Berlin, 12587, Germany.*

[6]*Institute for Biochemistry and Biology, Potsdam University, Maulbeerallee 2, Potsdam, D-14476, Germany.*

[7]*Department F.-A. Forel for Environmental and Aquatic Sciences & Institute for Environmental Sciences, University of Geneva, 66 Boulevard Carl Vogt, Geneva 4, CH 1211, Switzerland.*

[8]*Department of Environmental Sciences, Faculty of Science, Toho University, 2-2-1, Miyama, Funabashi, Chiba, 274-8510, Japan.*

[9]*Oceanlab, University of Aberdeen, Main Street, Newburgh, Scotland, AB41 6AA, UK.*

[10]*Department of Biological Sciences, The University of Alabama, 300 Hackberry Lane, Tuscaloosa, AL 35487, USA.*

[11]*Department of System Ecotoxicology, Helmholtz Center for Environmental Research – UFZ, Permoserstrasse 15, 04318 Leipzig, Germany.*

[12]*Department of Conservation Biology, Helmholtz Center for Environmental Research – UFZ, Permoserstrasse 15, Leipzig, 04318, Germany.*

[13]*ECOLAB, Université de Toulouse, CNRS, INPT, UPS, Toulouse, France.*

[14]*Institute of Oceanography, National Taiwan University, No.1 Section 4, Roosevelt Road, Taipei, 10617, Taiwan.*

[15]*Research Center for Environmental Changes, Academia Sinica, No.128 Section 2, Academia Road, Nankang, Taipei, 11529, Taiwan.*

[16]*WasserCluster Lunz – Biological Station, Inter-University Centre for Aquatic Ecosystem Research, A-3293 Lunz am See, Austria.*

[17]*Faculty of Environmental Sciences and Natural Resource Management, Norwegian University of Life Sciences, P.O. Box 5003, NO-1432, Ås, Norway.*

[18]*Universidad Latina de Costa Rica, Campus San Pedro, Apdo, San Jose, 10138-1000, Costa Rica.*

[19]*BioPol ehf, Einbúastig 2, Skagaströnd 545, Iceland.*

[20]*Faculty of Natural Resource Sciences, University of Akureyri, Borgir v. Nordurslod, Akureyri, IS 600, Iceland.*

[21]*Sugadaira Montane Research Center, University of Tsukuba, 1278-294, Sugadaira-Kogen, Ueda, Nagano, 386-2204, Japan.*

[22]*Université Clermont Auvergne, UMR CNRS 6023 LMGE, Laboratoire Microorganismes: Génome et Environnement (LMGE), Campus Universitaire des Cézeaux, Impasse Amélie Murat 1, CS 60026, Aubière, 63178, France.*

[23]*Kinneret Limnological Laboratory, Israel Oceanographic & Limnological Research, P.O.Box 447, Migdal, 14950, Israel.*

[24]*Department of Biology, University of Utrecht, Padualaan 8, Utrecht, TB 3508, The Netherlands.*

Received 20 March, 2017; Revised: 9 June, 2017; accepted 10 June, 2017. *For correspondence. E-mail ramsyagha@gmail.com; Tel. +49 (30) 64 181 745; Fax +49 (30) 64 181 682.

[25] *Institute of Biology, Freie Universität Berlin, Königin-Luise-Straβe 1-3, Berlin, 14195, Germany.*
[26] *Department of Biological and Environmental Sciences, University of Gothenburg, Box 461, Göteborg, 405 30, Sweden.*
[27] *Gothenburg Global Biodiversity Centre, Box 461, Göteborg, SE-405 30, Sweden.*

Summary

Chytridiomycota, often referred to as chytrids, can be virulent parasites with the potential to inflict mass mortalities on hosts, causing e.g. changes in phytoplankton size distributions and succession, and the delay or suppression of bloom events. Molecular environmental surveys have revealed an unexpectedly large diversity of chytrids across a wide range of aquatic ecosystems worldwide. As a result, scientific interest towards fungal parasites of phytoplankton has been gaining momentum in the past few years. Yet, we still know little about the ecology of chytrids, their life cycles, phylogeny, host specificity and range. Information on the contribution of chytrids to trophic interactions, as well as co-evolutionary feedbacks of fungal parasitism on host populations is also limited. This paper synthesizes ideas stressing the multifaceted biological relevance of phytoplankton chytridiomycosis, resulting from discussions among an international team of chytrid researchers. It presents our view on the most pressing research needs for promoting the integration of chytrid fungi into aquatic ecology.

Introduction

Phytoplankton constitute the base of most aquatic food webs and play a pivotal role in biogeochemical cycles, accounting for more than half of the global carbon fixation (Falkowski, 2012). Phytoplankton can be infected by a number of parasites, which have the potential to regulate their abundance and dynamics and, thereby, modulate large scale ecological and/or biogeochemical processes. Parasitism constitutes an important evolutionary driver, which can promote genetic diversity in host populations and speciation (Hamilton, 1982; Weinbauer and Rassoulzadegan, 2004; Evison *et al*., 2013). Parasites are involved in most trophic links within aquatic food webs, and can contribute significantly to the transfer of carbon and energy between trophic levels (Amundsen *et al*., 2009). Moreover, diverse phytoplankton taxa are also increasingly used in aquaculture industry for the production of food supplements, biofuels and pharmaceuticals (Skjånes *et al*., 2013). Parasite epidemics can be especially devastating in such commercial scale monocultures, posing severe monetary risk for the algal industry (Carney and Lane, 2014).

Common parasites of phytoplankton include viruses, fungi, protists and pathogenic bacteria (Park *et al*., 2004; Gachon *et al*., 2010; Gerphagnon *et al*., 2015). Among these, viruses raised the most interest in the previous decades (Bergh *et al*., 1989) and their profound ecological implications were recognized soon after (Proctor and Fuhrman, 1990; Suttle *et al*., 1990; Bratbak *et al*., 1993; 1994; Fuhrman and Suttle, 1993; Fuhrman, 1999). In a similar way, we perceive that scientific interest towards fungal parasites of phytoplankton has gained momentum in recent years. This is in large part attributable to molecular environmental surveys revealing unexpected diversity of uncultured aquatic fungal organisms – i.e. the so-called Dark Matter Fungi (Grossart *et al*., 2016) – which is often dominated by members of the early diverging fungal phylum Chytridiomycota (Monchy *et al*., 2011; Jobard *et al*., 2012; Lefèvre *et al*., 2012; Comeau *et al*., 2016). Following initial work by Canter and Lund (Canter, 1946; Canter and Lund, 1948; 1951) and some later studies (Reynolds, 1973; Van Donk and Ringelberg, 1983), chytrids are raising renewed interest, as further evidence accumulates for their widespread distribution across climatic regions, in both marine and freshwater ecosystems (Lefèvre *et al*., 2007; Lepère *et al*., 2008; Wurzbacher *et al*., 2014; De Vargas *et al*., 2015; Gutiérrez *et al*., 2016; Hassett *et al*., 2017; Hassett and Gradinger, 2016).

Due to their inconspicuous morphological features, chytrids have been often misidentified as bacterivorous flagellates and their role as parasites or saprobes in aquatic ecosystems have thus often been neglected. However, some chytrid taxa are lethal parasites (i.e. parasitoids) and have the potential to inflict mass mortalities on their hosts, causing changes in phytoplankton size distributions, promotion of r-strategist hosts with fast turnover, delay or suppression of bloom formation and successional changes (Reynolds, 1973; Van Donk and Ringelberg, 1983; Van Donk, 1989; Rasconi *et al*., 2012; Gerphagnon *et al*., 2015; Gleason *et al*., 2015). Parasitism by chytrids mediates inter- and intraspecific competition (Rohrlack *et al*., 2015) and might promote diversity and polymorphisms in host populations (Gsell *et al*., 2013b). Chytrids are characterized by a free-living motile stage in the form of single-flagellated zoospores that are assumed to actively search for their hosts by chemotaxis (Canter and Jaworski, 1980; Muehlstein *et al*., 1988). Upon settlement on their host, chytrids penetrate the cell and develop rhizoids to extract nutrients from it. Encysted zoospores develop into epibiotic sporangia which, once mature, release new zoospores (Canter, 1967). Zoospores have been found to constitute a highly nutritional food source

for zooplankton and chytrids may hence establish alternative trophic links between primary and secondary production in pelagic ecosystems (Kagami *et al*., 2007b; Rasconi *et al*., 2011; Agha *et al*., 2016).

