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REVIEW

Fischerella thermalis: a model organism to study thermophilic diazotrophy, photosynthesis and multicellularity in cyanobacteria

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Abstract

The true-branching cyanobacterium *Fischerella thermalis* (also known as *Mastigocladus laminosus*) is widely distributed in hot springs around the world. Morphologically, it has been described as early as 1837. However, its taxonomic placement remains controversial. *F. thermalis* belongs to the same genus as mesophilic *Fischerella* species but forms a monophyletic clade of thermophilic *Fischerella* strains and sequences from hot springs. Their recent divergence from freshwater or soil true-branching species and the ongoing process of specialization inside the thermal gradient make them an interesting evolutionary model to study. *F. thermalis* is one of the most complex prokaryotes. It forms a cellular network in which the main trichome and branches exchange metabolites and regulators via septal junctions. This species can adapt to a variety of environmental conditions, with its photosynthetic apparatus remaining active in a temperature range from 15 to 58 °C. Together with its nitrogen-fixing ability, this allows it to dominate in hot spring microbial mats and contribute significantly to the de novo carbon and nitrogen input. Here, we review the current knowledge on the taxonomy and distribution of *F. thermalis*, its morphological complexity, and its physiological adaptations to an extreme environment.

Keywords *Fischerella* · *Mastigocladus* · Distribution · Thermophile · Nitrogen fixation · Photosynthesis · Multicellularity · Hot springs

Introduction

Taxonomic controversy and worldwide distribution in hot springs

Cyanobacteria are photosynthetic bacteria. They are important primary producers, and some of them can also fix atmospheric dinitrogen (N_2). These bacteria are widely distributed in terrestrial and aquatic environments, including oceans, rivers, lakes, hot springs, soils and deserts (Whitton and Potts 2002; Sánchez-Baracaldo 2015). They show a high degree of morphological diversity ranging from unicellular to filamentous and filamentous-branching forms (Rippka et al. 1979). Cyanobacteria of all forms are crucial in microbial mats from hot springs, as they provide the main input of carbon and nitrogen up to temperatures of nearly 70 °C (Ward et al. 1998; Bolhuis et al. 2014; Alcamán et al. 2015; Thiel et al. 2017; Alcamán-Arias et al. 2018).

Thermal microbial mats with a near-neutral pH are often dominated by the filamentous true-branching cyanobacterium *Fischerella thermalis* (also known as *Mastigocladus*

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laminosus). Its complexity is evident not only in the presence of secondary trichomes but also in the formation of specialized cells. These are (1) heterocysts, terminally differentiated cells for nitrogen fixation; (2) akinetes, dormant cells resistant to cold and desiccation and (3) necridia, dead cells that function for the release of (4) hormogonia, thin motile filaments (Komárek 2013).

The history of morphological descriptions and taxonomical changes for this organism is depicted in Table 1 and can be divided into three main periods: (1) the long morphological paradigm with ambiguous descriptions in both *Fischerella* and *Mastigocladus* genera (1837–2008), (2) the phylogenetical definition of monophyletic species (2008–2017) and (3) genomic species definition (2017–today).

The organism was first described by Hofrath Schwabe in 1837 from a hot spring in Karlovy Vary (Carlsbad, Czech Republic) as *Mastigonema thermale*, although in the same monograph he also described *Fischera thermale* (Schwabe 1837). Later on, Ferdinand Cohn continued the studies on the diversity of the Karlovy Vary algae and defined the genus *Mastigocladus* (Cohn 1862). His early drawing already shows remarkably well the main morphological features common to true-branching cyanobacteria (Fig. 1a). However, at the time, it was difficult to recognize that the genera *Fischerella* and *Mastigocladus* were identical. Frémy (1930) even assumed that a form he collected from soil was the same as *F. thermalis*. This mistake was perpetuated in the Geitler (1932) classification system and, as argued by Kastovský and Johansen (2008), the *Fischerella* genus was associated mainly with soil forms in the 20th century.

The division of the thermal strains into two different genera was used until 16S rDNA phylogenetic data became available. Phylogenetic reconstructions revealed that thermal strains form a monophyletic clade among true-branching cyanobacteria (Gugger and Hoffmann 2004; Kastovský and Johansen 2008). Kastovský and Johansen (2008), furthermore, suggested that the original and correct species name should be *M. laminosus*, given the fact that early *M. laminosus* and *F. thermalis* descriptions were based on the same

