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PRIMARY RESEARCH PAPER

A newly isolated chytrid fungus specialized in parasitizing heterocysts of the filamentous cyanobacterium *Dolichospermum* sp.

Xujian Xu · Minoru Kasada · Hans-Peter Grossart · Bas W. Ibelings · Silke Van den Wyngaert

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Abstract Chytridiomycota (chytrids) are aquatic fungal parasites characterized by a stage of free-swimming zoospores and that are known to infect many phytoplankton species, typically killing the host cell. We report a novel chytrid species strictly infecting heterocysts of the N_2-fixing cyanobacterium *Dolichospermum* sp. During a two-month Lake Stechlin (Germany) sampling campaign, two *Dolichospermum* morphotypes coexisted: coiled (dominant, chytrid infection found mainly on vegetative cells) and straight (rare, heterocysts targeted by the new chytrid). Phylogenetic and morphological analyses place this parasite into the phylum Chytridiomycota, order Lobulomycetales where it represents a novel lineage within a clade that includes uncultured parasites of algae and heliozoa. This is the first discovery of a cyanobacteria parasite within the order. Heterocyst-specific infection suggests a potential disruption of cyanobacterial N_2-fixation. By creating a conditionally relevant pathway between filamentous N_2-fixing cyanobacteria and zooplankton via chytrid zoospores, the 'trophic dead end' of large cyanobacteria may be temporarily alleviated during periods of nitrogen limitation. Though chytrid infections have been shown to re-shape aquatic food web structure through the so-called mycoloop, our study points to a specific nitrogen pathway via infection of heterocysts, which connects N_2-fixing cyanobacteria with

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X. Xu · B. W. Ibelings (✉)
Department F.-A. Forel for Environmental and Aquatic Sciences and Institute for Environmental Sciences, University of Geneva, Geneva, Switzerland
e-mail: Bastiaan.Ibelings@unige.ch

X. Xu
Dazhou Key Laboratory of Agricultural Resources Development and Ecological Conservation in Daba Mountain, Sichuan University of Arts and Science, Tashi Road 519, Dazhou 635000, China

M. Kasada
Graduate School of Life Sciences, Tohoku University, Sendai, Japan

M. Kasada · H.-P. Grossart · S. Van den Wyngaert
Department of Plankton and Microbial Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Zur alten Fischerhütte 2, 16775 Stechlin, Germany

H.-P. Grossart
Institute of Biochemistry and Biology, Potsdam University, Maulbeerallee 2, 14469 Potsdam, Germany

S. Van den Wyngaert
Department of Biology, University of Turku, Vesilinnantie 5, 20014 Turku, Finland

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the lake food web and thus is of potential importance for aquatic nitrogen cycling.

Keywords Fungal parasite · *Dolichospermum* heterocyst · Morphology · Phylogeny · Life cycle · Nitrogen fixation

Introduction

Fungi are morphologically, phylogenetically, and ecologically diverse members of all ecosystems (Grossart et al., 2019). Best estimations of the total number of fungal species on earth vary between 2.2 and 6 million (Hawksworth & Lucking, 2017; Baldrian et al., 2022), of which only about 3000 to 4000 species have been assigned to freshwater and marine ecosystems (Shearer et al., 2006; Jones et al., 2014). However, advanced DNA-sequencing technologies suggest the real number could be up to 10,000 individual species (Schmit & Mueller, 2007; Jones, 2011). Aquatic fungi are associated with various phytoplanktons and affect many features of the plankton community, but they have been largely overlooked compared to terrestrial fungi (Grossart et al., 2019).

Fungi in aquatic environments are mostly dominated by members of the early diverging fungal phylum Chytridiomycota (Comeau et al., 2016, Grossart et al., 2016, Van den Wyngaert et al., 2022), which are commonly called chytrids. Chytrids are characterized by the production of motile uniflagellate zoospores (Karling, 1942; Ibelings et al., 2004) and comprise both saprotrophs and parasites (Perrott, 1960; Sparrow, 1960). An increasing number of studies reveal that parasitic chytrids are ubiquitous and play an important role in aquatic ecosystems (Gleason et al., 2008, Kagami et al., 2014, Frenken et al., 2017, Ittner et al., 2018, Van den Wyngaert et al., 2022). Parasitic chytrids infect almost all major groups of phytoplankton (Sun et al., 2017, Haraldsson et al., 2018, Rad-Menéndez et al., 2018, Van den Wyngaert et al. 2018), for instance diatoms (Garvetto et al., 2019), green algae (Jeon et al., 2018; Strittmatter et al., 2020), dinoflagellates (Fernández-Valero et al., 2022), and cyanobacteria (McKindles et al., 2020). Chytrid infections are often highly host-specific even at the genotype level (Ibelings et al. 2004; Agha et al., 2018) and usually infections kill the host cell. They have the potential to end toxic harmful cyanobacterial blooms (Haraldsson et al., 2018) by infecting and killing in some instances > 90% of the entire cyanobacterial population (Rasconi et al., 2012). Filamentous phytoplankton like certain cyanobacteria, such as *Planktothrix* species, are considered low-quality food sources for grazers and are hard to ingest due to their long filamentous morphology. Interactions with chytrids, however, may lead to fragmentation of the long cyanobacterial filaments into shorter cell-chains that fall into the grazers' prey size range (Frenken et al., 2020a, 2020b). Moreover, chytrid zoospores due to their small body size and nutritious composition (Gerphagnon et al., 2019) can serve as a good food source for grazers (Kagami et al., 2005; Frenken et al., 2020a, 2020b). In this way, chytrid interactions may render blooms of "inedible", large cyanobacteria available to grazers and provide an additional trophic pathway for the efficient transfer of energy and organic matter to higher trophic levels (Thongthaisong et al., 2022). Moreover, chytrid infection poses highly specific selection pressures on host strains, through which genetic diversity of the host species can be maintained via succession of the host strains during chytrid infection (Gsell et al., 2013).

Dolichospermum is the new genus name for most of the planktonic forms of the genus *Anabaena* (Wacklin et al., 2009). *Dolichospermum*/*Anabaena* species, as well as other cyanobacteria form harmful algae blooms worldwide (Chernova et al., 2017; Huisman et al., 2018; Wan et al., 2019; Aubriot et al., 2020). This causes serious environmental and public health issues, for example, loss of aquatic biodiversity (Watson et al., 2015), animal kills (Wang et al., 2021), drinking water contamination (Clark et al., 2017) and human illness (Trevino-Garrison et al., 2015). The level of cyanobacteria bloom-induced deterioration of aquatic ecosystems resulting from eutrophication together with global warming has greatly increased (Paerl, 2018). Species of the *Dolichospermum* genus are generally filamentous organisms, some of which are capable of forming differentiated cell types under selecting environmental conditions. These comprise—in addition to standard vegetative cells—akinetes (Li et al., 1997), which serve as resting stages when environmental conditions turn unfavorable for growth and heterocysts, differentiated from vegetative cells, which are capable of dinitrogen fixation during nitrogen starvation (Haselkorn, 1978). Chytrid infections associated with

all three cell types have been observed, whereby two chytrid species were identified based on morphological observations, i.e., *Rhizosiphon crassum* Scherff. (Canter & Lund, 1951; Gerphagnon et al., 2013a, 2013b) growing on both vegetative cells and akinetes, and *Rhizosiphon akinetum* Canter (1954) infecting solely akinetes. Chytrid infections of heterocysts of *Anabaena smithii* (Komárek) M.Watan have also been documented (Takano et al., 2008). In 1963, Canter described and identified a chytrid infecting heterocysts of *Aphanizomenon* (Canter, 1963). However, since none of these chytrids have been isolated, phylogenetic characterization linked to the historical morphology-based identification could not be supported by DNA-sequencing methods.

