Filamentous anoxygenic phototrophic bacteria in water bodies of Middle and Lower Volga basin (European Russia): an overview

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Abstract. "Filamentous anoxygenic phototrophs" or "green non-sulfur bacteria" form a distinct lineage, order Chloroflexales (Chloroflexia: Chloroflexi) in broad polyphyletic group of anoxygenic phototrophic bacteria. Filamentous anoxygenic phototrophic bacteria have apparently important, though not fully understood functions in contemporary ecosystems and perhaps were much more significant in the Archaean. However, since their discovery in hot spring mats in the late 1960s, the extremophilic species of thermal, hypersaline and/or hyperalkaline habitats remain the most studied representatives of the lineage. In this overview, we show our representation of the history of finding and research of filamentous anoxygenic phototrophs, and current situation with their taxonomy, phylogeny and diversity. Some problems and uncertainties in these fields are also discussed. The special section is dedicated to the discovery and research of this group in the Volga River basin. Due to the small number of studies, the exact distribution of filamentous anoxygenic phototrophic bacteria in the region is unknown. For example, in small stratified lakes with high humic and/or ferruginous water in the basins of the Oka and the Upper Volga the development of planktonic filamentous anoxygenic phototrophs is very likely. Besides, the development of Chloroflexales, including new species, is quite expected in the microbial mats of saline rivers and hypersaline lakes of the Lower Volga region. Further progress in the field will be possible only with the use of molecular methods along or preferably in combination with traditional methods (polyphasic approach). Since their role in communities is far from elucidated, further studies of FAP may also provide new opportunities in the field of microbial biotechnology.

1. Introduction
The light energy reaching the Earth’s surface is the most common source of energy on our planet. Not only ecosystems of the illuminated zones of the biosphere, but also most part of underground and underwater ecosystems in the aphotic and twilight areas depend on substances and energy synthesized in the process of photosynthesis. Whilst there exist ecosystems that depend on the supply of reduced compounds from deep geological strata (e.g. ecosystems of hydrothermal vents of mid-ocean ridges and other areas of underwater volcanism, or microbial communities of the deep biosphere etc.), most of them either directly or indirectly required molecular oxygen generated by oxygenic phototrophs [1]. Although oxygen photosynthesis is currently prevalent, anoxygenic photosynthesis processes also continue to perform important functions in the modern biosphere.
In broad sense, photosynthesis encompasses any process of using light energy for the metabolism of living organisms. In the rhodopsin type of photosynthesis, light energy absorbed by the retinal molecule in the specialized pigment-protein complex (halo-, bacterio- or xantorhodopsin) causes its isomerization which is accompanied by transmembrane transfer of a proton or other cation resulting in the formation of an electrochemical gradient finally used for the ATP synthesis [2]. This mechanism is not associated with any redox reactions and, probably, it had been very important in the earliest times of the Earth's evolution in an excess of primordial organic matter.

The second, more complex, mechanism consists in the light-dependent transmembrane transfer of electrons (so called charge separation); it is carried out in specialized pigment-protein "reaction center" complexes, containing Mg-tetapyrrole pigments (chlorophylls or bacteriochlorophylls) as electron donors, carotenoids as auxiliary pigments, Mg- or H2-tetapyrroles as intermediate electron acceptors and quinones or Fe-S complexes as terminal electron acceptors [1,2]. After the charge separation, electrons are transferred through the electron transport chain, and eventually can be used to reduce CO2 to organic compounds, or can return back to the "donor" part of the reaction centers in "cyclic" electron transport. The transmembrane proton gradient required for the synthesis of ATP is generated beyond the reaction centers at certain stages of electron transfer. Such a mechanism of photosynthesis is called chlorophototrophic.

