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Floristic-taxonomic study of the Euglenophytes
Implications for their ecology, distribution and practical protection

Ph.D. Thesis

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■ ANNOTATION

This thesis is focused on study of photosynthetic euglenophytes (Euglenoida, Euglenophyceae, Euglenales) in the Czech Republic and Poland. In the introductory part, euglenoids are presented as a group of organisms along with their taxonomy, ecological role and their inhabited biotopes together with information about their distribution on global and local scales. The results and implications for the euglenoid ecology, distribution and practical protection are shown in the rest of the thesis. Main results are based on a long-term research (2007-2014) using several literature sources in combination with field sampling, which brings data about taxonomy, ecology and rarity of 284 taxa of the Czech Republic's algal flora with two new records of rare and interesting euglenoid taxa, *Trachelomonas bituricensis* var. *lotharingia* and *Trachelomonas saccasii*. The results of projects published in this thesis are focused on the study of (1) euglenoid ecology connected with small and shallow water bodies (former clay-pit pond in the eastern Poland, small mesotrophic pond in the southern part of the Czech Republic) which brings interesting data about euglenoids' ecological preferences; (2) taxonomic conclusions about common euglenoid species, *Trachelomonas caudata*, occurring in natural populations, where results show that several morphologically similar species probably form a part of "caudatae complex" and they are synonymous with *T. caudata*. Aggregate data on ecology and the distribution of euglenoids in the Czech Republic are used in the Red List of these algae as the beginning of the pilot work of the Red List of microscopic algae as a guideline for the practical protection of biotopes.

■ **DECLARATION** [In Czech]

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České Budějovice, 2nd January 2019

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Mgr. Josef Juráň

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*“Coming together is a beginning;
keeping together is progress;
working together is success.”
Henry Ford*

“THANK YOU ALL...”

... each of my colleagues, friends and family will find themselves in these few words. Everyone knows how supported me and what role played, even if it was just a minor role, I appreciate all of you for it!

■ LIST OF ARTICLES AND AUTHOR'S CONTRIBUTIONS

The thesis is based on the following papers (listed chronologically):

Juráň, J. 2016. *Trachelomonas bituricensis* var. *lotharingia* M.L. Poucques 1952, a morphologically interesting, rare euglenoid new to the algal flora of the Czech Republic. *Phytokeys* 61:81–91. (IF = 1.20)

Wołowski, K., Poniewozik, M. & **Juráň, J.** 2016. Morphological variability of loricae in *Trachelomonas caudata* complex (Euglenophyta). *Cryptogamie, Algologie* 37:97–108. (IF = 1.11)

In this paper Josef processed the basic morphometric analysis on the loricae, processed data statistically and participated in the writing of manuscript and the figures composition.

Juráň, J. 2017. The checklist of photosynthetic euglenoids (order Euglenales) of the Czech Republic: ecology, taxonomy, distribution. *Phytotaxa* 317:1–16. (IF = 1.19)

Juráň, J. & Couté, A. 2018. African *Trachelomonas saccasii* found in a European mesotrophic pond (Czech Republic). Implication for euglenoid biogeography and recommendations for euglenoid flagship species. *Phytotaxa* 334:201–214. (IF = 1.19)

Josef was the initiator of this project: he documented and characterized the target species, designed flagship species for further studies of the euglenoid biogeography and wrote the main part of the manuscript.

Poniewozik, M. & **Juráň, J.** 2018. Extremely high diversity of euglenophytes in a small pond in eastern Poland. *Plant Ecology and Evolution* 151:18–34. (IF = 1.14)

In this project Josef participated in field sampling in the 2014 season, euglenoids determination in optical and electron microscopy and on the writing of manuscript and the figures composition.

Juráň, J. & Kaštovský, J. (manuscript). Do we need Red List of microalgae? The procedure of compiling the Red List of microscopic algae of the Czech Republic – Euglenophyta model.

In this project, Josef had an original idea to propose criteria for evaluation of the level of threat of species of microalgae, he participated in the designation of methodology and testing of criteria on his own dataset about euglenoids, he wrote the main part of the manuscript.

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GENERAL INTRODUCTION

■ GENERAL INTRODUCTION

What are euglenoids?

Euglenoids are single-cell flagellates reproducing only by simple division with differences in their nutrition from osmotrophy or phagotrophy to mixotrophy and photoautotrophy. In addition, near all of them are free living, only several species are sessile, colonial or endozoic. These worldwide-distributed organisms inhabit most of freshwater habitats, a small number of taxa is living in brackish or marine biotopes and a small number of these organisms could be found in edaphon (Leander *et al.*, 2017, Leedale, 1967, Wołowski & Hindák, 2005).

Cells of euglenoids have characteristic eukaryotic structure with some alterations typical for this group of organisms. A common feature of all euglenoids is their surface – pellicula. Pellicula is built from the system of protein strips with developed structures, which allows the strips to engage with each other. The degree of interconnection of the pellicle strips determines cell rigidity – from a metabolic cell of some *Euglena* species to semi-rigid species and to fully rigid genera such as *Phacus* or *Monomorpha* (Barsanti & Gualtieri, 2006, Leedale, 1967). The number of pellicular strips, their orientation and arrangement have an evolutionary pattern (Esson & Leander, 2009, Leander *et al.*, 2001, 2007). A different number of flagella ordinarily realizes movement of the euglenoids; the number is varying from 1 to 7. What is typical for the photoautotrophic euglenoids from the order Euglenales is one emergent flagellum and second short flagellum, which is located in the reservoir. Flagella of euglenoids have a typical structure and is covered with mastigonemes (Leedale, 1967). Similarly, even though stigma was originally thought to be the photoreceptor, it has been later identified that the paraxonemal/paraflagellar body is a light responsive organelle, which is connected to the flagella. Euglenoids respond to light with photophobic responses – photokinesis and phototaxis. The light responses operate synergistically with gravitaxis, aerotaxis and other response (Häder & Iseki, 2017).

The nutrition of euglenoids is very varied. Some representatives of genera are osmotrophic (they absorb the organic molecules), several genera employ phagotrophy (consumption of particles, including other cells) via specialized feeding apparatus with two main nutrition strategies – bacterivory and eukaryovory, and quite a large number of euglenoids is phototrophic

(Leander *et al.*, 2017). Moreover, a phototrophic genus *Rapaza* with mixotrophy was recently found (Yamaguchi *et al.*, 2012). Photoautotrophic euglenoids contain in their cell chloroplast obtained during the event of secondary endosymbiosis from the green alga related to the prasinophyte genus *Pyramimonas* (Vanclová *et al.*, 2017). Chloroplasts consist of three membranes, thylakoids are usually grouped into threes forming lamellae, photosynthetic pigments are chlorophylls *a* and *b* with carotenes and xanthophylls; chloroplasts typically occur in a large amount per cell and show diversity of size, shape and morphology, some of them contain pyrenoid (Barsanti & Gualtieri, 2006). Some photoautotrophic species have secondarily lost their plastids, e.g. *Euglena longa* (Záhonová *et al.*, 2016), *E. quartana* (Marin, 2004, Hadariová *et al.*, 2018) and *Lepocinclis cyclidiopsis* (Bennett & Triemer, 2014). These species are representatives of secondary osmothrops (Vanclová *et al.*, 2017). The storage product of euglenoids is paramylon (β -1,3-glucan, a polysaccharide from glucose units), which is stored in the form of grains of the various sizes and shapes (Ciugulea & Triemer, 2010). According to Monfilis *et al.* (2011), paramylon can be used at the generic level to support major clades and generic relationships.

Several euglenoid genera, *Colacium*, *Euglena*, *Strombomonas* and *Trachelomonas*, are able to produce polysaccharide mucus (e.g. detailed study by Barnes *et al.*, 1986, Dunlap *et al.*, 1986, Rosowski & Willey, 1977; summed up in Leedale, 1967 or Linton *et al.*, 2017). Some *Euglena* species are able to produce resting palmeloid stadia or cyst, their formation is connected to the presence of the muciferous bodies under the pellicle of these species (Karnkowska *et al.*, 2015, Zakryś *et al.*, 2017). What is well studied is the formation of cysts by *Euglena agilis*. These cysts have morphology similar to *Trachelomonas loricae* (Hindák, 1982, 1986) and the later study of some neustonic *Euglena*-species was published by Hindák *et al.* (2000). Another studies suggested that mucocyst shape should be species-specific, as is shown in the study of euglenoid species with the stellate chloroplasts (Kosmala *et al.*, 2009). Other listed genera show a production of the extracellular muciferous projections – stalks or loricae. Formation of the muciferous stalks or “pillows” in the genus *Colacium* is well documented (e.g. Killen *et al.*, 1984, Rosowski & Kugrens, 1973, Rosowski & Willey, 1977, Willey 1984), *Colacium* forms these structures on several substrates, e.g. zooplankton, filamentous and planktonic algae (Starmach, 1983). In some species, these formations are incrustated with iron, e.g. “pillows” of *C. cyclopicola* or stalks of some *C. vesiculosum* eco-

morphs (Poniewozik, 2017). Genera *Strombomonas* and *Trachelomonas* form from the mucus loricae around their cells, these loricae contain a higher number of inorganic ions, e.g. silica, iron and manganese (e.g. Barnes *et al.*, 1986, Dunlap *et al.*, 1986, Pereira *et al.*, 2003, Poniewozik, 2017). Formation of the loricae and their composition is well studied (e.g. Leedale, 1975, Pringsheim, 1956, Singh, 1956a,b). Shape, surface and other features are species-specific and they are still the main characteristics in morphology-based taxonomy of these genera.