In this study, we report on a newly isolated parasitic chytrid which specifically infects the heterocysts of members of the *Dolichospermum* genus. We used light and fluorescence microscopy to characterize the morphological features of the chytrid and sequenced the large subunit 18S rDNA (28S) to infer its phylogenetic position. We quantified the prevalence of infection for different chytrids on *Dolichospermum* species during a field campaign in Lake Stechlin (Germany). Furthermore, we compared in detail the morphology of this novel chytrid with Canter's discovery from 1963, to support our conclusion that this chytrid is indeed a so far undiscovered parasitic fungus which is new to science. To our knowledge, this study is the first to report on the isolation, and morphological and phylogenetic characterization of this novel *Dolichospermum* heterocyst-infecting chytrid, which we were able to stably maintain under defined laboratory conditions.

Materials and methods

Host and chytrid isolation and cultivation

From September to October 2018, phytoplankton for isolation and cultivation of the cyanobacterial host and its chytrid parasites was sampled in Lake Stechlin, a eutrophied, oligo-mesotrophic lake in North-East Germany (53°10′N, 13°02′E). Sampling was conducted biweekly with 18 samples obtained in total. Concentrated plankton samples were collected using a phytoplankton net (mesh size: 10 µm, diameter: 25 cm) from 15 m depth to the surface to also include the deep chlorophyll maximum (DCM) where cyanobacteria were most abundant in Lake Stechlin. Forty ml of the plankton samples were fixed with 0.8-mL glutaraldehyde (0.5–1% final concentration) for quantitative analysis and the rest of the unfixed samples were used for host and chytrid isolation.

Isolation of the cyanobacterial host and its chytrid parasites was performed with the unfixed plankton samples. First, host cyanobacteria were isolated by picking individual filaments using an elongated glass pipette (10 µm in diameter). Following the protocol of(Van den Wyngaert et al., 2017), picked filaments were washed three times in 0.2-µL filtered MilliQ water droplets before transferring them into 24-well plates containing 1 mL of selected growth medium in each well. Different growth media including Z8, Chu-10, WC and Z media were selected and tested for culturing cyanobacteria of the genus *Dolichospermum*. Different *Dolichospermum* morphotypes, both straight and coiled, were picked repeatedly from fresh Lake Stechlin plankton samples for the purpose of establishing both stable cyanobacterial hosts and host-chytrid parasite co-cultures.

Chytrid isolates were obtained by picking infected *Dolichospermum* filaments using the same method as for the cyanobacterial host, but this time picking chytrid infected filaments which were as above transferred into 24-well plates containing 1 mL of each selected medium and a selection of different host filaments to maximize the likelihood that among the host strains one would be suitable for infection by the chytrid, see (Ibelings et al., 2004). After obtaining a stable host culture, infected filaments were then 3 times washed and transferred into fresh 24-well plates filled with fresh host cultures. After successful infection, the chytrid was transferred into 250-mL flasks containing 100 mL of the stable cyanobacterial host culture. From here on the preferred growth medium, modified Z8, without a nitrogen source was used. Polyclonal co-cultures of host-parasites were maintained by weekly transfers of 1 mL of the infected culture into flasks with 100 mL of fresh cyanobacterial host culture. Both cyanobacteria host alone and in co-culture with the chytrid were incubated and maintained at 22 °C and a 16-h/8-h light/dark cycle with a light intensity of 59 µmol photons m^{-2} s^{-1}.

Light and fluorescence microscopy

Suspensions of a highly concentrated host-chytrid co-culture were stained with calcofluor white (with a final concentration of 0.5–1%, Sigma-Aldrich, Switzerland) and incubated in the dark for 5 min before microscopic analysis (Gerphagnon et al., 2013a, 2013b). Stained samples were then mounted on glass slides and different life stages of the chytrid parasite were examined using an Olympus BX61 microscope (Olympus, Switzerland) with 100×, 200×, 400×, and 1000× magnifications. An Olympus XS30 (Olympus, Switzerland) camera was used to take micrographs of both chytrid sporangia and zoospores.

Host dynamics and prevalence of parasite infection quantification

Host dynamics and prevalence of chytrid infections were studied on net-concentrated plankton samples from the lake. For host dynamics, concentrated plankton samples were diluted 5 times for counting due to the high density of target cyanobacteria. One mL of a diluted plankton sample was pipetted into a counting chamber (Malassez, Germany), and different morphological types of *Dolichospermum* host filaments were counted and recorded separately (straight and coiled *Dolichospermum*, respectively) using an Olympus BX61 microscope. Fifty filaments per types were measured to obtain the average length and diameter of the different morphological types of host filaments. Concentration factors were calculated by dividing the total volume of the water sample filtered by the final sample volume collected through the phytoplankton net (Scofield et al., 2004). Volume of the total water sample filtered by the phytoplankton net was calculated as follows:

$$V = \pi r^2 h,$$

where, V = volume of the water sample filtered (L), r = radius of the phytoplankton net (m), h = sampling depth (m).

Population abundance was represented by the biovolume of all filaments counted (counting chamber) and calculated by the cylinder volume equation (filaments were considered as cylinders), where r and h are the radius and length of the filament, respectively.

For quantification of the prevalence of chytrid infection, concentrated plankton samples were used and prepared for counting as described above. A minimum of 100 *Dolichospermum* filaments were counted for each sample and the infected filaments were recorded separately for host morphotypes (straight and coiled) as well as per host cell type (vegetative cells, akinetes, and heterocysts).

Prevalence of infection, calculated as the proportion of infected filaments over the total number of filaments, was assessed at the overall population level (straight + coiled filament types combined), at the level of individual morphotypes (straight and coiled filament type, separately), as well as for the different cell types, vegetative, akinete and heterocyst separately.

DNA extraction, sequencing, and phylogenetic analysis

Two mL of a dense *Dolichospermum*-chytrid co-culture was centrifuged at 10,000 g for 30 min. The supernatant was discarded, and DNA was extracted from the remaining pellet using a slightly modified Hot-SHOT extraction method based on (Ishida et al., 2015). Briefly, 20-µL alkaline lysis buffer was added to the pellet and heated at 95 °C for 10 min. After cooling down on ice for 5 min, 20 µL of neutralizing buffer was added. The large subunit 18S rDNA gene of the chytrid isolate was amplified with primers LR0R-LR5, with sequences 5′-GTACCCGCTGAA CTTAAGC-3′ for LR0R and 5′-ATCCTGAGGGAA ACTTC-3′ for LR5, respectively (Vilgalys & Hester, 1990; Rehner & Samuels, 1994), using MyTaq Red DNA Polymerase (BIOLINE, Germany) with the following amplification conditions: 94 °C heating for 2 min, 32 cycles at 94 °C heating for 15 s, 53 °C heating for 15 s, 72 °C for 30 s, and a final extension step at 72 °C for 5 min (Van den Wyngaert et al., 2017). The PCR product was sequenced at Macrogen Europe using Sanger sequencing. Sequences were assembled using BioEdit (Hall et al., 2011) and deposited in GenBank (Accession number: PV714792).

To clarify the phylogenetic position of the chytrid isolate Dol-Heterocyst-01, a phylogenetic analysis was conducted using a dataset comprising 28S rDNA

sequences. In addition, we included three nanopore-generated long-read sequences of uncultured Lobulomycetales isolates which showed highest sequence similarity (and best BLAST hits) to our chytrid isolate. Sequences were aligned using MAFFT v7.487 (Katoh & Standley, 2013). 28S rDNA sequences were first aligned separately and the long-read sequences were added into the reference alignment using MAFFT with the "–addlong" option. The alignment was trimmed using trimAl (Capella-Gutiérrez et al., 2009) with the "gappyout" method. A maximum likelihood (ML) tree was inferred with IQ-TREE 2 (Minh et al., 2020). The best model of the alignment was examined using ModelFinder (Kalyaanamoorthy et al., 2017) implemented in IQ-TREE 2. According to the corrected Akaike information criterion (AICc), TN + F + I + G4 was the best-fit model. The tree was visualized with FigTree (https://github.com/rambaut/figtree) and edited with inkscape (https://www.inkscape.org).