In the oxygenic photosynthesis, water molecules serve as an oxidant (=electron donor), producing molecular oxygen. Anoxygenic photosynthesis uses other electron donors such as sulfides, ferrous iron or other inorganic and organic reduced compounds, or utilizes cyclic electron transfer to synthesize ATP without reducing CO2. Currently, anoxygenic photosynthesis is restricted to several phyla of the domain Bacteria and primarily to anoxic habitats. The most abundant anoxygenic phototrophs are sulfur- and non-sulfur purple bacteria (phototrophic species of various taxa of the phylum Proteobacteria), "green sulfur bacteria" (GSB) (phylum Chlorobi), "filamentous anoxygenic phototrophs" (FAP) (order Chloroflexales, phylum Chloroflexi), and heliobacteria (fam. Heliobacteraeae; phylum Firmicutes). Besides, it was found recently that several species of other phyla, Acidobacteria, Gemmatimonadota, and Ca. Eremiobacterota (WPS-2) are also capable of photoheterotrophic metabolism.

Although some phototrophic representatives have recently been found in other classes of the phylum Chloroflexi [4-7], almost all known phototropic species of Chloroflexi belong to the order Chloroflexales in the class Chloroflexia. FAP are multicellular filamentous anoxygenic chlorophototrophic organisms that are widespread in microbial mats. Other species are euplanktonic and inhabit metalimnia in stratified lakes in areas devoid of high concentrations of both oxygen and sulphides. However, due to the concentration of FAP research on extremophilic environments, data on their effects on freshwater ecosystems in the temperate zone are certainly incomplete.

In this brief review, we will try to describe the history of the study, taxonomy, and ecology of this group as a whole, as well as their known distribution in the Volga basin.

2. History of research

While purple and green sulfur bacteria were first discovered in the mid-19th and early 20th century, respectively, first representatives of Chloroflexales were found in hot spring mats of the Yellowstone National Park (USA) in the late 1960s [8]. One of the discovered "photosynthetic gliding bacteria" was soon formally described as Chloroflexus aurantiacus [9]; shortly thereafter, similar mesophilic species, Chlororhendes giganteum, Chn. spiroideum, Oscillatoria chrysea, and Osc. trichoides were described from the lake water and microbial mats of mesophytic habitats [10-12]. Almost until the end of the 20th century, these species, and the first "red" species, devoid of chlorosomes Heliothrix oregenonis Pierson et al. 1986, remained the only known representatives of "green non-sulfur bacteria", as the current phylum Chloroflexi was then called. The group therefore considered almost exclusively phototrophic. However, already in the 1980s, the proximity of the heterotrophic "flexibacterium" Herpetosiphon aurantiacus Holt and Lewin 1965 to this group was clarified [13].

By the end of the 20th century, the indications appeared that many heterotrophic filamentous bacteria of activated sludge (so-called Eikelboom-type bacteria) are related to this group [14]. Subsequently,
many of these bacteria were included in the classes Anaerolinea, Caldilinea, and Ktedonobacteria within Chloroflexi; later, several other classes with heterotrophic representatives were described. As a result, the initially almost exclusively phototrophic phylum became predominantly heterotrophic with a few phototrophic species. The order Chloroflexales itself has not undergone significant changes, remaining purely photosynthetic.

In the first decade of the 21st century, only two new phototrophic species of Chloroflexales, *Roseiflexus castenholzii* [15] and *Ca. Chlorothrix halophyla* [16] were described; the main efforts at this time were focused on the isolation and description of representatives of the new heterotrophic classes of Chloroflexi. Recently, however, a certain revival of interest in phototrophic species of Chloroflexi occurs, resulted in description of new species belonging both to class Chloroflexia (order Chloroflexales) [17-21, etc.] and to the other classes [4, 6, 7].

Simultaneously, various aspects of the biochemistry and physiology of this group and first of all, their photosynthesis, were studied; cultures of *Chloroflexus aurantiacus*, *Oscillochloris trichoides* (neotype, strain DG-6), *Chloronema sp.* (strain GNSB-1=UdG9001), *Roseiflexus castenholzii* and some other strains served as objects; subsequently many aspects of physiology of this group were determined on the basis of whole genome sequencing.