Despite accumulating evidence for their ecological importance, studies addressing phytoplankton-chytrid interactions are limited by the availability of model systems and empirical data. We know relatively little about the life cycles of chytrids, their phylogeny, and their host specificity and range. Information regarding their mechanisms of infection, as well as the co-evolutionary effects of chytrid parasitism on host populations is also missing. This paper aims to synthesize novel notions stressing the biological relevance of phytoplankton chytridiomycosis, keeping a focus on the immediate research needs. Our intent is not to recreate existing reviews on the topic (Ibelings *et al*., 2004; Gleason *et al*., 2008; 2011; 2014; 2015; Sime-Ngando, 2012; Kagami *et al*., 2014; Gerphagnon *et al*., 2015; Jephcott *et al*., 2015). Rather, we aim to (i) briefly highlight the profound and multifaceted impact of chytrid parasitism on phytoplankton dynamics, (ii) identify the current major gaps in knowledge and (iii) propose future directions to bridge them. We intend to stimulate experimentation in different aspects of the biology of chytrids and their hosts and, thereby, contribute integrating chytrid parasitism of phytoplankton into traditional aquatic (microbial) ecology.

Life-cycle and ecological strategies

Parasitic chytrids obtain their nutrients and energy from living organisms, mainly phyto- and zooplankton, whereas saprophytic taxa generally use other organic substrates (Longcore *et al*., 1999). Currently, chytrids are categorized as (i) *obligate parasites*, which need a living host to reproduce and complete their life cycle, e.g. *Rhizophydium planktonicum* parasitizes the diatom *Asterionella formosa* (Canter and Jaworski, 1978); (ii) *obligate saprophytes*, which can use a broad spectrum of organic materials as a substrate to reproduce and complete their life cycle, e.g. *Rhizoclosmatium globosum* grows on pollen, keratin, cellulose and chitin (Sparrow, 1960) and (iii) *facultative parasites*, which are able to infect and reproduce on living hosts, but are also able to exploit senescent hosts or other dead organic material, e.g. *Dinochytrium kinnereticum* is parasitic on weakened cells of the dinoflagellate *Peridinium gatunense*, but also grows saprophytic on pollen (Table 1) (Leshem *et al*., 2016).

However, it is not clear whether the degree of parasitism or saprophytism is bound to individual taxa, or if chytrids display a continuum of consumer strategies, ranging from obligate parasitic to obligate saprophytic life styles, depending on environmental conditions (Fig. 1). Some chytrids that exploit phytoplankton, can also be found on organic substrates (Alster and Zohary, 2007; Leshem *et al*., 2016). It is unclear whether these facultative parasites can only infect physiologically senescent hosts as an 'extension' of saprophytism, or whether they are also adapted to parasitism. Parasitism likely grants access to higher quality resources compared to most other (dead) organic substrates, but the costs associated with parasitism are usually high, given the necessity of evading host immune response (Frank, 1996; Schmid-Hempel, 2008). On the other hand, saprophytism in facultative parasites can serve as a survival strategy in the absence of a host. Exploring the continuum between parasitic and saprophytic lifestyles of chytrids and their trade-offs is still needed for a functional and ecological characterization of chytrid diversity.

The ecological role of chytrid hyper-parasites, taxa that infect other parasitic chytrids (e.g. *Chytridium parasiticum* or *Septosperma* spp.), represent a unique case (Gleason *et al*., 2014), which remains largely unknown (see *Top-down regulation of chytridiomycosis and trophic interactions*). To estimate the proportion of parasitic species relative to total chytrid diversity and to determine general patterns that can explain their life cycles and host range remains challenging. However, this would allow us to better understand their functional diversity and establish hypotheses about the divergence of chytrid lineages and the evolution of parasitism.

Chytrids combine asexual and sexual modes of reproduction (Doggett and Porter, 1996a), but so far sexual reproduction has only infrequently been documented (Canter and Lund, 1948; Van Donk and Ringelberg, 1983; Seto *et al*., 2017). Zoospores are produced asexually and can survive for only short periods of time (hours to a few days; Fuller and Jaworski, 1987) in absence of a suitable host. Therefore, some chytrid species probably rely on resting/resistant stages to survive periods of host absence (Doggett and Porter, 1996b). Studies on the abundance of resting spores in sediments and the water column, as well as the stimuli and/or mechanisms triggering resting spore formation and germination are still needed. This, together with accurate estimates of the lifetime of zoospores in the absence of hosts will help increase our understanding of the life cycles of chytrids and their survival strategies during periods of host absence in the water column.

Taxonomy and molecular phylogeny

Much effort has been devoted to unravelling the molecular phylogeny of chytrids and other zoosporic fungi. However, phylogenies in the early branches of the fungal tree still remain an open question. Traditionally, the taxonomic assignment of these organisms was based on morphology and host affiliation. Yet, identification by morphology alone has proven a difficult task, given their

Table 1. List of isolated parasitic chytrid taxa, including their life cycle strategy and host taxa.

Species	Life cycle strategies	Host(s)	Reference
Chytridiales			
Chytridium olla	Obligate parasite	*Oedogonium* spp.	Sparrow (1960), Vélez *et al*. (2011)
Dinochytrium kinnereticum	Facultative parasite	*Peridinium gatunense*	Leshem *et al*. (2016)
Phlyctochytrium planicorne	Facultative parasite	*Asterococcus* sp., *Cladophora* sp., *Cosmarium contractum* var. *ellipsoideum*, *Oedogonium* sp., *Peridinium cinctum*, *Rhizoclonium hieroglyphicum*, *Sphaerocystis schroeteri*, *Spirogyra* spp., *Staurastrum* spp., *Staurodesmus curvatus*, *Vaucheria* sp.	Canter (1961), Letcher and Powell (2005), Sparrow (1960)
Rhizophydium planktonicum	Obligate parasite	*Asterionella formosa*	Canter (1969), Seto *et al*. (2017)
Gromochytriales			
Gromochytrium mamkaevae	Obligate parasite	*Tribonema gayanum*	Karpov *et al*. (2014)
Lobulomycetales			
Chytridium polysiphoniae	Obligate parasite	Only macroalgal hosts described: *Acinetospora crinita*, *Ectocarpus* spp., *Feldmannia* spp., *Hincksia* spp., *Pilayella littoralis*, *Spongonema tomentosum*, *Myriotrichia clavaeformis*, *Haplospora globosa*, *Eudesme virescens*, *Carpomitra costata*, *Endarachne binghamiae*, *Scytosiphon lomentaria*,	Küpper *et al*. (2006), Müller *et al*. (1999).
Mesochytriales			
Mesochytrium penetrans	Obligate parasite	*Chlorococcum minutum*	Karpov *et al*. (2010)
Rhizophydiales			
Aquamyces chlorogonii	Facultative parasite	*Chlorogonium* spp., *Oedogonium cardiacum, Spirogyra* spp., *Tribonema bombycinum, Ulothrix subtilissima*, *Vaucheria* sp., *Zygnema* sp.	Barr (1973), Letcher *et al*. (2008), Sparrow (1960)
Dinomyces arenysensis	Obligate parasite	*Alexandrium* spp., *Ostreopsis* spp.	Lepelletier *et al*. (2014)
Gorgonomyces haynaldii	Facultative parasite	*Chlorogonium elongatum, Oedogonium* spp., *Spirogyra* spp., *Tribonema bombycinum, Ulothrix* spp., *Vaucheria* sp., *Zygnema* sp.	Barr (1973), Letcher *et al*. (2008), Sparrow (1960)
Protrudomyces laterale	Facultative parasite	*Ulothrix* spp., *Stigeoclonium* sp.	Barr (1973), Letcher *et al*. (2008), Sparrow (1960)
Rhizophydium globosum	Facultative parasite	*Cladophora lomerate, Closterium* spp., *Navicula* sp., *Penium digitus*, *Pinnularia viridis*, *Pleurotaenium trabecula*, *Spirogyra* sp., *Staurastrum* sp., *Ulothrix* sp.	Letcher *et al*. (2006), Sparrow (1960)
Rhizophydium megarrhizum	Obligate parasite	*Lyngbya* sp., *Oscillatoria* spp., *Planktothrix* sp.	Sønstebø and Rohrlack (2011), Sparrow (1960)
Staurastromyces oculus	Obligate parasite	*Staurastrum* sp.	Van den Wyngaert *et al*. (2017)
Order incertae sedis			
Zygorhizidium planktocnium	Obligate parasite	*Asterionella formosa*, *Synedra* spp.	Canter (1967), Doggett and Porter (1995), Seto *et al*. (2017)
Zygorhizidium melosirae	Obligate parasite	*Aulacoseira* spp.	Canter (1967), Seto *et al*. (2017)

small and inconspicuous thalli and considerable morphological variation under changing environmental conditions and substrates (Paterson, 1963).

The application of transmission electron microscope (TEM) techniques, allows for analysis and characterization of zoospore ultrastructural features and has proven to be a powerful tool for identification purposes (Beakes *et al*., 1988; 1993; Letcher *et al*., 2012), especially when integrated with molecular data (James *et al*., 2006). However, some studies have indicated that cryptic species might exist at both the genetic and ultrastructural levels (Letcher *et al*., 2008; Simmons, 2011).