Results

Cyanobacteria host and chytrid isolation and cultivation

Dolichospermum host strains were isolated from fresh plankton samples of Lake Stechlin. Initially, both types of *Dolichospermum* strains were isolated from the lake plankton samples indicating two different host strain morphotypes, i.e., a coiled and a straight type (Fig. 1A, B). Only, the *Dolichospermum* straight type could be successfully maintained under lab conditions, using a modified Z8 medium without any nitrogen source. Stably growing, straight *Dolichospermum* colonies were used to isolate the chytrid in the laboratory.

Chytrid infection on different cell types of two *Dolichospermum* host morphotypes were observed in the natural lake samples (Fig. 1C–H); however, only the chytrid infecting heterocysts of the straight *Dolichospermum* type (Fig. 1B) was successfully isolated and maintained stably under laboratory conditions. Nitrogen-free Z8 medium was used (Appendix—Supplementary Material 1) to stimulate heterocyst formation by the host filaments.

Morphology of chytrid life cycle stages

Zoospores of the chytrid strain (named Dol-Heterocyst-01) infecting the heterocysts of the straight *Dolichospermum* type are spherical, 6.15 ± 0.93 µm in diameter, and characterized by a single flagellum (Fig. 2A–D). Zoospores emerge from a single large lateral opening. Within the family Lobulomycetales—see below—there are both inoperculate and operculate species. In some species the operculum can also become easily detached and therefore difficult to observe (Fig. 2E–H). After release from the sporangium, zoospores become active and start to swim irregularly away from the sporangium to search, engage with and infect a new host cell. Once a host filament is detected, zoospores are repeatedly circling around the target, until final attachment to the host's cell surface. After attachment, the zoospore gradually grows into a mature sporangium, a spherical structure that produces new zoospores. After reaching maturation, sporangia break-open and release the new zoospores. Figure 3 shows the different life cycle stages of the chytrid.

Phylogeny of the isolated chytrid

The 28S rDNA sequence obtained from the isolated chytrid strain (Dol-Heterocyst-01) showed highest identity (87% sequence similarity) to an uncultured fungus, isolated from soil from Kungsängen Nature Reserve (Sweden) and three uncultured Lobulomycetales fungi, isolated from algal hosts *Spirogyra sp.* and *Melosira varians* and an unidentified heliozoan. The maximum likelihood (ML) tree based on 28S rDNA positions Dol-Heterocyst-01 within the order Lobulomycetales where it represents a novel lineage within a clade that includes uncultured Lobulomycetales parasites of algae and heliozoa (Fig. 4).

Lake Stechlin host dynamics and prevalence of infection

Throughout the sampling period, the *Dolichospermum* biovolume was decreasing, after a peak was already reached at the beginning of the sampling campaign in August 2018 (Fig. 5A: Overall). Coiled type *Dolichospermum* dominated during the sampling campaign, decreasing gradually toward a level that was, however, still higher than the straight

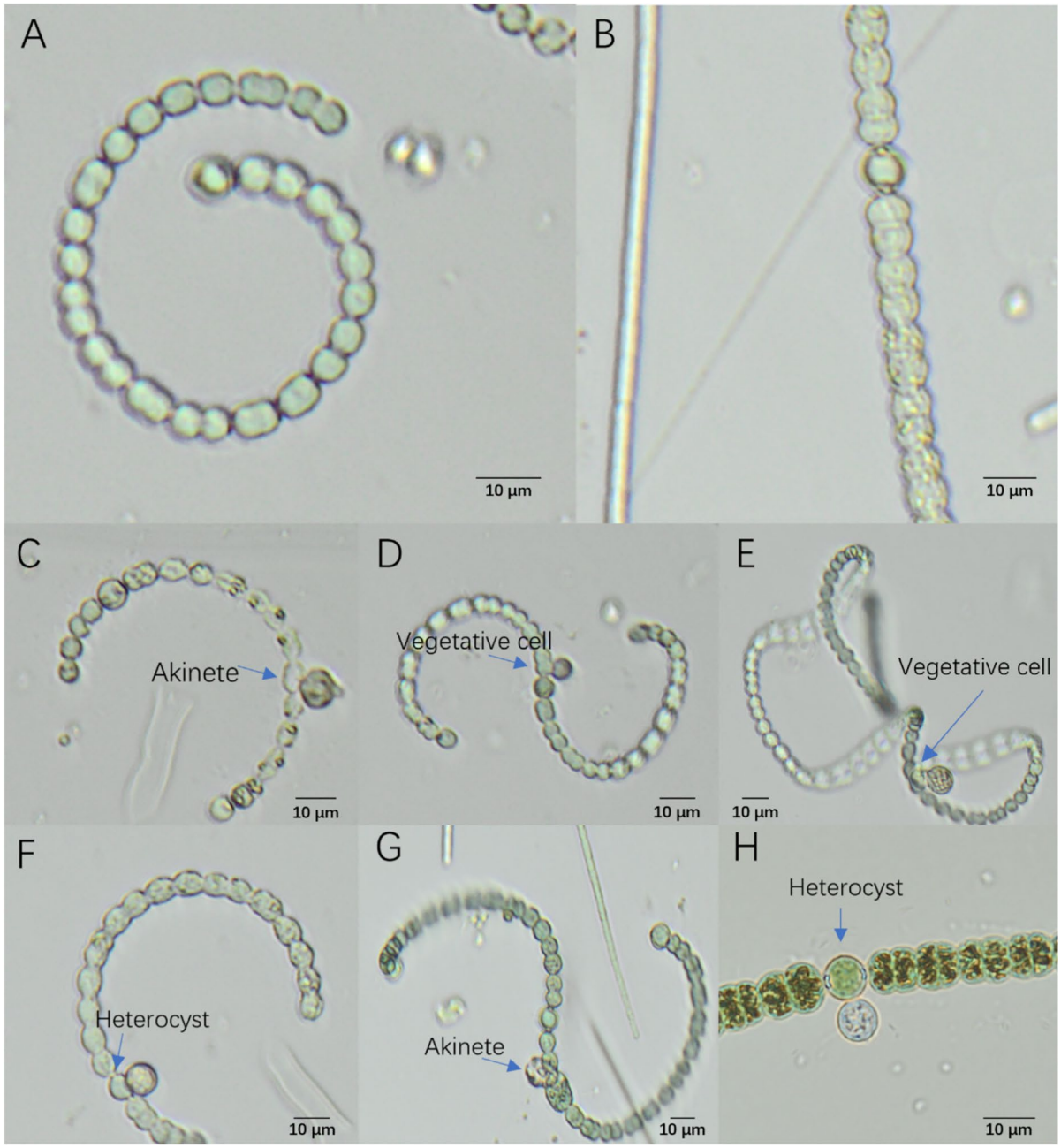

Fig. 1 Light microscopy of the two *Dolichospermum* morphotypes and their associated chytrid species. **A**, **B** Coiled and straight *Dolichospermum*, respectively. **C–G** Chytrids infecting different cell types of *Dolichospermum*. **H** Chytrid infecting a heterocyst of the straight *Dolichospermum* type. Scale bar: 10 µm

Dolichospermum type (Fig. 5A: Straight type and Coiled type, respectively).

Microscopic cell counts revealed that the coiled *Dolichospermum* type dominated in all samples, accounting for > 75% of the total number of *Dolichospermum* filaments. Overall, the filament ratio between the straight to coiled type was varying between 0.05 and 0.23, with the highest ratio observed in the sample collected on 18/10/2018 (Fig. 5B).

