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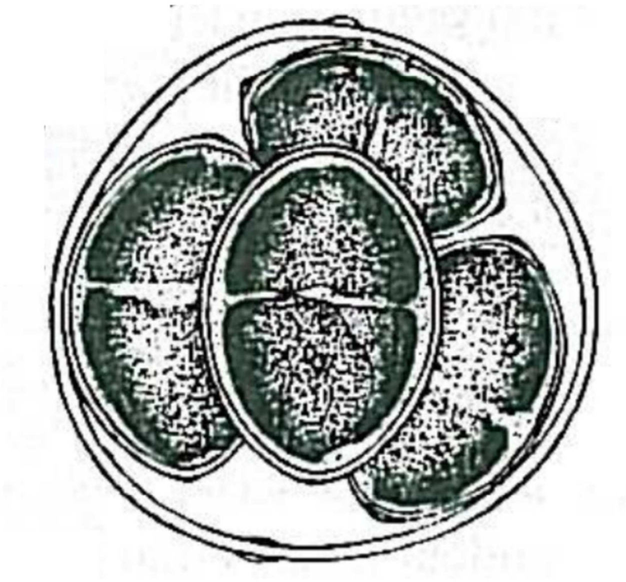

Systematic revision of the family Oocystaceae

Master thesis

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Abstract

The family Oocystaceae is a group of morphologically and ultrastructurally distinct green algae that constitute a well-supported clade in the class Trebouxiophyceae. Family possesses the clear delimitation, based on the presence of specific cell wall. Despite this, only few of the Oocystaceae members were examined using data other than morphological. After the establishment of molecular phylogeny, the generic classification of the family was called into question. The genus *Oocystis* proved to be paraphyletic; some of the taxa were excluded, and few other species newly redefined as members of this family. I investigated 53 strains assigned to the Oocystaceae using morphological, ultrastructural and molecular data (the SSU rRNA and the *rbcl* genes). *Oonephris obesa* and *Nephrocytium agardhianum* cluster within the Chlorophyceae and thus are no longer members of the Oocystaceae. Conversely, I confirmed transfer of the coenobial *Willea vilhelmii* to the Oocystaceae from the Scenedesmaceae. I placed another coenobial strain *Komarekia rotunda* to the Chlorellaceae. The division of the family into three subfamilies and into five morphological clades, and 2 new species combinations were suggested. On the basis of both molecular and morphological data, I expect further taxonomical changes in the genera *Crucigeniella*, *Crucigenia*, *Ecballocystis*, *Echinocoleum*, *Elongatocystis*, *Eremosphaera*, *Franceia*, *Lagerheimia*, *Neglectella*, *Oocystidium*, *Oocystis*, *Ooplanctella* and *Willea*.

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V Českých Budějovicích dne 13. 12. 2013 Lenka Štenclová

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Contents

1. Introduction

1.1. Why Oocystaceae - Current situation

Green algae with a coccal thallus were associated to the order Chlorococcales for decades. With the introduction of the molecular phylogeny, the taxonomic system has undergone significant changes. The order Chlorococcales dissolved and subsequently most coccal Chlorophytes are distributed within the classes Chlorophyceae and Trebouxiophyceae. The family Oocystaceae Bohlin clusters within the Trebouxiophyceae (Hepperle et al. 2000).

The delimitation of the family varied in several previous studies (Smith 1950, Fott 1967, Komárek and Fott 1983, Melkonian 1983). Komárek (1979) redefined the family as an independent unit on the basis of characteristic cell wall ultrastructure that is multi-layered with the cellulose fibrils in each layer perpendicular to that of the adjoining layer (Fig. 1). However, the ultrastructural data is available only for some genera: *Amphikrikos*, *Eremosphaera*, *Franceia*, *Lagerheimia*, *Makinoella*, *Neglectella Oocystis*, *Siderocystopsis* and recently examined *Ecballocystis* and *Ecballocystopsis* (Bowen 1965, Crawford and Heap 1978, Hegewald et al. 1978, Quader et al. 1978, Hegewald et al. 1980, Hegewald et al. 1999, Schagerl 1993, Schnepf et al.1980 and Xia et al. 2013).

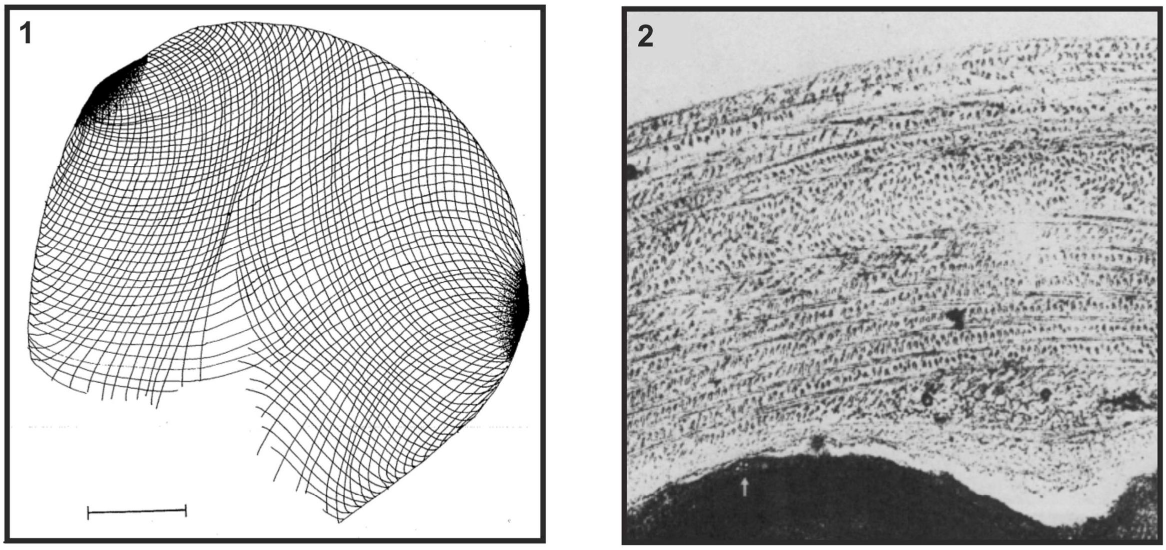

Figure 1: 1 Cell wall structure of the species ***Neglectella peisonis*** (Schagerl 1993) and 2 the cross-section of the cell wall of the species ***Oocystis solitaria*** (Quader 1983).

Differential characteristics of the family Oocystaceae according to Komárek (1979): cells +/- spherical, oval, elliptical to spindle-shaped, sometimes irregular; cell wall multilayered; propagation by autospores; daughter cells remain for some time to all their life enclosed in the expanded mother cell wall. Description: cells solitary or in 2-, 4-, 8-, 16- to multicellular colonies, daughter cells mainly in the expanded mother cell wall enclosed. Cells typically oval or elliptical, sometimes spherical, rhombic, spindle-shaped, bean-shaped or a little bit irregular. Inside the cell one, a few or many chloroplasts, mostly cup-shaped or girdle–shaped, sometimes radial or spongiomorph, with or without pyrenoid. Cell wall smooth, warty or spiny, often with polar thickness, multilayered. Propagation by autospores. Daughter cells usually stay longer time of all their life enclosed in the mother cell wall. Type genera *Oocystis* A. Braun. Three subfamilies were established; one with large cells, numerous chloroplast and smooth cell wall Eremosphaeroideae and two small celled subfamilies: Lagerheimioideae with spiny cell wall, and Oocystoideae with smooth cell wall (Komárek 1979).

Traditionall definition of the families, genera and species was based on morphology. Yet, microscopic coccal green algae possess only a limited amount of morphological traits and some of them occured far more than once in evolution. In some cases ultrastructural examination can help to recognize monophyletic and polyphyletic origins e.g. spines (Hegewald and Schnepf 2002, Pröschold et al. 2010). However, some features are doubtful e.g. cell shape (Luo et al. 2010, Bock et al. 2011). Therefore morphological species (and generic) concept can hardly work among the unicellular algae. Biological species concept by Mayr (1942) the most widely accepted criterion for species delimitation in eukaryotes can not by applied either, because many of the coccal green algae are considered as asexual, especially inside Trebouxiophyceae. Cryptic sexual reproduction was found by analysis of genomes of the Trebouxiophyta (e.g. Blanc et al. 2010). Nevertheless, only a few species were observed to propagate sexually (Kies 1967, Gonzalves and Mehra 1959, Iyengar and Ramanathan 1940, Iyengar and Ramanathan 1974) and the required circumstances are not explained. The phylogenetic species concept based on the simulation of the evolution, established by Mishler and Theriot (2000) proved to be sufficient for systematics of asexual green coccal algae.

With the establishment of the molecular phylogeny, the traditional generic concept of all families of Chlorophyta more or less changed (e.g. Wolf et al. 2003, Luo et al. 2010). Generic conception of Oocystaceae based on morphological data by Komárek and Fott (1983) proved to be inaccurate. Four species *Elakatothrix viridis, Nephrochlamys subsolitaria, Rhombocystis* *complanata,* and *Trochiscia hystrix* were transferred to the class Chlorophyceae (Buchheim et al. 2001, Krienitz et al. 2011). Spiny *Diacanthos belenophorus* moved inside Trebouxiophyceae to the genus *Micractinium* (Chlorellaceae) (Krienitz 2004). Within Oocystaceae two new genera formed: *Elongatocystis* and *Ooplanctella.* (Pažoutová et al. 2010, Krienitz and Bock 2011) Coenobial strains *Crucigeniella rectangularis, Ecballocystis hubeiensis, Ecballocystopsis dichotomus, Makinoella tosaensis, Tetrachlorella alternans* and *Willea vilhelmii* were newly redefined as members of the family (Hepperle et al. 2000, Krienitz et al. 2003, Krienitz and Bock 2011, Štenclová 2011, Xia et al. 2013). The expanded family also contains together clustering taxa *Amphikrikos* sp., *Quadricoccus ellipticus* and *Schizochlamydella capsulata* (Hepperle et al. 2000, Wolf et al. 2003, Krienitz and Bock 2011). Some authors suggest including three basal species - coenobial *Tetrastrum heteracantum and T. staurogeniiforme* and simply filamentous *Planctonema lauterbornii* inside the family (Krienitz and Bock 2011, Bock et al. 2013).

Taxa with irregular shape of the cell *Elakatothrix viridis*, *Rhombocystis complanata* and *Nephrochlamys subsolitaria* excluded from the family (Buchheim et al. 2001, Krienitz et al. 2011) suggest that the shape of the cell could be relevant to distinguish whether taxa include to the family. Coenobial taxa new to the family indicates that the family Oocystaceae have a wider definition, than previously expected (Krienitz et al. 2003). The family Oocystaceae needs a precise definition. (Komárek and Fott 1983.) All the changes and polyphyletic state of the *Oocystis* species suggest that a revision of all the family is required.

1.2. Objectives

1.2.1. Review

The first step is a comprehensive study of scientific literature of genera assigned to the family Oocystaceae and family Scenedesmaceae subfamily Crucigenoideae to reveal potential members of Oocystaceae. *Oocystis* A. Braun is the type genus of the family; therefore it was under particular interest and was examined on species level.

1.2.2. Delimitation

On the basis of the review, strains chosen to cover as much variability as possible were examined through polyphasic approach. I focused on detection of the delimitation of the family Oocystaceae.

1.2.3. Definition

Provide improved morphological definition of the family Oocystaceae on the basis of the multi-approach taxonomical revision is necessary.

1.2.4. Internal structure

I aim to compare inner structure proposed by Komárek and Fott (1983) with results of molecular phylogeny and morphological observation. Further, I focus on the importance of morphological characters: spines, mucilage covers with or without projections, granula on the surface of the cell wall, coenobia character of the cell arrangement, cell dimension and number of chloroplasts for the structure of the family.

1.2.5. Generic and species concept

Purpose of this study is also generic concept of the family and species concept inside some genera. Especially the genera *Crucigeniella*, *Crucigenia*, *Ecballocystis*, *Echinocoleum*, *Elongatocystis*, *Eremosphaera*, *Franceia*, *Lagerheimia*, *Neglectella*, *Oocystidium*, *Oocystis*, *Ooplanctella* and *Willea* are under interest.

1.3. Review

1.3.1. Coenobial genera

Algae that dispose crucigenoid coenobial morphology were traditionally assembled in the family Scenedesmaceae subfamily Crucigenoideae. Komárek (1974) described the complicated history of the taxonomy of this group and recognized inside subfamily Crucigenoideae genera *Crucigenia*, *Crucigeniella, Hofmania, Tetrachlorella, Tetrastrum, Westella*, and *Willea*. Komárek and Fott (1983) assigned to subfamily Crucigenoideae genera *Crucigenia, Crucigeniella, Didymogenes, Gilbertsmithia*, *Makinoella, Suxenella, Tetrachlorella, Tetrastrum, Westella* and *Willea.*

Some taxa from this group were moved inside Oocystaceae by molecular phylogeny. Hepperle et al. (2000) include genus *Tetrachlorella* Korshikov with the type species *T. alternans* (G.M.Sm.) Korshikov in Oocystaceae. According to Hindák (1980), belongs to this genera monotypic genus *Fotterella* R.Buck with the only species *F. tetrachlorelloides* R.Buck. Genus *Makinoella* Okada with only species *M. tosaensis* Okada was placed to the Oocystaceae by Okada (1949). Komárek and Fott (1983) considered the coenobial character of *Makinoella* and proposed its assignation to the family Scenedesmaceae subfamily Crucigenoideae. Both the molecular phylogeny and ultrastructure of the cell wall confirmed the original placement to the Oocystaceae (Hegewald 1999, Hepperle et al. 2000). Third coenobial taxon analysed in Hepperle et al. (2000), *Crucigeniella rectangularis* (Nägeli) Komárek is only one species of the genera *Crucigeniella* Lemmerm. in Oocystaceae. Tsarenko et al. 2002 proposed a transfer of the type species of *Willea, Willea irregularis* (Wille) Schmidle to the *Crucigeniella* as *Crucigeniella irregularis* (Wille) P.M. Tsarenko and D.M. John and Hegewald et al. (2010) recommended including *Crucigeniella* as well as morphologically similar *Willea* Schmidle to the Trebouxiophyceae.

Genus *Crucigeniella* seems to be polyphyletic. Bock et al. (2013) claim that some of the *Crucigeniella* species cluster together with some *Crucigenia* Morren species including type species *Crucigenia quadrata* Morren in the Chlorophyceae as a new lineage sister to the *Scenedesmus* Meyen *sensu lato* (Pažoutová, Bock and Krienitz, unpublished results).

Crucigenia proved to be polyphyletic, as well. *C. tetrapedia* (Kirchner) Kuntze clustered inside Trebouxiophyceae together with *Tetrastrum komarekii* Hindák, *T. triangulare* (Chodat) Komárek, *and T. punctatum* (Schmidle) Ahlstrom as a member of the re-established genus *Lemmermannia* (Chodat) C. Bock & Krienitz sister to the Botryococcus clade. New combinations *Lemmermannia tetrapedia* (Kirchner) Lemmerm. *L. komarekii* (Hindák) C. Bock & Krienitz, *L. triangularis* (Chodat) C. Bock & Krienitz, and *L. punctata* (Schmidle) C. Bock & Krienitz were created. *T. heteracantum* (Nordst.) Chodat and *T. staurogeniiforme* (Schröder) Lemmerm. remained in the genus *Tetrastrum* Chodat clustering near Oocystaceae or on the basis of the Oocystaceae (Bock et al. 2013)

On the basis of *Hofmania* Chodat, new genus *Komarekia* Fott was established by Fott (1981) with type species *K. appendiculata* (Chodat) Fott. Komárek and Fott (1983) classified all genus inside Scenedesmaceae. *K. lauterbornii* (Schmidle) Fott was signed as synonymic to the *Crucigenia lauterbornii* (Schmidle) Schmidle (Komárek and Fott 1983), then proved it to be a member of Chlorellaceae and left without taxonomical conclusion (Bock et al. 2013).

Westella De Wild. with the type species *W. botryoides* (West) De Wild. clustered in the family Scenedesmaceae subfamily Scenedesmiodeae (Hegewald et al. 2010) whereas *Didymogenes* Schmidle with the type species *D. palatina* Schmidle was transferred to the family Chlorellaceae (Pröschold et al. 2010). Two *Suxenella* P.Srivast. & M.Nizamuddin species were splitted into the genera *Crucigenia* as *C. mitrii* G.L.Tiwari & D.C.Pandey (Tiwari and Pandey 1971) and *Asterarcys* A.Comas Gonzales as *A. quadricellulare* (Behre) Hegewald & Schmidt (Hegewald et al. 2010). Coenobial monotypic genus *Gilbertsmithia* M.O.P.Iyengar with type *G. grandes* M.O.P.Iyengar stays designated to Scenedesmaceae Crucigenoideae (Komárek and Fott 1983) and genus *Ettliella* Hindák with the type species *E. tetraspora* Hindák inside Oocystaceae (Hindák 1988).

1.3.2. Genera with large dimension and numerous chloroplasts

Subfamily Eremosphaeroideae *sensu* Komárek and Fott (1983) includes three genera with numerous chloroplast and large cell dimensions: *Eremosphaera, Excentrosphaera* and *Oocystaenium*. *Oocystaenium* Gonzalves & K.R.Mehra with only one species *O. elegans* Gonzales & K.R.Mehra, and one species of genus *Eremosphaera* De Bary with the type *E. viridis* De Bary were observed to reproduce sexually by homothallic oogamy (De Bary 1858, Gonzales and Mehra 1959). *E. viridis* so far only one sequenced member of this group clusters on the basis of the family Oocystaceae (Pažoutová et al. 2010). *Excentrosphaera* G.T.Moore is monotypic genera with the type species *Ex. viridis* G.T.Moore.

Similar morphology as Eremosphaeroideae possesses genus *Neglectella* Voden. & Benderl. described by Vodenicarov and Benderliev (1971) with the type species *N. eremosphaerophila* Voden. & Benderl.. The genus was revised and on the basis of morphology split into three genera: *Neglectella, Neglectellopsis* and *Skujaster* (Vodenicarov 1989). An ultrastructural study executed by Schagerl 1993 on *Neglectella peisonis* Schagerl confirmed the genera *Neglectella* inside Oocystaceae. Affiliation of the monotypic genus *Skujaster* Voden. with the type species *Skujaster asteriferus* (Fott) Voden. and *Neglectellopsis* Voden. with only one species *Neglectellopsis skujae* Voden. is not resolved.

Another two monotypic genera: *Oonephris* Fott (type species *O. obesa* (West & G.S.West) Fott) and similar *Chondrosphaera* Skuja with the type of the genus *C. lapponica* Skuja belongs to this group according the morphology.

1.3.3. Spiny genera

Komárek and Fott (1983) summarized all spiny genera of the family Oocystaceae to the subfamily Lagerheimioideae. Subfamily comprised 11 genera *Chodatellopsis, Cryocystis, Dendrocystis, Diacanthos, Franceia, Lagerheimia, Pilidiocystis, Pseudobohlinia, Mycotetraedron, Micracantha, Trochiscia.* Authors notice that genus *Mycotetraedron* Hansg. dispose of doubtful validity and propose its elimination from Chlorococcales. Komárek and Fott (1983) also remarked that the genera *Pseudobohlinia* Bourr., *Micracantha* Korshikov and *Cryocystis* Kol are morphologically similar to the Xanthophyceaean algae *Gaumiella bellifontana* Bourr..

