

Ever since Antonie van Leeuwenhoek's discovery of the light microscope in the 17th century humans have been fascinated by the world of microorganisms. For more than 300 years researchers have been laboriously and with determination describing and trying to understand these commonly occurring organisms that have great influence on our existence. Meanwhile large-scale contemporary sampling campaigns such as TARA (<http://oceans.taraexpeditions.org>) or BIOMARKS (<http://www.biomarks.eu>) reveal just how limited our knowledge is of the overall biodiversity of protists, their spatial distributions and temporal dynamic.

One of the least researched groups of microorganisms is the autotrophic euglenids, single-cell algae, that appeared more than a billion years ago. All green euglenids are mixotrophs (despite their ability to conduct photosynthesis, they also feed by means of osmotrophy) and therefore appear in large numbers in eutrophic and hypereutrophic waters (e.g. fish-, field-, farm- or urban ponds) and although their role is not yet fully identified there is evidence that they partake in self-cleaning of waters. In recent years reports of toxic species (*Euglena sanguinea*) have appeared. By producing ichthiotoxins, it led to mass deaths of fish in fish breeding ponds in Northern Carolina causing huge material losses. Research on paramylon (storage material) in *Euglena gracilis* showed its cancer countering and regenerative activities of liver and skin cells in experimental animals. The cells of another species (*Euglena viridis*) have been revealed to possess active antibacterial compounds. Wax esters accumulated during mitochondrial anaerobic fermentation has potential as a biofuel. Euglenids accumulate a large amount of nutraceuticals, cosmeceuticals so their biomass has a large nutritional value. The given examples show only to a small extent the great capabilities of those unique organisms that could be used by mankind. Unfortunately further studies of euglenids have so far been very difficult, laborious and time-consuming due to the large number of described species (more than a thousand), problems with identification and a lack of reliable information concerning their ecology or biodiversity. The application of the efficient next-generation sequencing method (NGS) combined with advanced bioinformatic tools is an unprecedented opportunity to change this situation thanks to provision of huge amounts of new data in a relatively short time. So far molecular studies of species diversity and richness assessment in environmental samples have shown that eukaryotic microorganisms are far more diverse than has been previously thought. However the true scale and the distribution of this diversity is still unknown, and so are its patterns, spatial and temporal dynamics and ecological role. Most contemporary field studies of eukaryotic microorganisms have concerned oceans and despite the global distribution and ecological importance of smaller, shallower and often ephemeral freshwater ecosystems there is almost no information on the diversity of small protists in them. Furthermore several groups of eukaryotic microorganisms – among them euglenids which are frequently dominant in freshwater ecosystems – are known to be underrepresented in the environmental sequencing surveys. This is probably due to their highly divergent and long V4 18S rDNA sequences, that are commonly used as DNA barcodes for molecular species identification and in euglenids are difficult to amplify by universal methods. Therefore an individual approach for metabarcoding studies of that group is proposed in our project. We plan the use of a customized DNA barcode marker (V2 region of nSSU rDNA) which enables identification of autotrophic euglenid species in environmental samples. Analysis of their biodiversity and species richness in different ecosystems will also be conducted. To realize this aim plankton samples will be taken several times during the vegetative season over the span of three years from 12 selected bodies of water representing the two types of ecosystems most typical for autotrophic euglenids (fish-, farm-, urban- and field ponds) differing in both the physico-chemical parameters of the water (the amount of organic matter and mineral substances) and location (Mazovia, Mazurian Lake District, Wielkopolska). During each sampling basic water parameters will be measured. Subsequently the species identification based on molecular features, next generation sequencing and advanced bioinformatics tools will be conducted to assess the biodiversity and species richness in analyzed ecosystems. The results will be compared with the species composition obtained by the traditional morphological approach taking into account the water parameters, type of water body and its location using statistical analysis.

We expect that the planned research will result in (1) better and faster recognition of species richness, dominant species and biodiversity of autotrophic euglenids in eutrophic environments and definition of their dynamics and the factors affecting them, (2) identification of bioindicators existing in such extreme ecosystems which may prove useful in biomonitoring. We also expect to (3) discover euglenid species new to both Poland and science, (4) recognize genetic diversity of green euglenids representing various phylogenetic lines, especially intraspecific diversity of many clads on phylogenetic trees, at present represented merely by single sequences, (5) discover new phylogenetic lines (genus) of autotrophic euglenids. The studies will be carried out in Poland only, but taking into account the fact that euglenids are cosmopolitan (like most of microorganisms) the results obtained will have universal character.