Modern concepts of bacterial photosynthesis were formed in the 1990s, when it became generally accepted that there are two types of reaction centers, "iron-sulfur", similar to photosystem I of plants, and "quinone", similar to their photosystem II. Reaction centers of the Chloroflexales were found to belong to type II, that is, they have quinone terminal acceptors [3], namely menaquinones [22]. It was found that during autotrophic growth, *Clf. aurantiacus* uses a specific mechanism for the formation of organic matter, 3-hydroxypropionate bicycle [23]; however, *Osc. trichoides* applies for CO₂ fixation ribulose-bisphosphate pathway [24], common for all oxygenic phototrophs. It was found also that *Clf. aurantiacus* lack membrane electron-transport complex bc₁ (or bf) which is common for most bacteria. Instead of it, alternative complex III (ACIII) performs its functions [25]; however, *Osc. trichoides* contains genes encoding both complexes [26]. The features of the pigment-synthesis enzymes, and of light-harvesting complexes (chlorosomes) in "green" Chloroflexales, and membrane chlorophyll-protein complexes in "red" *Roseiflexus* were also researched in detail, summarizing in [1-3, 27].

The biochemical and genetic characteristics of filamentous anoxygenic phototrophs are unusual and do not occur in a similar combination in other phototrophic groups; therefore, until recently, many considered Chloroflexales to be one of the oldest, if not the most ancient, group of phototrophs [28]. However, in recent years, a different paradigm has prevailed, according to which most, if not all components of the photosynthetic apparatus and some other traits were acquired by Chloroflexales at different times and from different groups of bacteria through horizontal genetic transfer [5, 29]. However, the range of assumed horizontal transfer is extremely wide compared with any other prokaryotic phylum. We suppose that the unusual properties of Chloroflexi may not be a consequence of horizontal transfer, but an extremely strong selection pressure towards greater specialization, which led the initially mixotrophic group to a strong reduction of the photosynthetic machinery and its complete loss in most branches.

### 3. Taxonomy, phylogeny and diversity

The order Chloroflexales comprised both anaerobic and (micro)aerobic species of multicellular filamentous bacteria, devoid of flagella, but usually capable of sliding movement. Despite most of them stain Gram-negative, they are either devoid the outer membrane, or lack lipopolysaccharides [29, 30], the feature linking them with other members of Terrabacteria supergroup.

The species of the order are, at most, photoorganotrophic and mixotrophic, with different potential for chemoheterotrophic and lithoautotrophic metabolism. The first isolated strain of *Chloroflexus aurantiacus* is capable of photoheterotrophic and aerobic chemoheterotrophic growth; it almost unable to grow photolithothrophically. Other strains, isolated from sulfidic environments can more or less efficiently grow photoautotrophically using H₂ and/or sulfides. In contrast, such species as *Chlorothrix halophyla* and *Oscillochloris* spp. hardly able of aerobic heterotrophic growth at all, but instead they are
highly adapted to photoautotrophy under strong reductive conditions. However, these species are difficult to isolate and maintain in cultures due to their complex metabolic requirements and possible auxotrophy.

According to the generally accepted taxonomic scheme (LPSN – List of Prokaryotic names with Standing in Nomenclature; https://lpsn.dsmz.de), the class Chloroflexia is currently divided into two orders, the heterotrophic Herpetosyphonales and the phototrophic Chloroflexales. The latter includes two suborders, Chloroflexinea and Roseiflexinea (table 1). Chloroflexinea, in turn, is divided into the families Chloroflexaceae and Oscillochloridaceae.