The type species of the genus *Trochiscia* Kützing was not established. Komárek and Fott (1983) argued that the genus needs thorough revision and distinguished only four species (*T. aciculifera* (Lagerh.) Hansg., *T. hystrix* (Reinsch) Hansg., *T. granulata* (Reinsch) Hansg. and *T. americana* Kol) from the many of described taxa of *Trochiscia*. One member of the genera *Trochiscia hystrix* was sequenced and found its position among Volvocales (Buchheim et al. 2001). *Diacanthos* Korshikov monotypic genera with the type species *D. belenophorus* Korshikov was removed from Oocystaceae (Komárek and Fott 1983) to the family Chlorellaceae, as a new combination *Micractinium belenophorum* (Korshikov) Pröschold, C. Bock, W.Luo & Krienitz (Pröschold et al. 2010).

So far only one taxa of morphologically integrated group *Lagerheimia* Chodat, *Franceia* Lemmerm. and *Chodatellopsis* Korshikov - *Lagerheimia genevensis* (Chodat) Chodat the type species of the genus *Lagerheimia* Korshikov was sequenced. Affiliation to the Oocystaceae was confirmed (Krienitz et al. 2003). Individual genera, as well as species inside genera, are distinguished according to the placement, number and morphology of the spines. Spines of *Lagerheimia* and *Franceia* dispose of the unique ultrastructure composed of a fibrillar axis covered by amorphous matter. (Hegewald et al. 1980). Komárek and Fott (1983) noticed that spines of *Dendrocystis* M.O.P.Iyengar possesse a different ultrastructure, therefore, its position is unclear.

Komárek and Fott (1983) suggest that to the genus *Pilidiocystis* Bohlin with the type species *Pilidiocystis endophytica* Bohlin should be assigned *Ourococcus multisporus* B.Bisch. & H.C.Bold as *Pilidiocystis multispora* (B.Bisch. & H.C.Bold) H.Ettl & G.Gärtner. No molecular data is available to date.

1.3.4. Granulated genera

According to Komárek and Fott (1983) only two granulated genera *Granulocystis* Hindák (type species *Granulocystis verrucosa* (Y.V.Roll) Hindák) and *Granulocystopsis* (type species *G. pseudocoronata* Korshikov) belong to the family Oocystaceae. Heynig (1991) established new genus *Oocystopsis* Heynig with only one member *Oocystopsis granulata* (Hortob.) Heynig. Further granulated taxa were assigned to the family by molecular phylogeny. Granulated algae *Siderocelis* (Naumann) Fott (type species *Siderocelis minor* (Naumann) Fott) was placed to the Chlorellaceae the subfamily Siderocelidoideae Komárek and Fott (1983) with also granulated *Amphikrikos* Korshikov with the type species *Amphikrikos minutissimus* Korshikov. Affiliation of *Amphikrikos* to the Oocystaceae confirmed molecular phylogeny and ultrastructure (Crawford and Heap 1978, Hepperle et al. 2000).

Komárek and Fott (1983) classified genus *Quadricoccus* Fott with the type species *Quadricoccus verrucosus* Fott to the Botryococcaceae. Molecular analyses incorporated it to the Oocystaceae (Krienitz and Bock 2011). Komárek and Fott (1983) included *Siderocystopsis* Swale genus described on the basis of *Siderocystis fusca* Korshikov (*Siderocystis* Korshikov is currently not recognized genera) with type species *Siderocystopsis fusca* (Korshikov) Hegewald & Schnepf to the Micractiniaceae. Molecular analyses assigned *Siderocystopsis punctifera* (Bolochonzew) Hegewald & Schnepf to the Oocystaceae (Pröschold et al. 2010) in accord with ultrastructural observation (Schnepf et al. 1980).

Sestosoma Hortob. with only one species *Sestosoma villosum* Hortob. bears analogous morphology. Its cell wall is irregularly littered with the wrinkled processes of variable length. Incrustations producing *Gloeotaenium* Hansg. (type species *G. loitlesbergereanum* Hansg.) can be also assigned to the group (Komárek and Fott 1983).

1.3.5. Mucilage producing genera

Palmeloid genus *Coenochloris* Korshikov seems to be deeply polyphyletic. Type species *C. pyrenoidosa* Korshikov and *C. signiensis* (Broady) Hindák clustered within Chlorophyceae (authors unpublished data). *C. Planoconvexa* Hindák clustered within Oocystaceae and was recently combined as *Ooplanctella planoconvexa* (Hindák) Pažoutová, Škaloud & Nemjová to the new genus *Ooplanctella* Pažoutová, Škaloud & Nemjová (Pažoutová et al. 2010). Pažoutová et al. (2010) demonstrated mucilage envelope with projections typical for the species. Krienitz and Bock (2011) assigned *Ooplanctella* to one strain of *Oocystidium* sp. Korshikov, in accordance with the morphology, *Oocystidium polymammilatum* Hortob. exposes similar a mucilage cover with projections. Hindák and Horecká (1987) combined *Oocystidium polymammilatum* as *Echinocoleum polymammilatum* (Hortob.) Hindák & Horecká on the basis of similarity of the mucilage cover with *Echinocoleum elegans* Jao & Lee the type species of *Echinocoleum* Jao & Lee.

1.3.6. Other genera

Ecballocystis Bohlin with the type species *E. pulvinata* Bohlin and *Ecballocystopsis* M.O.P.Iyengar with the type species *E. indica* M.O.P.Iyengar preliminary assigned to Chlorodendraceae (Fritsch 1956) or Botryococcaceae (Komárek and Fott 1983) were to the Oocystaceae placed by molecular phylogeny and ultrastructure (Xia et al. 2013). Wolf et al. (2003) placed to Oocystaceae *Schizochlamydella capsulata* (Guillard, H.C.Bold & MacEntee) S.Watan.. Some authors suggest including simply filamentous algae *Planctonema* Schmidle with the type species *Planctonema lauterbornii* Schmidle to the Oocystaceae (Krienitz and Bock. 2011).

Komárek and Fott (1983) included four genera with atypical or irregular shape of the cell to the family. *Nephrochlamys* Korshikov with the type species *N. subsolitaria* (West) Korshikov and *Rhombocystis* Komárek with the type species *R. complanata* Komárek was excluded by molecular phylogeny (Krienitz et al. 2011). Monotypic genus *Fusola* J.Snow with the type species *Fusola viridis* J.Snow is a basionym of *Elakatothrix viridis* (J.Snow) Printz. It was analyzed in (Buchheim et al. 2001) and placed to the Volvocales. *Juranyiella* Hortobagyi with the type species *J. javorkae* (Horto.) Hortob. (by Hindák 1978 assigned to the genus *Nephrochlamys*) and *Nephrocitium* Nägeli (type species *N. agardhianum* Nägeli) were not examined by molecular phylogeny. Edelstein and Prescott (1964) established new genus *Rayssiella* Edelst. & Prescott with the type species *R. hemisphaerica* Edelst. & Prescott and listed it to the Oocystaceae. Authors discussed the similarity with the genus *Nephrocytium*.

1.3.7. Genus *Oocystis* A. Braun

Genus *Oocystis* A. Braun is under particular interest of this thesis. *Oocystis* proved to be a wide genus with enormous variability Komárek and Fott (1983). Serious changes occurred since the genus was established; therefore, species originally assigned to the *Oocystis* are now widespread in various genera. *Oocystis eremosphaeria* G.M.Sm. and *Oocystis gigas* W.Archer belong to the genus *Eremosphaera* De Bary. *Oocystis asterifera* Skuja is recognized as *Skujaster asteriferus* (Fott) Voden. Spiny *Oocystis echidna* Bohlin is now *Franceia echidna* (Bohlin) Bourr., *Oocystis polychaeta* Schirsch. *Franceia polychaeta* (Schirsch.) Korshikov and *Oocystis ciliata* Lagerh. *Lagerheimia ciliata* (Lagerh.) Chodat. *Oocystis verrucosa* Y.V.Roll is basionym of *Granulocystis verrucosa* (Y.V.Roll) Hindák. *Oocystis pseudocoronata* Korshikov is a synonym of *Granulocystopsis decorata* (Svirenko) P.M.Tsarenko. *Oocystis ornata* Fott is recently recognized as *Siderocelis ornata* (Fott) Fott. (Komárek and Fott 1983).

Oocystis alpina Reisigl was newly combined to the genus *Ecdysichlamys* as *Ecdysichlamys alpina* (Reisigl) Komárek & Comas (Komárek and Comas 1984). Hindák 1988 established new genus *Catenocystis* Hindák with the type species *Catenocystis cohaerens* (Skuja) Hindák on the basis of *Oocystis cohaerens* Skuja. *Oocystis granulata* Hortob. is according to Heynig (1991) member of new genus *Oocystopsis* Heynig *Oocystopsis granulata* (Hortob.) Heynig. *Oocystis ecballocystiformis* M.O.P.Iyengar was excluded and established as a new combination *Elongatocystis ecballocystiformis* (M.O.P.Iyengar) Krienitz & C.Bock by Krienitz and Bock 2011. Hepperle et al. (2000) suggest that *O. solitaria* Wittrock should be excluded on the basis of molecular phylogeny and the morphological dissimilarity with the type species of *Oocystis O. naegelii* A. Braun.

According to the original incomplete description *O. naegelii* does not possess pyrenoid, and its descriptions vary in several observation. Skuja (1964) found only one or two chloroplasts per cell on the type material which does not exhibit pyrenoid. Therefore Řeháková (1969) suggested as a type of the genus *O. lacustris* Chodat. Despite, subsequent studies (e.g. Komárek and Fott 1983) recognize *O. naegelii* as the type, on the basis of Skujas (1964) thorough examination. The presence of the pyrenoid considered as valuable trait Hindák (1988) following the concept of Lemmermann (1903) and proposed the split of the genus to the genera *Oocystis* and *Oocystella* Lemmerm. (type species *Oocystella natans* Lemmerm.). He transferred 13 *Oocystis* species that possessed a pyrenoid to the genus *Oocystella* and found 4 new species of the genus *Oocystella: O. carpatica* Hindák, *O. oogama* Hindák, *O.*

piscinalis Hindák and *O. radiosa* Hindák. On the contrary, Komárek and Fott (1983) and followers (e.g. John and Tsarenko 2002) recognize only one genus *Oocystis* (Table 1).

Table 1: Comparison of the conception of *Oocystis/Oocystella* species according to Hindák and Komárek and Fott. *Species excluded from *Oocystis*. ** Does not contain 16 doubtful (incompletely described or difficult identifiable) taxa.

***Oocystis sensu* Hindák 1988**	***Oocystella sensu* Hindák 1988**		***Oocystis sensu* Komárek and Fott 1983**
O. apiculata	*O. bispora*		*O. apiculata*
*O. ecballocystiformis**	*O. borgeii*		*O. bispora*
O. elliptica	*O. carpatica*		*O. borgei*
O. hunanensis	*O. heteromucosa*		*O. cohaerens* *
O. irregularis	*O. lacustris*		*O. ecballocystiformis**
O. kumanensis	*O. marssonii*		*O. elliptica*
O. marina	*O. natans*		*O. heteromucosa*
O. naegelii	*O. nephrocytioides*		*O. hunanensis*
O. novae-semliae	*O. oogama*		*O. irregularis*
O. pelagica	*O. panduriformis*		*O. kumanensis*
O. pusilla	*O. parva*		*O. lacustris*
O. pyriformis	*O. piscinalis*		*O. marina*
O. rupestris	*O. radiosa*		*O. marssonii*
O. socialis	*O. rhomboidea*		*O. natans*
	O. solitaria		*O. naegelii*
	O. submarina		*O. nephrocytioides*
	O. tainoensis		*O. novae-semliae*
			O. panduriformis
			O. parva
			O. pelagica
			O. pusilla
			O. pyriformis
			O. rhomboidea
			O. rupestris
			O. socialis
			O. solitaria
			O. submarina
			O. tainoensis

2. Materials and Methods

2.1. Material

I obtained isolated single-species algae strains (axenic or with bacterial/fungus contamination) from the public collections Culture Collection of Autotrophic Organisms Academy of Sciences of the Czech Republic in Třeboň (CCALA), A National Center for Marine Algae and Microbiota (NCMA formerly CCMP), Culture Collection of Algae of the Charles University of Prague (CAUP), Culture Collection of Algae at the University of Gottingen (SAG), Coimbra Collection of Algae (ACOI), Culture Collection of Algae and Protozoa (CCAP) and from the private collection of Marvin.W. Fawley, Christina Bock, Lothar Krienitz and Marie Pažoutová (Appendix 1).

Undetermined strains provided from the private collections were designated following Komárek and Fott (1983). Determined strains from the public collections were authenticated according to Komárek and Fott (1983) to revise incorrect strain determination. Appropriateness of the names of the taxa in the thesis was checked by Index Nominum Algarum and the forms of authors names by The International Plant Names Index.

2.2. Cultivation

I kept strains in bottles with solid BBM medium (Bischoff and Bold 1963) (paved with 1,5% agarose) under standard cultivating conditions: irradiance 22 μmol m^{-2} s^{-1} and constant temperature 16.°C. Some strains were additionally cultivated in a liquid medium, because of better conservation of significant morphological traits: mucilage covers and spines.

2.3. Morphology

All strains were repeatedly observed by light microscope Olympus BX to capture all stages of algal life cycle. Magnifications 400x and 1000x with immersion oil were used. I stained strains by methylene blue to detect potentially attractive cell walls and mucilage covers. I made morphological descriptions of fifty observed strains. (Appendix 3) The observation was documented by camera Olympus DP 71, a part of the microscope, using software DP controller DP manager. Picture plates were constructed by the graphic program CorelDraw X6. (Appendix 4)

2.4. Ultrastructure

Fourteen strains were chosen for transmission electron microscopy (TEM), to observe cell wall ultrastructure. Samples were prepared by workers of Laboratory of Electron Microscopy - Institute of Parasitology. Samples were washed with 0.05 M phosphate buffer and postfixed with 2% osmium tetroxide in 0.05 M phosphate buffer at room temperature for 2 hours. Then was samples repeatedly washed with 0.05 M phosphate buffer. Cells were dehydrated with a concentration gradient of isopropanol solutions, and poured in the Spurr’s resin (Spurr 1969) afterwards, with propylene oxide as an intermediate stage. Thin sections were stained with uranyl acetate and lead citrate. I observed the prepared samples in a Jeol JEN 1010 transmission electron microscope at an accelerating voltage of 80 kV. I made picture plates documenting the examination by CorelDraw X6 (Figs. 2-3).

2.5. Molecular data

DNA was extracted using Invisorb Spin Plant Mini Kit (Invitek) following the official instructions, and by modified xanthogenate-SDS buffer extraction protocol with the addition of 3% PVPP and PEG-MgCl2 precipitation Yilmaz et al. (2009). This method was devised for cyanobacteria, however, it works perfectly for green algae, either for mucilage producing strains and strains with a rigid cell wall.

I chose SSU rRNA gene and *rbcl* gene for my analysis. Both genes dispose of the appropriate number of the polymorphism on the family and generic level. There are also a large number of the sequences of the SSU rRNA gene in the public database NCBI (http://www.ncbi.nlm.nih.gov/). For the *rbcl* gene dispose NCBI at least of some data, and moreover it is a housekeeping gene.

I have amplified both genes with PCR reaction consisting of 10 ng of template DNA with 2,5 pmol of forward and reverse primer and 10µl Plain PP Master Mix (Top Bio, the Czech Republic) using cyclers XP Cycler - Bioer T 300 Thermocycler - Biometra. Primer combination for amplification SSU rRNA was NS1F – ITS4R or 1650R, when to obtain unfragmented gene was possible, and combination of NS1F – 1150R and 1170F – ITS4 or 1650R, when the first choice was not successful. Program information: prefatory denaturation: 95°C, 1min, (denaturation: 95°C, 1min; annealing 52-55°C, 1min; elongation 72°C, 3min) 35x, final elongation: 72°C, 10 min. The temperature of the annealing was estimated of Tm of both primers (Checked by OligoAnalyzer 3.1 - Integrated DNA Technologies). Amplification of the *rbcl* gene was successful using primer combination

ORB1F – ORB1R. Program information: prefatory denaturation: 95°C, 1min, (denaturation: 95°C, 1min; annealing 52°C, 1min; elongation 72°C, 3min) 35x, final elongation: 72°C, 10min.

Successfully amplified DNA was proved on gel electrophoresis 1% agarose gel in TBE buffer. DNA stained by GEL RED was visualized by UV transilluminator ULTRA LUM. INC – gel imager with software Scion VisiCapture. PCR products were refined JetQuick PCR Purification Kit (Genomed). The manufacturer's instructions were followed.

Samples for sequencing were analysed by the Laboratory of Genomics, Biology Centre of the Academy of Sciences of the Czech Republic, České Budějovice (with the sequence analyser ABI PRISM 3130 XL, Applied Biosystems, Life Technologies Corp., CA, USA) or processed by commercial companies Macrogen and SeqMe. All primer information is written in table 2.

Table 2.: Primers used in the present study. Primers used only for sequencing are marked by an asterisk. Tm was checked by OligoAnalyzer 3.1 (Integrated DNA Technologies).

Gen	**Name**	**Sequence**	**F/R**	**Tm**	**References**
rbcl	ORB1F	CCACAAACTGAAACAAAAGCA	F	48,5	Košnar unpublished
rbcl	ORB1R	CCTTGGGGTAATGCTCCAG	R	53,2	Košnar unpublished
SSU	NS1F	GTAGTCATATGCTTGTCTC	F	47.2	Friedl unpublished
SSU	402F*	GCTACCACATCCAAGGAAGGCA	F	59.5	Katana et al. 2001
SSU	1150R	ACGCCTGGTGGTGCCCTTCCGT	R	68.1	Pažoutová et al. 2010
SSU	1170F	CTGTGGCTTAATTTGACTCAACACG	F	56.6	Pažoutová et al. 2010
SSU	1500AF*	GCGCGCTACACTGATGC	F	57.3	Helms et al. 2001
SSU	1650R vivi	TCACCAGCACACCCAAT	R	54.2	Kipp 2004
ITS	ITS1F*	TCCGTAGGTGAACCTGCGG	F	59.5	White et al 1990
ITS	ITS4R	TCCTCCGCTTATTGATATGC	R	52.1	White et al 1990

2.6. Alignment

I fold each sequence of both genes by SeqAssem (Hepperle 2004). The approximate position of each strain was checked by BLAST against all sequences contained in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Alignments consisted of authors sequences and sequences obtained from the public database GenBank - NCBI.

Alignments for outside Oocystaceae excluded taxa were assembled following the previous studies (Bock et al. 2011, Bock et al. 2013, - Chlorellaceae, Fučíková et al. 2011, Fučíková and Lewis 2012, Fučíková et al. 2013 - Sphaeropleales and Buchheim 2001 et al., Nakada et al. 2008 - Volvocales) to select suitable sequences that cover the main lineages of each examined group and reasonable outgroup. SSU rRNA gene was sufficient to determinate approximated position of this taxa. Alignments of the Oocystaceae (the SSU rRNA gene alignment, the *rbcl* gene alignment and concatenated SSU rRNA + *rbcl* dataset) include all reasonable sequences of the Oocystaceae from the GenBank and sequences used as outgroup (Chlorellaceae). All analysed sequences are recorded in the table (Appendix 5). Datasets was aligned using ClustalW (Larkin et al. 2007) and edited manually in Mega 5.2.2 (Tamura et al. 2011).