Phylogeny of the euglenoids

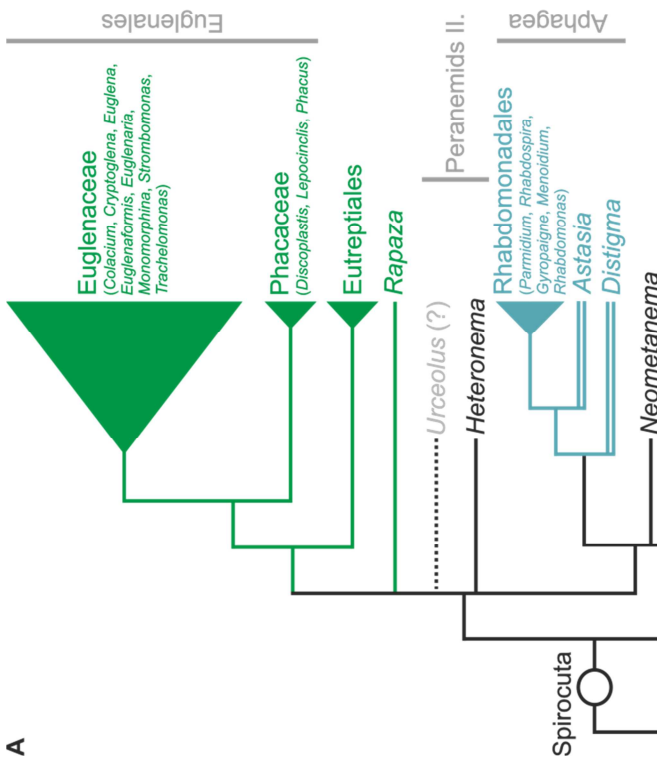
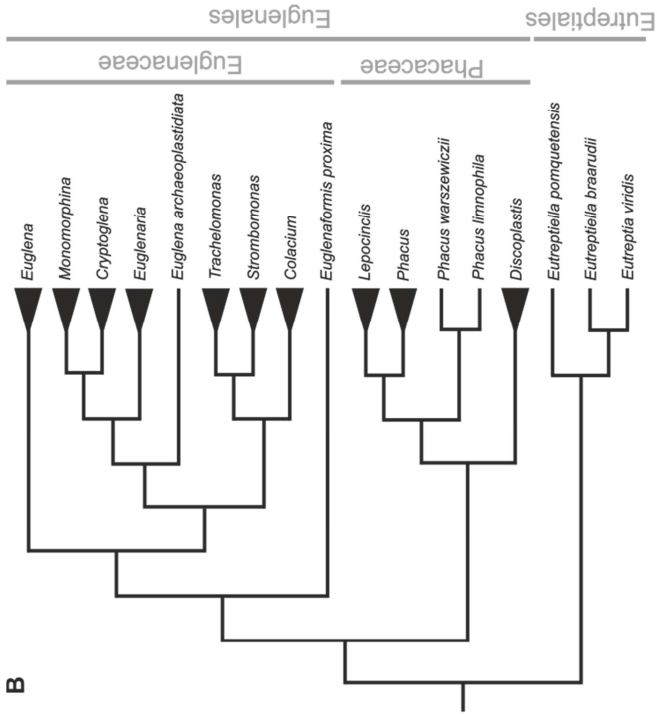
Traditional view of the euglenoids relationships was based especially on the number of flagella and the nutrition. Alternatively, another morphological features were designed by Leedale (1967) and euglenoids were divided into six main orders: Euglenales, Eutreptiales and Euglenamorphales which contain mainly photosynthetic genera and some colourless species. The rest of colourless genera are from the orders Rhabdomonadales (osmotrophic genera), Sphenomonadales (osmotrophic or phagotrophic genera) and Heteronematales (phagotrophic genera with feeding apparatus). Series of molecular studies show that relationships between euglenoids are not as clear as it seems according to morphologically based taxonomic studies. Euglenoids are close relatives to the kinetoplastids and diplomonids. Together these organisms form a group Euglenozoa (Triemer & Farmer, 2007, Cavalier-Smith, 2016) and current knowledge of the evolutionary relationships of euglenoids is shown in the Figure 1. Photoautotrophic euglenoids are a monophyletic group with the basal mixotrophic genus *Rapaza* (Karnkowska *et al.*, 2015, Kim *et al.*, 2015). Photoautotrophic euglenoids, together with some colourless groups (Peranemids, Anisonemids, Aphagea group and *Neometanema*), belong to the bigger group Spirocuta with spiral pellicle strips arrangement as synapomorphy (Cavalier-Smith, 2016). Phylogenetic relationships of the colourless groups seem to be a little bit complicated as is visible in the Figure 1A, in which some genera are polyphyletic (e.g. *Astasia* and *Distigma*). The position of several groups is not clear and some genera still miss molecular data for the evaluation of their position in this tree (e.g. *Urceolus* and *Sphenomonas*).

From the point of view of this dissertation, it is more fundamental and interesting to describe a situation within photoautotrophic euglenoids (see Figure 1B). Photosynthetic euglenoids form monophyletic lineage divided into two

orders: Eutreptiales including marine and brackish genera – *Eutreptiella* and *Eutreptia* – with characteristic two or four flagella (Bicudo & Menezes, 2016, Yamaguchi *et al.*, 2012) and Euglenales with the dominance of freshwater species belonging to the common algal communities, e.g. plankton, mephyton and neuston (Wołowski & Hindák, 2005). In this order, Kim *et al.* (2010) established new family Phacaceae including genera *Discoplastis*, *Lepocinclis* and *Phacus*, the rest of euglenales genera belong to the family Euglenaceae (name designed by Dujardin, 1841, confirmed by Silva, 1980). A common feature of genera from Phacaceae family is the presence of small discoid chloroplasts without pyrenoids and 32 pellicle strips (Kim *et al.*, 2010, Leander *et al.*, 2001, 2007). *Phacus* and *Lepocinclis* have similarities in pellicle strips arrangement and of dimorphic paramylon grains (Monfilis *et al.*, 2011). *Discoplastis* seem to be a monophyletic sister group to the *Phacus* and *Lepocinclis* (Karnkowska *et al.*, 2015, Triemer *et al.*, 2006). Two *Phacus* species (*P. warszewiczii* and *P. limnophila*) branched independently from other species of the genus and they are characterized by morphological differences: *P. warszewiczii* has longitudinally twisted cell body having here curved ridges; *P. limnophila* has elongated spindles shape and two long, straight, rod-shaped paramylon grains (Kim *et al.*, 2015).

In the Euglenaceae group, several lineages have developed – *Euglenaformis proxima* as basal organisms of this group, *Trachelomonas-Strombomonas-Colacium* lineage, *Monomorphina-Cryptoglena-Euglenaria* lineage and *Euglena*-lineage (Karnkowska *et al.*, 2015).

► **Figure 1.** Phylogenetic relationships within euglenoids and related organisms. (A) Current knowledge of the evolutionary tree of euglenoids, based primarily on SSU rRNA gene phylogenies, photoautotrophic taxa are shown in green, primary osmotrophs in blue, “typical” phagotrophic taxa in black, and symbiontids in orange, taxa shown in gray are important taxa whose positions are inferred from morphological information (no molecular data are currently available), double lines on a branch represent paraphyletic groups (according to Leander *et al.*, 2017, modified and simplified); (B) Schematic diagram of phylogenetic relationship of the phototrophic euglenoids, based on multigenes (nSSU, nLSU, cpSSU, hsp90 and psbO) Bayesian analysis (according to Karnkowska *et al.*, 2015, modified and simplified).



The group including loricate genera *Trachelomonas* and *Strombomonas* together with *Colacium* seems to be monophyletic. This relationship is not supported only by the molecular data (Karnkowska *et al.*, 2015, Ciugulea *et al.*, 2008), but there is also morphological synapomorphisms: 40-48 pellicle strips (Leander *et al.*, 2001, 2007) and the production of extracellular mucosal structures – loricae and stalks (see above). Divergency between *Trachelomonas* and *Strombomonas* was studied by Deflandre (1930), who established *Strombomonas* as a new independent genus based on the differences of lorica morphology. According to the results of Marin *et al.* (2003), these genera were synonymous, but the work of Ciugulea *et al.* (2008) supported the hypothesis about independence of these genera and their sister position.