Table 1 Timeline of *Fischerella thermalis* taxonomy

Year	Description of taxonomical changes	References
1837	Description of *Mastigonema thermale* and *Fischera thermale* from Karlovy Vary, Czech Republic	Schwabe 1837
1862	Description of *Mastigocladus laminosus* from Karlovy Vary, Czech Republic	Cohn 1862
1885	*Mastigocladus laminosus* Cohn 1862 is transferred to *Hapalosiphon laminosus* (Cohn) Hansgirg 1885	Hansgirg 1885
1886	Name adaptation and proposal of subgenus *Fischerella* (Bornet and Flahault 1886)	Bornet and Flahault 1886
1887	Starting point of genus *Hapalosiphon* Nägeli in Kützing ex Bornet and Flahault 1887	Bornet and Flahault 1887[a]
1887	Thermal descriptions asigned to *Hapalosiphon laminosus* (Cohn) Hansgirg 1885 in the starting point publication of Bornet and Flahault (1887)	Bornet and Flahault 1887
1895	Post-starting point of *Fischerella thermalis* (Schwabe) Gomont 1895	Gomont 1895[a]
1898	*Hapalosiphon laminosus* species is elevated to genus level. Post-starting point of *Mastigocladus laminosus* Cohn in Kirchner 1898	Kirchner 1898[a]
1930	Fremý assumed that a form he collected from soils was the same as *F. thermalis*	Frémy 1930
1932	Fremý drawing used for *F. thermalis* identification. Beginning of non-thermal *F. thermalis* descriptions	Geitler 1932
1936	Non-thermal reports classified as *Hapalosiphon laminosus*	Frémy 1936
1979	*Mastigocladus* and *Fischerella* genera are recognized, but strains are assigned to *Fischerella* genus	Rippka et al. 1979
1981	Reclassification of *Fischerella*, *Hapalosiphon* and *Mastigocladus* within *Stigonema* genus	Drouet 1981
1990	*Fischerella* and *Mastigocladus* genera are classified in different families	Anagnostidis and Komárek 1990
2004	*Mastigocladus*, *Fischerella* and *Hapalosiphon* should be collapsed into one genus (by phylogeny)	Gugger and Hoffmann 2004
2008	Thermal strains conform one species and adopt *Mastigocladus laminosus* nomenclature (by phylogeny)	Kastovský and Johansen 2008
2017	Description of four *Fischerella* species inside genus by ANI (genomic taxonomy)	Walter et al. 2017
2018	Thermal strains conform monophyletic species *F. thermalis* (genomic taxonomy)	Alcorta et al. 2018
2018	*F. thermalis* and *F. muscicola* species for thermal members (genomic taxonomy)	Parks et al. 2018

[a]Post starting points for species proposal

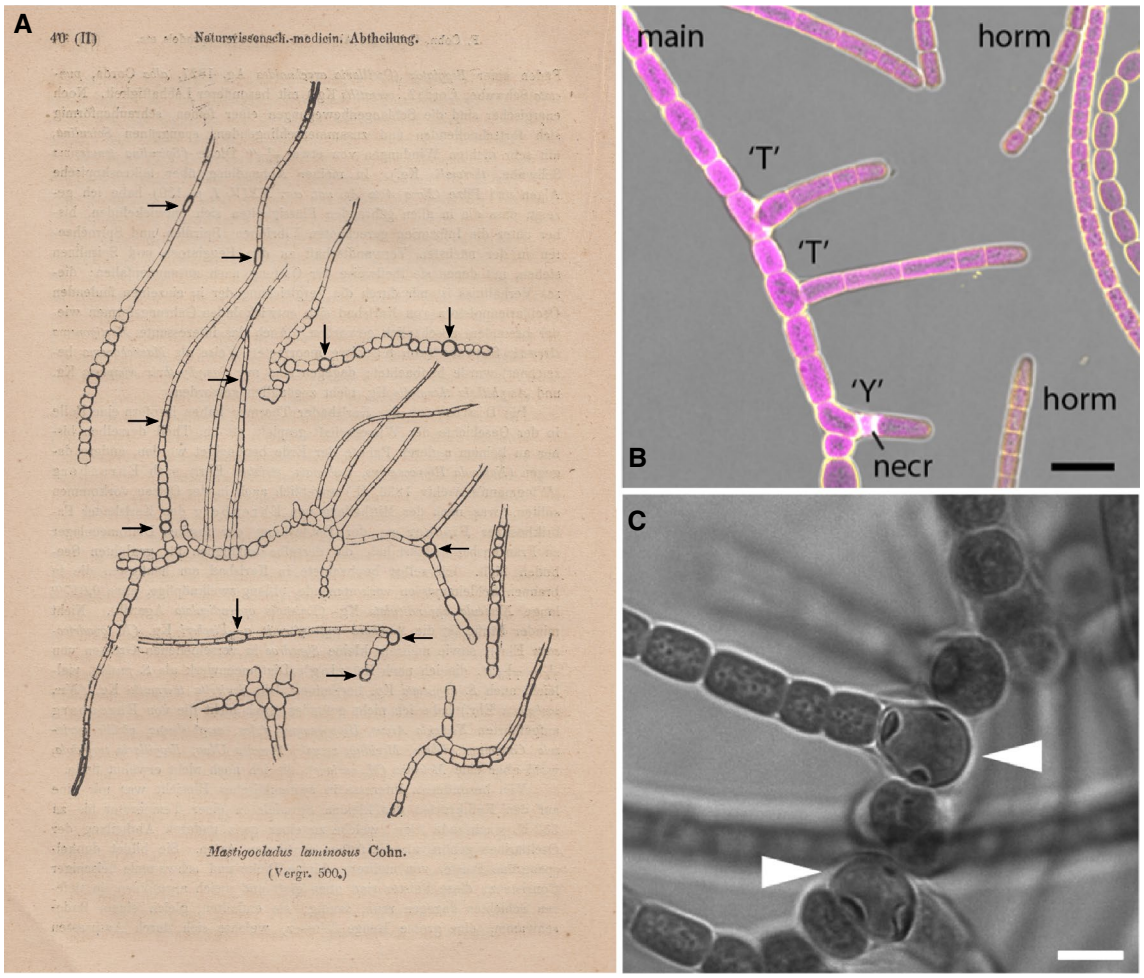

Fig. 1 Morphological characteristics of *Fischerella thermalis* as shown by Cohn in 1862 (Cohn 1862) (**a**) and in recent studies (**b**, **c**) (Nürnberg et al. 2014; Antonaru and Nürnberg 2017). **a** Drawing by Cohn showing the variation in cell and filament morphology. The cells with a thick envelope are most likely heterocysts (arrow). **b** Confocal fluorescence image showing the different types of branching (T and Y), necridia (necr) and hormogonia (horm). The image shows an overlay of chlorophyll fluorescence (magenta), FM1-43 FX fluorescence of the cytoplasmic membrane (yellow) and the bright-field view (grey). Scale bar, 10 µm. **c** Bright-field image of heterocysts at the branching start. Note the presence of three cyanophycin plugs in the region next to the neighboring cells. Scale bar, 5 µm. (Images from Cohn 1862; Nürnberg et al. 2014; Antonaru and Nürnberg 2017)