Ultrastructural analyses require concentrated suspensions of zoospores, but given the limited number of chytrids strains available in culture (especially obligate parasitic chytrids), relatively few taxa are currently available for study. However, the use of molecular tools for identification of sequences originating from environmental DNA by reference to sequence databases (Hibbett *et al*., 2016) can overcome many limitations of traditional microscopic

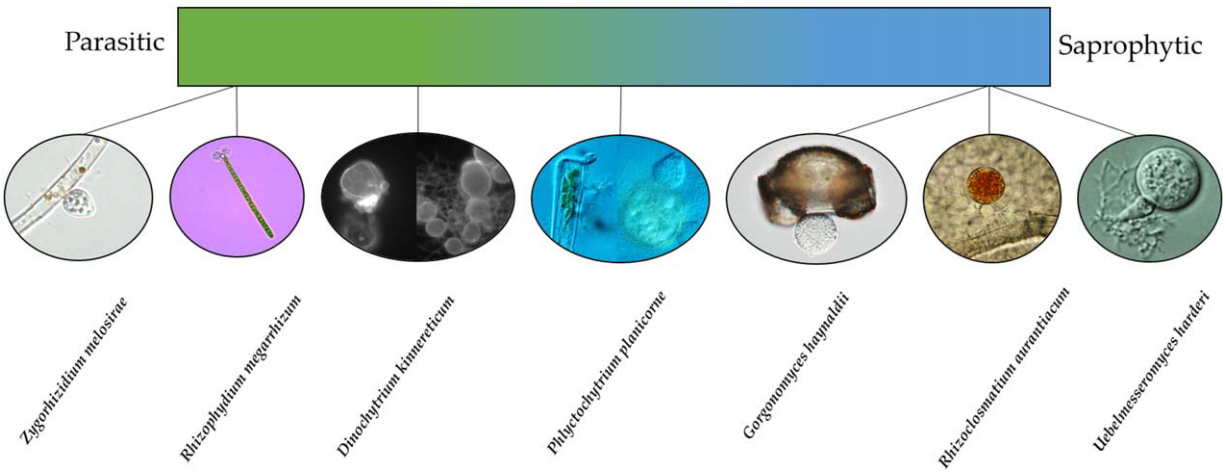

Fig. 1. Examples of chytrid taxa with different consumer strategies ranging from parasitic to saprophytic. From left to right: *Z. melosirae* parasitizing *Aulacoseira granulata* (Kensuke Seto), *R. megarrhizum* parasitizing *P. rubescens* (Thijs Frenken), *D. kinnereticum* parasitizing *P. gatunense* (left) and growing on pollen (right) (Tamar Leshem), *P. planicorne* parasitizing an unindentified diatom (left) and growing on pollen (right) (Martha J. Powell), *G. haynaldii* growing on pollen (Kensuke Seto), *R. aurantiacum* growing on chitin (Martha J. Powell), *U. harderi* growing on agar (Martha J. Powell and Peter Letcher). [Color figure can be viewed at wileyonlinelibrary.com]

approaches, not only to discover, classify and name fungal species according to their phylogenetic relationships and taxonomy, but also to perform ecological studies. In this context, two key considerations must be taken into account: (i) there does not appear to be a universal genetic marker able to discriminate among distant taxa, and simultaneously provide adequate resolution to identify organisms at the species level, and (ii) current representation of Chytridiomycota, and especially parasitic chytrids, in sequence databanks is limited.

The nuclear rRNA gene region, consisting of three genic markers evolving at different rates, has been instrumental for fungal identification by molecular barcoding. First, the small ribosomal subunit (SSU), which can be aligned across the breadth of the phylum level due to its conservative nature, allows the placement and identification of a broad and divergent range of taxa. Such analyses can result in phylogenies with strongly supported lineages, but may suffer a poorly supported backbone due to many polytomies with little or no indication of relative relationships among clades (Letcher *et al.*, 2008; Wakefield *et al.*, 2010; Longcore and Simmons, 2012). Hence, the SSU can provide an adequate molecular framework at the phylum level for Chytridiomycota, but a higher resolution can only be achieved using other markers.

Second, and to the goal of achieving higher resolution, the large ribosomal unit (LSU) has proven a promising genetic marker for chytrids delineation, as it exhibits more variability than the SSU. Thus, it has been used to delineate new orders such as Rhizophydiales, Rhizophlyctidales, Cladochytriales, Lobulomycetales and Polychytriales, and to confirm existing orders (Spizellomycetales, Chytridiales), and for delineation at family, genus and species level (Davis *et al.*, 2015; Letcher *et al.*, 2015b; Powell *et al.*, 2015; Leshem *et al.*, 2016).

Third, of the rRNA markers, the intergenic transcribed spacer (ITS) has been proposed as the most suitable molecular marker for fungal barcoding (Schoch *et al.*, 2012). Yet, for the early diverging Chytridiomycota, the unconstrained and rapidly evolving ITS1 and ITS2 portions of the ITS region are difficult to align, and may suffer saturation (i.e. reduced signal of sequence divergence rate), thereby ruling out its use as the only marker for phylogenetic studies. However, the ITS region has been successfully used in conjunction with LSU to delineate closely related taxa, which was not possible using the LSU alone (Letcher *et al.*, 2006; 2015a; Vélez *et al.*, 2013). Consequently, resolution in phylogenetic studies of Chytridiomycota would benefit from combining more than one molecular marker.

Recent developments in sequencing technologies pave the way for promising new alternatives such as the use of the complete ribosomal operon. This long read can be readily covered by novel sequencing methods like Pacific Biosciences (Rhoads and Au, 2015) and Oxford Nanopore (Laver *et al.*, 2015). Additionally, phylogenetic analyses could be complemented by the use of other novel fungal markers such as the elongation factor TEF1α and the single-copy protein-coding gene RPB2 (Stielow *et al.*, 2015; Větrovský *et al.*, 2016). Another promising approach could involve the development of a large number of new candidate loci from sequencing different chytrid genomes from divergent lineages (e.g. through single cell genomics) and the development of

Table 2. Current number of sequences of Fungi and Chytridiomycota across various databases and according to different molecular markers (April 2017).

Database	Marker	Fungi	Chytridiomycota	Percentage
GenBank	SSU	546 728	1243	0.23
GenBank	ITS	983 576	978	0.10
GenBank	LSU	507 270	1097	0.22
Silva Ref128	SSU	23 721	862	3.63
Silva Ref128	LSU	2925	124	4.24
UNITE[a] **v7.1**	ITS	21 607	124	0.57
RDP[b]	LSU	8993	249	2.77

a. Representative sequences for 97% similarity clustering.

b. Training set 11.

specific primers for these regions (Gawad *et al*., 2016; Rutschmann *et al*., 2016).

The second major constraint for the taxonomy of this group is a general lack of representatives, especially parasitic species (or those described as such), in sequence databases. A survey of the most important databases for fungal taxonomic assignment reveals that Chytridiomycota represent between 0.1 and 4% of the fungal sequences, where the number of those that are parasitic species is difficult to estimate, but not larger than a few dozen (Table 2). It is therefore not surprising that some species of parasitic chytrids were recently found to be related to sequences of novel lineages only characterized by environmental sequences (Karpov *et al*., 2014; Seto *et al*., 2017). The use of culture-independent molecular methods, e.g. single cell/colony/spore PCR (Ishida *et al*., 2015), as well as sequencing of bulk phytoplankton samples, will likely improve chytrid representation in future sequence databases.

Mechanisms of infection

The process of chytrid infection has been primarily documented by microscopic observations. However, the underlying mechanisms still remain largely unknown. In general, infection consists of four main phases comprising (i) attraction of zoospores to a host; (ii) interactions on the host's surface leading to chytrid encystment (i.e. attachment); (iii) germination and formation of infection structures by the parasite and penetration of host cell wall and (iv) maturation of infection, during which new zoospores are formed and finally released.

Observations that some chytrids are unable to complete their infection cycle in darkness, or at very low light intensities, indicate that chemical cues driving attraction of zoospores to their host, and host recognition might be closely related to photosynthetic exudates (Barr and Hickman, 1967; Canter and Jaworski, 1981; Bruning, 1991b). This idea is further supported by a lowered ability of a chytrid taxon to infect its diatom host in the presence of photosynthesis-inhibiting compounds, such as herbicides (Van den Wyngaert *et al*., 2014). A range of phytoplankton exudates, including photosynthesis by-products, have been reported as attractants for different zoosporic parasites. These compounds include amino acids, saccharides and other carbohydrates (Halsall, 1976; Orpin and Bountiff, 1978; Mitchell and Deacon, 1986; Muehlstein *et al*., 1988; Donaldson and Deacon, 1993; Moss *et al*., 2008). Whole-cell extracts and mixtures of carbohydrates (xylose, ribose, rhamnose, mannose, fucose, glucose and arabinose) attracted more zoospores as compared to single compounds alone (Scholz *et al*., 2017), suggesting that multiple attractants drive chemotaxis and that they act synergistically. Altogether, this suggests that taxis in zoosporic parasites might not be specific in terms of host selection and is consistent with observations that zoospore attachment to hosts can be reversible in some taxa (Doggett and Porter, 1995).