| Suborder         | Family             | Genus          | Species                  |
|------------------|--------------------|----------------|--------------------------|
| Chloroflexinea   | Chloroflexaceae    | Chloroflexus   | Cfl. aurantiacus          |
|                  |                    |                | Cfl. aggregans           |
|                  |                    |                | Cfl. islandicus          |
| Oscillochloridaceae | Oscillochloris     | Osc. trichoides|                         |
|                  |                    | Osc. chrysea   |                          |
|                  | Chloronema         |                | Ca. Osc. fontis          |
| insertae sedis   |                    |                | Ca. Osc. kuznetsovii     |
| Roseiflexinea    | Roseiflexaceae     | Roseiflexus    | Roseiflexus castehholzii |
| insertae sedis or not validly published | Heliothrix |                | Heliothrix oregonensis   |
|                  | "Kouleothrix"      |                | Ca. Chloranaerofilum     |
|                  | "Kouleothrix aurantiaca" |              | "Ca. Chloranaerofilum corporosum" |

* – despite its correction to "Chlorothicoides" by Clappenbach and Pearson (2004), the name “Chlorothrix” is still overwhelming in the literature.

b – Eikelboom Type 1851 [32]; it was never formally described under the name Kouleothrix aurantiaca in microbiological publications, but named so in Genbank and was widely cited subsequently under this name.

c – the name is mentioned in [4] as "isolate similar to Oscillochloris sp. has been obtained in enrichment cultures, and tentatively named "Candidatus Chloranaerofilum corporosum" without formal description, and further taxonomic publications did not follow.

Candidate genera Chlorothix, Chloroploca, and Viridilinea were not assigned to any family, so they are designated as incertae sedis; but the original description of Ca. Lithoflexus emphasizes its proximity to the Roseiflexus clade [21], so it should be placed into Roseiflexaceae. According to all available nucleotide data, Ca. Chloroploca and Viridilinea form a monophyletic clade, which in different phylogenetic cladograms belongs either to Chloroflexaceae, in most phylogenetic trees constructed using 16S rRNA gene, or to the Oscillochloridaceae according to the data inferred from the concatenated amino acid sequences of the core proteins [18-20]. Another unusual species, Ca. Chlorolinea photomethanotrophicum, has been recently described from an iron-rich hot spring in Japan with unique iron-oxidizing microbial community. Although this species is known only from its metagenome-assembled genome (MAG), the authors arued for its ability to phototrophic oxidation of methane. This species belongs to the Chlorolinea/Viridilinea branch in the Chloroflexilinea suborder [31].

Phylogenetic position of the genera Ca. Chlorothrix and Heliothrix is even more uncertain. They were included into the suborder Chloroflexinea and family Roseiflexinea, respectively, solely on the basis of the pigment composition. But in most published phylogenetic trees, they both branched deeply inside Chloroflexinea and outside of known families. This may be illustrated by the phylogenetic tree
of all known species and candidate species of Chloroflexales and several cultured strains and uncultured clones built on the basis of sequences of their 16S rRNA genes shown at figure 1.

Figure 1. Phylogenetic tree of the class Chloroflexia, constructed by the Maximum Likelihood algorithm based on a comparison of the partial nucleotide sequences of the 16S rRNA gene from the Genbank database. The statistical significance of branching is obtained after 1000 replicates. Representatives of the classes Anaerolinea and Thermomicrobia are used as outgroups.

According to the tree, Ca. Chlorothrix and Heliothrix together form a separate clade ("Chlorothrix clade") that is sister to the Chloroflexilineae and unrelated to the Roseiflexilineae clade. Therefore, this clade probably has the rank of new family or even a suborder with two new families; its valid description required detailed analysis, including multigenic protein phylogeny.

Genus Heliothrix is apparently an endemic of the thermal springs of the Rocky Mountains; in public databases there is single sequence related to Heliothrix oregonensis at the genus level, the clone SM1D03 from Mammoth pool in Yellowstone National Park, USA (accession No. AF445672); all other 16S RNA sequences in Genbank have similarities <91%. Unfortunately, its type culture is lost, and
neotyping is not possible in absence of pure culture. Therefore, to clarify its taxonomic affiliation and metabolic capabilities, a complete or draft reference genome is needed, preferably from the original isolation site of this species. Candidate genus *Chlorothrix*, in contrast, have numerous related uncultivated sequences isolated from different saline, hypersaline and terrestrial habitats.