2.7. Molecular phylogeny

The phylogenetic trees were inferred for all datasets using algorithms Maximum likelihood by PHYML 3.0 (Guindon et al. 2010). Generalised time-reversible (Tavaré 1986) model of evolution with gamma distribution and invariable sites (GTR+Γ+I) was chosen. The gamma shape parameter α, as well as, the proportion of invariable sites was estimated from the data set. Nonparametric bootstrap support was calculated (100 repetition) to distinguish the probability of the branches. Secondly I used Bayesian inference by Mr.Bayes 3.2.2. (Ronquist et al. 2012). Two runs with both four MCMC chains were executed with setting of the default parameters for 2 000 000 (simple dataset) or 3 000 000 (concatenated dataset) generations. Two analysis of concatenated alignment was executed: the first without partitions and the second with partitions. For the second one, four partitions were established, one for 18S and three for *rbcl*, separated by codon position. Posterior probabilities of branches were recorded.

3. Results

All the results of this thesis are summarized in Appendix 2.

3.1. Morphology

5 strains names were revealed as incorrect when authenticated according to Komárek and Fott (1983), therefore changes were made (Appendix 1).

For all 50 observed strains, I made morphological descriptions (Appendix 3). Photos of these 50 strains were made by author and photos of two strains were obtained from Marie Pažoutová. I chose four photos of those 52 strains and made picture plates (Appendix 4). I have not documented strain CCMP 245 *Schizochlamydella capsulata* (Guillard, H.C.Bold & MacEntee) S.Watan.. Original images from the CCMP collection are shown. Picture plates demonstrated a comparison of strains examined in this thesis with strains investigated in previous studies. Some additional pictures from public resources were used. Three plates compare strains excluded from Oocystaceae compared with relation algae. One plate shows basal strains in with doubtful assignation to the Oocystaceae. One plate shows group *Oocystis sensu lato* and the rest of the plates represent strains with relevant morphological traits.

3.2. Ultrastructure

I examined the structure of the cell wall for 14 strains by transmission electron microscopy. Cell wall of the three strains SAG 2082 *Komarekia rotundata* (Teiling) Fott, SAG 34.81 *Nephrocytium agardhianum* Nägeli and CCALA 398 *Oonephris obesa* (West & G.S.West) Fott does not possesses an *Oocystis*-like ultrastructure (Fig. 2).

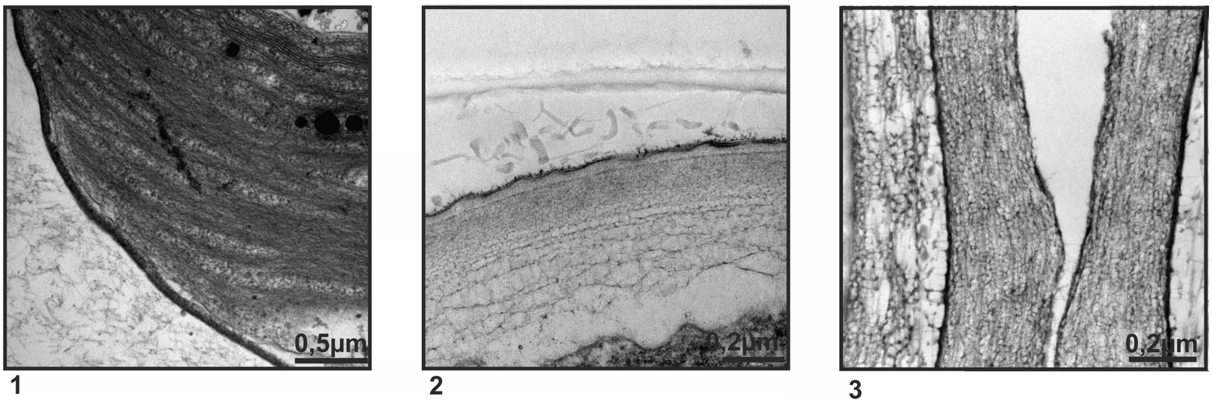

Figure 2: TEM examination of the cross-section of the cell wall. 1. SAG 2082 ***Komarekia rotundata***, 2. SAG 34.81 ***Nephrocytium agardhianum***, 3. CCALA 398 ***Oonephris obesa***.

Examination of the ultrastructure of *Planctonema* Schmidle and *Tetrastrum* Chodat strains did not reveal *Oocystis*-like structure of the cell wall (Fig. 3).

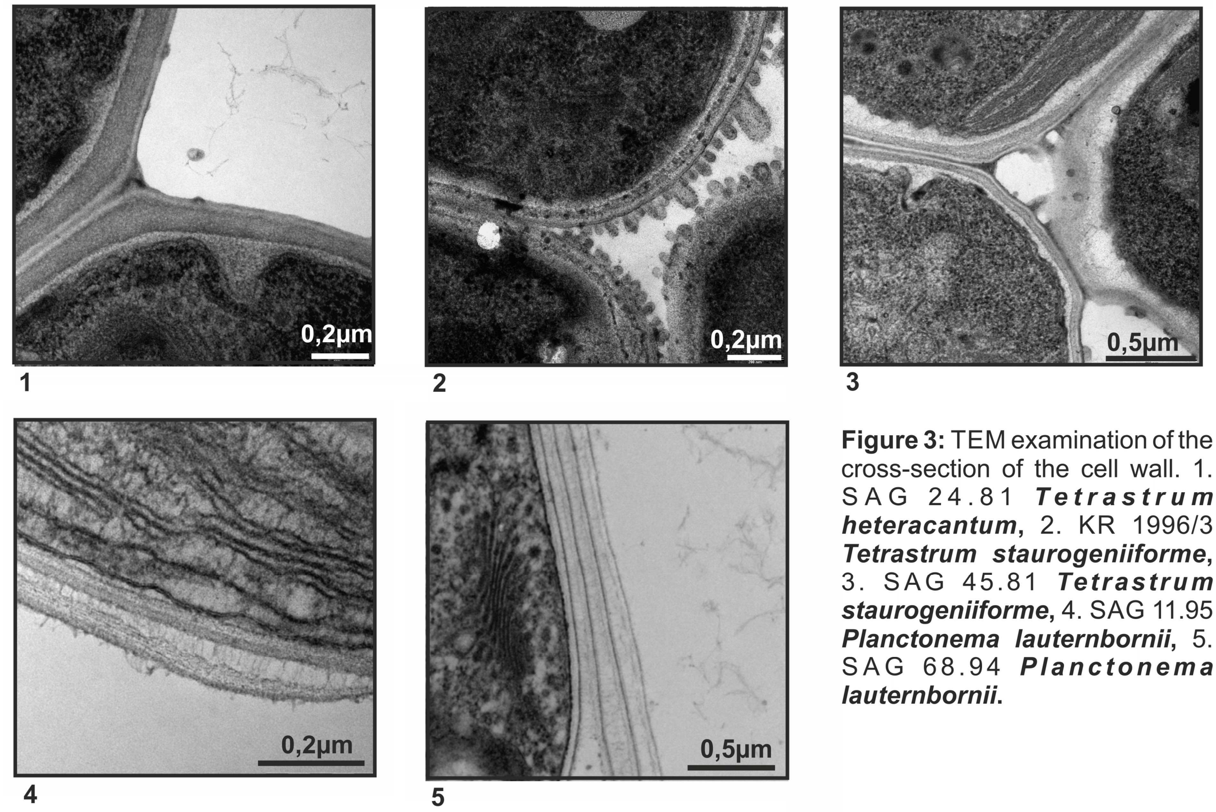

Figure 3: TEM examination of the cross-section of the cell wall. 1. SAG 24.81 ***Tetrastrum heteracantum***, 2. KR 1996/3 ***Tetrastrum staurogeniiforme***, 3. SAG 45.81 ***Tetrastrum staurogeniiforme***, 4. SAG 11.95 ***Planctonema lauternbornii***, 5. SAG 68.94 ***Planctonema lauternbornii***.

Strains SAG 2081 *Crucigeniella rectangularis* (Nägeli) Komárek, SAG 56.81 *Granulocystis verrucosa* (Y.V.Roll) Hindák, CCALA 961 *Makinoella tosaensis*, Okada, Tow 6/3 P-1ou *Oocystis* cf. *parva*. W.West & G.S.West, SAG 42.81 *Tetrachlorella alternans* (G.M.Sm.) Korshikov and CCALA 515 *Willea vilhelmii* (Fott) Komárek bears the *Oocystis*-like ultrastructure (Fig. 4).

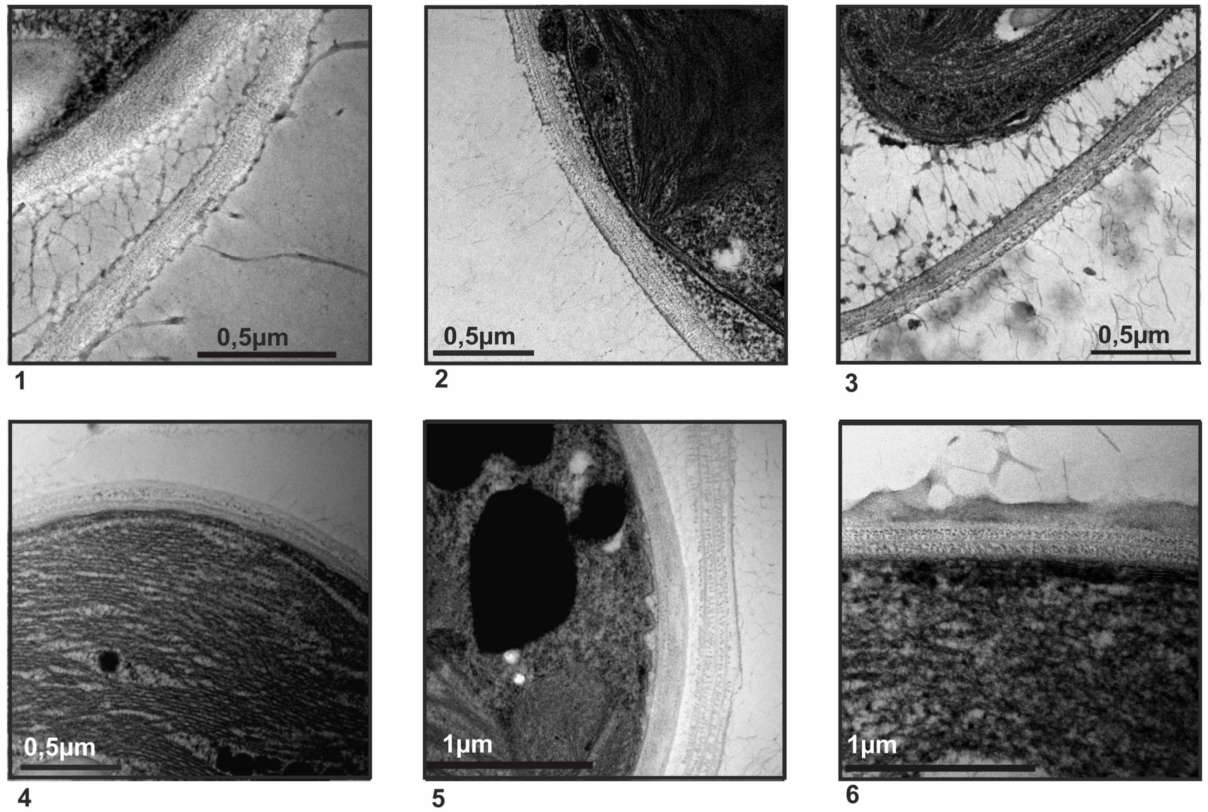

Figure 4: TEM examination of the cross-section of the cell wall. 1. SAG 2081 ***Crucigeniella rectangularis***, 2. SAG 56.81 ***Granulocystis verrucosa***, 3. CCALA 961 ***Makinoella tosaensis***, 4. Tow 6/3 P-1ou **Oocystis cf. parva,** 5. SAG 42.81 ***Tetrachlorella alternans***, 6. CCALA 515 ***Willea vilhelmii***.

3.3. Molecular phylogeny

SEQUENCES

Totally I obtained 27 new sequences of SSU rRNA gene and 48 new sequences of *rbcl* gene (Appendix 2). 4 SSU rRNA sequences are obtained from previous authors study (Štenclová 2011) and the rest from the public database GenBank of NCBI.

ALIGNMENT

Six datasets were aligned. Alignments based on SSU rRNA gene sequences were made for taxa excluded from the family Oocystaceae. The final alignment of the Chlorellaceae comprises 1552bp, alignment of Sphaeropleales 1661bp and Volvocales 1710bp. 3 *rbcl* sequences of strains excluded from Oocystaceae were not analysed.

Three datasets were made of sequences of Oocystaceae. The final alignment of SSU rRNA gene includes 1568bp, the *rbcl* alignment 1108bp and the concatenated alignment of both genes 2676bp.

PHYLOGENETIC TREES

Phylogenetic trees were constructed for each dataset, and phylogenetic position of all strains was found. Three strains are moleculary distant from the family Oocystaceae. All other strains make monophyletic cluster.

KOMAREKIA ROTUNDATA SAG 2082

Strain SAG 2082 *Komarekia rotundata* (Teiling) Fott clustered within Chlorellaceae sister to *Crucigenia lauterbornii* (Schmidle) Schmidle (Fig. 5).

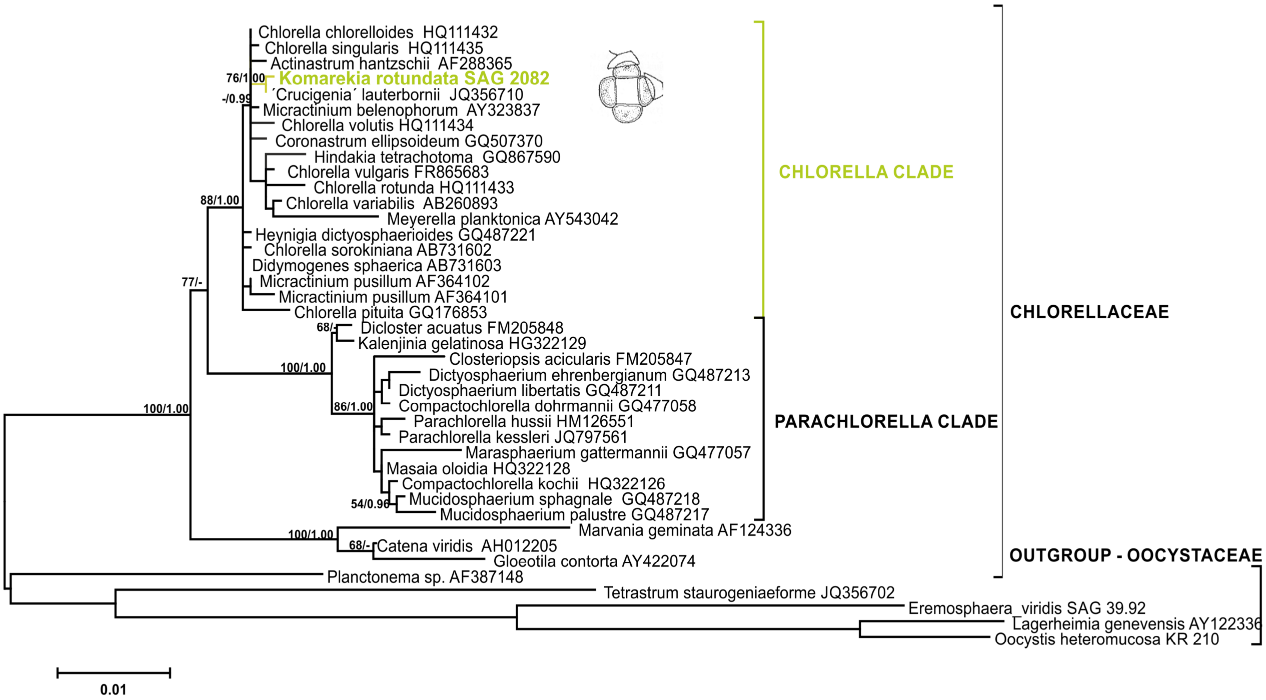

Figure 5: Phylogenetic analyses of SSU rRNA gene sequences of members of **Chlorellaceae** with Oocystaceae as outgroup. Numbers at the branches indicate bootstrap support from maximum likelihood (ML, 1000 replicates) and Bayesian posterior probabilities (BI). Support ≥50% for ML and ≥0.95 for MB is shown. ML/BI. Drawing of *Crucigenia lauterbornii* is shown.

NEPHROCYTIUM AGARDHIANUM SAG 34.81

SAG 34.81 *Nephrocytium agardhianum* Nägeli was placed in the Sphaeropleales *incertae sedis* as the sister to the taxon *Pseudomuriella* N.Hanagata sp. (Fig. 6).

Figure 6: Phylogenetic analyses of SSU rRNA gene sequences of members of **Sphaeropleales** with Volvocales as outgroup. Numbers at the branches indicate bootstrap support from maximum likelihood (ML, 1000 replicates) and Bayesian posterior probabilities (BI). Support ≥50% for ML and ≥0.95 for MB is shown. ML/BI. Drawing of *Nephrocytium agardhianum* is shown.

OONEPHRIS OBESA CCALA 398

CCALA 398 *Oonephris obesa* (West & G.S.West) Fott clusters as a sister to *Cylindrocapsa geminella* Wolle within the Volvocales Treubarinia clade *sensu* Nakada et al. (2008) (Fig. 7).

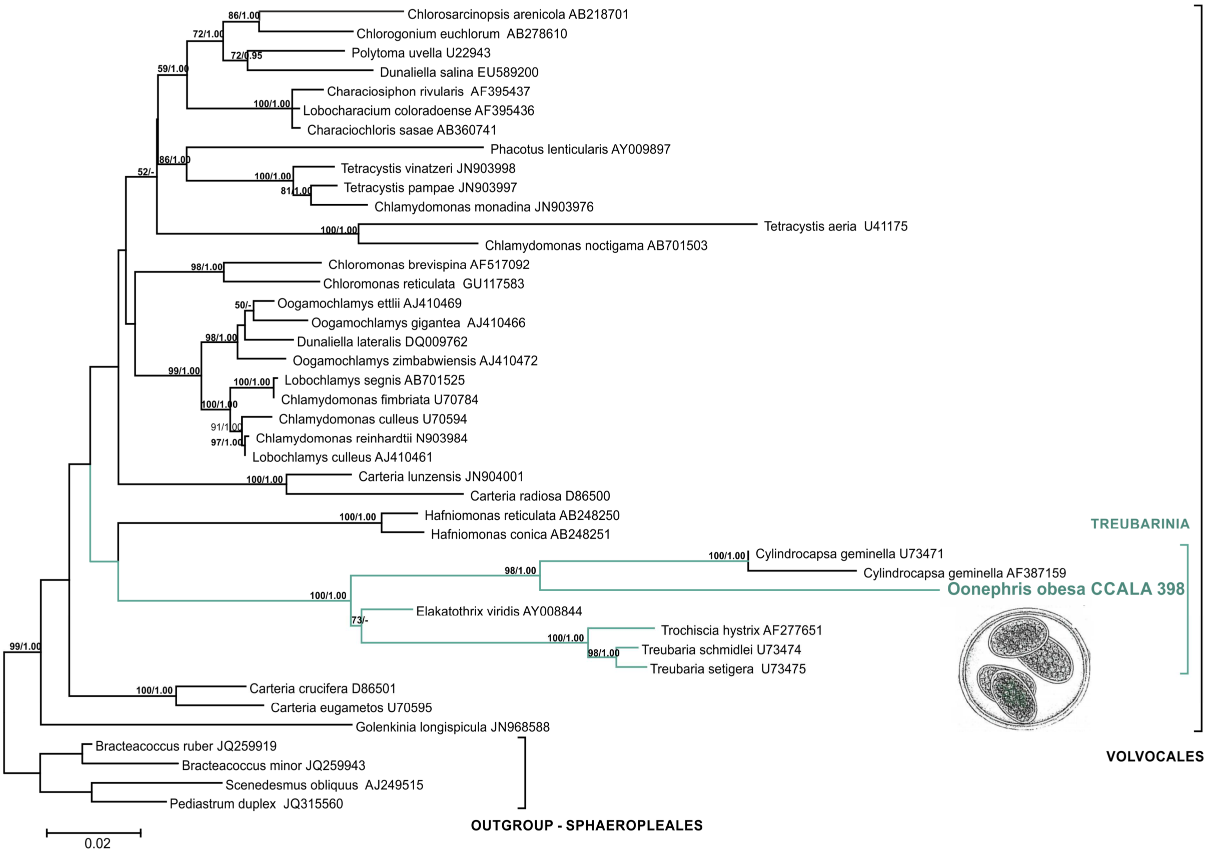

Figure 7: Phylogenetic analyses of SSU rRNA gene sequences of members of **Volvocales** with Sphaeropleales as outgroup. Numbers at the branches indicate bootstrap support from maximum likelihood (ML, 1000 replicates) and Bayesian posterior probabilities (BI). Support ≥50% for ML and ≥0.95 for MB is shown. ML/BI. Drawing of *Oonephris obesa* is shown.