Upon encounter, zoospores encyst on suitable hosts. Parasite-host recognition traits are likely mediated by chemical interactions at the host's surface and arguably constitute one of the determining factors controlling host-parasite compatibility. Knowledge of other zoosporic parasites (e.g. oomycetes) suggests lectin-carbohydrate interactions as likely chemical mechanisms driving zoospore encystment (Hinch and Clarke, 1980; Jacobson and Doyle, 1996; Levitz, 2010; Petre and Kamoun, 2014), as well as interactions with antibodies or exopolysaccharides in the host mucilage. Particularly in the chytrids *Entophlyctis apiculata* and *Zygorhizidium planktonicum*, adhesive materials between fungal and host cells were observed by TEM (Beakes *et al*., 1992; Shin *et al*., 2001). Analysing host and parasite surface characteristics using laboratory chytrid-phytoplankton systems is needed to elucidate the triggers of zoospore encystment. In particular, comparative studies of conspecific susceptible and resistant host isolates can potentially help to pinpoint cellular surface traits that determine host-parasite compatibility.

Upon zoospore encystment on the host cell, a germ tube is formed which, in most cases, penetrates the host cell immediately after germination. Rhizoids are then produced, which expand through the host cell, enabling transfer of material into the host cell (Gromov *et al*., 1999; Shin *et al*., 2001; Van Rooij *et al*., 2012; Karpov *et al*., 2014; Lepelletier *et al*., 2014). However, host penetration mechanisms likely differ between host species. For instance, diatom infecting chytrids use a germ tube that enters the host cell through the girdle region of the frustule (Van Donk and Ringelberg, 1983; Beakes *et al*., 1992), whereas in other algal hosts, the germ tube penetrates the cell through the mucilage surrounding the host (Canter, 1950; Canter and Lund,

1951), or directly through the cell wall in absence of such mucilage (Gromov *et al*., 1999; Shin *et al*., 2001; Karpov *et al*., 2014; Lepelletier *et al*., 2014). Despite these observations, the underlying molecular mechanisms of the penetration process are largely unknown. It has been shown that some fungal plant pathogens degrade enzymatic polysaccharides of the host cell wall (Jones *et al*., 1972) and penetrate the cell by using the internal turgor pressure of the plant (Howard and Ferrari, 1989). More studies should be performed to observe successive stages of the infection process (encystment to penetration) including the study of structures by scanning electron microscopy (SEM) and TEM.

Regarding the colonization of host cells, it has been shown that zoosporic plant pathogens, such as oomycetes, deliver effector proteins inside the cells to facilitate host colonization (Petre and Kamoun, 2014). One class of secreted pathogen effectors comprises the modular CRN (Crinkling and Necrosis) family of proteins that alter the host cell physiology by targeting and cleaving DNA. CRN proteins contain a conserved N84 terminal domain specifying translocation into host cells and diverse C-terminal regions harbouring effector functions (Stam *et al*., 2013). Notably, CRN proteins have been identified in the genome of the amphibian chytrid *Batrachochytrium dendrobatidis* (Joneson *et al*., 2011). The presence of CRNs genes in phylogenetically distant eukaryotic pathogens suggests that eukaryotic effectors might display a conserved mode of action and might also be present in phytoplankton-infecting chytrids. The use of transcriptomic, proteomic and metabolomic approaches will contribute to a mechanistic understanding of all infection phases, which is crucial for unravelling the bases of host-parasite specificity.

Host specificity and range

Host specificity, defined as the extent to which parasites can exploit different host species, is a fundamental trait of parasites both from an ecological and evolutionary perspective (Poulin *et al*., 2011). Most field studies have concluded, solely based on morphological identification of phytoplankton-chytrid pairs, that these interactions are highly species-specific (Holfeld, 1998; Rasconi *et al*., 2012) and that some chytrids are even specialized on specific cell types or even proteins (Marantelli *et al*., 2004; Vélez *et al*., 2011; Gerphagnon *et al*., 2013). Molecular analyses based on single spore/cell PCR revealed the presence of specialists, but also of generalists capable of infecting multiple host species (Ishida *et al*., 2015). Cross-infection assays under laboratory conditions often expose an even more complex picture, with some chytrids infecting specific host strains only (Canter and Jaworski, 1979; De Bruin *et al*., 2004) and others capable of infecting different species, although within single host species both susceptible and resistant strains occur (Gromov *et al*., 1999; Gutman *et al*., 2009; Lepelletier *et al*., 2014).

Our current knowledge of host range and chytrid specificity is greatly biased by the fact that morphological identification often does not provide enough resolution to identify chytrids (and sometimes also phytoplankton) at the species level (Letcher *et al*., 2008; Van den Wyngaert *et al*., 2015). This potentially masks several hidden host-chytrid interactions and their dynamics. As seen in many other host-parasite systems, it is likely that within a single chytrid species both specialist and generalist strains coexist (Koehler *et al*., 2012). Extrapolations of results from cross-infection assays between single chytrid and host strains to the population level have, therefore, to be taken with caution. Moreover, whereas most infection assays have been conducted under constant environmental conditions (De Bruin *et al*., 2008; Gutman *et al*., 2009; Lepelletier *et al*., 2014), temperature can alter host-genotype specific susceptibility to chytrid infection (Gsell *et al*., 2013a), implying that heterogeneous environments might provide different outcomes in specificity tests (Wolinska and King, 2009).

Similarly to the continuum between saprophytic and parasitic consumer strategies (see *Life-cycle and ecological strategies*), the occurrence of generalist and specialist parasitic chytrids raises questions about the conditions promoting different strategies. Commonly assumed costs associated with generalists have not been investigated yet in parasitic chytrids. Elucidating the mechanisms underlying host specificity and their associated costs will allow formulation of more targeted hypotheses about the conditions that promote specialist or generalist strategies. For example, if host specificity does not operate at the attraction stage, specialists are expected to suffer more from a 'dilution effect' (i.e. reduced host densities) under conditions of high host diversity, since generalists may have higher probability to encounter suitable hosts (Keesing *et al*., 2010; Alacid *et al*., 2016).

Whereas field studies capture the 'contextual' host range and specificity of chytrids in their natural settings, experimental cross-infection assays can capture the potential host range. By examining the different steps of the infection process across a range of potential host species and environmental conditions, we can test which infection steps drive specificity and contribute to shaping host ranges, as well as to what extent genetics and environment determine and modulate host and parasite compatibility (Ebert *et al*., 2016). Such assays are important for making predictions on the spread and persistence of chytrids in novel environments – as driven by climate change – but also in mass cultivation systems.

Host-parasite co-evolution and host diversity

Maintenance of genetic diversity in populations has been linked to strong reciprocal selection between hosts and their parasites, resulting in co-evolution. Host-parasite co-evolution can occur through successive fixation of beneficial mutations (selective sweeps) or through sustained genotype frequency oscillations as parasites adapt to the most common genotypes, conferring a selective advantage to rare genotypes (Red Queen dynamics) (Woolhouse *et al*., 2002). While selective sweeps lead to fast evolution in genes but low levels of genotype standing variation, Red Queen dynamics lead to long-term maintenance of genotype diversity. To show potential for co-evolution, we need evidence for (i) strong reciprocal selective pressure, (ii) genotype-specific infectivity and resistance and (iii) a genetic basis for differences in infectivity and resistance. Conclusive proof of co-evolution in chytrid-phytoplankton systems is lacking, but some of the above points are supported. Phytoplankton-infecting chytrids are often obligate parasites (but see *Life-cycle and ecological strategies*), and phytoplankton hosts cannot recover from infection, resulting in strong reciprocal selection pressure. Host geno- and/or chemotypes (i.e. differentiated by cellular oligopeptide fingerprints) can differ in resistance (Sønstebø and Rohrlack, 2011; Gsell *et al*., 2013b). Experimental evolution of chytrids shows fast adaptation in genetically homogeneous host cultures, but not in heterogeneous ones, indicating that host genetic diversity restricts parasite evolution (De Bruin *et al*., 2008). Genotype-specific differences in parasite infectivity, however, remain understudied.