Genus *Euglena*, according to the traditional morphology-based concept, seemed to be polyphyletic and it was supported by several studies using molecular methods (Karnkowska-Ishikawa *et al.*, 2011, 2012, 2013, Kosmala *et al.*, 2005, 2009, Marin *et al.*, 2003, Wang & Chen, 2004, Zakryś *et al.*, 2001, 2002, 2004). As the results of these studies showed, several new genera from this genus were separated, e.g. *Discoplastis* (Triemer *et al.*, 2006), *Euglenaformis* (Bennet *et al.*, 2014) and *Euglenaria* (Linton *et al.*, 2010), and several species were moved to another genera, e.g. *Euglena acus*, *E. oxyuris*, *E. spirogyra*, *E. tripteris* to the genus *Lepocinclis* (Marin *et al.*, 2003) and *Euglena limnophila* to the genus *Phacus* (Linton *et al.*, 2010). As well as two species of *Euglena* that fall outside *Euglena* clade: *E. archaeoplastidiata* and *E. velata* (Karnkowska-Ishikawa *et al.*, 2012, Kim *et al.* 2010, 2015, Kim & Shin, 2008).

Common features for the monophyletic lineage of *Monomorphina* and *Cryptoglana* genera are rigid pellicle from small number (15-16) of broad pellicle strips and cell containing only one plastid (Leander *et al.*, 2001, 2007).

Genus *Euglenaria* seems to be monophyletic with some morphological features (e.g. lobate plastids with diplopyrenoids) similar to those other *Euglena* species, but there is a distinction in molecular signatures in nuclear SSU rDNA sequences (Linton *et al.*, 2010, Karnkowska-Ishikawa *et al.*, 2012). According to Leander *et al.* (2017), the phylogenetic position of that *Euglenaria*-lineage is not well resolved.

Euglenoids taxonomy – main problems and tasks in Euglenales

The research of euglenoids and all other microorganisms is connected to the development of a microscope and its gradual improvement since the 16th century, which is traditionally associated with Antoni van Leeuwenhoek (1632-1723) or Robert Hooke (1635-1706). The first mention of observation of *Euglena* is attributed to Antoni van Leeuwenhoek – “These animalcules had divers colours, some being whitish and transparent; others with green and very glittering little scales; others again were green in the middle, and before and behind white; others yet were ashen grey” (Dobell, 1932). The first description of the photosynthetic euglenoids was made by Ehrenberg at the beginning of the 19th century – *Euglena* (Ehrenberg, 1830), *Cryptoglena* (Ehrenberg, 1832), *Colacium* (Ehrenberg, 1834) and *Trachelomonas* (Ehrenberg, 1834). During the 19th century, other traditional genera were described – *Phacus* (Dujardin, 1841), *Lepocinclis* (Perty, 1849), *Monomorphina* (Mereschkowsky, 1877) and *Ascoglena* (Stein, 1878), the last traditional genus *Strombomonas* was described at the beginning of the 20th century by Deflandre (1930). Studies of euglenoids, as well as other algal or protistian groups were based on morphological features, which were the key factor of the taxonomic studies of euglenoids during the whole 19th and most of the 20th century. During that relatively long research period, a huge number of species and intraspecific taxa in these listed genera were described. According to the AlgaeBase (Guiry & Guiry, 2018), a common-used source about algal diversity and taxonomy, there was 1553 species described in these nine genera from which about 60 % have been flagged as accepted taxonomically, on the intraspecific level 1956 taxa are in the database listed. These numbers suggest that the question of taxonomic position or validity of a huge number of species and infraspecific taxa is still a challenge for the further research. This problem of a high number of described species and taxa was simply commented by Pringsheim (1953), who explains that certain authors could not resist the temptation to give a name to every minor deviation from the typical form previously described or prevailing at same or similar places. The huge morphological variability could be probably explained by using two main factors: (1) the impact of environmental conditions to the euglenoid morphology and (2) the role of the ontogeny stage in which species is observed.

Several studies support the theory of the environmental impact on the euglenoid morphology. Conforti (1998) studied morphological changes of several euglenoids (*Lepocinclis acus*, *L. spirogyroides*, *Monomorphina pyrum*,

Phacus curvicauda and *P. tortus*) in response to the organic enrichment of laboratory cultures of these algae. Results show cell deformation, the extraordinary accumulation of paramylon grains and the development of larger paramylon grains in higher frequency than which is typical for euglenoids from enriched cultures. An organic enrichment of medium has a significant impact on shortening and widening of cells of *Lepocinclis acus*, observed in cultures by Conforti *et al.* (2017). In addition, similar results were obtained in the study realized by Nannavecchia *et al.* (2014) on the culture of *Phacus brachykentron*. Moreover, similar results have been shown in studies on a natural condition, e.g. Bauer *et al.* (2012) designed bioassay based on *Lepocinclis acus* and *Acutodesmus acutus* as model organisms in order to study the impact of the chemical and textile industry on the environment of river in Argentina. Their results, again, show significant increase in the cellular volume for species together with abnormal shape in the studied sites.

Second important factor which could explain a huge number of described species and infraspecific taxa is the variability of euglenoids during their ontogeny. The morphological variability of the several species of the genus *Trachelomonas* was studied by Pringsheim (1953) and Singh (1956a,b). Their results show that the *Trachelomonas* species studied in the clonal cultures exhibit a morphological variability on their monads as well as their loricae during the life cycle. Authors conclude that this variability is a rather complicating factor in describing new species from natural populations, because descriptions of new taxa were based on few individuals from the population without deeper study of their life cycle at all. Owing to the modern research, the role of ontogeny is well documented in the work focused on the common euglenoid species *Monomorphina pyrum* by Kosmala *et al.* (2007), who provided a revision of *M. pyrum* and morphologically similar taxa using a morphological-molecular approach and one of the results was synonymization of several *Monomorphina* taxa with *M. pyrum*. Authors support their results by detailed discussion about reasons why such a high number of morphologically similar organisms were described by different authors as separate species. The description of these species was based on differences from the “typical” *M. pyrum*, e.g. the presence of two chloroplasts, the absence of large paramylon grains, and the degree of cell flatness. Authors connect this description with the observation of *M. pyrum* in different stages of its life cycles and support this claim with a detailed description of changes in *M. pyrum* during ontogeny. The

second study connected with the variability of euglenoids during their ontogeny is the study of natural populations of the common *Trachelomonas caudata* species (Wołowski, *et al.*, 2016, **PAPER IV**). Authors used basic morphometrical data in the combination with the literature sources to compare *T. caudata* with morphologically similar taxa (*T. caudata* f. *pseudocaudata*, *T. fusiformis*, *T. allorgei*, *T. mollesta* and *T. bernardinensis*) – as a result of this comparison, authors claim that all studied species should be synonymized with the *T. caudata*. According to this study, we could assume that the description of these species was connected with the observed stage of *T. caudata* life cycle as was shown in the previous study about *Monomorpha pyriformis*.

In the past, several authors tried to solve some problems of the euglenoid taxonomy and several revisions were published, e.g. Chu (1947), Gojdics (1953), Pochmann (1942) or Pringsheim (1956). In addition, some revisions and taxonomical conclusions are part of monographs focused on euglenophytes, e.g. Huber-Pestalozzi (1955), Wołowski (1998) and Yamagishi (2013, 2016). Furthermore, all of these revisions are based on morphological features comparison using data from optical or electron scanning microscope. Additionally, they are sometimes supplemented with information about the structure of some organelles using cytochemical reactions or ultrastructure using transmission electron microscope. Despite the large number of taxonomic works, the situation in the taxonomy of euglenophytes is still not satisfactorily solved in many cases.

The solution to these taxonomic problems came with methods of molecular phylogenetics at the turn of the 20th and the 21st centuries. Zakryś *et al.* (2002) resolved problem of *Euglena geniculata* and *E. myxocylindracea*, which are according to authors' results genetically and morphologically identical; Marin *et al.* (2003) studied euglenoids phylogeny based on SSU rDNA sequences, authors renewed the genus *Monomorpha*, which was a sub-part of the genus *Phacus* and made changes in the taxonomic position of common worldwide members of the genus *Euglena* (*E. acus*, *E. oxyuris*, *E. spirogyra*, *E. tripteris*) to the genus *Lepocinclis*; Shin & Triemer (2004) focused on a type of species of the genus *Euglena* – *E. viridis*; Zakryś *et al.* (2004) studied isolates of the common *Euglena agilis* using combination of ITS2 of extrachromosomal rDNA and the chloroplast SSU rDNA sequences. During the last ten years, several revisions were made in the number of euglenoid genera, e.g. *Euglena* sensu lato (Bennet *et al.*, 2014, Karnkowska-Ishikawa *et al.*, 2011, 2012, 2013,

Kosmala *et al.*, 2009, Linton *et al.*, 2010, Triemer *et al.*, 2006), *Lepocinclis* (Kosmala *et al.*, 2005), *Monomorphina* (Kosmala *et al.*, 2007b, Nudelman *et al.*, 2005) and *Phacus* (Karnkowska-Ishikawa, *et al.*, 2010, Kosmala *et al.*, 2007a, Łukomska-Kowalczyk, 2015). The question about cryptic species diversity is studied and discussed in several works, Kim *et al.* (2013a, b) described quite a high number of cryptic species in the genus *Monomorphina* and *Cryptoglena*; Kim & Shin (2014) studied the cryptic diversity in the genus *Phacus* with the description of seven new species and the work about morphological and genetic diversity of *Euglena deses* group with an emphasis on cryptic species was published by Kim *et al.* (2016).