OOCYSTACEAE

All remaining strains made monophyletic clade with the strong support. Trees made from sequences of SSU rRNA gene, of *rbcl* gene and of the concatenated alignment of the both genes is shown on Figs. 8-10. Three subfamilies Eremosphaeroideae, Makinelloideae and Oocystoideae and 5 morphological clades are presented.

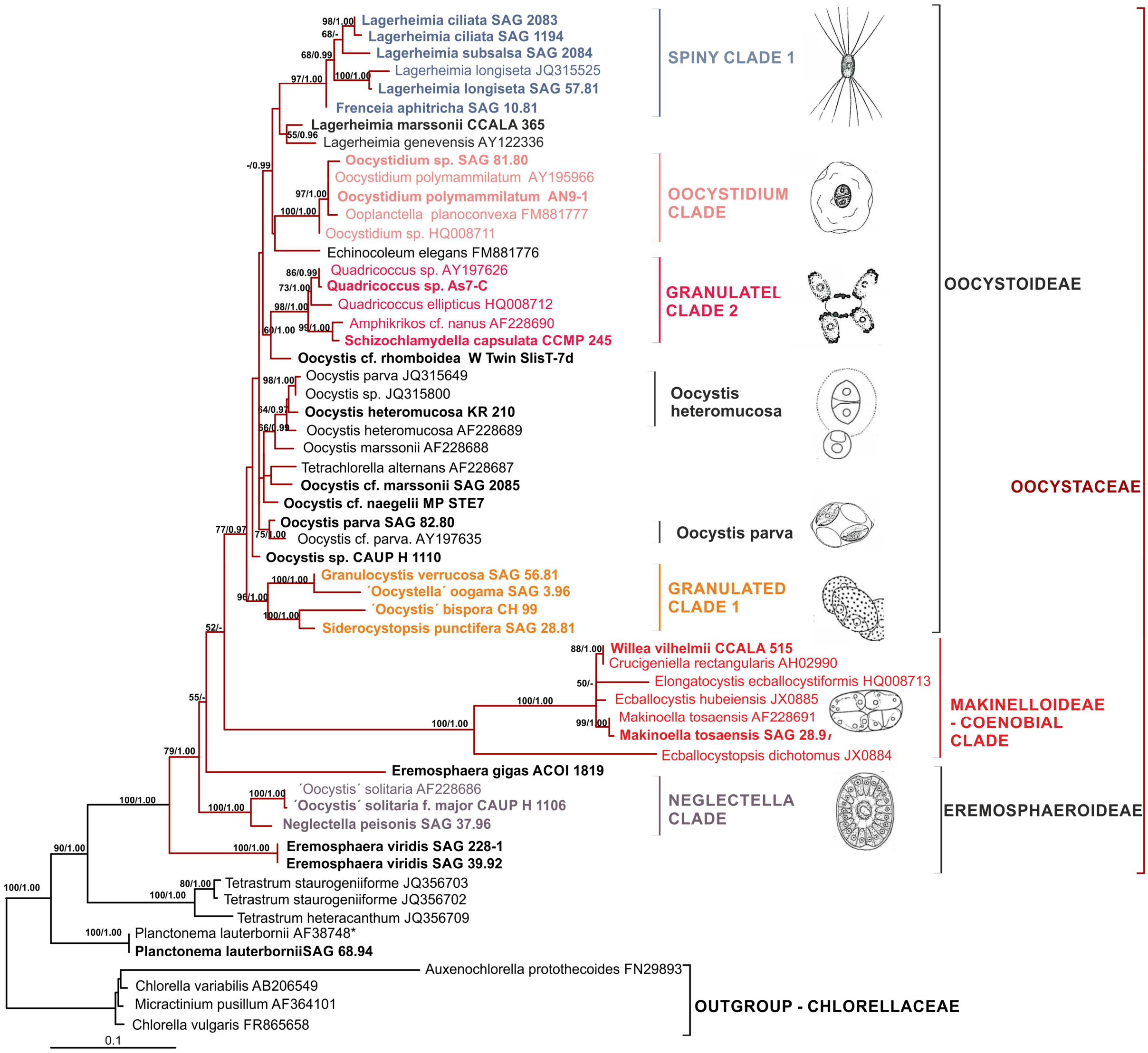

Figure 8: Phylogenetic analyses of **SSU rRNA** gene sequences of members of Oocystaceae with Chlorellaceae as outgroup. Numbers at the branches indicate bootstrap support from maximum likelihood (ML, 100 replicates) and Bayesian posterior probabilities (BI). Support ≥50% for ML and ≥0.95 for MB is shown. ML/BI (Bold taxa = one sequence added.). Drawings demonstrate typical morphology of the individual clades.

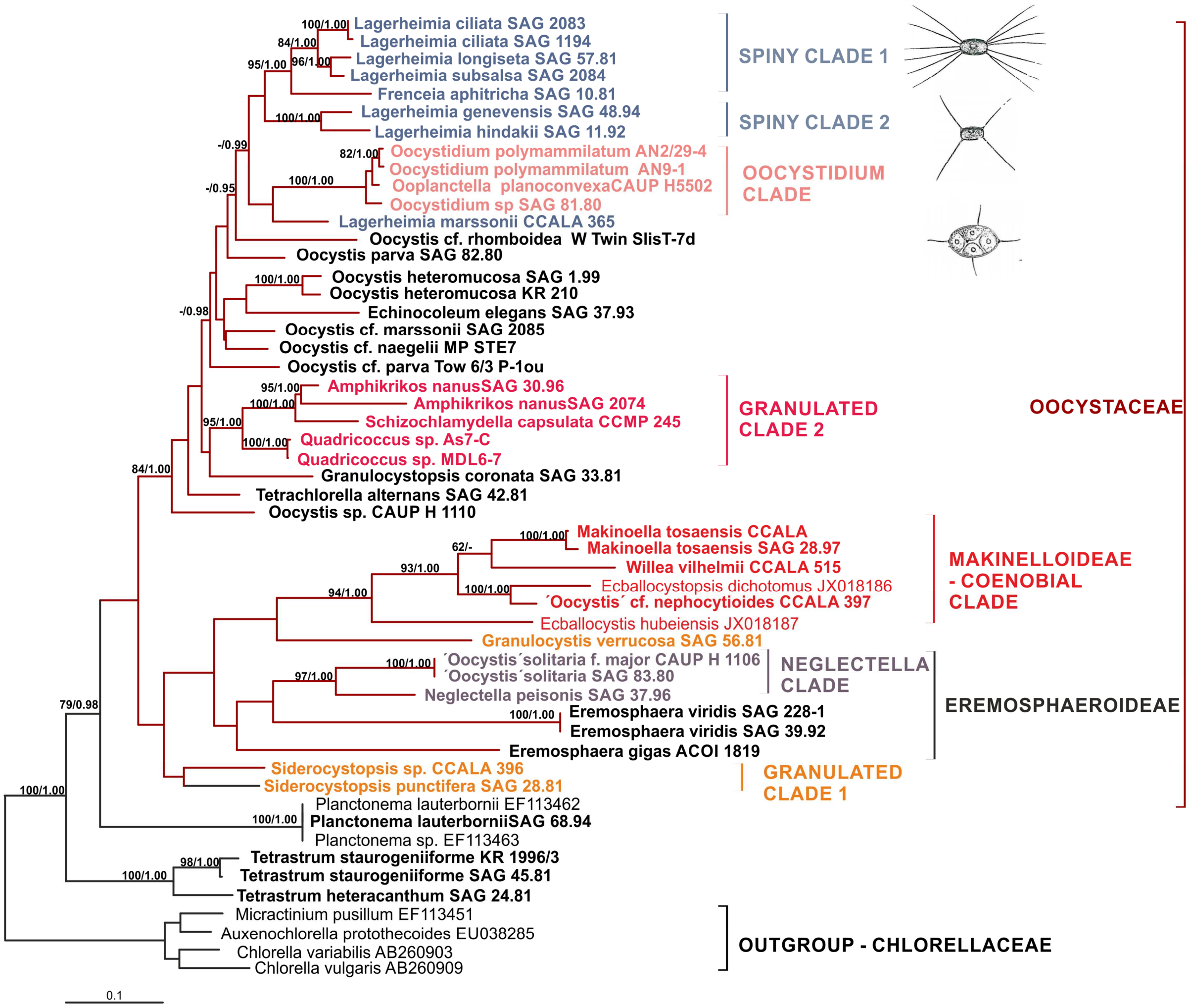

Figure 9: Phylogenetic analyses of ***rbcl*** gene sequences of members of Oocystaceae with Chlorellaceae as outgroup. Numbers at the branches indicate bootstrap support from maximum likelihood (ML, 100 replicates) and Bayesian posterior probabilities (BI). Support ≥50% for ML and ≥0.95 for MB is shown. ML/BI. (Bold taxa = one sequence added.) Drawings demonstrate typical morphology of the individual *Lagerheimia* lineages.

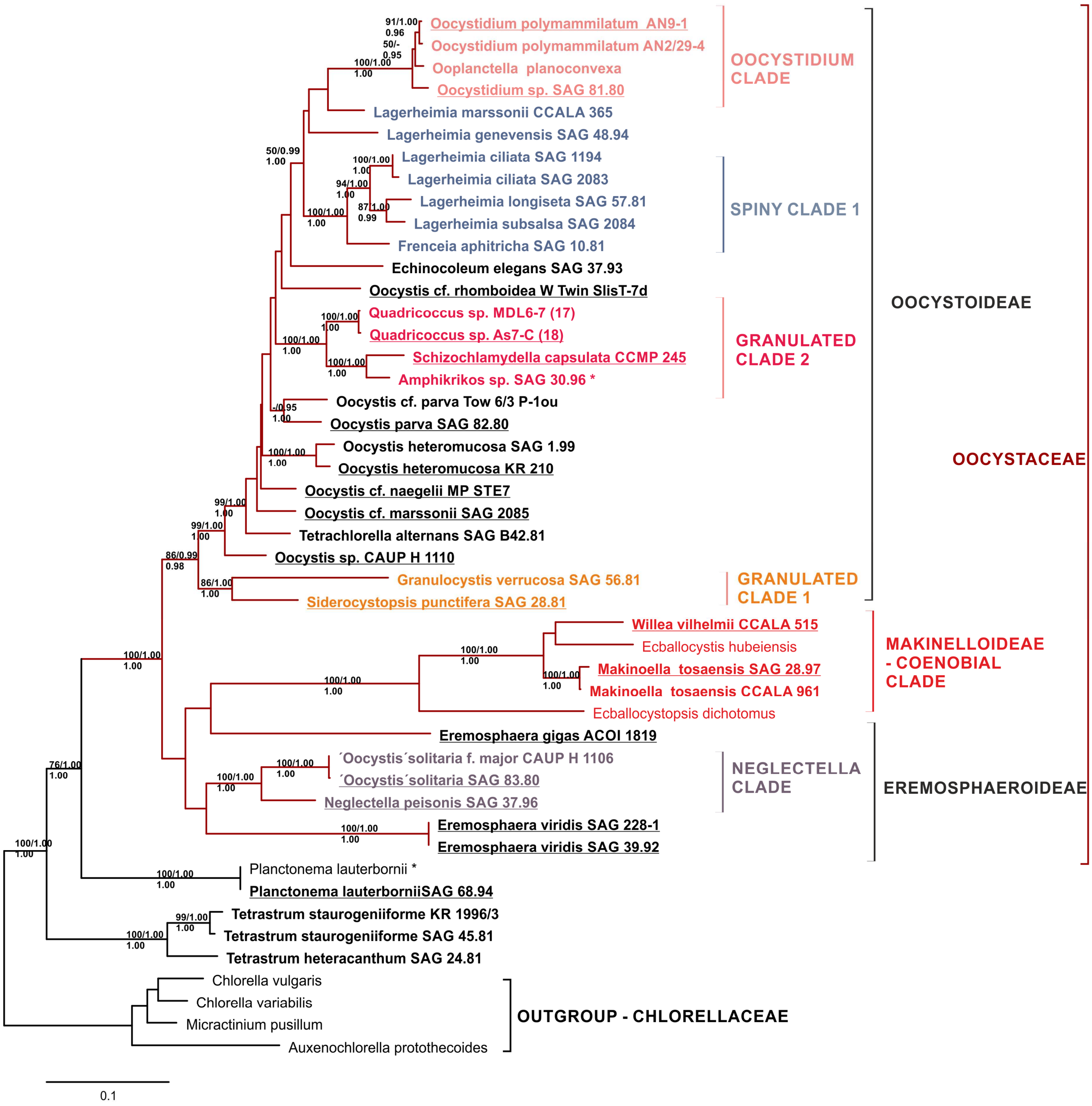

Figure 10: Phylogenetic analyses concatenated dataset of **SSU rRNA** and ***rbcl*** sequences of members of Oocystaceae with Chlorellaceae as outgroup. Numbers at the branches indicate bootstrap support from maximum likelihood (ML, 100 replicates) of unpartitioned alignment and Bayesian posterior probabilities of unpartitioned (BI) and partitioned (PBI) alignment. Support ≥50% for ML and ≥0.95 for MB is shown ML/BI/PBI. (Bold and underlined taxa = both sequences added, bold = one sequence added.)

4. Discussion

4.1. Morphology

Strains names were corrected according the Komárek and Fott (1983) and appropriate changes were made (Appendix 1). 5 names were changed, 3 completed and 9 unknown strains determined. 5 strains were determined only to the genus, when the presence of one or more determining traits was missing or uncertain. Two nearly identical observed strains of genus *Quadricoccus* Fott were defined as *Quadricoccus* sp. due an unclear state of cell arrangement. Name *Oocystidium* sp. Korshikov was established for strain with uncertain presence of mucilage projections of mucilage cover. Only *Siderocystopsis* sp. Swale was chosen for granulated strain because the arrangement of the granula on the surface of the cell wall was not sufficiently investigated. *Oocystis* sp. A.Braun was chosen because of the high variability of cell dimensions and shape of the cell.

Some of these complications were probably caused by deformations maintaining them under culture conditions. Cultivation can influence spine production of spiny algae. Two spiny strains (CCALA 365 *Lagerheimia marssonii* Lemmerm. and SAG 48.94 *Lagerheimia genevensis* (Chodat) Chodat were examined many times to capture spines and spiny cells of SAG 11.92 *Lagerheimia hindakii* Hegewald & Schmidt were not detected at all. It could be caused by the small dimension of cells and spines of this algae. Spines should be better examined by scanning electron microscopy as executed Hegewald et al. (1980). Hindák (1978) discussed the elimination of the coenobial organization during cultivation. Two observed strains of genus *Quadricoccus* Fott lack the arrangement in the typical tetrads in accord with observation of *Quadricoccus* performed by Krienitz and Bock (2011). According to Pažoutová et al. (2010) the mucilage is not detectable at standard conditions. In strains of the genus *Oocystidium* Korshikov were visible. Bock and Krienitz (unpublished results) assumed that for some coccal green algae (*Dictyosphaerium granulatum* Hindák and *Raphidocelis div. spec.*) granula are only phenotypic features.

Importance of authentication of the species names demonstrates changes made in the present study. Strain SAG 2083 labeled as *Lagerheimia longiseta* (Lemmerm.) Printz were corrected to *L. ciliata* (Lagerh.) Chodat. These species differ only in the range of the spines and according to Hindák (1978) both species represent the same algae.

Granulated strain CCALA 396 labelled as *Oocystis* cf. *lacustris* Chod. bears granules that are not in accord with the descriptions of the species. New name *Siderocystopsis sp.* for the strain was designated according to Komárek and Fott (1983). Strain CCMP 245 *Schizochlamydella capsulata* (Guillard, H.C.Bold & MacEntee) S.Watan. was not authenticated. Description of the species does not mention granule on the surface of the cell visible on the photo of the strain from NCMA (CCMP). Verification of the determination of the strain is required. Original descriptions of the granulated strain CB 99 *Oocystis bispora* Komárek do not mention granula. Species *Oocystis bispora* is uncompromisingly typical by its propagation by 2 autospores; therefore no doubt is about itsdetermination.

Strain SAG 81.80 *Oocystidium* sp. was incorrectly determined as *Oocystis lacustris* Chodat which seems to be a common mistake in the literature (Řeháková 1969, Stoyneva 2007). Additional 2 *Oocystis* strains were determined inaccurately, and one designation is uncertain.

4.2. Ultrastructure

Excluded strains do not possess *Oocystis*-like ultrastructure of the cell wall as expected. The cell walls of two basal genera *Tetrastrum* Chodat and *Planctonema* Schmidle were examined to determine, whether it is proper to include these genera into the family Oocystaceae. The desired ultrastructure was not found; accordingly there is no reason to assign them to the family.

Six studied strains of Oocystaceae SAG 2081 *Crucigeniella rectangularis* (Nägeli) Komárek, SAG 56.81 *Granulocystis verrucosa* (Y.V.Roll) Hindák, CCALA 961 *Makinoella tosaensis* Okada, Tow 6/3 P-1ou *Oocystis* cf. *parva* W.West & G.S.West, SAG 42.81 *Tetrachlorella alternans* (G.M.Sm.) Korshikov and CCALA 515 *Willea vilhelmii* (Fott) Komárek beard the multi-layered structure with cellulosic fibrils arranged crosswise to that in the adjoining layer. In comparison with preliminary research (Bowen 1965, Crawford and Heap 1978, Hegewald et al. 1978, Quader et al. 1978, Hegewald et al. 1980, Schnepf et al. 1980, Schagerl 1993, Hegewald et al. 1999 and Xia et al. 2013) is evident that cell wall of taxa with large cells as *Eremosphaera* De Bary, *Oocystis solitaria* Witrock and *Neglectella peisonis* Schagerl composed of larger amounts of layer, then cell wall of small celled algae as *Willea vilhelmii* or *Granulocystis verrucosa* and especially *Oocystis* cf. *parva*.

4.3. Molecular phylogeny

SEQUENCES

Two combinations of primers used for the PCR amplification of all gene were needed as well as two combination of primers for fragmented PCR. Sequences of 6 strains were not obtained, despite several tried primer combinations.

One possible complication is occurrence of numerous introns in sequence of SSU rRNA gene enormously extending the sequence. For example, sequence of the strain Hegewald 1995-26 *Amphikrikos* cf. *nanus* (Fott and Heynig) Hindák includes 5 introns and complete sequence is 3600bp long (Hepperle et al. 2000); sequence of strain SAG 37.93 *Echinocoleum elegans* Jao & Lee possess 6 introns and sequence 4257bp long (Pažoutová et al. 2010). The other possibility is an intron or some mutation in place of the annealing of the primer. Mutation hypothesis supports rapid mutation rate among SUU rRNA gene of Oocystaceae as compared with the rest of Trebouxiophyceae.