population obtained from Karlovy Vary hot spring. Besides, the morphological description bounded to the thermal environment was more related to *M. laminosus* than *F. thermalis* (both summarized in Komárek 2013). The thermal strains form densely entangled, branching filaments ~ 4–8 µm wide. Secondary trichomes usually branch out perpendicularly to their origin (T-type), or—rarely—at an acute angle (Y-type). Newly formed branches are usually narrower than the main trichomes and show a characteristic tapering at the end (see Fig. 1b).

To solve the *Mastigocladus* vs. *Fischerella* nomenclature problem, Kastovský and Johansen (2008) proposed three different approaches. First, if true-branching cyanobacteria described as *Mastigocladus* and *Fischerella* (as well as *Hapalosiphon*, *Westiellopsis,* and *Nostochopsis*) are congeneric, they should be joined in one genus under the name *Hapalosiphon* Nägeli ex Bornet and Flahault (1886), as it was the first to be mentioned in a scientific publication. Alternatively, the name *Fischerella laminosa* should be used only for thermal strains, and all other freshwater *Fischerella* species should be transferred to different genera. As a third possibility, the name *F. thermalis* should be rejected. For this last option, one could use *M. laminosus* for all thermal strains and instead define *Fischerella muscicola* Gomont 1895 as the type species for the *Fischerella* genus.

More recently, the genomic analyses of Walter et al. (2017) and Alcorta et al. (2018) showed > 95% average nucleotide identity between *F. thermalis* genomes. This is within the genomic threshold for the same species (Richter and Rosselló-Móra 2009). The species also showed ~ 87%

identity to one *Hapalosiphon* and to six non-thermal *Fischerella* strains, placing them within the same genus (Richter and Rosselló-Móra 2009). These results put the nomenclature solutions proposed by Kastovský and Johansen (2008) in a new light. Accordingly, *Hapalosiphon* should be the genus name for these congeneric strains given its early description in the literature, as seen by first publication dates for the *Hapalosiphon*, *Fischerella* and *Mastigocladus* genera in Table 1. Notably, Frémy studied prominently this hot spring cyanobacteria under the *Hapalosiphon laminosus* Hansg species name (Frémy 1936) which is currently considered a synonym of *M. laminosus* Cohn ex Kirchner (Komárek 2013). However, this would be difficult to resolve considering the complexity of the cyanobacterial taxonomy, the high number of published articles using both species names and the growing size of bioinformatics databases.

The taxonomy of Cyanobacteria is continuously changing, as more information becomes available. The latest classification by Komárek et al. (2014) uses morphological characteristics in combination with phylogenetic data from 31 conserved proteins to suggest that *F. thermalis* belongs to the order *Nostocales* (monophyletic group characterized by the ability to differentiate heterocysts), family *Hapalosiphonaceae* and genus *Fischerella*. Up to now, their proposed classification is the most widely used one and the one closest to the NCBI taxonomy database. However, the growing number of available genome sequences has reshaped the tree of life, including cyanobacteria. For instance, Parks et al. (2018) have calculated a tree based on 120 single-copy genes and relative evolutionary divergence values for all bacterial and archaeal genomes. Rather than using morphological and ecological characteristics to distinguish phylogenetic orders, they established thresholds for assigning node length to specific taxonomic levels. Thus, in the genome taxonomy database (Parks et al. 2018), *F. thermalis* species have been included in the proposed phylum *Cyanobacteriota*, class *Cyanobacteriia*, order *Cyanobacteriales*, family *Nostocaceae* and genus *Fischerella*.

Distribution

Morphological studies from 1837 until today have reported the presence of *F. thermalis* in a variety of hot springs found in at least 26 countries across the globe (see Table S1 and Fig. 2). In addition, 16S rRNA sequences from environmental samples and isolated strains allowed insights into the distribution and phylogenetic relationships of *F. thermalis* isolates (e.g. Miller et al. 2007). In the NCBI database there are currently 214 *F. thermalis*-related sequences (16S rDNA partial sequences and genomes; revised in January 2019),