The effort to understand the taxonomy and phylogenetic relationships of euglenoids also brings new methods. Bennet & Triemer (2012) designed new methods for obtaining nuclear gene sequences from field samples. Authors use this method successfully in the study of position of *Lepocinclis horridus* (formerly *Phacus horridus*) and *Lepocinclis helicoideus* (formerly *Euglena helicoideus*). This method could solve the problem with the complicated or impossible cultivation of some species and the inability to work with cultures of these organisms. Łukomska-Kowalczyk *et al.* (2016) focused on the use of barcoding in the photosynthetic euglenoids and, as a result of their studies, authors suggested two molecular markers (COI and 18S rDNA) as potential DNA barcodes. Despite a significant progress in this area, there is still a relatively high number of species complexes together with some genera, e.g. *Colacium*, *Strombomonas* and *Trachelomonas*, where molecular data is insufficient or fully missing – these problems bring tasks and challenges for further researches.

Ecological role of euglenoids and their inhabited biotopes

Euglenoids are a common part of planktonic communities in almost all freshwater biotopes and their occurrence is, in general, connected to high-nutrient contents. According to Reynolds *et al.* (2002), euglenoids are typical members of small organic pond communities with high biochemical oxygen demand. In these communities, euglenoids are present together with green alga *Gonium* or chyrophyte genus *Synura*. Additionally, we could find euglenoids in another enriched habitats, e.g. in shallow enriched lakes, ponds and rivers together with chlorococcal algae, in shallow enriched turbid waters together with diatom *Synedra acus* or in habitats with shallow mixed layers in enriched

conditions together with chlorococcal algae (Reynolds *et al.*, 2002). These generally valid claims are well shown on the work focused on euglenoid biodiversity in a small and very shallow former clay-pit pond in Poland (Poniewozik & Juráň 2018, **PAPER V.**). Authors studied this biotope, assessed during the period 2002–2004 and in 2014, and reported 63 euglenophyte taxa from this pond, where euglenophytes, with a few exceptions, were the only group inhabiting the pond. Results of **PAPER V.** (Poniewozik & Juráň, 2018) show that the genus *Trachelomonas* was the most numerous and diverse. This observation was unexpected due to physical chemical conditions in the pond during the study (high-nutrient water, especially rich in ammonium salts), which is more characteristic for species of genera *Euglena* and *Phacus*. According to the literature, *Trachelomonas* prefers less fertile and less polluted conditions and, in addition, Reynolds *et al.* (2002) present the genus *Trachelomas*, which is a typical representative of planktonic communities of shallow mesotrophic lakes. This phenomenon is explained by authors who claim that euglenoids may survive in an unfavourable environment due to fast reproduction, cysts formation and mixotrophy as a mode of nutrition, according to results of Płachno *et al.* (2015).

There is common knowledge that the species richness of any pond is a function of the number of processes operating at the different spatial and temporal scales, e.g. factors such as colonization and extinction together with abiotic and biotic interactions in the local environment (Brönmark & Hansson, 2009) and the structure of a community is the result of all of these factors together. There is common knowledge that the decline of species richness and nutrient enrichment are two of the most driving forces of changes in ecosystems on global scale (O'Connor *et al.*, 2015). The main factor associated with the decline of species richness is eutrophication, which is a complex process associated with not only a change in algal biomass but also with a change in biodiversity, connected especially with increasing amounts of total nitrogen and phosphorus in freshwater habitats (Gilbert, 2017). The effect of increasing phosphorus concentration on species richness is shown by Jeppesen *et al.* (2000) using data from 71, mainly shallow, Danish lakes. Their results show that with the increase of total phosphorus, a significant decline is observed in the species richness of zooplankton, submerged macrophytes and unimodally related species richness to total phosphorus for fish, phytoplankton and floating macrophytes (with maximum at 0.1-0.4 mg.l⁻¹ of phosphorus). In the comparison of the

oligotrophic and eutrophic freshwater habitats' species richness, it is common knowledge that oligotrophic biotopes contain low biomass of algae, but the community is made up of a large number of species with characteristic genera and species from Chlorophyceae, Desmidiaceae, Bacillariophyceae and Chrysophyceae. On the other hand, eutrophic biotopes have large biomass of algae with a small species richness dominated by Cyanobacteria and green algae s.l. (Lampert & Sommer, 2007, Rawson, 1956, Sigee, 2005). According to Della Bella *et al.* (2009), its nutrient availability was described as the major predictor of species distributions and the highest macrophyte diversity was observed in mesotrophic or slightly eutrophic ecosystems, so we might assume the same trend in the case of algae and cyanobacteria in these types of habitats.

As results of work of Poniewozik & Juráň (2018, **PAPER V.**) suggest, the size of a habitat and the presence of vegetation macrophytes should influence the species richness of the locality. This information supports several studies, e.g. those focused on the influence of emergent macrophytes, focused on phytoplankton dynamics in a shallow polymictic tropical lake (Gebrehiwot *et al.*, 2017), the impact of macrophyte-created habitats on microalgae and zooplankton in small water bodies (Celewicz-Goødyn & Kuczyńska-Kippen, 2017), the role of small water ponds as reservoirs of algae species richness (Paczuska & Paczuski, 2015) or the way a habitat size and its isolation could contribute to the species richness (Scheffer *et al.*, 2006). These claims are supported by a studies from the small mesotrophic pond with rich water plant communities in the southern part of the Czech Republic. Results of these studies show the high species richness of algae in this habitat, especially from the euglenoid genus *Trachelomonas*, moreover, two rare (or little-known) taxa of the *Trachelomonas* genus were recorded in this mesotrophic locality – *T. bituricensis* var. *lotharingia* with only several findings across the world (Juráň, 2016, **PAPER II**) and the second world' finding of *T. saccasii* after its original description in Africa (Juráň & Couté, 2018, **PAPER III**).

Several euglenoid species are connected with the formation of neustonic films, communities of planktonic organisms living at the air-water interface. Formations of these films are well-known in the case of *Euglena sanguinea* which produces typical red blooms on the water surface due to the content of several types of carotenoids in *Euglena* cells (Deli *et al.*, 2014). These carotenoids seem to work as a protection against injury by ultraviolet light (Lackey, 1968). These blooms are reported across the world, e.g. Brasil (Xavier

et al., 1991), United States (Davis, 1956), Himalaya and Nepal (Khan, 1993, Mandal, 2016), and Nigeria (Kadiri, 2011). Reports of these blooms of *Euglena sanguinea* are reported in the Czech Republic too (Juráš, 2017). Even though the *Euglena sanguinea* is well known for the mentioned phenomena, there are several other euglenoid species which are able to form some blooms, e.g. *Euglena pascheri*, *Lepocinclis ovum* (Ligęza & Wilk-Woźniak, 2011), *Trachelomonas volvocina* (Komárková, 1977) and *Euglena granulata* (Bednarz, 1974). Wołowski *et al.* (2017) provide further examples of bloom-forming euglenoid species from Poland and Thailand (*Euglena clara*, *E. hemichromata*, *E. geniculata*, *Euglenaria anabaena*, rare bloom of *Phacus triqueter*, *Trachelomonas volvocinospsis*, *T. rugulosa* etc.).

Even though euglenoids living in the soil biota – the edaphone – are somewhat untraditional community, still, they might be found in this environment as well. These euglenoids have a specific cell shape - narrow but long cells – and typical snake – or worm-like movements, which help these algae to move in the mud between detritus particles and in the moist soil. This ecology is specific for *Euglena deses* (including former species *E. intermedia*) and *E. mutabilis* (Kusel-Fetzmann & Weidinger, 2008).

Euglenoids were even able to inhabit extreme environments, e.g. acidic, hot or heavy metals containing biotopes. *Euglena mutabilis* is a common species in several types of peat-bog biotopes with lower pH (Gojdics, 1953, Starmach, 1983). Concurrently, it is able to live in biotopes with very low pH, with the range 2-3: biotopes developed after the finishing of pyrite containing slates minig (e.g. Kuberová & Lederer, 2001, Lessmann *et al.*, 2000, Wołowski *et al.*, 2008, 2013). Due to biological effects of high H⁺ concentrations in acid environments, algae have theoretically a choice between strategies of stress avoidance (e.g. a low permeability of the plasma membrane for H⁺, a high H⁺ export capacity or high buffer capacity) or stress tolerance in the acidic environments (Gimmler, 2001). The description of new species of the genus *Euglena* from the acidic hot mud pool in a volcanic area of Costa Rica is unique. This new species, *E. pailasensis*, is closely related to *E. mutabilis* (Sittenfeld *et al.*, 2002, Sánchez *et al.*, 2004). In many cases, living in an extreme environment is connected with the heavy metals content. Several studies show that euglenoids, especially species of the genus *Euglena*, which are commonly used for this laboratory studies, are able to live in the medium containing heavy metals, e.g. reported by Ferroni *et al.* (2004), Lira-Silva *et al.* (2011) and

Rehman (2011). Moreno-Sánchez *et al.* (2017) summarized that utilization of heavy metals using thiol-metabolites (e.g. phytochelatins) and their further compartmentalization into chloroplasts and mitochondria are the best understood mechanisms for their accumulation in *E. gracilis* and, consequently, that this species is suitable to be used in bioremediation processes of water wastes polluted with heavy metals. Resistance of euglenoids to the presence of heavy metals is not only known from laboratory experiments, Płachno *et al.* (2015) reported from the Graniczna Woda stream in Poland, which is highly contaminated by thallium and other heavy metal compounds (e.g. cadmium, lead or zinc), 66 algae taxa representing five groups (Cyanobacteria, xanthophytes, euglenoids, chlorophytes and diatoms) among which euglenoids prevail. Authors discussed that it is unclear whether algae of this biotope have a broad tolerance to these conditions or they are adapted to this environment and they are representing some new “eco-morphs” or “eco-forms” evolved from typical forms.