Rbcl sequences were far easier to amplify than sequences of SSU rRNA gene. Only 5 strains were not amplified. Lack of *rbcl* sequences of CCAP 274/3 *Elongatocystis ecballocystiformis* and SAG 2181 *Crucigeniella rectangularis* (Nägeli) Komárek is caused by death of the strains before successful DNA isolation. Content of the further work is obtaining sequences of both genes of all strains by trying other primer combinations.

COMPARISION OF THE TREES

Phylogenetic trees of two different genes were constructed: SSU rRNA tree, *rbcl* tree and tree based on concatenated alignment of both genes. The level of resolution of each gene differs, therefore, concatenated tree disposes of the best support.

Sample sorting of both genes differs, yet topologies do not exclude each other except the situation on the basis of the trees. SSU rRNA gene analysis show *Tetrastrum* Chodat closer then *Planctonema* Schmidle to the family Oocystaceae. *Rbcl* gene and concatenated trees propose *Planctonema* closer then *Tetrastrum.* (Figs. 8 – 10)

Especially the tree of the SSU rRNA gene is characterized by disproportionately different lengths of the branches. Strains of Eremosphaeroideae and especially Makinoelloideae possess extremely long ones and the strains inside Oocystoideae, especially in the group

Oocystis sensu lato short ones. In the tree constructed on the basis of the *rbcl* gene sequences are not the differences so significant as in the SSU rRNA tree.

Neither tree was able to distinguish relationships within strains of *Oocystis sensu lato* group. Additional strains and more sequenced genes can help resolve the situation inside the group. As suitable gene, I propose ITS region consisted of ITS1, 5,8S rRNA and TIS2 region or some housekeeping gene, which will be designated on the basis of the intended research of the respected literature.

Some relations among subfamily Eremosphaeroideae are not determined either. Tree of SSU rRNA gene showed it as paraphyletic and trees of *rbcl* gene and concatenated dataset as monophyletic. However, neither state is well supported. In the subfamily occur long branches caused probably due to the incomplete lineage sorting. Long branches entail numerous homoplasies and therefore long branch attraction occurs, especially in maximum likelihood analyses. Long branch attraction complicates clear reconstruction of the phylogeny. Long branch attraction was noticeable in the case of the strain ACOI 1918 *Eremosphaera gigas* (W.Archer) Fott & Kalina. Its position varies according to the type of analyses. ML analysis assigned it to the Makinelloideae without support; BI to the *Neglectella* clade moderately supported. Advanced taxa selection will probably break the long branches and provide explicit results.

4.4. Delimitation

Three morphologically and ultrastructurally Oocystaceae unlike taxa SAG 2082 *Komarekia rotundata* (Teiling) Fott, SAG 34.81 *Nephrocytium agardhianum* Nägeli and CCALA 398 *Oonephris obesa* (West & G.S.West) Fott were excluded from the family. All three taxa lack *Oocystis*-like ultrastructure of the cell wall (Fig. 2).

Komarekia rotundata new member of the family Chlorellaceae clusters with sufficient support as a sister to the *Crucigenia lauterbornii* (Schmidle) Schmidle analyzed by Bock et al. (2013). The authors left their finding without taxonomical conclusion. In this thesis, I recommend including both taxa in the genus *Komarekia* Fott as *Komarekia lauterbornii* (Schmidle) Fott and *Komarekia rotundata* (Teiling) Fott as proposed Fott (1981). Morphological similarity of both taxa is demonstrated by Fig. 11 (Appendix 4).

Nephrocytium agardhianum is placed in the Sphaeropleales *incertae sedis* as the sister to *Pseudomuriella* N.Hanagata sp.. These two taxa present new separated lineage within

Sphaeropleales. Without any more data, I cannot do taxonomical conclusion. Morphological comparison of some Sphaeropleales species is shown on the Fig. 12 (Appendix 4).

Oonephris obesa clusters as a sister to *Cylindrocapsa geminella* Wolle within the Volvocales clade Treubarinia *sensu* Nakada et al. (2008). Group disposes of disproportionately long branches (Fig. 7). See Fig. 13 (Appendix 4). for comparison of the morphology of these *Oonephris obesa* and *Cylindrocapsa geminella*.

The remaining strains constitute monophyletic clade inside Trebouxiophyceae including coenobial *Willea vilhelmii* (Fott) Komárek originally placed in Scenedesmaceae (Komárek and Fott 1983) and previously transferred to the Oocystaceae (Štenclová 2011). Ultrastructure of the cell wall similar to other members of the Oocystaceae supports the position of *Willea vilhelmii* (Fig. 4).

Whether to include to the family two basal genera *Planctonema* Schmidle and *Tetrastrum* Chodat, is controversial. The presence of multi-layered cell wall was not confirmed (Fig. 3). Both genera also dispose of differences in some morphological features (Fig. 14, Appendix 4). *Planctonema lauterbornii* Schmidle has a filamentous thallus and does not reproduce by autospores, but by fragmentation of the filament (Schmidle 1903). Parietal chloroplast and the cylindrical cell shape are in agreement to the family definition. In contrary *Tetrastrum* species propagate with autospores and make crucigenoid coenobial as some taxa in coenobial clade of Oocystaceae. Cell shape is similar to the *Crucigenia* Morren species and structure typically *Chlorella*-like with one parietal chloroplast and small rounded pyrenoid. There is a possibility that both genera belong to two separate lineages.

4.5. Definition

In accord with previous molecular studies (Buchheim et al. 2001, Krienitz et al. 2011), changes presented in this thesis indicate that the definition of the family regarding the shape of the cell is rather robust. All analysed members of the family possess lemon-like, oval, cylindrical or nearly spherical cell shape. No irregular-shaped taxa stayed inside family, so far. Conversely, coenobial and pseudo filamentous members, new to the family, show larger diversity of the cell arrangement. The family stays variable in traits cell dimensions and number of chloroplasts. However, it seems to be one of the criteria for internal division of the family.

4.6. Internal structure

SUBFAMILIES

Komárek and Fott (1983) divided family Oocystaceae into the three subfamilies: Eremosphaeroideae, Lagerheimioideae and Oocystoideae. Molecular phylogeny combined with a morphology of the cell support different concept consisted of three subfamilies **Eremosphaeroideae, Oocystoideae** and **Makinelloideae** *subfamily nova.* The name of the subfamily was chosen on the basis of clear defined species *Makinoella tosaensis* Okada. Taxonomic relations around species of *Crucigeniella* Lemmerm. and *Willea* Schmidle, as well as *Ecballocystis* Bohlin and *Elongatocystis* Krienitz & C.Bock, is not clear.

Subfamily **Makinelloideae** well supported by molecular phylogeny represent coenobial clade comprised of strains SAG 2081 *Crucigeniella rectangularis* (Nägeli) Komárek, CCALA 515 *Willea vilhelmii* (Fott) Komárek, CCALA 397 *Oocystis cf. nephrocytioides* Fott & Čado, CCAP 274/3 *Elongatocystis ecballocystiformis* Krienitz and C. Bock, *Ecballocystis hubeiensis* Hu & Liu, *Ecballocystopsis dichotomus* Hu & Bi, SAG 28.97 *Makinoella tosaensis* Okada and CCALA 961 *Makinoella tosaensis.* Main part of the strains were preliminary classified as members of Scenedesmaceae Crucigenoideae. One ex-crucigenoid coenobial algae SAG 42.81 *Tetrachlorella alternans* G.M.Sm.) Korshikov was not included. *Tetrachlorella* Korshikov varies from other coenobial taxa of Oocystaceae in the spindle shaped cells. The rest of the strains possesses oval to elliptical cells with rounded ends. *Tetrachlorella* is included into the group *Oocystis sensu lato* comprised of *Oocystis* species with unresolved relations.

In addition to the coenobial strains, three strains of different morphology were included into the subfamily Makinelloideae: *Ecballocystis hubeiensis*, *Ecballocystopsis dichotomus* and *Elongatocystis ecballocystiformis.* All three strains possess long cylindrical cell organized in tetrads and enclosed in the mother cell wall. The tetrads of *Ecballocystis hubeiensis* and *Ecballocystopsis dichotomus* are arranged into simple filaments. Morphological characters of the subfamily Makinelloideae are visualized on the Fig. 15 (Appendix 4).

Subfamily **Eremosphaeroideae** contains two genera *Neglectella* Voden. & Benderl. (included *Neglectella solitaria* (Witrock) Štenclová *combination nova*) and *Eremosphaera* De Bary. Monophyly is not significantly supported by molecular analyses. It can be caused by narrow species sample accompanied by long branches and their attraction probably caused by homoplasies. Incomplete lineage sorting in common problem in phylogeny to sequence additional taxa is recommended. Still, clear morphological delimitation based of presence of numerous chloroplasts and large size of the cells supports recognition of the subfamily. Morphological characteristics of the subfamily are shown on the Fig. 16 (Appendix 4).

Newly defined subfamily **Oocystoideae** includes Oocystoideae (except *Neglectella* Voden. & Benderl.) and Lagerheimioideae *sensu* Komárek and Fott (1983). It is divided to four morphologically and phylogenetically well defined clusters. *Oocystidium* clade, spiny clades 1 and 2, granulated clade 1 and 2. Remaining strains assigned to the genus *Oocystis* and *Tetrachlorella* were entitled as *Oocystis sensu lato* group.

SPINY CLADES

Group containing taxa preliminary in Lagerheimioideae *sensu* Komárek and Fott (1983). Some analysis shows it as monophyletic other as paraphyletic. Neither state is well-supported. Even if monophyly of the spiny taxa is not supported by molecular phylogeny, its existence as classification unit is acceptable. Some paraphyletic taxa have a predictive value because the members of such group share a common evolution, unlike the polyphyletic taxa (Hörandel and Stuessy 2010). Moreover, the group is supported by strong synapomorphy: spines. Variability of spiny strains is demonstrated on the Fig. 17 (Appendix 4). Analysis of *rbcl* gene sequences distinguishes **two spiny clades**.

GRANULATED CLADES

For Oocystaceae, granula seems to be systematical trait, despite suggestion of Bock and Krienitz (unpublished results) that for some coccal green algae are granula only phenotypic features. Granulated strains inside Oocystaceae are not monophyletic but form **two granulated clades**. Variability of granulated strains is demonstrated on the Fig. 18 (Appendix 4).

Granulated clade 1 consisted of strains with spindle-shaped or elliptical cells with granula irregularly arranged on all cell surfaces. Clade includes 5 strains: CCALA 396 *Siderocystopsis sp*. Chodat, SAG 28.81 *Siderocystopsis punctifera* (Bolochonzew) Hegewald & Schnepf, SAG 56.81 *Granulocystis verrucosa* (Y.V.Roll) Hindák, SAG 3.96 *Oocystella oogama* Hindák and CB 99 *Oocystis bispora* Komárek. *Oocystis bispora* clusters sister to *Siderocystopsis punctifera* (type species) and *Oocystella oogama* sister to *Granulocystis verrucosa*. Further taxonomical changes among this group are supposed. Granulated clade 1 is not supported by maximum likelihood analysis of *rbcl* gene. Bayesian inference put three strains CCALA 396 *Siderocystopsis sp.* Chodat, SAG 28.81 *Siderocystopsis punctifera* (Bolochonzew) Hegewald & Schnepf and SAG 56.81 *Granulocystis verrucosa* (Y.V.Roll) Hindák together with posterior probability of 0.97. Differences are caused by long branch attraction the result of incomplete lineage sorting.

Granulated clade 2 consisted of strain with oval to nearly spherical shape of the cell and subpolar arrangement of granula. Clade contains *Quadricoccus* spp., two strains of the species *Amphikrikos nanus* and CCMP 245 *Schizochlamydella capsulata* (Guillard, H.C.Bold & MacEntee) S.Watan. with strong support. Clear on morphology based affiliation of the strain SAG 33.81 *Granulocystopsis coronata* (Lemmerm.) Hindák to the clade stays without molecular support.

OOCYSTIDIUM Koršikov **CLADE**

Well supported *Oocystidium* Koršikov clade includes *Oocystidium* species and *Ooplanctella planoconvexa* (Hindák) Pažoutová, Škaloud & Nemjová. Shared morphological traits as the wide mucilaginous envelope support natural character of the clade. Unexpected is pending position of SAG 37.93 *Echinocoleum elegans* Jao & Lee that possesses a similar mucilaginous envelope. Its relationship with the *Oocystidium* clade and other Oocystaceae remains unclear, despite the phylogenetic analyses based of sequences of two genes. Analyse of SSU rRNA gene and combined dataset shows its position close to the *Oocystidium* clade (SSU rRNA) or to the group of *Oocystidium* clade with the spiny strains (combined dataset); though without significant support. Similar mucilage cover with the projections was also described for *Oocystis lacustris* Chodat (Řeháková 1969) and *Lagerheimia ciliata* (Lagerh.) Chodat (Hindák 1978). *Lagerheimia* Chodat strains are close to *Oocystidium* clade according the molecular phylogeny. Relationship of the *Oocystis lacustris* and *Oocystidium* sp. is not resolved. Variability of mucilage forming strains is demonstrated on the Fig. 19 (Appendix 4).

OOCYSTIS SENSU LATO GROUP

The rest of the strains are assigned to the genus *Oocystis* A. Braun. Position of the strains remains unclear except two clades: *Oocystis heteromucosa* Hegewald clade and *Oocystis parva* W.West & G.S.West clade.

4.7. Generic and species concept

Inside some genera species concept changed or is expected to be changed in the close future.

WILLEA Schmidle

Additionally to the traditional species of genus *Willea* Schmidle *Willea vilhelmii* (Fott) Komárek and *Willea irregularis* (Wille) Schmidle, Gonzales et al. (1984) established species *Willea komarekii* A.Comas Gonzales. Genera *Willea* and *Crucigeniella* Lemmerm. share similar cell morphology and arrangement into the crucigenoid coenobia and both were traditionally assigned into the family Scenedesmaceae subfamily Crucigenoideae. *Crucigeniella rectangularis* (Nägeli) Komárek and *Willea vilhelmii* cluster inside Oocystaceae as sisters to each other with satisfied support. John and Tsarenko (2002) proposed the transfer of *Willea irregularis* (Wille) Schmidle to the genus *Crucigeniella as Crucigeniella irregularis* (Wille) P.M. Tsarenko and D.M. John. The type species *Crucigeniella lunaris* Lemmerm. (now *Crucigenia lunaris* (Lemmerm.) Wille) has to be sequenced to make taxonomical conclusion of the situation.

ECBALLOCYSTIS Bohlin and **ECBALLOCYSTOPSIS** M.O.P.Iyengar

Genus *Elongatocystis* Krienitz & C.Bock with the type species *Elongatocystis ecballocystiformis* Krienitz & C.Bock recently established by Krienitz and Bock (2011) possesses similar morphology as genus *Ecballocystis* Bohlin. *Elongatocystis ecballocystiformis* clusters close to *Ecballocystis hubeiensis* Hu & Liu, however, distant from *Ecballocystopsis dichotomus* Hu & Bi that represents different lineage (Xia et al. 2013). The strain CCALA 397 designated as *Oocystis* cf. *nephrocytioides* Fott & Čado probably belongs to the genus *Ecballocystopsis* M.O.P.Iyengar. To resolve relationship among genera *Elongatocystis, Ecballocystis* and *Ecballocystopsis* is necessary to sequence additional members of the genus *Ecballocystis* including the type species *E. pulvinata* Bohlin and *Ecballocystopsis* including the type species *E. indica* M.O.P.Iyengar.

NEGLECTELLA Voden. & Benderl.

In this thesis, I propose transfer of *Oocystis solitaria* Wittrock inside the genus *Neglectella* Voden. & Benderl. as a new combination *Neglectella solitaria* (Wittrock) Štenclová *combination nova.* Molecular phylogeny and morphological similarities arrangement of numerous chloroplasts, cell dimensions, shape of the cell and staying in the mother cell walls. The ecology of *Oocystis solitaria* littoral of the acidic freshwaters is also closer to the *Neglectella* littoral of the acidic freshwater than to the *Oocystis*, planktonic in freshwater

(Schagerl 1993). Synonymity of *Neglectella solitaria* var. *major* with *Neglectella solitaria* was proposed on the basis of morphology and molecular phylogeny.

Neglectella solitaria (Wittrock) Štenclová *combination nova*

Basionym: *Oocystis solitaria* Wittrock in Wittrock and Nordsted (1889, p. 24, iconotype figs 1-5). Homotypic Synonym: *Oocystella solitaria* (Wittrock) Hindák, Heterotypic Synonyms: *Oocystis solitaria* var. *notabile* West & G.S.West, *Oocystis crassa* Wittrock, Heterotypic Synonym: (established in present study) *Oocystis solitaria* var. *major* (Wille) P.M.Tsarenko.

EREMOSPHAERA De Bary

On the basis of morphological observation, *Eremosphaera gigas* (W.Archer) Fott & Kalina will be probably included into *Neglectella* Voden. & Benderl.. The cell structure, especially chloroplast arrangement, differs from the *Eremosphaera viridis* De Bary whose cells contain large central vacuole traversed by radial strands of cytoplasm with chloroplasts which connect central nucleus to periphery part of the cell with numerous chloroplasts. No such structure was found in *Eremosphaera gigas*. Arrangement of the chloroplast is *Neglectella*-like: stacked in the surface layer of the cell. Long branches complicate reconstruction of the phylogeny of all Eremosphaeroideae. Data of additional strains of thegenera *Eremosphaera* and *Neglectella*, principally *N. eremosphaerophila* Voden. & Benderl., type species of the genus is required for better taxonomical revision.

LAGERHEIMIA Chodat and **FRANCEIA** Lemmerm.

Eight spiny strains were analysed in the present thesis. Molecular phylogeny combined with morphology supports three types of spiny group: *L. longiseta* (Lemmerm.) Printz clade – spiny clade 1, *L. genevensis* (Chodat) Chodat clade – spiny clade 2, and separated strain of *L. marssonii* Lemmerm..

Inside **spiny clade 1** clustered four species *Franceia amphitricha* (Lagerh.) Hegewald, ***L. ciliata*** (Lagerh.) Chodat, *L. subsalsa* Lemmerm., and *L. longiseta* (Lemmerm.) Printz form a monophyletic clade according all three trees. All clade is characterised by plurality of spines and different species are distinguished by their length, number and placement.

Spiny clade 2 consisted of strains *Lagerheimia genevensis* (Chodat) Chodat and *L. hindakii* Hegewald & Schmidt clusters together as shown on *rbcl* tree. Synapomorphy of the clade is arrangement of spines, two on each pole of the cell placed subpolar. The placement of

Lagerheimia marssonii disposes of poor support. This alga differentiated of both spiny clades by a few spines arranged polarly and equatorial.

There are several possibilities of classification of this situation. One of the possibilities is to recognize one large genera *Lagerheimia sensu lato* that includes all these spiny taxa. In the case of paraphyly accept it despite the paraphyly. Second possibility is to include spiny species and *Oocystidium* clade into one group. Both group together clusters as well-supported monophyletic clade. These actions disagree to the recent trend to establish small genera that differentiate only in few features and include a small number of species (Luo et al. 2010). Genus *Lagerheimia* will be presumably split into two or three genera. Spiny clade 2 contains type species *of Lagerheimia L. Genevensis*; therefore it will remain *Lagerheimia.* Spiny clade 1 represents another genus, probably *Franceia* Lemmerm. According to one of the included strains (SAG 10.81 *Franceia amphitricha* (Lagerh.) Hegewald). The sequencing more species of both genera *Lagerheimia* nad *Franceia*, including the type species *Franceia ovalis* (Francé) Lemmerm., is recommended for the resolution of this taxonomical issue. More data can help to resolve the position of *Lagerheimia marssonii*.