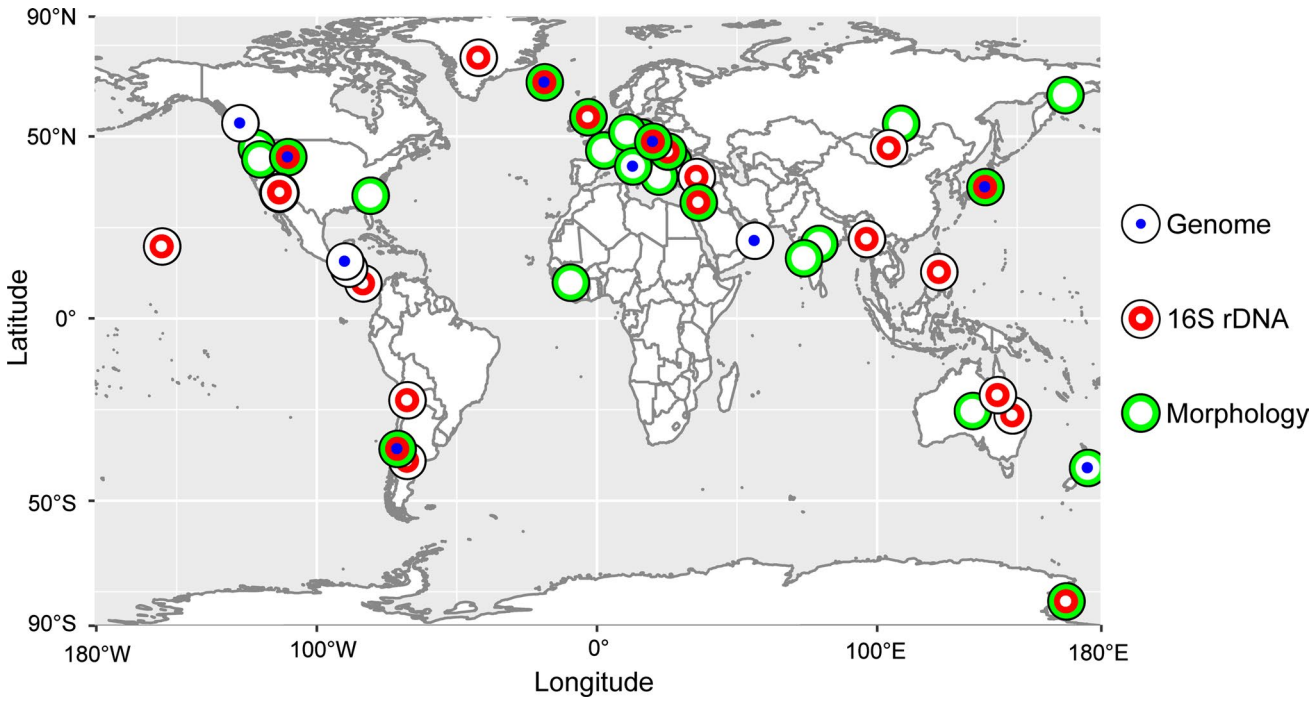

Fig. 2 Map of the worldwide distribution of *Fischerella thermalis*. The distribution of *F. thermalis* is shown based on morphological studies (outer circle), 16S rDNA sequences (middle circle) and genomes (centric circle). Sequence information was retrieved from the NCBI database in January 2019. The location, access numbers and references are listed in Table SI. Location of capital cities was used as a shorthand target for labeling in each country, with the exception of Antarctica, Argentina, Australia, Chile, Greenland, India, Russia and the USA

associated with 26 published articles (see Table S1 and Supplementary Material). Overall, these studies revealed the near-ubiquitous presence of *F. thermalis* in microbial mats of hot springs worldwide, along a thermal gradient up to 60 °C (e.g. Miller et al. 2006; Lacap et al. 2007; Roeselers et al. 2007; Boomer et al. 2009; Soe et al. 2011; Mackenzie et al. 2013, Alcamán et al. 2015).

Besides natural environments, anthropogenic habitats may also host thriving populations of *F. thermalis*. For instance, today there are still hot spring microbial communities growing in the middle of the city of Karlovy Vary (Fig. S1A; the same site from which Schwabe in 1837studied *F. thermalis* for the first time). *F. thermalis* has even been isolated from cooling reservoirs (> 50 °C) in the nuclear reactor in Savannah River (USA), and sampled strains have shown the capacity to mitigate high nutrient concentrations in laboratory experiments (Weissman et al. 1998).

Multicellularity and ultrastructural characteristics

The ultrastructure of *F. thermalis* was studied in detail by Stevens and co-workers (Nierzwicki et al. 1982, 1984; Stevens et al. 1985). These authors observed stark differences between young and old trichomes. In the cells of young, thin trichomes, the central portion of the cell (about half of the volume) contains genomic DNA, ribosomes, and carboxysomes. Meanwhile, in peripheral portions, there are thylakoids with lipid-body-like structures and cyanophycin granules as a dynamic nitrogen reserve. These are arranged in tight parallel pairs, with antenna complexes (phycobilisomes) between them.

However, in rounded, older cells, there is less distinction between the thylakoid and cytoplasmic portions, with less well-organized thylakoid membranes. These older cells tend to have more lipid-body-like structures, carboxysomes and newly formed polyphosphate bodies, while cyanophycin granules are rare and the ribosomal content is reduced. This was suggested to reflect a shift from an active growth state to a resting state (Nierzwicki et al. 1982). More recently, Gonzalez-Esquer et al. (2016) analyzed four *Fischerella* genomes (including *F. thermalis* JSC-11) and found protein domains related to these ultrastructure features: one for carboxysome shell proteins (PFAM03319); two for type-5 and 54 for type-1 glycosyltransferases related to the synthesis of glycogen granules (PFAM08323 and PFAM00534, respectively); 13 for mur-ligase domains related to cyanophycin synthetases (PFAM08245, PFAM02875 and PFAM13335) and two polyphosphate kinase domains for polyphosphate granules production (PFAM02503).

Filaments grow by cell division. The cell division process in *F. thermalis* was found to be similar to other *Fischerella* species (Nierzwicki et al. 1982). This process involves the invagination of the cytoplasmic membrane and the peptidoglycan layer by polar mesosome-like structures until the cytoplasm is transected by a cross-septum of peptidoglycan, which later begins to thicken on both ends. Then, the septum splits down in the middle. The invagination continues until the daughter cells are separated (Nierzwicki et al. 1982). This process occurs for all cells irrespective of their branching type and is only superficially different with respect to the angle of cell elongation (Golubic et al. 1996; Nürnberg et al. 2014).