Various biotic interactions into which euglenoids enter are quite interesting. It is commensalism of species of the genus *Colacium* with zooplankton which is probably well-known. Also, this interaction is quite well studied (e.g. Chiavelli *et al.*, 1993, Al-Dhaheri & Willey, 1996, Dubovskaya *et al.*, 2005, Zalocar *et al.*, 2011). Interesting results about the interaction between *Colacium vesiculosum* and its hosts were brought by the study conducted by Zalocar *et al.* (2011) concerning a shallow lake in Argentina – prevalence of *Colacium* is higher in adult crustaceans than in their larvae and juveniles and the attachment sites on the exoskeleton were found to be the portions of the body which have a higher probability of encounter with epibionts during locomotion and feeding (e.g. antennae and thoracic legs in copepods, and thoracic legs and postabdomen in cladocerans). The second well documented example of the euglenoids’ commensalism is an interaction between the carnivorous plants of the genus *Utricularia*. One of the first summarizing works about this phenomenon was published by Hegner (1926) and, since then, the interest in this interaction has been persistent until today (e.g. Alkhalaf *et al.*, 2008, Díaz-Olarte *et al.*, 2009, Mette *et al.*, 2000, Peroutka *et al.*, 2008, Płachno *et al.*, 2012, 2014). As well as species succession is recorded in traps of the *Utricularia*, e.g. Płachno *et al.* (2012) found in young traps *Scenedesmus* spp. and *Characiopsis* sp. as the most abundant algae, while *Scenedesmus* spp. and the palmelloidal form of *Euglena* spp. were dominated in the old traps. More interesting and

probably still not adequately resolved is a question about the role of euglenoids in the traps of *Utricularia* plants - is it really mutualism or facultative parasitism? This question was only shortly commented on by Adamec & Komárek (1999a,b) with the reference to R. Jobson's work, in which the author assumes that a plant with euglenoids in their traps grow worse than a plant without euglenoids. In connection to biotic interactions, it is necessary to mention the production of ichthyotoxin – euglenophycin (firstly characterized by Zimba *et al.*, 2004). This toxin was found in several euglenoid species (Zimba *et al.*, 2010, 2017). The presence of this toxin in an environment was linked to the mass death of fish with an unknown cause (e.g. Zimba *et al.*, 2010, Kadiri, 2011, Daoudi *et al.*, 2013, Ramham *et al.*, 2012). Toxicological properties of euglenophycin are not known yet, only its cytotoxicity has been demonstrated on two cancer-cell lines (Wahome *et al.*, 2014) – it has led some authors to the belief that this toxin could be used in the fight against cancer.

As it is obvious from the previous section, euglenoids are the group of algae which is able to settle in a huge scale of habitats; as with other groups of Cyanobacteria and eukaryotic algae, there exists euglenoid species with wide ecological valency as well as species connected to the specific habitats – these species could be understood as bioindicators (Bellinger & Sigee, 2015) or flagship species for monitored habitat. Many of these habitats are very often threatened by anthropogenic and environmental impacts. These endangering factors are in the work of Janssen *et al.* (2016) focused on European terrestrial and freshwater biotopes. Protection of habitats with rich microbial communities (especially protists or algae) is a relatively complex task which is complicated due to missing methodologies and guidelines. Common methodologies belonging under the IUCN Red List (IUCN, 2013) are not applicable to microscopic organisms. This gap has led to the development of various methodologies for conservation microalgae and their biotopes (e.g. Brodie *et al.*, 2007, 2009, Kondratyeva, 2003, Palamar-Mordvintseva *et al.*, 2000, Stoyneva-Gärtner *et al.*, 2015). In addition, Juráň & Kaštovský (**PAPER VI**) propose a redlist of microalgae with the reflection of autecology of listed species with the combination of flagship species for endangered biotopes and try to show a possible way to prepare a useful and practical guideline for the protection of microalgae and their biotopes. Euglenoids are used as an unusual model group in this redlist. The results of this study are containing several euglenoid species and their connection with the potentially endangered biotopes.

The distribution of euglenoids and protist at all – global and local scales

Euglenoids, as well as another algal and protistian group, have a global distribution pattern. Notwithstanding, there are several questions connected with the euglenoids' distribution on the species level – the existence of worldwide distributed species and, on the other hand, there are theories about endemic species. With these tasks, two main distributional theories about microorganisms are connected – ubiquity theory (Finlay *et al.* 1996, 2004, Finlay & Fenchel 2004, Fenchel & Finlay 2004) and the theory of moderate endemism (Foissner 1999, 2006, 2007).

Finlay's concept (Finlay *et al.* 1996, 2004, Finlay & Fenchel 2004, Fenchel & Finlay 2004) states that similar morphospecies of protists inhabit similar habitats and exhibit a global distribution, provided there are no geographical barriers. This theory is classically paraphrased as “everything is everywhere”. The ubiquitous distribution of microorganisms is explained by the high abundance of individuals within morphospecies, high rates of their migration and low or unknown rates of allopatric and non-allopatric speciation (Foissner, 2009). Several studies supported this theory, e.g. Finlay & Clarke (1999), Fenchel & Finlay (2004), Finlay & Fenchel (2004) and Řežáčová & Neustupa (2007). Finlay & Clarke (1999) studied species of the genus *Paraphysomonas* in 0.1 cm² of sediment from a freshwater pond in England; authors perhaps that all species in the chrysophyte genus *Paraphysomonas* are ubiquitous and supported this theory with their results when they identified 78 % of all species of this genus from studied samples. Similar results were brought by the study conducted by Řežáčová & Neustupa (2007) who studied species of chrysophyte genus *Mallomonas* in the alluvial plain and river Lužnice in the Czech Republic. As the result of the study, they recorded in their investigated localities about 86.5 % of species of *Mallomonas* previously reported in all types of freshwater biotopes in the country. The study of two localities (freshwater pond with 1278 eukaryotic species and shallow marine bay with 785 eukaryotic species) provided by Fenchel & Finlay (2004) and Finlay & Fenchel (2004) were performed in order to examine these records and establish the extent of global coverage of recorded species; results of these studies support the ubiquity theory of microscopic organisms smaller than 1 mm – these organisms occur worldwide wherever their required habitats are realised.

Foissner (Foissner 1999, 2006, 2007) goes with his theory of moderate endemism in the opposite way than Finlay's concept – the theory simply says:

“not everything is everywhere”. This theory works with the hypothesis that some species could have global distribution, but other species have limited geographical distribution. For supporting of this hypothesis, Foissner’s theory used flagship species – morphologically clearly defined and practically unchangeable species with limited geographical distribution. Tyler (1996) discussed the endemism of algae on the examples from an Australian algal flora. He shows several taxa noted as “flagship species” – organisms which are of such a distinctive appearance or novelty that, according to Tyler (1996), there is a little doubt about their endemism and their existence increases the probability of less-distinguished species to be also endemic. In the case of a general validity of the ubiquitous distribution of microorganisms, these species would have to be found in required habitats across the globe and due to their morphological uniqueness, clear records would have to be published about them. The theory of moderate endemism is based on the following assumptions: the absolute abundance of individuals within morphospecies is low in the majority ($\geq 90\%$) of species, high only in some euryoecious species, furthermore, rates of migrations are low for most of the rare species (high only for some euryoecious species) and high rates of non-allopatric speciation (Foissner, 2009). Probably the main problem of ubiquity theory is an undersampling in several groups of microorganisms across the world – this essential knowledge gap complicates the generalized application of Finlay's theory, who discussed this problem briefly in Finlay & Fenchel (2004) for some groups of protists. Foissner uses this gap for supporting his theory of moderate endemism and supported it with several studies, especially the study about diversity of free-living ciliates (Foissner, 1999). Results of this study show two main problems in understanding diversity and the distribution of microorganisms: (1) Foissner found an average of one new species in almost every carefully analysed sample; (2) about one-half of ciliate species (cca 1000 species) were undescribed – new for science. Additionally, Foissner (1999) claims that there are protists with a restricted geographical distribution and only a tiny fraction of the potential habitats has ever been investigated for protists – all of these results show a huge gap in distributional knowledge about protists and the problematic application of ubiquitous theory due to this gap. Simultaneously, these gaps bring problems to the moderate endemism model too.