To understand how co-evolution shapes host-parasite dynamics and diversity, we need insight into the extent of specificity in phytoplankton-chytrid relationships and the genetics underlying infectivity and resistance. Moreover, alternative mechanisms affecting co-evolutionary trajectories need to be evaluated, e.g. fluctuating selection in variable environments (Wolinska and King, 2009) or selection for facultative parasites in non-host refuges. Effects of spatiotemporal variation in competition between parasites (multiple infections) raise questions on the importance of priority effects, i.e. effects caused by the first-infecting parasite. The occurrence of chytrids infecting subsets of genotypes across several host species (M. Kagami, pers. comm.) allows exploration of co-evolutionary trajectories leading to subdivision of host species and possibly sympatric speciation. Before we can gauge the potential for co-evolution in the field, we need to map the extent of refuges for parasites and hosts (see *Environmental refuges*) reducing reciprocal selection and therefore slowing co-evolution.

To disentangle the mechanisms of co-evolution in phytoplankton-chytrid model systems, we need experiments that test evolutionary responses based on mutations (selective sweep scenario) and/or standing genetic variation (Red Queen scenario). Further efforts are needed to assess spatial and temporal co-evolutionary trajectories through local adaptation experiments (Greischar and Koskella, 2007) or experiments on asymmetric evolution, i.e. one of the antagonists is not allowed to evolve (Schulte *et al*., 2010). As the genetic basis for differences in infectivity and resistance remain unresolved, proteomics of infected and uninfected cultures may help to identify proteins involved in the response to infection and elucidate the nature of host defence and resistance. Modelling host-parasite interactions can help to constrain expectations for different co-evolution scenarios when exploring the effect of specialist/generalist or obligate/facultative parasitism (or graduations thereof) on the maintenance of host genetic diversity, and, conversely, the effect of host genetic diversity on disease spread (King and Lively, 2012).

Host defence and parasite counter-defence

Host defences can be classified in three main groups: barrier defences, immune defences and behavioural defences. Barrier defences guard against the entry of the parasite into the cell prior to contact with the immune defences (Parker *et al*., 2011). The genetic and biochemical mechanisms of zoospore encystment remain essentially unknown (but see *Mechanisms of infection*). Within a single host species, zoospores encyst on certain strains only (Sønstebø and Rohrlack, 2011), indicating that host surface traits may grant resistance in some cases. In turn, parasites often evade barrier defences by molecular mimicry of host receptors and, therefore, host and parasite active binding sites often show convergent evolution (Sikora *et al*., 2005). However, whether this is the case for chytrids remains currently unknown.

Once barrier defences are overcome, chytrids encounter host immune defences. Although defence mechanisms likely differ between host organisms, some strategies have been identified. A first type of defence is hypersensitivity, a particular type of apoptosis, which requires the host cell to detect infection in an early stage. Laboratory work showed a hypersensitive response of *A. formosa*, which kills the chytrid parasite before it can complete its life cycle (Canter and Jaworski, 1979). Since hypersensitivity kills the infected host cells to protect the unaffected ones, this type of defence suggests that host cells within a population (or at least, within the susceptible subset of the population) collaborate to fight off parasites (Franklin *et al*., 2006). A second type of immune defence is related to the production of defensive

chemical compounds. Planktonic cyanobacteria produce a wide range of bioactive oligopeptides (Agha and Quesada, 2014), that display numerous enzyme inhibitory properties and could contribute to antiparasite defences (Rohrlack *et al*., 2013). Also, Pohnert (2000) found that phytoplankton cells release potent fungicides from their cells when mechanically wounded. Whether these repel chytrid parasites has yet to be tested, but if they do, they may also protect host cells that are in tight proximity to the cell that is under attack. The third type is behavioural defence. For instance, by utilizing a buoyancy regulation system, the cyanobacterium *Planktothrix* migrates and accumulates in the metalimnion of clear-water lakes, where low temperatures and light render an environmental refuge against chytrid infection (Kyle *et al*., 2015). This is analogous to the 'Cheshire cat' escape strategy of the coccolitophore *Emiliania huxleyi* in response to viral infection, whereby the usual diploid host phenotype transforms temporarily into a haploid phenotype, which is invisible to the virus (Frada *et al*., 2008).

The major problem to directly characterize these defence strategies is that chytrids infecting phytoplankton remain black boxes, both biochemically and genetically. Gathering information on the molecular basis of chytrid infection is hence urgently needed to systematically search for host defence and chytrid counter defence mechanisms and characterize them.

Environmental refuges

Environmental refuges in host-parasite interactions are little understood but thought to be important in shaping co-evolution (Wolinska and King, 2009). Chytrid escape from low host density conditions is possible through a 'host-free' stage in their life cycle (Leung *et al*., 2012), for example by switching to saprophytic interactions (Gleason *et al*., 2008) or the formation of resting stages (Doggett and Porter, 1996b; Ibelings *et al*., 2004). Hosts, in turn, may escape the worst of an epidemic by 'taking shelter' where conditions are not favourable for infection. Besides the active migration of the cyanobacterium *Planktothrix* to colder metalimnetic depths to escape infection, Bruning (1991b) demonstrated that a diatom-infecting chytrid displays greatly reduced capacity for epidemic development under conditions of low temperature and irradiance. The existence of a cold water host refuge ($<$ 1–2°C) was confirmed by Gsell *et al*. (2013a). This study also found evidence for a warm water refuge above 20°C, where *Zygorhizidium* sporangia no longer fully matured. Warmer winters are expected to cause a gradual disappearance of a cold 'window of opportunity' for an early, parasite-free development of *Asterionella*. Early chytrid infections, although at low prevalence, may prevent the host from blooming, and thereby, hamper the opportunity of the parasite to reach epidemic levels of infection (Ibelings *et al*., 2004). So, perhaps paradoxically, the loss of cold water refuges may ultimately be detrimental to this particular parasite.

Despite these observations, many open questions exist: Can the above observations be generalized to other phytoplankton taxa? What is the relative role of refuges for stabilizing the interaction between host and parasite? If the host fully relies on refuges for seasonal development, how will global drivers of lake-ecosystem change affect the persistence and seasonal succession of phytoplankton? Beside their obvious importance for disease prevalence, infection refuges are arguably important modulators of parasitic pressure on host populations, where co-evolutionary processes decelerate or even cease (Kyle *et al*., 2015; Rohrlack *et al*., 2015). How does this affect eco-evolutionary feedbacks between host and parasite? On top of this, we still have inadequate understanding of the nature of chytrid specificity, infectivity and host defence and their modulation by environmental factors – as formalized by the disease triangle concept (Stevens, 1960).

Many of these questions can be approached using host-parasite isolates to undertake laboratory experiments under controlled conditions. For example, in order to study the basis of reduced/absence of infections under cold or low light conditions occurring e.g. in deep stratified lakes, conditions of temperature and light refugia can be reproduced in the laboratory. Since the rate of photosynthesis by hosts is both light and temperature dependent, cold and low light conditions might result in reduced excretion of dissolved organic carbon (DOC) by the host which might in turn limit the chemotactic ability of chytrid zoospores to locate and infect their hosts. To explore this idea, experiments could be performed, where host taxa putatively exploiting these refuges (e.g. *Planktothrix rubescens*) are grown under a range of environmental conditions representing different depths of deep lakes, where irradiance and temperature decrease, and nutrient availability increases with depth. By mimicking their environmental conditions in the laboratory and using different established host-parasite isolates, the role of environmental refugia on phytoplankton-chytrid interactions can be further elucidated.

Ecological stoichiometry of chytrid infections

Planktonic organisms experience dynamic changes in resource availability at different temporal and spatial scales, not only as a result of seasonality or changes in mixing regimes, but also due to climate change and anthropogenic impacts (Behrenfeld *et al*., 2006; Berger *et al*., 2014). Shifts in the availability of nutrients affect phytoplankton