The cells of *F. thermalis* maintain connections after division. Nürnberg et al. (2014) showed that fluorescent dyes could be transferred along entire filaments, in both the main trichome and the branches. Furthermore, the authors identified the presence of SepJ (or FraG), a protein involved in intercellular communication and filament integrity (Flores et al. 2007; Nayar et al. 2007; Mullineaux et al. 2008; Nürnberg et al. 2014). Immunofluorescent labeling studies confirmed its localization in distinct spots in the cell septa of wide and narrow trichomes, as well as in branching points. Furthermore, electron micrographs of ultra-thin sections showed structures connecting the cells, which have been termed septal junctions (previously known as microplasmodesmata or septosomes) (Mariscal 2014, and references therein). Additional electron micrographs of isolated peptidoglycan sacculi revealed circular perforations (termed nanopores) in the septal walls. These are likely to accommodate the septal junctions (Marcenko 1962; Lehner et al. 2013; Flores et al. 2019 and references therein). Cell–cell adhesion, intercellular communication, and terminal cell differentiation define multicellularity in Cyanobacteria (Flores and Herrero 2010), but the ability of *F. thermalis* to divide into a second plane and form branches adds another layer of complexity.

It is still unknown which genes are required for multicellularity in the cyanobacteria. A comparative genomics study by Stucken et al. (2010) suggested that only 20 genes are necessary to form a filament. Earlier, it was hypothesized that branch formation could be under single-gene control. However, when the first *Fischerella* genomes were reported, no branching gene was revealed (Dagan et al. 2013; Shih et al. 2013). It was found, however, that genomes of true-branching strains are enriched in genes involved in the transduction of signals and transcription (Shih et al. 2013), suggesting that regulatory elements might be essential for the morphological transition.

More recently, Koch et al. (2017) investigated branching by differential RNA sequencing (5′-dRNA-seq). A branchless phenotype formed in *F. thermalis* PCC 7521 (and a closely related species *F. muscicola* PCC 7414) when the cyanobacteria were grown photoheterotrophically with sucrose. The comparison of the transcription start sites (and, indirectly, transcripts) between the two phenotypes revealed higher amounts of transcripts from genes such as

*fra*C and *mre*B, which are known to be important for the formation of septal junctions and elongasome complexes (Koch et al. 2017). Accordingly, Koch et al. (2017) suggested the 'plasticity first' evolutionary scenario for branch-formation. This states that phenotypic variation induced by transcriptional changes plays a more important role in the process of developing a branching phenotype than the acquisition of new genes. It is worth mentioning that, although not genetically confirmed, Singh and Tiwari (1969) could induce true-branching in the non-branching cyanobacterium *Nostoc linckia* by random mutagenesis using ultraviolet irradiation.

Photosynthesis

Cyanobacterial photosynthesis is a process divided into four steps: (1) The light-dependent water-splitting by photosystem II (PSII); (2) the transfer of electrons to the cytochrome b_6f complex; (3) the light-dependent electron transfer from cytochrome b_6f to photosystem I (PSI), and (4) the cytochrome b_6f-PS I cyclic electron flow or the reduction of $NADP^+$. The protein complexes involved in the photosynthetic process of *F. thermalis* have been widely studied in terms of functionality and structure partly due to their higher stability and tendency to crystallize (e.g. Schirmer et al. 1985; Duerring et al. 1990; Almog et al. 1991; Reuter et al. 1999 and Kurisu et al. 2003). Several studies focused on the main components of this process and are listed below:

Photosystem II

Like many biological processes, photosynthesis is affected by temperature. High temperatures decrease chlorophyll content (Chalanika De Silva and Asaeda 2017). They also lower transcript levels of genes encoding for photosystem II subunits PsbA and PsbD (Rowland et al. 2010; Singh et al. 2006; Suzuki et al. 2006), which affects the water-splitting site of this enzyme (Yamashita and Butler 1968). However, thermophilic strains have evolved to fit their environment. In *F. thermalis,* the O_2-evolution rates (a marker of PSII activity) depend on the studies and the strains (Bohler and Binder 1980; Ramesh et al. 2002). There are higher reported levels between 40 and 55 °C, but in most strains, they consistently decrease above 55 °C. Transcript levels of PSII-genes of *F. thermalis* populations in the Chilean hot spring of Porcelana were highest, between 48 and 58 °C and they decreased sharply at 66 °C (Alcamán-Arias et al. 2018). Altogether, these results suggest an optimal temperature range of ~ 45–55 °C for *F. thermalis* and a strong negative effect of higher temperatures on PSII activity.

Phycobilisomes

Phycobilisomes are light-harvesting protein complexes associated mainly with PSII (Watanabe and Ikeuchi 2013). They allow cyanobacteria to absorb light in a spectral range that is not covered by chlorophylls and thereby reduce the green gap (Reuter and Nickel-Reuter 1992; Watanabe and Ikeuchi 2013). In the case of *F. thermalis*, their architecture has been well described (Glauser et al. 1992; Nies and Wehrmeyer 1981; Reuter and Nickel-Reuter 1992). Similar to phycobilisomes in other filamentous cyanobacteria like *Anabaena,* they consist of a trimeric core and up to eight rods. Their conformation changes in response to the environmental conditions. Different forms have been defined as "minimal", "intermedial" and "maximal", depending on the number of components involved (Reuter and Nickel-Reuter 1992). However, phycobilisome antennas are highly adaptable to different light and temperature conditions without major conformational changes (Reuter and Nickel-Reuter 1992).