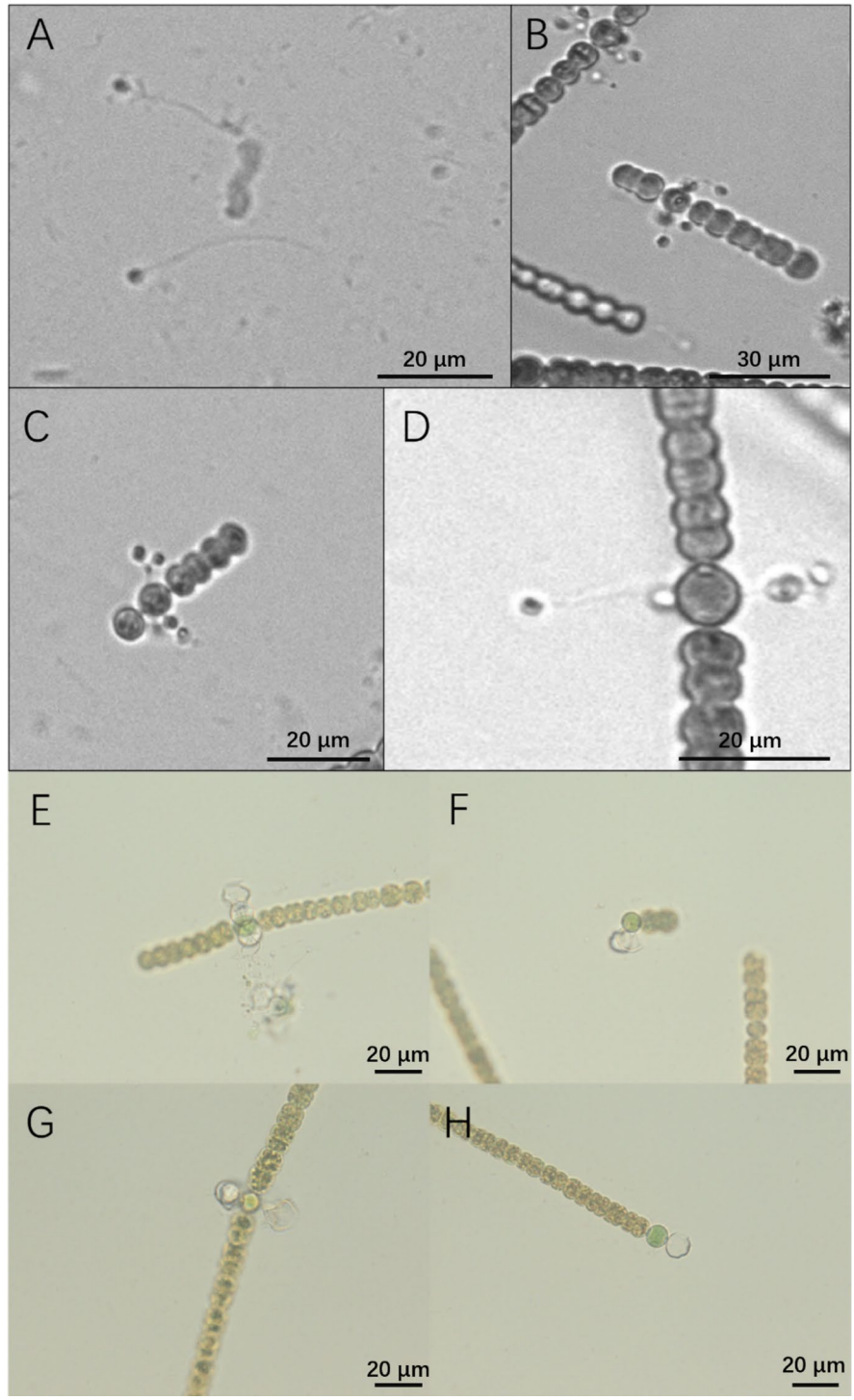

Fig. 2 Zoospores and opening position on sporangia upon zoospore discharge. **A**–**D** Zoospores and attachment to host cells. **E**–**H** Single opening at different locations of the sporangia for zoospore discharge. Scale bar: 20 µm

Overall, prevalence of chytrid infection at the filament level for the *Dolichospermum* population was consistently below 4% throughout the entire sampling period, with a maximum of 3.7% at the beginning of the sampling campaign when host filament number were highest (Fig. 6A: Overall). At this time prevalence of infection at the

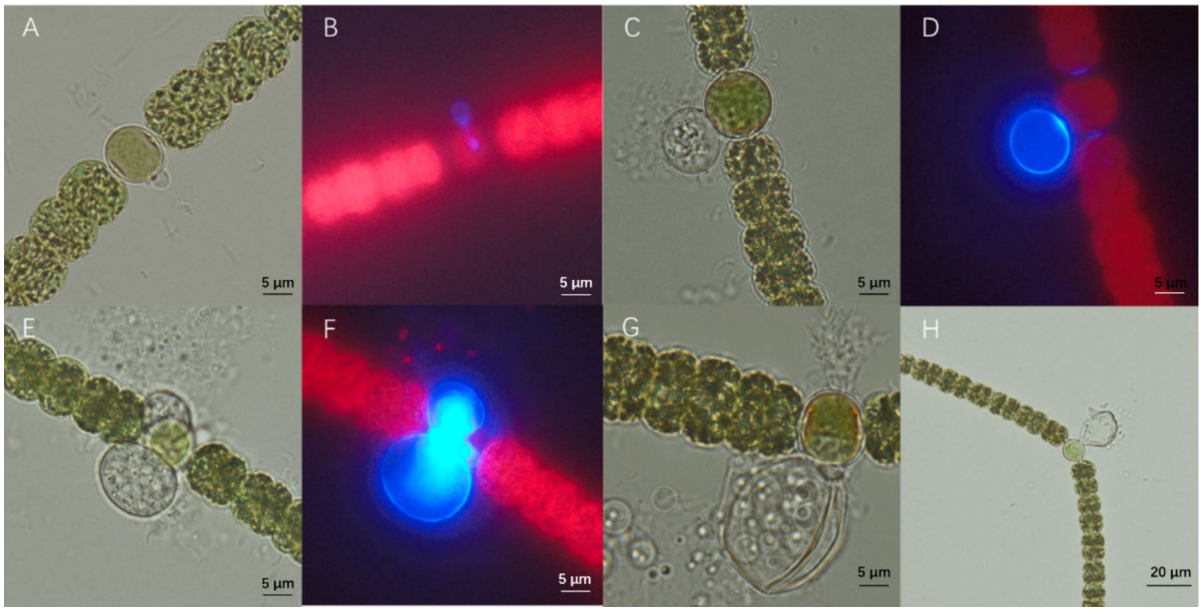

Fig. 3 Light and fluorescence microscopy of the life cycle stages of the isolated chytrid (with and without Calcofluor White staining). **A**, **B** zoospores attachment, **C**, **D** immature sporangium, **E**, **F** mature sporangia, **G** zoospore release, and **H** empty sporangium. **A**, **C**, **E**, **G**, **H** light microscopy; **B**, **D**, **F** fluorescence microscopy with Calcofluor White staining, excitation and emission at 405 and 435 nm, respectively. Scale bar: 5 µm for **A**–**G** and 20 µm for **H**

overall population level was higher for the dominant coiled than for the sub-dominant straight host morphotypes.

Prevalence of infection at the filament level for the coiled host type, which accounts for 75% of the entire host population, followed a similar trend as the prevalence of infection for the overall host population (Fig. 6A: Coiled type). Infection prevalence for the straight morphotype was low, given the small abundance of these straight filaments in the overall host population.