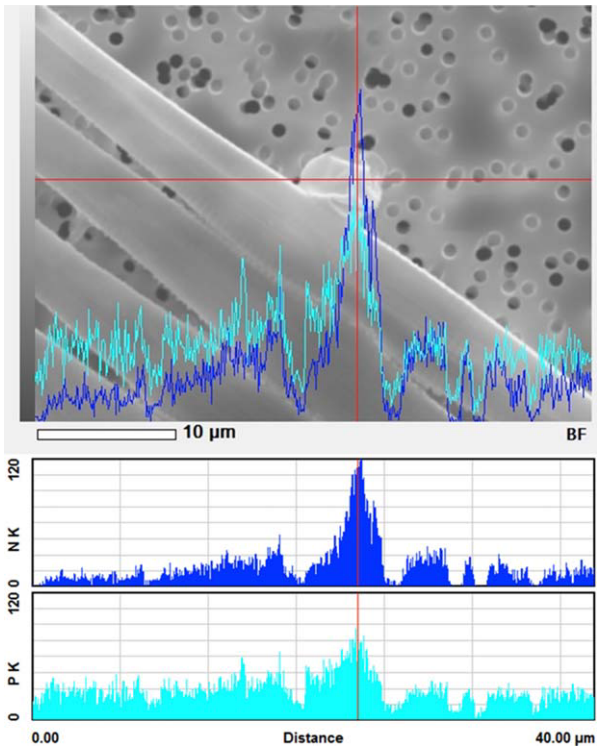

Fig. 2. SEM image of the diatom *Fragilaria crotonensis* infected by the chytrid strain FRA-CHY1, isolated from Lake Stechlin (Germany) in March 2015. Elemental energy intensities of N (dark blue) and P (light blue) of the diatom and the chytrid sporangium along a transect (horizontal red line) are shown. The graph within and below the SEM image indicates higher N and P contents in the chytrid sporangium compared to its diatom host. Strains were isolated by Silke Van den Wyngaert, IGB, Stechlin. SEM image and energy dispersive x-ray (EDX) analyses, using a SEM microscope (JEOL 6000) equipped with an EDX-system were performed by Reingard Rossberg and Stella A. Berger, IGB Stechlin. [Color figure can be viewed at wileyonlinelibrary.com]

growth and its elemental composition, which may in turn propagate to higher trophic levels (Sterner and Elser, 2002; Berger *et al*., 2006; Van de Waal *et al*., 2010; De Senerpont Domis *et al*., 2014). Specifically, heterotrophs tend to have higher nutrient demands as compared to phytoplankton, reflected by lower C:P and C:N ratios (Vrede *et al*., 1999; Hessen *et al*., 2013). Such stoichiometric mismatches may become a bottleneck for the transfer of carbon and nutrients to higher trophic levels (Urabe *et al*., 2003; Elser *et al*., 2010).

Table 3. Molar C:N and C:P ratios of zoospores of the chytrid *Rhizophydium megarrhizum* and its cyanobacterial host (*Planktothrix rubescens* NIVA-CYA97/1) grown under nutrient replete conditions (Frenken *et al*. 2017).

	C:N ratio (molar)	C:P ratio (molar)
Chytrid average (SE), $n = 4$	4.75 (0.02)	59.9 (0.9)
Cyanobacteria average (SE), $n = 4$	4.39 (0.01)	48.3 (1.6)

Table 4. Maximal and average elemental energy intensities of N and P signals recorded on the chytrid sporangium (intersection of red lines in Fig. 2) and its diatom host.

Peak transect	N intensity	P intensity
Chytrid peak	111	85
Chytrid average (SE), $n = 67$	55 (0.6)	52 (1.7)
Diatom average (SE), $n = 191$	16 (0.6)	31 (0.6)

In analogy to zooplankton grazing, chytrid infections may be stoichiometrically constrained, where the outcome of infection depends on the overlap in stoichiometric requirements of the parasite with its host (Aalto *et al*., 2015). Although chytrids have been shown to be an important nutritional component of the zooplankton diet (Kagami *et al*., 2004; 2007a; 2014; Grami *et al*., 2011; Rasconi *et al*., 2014), surprisingly little is known about the elemental composition of chytrids and their zoospores and, therefore, the stoichiometry of chytrid infections. Initial elemental analyses by single cell SEM-based techniques indicate that chytrid sporangia contain more nutrients (P and N) than its algal host (Fig. 2, Table 4. Furthermore, analyses on zoospore suspensions indicated relatively low C:N and C:P ratios (Table 3; Frenken *et al*., 2017). Low carbon to nutrient ratios may be attributed to relatively high amounts of nucleic acids and lipids, including fatty acids and sterols (Barr and Hadland-Hartmann, 1978; Beakes *et al*., 1988; Elser *et al*., 1996; Kagami *et al*., 2007b) and indicate that chytrids have high phosphorus (Kagami *et al*., 2007b), and nitrogen requirements (Frenken *et al*., 2017).

Net effects of nutrient limitation on chytrid epidemics will depend on changes in host growth rates relative to its chytrid parasite (Bruning and Ringelberg, 1987; Van Donk, 1989; Bruning, 1991a) which may result, according to model simulations, in different alternative stable states: one with only the host and one allowing host and parasite coexistence (Gerla *et al*., 2013). A further understanding of the ecological stoichiometry of chytrid infections and its role in aquatic food webs requires additional analyses of chytrid elemental composition and their interaction with host stoichiometry.

Top-down regulation of chytridiomycosis and trophic interactions

While chytrid parasites can exert strong top-down control on phytoplankton, chytrids themselves can also be used as prey in two different ways: they can either be grazed upon by zooplankton, or they serve as a host themselves for hyper-parasites.

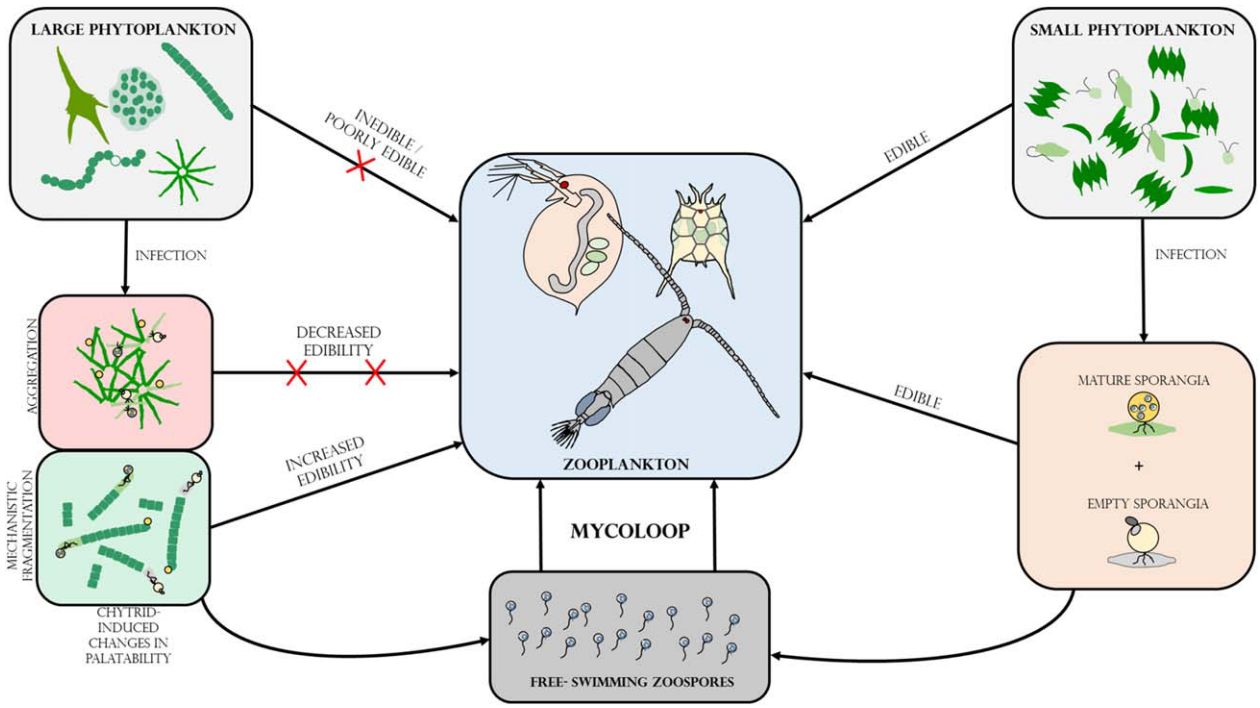

Fig. 3. Schematic representation of the chytrid-mediated trophic links between phytoplankton and zooplankton (mycoloop). While small phytoplankton species can be grazed upon by zooplankton, large phytoplankton species constitute poorly edible or even inedible prey. Chytrid infections on large phytoplankton can induce changes in palatability, as a result of host aggregation (reduced edibility) or mechanistic fragmentation of cells or filaments (increased palatability). First, chytrid parasites extract and repack nutrients and energy from their hosts in form of readily edible zoospores. Second, infected and fragmented hosts including attached sporangia can also be ingested by grazers (i.e. concomitant predation). [Color figure can be viewed at wileyonlinelibrary.com]

Chytrids have been shown to constitute a key nutritional component of the zooplankton diet (Kagami *et al*., 2004; 2007a; 2014; Grami *et al*., 2011; Rasconi *et al*., 2014). This pictures a three-way trophic link between algal primary producers, zooplankton and chytrid parasites (Fig. 3) and implies a potential role of zooplankton as an important top-down control agent of chytrid infections (Kagami *et al*., 2004; 2007a; Schmeller *et al*., 2014). Also, these interactions might profoundly affect phytoplankton seasonal dynamics and composition. For example, during blooms of edible algae or other hosts, zooplankton can affect chytrid prevalence and transmission by (i) grazing on the host and/or on the parasite, affecting the chance of host and parasite encounter and (ii) grazing on edible phytoplankton, thereby promoting the dominance of larger inedible phytoplankton species, ultimately reducing the availability of suitable food sources for zooplankton. However, if inedible phytoplankton become infected, produced zoospores can provide an alternative suitable food source to zooplankton, potentially re-coupling primary and secondary production, through the so-called mycoloop (Kagami *et al*., 2007a,b; Agha *et al*., 2016; Frenken *et al*., 2016). In addition, there are indications that chytrid infections could modify host palatability to zooplankton. For example, large filamentous cyanobacteria get fragmented as a result of infection and might become more edible to zooplankton (Gerphagnon *et al*., 2013; Agha *et al*., 2016), while infected diatom colonies may aggregate and become less edible (Kagami *et al*., 2005). Additional efforts are needed to better characterize and quantify zooplankton-chytrid-phytoplankton interactions and assimilate them in an ecological context, including their consequences for trophic linkages in aquatic food webs.