Time is an essential factor in the resistance to high temperatures. Zhao and Brand (1989) observed that *F. thermalis* cultures subjected to temperatures between 60 and 65 °C for a few minutes decreased the absorption of their phycobilisomes while chlorophyll and carotenoids were not affected. On the other hand, experiments carried out by Radway et al. (1992) on *F. thermalis* demonstrated that both phycobilisomes and chlorophyll are reduced by 20% when cultures are exposed for at least 1 h to temperatures between 60 and 65 °C. These authors also demonstrated that strains subjected to 65 °C, were bleached and were not able to continue growing even when cultivated at lower temperatures again. At 70 °C, the effect was stronger, and the cultures showed irreversible damage after only half an hour of heat shock (Radway et al. 1992).

Cytochrome b_6f

The cytochrome b_6f complex transfers electrons between PSII and PSI. The structure of the cytochrome b_6f from *F. thermalis* was one of the first to be resolved by crystallography (Baniulis et al. 2008; Kurisu et al. 2003). It showed structural similarity to other cytochrome b complexes such as the core of mitochondrial cytochrome bc_1 and is highly conserved among photosynthetic organisms, even among species as distantly related as *F. thermalis*, spinach and the green algae *Chlamydomonas reinhardtii* (Baniulis et al. 2008; Whitelegge et al. 2002). By the structural conservation, the high stability and the tendency of thermophilic proteins to crystallize; the cytochrome b_6f of *F. thermalis* has been widely used to understand molecular mechanisms of electron flow (reviewed in Allen 2004).

Photosystem I

Electrons are subsequently transferred from cytochrome b_6f to PSI, which has a crucial role in increasing ATP yield by cyclic electron flow (Larkum et al. 2017). The PSI of *F. thermalis* was one of the first to be crystallized, and it showed a monomeric and a trimeric conformation (Almog et al. 1991). However, a recent study by Li et al. (2019) suggests that depending on the growth condition *Fischerella* can form PSI tetramers, an adaptation that appears to be widespread among heterocyst-forming cyanobacteria. Furthermore, the PSI of *F. thermalis* shows high thermostability, with 55 °C as its optimal assembly temperature (Lushy et al. 2000). Ferredoxin, the final electron acceptor, can even tolerate temperatures up to 65 °C (Fish et al. 2005).

Carbon fixation and growth at high temperatures

F. thermalis is a major primary producer in hot springs. As early as 1903, ex situ populations from the Karlovy Vary hot spring were shown to grow at 49 °C with the assimilation of CO_2 (Löwenstein 1903). However, this thermostability is lost during long-term adaptation to lower temperatures (Löwenstein 1903). Previous studies from the first half of the 20th century showed the worldwide distribution and the optimal (~ 45–50 °C) and maximum (55–60 °C) growth temperatures estimated of this cyanobacterium (e.g. Löwenstein 1903; Frémy 1936, and references therein). Later, Schwabe determined a ~ 60 °C upper temperature limit for cultures from different locations, stablishing also that the limit could vary depending on the original hot spring geochemistry (Schwabe 1960). Brock and Brock (1966) studied microbial mats from Yellowstone National Park using chlorophyll measurements and found that *F. thermalis* does not grow over 55 °C. Several other studies confirmed an upper temperature growth limit of 57–58 °C in culture (Schwabe 1960; Muster et al. 1983; Miller et al. 2007; Finsinger et al. 2008; Alcamán et al. 2017). Although slow growth of *F. thermalis* at 15 °C has been reported (Radway et al. 1992), its productive temperature range is considered to be from 25 to 58 °C (e.g. Finsinger et al. 2008; Stal 2017).

However, it should be noted that optimal temperatures may differ depending on the characteristic that is being measured. In Mushroom hot spring at Yellowstone National Park, the maximum photosynthetic yield of *F. thermalis* was measured between 41.6 and 48.3 °C, while higher biomass production was found between 44 and 55 °C (Brock 1967; Muster et al. 1983). In Porcelana hot spring, up to 90% of the transcriptional activity related to the Calvin–Benson–Bassham cycle can be attributed to *F. thermalis* at 48 and 58 °C during daytime. The highest carbon (^{13}C) assimilation rates were found also in these temperatures, suggesting that *F. thermalis* is the major primary producer in this environment (Alcamán-Arias et al. 2018). Although the transcriptional activity of *F. thermalis* was found at 66 °C, it was scarce, indicating a low or null carbon input of *F. thermalis* to the microbial community over 60 °C (Alcamán-Arias et al. 2018).

Nitrogen fixation

Nitrogen fixation represents an evolutionary advantage in the often nitrogen-limited waters inhabited by *F. thermalis* (Miller et al. 2006; Alcamán et al. 2015). For instance, *F. thermalis* from a New Zealand hot spring was the first branching, heterocyst-forming cyanobacterium in which nitrogen fixation was observed (Fogg 1951). However, it was not until 1969 that heterocysts were identified as the actual sites for nitrogen fixation (Stewart et al. 1969). Heterocysts are characterized by having a thick, multilayered envelope to reduce oxygen permeability and protect the O_2-sensitive nitrogenase (Awai et al. 2009). Additionally, heterocysts dismantle their O_2-producing PSII and increase the rate of respiration (Wolk et al. 1994). The development of the heterocyst under nitrogen deprivation is mediated by the transcriptional regulator HetR whose protein crystal structure was first elucidated for *F. thermalis* (Kim et al. 2011). Regarding heterocyst position, *F. thermalis* is unusual. Even a cell at a branching start can differentiate, resulting in the formation of a three-pored heterocyst with three cyanophycin plugs in the neck areas (Fig. 1c; Nürnberg et al. 2014).