Figure 6B shows the prevalence of chytrid infection within the individual subtypes of the *Dolichospermum* host, straight and coiled. Prevalence of chytrid infection on the straight type host was never above 1% of the total (see Fig. 6A), but when expressing infection prevalence just on basis of the straight host subtype only, infection clearly exceeded the maximum level for the dominant coiled type and peaked at 8.8% in late September, compared to nearly 4% for the coiled subtype in late August.

Furthermore, the prevalence of chytrid infection was determined for the different *Dolichospermum* cell types, i.e., vegetative cells, heterocysts, and akinetes, once again at both overall population and individual morphotype levels. Overall, at the population level, vegetative cells showed the highest level of chytrid infections, with a maximum percentage of 2.5% of all vegetative cells being infected at the beginning of the sampling period (Table 1: Overall population). At that time, vegetative cells accounted for > 50% of the total chytrid infection detected in the plankton sample (Fig. 7). Chytrid infections on akinetes only occurred for a short period at the beginning of the sampling period. In contrast, chytrid infection on the heterocyst cells occurred throughout the entire sampling period, but remained below 0.5% for most of the sampling points, except for 1.12% at the first sampling day (Table 1: Overall population, H). Yet, on two sampling days in October 100% of the observed infections was of heterocysts only. As before straight morphotypes were infected more than coiled ones, at least for vegetative cells and heterocysts. Whereas prevalence of infection of the akinetes was always very low, none of the akinetes of straight filaments were infected, only a few in coiled ones.

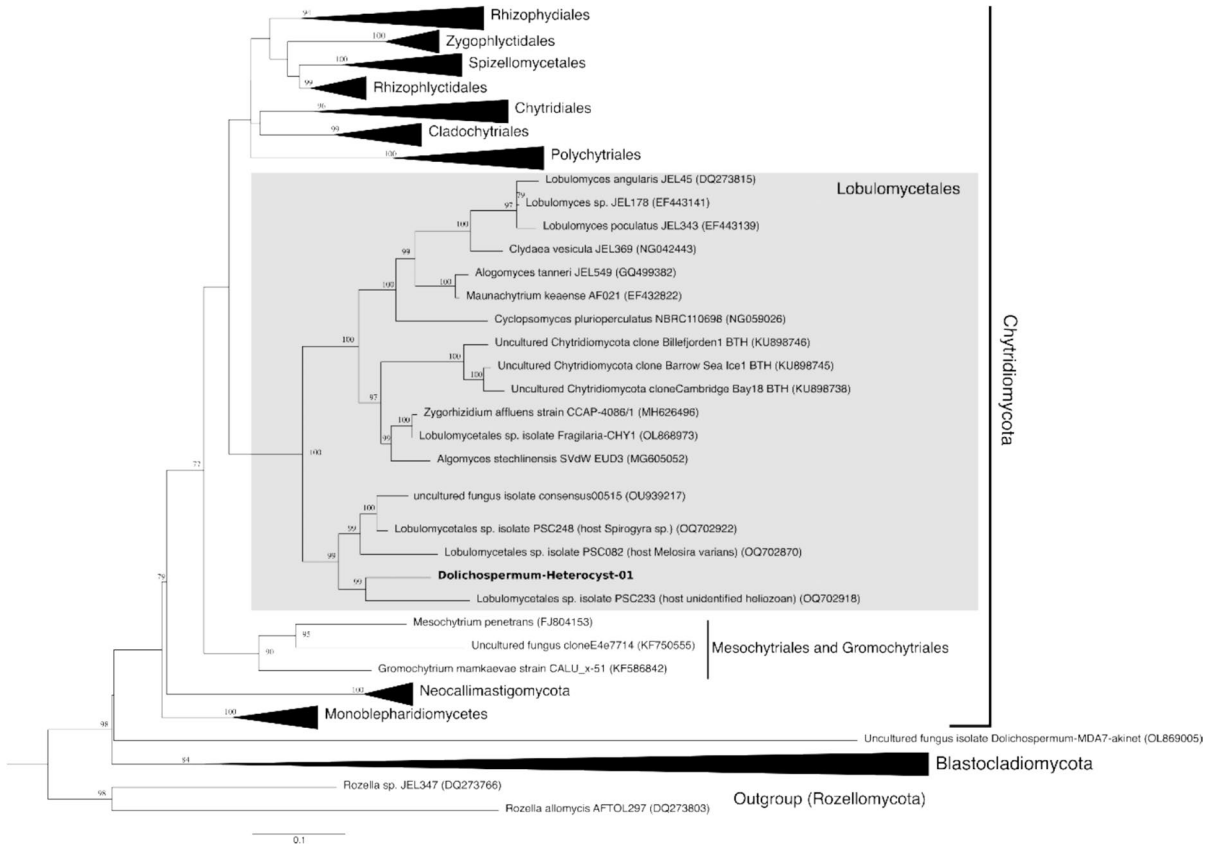

Fig. 4 Maximum likelihood tree of cultured and environmental sequences of *Chytridiomycota* using 28S rDNA sequences showing the position of Dol-Heterocyst-01 within the order *Lobulomycetales*. *Rozellomycota* was selected as an outgroup. GenBank accession numbers are given in parenthesis. Only bootstrap support ≥ 70% is shown

Discussion

Morphological and molecular identification

In 1963, Canter (1963) described a chytrid infecting the heterocyst of *Aphanizomenon flos-aquae* (L.) Ralfs, named *Chytridium cornutum*Braun, which in morphology shares a great deal of similarities with the chytrid that we describe here (Appendix—Supplementary Material 2). However, these two chytrids do not match exactly: both chytrids infect heterocysts, except their targeted host species are different: *Aphanizomenon* for *Chytridum cornutum* and *Dolichospermum* for the new chytrid described here (Appendix—Supplementary Material 3). Our chytrid isolate has been tested with other *Dolichospermum* and *Anabaena* species, and it can only infect the straight *Dolichospermum* host type. Indeed, it is known that even for one specific host x chytrid species pair there are always non-matching couples at the strain level (Ibelings et al., 2004). Our conclusion whether we describe a new to science chytrid here remains inconclusive, as we did not perform any infection test with *Aphanizomenon flos-aquae* and there are no sequencing data for *Chytridium cornutum*. Formation of the flattened apex with a small lid of the sporangia (observed in *Chytridium cornutum*) was never found in the sporangia of the newly isolated chytrid, and the discharge of zoospores is more of a bursting process (i.e., all zoospores are released together rather than what Canter described in her work: "emerging one by one through the apex." (3) For *Chytridium cornutum*, the sporangium's cell wall does not collapse after zoospore discharge, whereas for the chytrid we found here, the sporangium's cell wall collapses after zoospore release (Fig. 3H). No "bubble-like"

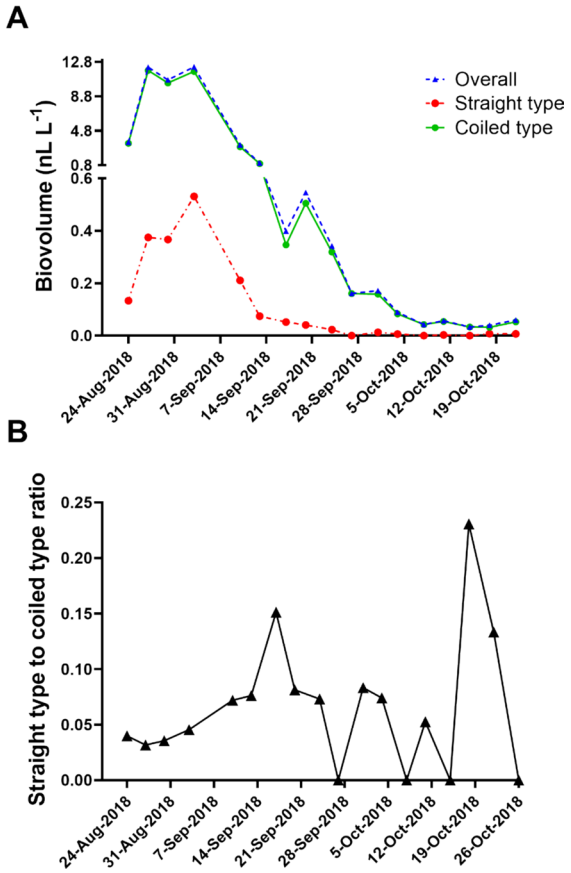

Fig. 5 *Dolichospermum* succession. **A** Morphotype succession on biovolume, blue-dotted line for overall population, red-dotted line for the straight type, and green line for the coiled type. **B** Ratio of straight to coiled type of host filaments