There are several algal groups, e.g. diatoms, silica-scaled chrysophytes and desmids – with well-studied biogeography, e.g. comprehensive studies of

(e.g. Kristiansen, 1996, Foissner & Hawksworth, 2009, Fontaneto, 2011). Concerning other algal groups, there are still present some gaps and their biogeography is still a challenging task. Similarly, this is the case of euglenoids too. The first attempt to affect biogeography of euglenoids was made by Hisoriev (2001) who tried to make a comparison of local floras and made some conclusion, including the presentation of the number of endemic species (see Figure 2), and, in addition, all phytogeographical regions show quite a high number of endemic species (in the sense that they are not listed in another way than the one from compared floras). Hisoriev's results point out the one of biogeographical problems – endemism of the microscopic organisms. Data based on literature and the exhibited high portion of endemic species should be interpreted in the other way. There are two main questions: "Are all the listed species endemic?" and "Could some species just be rare or overlooked?"

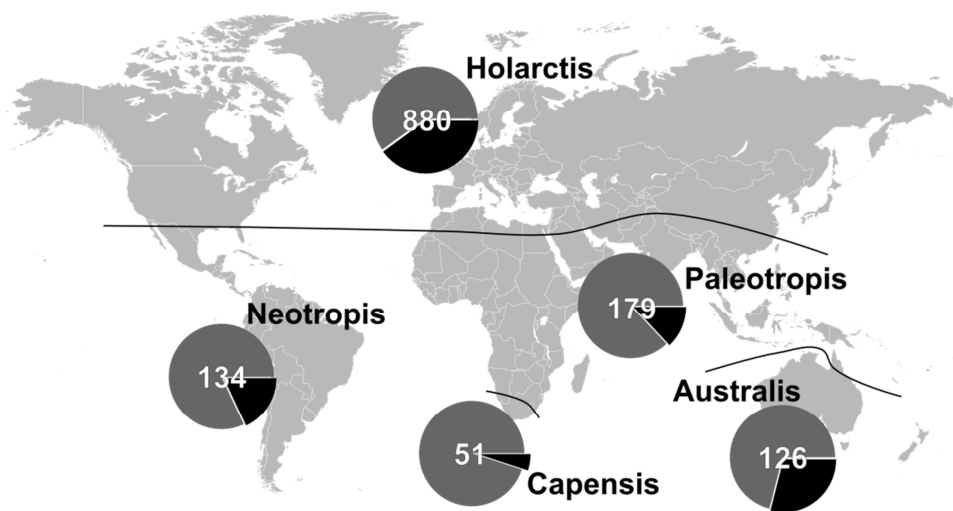


Figure 2. Phytogeographical regions with numbers of euglenoids recorded from it. Circular charts show the proportion of endemic species (*black part*) from the total number of species in the region (according to Hisoriev, 2001, for details see the text).

Two papers based on floristic data from the Czech Republic focus on this phenomenon. The record of morphologically quite well-defined taxa *Trachelomonas bituricensis* var. *lotharingia* (Juráň, 2016, **PAPER II**) shows that the rarity of species is quite a questionable characteristic due the poor

knowledge of species distribution, taxonomy and ecology. For this kind of taxa, which have insufficient knowledge of listed properties, a more predictable term has been proposed – “little-known species”, according to Molina & Marcot (2007). In the case of *T. bituricensis* var. *lotharingia*, there is a contradiction in its taxonomy due to its certain similarity with other species and missing molecular data for comparison. The detailed discussion on biogeography of the euglenoids took place in the work based on finding a species of *Trachelomonas saccasii*, described from Africa, in the European mesotrophic pond (Juráň & Couté, 2018, **PAPER III**) – finding of this species highlights current knowledge gaps regarding the biogeography and worldwide distribution of euglenoids. Authors propose several euglenoid species with well-defined morphology and with a limited geographical distribution (*Colacium epiphyticum*, *C. minimum*, *Lepocinclis crassicollis*, *Phacus plicatus*, *Trachelomonas argentinensis*, *T. hemispherica* and *T. magdaleniana*) as potential flagship species for the better understanding of the euglenoids biogeography.

To understand the distribution of algae on the global scale, it is essential to have knowledge about the distribution on the local scale. There are several publications and checklists about local floras published across the world. As the result of the long-term tradition of algal floristic survey in the Czech Republic, several comprehensive works about our algal flora have been published (Hansgirg, 1892, 1899, Poulíčková *et al.*, 2004, Lhotský & Rosa, 1955). However, these works are becoming not to be up to date due to further publishing of data and, above all, due to various taxonomical changes, etc. In the last year, a demand for updated and commented check-lists that can be used for the comparison of new finds from our state, ecological studies and practical nature conservation has been presented. Current Czech works summarize our knowledge of algae flora of cyanobacteria (Kaštovský *et al.*, 2010), desmids (Šťastný, 2010), charophytes (Caisová & Gąbka, 2009), silica-scaled chrysophytes (Škaloud *et al.*, 2013), red algae (Kučera *et al.*, 2008) and euglenoids (Juráň, 2017, **PAPER I**). The commented check-list of euglenoids brings results of the floristic survey based on a floristic examination of more than 200 sites during the years 2008–2014 in combination with the literature data recorded from the end of the 19th century to the present. Moreover, with 284 euglenoid taxa in 11 genera, the check-list eventually represents a practical guide for understanding the diversity of euglenoids in the Czech Republic, reflecting their actual taxonomy, colonized habitats, the life strategy, the level of

rarity, and, in addition, this type of work also plays a vital role in the practical conservation of biotopes as is shown in the work of Juráň & Kaštovský (**PAPER VI**).

Objectives and content of the thesis

Nowadays, it seems that the scientific focus on euglenoids, as well as in the case of other groups of algae and microorganisms in general, has now begun to move away from classical ecological floristic and morphology-based taxonomic works to issues using methods of molecular biology (Leander *et al.*, 2017). Despite this methodological progress, a lot of “white spots” remain within our knowledge of euglenoids and their ecology, distribution and also taxonomy – these gaps in knowledge bring new challenges and questions for future research.

This thesis aims to contribute to knowledge about the distribution of euglenoids and their ecology together with possible overlaps with practical nature protection. This thesis is composed of three main chapters corresponding to the previously mentioned topics.

CHAPTER 1 focuses on the euglenoids distribution on local and global levels. Information about the distribution of euglenoids on a local level is shown in **PAPER I**, which represents an actual commented checklist from the Czech Republic. Data for compilation of this list come from the literature data recorded from the end of the 19th century to the present together with a floristic survey of more than 200 localities across the Czech Republic during the years 2008–2014. The presented checklist included ecological characteristics (colonized habitats, life strategy, level of rarity) for each taxon too. Another two papers included to this chapter are dedicated to the distribution of rare species of euglenoids *Trachelomonas bituricensis* var. *lotharingia* (**PAPER II**) and *Trachelomonas saccassii* (**PAPER III**), both reported from a small mesotrophic pond in the southern part of the Czech Republic. Records of these taxa are documented by means of light microscopy and scanning electron microscopy and, in addition, distributional data are compared with literature. In the case of *T. saccassi* (**PAPER III**), methods of studying the distribution of euglenoids on a global scale are proposed.

The issue of ecology of euglenoids is part of **CHAPTER 2**. In **PAPER IV**, there is a study of morphological variability of *Trachelomonas caudata* in natural populations based on basic morphometry and statistic evaluation of the

loricas parameters using data from the light and scanning electron microscopy with comments connected to the taxonomy of *T. caudata* and morphologically similar species. **PAPER V** includes results of the floristic study of euglenoids in a small clay-pit in Poland using the data about water chemistry, taxa abundances and basic statistics for the study of a rich biodiversity of euglenoids in this type of biotope. In addition, listed taxa were documented by using light microscopy and scanning electron microscopy.

CHAPTER 3 is made up of methodological **PAPER VI** focused on making of a consensus for using microalgae as a part of the biotope evaluation for the practical nature protection. The paper summarizes data from the published Red Lists from European countries and, in the methodological part, there is a scheme for the evaluation of species' level of threat together with a detailed discussion and a list of potential problems associated with species' evaluation, which is accompanied by suggestions for adjustments and solutions to these problems.

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CHAPTER 1
EUGLENOID DISTRIBUTION

PAPER I.

The checklist of photosynthetic euglenoids (order Euglenales) of the Czech Republic: ecology, taxonomy, distribution

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Abstract

This study brings results of a floristic survey of photosynthetic euglenoids. Data in this survey come from a floristic examination of more than 200 localities during the years 2008–2014 in combination with the literature data recorded from the end of 19th century to the present. In this work, 284 euglenoid taxa which belong to the genera *Colacium* (6 taxa), *Cryptoglana* (2 species), *Discoplastis* (1 species), *Euglena* (48 taxa), *Eugleniformis* (1 species), *Euglenaria* (3 species), *Lepocinclis* (36 taxa), *Monomorphina* (7 species), *Phacus* (71 taxa), *Strombomonas* (16 taxa) and *Trachelomonas* (93 taxa) are listed. Four categories of rarity were designed—common (52 taxa), rare (44 taxa), very rare (120 taxa) and data deficient (68 taxa). In checklist, ecological characteristics (colonized habitats, life strategy, level of rarity) of taxa are shown. Number of species is compared with several works from European countries (Hungary, Great Britain, Poland, Romania, Slovak Republic, Slovenia and Ukraine). Taxonomic notes and new combinations reflecting recent works based on molecular-morphology are made and discussed. Results of this study bring next part to the knowledge of the Czech Republic algal flora.