The taxonomic position of the planktonic genus *Chloronema* in also uncertain. *Chloronema giganteum* remains the only validly described species of planktonic Chloroflexales; the second simultaneously described species, *Chloronema spiroideum* [10] remained unrecognized by the International Committee on Systematics of Prokaryotes after the taxonomic revision of 1980, which established the starting point of modern prokaryotes taxonomy. The type strain of *Chloronema giganteum* was not assigned; for some time, the strain *Chloronema* sp. Gnsb1 [33] isolated from Little Long Lake (Wisconsin, USA) was used as a reference strain, but its characteristics significantly differed from the original description by Dubinina and Gorlenko [10].

However, along with *Chloronema* sp. Gnsb1, Gich et al [33] obtained 18 short partial SSU rDNA sequences from uncultured planktonic Chloroflexi clones, that were found to be polyphyletic by phylogenetic analysis. Nine of the clones are outside of class Chloroflexia and are most probably heterotrophic. Ten remaining sequences, however, also did not form a monophyletic group; eight of them form two separate branches inside the Oscillochloridaceae radiation suggesting a new genus within the family. The rest two clones (GNSB-6 and GNSB-7) formed a sister group to the whole class Chloroflexia.

Our analysis of 16S RNA amplicon libraries obtained from lakes of the Experimental Lake Area in Canada [34] and Evo forest area in southern Finland [35] have shown that among the OTU found in these lakes, 6 and 4 OTU, respectively, belong to Chloroflexales. These 10 OTU as well as the Chloroflexales from the USA lakes [33] did not form a monophyletic group (figure 2). So, the main conclusion remains the same: planktonic Chloroflexales do not form a monophyletic group and instead are scattered throughout the order; and the position of the organism that was originally described as *Chloronema giganteum* remains uncertain. A recent description of Ca. *Chlorohelix alloauthotrophica* ("Ca. Chloroheliales": Chloroflexi) [7], filamentous planktonic phototroph, morphologically more similar to the original description of spirale form of *Chloronema giganteum* than *Chloronema* sp. GNSB-1, further complicated the problem of planktonic Chloroflexales.

4. Ecology

4.1. Microbial mats

Most of Chloroflexales are inhabitants of (cyano)bacterial mats, its species are found by molecular genetic methods in almost any studied mat communities. However, majority of the described species are dwellers of extreme habitats; only *Oscillochloris* spp. (excluding Ca. *Osc. kuznetsovii*) prefer mesophilic temperature conditions and low to moderate salinity; the rest are either thermophilic (*Chloroflexus, Chloranaerophilus, Heliochloris, Roseiflexus*) or require high salinity (*Chlorothrix halophila*) and/or elevated alkalinity (*Viridilinea* spp., *Chloroploca asiatica, Lithoflexus mexicanus*).

Chloroflexales are an essential constituent of microbial mat communities due to their wide environmental plasticity. Most of them tolerate both oxygen and sulfide, at least at low concentrations; all are probably mixotrophs, capable of both photoheterotrophic (and even photolithoautotrophic) and organoheterotrophic metabolism, with different species preferring different positions at the photo/heterotrophy scale. For many studied species, H₂-dependent photolithothrophy was reported in culture conditions [36]; sulfide-dependent photosynthesis was also demonstrated for some species like *Osc. trichoides* and Ca. *Chlorothrix halophyla* and some strains of *Chloroflexus* spp.
Figure 2. Phylogenetic position of planctonic Chloroflexaceae clones in the suborder Chloroflexilinea. Clones "ELA 111314 OTU..." are from lakes of Experimental Lake Area (Ontario, Canada) [34]; clones "EvoLake...", from Evo area (Finland) [35]; clones ENRICH_116 and ENRICH_123 are from water column of Lake Baker and Lake Mud (Michigan, USA), respectively. 1, 2 3 – indicate monophyletic branches of planctonic FAP. Roseiflexus castenholzii is used as outgroup.