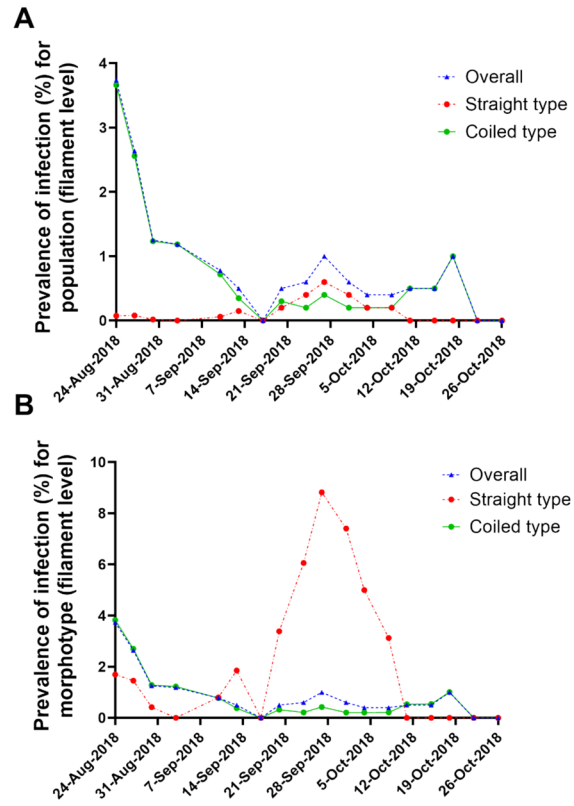

Fig. 6 Prevalence of infection at the filament level for overall population and individual morphotypes. **A** Prevalence expressed for the whole population. **B** Prevalence expressed per individual host morphotypes. Blue-dotted line: Overall population. Red-dotted line: Straight type. Green line: Coiled type

structure inside the infected heterocyst cell was found for the new chytrid (Fig. 3C, D) compared to Canter's chytrid. (5) *Chytridium cornutum* has a sexual reproduction process which results in the formation of resting spores; however, for the new chytrid, sexual reproduction stages were never found.

Phylogeny

Phylogenetic analysis places Dol-Heterocyst-01 within the order Lobulomycetales marking it the first discovered cyanobacteria parasite in this order. Lobulomycetales was described in 2009 as a new order (Simmons et al., 2009) within the phylum Chytridiomycota, at that time, it was one of the smallest orders which included four genera and five species. More species were discovered and identified later. In 2015, one additional chytrid species discovered from soil was identified and included in this order (Seto & Degawa, 2015). In 2018, several parasitic species of diatoms and green algae were included and formed a novel clade SW-I sensu Van den Wyngaert et al. (2018). Recently, Seto and colleagues (2023) identified an additional clade with novel parasite lineages within Lobulomycetales and our strain clustered within this group, being most closely affiliated with a parasite isolated from an unidentified heliozoan. This is the first discovery of a cyanobacteria parasite within the order Lobulomycetales. Broader taxon sampling that is more isolates and inclusion of data on the zoospore ultrastructure are needed to confirm if its taxonomic position needs the erection of a new order or whether it presents a novel clade within the order Lobulomycetales. Comparison with other

Table 1 Prevalence of infection range for three cell types (H: heterocyst, V: vegetative cell, A: akinete) at both overall population and individual morphotype, straight and coiled, levels

Prevalence of infection range (%)								
Overall population			Straight type			Coiled type		
H	V	A	H	V	A	H	V	A
0.07–1.12	0.2–2.47	0.02–0.29	0.27–3.7	0.54–8.82	0	0.07–1.13	0.21–2.54	0.02–0.31

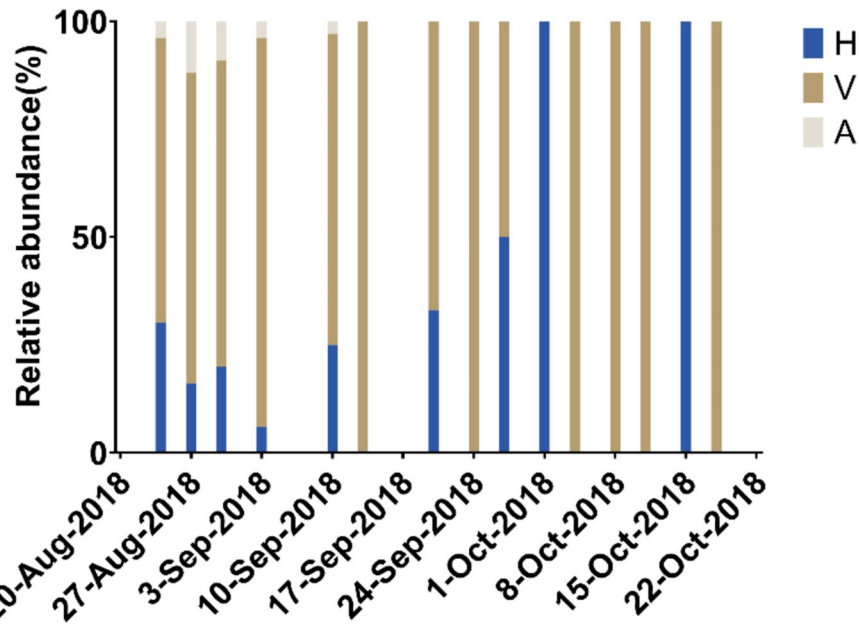

Fig. 7 Relative abundance (%) of chytrid infection on different cell types at overall population level. H: heterocyst, V: vegetative cell, A: akinete

chytrid species infecting *Dolichospermum* identified by (Van den Wyngaert et al., 2022) showed that Dol-Heterocyst-01 is not related to any of the previously known and described zoosporic fungi that are associated with akinetes and vegetative cells of *Dolichospermum*.

Sequencing results of Dol-Heterocyst-01 were also compared with two single-cell sequences of heterocyst-associated fungi of species from the genus *Aphanizomenon* (Appendix—Supplementary Material 4) and *Dolichospermum* (isolated from Lake Stechlin in August 2017 and August 18, respectively). Sequencing similarity was found at a level of 99% between these two heterocyst-associated fungi, which shows a very high possibility of these being closely related species or different strains of the same species. However, both are very different from Dol-Heterocyst-01, with 83% sequencing similarity. These two additional heterocyst-associated sequences showed the highest identity with an uncultured *Rozellomycota* sequence that was isolated from a single cell of the diatom *Aulacoseira* (Kagami et al., 2021). Since *Rozellomycota* species have so far only been described as parasites of oomycetes or other fungi like chytrids, the sequences are likely to represent a hyperparasite of those parasitic chytrids that are infecting heterocysts of cyanobacteria. Hyperparasitism may be another mechanism of infection control on cyanobacterial or algal hosts and may help to explain interannual variability of infection prevalence within and between lakes. The underexplored diversity and complexity of the phytoplankton-associated zoosporic fungal community awaits further evaluation.