Chytrids can also serve as a host for hyper-parasites (Gleason *et al*., 2014). Hyper-parasitism may reduce disease risk in phytoplankton host populations. For example, the parasitic chytrid *Zygorhizidium affluens* infecting the diatom *A. formosa* is frequently found hyper-parasitized by another early diverging fungus: *Rozella parva* (Canter, 1969). Hyper-parasitism of the primary parasite may reduce or suppress the output of spores and therefore arguably results in a reduced parasitic pressure on phytoplankton (Canter-Lund and Lund, 1995). Similarly, it is likely that chytrids (like their hosts) are targeted by viral infections, although this research area is virtually unexplored. Parasites, predators and hyper-parasites interact and dynamically shape the phytoplankton community structure. We need to disentangle this complex matrix of multipartite interactions and

integrate them with the effects of abiotic variables, which, altogether, modulate the composition, density and dynamics of the planktonic communities. Since the majority of experiments have been conducted using a single host, chytrid or grazer, interactions at the community level remain largely unexplored. This makes it hard to predict if (and when) top-down control by predators and/or hyper-parasites can override bottom-up mechanisms.

Inclusion of chytrids in food web models

Food web models help to reveal and clarify mechanisms behind food web dynamics (e.g. the effect of parasites on population stability), infer cause-effect relationships between multiple components, estimate standing stocks and fluxes of materials and/or forecast the future status of food webs (e.g. parasite infection rates 1 year later).

Theoretical models, such as mass-balance and node food webs, are helpful tools to describe and quantify energy and matter flows via directional trophic linkages. To properly describe trophic food webs, all matter and/or energy flows among nodes need to be known and quantified. However, natural ecosystems are complex and some compartments, such as parasites, are cryptic and difficult to measure directly, preventing a full characterization of all fluxes (Niquil *et al*., 2011). Inverse analysis (Vézina, 1989) is a method based on the mass-balance principle, which allows calculating flows that are not measured directly using linear equations and ecological constraints (Vézina *et al*., 2004; van Oevelen *et al*., 2010). For example, inverse analysis was applied successfully to show that chytrid parasites contribute to longer carbon path lengths and loop strength, higher levels of activity and specialization, lower recycling and enhanced stability of the pelagic food web (Grami *et al*., 2011; Rasconi *et al*., 2014).

Alternatively, empirical dynamic modelling approaches, including linear [e.g. multivariate autoregressive models (MAR models; e.g. Hampton *et al*., 2013)], or nonlinear models [for instance convergent cross mapping (CCM, e.g. Sugihara *et al*., 2012)], are used to infer interactions between food web and environmental components by regression structure or causal-effect relationship, and for short-term forecasting. The advantage of these models compared to theoretical ones is that no specific assumptions on the underlying driving mechanisms are required. MAR models use long-term data of aggregated taxonomic, trophic or trait-based groups to infer direction and strength of interactions, not only between trophic links, but also between groups connected by indirect interactions (e.g. competition or facilitation). The resulting interaction matrix allows derivation of network stability metrics (Ives *et al*., 1999) and can be passed on to network analysis (Gsell *et al*., 2016).

Models have contributed to a better understanding of the quantitative importance of chytrids in trophic food webs (Grami *et al*., 2011; Kagami *et al*., 2014), however, they still show limitations. Inverse models provide only a snapshot of the natural complexity, illustrating steady-state webs for a chosen time period, but do not integrate temporal evolution nor allow describing complex dynamics like host-parasite interactions (Miki *et al*., 2011). In turn, empirical dynamic models require good quality long-term datasets with a time resolution matching the relevant rates of the biological process in question, i.e. grazing or infection. Hence, they are still limited by the current lack of datasets showcasing long-term dynamics of chytrid infections. Moreover, the interpretation of results from linear models (e.g. MAR models) is not always straightforward. Regression approaches carry a risk of yielding spurious relationships. In addition, linear models assume that the system is linearly fluctuating around the neighbourhood of a stable equilibrium. Instead, nonlinear empirical models (e.g. CCM) are better at excluding spurious relationships and can be also applied to chaotic systems.

Despite current limitations, modelling can contribute to unravelling the influence of parasites on the structure of host populations and its consequences for the rest of the food web, including estimations of the efficiency of matter and energy transfer from hosts to higher trophic levels. Improved methodologies will contribute to more accurate quantifications of ecological processes, which will improve model parameterization. By identifying more realistic ecological constraints, possible model solutions and their associated uncertainties can be effectively reduced, making it possible to draw more generalizable conclusions when comparing models issued for different ecosystems.

Technical and methodological challenges

Despite recent progress, we still have minimal understanding of many fundamental aspects of plankton chytridiomycosis. In spite of their multidisciplinary nature, we perceive that most research gaps we highlight are affected by three main constraints that greatly hamper a deeper knowledge on the biology of chytrid parasites and its implications in plankton ecology.

The first constraint is the *lack of available chytrid-phytoplankton isolates*. Most hypotheses shaping current scientific notions about the importance of chytridiomycosis in ecological processes stem essentially from experimental work with the few available laboratory isolates. Establishing chytrid-host cultures is not an easy task, but so far little effort has been devoted to isolation and

cultivation of parasitic chytrids, probably due to the lack of interest this topic raised among aquatic ecologists in the past. To remedy this situation, the application of automated single-cell sorting using flow-cytometry can potentially facilitate the isolation of host-parasite pairs, not only for taxonomic purposes, but also for cultivation. Experimental work with isolates is essential to study chytrid biology and that of their hosts in its numerous facets, such as the ecophysiology of chytrid infection, or its underlying mechanisms at the cellular level. For example, isolates can be used to undertake chemotactic assays based on live-cell imaging coupled with microfluidics (Rusconi *et al*., 2014; Scholz *et al*., 2017) to determine zoospore swimming properties or chemotaxis in response to biotic or abiotic factors. Formulation of hypotheses about the interaction between chytrids and other trophic levels (e.g. zooplankton), including food quality and stoichiometric aspects, also demands laboratory work with chytrid isolates. Analyses of chytrids and their hosts using a combination of SEM and x-ray microanalyses can provide accurate estimates of the stoichiometry of host and parasite (Fig. 2, Table 4), whereas the use of stable isotope probing (SIP) and nanoscale secondary ion mass spectrometry (NanoSIMS) can be a powerful tool to quantify both substrate utilization by parasite, and transfer to upper trophic levels by predators (e.g. *Daphnia*). Lastly, organismal systems based on chytrids and their hosts constitute valuable tools to undertake experimental evolution assays (e.g. De Bruin *et al*., 2008) to test evolutionary hypotheses on host-parasite co-evolution and make predictions about the impact of such evolutionary processes in natural communities.

The second constraint is the irrefutable fact that *chytrid parasites represent genetic black boxes*. The lack of a sequenced genome from chytrid isolates represents one of the current most important burdens in chytrid research, as it prevents the application of proteomic and transcriptomic approaches, which would in turn provide indispensable insights into the mechanisms of infection. Genome sequencing of chytrid parasites will also contribute to the development of improved molecular markers for phylogeny (i.e. markers that provide both discriminatory power among distant taxa and high resolution at the species level) and quantification (i.e. single-copy genes suitable for qPCR applications). In addition, comparative studies between chytrids with different host ranges and preferences will help to identify the molecular basis of host-parasite specificity. These can in turn provide insights into the process of host and parasite co-evolution, while rendering suitable molecular markers to track matching host and parasite genotypes in the wild. A collaborative action among the scientific community could rapidly change this situation, opening new exciting experimentation possibilities.