OOCYSTIDIUM Korsikov

Two strains determined as *Oocystidium polymammilatum* Hortob. Were included into *Oocystidium* Korsikov clade with two strains marked as *Oocystidium* sp.. Hindák and Horecká (1987) transferred *Oocystidium polymammilatum* newly combined as *Echinocoleum polymammilatum* (Hortob.) Hindák and Horecká into the genus *Echinocoleum* Jao end Lee. Phylogenetic analyses in this thesis rejected close relationship; therefore I recommend old combination *Oocystidium polymammilatum* Hortob.

Inside the cluster of *Oocystidium* species appeared strain *Ooplanctella planoconvexa* (Hindák) Pažoutová, Škaloud & Nemjová 2010 (Pažoutová et al. 2010). Analyses reveal tight relationship of *Ooplanctella* and *Oocystidium* strains. I suggest this clade probably represents one genus. On the basis of the molecular phylogeny and morphological similarity of mucilage covers, I suggest new combination *Oocystidium planoconvexum* (Hindák) Štenclová.

Oocystidium planoconvexum (Hindák) Štenclová *combination nova*

Basionym: Coenochloris planoconvexa Hindák (1977, p. 22, iconotype Pl. 5 fig. 1). Epitype: The strain CAUP H5502 permanently cryopreserved at the Culture Collection of algae of the

Charles University in Prague, Czech Republic (CAUP) (Pažoutová et al. 2010). Homotypic Synonym: *Ooplanctella planoconvexa* (Hindák) Pažoutová, Škaloud & Nemjová.

OOPLANCTELLA Pažoutová, Škaloud & Nemjová

Genus *Ooplanctella* and species *Ooplanctella planoconvexa* (Hindák) Pažoutová, Škaloud & Nemjová as well as *Coenochloris planoconvexa* Hindák is proposed to be recognized as synonymous to *Oocystidium* Korsikov and *Oocystidium planoconvexum* (Hindák) Štenclová.

ECHINOCOLEUM Jao & Lee

By rejecting of the combination *Echinocoleum polymammilatum* (Hortob.) Hindák and Horecká in the genus *Echinocoleum* Jao & Lee remain *Echinocoleum elegans* Jao & Lee and recently described *Echinocoleum flocculosum* Wujek (Wujek 2012). Species differ of all Oocystaceae species by its spherical shape and its affiliation to the genus *Echinocoleum* is doubtful.

OOCYSTIS A. Braun and **OOCYSTELLA** Lemmerman

First clade contains the strains SAG 82.80 *Oocystis parva* W.West & G.S.West and Tow 6/3 P-1ou *Oocystis* cf. *parva* that cluster sister to each other.

Second cluster consisted of four strains. Two strains designated as *Oocystis parva* and *Oocystis* sp. (not authenticated) cluster together with two strains of *Oocystis heteromucosa* Hegewald. In this clade appeared authentic strain SAG 1.99 *Oocystis heteromucosa*. I propose all the members of the clade are representatives of the species *Oocystis heteromucosa*. The Strain KR 96/10 labeled as *Oocystis marssonii* Lemmerm. Clusters as a sister to the clade. Strain was sequenced by Hepperle et al. (2000), and its determination was not verification.

Another strain SAG 2085 designated as *Oocystis heteromucosa* is was labeled as *Oocystis* cf. *marssonii* when authenticated following to Komárek and Fott (1983). On the contrary, strain CAUP H 1110 labeled as *Oocystis marssonii* is probably different species. Strain was labeled as *Oocystis sp*.

Characteristics of the strain STE 7 responds to the characteristics of species *Oocystis naegelii* except the presence of the pyrenoid. In some taxa, it is problematic recognize whether a taxon bears a pyrenoid. In some species (*Makinoella tosaensis* Okada Hegewald et al. 1999) pyrenoid is presented, yet not clearly visible in the light microscope. Examination under TEM is recommended. Skuja (1964) found in the cell of the type species of *Oocystis O. naegelii* chloroplasts both with and without pyrenoid. Strain was labelled as *Oocystis* cf. *naegelii*.

Complex examination of the presence of pyrenoid is necessary, because Hindák (1988) suggested splitting the genus into two genera, *Oocystis* and *Oocystella* on the basis of possession of pyrenoid. *Oocystis* species without pyrenoid is not available in the public culture collections. The main future aim is to isolate additional strains of the genus *Oocystis*, especially the *Oocystis* species without pyrenoid (if exist) including the type species *Oocystis naegelii*, and on the basis of molecular and morphological data provide comprehensive revision of the genus.

So far I propose to transfer *Oocystis solitaria* Wittrock to the genus *Neglectella* Voden. & Benderl. On the basis of molecular, morphological and ecological similarity in accord with the previous suggestions (Schagerl 1993, Hepperle et al. 2000). *Oocystis bispora* Komárek and *Oocystella oogama* Hindák clustered outside *Oocystis sensu lato* group within Granulated clade 1; therefore is appropriated to exclude both species from *Oocystis* and *Oocystella* group. Strain labelled as *Oocystis* cf. *nephrocytioides* occurred inside the Coenobial clade. Its determination as all description of the taxa *Oocystis nephrocytioides* is uncertain, and both need a revision. It is certain that the strain after molecular analyses does not belong to the genus *Oocystis*.

Strain of real *Oocystis lacustris* was not sequenced so far, because two examined strains of *´Oocystis lacustris´* were incorrectly determined. This species is under interest and particularly its relationship to the morphologically close genus *Oocystidium.*

The wrong determined taxa shows that distinct characteristics to recognize individual species are missing. Komárek and Fott (1983) stated 28 and 16 incompletely described species, sometimes with several varieties and according to the authors determination key, individual *Oocystis* species often are distinguished only by one trait. Řeháková (1969) pointed out the wide synonymity among the *Oocystis* species. Conversely, Komárek and Fott (1983) notice that is presumable that some not distinctly described species e.g. *Oocystis marssonii* Lemmerm. And *Oocystis lacustris* Chodat can comprise few species. Stoyneva (2007) demonstrated the wide morphological variability of the *Oocystis* species on the species *Oocystis lacustris* and complicated state inside the genus on the example of species *O. borgei* J.Snow, *O. lacustris, O. marssonii, O. nephrocytioides* Fott & Čado, *O. parva* W.West & G.S.West.

All these facts indicate that the situation inside the genus *Oocystis* is complicated even among typical *Oocystis* species and moreover, was demonstrated that *Oocystis* was traditionally used as collecting genus for spindle-shaped and oval-shaped algae now assigned to the various genera. True *Oocystis* is rather smaller genus than was traditionally expected. The comprehensive revision of the genus *Oocystis* is essential.

5. Conclusion – taxonomy and systematic of green coccal algae

Green coccal algae dispose of a wide variability and numerous described (and undescribed) species. Only a fraction of them were sequenced so far. Molecular phylogeny proved to be a useful tool to find out cryptic diversity within species and genera (Boyer et al. 2002) or reveal polyphyletic origin of genera designated on common morphological traits. The result is the disintegration of traditionally defined species and generic concept and establishment of small genera, as shown e.g. in genera *Dictyosphaerium* Nägeli (Krienitz et al. 2010) and *Chlorella* Beyerinck (Bock et al. 2011). Small genera appropriately reflect molecular variability among taxa and seems to be more natural. Oocystaceae will be probably divided into smaller genera as demonstrated on genera *Oocystis* A. Braun and *Lagerheimia* Chodat.

The situation inside Oocystaceae was shown to be similarly confused as the situation in other families of Chlorophyta. Serious problems occur in the reconstruction of the generic and species concept of the family. The type material is not available in most cases, and the strains are often wrongly determined, or is difficult to determine them, because of the confused descriptions of the species; therefore the majority of the relations of the family stays undefined.

The main results of the thesis is the changed internal structure of the family Oocystaceae and partly revised generic concept, as well as species concept of some genera, especially type genus of the family – *Oocystis* A. Braun. Two new combinations were established, and prospective taxonomical revisions of several genera were proposed. The present study is the basis for future research.

6. References

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The used line drawings are taken from the Komárek and Fott (1983).

7. Appendix

7.1. Appendix 1: List of the strains

Table 4: review of the strains names. The strains with changed names according to the morphological observation are labeled by bold font. Changes made after analyses are presented in the third column. Ex = excluded from *Oocystis/Oocystella*.

STRAIN	ORIGINÁL NAME	DETERMINATION	TAXONOM. CHANGES
SAG 30.96	***Amphikrikos sp.***	***Amphikrikos nanus***	
SAG 2074	***Amphikrikos sp.***	***Amphikrikos nanus***	
SAG 2081	*Crucigeniella rectangularis*	*Crucigeniella rectangularis*	
SAG 37.93	*Echinocoleum elegans*	*Echinocoleum elegans*	
CCAP 274/3	*Elongatocystis*	*Elongatocystis*	
ACOI 1819	*Eremosphaera gigas*	*Eremosphaera gigas*	
SAG 228-1	*Eremosphaera viridis*	*Eremosphaera viridis*	
SAG 39.92	*Eremosphaera viridis*	*Eremosphaera viridis*	
SAG 10.81	*Franceia amphitricha*	*Franceia amphitricha*	
SAG 56.81	*Granulocystis verrucosa*	*Granulocystis verrucosa*	
SAG 33.81	*Granulocystopsis coronata*	*Granulocystopsis coronata*	
SAG 2082	*Komarekia rotundata*	*Komarekia rotundata*	
SAG 1194	***Lagerheimia longiseta***	***Lagerheimia ciliata***	
SAG 2083	*Lagerheimia ciliata*	*Lagerheimia ciliata*	
SAG 48.94	*Lagerheimia genevensis*	*Lagerheimia genevensis*	
SAG 11.92	*Lagerheimia hindakii*	*Lagerheimia hindakii*	
SAG 57.81	*Lagerheimia longiseta*	*Lagerheimia longiseta*	
CCALA 365	*Lagerheimia marssonii*	*Lagerheimia marssonii*	
SAG 2084	*Lagerheimia subsalsa*	*Lagerheimia subsalsa*	
SAG 28.97	*Makinoella tosaensis*	*Makinoella tosaensis*	
CCALA 961	*Makinoella tosaensis*	*Makinoella tosaensis*	
SAG 37.96	*Neglectella peisonis*	*Neglectella peisonis*	
SAG 34.81	*Nephrocytium agardhianum*	*Nephrocytium agardhianum*	
SAG 3.96	*Oocystella oogama*	*Oocystella oogama*	*Oocystella oogama* ex
AN9-1	**-**	***Oocystidium***	
AN2/29-4	**-**	***Oocystidium***	
SAG 81.80	***Oocystis lacustris***	***Oocystidium*** **sp.**	
CH 99	**-**	***Oocystis bispora***	***Oocystis bispora*** ex
SAG 1.99	*Oocystis heteromucosa*	*Oocystis heteromucosa*	
KR 210	**-**	***Oocystis heteromucosa***	
SAG 2085	***Oocystis heteromucosa***	***Oocystis*** **cf.** ***marssonii***	
MP STE7	**-**	***Oocystis*** **cf.** ***naegelii***	
CCALA 397	*Oocystis cf. nephrocytioides*	*Oocystis cf. nephrocytioides*	*Oocystis cf.nephrocytioides* ex
SAG 82.80	*Oocystis parva*	*Oocystis parva*	
SAG 83.80	*Oocystis solitaria*	*Oocystis solitaria*	*Neglectella solitaria* ex
CAUP H	*Oocystis solitaria* var. *major*	*Oocystis solitaria* var. *major*	*Neglectella solitaria* ex
Tow 6/3 P-	**-**	***Oocystis*** **cf.** ***parva***	
W Twin	**-**	***Oocystis*** **cf.** ***rhomboidea***	

CAUP H	***Oocystis marssonii***	***Oocystis*** **sp.**	
CAUP H	*Ooplanctella planoconvexa*	*Ooplanctella planoconvexa*	*Oocystidium planoconvexum*
CCALA 398	*Oonephris obesa*	*Oonephris obesa*	
SAG 11.95	*Planctonema lauterbornii*	*Planctonema lauterbornii*	
SAG 68.94	*Planctonema lauterbornii*	*Planctonema lauterbornii*	
MDL6-7	**-**	***Quadricoccus*** **sp.**	
As7-C	**-**	***Quadricoccus*** **sp.**	
CCMP 245	*Schizochlamydella capsulata*	*Schizochlamydella*	
SAG 28.81	*Siderocystopsis punctifera*	*Siderocystopsis punctifera*	
CCALA	***Oocystis cf. lacustris***	***Siderocystopsis sp.***	
SAG 24.81	*Tetrastrum heteracantum*	*Tetrastrum heteracantum*	
SAG 45.81	*Tetrastrum staurogeniiforme*	*Tetrastrum*	
KR 1996/3	*Tetrastrum staurogeniiforme*	*Tetrastrum*	
SAG 42.81	*Tetrachlorella alternans*	*Tetrachlorella alternans*	
CCALA	***Willea sp.***	***Willea vilhelmii***	

7.2. Appendix 2: Table of results

Table 5: assumed results of the strains examined present thesis. Des. = description, Plate = picture plate, Ultra = examined ultrastructure of the call wall, SSU = obtained SSU rRNA sequence, *Rbcl* = obtained *rbcl* gene sequence. P = present thesis. B = authors bachelor thesis. * Picture obtained from Marie Pažoutová or NCMA.

STRAIN	**NAME**	**Des.**	**Plate**	**Ultra**	**SSU**	***Rbcl***
SAG 30.96	*Amphikrikos nanus*	P	P	-	-	P
SAG 2074	*Amphikrikos nanus*	P	P	-	-	P
SAG 2081	*Crucigeniella rectangularis*	P	P	P	-	-
SAG 37.93	*Echinocoleum elegans*	-	P*	-	-	P
CCAP 274/3	*Elongatocystis ecballocystiformis*	P	P	-	-	-
ACOI 1819	*Eremosphaera gigas*	P	P	-	P	P
SAG 228-1	*Eremosphaera viridis*	P	P	-	P	P
SAG 39.92	*Eremosphaera viridis*	P	P	-	P	P
SAG 10.81	*Franceia amphitricha*	P	P	-	P	P
SAG 56.81	*Granulocystis verrucosa*	P	P	P	P	P
SAG 33.81	*Granulocystopsis coronata*	P	P	-	-	P
SAG 2082	*Komarekia rotundata*	P	P	P	P	P
SAG 1194	*Lagerheimia ciliata*	P	P	-	P	P
SAG 2083	*Lagerheimia ciliata*	P	P	-	P	P
SAG 48.94	*Lagerheimia genevensis*	P	P	-	-	P
SAG 11.92	*Lagerheimia hindakii*	P	P	-	-	P
SAG 57.81	*Lagerheimia longiseta*	P	P	-	P	P
CCALA 365	*Lagerheimia marssonii*	B	P	-	B	P
SAG 2084	*Lagerheimia subsalsa*	P	P	-	P	P
SAG 28.97	*Makinoella tosaensis*	P	P	P	P	P
CCALA 961	*Makinoella tosaensis*	P	P	-	-	P
SAG 37.96	*Neglectella peisonis*	P	P	-	P	P
SAG 34.81	*Nephrocytium agardhianum*	P	P	P	P	P
SAG 3.96	*Oocystella oogama*	P	P	-	P	-
AN9-1	*Oocystidium polymammilatum*	P	P	-	P	P
AN2/29-4	*Oocystidium polymammilatum*	P	P	-	-	P
SAG 81.80	*Oocystidium* sp.	P	P	-	P	P
CH 99	*Oocystis bispora*	B	P	-	B	-
SAG 1.99	*Oocystis heteromucosa*	P	P	-	-	P
KR 210	*Oocystis heteromucosa*	B	P	-	B	P
SAG 2085	*Oocystis* cf. *marssonii*	P	P	-	P	P
MP STE7	*Oocystis* cf. *naegelii*	P	P	-	P	P
CCALA 397	*Oocystis cf. nephrocytioides*	B	P	-	-	P
SAG 82.80	*Oocystis parva*	P	P	-	P	P
Tow 6/3 P-1ou	*Oocystis* cf. *parva*	P	P	P	-	P
W Twin SlisT.	*Oocystis* cf. *rhomboidea*	P	P	-	P	P
SAG 83.80	*Oocystis solitaria*	P	P	-	-	P
CAUP H 1106	*Oocystis solitaria* var.*major*	B	P	-	P	P
CAUP H 1110	*Oocystis* sp.	B	P	-	P	P
CAUP H 5502	*Ooplanctella planoconvexa*	-	P*	-	-	P

CCALA 398	*Oonephris obesa*	P	P	P	P	P
SAG 11.95	*Planctonema lauterbornii*	P	P	P	-	-
SAG 68.94	*Planctonema lauterbornii*	P	P	P	P	P
MDL6-7	*Quadricoccus* sp.	P	P	-	-	P
As7-C	*Quadricoccus* sp.	P	P	-	P	P
CCMP 245	*Schizochlamydella capsulata*	-	P*	-	P	P
SAG 28.81	*Siderocystopsis punctifera*	P	P	-	P	P
CCALA 396	*Siderocystopsis sp.*	B	P	-	-	P
SAG 24.81	*Tetrastrum heteracantum*	P	P	P	-	P
SAG 45.81	*Tetrastrum staurogeniiforme*	P	P	P	-	P
KR 1996/3	*Tetrastrum staurogeniiforme*	P	P	P	-	P
SAG 42.81	*Tetrachlorella alternans*	P	P	P	-	P
CCALA 515	*Willea vilhelmii*	B	P	P	B	P

7.3. Appendix 3: Morphological descriptions

SAG 30.96 ***Amphikrikos nanus***: Cells oval to cylindrical with the rounded ends without thickness of the cell wall. Granula arranged into the ring in the equatorial of the cells. In cells 1 parietal pyrenoid-bearing chloroplast. Propagation by 4 autospores. Cell breadth 2-4 µm; cell length 3-7 µm.

SAG 2074 ***Amphikrikos nanus***: Cells oval to cylindrical with the rounded ends without thickness of the cell wall. Granula arranged into the ring in the equatorial of the cells. In cells 1 parietal pyrenoid-bearing chloroplast. Propagation by 4 autospores. Cell breadth 2-4 µm; cell length 3-6 µm.

SAG 2081 ***Crucigeniella rectangularis***: Cells elliptical with the rounded ends without the cell wall thickness, arranged in the typical flat crucigenoid coenobia. Each cell contains 1 parietal chloroplast with the extensive pyrenoid. The small rhombic gap occurs between the tetrad of the cells. The cells propagate by 4 autospores (daughter coenobia). Cell breadth 5-7 µm; cell length 7-10 µm.

CCAP 274/3 ***Elongatocystis ecballocystiformis***: Cell long cylindrical to oval with rounded ends without cell wall thickness. Cell breadth 3-8 µm; cell length 13-28 µm. 4 cells coenobial-like enclosed in mother cell wall. 1-4 chloroplasts with pyrenoid per cell. Propagation observed by 4 autospors.

ACOI 1819 ***Eremosphaera gigas***: Spherical cells occur mainly in pairs enclosed inside the spindle-shaped mother cell wall. Numerous chloroplasts arranged at the surface of the cell wall. Propagation by 2 autospores that stay longer time inside the mother cell wall. Cells have in diameter 75-110 µm.