Host growth dynamic and prevalence of infection

In the early twenty-first century, Lake Stechlin experienced a rapid invasion of heterocystous cyanobacteria, including a number of species from *Dolichospermum* and *Apanizomenon* (Selmeczy et al., 2016). In the recent decade, Lake Stechlin has a general annual phytoplankton distribution pattern of either unimodal, one peak in spring, or bimodal, with a second, lower biomass peak in late summer following the spring peak. More recently, the bimodal pattern has turned to form a higher peak in summer with cyanobacteria as the dominant group different from the previously observed diatom spring peak. This change in phytoplankton dynamics is presumably linked to the shift in the lake's trophic state from formerly oligo-mesotrophic to currently eutrophic. Over the entire sampling period in late summer 2018, growth of both *Dolichospermum* morphotypes—coiled and straight—were decreasing, and the only period of population growth occurred already in the first two weeks of the sampling campaign. Two peaks were observed, one on August 24, 2018 and on September 03, 2018. Comparison of Fig. 6A and B revealed that the coiled *Dolichospermum* type dominated. This is in line with a survey of the *Dolichospermum* development in late summer 2011 in Lake Stechlin, where the coiled *Dolichospermum* type was dominant (Dadheech et al., 2014). A similar outcome was also reported by (Weisbrod et al., 2020), who studied the short-term bloom dynamics of *Dolichospermum* in an oligo- to mesotrophic reservoir in southwest Germany and found that the coiled type was dominant during the later summer bloom, when heavy chytrid infection (close to 50% prevalence) led to a decrease of the straight *Dolichospermum* type. Chytrid infection could also be a driving factor in Lake Stechlin as we found that compared to the coiled *Dolichospermum* type, more of the straight host type was infected by the chytrid, yet still at low to at best moderate infection levels (Naselli-Flores et al., 2021). Although prevalence of infection in Lake Stechlin was much lower than in the other German lake, even a low amount of parasite infection can exert additional pressure on the straight morphotype, thereby weakening its competitiveness in comparison to the dominant coiled one. Weisbrod, Riehle et al. link reduced competitiveness also to enhanced production of bioactive compounds, including microcystin, by the highly infected *Dolichospermum* morphotypes (Weisbrod et al., 2020).

Prevalence of chytrid infection for all three cell types was measured directly by counting the infected filaments. Most of the chytrid infections were found on the coiled host type. However, the overall maximum infection rate was low (3.7%) during the sampling campaign, which is very different from a previous study with the same host—but a different chytrid—that revealed over 90% of the *Dolichospermum*/*Anabaena* population can be infected in Lake Aydat (Rasconi et al., 2012). Later studies on the same lake, e.g., (Gerphagnon et al., 2017) did not find such high infection levels, while Wierenga and Ibelings (unpublished data) failed to find any chytrid infections despite dense *Dolichospermum* blooms in Lake Aydat (France). Obviously host density is a key factor for parasite success (Ibelings et al., 2004), but environmental factors modulate this. Nutrients are among the factors that can change the infection dynamics (Bruning & Ringelberg, 1987). Lake Stechlin is a recently eutrophied oligo-mesotrophic lake, but epilimnetic nutrient levels may still not be high enough to support a dense cyanobacteria bloom in the epilimnion, resulting in a low density of the host, making it difficult for zoospores to find their host cells during their infective lifetime. Other factors such as light and temperature can also influence chytrid infection rates by affecting both chytrid and phytoplankton host development (Bruning, 1991a, 1991b, 1991c). Ibelings et al. (2011) studying long-term trends in chytrid infection of the diatom *Asterionella formosa* Hassall in Lake Maarsseveen, The Netherlands, highlight the complexity of unraveling the disease triangle between host, parasite, and a changing lake environment.

Within the population, more straight host morphotypes were infected, even though the coiled host morphotype dominated. This indicates that straight filaments are somehow more sensitive to chytrid infection, for which we have no good explanation at present. Most infections were found on vegetative cells. Chytrid infections on heterocyst cells were the next frequently observed type, while chytrids infecting akinetes were rarely found. A likely explanation could simply be that there are more vegetative cells compared to heterocysts and those compared to akinetes, resulting in more frequent infections of vegetative cells, followed by heterocysts. Density of

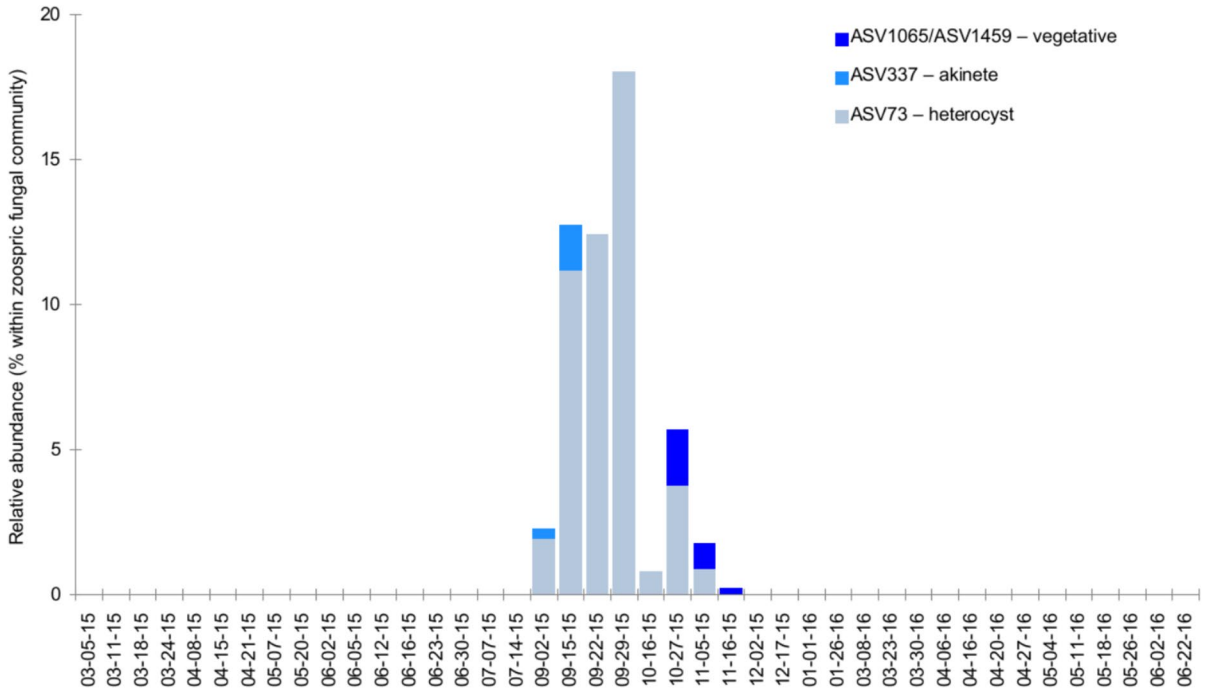

Fig. 8 Relative abundance of parasitic chytrids (Amplicon Sequence Variants, ASV) associated with different cell types of *Dolichospermum* in Lake Stechlin, 2015–2016

heterocysts was maximal two per filament, while for akinetes were less than 1 per filament. Moreover, akinetes were observed only at the beginning of the sampling period, indicating that in August conditions for *Dolichospermum* in Lake Stechlin were sub-optimal, possibly caused by low nutrient levels or unfavorably high temperatures. The study from (Gerphagnon et al., 2017) discussed how infection of *Dolichospermum macrosporum* (Kleb.) Wacklin, L.Hoffm. & Komárek by the *Rhizosiphon crassum* peaked at 98% in 2007, whereas a few years later this chytrid was fully replaced by *Rhizosiphon akinetum*, which was observed to infect as much as 47% of the akinetes in the host population. Over time, this shift in parasitism was coupled to a shift in the peak period for *Dolichospermum* infection, from the end of August to the end of October. Additionally, bloom duration was decreased from one month to one week, narrowing the window for successful infection. Taking out akinetes at a large scale through chytrid infection clearly reduces the size of the overwintering *Dolichospermum* population and therewith the size of the inoculum for the new cyanobacterium bloom. The (Gerphagnon et al., 2017) study underlines how interlinked shifts in size of the host bloom, the nature and intensity of infection, and—unavoidable—differences in environmental conditions between years all play out to determine interannual cyanobacterium chytrid dynamics in a lake. This is also supported by comparing our 2018 data with the occurrence of *Dolichospermum* chytrids associated with the different *Dolichospermum* cell types in a seasonal dataset from 2015 to 2016 of Lake Stechlin (Van den Wyngaert et al., 2022), in Fig. 8. Despite much higher host abundance of *Dolichospermum* in summer, chytrids associated with different cell types were only present during the autumn bloom (same period as in this study 2018), with the highest relative abundance reached by heterocyst-associated chytrids, which accounted for up to 20% of the total zoosporic fungal community.