Chloroflexales, however, are rarely encountered by microscopic examinations of microbial mats. The main reason for this is their morphological similarity with other filamentous prokaryotes, primarily filamentous cyanobacteria of the families Pseudanabaenaceae, Leptolyngbyaceae (Synechococcales), Coleofasciculaceae and Microcoleaceae (Oscillatoriales) etc. Size characteristics of Chloroflexales species are so similar to small filamentous cyanobacteria that they look almost the same even when microscoping their colonies on solid substrates, while small cell size makes it difficult to distinguish their color. In the mid-1970s, Gorlenko and Pivovarova [11] suggested that many yellow-green colored cyanobacterial species described solely by morphological characteristics may be in fact representatives of Chloroflexales. They listed more than 20 species and subspecies, whose taxonomic affiliation deserved special research; unfortunately, these studies were never carried out.
4.2. Planktonic Chloroflexales
First described in the USSR from small Karelian lakes (lamba, from "lampi", pond in Finnish) on the first wave of interest to the "green non-sulfur bacteria" [10], *Chloronema giganteum* remains the only validly described species of planktonic Chloroflexales. Since than planktonic Chloroflexii are found in many other lakes, and in general, develop mainly at the oxic/anoxic border in lakes having low water mineralization (TDS) and low sulfate content, and usually elevated water color and iron content [10; 37-39 eqs.]. Microphotographies in [40] clearly demonstrate the variability of planktonic Chloroflexi.

Despite the development of molecular methods in the aquatic microbial ecology, our present concept of the planktonic Chloroflexales is still morphological at best; at present (18-3-2021) only 94 sequences are assigned to genus *Chloronema* in the SSU RNA sequence database [https://www.arb-silva.de/], and even among them a significant part was isolated from hot springs, wet soil, aerosols, and even from clinical material. Thus, the diversity and ecological distribution of the genus *Chloronema* remains mostly unclear.

In the end of the 20th century, a group of researchers from the Microbiology Laboratory of Institute of Aquatic Ecology, University of Girona (Spain), established that planktonic Chloroflexales develop and form maxima between oxycline (oxygen gradient zone) and redoxcline (zone of redox potential gradient) [40], and that they usually occur below the metalimic maximum of cyanobacteria and eukaryotic algae, simultaneously with species of purple sulfur bacteria (Chromatiaceae), but above the maximum of Chlorobiaceae [39]. The only representatives of Chlorobiaceae capable and/or even preferring to coexist with planktonic FAPs were the constituents of phototrophic consortia (symbiotic complexes) of green sulfur bacteria with immobile (*Chloroplana vacuolata*) and motile (*Chlorochromatium, Pelochromatium*) heterotrophic bacteria [41].

5. Filamentous anoxygenic phototrophs in the ecosystems of the Volga basin
The first detection of phototrophic Chloroflexi in the Volga basin occurred in 1975, when V M Gorlenko isolated enrichment cultures of mesophilic gliding phototrophic bacteria from lakes Chernyi Kichier and Konon’er (Mari ASSR, now the Republic of Mari El) [42]. By the complex of morphological characteristics, he assigned them to genus *Chloroflexus* as *Cfl. aurantiacus* var. *mesophilus*. However, since all known members of *Chloroflexus* genus are thermophilic, this organism probably represents a separate group of Chloroflexales [43]. Then a new species of "green non-sulfur bacteria", *Oscillochloris trichoides* comb. nov. Gorlenko and Korotkov, 1979 has been described from Lake Sernoe in Sernovodsk, Kuibyshev area (now Samara area) [12]. Another species of *Oscillochloris*, *Osc. chrysea*, was also detected on the fouling slides from the lake. And, finally, planktonic *Chn. giganteum* was found in mass quantities in the unique ferruginous meromictic Lake Kuznetchikha (republic Mari El) [37]. At this point, the initial, heroic, period of studying filamentous anoxygenic phototrophic bacteria in the Volga basin ended, and their further studies were transferred mostly to extreme habitats.