SAG 228-1 ***Eremosphaera viridis***: Cells large and spherical with thick cell wall. Numerous discoid chloroplasts with pyrenoid are arranged at the surface of the cell and inside plasmatic strings crossing cell and holding the nucleus. Rest of the cell is filled by a vacuole. Cell propagate by 2-4 autospors. Oogamy was not observed. Cell in diameter 90-150 µm.

SAG 39.92 ***Eremosphaera viridis***: Cells large and spherical with thick cell wall. Numerous discoid chloroplasts with pyrenoid are arranged at the surface of the cell and inside plasmatic strings crossing cell and holding the nucleus. Rest of the cell is filled by a vacuole. Cell propagate by 2-4 autospors. Oogamy was not observed. Cell diameter 120-160 µm.

SAG 10.81 ***Franceia amphitricha***: Cells elongated, oval to spindle-shaped with the rounded ends without polar thickness of the cell wall. All surface of the cell wall cover spines. Cells often in groups of 2-4 cells enclosed in a mucilaginous mother cell wall. In cells 1-2 parietal chloroplasts wit pyrenoid. Cell breadth 4-8 µm; cell length 7-13 µm.

SAG 56.81 ***Granulocystis verrucosa***: Cells clearly spindle-formed with tipped ends. Cell wall on the poles thickened bears granula irregularly widespread on all surface. 1-2 pyrenoid-bearing chloroplast per cell. Propagation by 4 autospores that stay for a longer time enclosed in the broadened mother cell wall and are released by its mucilaginous. Cell breadth 4-15 µm; cell length 7-21 µm.

SAG 33.81 ***Granulocystopsis coronata***: Cells cylindrical with rounded ends without polar thickness. On the cell wall surface, occur granula arranged on the poles of the cells. Cells contain 1 chloroplast with a pyrenoid. Propagation by 4, rarely 2 autospores. Daughter cells are not enclosed in the mother cell wall for a long time. Cell breadth 3-5 µm; cell length 5-8 µm.

SAG 2082 ***Komarekia rotundata***: Cells nearly spherical to wide elliptical with rounded ends without thickness on the poles. In each cell, one chloroplast with big pyrenoid, that fills almost all cell; before autosporulation divided into 4 chloroplast. Propagation by 4 autospores containing 1 chloroplast. Cells arranged in four-celled coenobia. Cell breadth 6-10 µm; cell length 8-12 µm.

SAG 1194 ***Lagerheimia ciliata***: Cells broadly elliptical to slightly spindle-shaped without cell wall thickness. 4-5 thick short spines on each pole. In the young cells 1 and the adult cells 2 chloroplasts; before autosporulation 4 chloroplasts. Pyrenoid in each chloroplast. Propagation by 2 or 4 autospores shortly released from the mother cell wall. Only free cells possess spines. Cell breadth 7-15 µm; cell length 8-17 µm.

SAG 2083 ***Lagerheimia ciliata***: Cells broadly elliptical to slightly spindle-shaped without cell wall thickness. 4-5 thick short spines on each pole. In the young cells 1 and the adult cells 2 chloroplasts; before autosporulation 4 chloroplasts. Pyrenoid in each chloroplast. Propagation by 2 or 4 autospores shortly released from the mother cell wall. Only free cells possess spines. Cell breadth 6-15 µm; cell length 7-17 µm.

SAG 48.94 ***Lagerheimia genevensis***: Cells elliptical to oval with rounded ends without apical thickness of the cell wall, with 2 spines on each pole placed little subapical. In the cells 1 parietal chloroplast with a pyrenoid. Propagation by 4 autospores. Cell breadth 2-6 µm; cell length 3-11 µm.

SAG 11.92 ***Lagerheimia hindakii***: Cells small and oval to spindle-shaped with rounded ends without apical thickness of the cell wall. Spines were not detected. In the cells 1 parietal chloroplast with a pyrenoid. Propagation by 4 autospores. Cell breadth 2-4 µm; cell length 3-7 µm.

SAG 57.81 ***Lagerheimia longiseta***: Cells broadly elliptical to slightly spindle-shaped without cell wall thickness. 6-8 long spines on each pole. In the young cells 1 and the adult cells 2 chloroplasts; before autosporulation 4 chloroplasts. Pyrenoid in each chloroplast. Propagation by 2 or 4 autospores shortly released from the mother cell wall. Young cells inside mother cell wall bear spines. Cell breadth 6-11 µm; cell length 7-15 µm.

CCALA 365 ***Lagerheimia marssonii***: Cell shape oval to spindle-shaped, cell wall with a thickness on the poles, cell occur solitary. Spines arranged on the poles (one on each) and a few on the equatorial plane. Each cell with only one parietal chloroplast with the pyrenoid, two or four chloroplasts before autosporulation. Autospores two or four early released from the mother cell wall by its rupture. Mucilage covers not detected. Cell breadth 4-8 µm; cell length 6-12 µm.

SAG 2084 ***Lagerheimia subsalsa***: Cells oval (young cells) to spindle-shaped, with papilla-like polar thickness (old cells). On each pole mostly two, sometimes 3 or 4 short spines. In young cells 1, in adult cells 2 and before autosporulation 4 parietal chloroplasts with visible pyrenoid. Autospores mainly 4, stay for a longer time inside the wide envelope of mother cell wall. Young cells inside mother cell wall bear spines. Cell breadth 3-7 µm; cell length 8-15 µm.

SAG 28.97 ***Makinoella tosaensis***: Young cells spherical, older cells wide oval with the rounded ends and without the cell wall thickness. Cells arranged in 4-celled coenobia. Propagation by 4 autospores forming daughter coenobia. In each cell, 2-more chloroplasts with pyrenoid. Cell breadth 14-17 µm; cell length 20-29 µm.

CCALA 961 ***Makinoella tosaensis***: Young cells spherical, older cells wide oval with the rounded ends and without the cell wall thickness. Cells arranged in 4-celled coenobia. Propagation by 4 autospores forming daughter coenobia. In each cell, 2-more chloroplasts with pyrenoid. Cell breadth 15-17 µm; cell length 19-30 µm.

SAG 37.96 ***Neglectella peisonis***: Cells typically spindle-shaped to lemon-shaped with pointed ends with the cell wall thickness crossed by the channels. Cell solitary or associated for 2 in the mother cell wall. In the each cell numerous chloroplast with pyrenoid arranged at the surface of the cell wall. Propagation was observed by 2 autospores. Cell breadth 22-31 µm; cell length 39-54 µm.

SAG 34.81 ***Nephrocytium agardhianum***: The roll-shaped or moon-shaped cells with the rounded ends and without cell wall thickness arranged in 2, 4, or 8 celled colonies enclosed in the enlarged mother cell wall. In each cell 1 parietal chloroplast filling nearly all cell. Pyrenoid is massive and rounded. Propagation by 2, 4 or 8 autospores. Cell breadth 6-10 µm; cell length 10-26 µm.

SAG 3.96 ***Oocystella oogama***: Cell spindle-shaped with pointed ends with papilla-like polar thickness of the cell wall. Cell wall irregularly covered with numerous granula. Cells solitary or in 2-4 celled colonies. Propagation by 2 or 4 autospores releasing from the mother cell wall by its mucilaginous. The rests of the cell walls occurred often in the culture. Cell breadth 4-6 µm; cell length 6-9 µm. Oogamy was not observed.

AN9-1 ***Oocystidium polymammilatum***: Cells oval to spindle-shaped without the cell wall thicknesses. Cell arranged solitary or in pairs or tetrads in the mucilage covers, sometimes multi-layered with a lobed border. In the cells 1-2 parietal pyrenoid-bearing chloroplasts. Propagation by 2 or 4 autospores that are shortly released from the mother cell wall. Cell breadth 4-6 µm; cell length 7-10 µm.

AN2/29-4 *Oocystidium polymammilatum*: Cells oval to spindle-shaped without the cell wall thicknesses. Cell arranged solitary or in pairs or tetrads in the mucilage covers, sometimes multi-layered with a lobed border. In the cells 1-2 parietal pyrenoid-bearing chloroplasts. Propagation by 2 or 4 autospores that are soon released from the mother cell wall. Cell breadth 3-6 µm; cell length 6-11 µm.

SAG 81.80 *Oocystidium* sp.: Cells oval to spindle-shaped without the cell wall thicknesses. Cells are arranged solitarily, or in the pairs or tetrads, in the mucilage covers. In the cells 1-2 parietal pyrenoid-bearing chloroplasts. Propagation by 2 or 4 autospores that are shortly released from the mother cell wall. Cell breadth 3-5 µm; cell length 5-10 µm.

CH 99 *Oocystis bispora*: Cells solitary or in pairs. Small cells elliptical or spindle shaped with pointed ends and small polar thickness. In cells presented one chloroplast with (not always distinctly visible) pyrenoid. Autospores observed only 2, sometimes staying in the mother cell wall are released by its rupture. Cell breadth 2-4 µm; cell length 4-7 µm.

SAG 1.99 *Oocystis heteromucosa*: Cells widely oval with polar tip shaped thicknesses, cell breadth 5-9 µm; cell length 8-18 µm. Adult cells contain 1-4 parietal chloroplasts each with one distinct pyrenoid. Cells solitary or most frequently, making two or four celled colonies bounded by enlarged mucilaginous mother cell wall. Cells inside colonies free. Reproduction most often by 2 or 4 autospores. Daughter cells stay closed in mother cell wall for a long time, and release by mucilaginous of the mother cell wall.

KR 210 *Oocystis heteromucosa:* Cells widely oval with polar tip shaped thicknesses, cell breadth 4-10 µm; cell length 8-21 µm. Adult cells contain 1-4 parietal chloroplasts each with one distinct pyrenoid. Cells solitary or most frequently, making two or four celled colonies bounded by enlarged mucilaginous mother cell wall. Cells inside colonies free. Reproduction most often by 2 or 4 autospores. Daughter cells stay closed in mother cell wall for a long time, and release by mucilaginous of the mother cell wall.

SAG 2085 *Oocystis* cf. *marssonii*: Cells oval to elliptical with slightly thickened cell wall on the poles. 2-8 cells arranged in regular *Oocysti*s-like colonies enclosed by broad elliptical mother cell wall. Mother cell wall and older cells have sometimes on the poles small tips. In the cells 2-4 parietal chloroplasts with pyrenoid, in autospores 1-2 chloroplasts. Propagation by 2-8 autospores. Cell breadth 10-15 µm; cell length 15-24 µm.

MP STE7 *Oocystis* cf. *naegelii*: Cells spindle-shaped to lemon-shaped with pointed cell wall thickness on the ends of the cell. Cells usually 2-4 in the mucilaginous mother cell wall enclosed. Inside the cells occur 1-4 parietal chloroplasts with poorly visible pyrenoid. Propagation by 2-4 autospores. Cell breadth 8-15 µm; cell length 12-26 µm.

CCALA 397 *Oocystis cf. nephrocytioides*: Cell in colonies 2 or 4. Cell shape is long elliptical, sometimes asymmetrical with rounded ends without polar thickness. 1-4 parietal chloroplasts with massive pyrenoid occur per cell. Autospores mainly 4 staying for some time enclosed in mother cell wall. Cell breadth 7-10 µm; cell length 9-18 µm.

SAG 82.80 *Oocystis parva*: Cells oval to spindle-shaped with slightly tipped thickened cell wall on the poles. Vegetativ cells with 1 pyrenoid-bearing chloroplast. Propagation by 2 or 4 autospores. Daughter cells stay enclosed for a longer time in the enlarged mother cell wall. Cell breadth 2-7 µm; cell length 5-10 µm.

Tow 6/3 P-1ou *Oocystis* cf. *parva*: Cells oval to spindle-shaped with slightly tipped thickened cell wall on the poles. Vegetativ cells with 1 pyrenoid-bearing chloroplast. Propagation by 2 or 4 autospores. Daughter cells stay enclosed for a longer time in the enlarged mother cell wall. Cell breadth 2-7 µm; cell length 5-11 µm.

W Twin SlisT. *Oocystis* cf. *rhomboidea*: Cells oval to elliptical with rounded ends without cell wall thickness. In cells 1 chloroplast with not clear visible pyrenoid. Propagation by 2 or 4 autospores often enclosed for more generation in the multiple mother cell walls. Cell breadth 3-4 µm; cell length 6-10 µm.

SAG 83.80 *Oocystis solitaria*: Cell shape spindle-like or lemon-like with considerable thickness on both poles crossed by channels. Cells solitary or arranged in 2-8celled colonies. Cells closed inside the enlarged mother cell wall for a longer time, sometimes for more generations, are released by its rupture. Numerous chloroplasts are parietal with irregular shape with clearly detectable pyrenoid. Autospors at most 2, sometimes 4 or 8 with more chloroplasts. Identical to *Oocystis solitaria*. Cell breadth 10-30 µm; cell length 21-52 µm.

CAUP H 1106 *Oocystis solitaria* var. *major*: Cell shape spindle-like or lemon-like with significant thickness on both poles crossed by channels. Cells solitary or arranged in 2-8celled colonies. Cells closed inside the enlarged mother cell wall for a longer time, sometimes for more generations, are released by its rupture. Numerous chloroplasts are parietal with irregular shape with clearly detectable pyrenoid. Autospors at most 2, sometimes 4 or 8 with more chloroplasts. Identical to *Oocystis solitaria*. Cell breadth 8-28 µm; cell length 15-50 µm.

CAUP H 1110 ***Oocystis*** **sp.**: Cell solitary with spindle-like, oval or elliptical shape. Young cells contain 1, adult 2-4 parietal chloroplasts with 1 pyrenoid. Propagation by 2-8 autospores. Cell breadth 4-14 μm; cell length 8-26 μm.

CCALA 398 ***Oonephris obesa***: Cells nearly spherical to broadly oval with rounded ends without cell wall thickness. Cells solitary or 2 or 4 enclosed in thick mother cell wall. Chloroplast spongiomorph with the big central pyrenoid and many smaller widespread for the chloroplast. Propagation by 2 or 4 autospores. Cell breadth 15-23 μm; cell length 18-36 μm.

SAG 11.95 ***Planctonema lauterbornii***: Cells long oval to cylindrical arranged in simple filament. Chloroplast 1 with not clearly visible pyrenoid. 2 chloroplasts observed in dividing cells. Filametns grow by transversal dividing and propagate by fragmentation of the filaments. Cell breadth 3-5 μm; cell length 6-8 μm.

SAG 68.94 ***Planctonema lauterbornii***: Cells long oval to cylindrical arranged in simple filament. Chloroplast 1 with not clearly visible pyrenoid. 2 chloroplasts observed in dividing cells. Filametns grow by transversal dividing and propagate by fragmentation of the filaments. Cell breadth 2-5 μm; cell length 4-8 μm.

MDL6-7 ***Quadricoccus*** **sp.**: Cell oval to elliptical with rounded ends without cell wall thickness. Arrangement in the typical tetrads close to mother cell wall is missing in the culture. 1-2 parietal chloroplasts per cell. Propagation by 4 autospores, releasing by the rupture of the cell wall. Lot of the rests of the cell wall occur in the culture. Granula was not studied in detail. Cell breadth 2-4 μm; cell length 4-6 μm.

As7-C ***Quadricoccus*** **sp.**: Cell oval to elliptical with rounded ends without cell wall thickness. Arrangement in the typical tetrads close to mother cell wall is missing in the culture. 1-2 parietal chloroplasts per cell. Propagation by 4 autospores, releasing by the rupture of the cell wall. Lot of the rests of the cell wall occur in the culture. Granula was not studied in detail. Cell breadth 3-4 μm; cell length 4-8 μm.

SAG 28.81 ***Siderocystopsis punctifera***: Solitary cells oval to elliptical with cell wall thickness on the poles. Numerous granula coverall surface of the cell wall. In the cells 1 parietal chloroplast with presented pyrenoid. Propagation by 4 autospores. Cell breadth 3-4 μm; cell length 5-7 μm.

CCALA 396 ***Siderocystopsis sp.***: Cell solitary or arranged in groups of 2-8, cell shape oval to spindle-shaped conspicuously thickened in poles. Cell wall irregularly granulated. In cells 1-4 parietal chloroplasts with one pyrenoid. Propagation by 2-4 autospores, which stays for some time in wide mother cell wall. Cell breadth 6-15 μm; cell length 3-9 μm.

SAG 24.81 ***Tetrastrum heteracantum***: Cells bun-shaped arranged in the tetrads. Bristles with various length occurred on the surface of the cell wall. Each cell contains 1 chloroplast with rounded pyrenoid filling almost all cell. Cells propagate by 4 autospores arranged to the daughter coenobia. Cell breadth 2-4 µm; cell length 2-5 µm.

SAG 45.81 ***Tetrastrum staurogeniiforme***: Cells bun-shaped arranged in the tetrads**.** Short stumps occurred on the surface of the cell wall. Each cell contains 1 chloroplast with rounded pyrenoid filling nearly all cell. Cells propagate by 4 autospores arranged to the daughter coenobia. Cell breadth 2-6 µm; cell length 2-6 µm.

KR 1996/3 ***Tetrastrum staurogeniiforme***: Cells bun-shaped arranged in the tetrads**.** Short stumps occurred on the surface of the cell wall. Each cell contains 1 chloroplast with rounded pyrenoid filling nearly all cell. Cells propagate by 4 autospores arranged to the daughter coenobia. Cell breadth 2-6 µm; cell length 2-6 µm.

SAG 42.81 ***Tetrachlorella alternans***: Cells clearly spindle-shaped with pointed ends with thickness of the cell wall. Cells arranged in the regularly tetrads joined by the mother cell wall. The tetrads usually formed 4-32 celled coenobia. The old cell walls changed into irregularly arranged mucilage. In each cell occur one parietal chloroplast with the pyrenoid. Propagation by 4 autospores produced by two consecutive divisions. Cell breadth 5-11 µm; cell length 13-18 µm.

CCALA 515 ***Willea vilhelmii***: Cells elliptical to cylindrical with rounded ends without cell wall thickness.. Two celled unit with cells connected lengthwise arranged to the flat and rigid 4-16 celled coenobia. The four-celled (sub)coenobia have a small rhombic gap. Older cells are bounded by wide mucilage envelopes. Each cell contains one parietal chloroplast; in older cells were more chloroplasts observed. Pyrenoid occurs in every chloroplast and is massive and clear visible. Cells reproduce by 2-4 autospores that stay for a long time enclosed in the mother cell wall. Cell breadth 9-13 µm; cell length 4-5 µm.

7.4. Appendix 4: Photographic documentation of the individual strains

Fig. 11: Morphological comparison of *Komarekia rotundata* and *Crucigenia lauternbornii*. 1 SAG 2082 ***Komarekia rotundata***, 2 ***Crucigenia lauternbornii*** (Bock et al. 2013). Scale bar indicates 20µm 1 and 10µm 2.