The third constraint is the lack of *assimilation of hypothesized ecological implications of chytridiomycosis into the context of natural ecosystems*. Information about the diversity of chytrids, their dominant strategies (saprophytism/parasitism and generalism/specialism), and the environmental conditions promoting them, can only be inferred from increased sampling of their natural habitats. However, sampling strategies have to be designed to provide enough temporal resolution to address rapid chytrid dynamics, enabling a better understanding of their life-cycles. Similarly, digital picture based techniques such as FlowCam (FluidImaging, USA) can provide high-throughput, near real-time identification of phytoplankton-chytrid interactions of live samples. In addition, FlowCam in combination with fluorescence staining techniques [e.g. fluorescein diacetate (FDA), SYTOX Green], can be used to estimate prevalence of infection and host viability directly from environmental samples (Dorsey *et al*., 1989; Franklin *et al*., 2012). Assisted by flow cytometry and cell sorting applications, single cell (cell, colony or spore) molecular approaches can likewise contribute to the characterization and quantification of chytrids on phytoplankton, thereby facilitating the study of their ecological relationships (Ishida *et al*., 2015; Maier *et al*., 2016). With regard to trophic interactions, further direct and model-based quantifications of the relative contribution of chytrid-mediated trophic transfers up the food web are still needed to integrate current experimental hypotheses about the role of chytrids as alternative conveyors of matter and energy between primary and secondary production. Lastly, from an evolutionary perspective, if we can elucidate the traits determining chytrid-host compatibility, these can be used as markers to track matching chytrid and host genotypes in the wild. This would allow studying the intensity of chytrid-mediated selective pressure and its contribution to the maintenance of diversity in host populations, as well as empirically testing different evolutionary scenarios (e.g. Red Queen hypothesis, selective sweeps) in natural settings directly.

The above methodological needs, although hampering progress at present, can in most cases be easily overcome. We believe that the scientific community can greatly profit from allocating efforts to resolve them, as it will unlock exciting new avenues for further experimentation that can largely contribute to the integration of chytrid parasites into traditional plankton ecology.

Conclusion

This paper identifies major research gaps in different aspects of the biology of chytrid parasites, as well as their

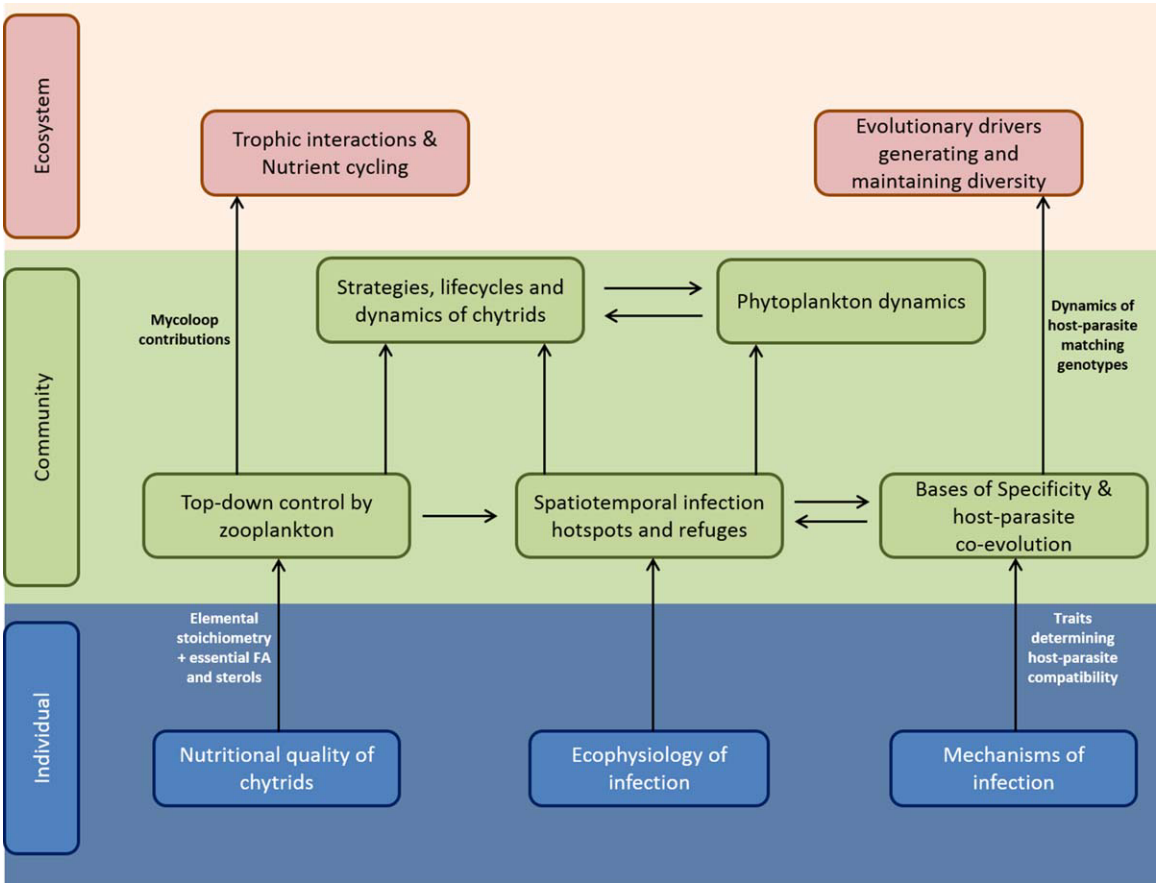

Fig. 4. Schematic representation of the research areas at different organizational levels and their interrelation. For explanation see text (see *Conclusion*). [Color figure can be viewed at wileyonlinelibrary.com]

role in the functioning of aquatic ecosystems. Our synthesis shows that the effects of chytrid parasitism on phytoplankton occurs at different scales, ranging from the individual organism, to the community and whole ecosystem levels, integrating physiological, ecological and evolutionary processes. To conclude, we provide our view on the different research aspects of plankton chytridiomycosis and how they relate to each other across complexity levels (Fig. 4), which illustrates the idea that progress in certain aspects can enable or stimulate development in others.

At the individual level, three main research areas can be identified. First, elucidating the mechanisms of chytrid infection is crucial to identify the basis of host resistance, specificity and host-parasite compatibility. Increased molecular and phylogenetic characterization of chytrid parasites and their hosts will allow the development of specific molecular markers that can resolve parasite and host cryptic diversity and thus contribute to a better understanding of chytrid ecological strategies and phytoplankton seasonal dynamics. Thereby, tracking the dynamics of matching host and parasite genotypes cycling in nature would be possible, which would allow researchers to empirically address the role of parasites as evolutionary drivers of the maintenance of genetic diversity at the ecosystem level. Second, ecophysiological investigations of chytrid infections will help us to identify potential ecological refuges with putative relevance for both chytrid life cycles and the dynamics of their hosts. In turn, by identifying infection refuges, we can delineate infection hot- and cold-spots and explore their role as modulators of co-evolutionary processes. Lastly, characterization of chytrids in terms of their nutritional value from the elemental stoichiometry and biochemical perspectives, together with data on the intensity, frequency and relative importance of top-down control of chytridiomycosis by zooplankton, can contribute to our understanding of the interrelation between parasitism and predation and its feedback on chytrid epidemics and plankton dynamics. This will result in more accurate estimates of parasite-driven transfer of carbon and nutrients through the food web and their contribution to total nutrient (re)cycling at the ecosystem level.

Despite the need for progress in these research areas, we currently face methodological limitations that, although may be easily overcome, hamper further advances in the field. The scarcity of isolated chytrid-

host cultures, the lack of genomic information on chytrid parasites of phytoplankton, and the marginal incorporation of chytridiomycosis-related research questions in field investigations represent the most important ones. However, new methodologies and techniques from other research fields are waiting to be implemented in chytrid research and can be used to overcome most these burdens. Therefore, we expect new exciting research avenues will open in the near future, leading to the integration of chytrid parasitism into aquatic ecology.

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Author contributions

Contributions to the individual sections: 1. Introduction: RA, TF; 2. Taxonomy and Molecular Phylogeny: KRJ, PML, KS, CW, FCK, MK, SVdW, ECB; 3. Life cycle and strategies: HPG, MK, FCK, SVdW, AS, AR; 4. Host specificity and range: SVdW, HPG, PML, KRJ, KS, MK, AS, JW, AR, EA; 5. Mechanisms of infection: RA, BS, KS; 6. Host-parasite co-evolution and host diversity: ASG, BWI, TR, JW, SVdW, RA; 7. Host defence and parasite counter defence: RA, TR; 8. Environmental refuges: BWI, TR; 9. Ecological stoichiometry: DVdW, EVD, TF, SAB; 10. Top-down control: MK, RA, TF, MG, DSS, AL, JCN, TSN; 11.Inclusion of chytrids in food web models: SR, ASG, TM, MK, JCN; 12.Technical and methodological challenges: KRJ, RA, HPG, ASG, AR, EA, SVdW, JCN, SAB; 13.Conclusion: RA, TF. RA and TF edited the manuscript. All authors commented on the manuscript and approved its final version.

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