In 2004, we found benthic Chloroflexales in the microbial mats of several sulfide springs and the reservoirs formed by them in the northeastern part of the Samara area (including Lake Sernoe, the type habitat of *Osc. trichoides*), and identified them by morphology as species of *Oscillochloris* [44]. In further studies of these springs in 2009 and 2013, the wide filaments corresponding by morphology to *Osc. chrysea* were not detected, but forms similar to *Osc. trichoides* continued to encountered in most samples.

In 2020, we studied the diversity of 16S-RNA genes in the microbial mats of the Solodovka wetland, one of the sulfide waterbodies in the northeastern Samara area. According to the results (in preparation), organisms distant from all known Chloroflexales genera (similarity <90%) prevail in the studied mat samples among the phototrophic Chloroflexi, while clones related to *Osc.trichoides* are present in samples only in minor quantities.

In 2005, the development of *Chn. giganteum* have been found in the plankton of the karst lake in the middle course of the Ik River [45]; then we detected this species in several lakes of the Raifa area of the Volzhsko-Kamsky nature reserve [46], and later in the Lake Kuznechikha [47], Lake Zelenoe (Vuver-Er) [48] and in several other polyhumic lakes of the Mari Polesye (unpublished data). In all cases,
however, the identification of these species was carried out on the basis of morphological characteristics in natural material. Attempts to cultivate the planktonic forms of Chloroflexales failed even at the enrichment stage, and enrichment cultures from microbial mats were quickly lost during subculturing.

The vertical distribution of planktonic filamentous phototrophs in studied lakes of the Volga basin has always followed the pattern established by Abella and coworkers [39-41]. In all cases, "Chloronema" was found in the intermediate zone between the layer of oxygen exhaustion and the redox step; its distribution coincides with Chlorobiaceae consortia and several small-celled Chromatiaceae, but the relation was unilateral, meaning that the presence of the consortia or/and Chromatiaceae were not predicting the presence of Chloroflexales.

Unfortunately, in the course of the literature search, we have not found any publications on the presence of filamentous anoxygenic phototrophic bacteria in the water bodies of the Upper Volga and the southern part of the Volga basin. Probably, our group is currently the only team conducting research on anoxygenic phototrophic bacteria in the Volga basin.

6. Further perspective and conclusion
Filamentous anoxygenic phototrophic bacteria have apparently important, though not fully understood functions in the ecosystems in which they are present. Although they are generally less common and/or diverse than purple or green sulfur bacteria (Chromatiaceae and Chlorobiaceae), there are a number of ecosystems in which they dominate over all other anoxygenic phototrophs and even compete with cyanobacteria.

The Volga basin is a vast territory with a significant gradient of natural conditions both in the latitudinal direction and from west to east. These gradients, of course, also determine the abiotic conditions in the water bodies of the region. The northern and northwestern parts of the basin are characterized by a high density of small lakes with high humus water, most of which are deep and stratified in summer. In such lakes in the basins of the Oka and the Upper Volga, the development of planktonic filamentous anoxygenic phototrophs is very likely.

The development of Chloroflexales, including new species, is quite expected, for example, in the microbial mats of saline rivers and hypersaline lakes of the Lower Volga region. However, it should be emphasized that, since the prokaryotic communities of "non-extreme" microbial mats are largely unexplored, their study can also yield new and unexpected findings.

However, the perspectives of purely morphological methods in aquatic microbial ecology have been exhausted. Further progress in the field will be possible only with the use of molecular methods either alone or in combination with traditional methods (polyphasic approach). 16S-RNA-barcoding will make it possible to clarify the biogeography of prokaryotic species, which seems to be very important and relevant to the phototrophic Chloroflexi, which consists of a few widespread species. This may be especially fruitful for the study of planktonic phototrophic Chloroflexi, since they rise many questions considering both old [32] and new [7] data. Metagenome-assembled-genome (MAG) construction become de facto the only way to describe new candidate species, and also may be extremely useful as a source of information about species physiology, which is especially important given the problems of cultivation of most Chloroflexales.

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