Nitrogen fixation is closely connected to the process of intercellular communication. Heterocysts are terminally differentiated cells that rely on neighboring vegetative cells for fixed carbon (in the form of sugars) in exchange for fixed nitrogen. Therefore, cyanobacteria need to regulate heterocysts formation. This process has been best investigated in other filamentous cyanobacterial models such as *Anabaena* and *Nostoc* (Wolk et al. 1994; Herrero et al. 2016). *Anabaena* and other filamentous cyanobacteria regulate nitrogen fixation by forming various (semi-) regular patterns. Although these patterns were thought to be non-regular in *F. thermalis* (Nierzwicki-Bauer et al. 1984), it has been recently shown that a semi-regular spacing pattern exists, and is dependent on cell morphology and the age of the culture (Antonaru and Nürnberg 2017).

F. thermalis heterocysts have been suggested to control the influx of gas from vegetative cells via septal junctions, providing a balance between the need for N_2, the inhibition by O_2 and the need for O_2 for respiration in the dark as short-term responses (Walsby 2007; Stal 2017). Long-term heterocyst envelope adaptations are reflected in genomes of *F. thermalis* strains. The deletion of two heterocyst envelope genes in downstream strains of White Creek hot spring

(temperature generalists) resulted in higher N_2-fixation rates in microoxic conditions. These could be related to the divergence of the populations (see below; Sano et al. 2018).

The first field studies of nitrogen fixation in *F. thermalis* were carried out in hot springs located in Alaska and Yellowstone National Park (USA) by Billaud (1967) and Stewart (1970). Stewart (1970) reported nitrogen fixation rates up to 54 °C, by using acetylene reduction assays and stable isotope measurements ($^{15}N_2$). The optimal fixation rate in situ for *F. thermalis* was measured at 42.5 °C. Similar results were obtained by Wickstrom (1980), confirming that nitrogen fixation in mats where *F. thermalis* is abundant occurs up to 55 °C. More recently, the study by Alcamán et al. (2015) reported in situ nitrogen fixation at 58 °C. Then, it can be concluded that the upper temperature limit for nitrogen fixation in *F. thermalis* mats is ~ 60 °C.

Similar results to in situ rates were obtained by using the marker gene *nif*H. The *nifH* gene encodes the α-subunit of the nitrogenase enzyme complex and thus can be used to identify nitrogen-fixing bacteria. The detection of *F. thermalis nifH* gene sequences at temperatures of ~ 50 °C in White Creek hot spring mats (Miller et al. 2006) and of ~ 58 °C in Porcelana hot spring mats (Alcamán et al. 2015) supports the upper temperature limit of nitrogen-fixation determined by in situ measurements. Furthermore, both in situ and in vitro methods, confirmed that for *F. thermalis* the N_2 fixation process is restricted to the daytime period (Miller et al. 2006; Alcamán et al. 2015, 2017). Overall, only a single report of *F. thermalis* without the ability to differentiate heterocyst has been described, and it corresponds to a strain isolated from Mt. Erebus, Antarctica (Melick et al. 1991).

Evolution

The evolutionary history of *F. thermalis* was first studied in detail by Miller et al. (2007), by sequence comparison of the V3–V8 region of the 16S rRNA gene (950 bp). The authors showed that hot spring strains from around the world share > 97% sequence identity with an estimated common origin at about 47–95 million years ago. The origin and global dispersion of these cyanobacteria have been suggested to match with the Paleocene-Eocene thermal maximum, which was marked by a 5–10 °C increase in the atmospheric temperature (Fricke and Wing 2004). The higher temperature tolerance range (15–58 °C) of these cyanobacteria could have supported their dispersal.

Microdiversity within *F. thermalis* species is evident at local scales. Significant research has been done on strains collected from a 1.5 km stretch of White Creek hot spring, across a 39–54 °C temperature gradient (Miller et al. 2009). Isolates from upstream temperatures (> 46 °C) behave like "ecological generalists", growing similarly well at 37 °C and 55 °C. Meanwhile, strains isolated downstream in the gradient are considered "ecological specialists". They produce 20% more biomass at 37 °C, compared to upstream, thermophilic strains. This divergence was supported by the study of Wall et al. (2014), where the authors found that specific fast-evolving genomic regions were shared between thermophilic strains. Among them was an expression island of heterocyst envelope polysaccharides. Low-temperature populations showed a deletion of two genes important for heterocyst envelope polysaccharide biosynthesis, suggesting possible consequences in envelope properties (Wall et al. 2014). Hutchins and Miller (2017) also observed specific mutations in genes such as *aps*K (adenylylsulfate kinase) and multiple cytochrome oxidases, suggesting they could be related to different yields in nitrogen fixation at different temperatures by yet unknown mechanisms.

Recently, Alcorta et al. (2018) also observed divergence in *F. thermalis* along a temperature gradient (46–61 °C) in Porcelana hot spring. Sampled populations clustered in three different groups based on proteomic results from MALDI-TOF mass spectrometry. Two of these groups corresponded to high-temperature isolates (54 and 61 °C). They showed a higher abundance of photosystem- and phycobilisome-linker proteins and, at the genetic level, had a *nif*H gene polymorphism distinguishing them from lower temperature strains.