Keywords

algae, Euglenophytes, floristics, microalgae

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PAPER II.

***Trachelomonas bituricensis* var. *lotharingia* M.L. Poucques 1952, a morphologically interesting, rare euglenoid new to the algal flora of the Czech Republic**

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Abstract

This report describes the discovery of the rare euglenoid taxon *Trachelomonas bituricensis* var. *lotharingia* in a small mesotrophic pond in the Czech Republic. Only limited data are available on the distribution of this taxon as same as for typical variety of *Trachelomonas bituricensis*, even though this taxon is morphologically very well defined. I provide a brief discussion of the taxonomic validity of this taxon based only on morphological features, which are characteristic for the taxonomy of the genus *Trachelomonas*. This finding is completely new for the algal flora of the Czech Republic. This report provides new information about the worldwide distribution of this taxon and its ecology.

Keywords

Czech Republic, floristics, microalgae, *Trachelomonas*

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PAPER III.

African *Trachelomonas saccasii* found in a European mesotrophic pond (Czech Republic). Implication for euglenoid biogeography and recommendations for euglenoid flagship species

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Abstract

This work reports the identification of *Trachelomonas saccasii* in a small mesotrophic pond in the Czech Republic. *Trachelomonas saccasii* was originally described from a small lake in Ivory Coast (West Africa) in 2009. This report from the Czech Republic is the second known record of this taxon after the initial description of this species. *Trachelomonas saccasii* represents a new species of euglenoid for the Czech Republic and Europe. This finding highlights the current knowledge gaps regarding the biogeography and worldwide distribution of euglenoids. The first aim of this study is to describe the identification of *Trachelomonas saccasii* using light and scanning electron microscopy and to compare it with the description of the original material from Africa. Second, we discuss the current knowledge of euglenophyte biogeography and propose methods that could be used to explore biogeographical distributions using floristic databases. These methods could be useful especially for the following flagship species: *Colacium epiphyticum*, *C. minimum*, *Lepocinclis crassicollis*, *Phacus plicatus*, *Trachelomonas argentinensis*, *T. hemispherica* and

T. magdaleniana. This study provides new data on the global distribution of euglenoids and proposes possible strategies to obtain further information about the global distribution of this group of microorganisms.

Key words

Euglenophytes, *Trachelomonas*, Biogeographical distribution, Mesotrophic pond

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CHAPTER 2
EUGLENOID ECOLOGY

■ PAPER IV.

Morphological variability of loricae in *Trachelomonas caudata* complex (Euglenophyta)

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Abstract

This study documented lorica ultrastructure variation in *Trachelomonas caudata* and related taxa from natural populations. On that basis, inaccuracies in the taxonomic system of euglenoid *Trachelomonas* are pointed out. Loricae of *T. caudata* and taxa very similar to it were examined by light and scanning electron microscopy and by energy-dispersive X-ray spectroscopy (EDS). The latter observations showed similarity of chemical composition between loricae of different putative taxa, with silicon and iron as the main components, and the absence of a chemical element considered to be a very important component of trachelomonad envelopes – manganese. All observed morphotypes were classified in one complex based upon envelope configuration, and the “caudatae complex” was established. Our morphological analyses led us to conclude that f. *pseudocaudata* of *T. caudata* should be subsumed in the typical form or treated as a synonym of *T. caudata*, as the morphological differences between them are within the typical range of phenotypic variability of species from natural environments. The same view applies to the species *T. bernardinensis*, *T. fusiformis*, *T. allorgei* and *T. molesta*, which should be considered synonyms of *T. caudata*.

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■ PAPER V.

Extremely high diversity of euglenophytes in a small pond in eastern Poland

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Background and aims – Phytoplankton samples were taken from a periodic, small and very shallow former clay-pit pond in eastern Poland near Lublin city. Diversity of the euglenophyte community was assessed during the period 2002–2004 and in 2014.

Methods – Water samples were collected with a 20 µm plankton net and with a slime aspirator (20 ml capacity) from the surface of the bottom. One aliquot of each sample was fixed for SEM observation; the fresh part of the sample was analysed using light microscope. Physical and chemical properties of the water (pH, temperature, conductivity, phosphates and ammonium salt contents) were measured. Diversity indices were calculated (Shannon-Wiener, evenness, Margalef and Simpson) and UPGMA cluster analysis was applied to discern differences among euglenoid assemblages.

Key results – In total, 63 euglenophyte taxa were found. The euglenophyte community was dominated by species belonging to the *Trachelomonas* genus (29 taxa). The most abundant and constant component were widespread and common species such as *T. caudata*, *T. hispida*, *T. intermedia*, *T. volvocina* and

T. volvocinopsis. They usually occurred in very high densities. We also found some rarely reported euglenophytes including *Euglena granulata*, *Trachelomonas lemmermannii*, and *T. sydneyensis*.

Conclusions – Euglenophytes were, with a few exceptions, the only group inhabiting the pond. Representatives of other groups such as diatoms or Scenedesmus species were recorded only occasionally. Nearly half of the taxa that were recorded in the first period (2002–2004) were found again after ten years. Of special concern was taxa belonging to the genus *Trachelomonas*. Trachelomonads, although known to represent taxa preferring waters moderately rich in nutrients, were very numerous in the investigated pond, which was enriched in phosphorus and nitrogen compounds.

Key words – Diversity, euglenoids, small water bodies, *Trachelomonas*.

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CHAPTER 3
PRACTICAL CONSERVATION IMPACT

■ PAPER VI.

Do we need Red List of microalgae? The procedure of compiling the Red List of microscopic algae of the Czech Republic – Euglenophyta model

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submitted to Biodiversity and Conservation

Keywords

conservation, redlist, flagship species, algae, microalgae, euglenoids

Abstract

Nature, species and biodiversity conservation should be a priority of each country. Publishing of Red Lists of animals or higher plants is quite common, but practical protection of algae and especially microalgae is quite a complex task with several complications. These complications are the result of our quite poor knowledge about biodiversity (taxonomy, phylogeny) of microalgae together with insufficient data about worldwide distribution of microalgae taxa (undersampling). The need to protect microorganisms, microalgae and heterotrophic protists is a crucial matter due to their role in bioindication and evaluation of the quality of their habitats. That is why it is necessary to have a Red List of these organisms as a practical manual for the protection of their habitat and, subsequently, also to have it as a basis for the protection of these organisms. Generally, microorganisms must be viewed not as rare species (in terms of their poor knowledge about distribution, ecology and taxonomy), but as little-known species and a Red List would reflect this idea. We propose a Red List of microalgae, using euglenophytes as a model group, with the reflection of taxonomy and autecology of listed species with linking to endangered habitats: habitat protection must be understood as a synonym for species conservation.

This proposal is the “starting point” for the evaluation of microalgal biodiversity of the Czech Republic and for deducting conclusions that can be used in the practical habitat conservation.

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SUMMARY OF THE RESULTS

SUMMARY OF THE RESULTS

The main objective of this thesis is to contribute to better knowledge of euglenoids diversity, ecology and distribution. It aims at the improvement of the current state of knowledge of the euglenoids distribution, especially on local scale together with some ecological consequences with an overlap in the practical protection of biotopes.

Euglenoids are organisms that inhabit mostly all water biotopes, sometimes quite extreme; some representatives may be found in wet soil. The study focuses primarily on the material from the Czech Republic together with some material from Poland. The material examination was carried out on live material or on fixed specimens using the common methods of light microscopy and scanning electron microscopy, additional environmental data have been used for making some ecological conclusions and the obtained results were discussed in detail with the published data.

Results of the paper included in **CHAPTER 1** about the euglenoids distribution bring data about these organisms on a local a global scale. In the compiled checklist published in **PAPER I**, there are 284 euglenoid taxa reported from the Czech Republic which belong to the genera *Colacium* (6 taxa), *Cryptoglena* (2 species), *Discoplastis* (1 species), *Euglena* (48 taxa), *Euglenaformis* (1 species), *Euglenaria* (3 species), *Lepocinclis* (36 taxa), *Monomorphina* (7 species), *Phacus* (71 taxa), *Strombomonas* (16 taxa) and *Trachelomonas* (93 taxa). Data presented with the additional concern for ecological preferences of individual taxa represent a first comprehensive set of this group in the Czech Republic. Other papers reported rare taxa with probably limited geographical distribution – *Trachelomonas bituricensis* var. *lotharingia* (**PAPER II**) and *T. saccasii* (**PAPER III**). The results of these works bring new records of an occurrence of these species and, simultaneously, outline the issues surrounding the global distribution of euglenoids. The main impact of the study of *T. saccasii* was a designation of well morphologically defined and unmistakable flagship species (*Colacium epiphyticum*, *C. minimum*, *Lepocinclis crassicollis*, *Phacus plicatus*, *Trachelomonas argentinensis*, *T. hemispherica* and *T. magdaleniana*) for further study of the euglenoid biogeography.