Potential ecological consequences of the newly isolated chytrid

Control of harmful algae blooms

Certain cyanobacteria species produce heterocysts to perform nitrogen fixation, supporting their growth in a nitrogen deficient environment. Vegetative cell growth of N_2-fixing cyanobacteria thus depends on the nitrogen supply from the heterocysts in the filament. In oligotrophic lakes, despite the low nitrogen and phosphorus nutrient status (two key resources promoting cyanobacteria blooms when availability is plentiful), harmful algal blooms (including cyanobacteria) can still form, and this has been frequently reported in recent studies (Reinl et al., 2021). Many of the species found forming harmful algal blooms in oligotrophic lakes belong to N_2-fixing cyanobacterial genera, such as *Dolichospermum*/*Anabaena*. Generally, chytrid infection is lethal to the cyanobacteria host and has the potential to cease the bloom when prevalence of infection is high enough to offset cyanobacterial cell division (Harris et al., 2024). The chytrid we isolated and describe here infects *Dolichospermum* heterocysts, but it does not kill the host instantly, instead, it slowly deprives the host of N in absence of ammonium or nitrate in the environment. Infection arrests and finally seem to impede the heterocyst's ability to fix nitrogen. For the later infection stages, microscopic observations show that heterocysts can physically become disassociated from the vegetative cells in the filament. This risks to break away the heterocyst from the filament, so that the vegetative cells are robbed of their heterocyst nitrogen supply. Apparently chytrid infection weakens the connection between heterocysts and vegetative cells, causing fragmentation of cyanobacteria filaments. Without any N-input from the heterocysts, vegetative cells cannot maintain growth. Therefore, chytrid infections of heterocysts when sufficiently abundant could arrest the *Dolichospermum* bloom development.

Alteration trophic pathway

For a long time, cyanobacteria blooms have been considered as a trophic dead end, due to their colonial shape or long filament size, which are difficult to be consumed by grazers, and their toxicity (Jang et al., 2003; Wilson et al., 2006; Ger et al., 2016). Recent studies, however, show that grazers can feed on large cyanobacteria after being fragmented by chytrid infection, and on chytrid zoospores, which have proved to be a high-quality food for grazers (Rasconi et al., 2020) and which funnel resources from large inedible algae to readily grazable zoospores (Agha et al., 2016; Frenken et al., 2020a, 2020b). The chytrid we discussed here is also able to fragment the host filament (heterocysts-vegetative cell separation), which in theory could also enhance grazing capacity and contribute to food web energy transfer (Thongthaisong et al., 2022).

Additional nitrogen cycling

Seasonal stratification in lakes amplifies N-limitation toward the end of the growing season, as thermal stratification restricts vertical mixing and depletes surface-layer nutrients. This creates a critical window where heterocyst activity becomes essential for cyanobacterial survival and promotes the type of chytrid infections observed in our study. Climate change-driven intensification of lake stratification may further exacerbate seasonal N-limitation, favoring diazotrophic cyanobacteria and their associated chytrid parasites. This could amplify the ecological significance of heterocyst-targeting infections, with cascading effects on cyanobacterial bloom dynamics and nutrient cycling in eutrophic systems. As highlighted by Paerl (2018), eutrophication and climate warming are shifting cyanobacterial dominance from spring diatom blooms to summer cyanobacterial blooms, often dominated by N_2-fixers. This shift prolongs the window of N-limitation, increasing opportunities for chytrid–host interactions.

Nitrogen cycling is crucial to aquatic ecosystems. In the aquatic N-cycle, N2-fixation by cyanobacteria plays a significant role. For instance, in marine ecosystem, nitrogen fixation supplies around 50% of the bioavailable nitrogen needed (Wen et al., 2022). Chytrids infecting heterocysts have the potential to change the metabolism of the heterocysts, which might weaken the heterocysts' ability to fix nitrogen. This is supported by results from an experimental study, showing a near sevenfold decrease of nitrogen fixation by *Dolichospermum* upon infection; Xu et al., *submitted*. Infection with the new chytrid had no impact on host growth when N is supplied in the

growth medium, however without N-supply, forcing *Dolichospermum* to rely on fixing atmospheric dinitrogen the *Dolichospermum* culture collapses. Infection of heterocysts could move the fixed nitrogen in these specialized cells into chytrid zoospores and then transfer this from the benefit of grazers, a kin idea developed for the mycoloop (Kagami et al., 2007). Infection of heterocysts may also weaken filament integrity, causing dissociation of heterocysts from vegetative cells. This physical disconnection could increase the vulnerability of heterocysts (rich in fixed nitrogen) to grazing, even if direct infection of these cells is rare. Future studies should investigate whether heterocysts act as a supplementary nitrogen source for zooplankton following filament fragmentation.

Conclusion and future perspectives

In conclusion, based on our genetic analysis, the described chytrid most likely represents a newly discovered species, currently placed in the order *Lobulomycetales*, within the class *Chytridiomycetes* from the phylum *Chytridiomycota*. The new chytrid species shows differences in a number of morphological traits from earlier descriptions of the heterocyst-infecting chytrid species *C. cornutum* (Canter, 1963). However, further studies need to verify whether the new chytrid has the capacity to also infect heterocysts from the cyanobacterium *Aphanizomenon* spp. Other chytrid species that infect *Dolichospermum* species in Lake Stechlin were observed, unfortunately, their isolation was not successful. The discovery of a new chytrid that is able to infect a specialized cell, the heterocyst, which is so distinct of diazotrophic cyanobacteria, opens up new avenues for research that would further elucidate how changing environmental conditions, like eutrophication or re-oligotropication, interact with generalist or more specialized chytrids that infect bloom forming cyanobacteria. This is exciting given the global interest in cyanobacterial blooms. Also, the renewed observation that prevalence of infection is so distinct between filament morphotypes—straight vs coiled—offers the possibility to study and better understand which traits offer resistance against fungal infection. Likewise, a better comprehension of the impressive interannual variation between generalist—infecting vegetative cells—and specialist—infecting akinetes and heterocysts—chytrids may offer knowledge on host–parasite interactions that go beyond the specifics reported here. More in particular, further studies are required to illustrate how this chytrid and its host interact: (a) how does the chytrid detect and identify its host? (b) Which membrane structures are involved in the attachment phase? (c) What is the "feeding" structure of the sporangia when taking up nutrients from the host? (d) Which morphological and physiological changes does the heterocyst undergo while being infected? (e) Whether or not zoospores of this chytrid are a good food source for zooplankton, for example *Daphnia*, in particular providing nitrogen that was fixed by the cyanobacterial heterocysts?

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Author contributions XX wrote the original draft. HPG, BWI, and SVW supplied comments to the manuscript. XX and MK collected the samples. XX and SVW analyzed the samples.

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Data availability Data are available from the authors upon reasonable request. Sequence data is deposited in GenBank under the accession numbers PV714792-PV714795.

Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval Not applicable.

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