In the same study, Alcorta et al. (2018) focused on three metagenome-assembled-genomes (MAGs) of *F. thermalis*. These were recovered from three separate populations across three temperatures (48, 58 and 66 °C). 97% of orthologous genes were identical between the 58 and the 66 °C populations. However, this number was reduced to 80% when compared to populations at 48 °C. In addition, in the 66 °C MAG, exclusive genes related to metabolism and the processing of genetic information were more abundant. These observations suggest the presence of specialized ecotypes of *F. thermalis* in the thermal gradient of Porcelana hot spring (Alcorta et al. 2018).

Accordingly, in Miller et al. (2009) and Alcorta et al. (2018) it is suggested that *F. thermalis* is leading a process of sympatric evolution. This refers to the genetic divergence of populations from a common ancestor while they continue to inhabit the same location. In the case of *F. thermalis*, the driving force is represented by inhabiting different niches along the thermal gradient. The balancing selection (the maintenance of polymorphic alleles over time) has a crucial role in this divergence and has been rarely observed in other bacteria (Sano et al. 2018).

Another example of balancing selection in *F. thermalis* might occur in the far-red gene cluster. This ~ 20-gene cluster encodes alternative phycobilisome and photosystem components, together with an enzyme synthesizing the alternative long-wavelength chlorophyll, chlorophyll *f* (Gan et al. 2014; Ho et al. 2016). Chlorophyll *f* photosynthesis has been recently discovered to expand the usable photosynthetic

Fig. 3 **a** *apc*E2 phylogeny of the *Fischerella* genus. ApcE2 is a phycobilisome-membrane linker used for far-red photosynthesis. There is a clear geographical effect on strain distribution. This geographical effect is also visible at higher magnifications, with most sequences from Yellowstone forming specific clades. 'Upstream' and 'downstream' Yellowstone clusters (grey box) represent identical sequences from multiple strains. The outgroup location of *Fischerella* sp. NIES-4106 might be explained by the fact that this cyanobacterium carries *apc*E2 on a plasmid. This might lead to higher rates of evolution. Multiple alignment: Clustal Omega, ten iterations. Phylogeny built with RaxML on the CIPRES webserver. Settings: GTRGAMMA, 100 bootstrap replicates. Rootpoint chosen to match previous apcE2 trees. Tree edited with iTOL and Inkscape. **b** Partial amino acid sequence comparison of ApcE2 sequences (21 aa) of 'Upstream' and 'downstream' Yellowstone genomes and other cyanobacterial sequences. The mutation in aa 558 in 'downstream' sequences is highlighted

spectrum into the near-infrared and is also found in a wide range of morphologically diverse cyanobacteria other than *F. thermalis* (Gan and Bryant 2015). No fully sequenced *Fischerella* genome is known, at present, to lack the genes for far-red acclimation. This is important because chlorophyll *f* photosynthesis has generally been assumed to be a rare adaptation (Gan and Bryant 2015). There are few sequences published, allowing very limited evolutionary inferences; however, the abundance of *Fischerella* sequences allows evolutionary studies of the far-red phenotype at an unprecedented resolution.

A phylogenetic analysis of the *apcE2* (far-red phycobilisome-membrane linker) sequences from the *Fischerella* genus is found in Fig. 3a. By looking at closely related strains from one locality (such as the recently sequenced WC strains from White Creek), one can see evolution in action over short timescales of tens of millions of years. Of particular interest are the 'upstream' and 'downstream' groups of Yellowstone. Sano et al. (2018) argued that the downstream, or specialist mesophilic strains, are descendant from the upstream thermophilic strains. In the present study, we also found one nucleotide difference between the upstream and a subset of downstream Yellowstone *apcE2* sequences, with the downstream mutation (Y → C) being distinct both from the upstream allele and from all other *apc*E2 alleles. This significant tyrosine in position 558 is highly conserved, not only among all the known far-red *apc*E2 sequences but among multiple conventional ApcE linker sequences, even in species as distantly related as the marine *Synechococcus* sp. KORDI-49 (Fig. 3b). However, single-nucleotide polymorphisms show evidence of being horizontally transmitted between Yellowstone groups, supporting the 'divergence with gene flow' model previously proposed by Sano et al. (2018).

Conclusions

Thermophilic true-branching cyanobacteria have been described in hot spring microbial mats since two centuries ago, but their taxonomy remains controversial. Phylogenetic analyses demonstrate that *Fischerella thermalis* is a monophyletic, thermophilic species of the genus *Fischerella*. It is present worldwide in hot springs up to 60 °C. This review aimed to synthetize the taxonomic literature for this species and thereby providing the basis for further systematic and nomenclature studies.

Ecologically, *F. thermalis* has been used as a model for bacterial biogeography. It is a keystone organism of the hot spring food chain and has appropriate adaptations. The heavily documented evolutionary history of this cyanobacterium, as well as the extensive in situ and laboratory studies focusing on its thermostability have made *F. thermalis* a model organism for studying many biological processes. These include photosynthesis, nitrogen fixation and even bacterial cell division and differentiation. Its branching process marks it as one of the most complex bacteria.

Many aspects of the biology of *F. thermalis*, remain to be uncovered in future studies. These include its evolution along a thermal gradient, biophysical aspects of thermostability, and regulation of cell division, among others. *F. thermalis* is a clear example of the cellular complexity that bacteria can achieve, and its adaptation to higher temperatures makes it especially suitable for laboratory studies on the functional and structural characterization of proteins.

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Compliance with ethical standards

Conflict of interest Authors declare they do not have conflict of interest.

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