The paper in **CHAPTER 2** focuses on the euglenoids ecology and brings two interesting results. In **PAPER IV**, there is a documented lorica ultrastructure variation in *Trachelomonas caudata* and related taxa from natural

populations. Results of this ecological study have taxonomic consequences showing that in the case of euglenoids, the impact on their morphology, besides the environmental effect, may also influence the phases of their ontogenesis. According to results of the morphological analyses, there is a conclusion that *Trachelomonas caudata* f. *pseudocaudata*, *T. fusiformis*, *T. allorgei* and *T. molesta* should be considered synonyms of *T. caudata*. Results of **PAPER V** are about diversity of the euglenoids community in a small and very shallow former clay-pit pond in eastern Poland where euglenoids show extreme biodiversity (63 taxa in total) with dominating species belonging to the *Trachelomonas* genus (29 taxa), although it is a nutritionally rich habitat in which a much lower biodiversity could be assumed.

The last **CHAPTER 3** about the practical nature conservation in the connection with microalgal species propose a methodology for the evaluation of a level of threat of microalgae, which is tested on euglenoids as a model group. This proposal is designed as a template that could be used, with some modification, for all microalgal species and their biotopes. The evaluation step highlights that conservation must reflect: taxonomy and potentially possible determination, information about ecology together with habitat preferences of species, and an effect of “expert weight”. This methodology is a “starting point” for the compilation of Red List of microalgae in the Czech Republic and, in the future, it could offer a practical guide for the conservation of habits and their biodiversity.

Altogether, this thesis contains data about a commonly worldwide distributed group of algae with a high number of species and infraspecific taxa. The results of this thesis include original data on distribution, ecology and taxonomy of euglenoids, and, at the same time, propose the way of use of algae in the practical conservation of nature in the form of a synthesis of the present knowledge about this group of algae. Although the presented results represent only a quite small portion of contribution to the recent knowledge about euglenoids, they mainly bring future research topics associated with this algal group, especially the studies of diversity of oligo- and mesotrophic localities, understanding biogeography of euglenoids together with the need for further species revisions using modern molecular methods. Focusing on these research themes, we will be able to experience the more or less hidden biodiversity of these organisms.

CURRICULUM VITAE

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Education

2007-2010

Bachelor study in Biology (Bc.)

Faculty of Science, University of South Bohemia in České
Budějovice, Czech Republic

*Bc. Thesis: Euglenophytes of the Czech Republic in view of South
Bohemia and the Bohemian Forest [in Czech]*

2010-2013

Master study in Botany (Mgr.)

Faculty of Science, University of South Bohemia in České
Budějovice, Czech Republic

*Mgr. Thesis: Pilot study on the issue of Euglenophyta in the Czech
Republic [in Czech]*

since 2013

Doctoral study in Botany (Ph.D.)

Faculty of Science, University of South Bohemia in České
Budějovice, Czech Republic

*Ph.D. Thesis: Floristic-taxonomic study of the Euglenophytes.
Implications for their ecology, distribution and
practical protection*

Scientific employment

since 2012

Faculty of Science, University of South Bohemia in České
Budějovice, Czech Republic

2013-2014

Institute of the Soil Biology, Biology Centre CAS, České
Budějovice, Czech Republic

since 2013

Institute of Botany, Czech Academy of Science, Třeboň, Czech
Republic

Conferences

- 2015 34th International Conference of the Polish Phycological Society, Rzeszów-Polańczyk, Poland
Posters: Do we need a redlist of algae? A way to compile of the red list of algae and cyanobacteria of the Czech republic - Euglenophyta model
Culture Collection of Autotrophic Organisms (CCALA) - past, present and future
- 2016 46th Jírovec's Protozoological Days of Czech Society for Parasitology, Bítov, Czech Republic
Poster: Do We Need to Protect Microalgae and Protists at All?
- 2017 36th International Conference of the Polish Phycological Society, Lublin-Kazimierz Dolny, Poland
Poster: What do you we know about Euglenophytes biogeography and how to find more?
- 2017 36th Annual Meeting of the European Culture Collections' Organisation, Brno, Czech Republic
Poster: Culture Collection of Autotrophic Organisms (CCALA) - past, present and future
- 2018 37th International Conference of the Polish Phycological Society, Kraków-Dobczyce-Jałowcowa Góra
Lecture: Do we need a Red List of microalgae? A way to compile the Red List of microscopic algae of the Czech Republic: the Euglenophyta model

International cooperation

- 2014 Department of Phycology, W. Szafer Institute of Botany PAS
via Erasmus Programme (April-July)
Cooperation with prof. dr hab. Konrad Wołowski and dr Małgorzata Poniewozik

Teaching activities

- since 2013 Phycology
practices

2013	Introductory botany, phycology and mycology <i>practices</i>
since 2014	Advanced Phycology Seminar <i>practices</i>
2013 & 2017	Field Work I <i>practices – algological part of the course</i>
2014 & 2016	Basic Phycological Methods <i>lectures & practice – parts about sampling and culturing techniques</i>
2016	Introduction to Ecology of Algae and Cyanobacteria <i>lectures & practices parts about algal biogeography, biotic interactions and bioindication</i>
2016	Algal Culturing Techniques <i>course leader – lectures and practice</i>
2018	Biogeography of Protistian Organisms <i>course leader – lectures</i>
2018	Biological Laboratory Techniques <i>practices –part of the course focus on diatoms</i>

Publications (chronologically)

In peer-reviewed scientific journals

Juráň, J. & Kaštovský, J. (*manuscript*). Do we need Red List of microalgae? The procedure of compiling the Red List of microscopic algae of the Czech Republic – Euglenophyta model.

Juráň, J., & Couté, A. 2018. African *Trachelomonas saccasii* found in a European mesotrophic pond (Czech Republic). Implication for euglenoid biogeography and recommendations for euglenoid flagship species. *Phytotaxa* 334:201–214.

Poniewozik, M., & **Juráň, J.** 2018. Extremely high diversity of euglenophytes in a small pond in eastern Poland. *Plant Ecology and Evolution* 151:18–34.

Juráň, J. 2017. The checklist of photosynthetic euglenoids (order Euglenales) of the Czech Republic: ecology, taxonomy, distribution. *Phytotaxa* 317:1–16.

Juráň, J. 2016. *Trachelomonas bituricensis* var. *lotharingia* ML Poucques 1952, a morphologically interesting, rare euglenoid new to the algal flora of the Czech Republic. *PhytoKeys* 61:81–91.

- Wołowski, K., Poniewozik, M., & **Juráň, J.** (2016). Morphological variability of loricae in *Trachelomonas caudata* complex (Euglenophyta). *Cryptogamie, Algologie* 37:97–108.
- Juráň, J.**, Hauer, T., Johansen, J. R., & Krienitz, L. 2015. (2365) Proposal to conserve the name *Cyanospira* G. Florenz. & al. (Cyanophyceae) against *Cyanospira* Chodat (Euglenophyceae). *Taxon* 64:845–846.

Books and book chapters

- Geriš, R., Škaloud, P., **Juráň, J.** & Lepšová-Skácelová, O. (*in press*). Chapter 14. Zelené řasy *sensu lato*. In Kaštovský, J. et al. *Atlas sinic a řas České republiky II*. powerprint, Praha.
- Juráň, J.** (*in press*). Chapter 2. Raphidophyceae. In Kaštovský, J. et al. *Atlas sinic a řas České republiky I*. powerprint, Praha.
- Juráň, J.** (*in press*). Chapter 9. Skryténky – Cryptophyta. In Kaštovský, J. et al. *Atlas sinic a řas České republiky II*. powerprint, Praha.
- Juráň, J.** (*in press*). Chapter 10. Obrněnky – Dinophyta. In Kaštovský, J. et al. *Atlas sinic a řas České republiky II*. powerprint, Praha.
- Juráň, J.** (*in press*). Chapter 11. Krásnoočka – Euglenophyta. In Kaštovský, J. et al. *Atlas sinic a řas České republiky II*. powerprint, Praha.
- Kaštovský, J., Hauer, T., Geriš, R., Chattová, B. **Juráň, J.**, Lepšová-Skácelová, O., Pitelková, P., Puzstai, M., Škaloud, P., Šťastný, J., Čapková, K., Bohunická, M. & Mühlsteinová, R. (*in press*). *Atlas sinic a řas České republiky I*. powerprint, Praha.
- Kaštovský, J., Hauer, T., Geriš, R., Chattová, B. **Juráň, J.**, Lepšová-Skácelová, O., Pitelková, P., Puzstai, M., Škaloud, P., Šťastný, J., Čapková, K., Bohunická, M. & Mühlsteinová, R. (*in press*). *Atlas sinic a řas České republiky II*. powerprint, Praha.

Popularizing articles in Czech journals

- Juráň, J.** & Kaštovský, J. 2016. Nový pohled na systém řas a jak ho učit? *Živa* 6:299–301.
- Kaštovský, J. & **Juráň, J.** 2016. Evoluce sinic a řas v moderním pojetí. *Živa* 6: CXXXIII–CXXXVI.
- Juráň, J.** 2015. Proč mít sbírku řas? *Botanika* 2015:18–19.
- Juráň, J.** 2015. Jak lépe poznat svět sinic a řas. *Botanika* 2015:20–21.

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