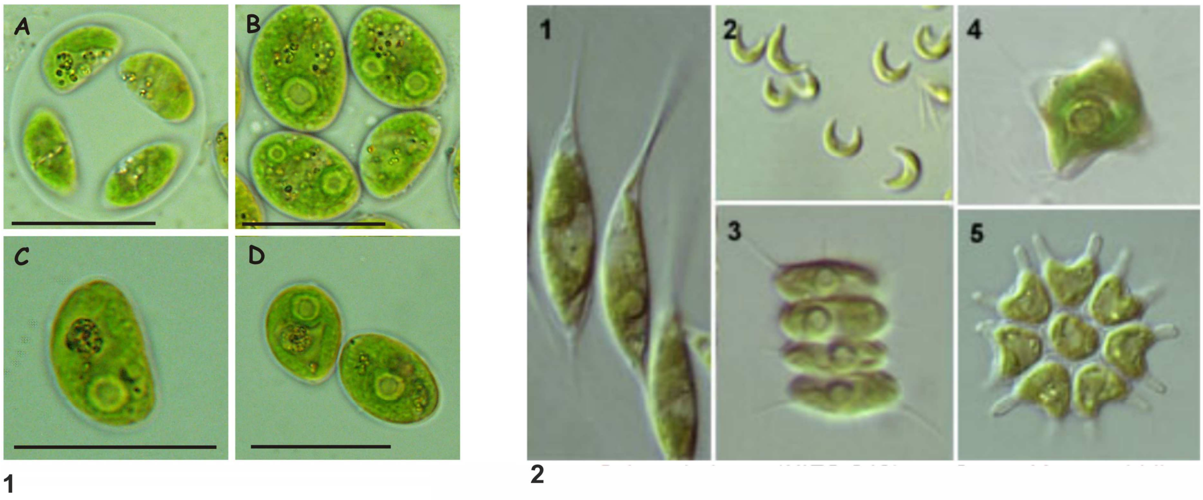


Fig. 12: Morphological comparison of algae assigned to **Sphaeropleales. 1** SAG 34.81 ***Nephrocytium agardhianum*, 2 Sphaeropleales (**1 NIES-246 ***Schroederia*** 2 NIES-480 ***Monoraphidium***, 3 NIES-798 ***Desmodesmus***, 4 NIES-232 ***Polyedriopsis***, 5 NIES-301 ***Pediastrum***) **http://www.shigen.nig.ac.jp/algae_tree/SphaeroplealesE.html .** Scale bar indicates 20µm.

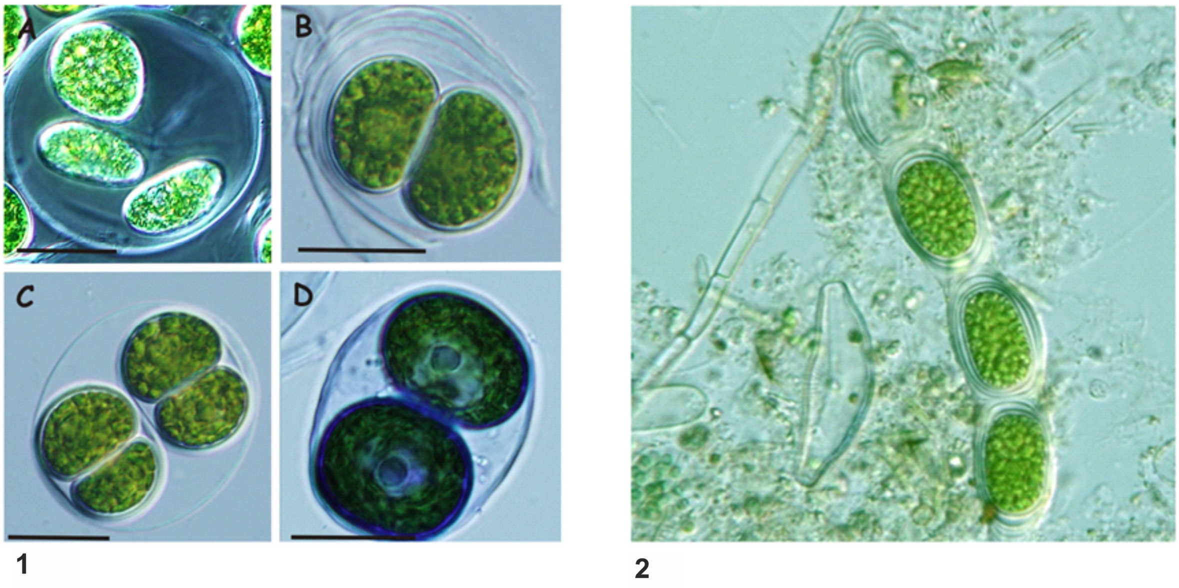


Fig. 13: Morphological comparison of two algae assigned to **Chlamydomonadales**. 1. CCALA 398 ***Oonephris obesa***. 2. ***Cylindrocapsa geminella*** http://protist.i.hosei.ac.jp/PDB7/PCD0009/htmls/01.html. Scale bar indicates 20µm.

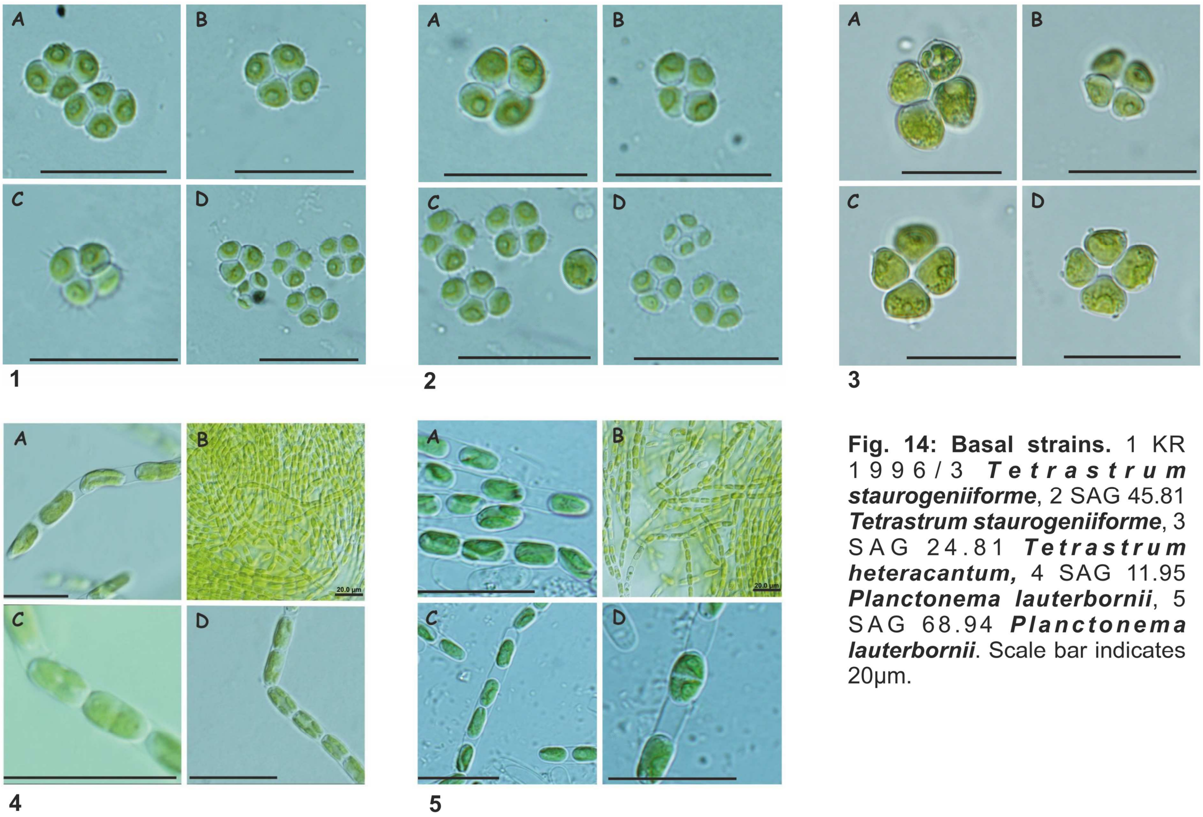


Fig. 14: Basal strains. 1 KR 1996/3 ***Tetrastrum staurogeniiforme***, 2 SAG 45.81 ***Tetrastrum staurogeniiforme***, 3 SAG 24.81 ***Tetrastrum heteracantum,*** 4 SAG 11.95 ***Planctonema lauterbornii***, 5 SAG 68.94 ***Planctonema lauterbornii***. Scale bar indicates 20µm.

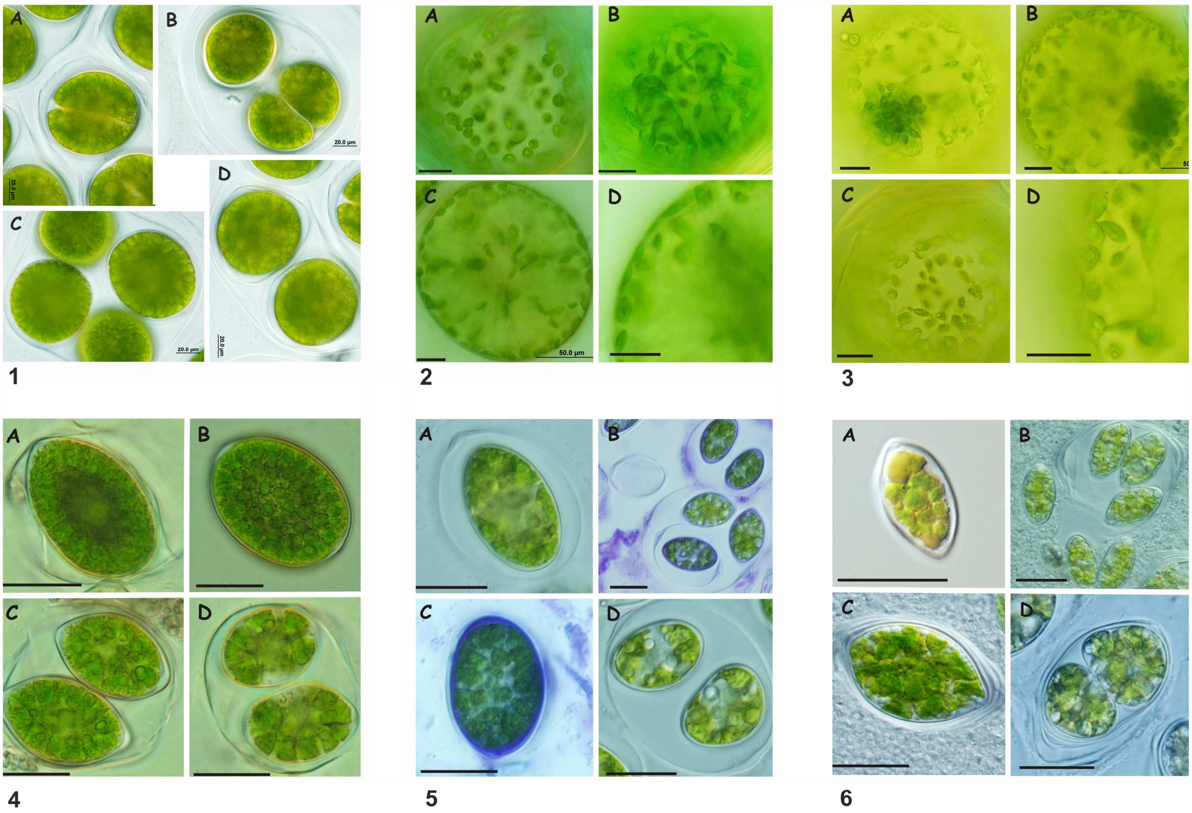
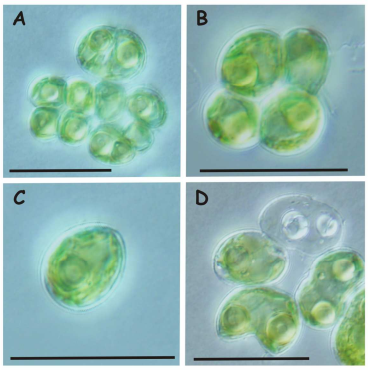
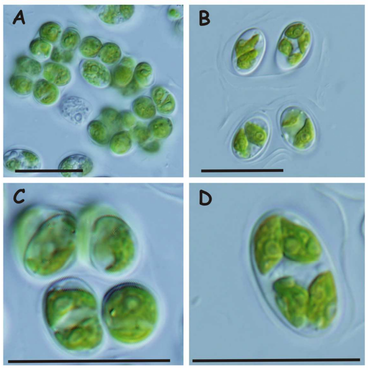
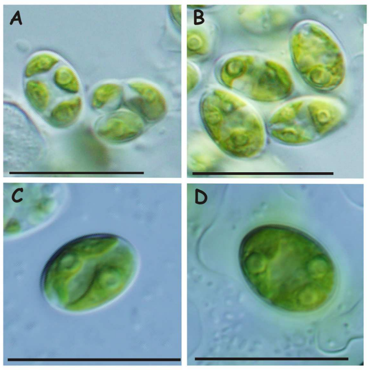
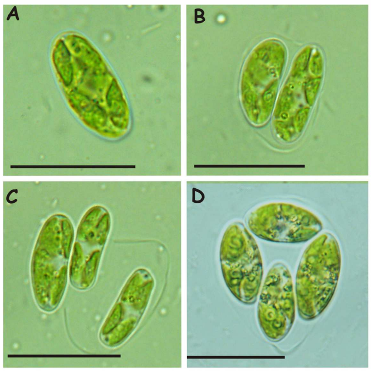


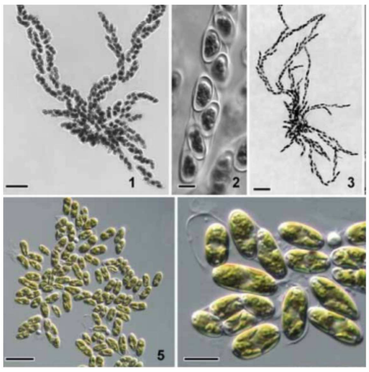
Fig. 15: Strains with **large dimensions** of the cell and numerous chloroplasts assigned to the subfamily Eremosphaeroideae. 1 ACOI 1819 ***Eremosphaera gigas***, 2 SAG 228-1 ***Eremosphaera viridis***, 3 SAG 39.92 ***Eremosphaera viridis***, 4 SAG 37.96 ***Neglectella peisonis***, 5 SAG 83.80 ***Oocystis solitaria***, 6 CAUP H 1106 ***Oocystis solitaria*. f. *major.*** Scale bar indicates 20µm.

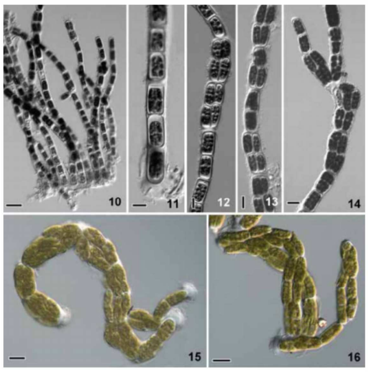


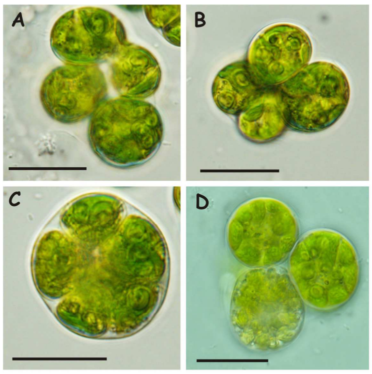












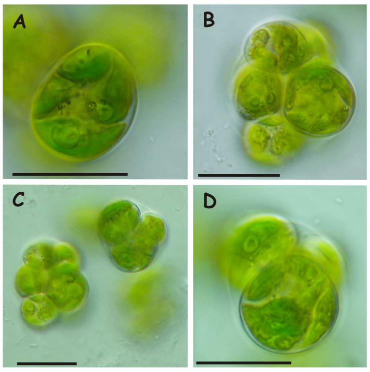


Fig. 16: Strains with **coenobial** arrangement oft the cells assigned to the subfamily Makinelloideae. 1 SAG 2081 ***Crucigeniella rectangularis***, 2 CCALA 515 ***Willea vilhelmii,*** 3 CCALA 397 ***´Oocystis´ cf. nephrocytioides,*** 4 CCAP 274/3 ***Elongatocystis ecballocystiformis***, 5 **Ecballocystis hubeiensis** (Xia et al. 2013), 6 **Ecballocystopsis dichotomus** (Xia et al. 2013), 7 CCALA 96 ***Makinoella tosaensis,*** 8 CCALA 961 ***Makinoella tosaensis.*** Scale bar indicates 20µm (1,2,3,4,7,8).

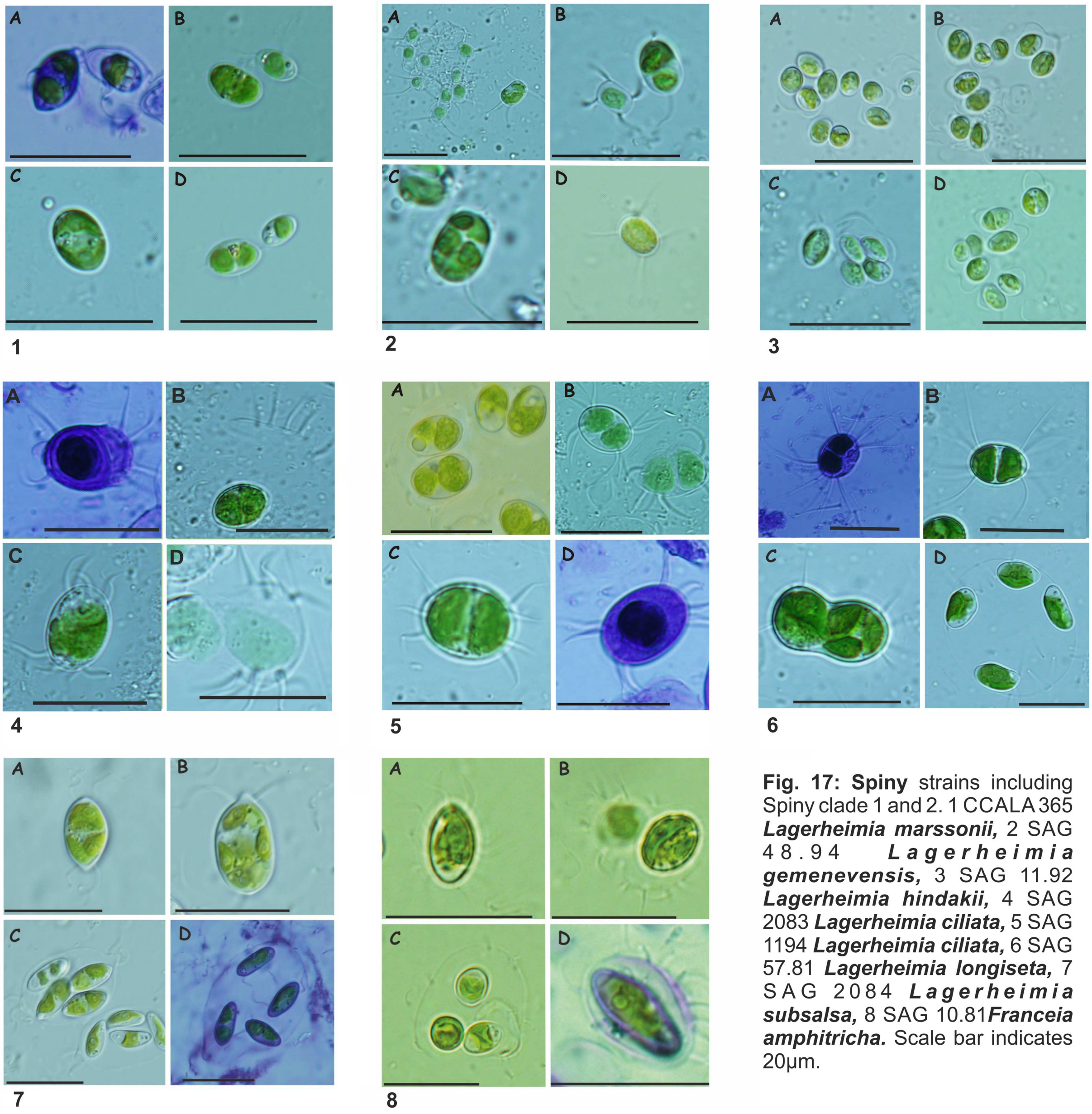


Fig. 17: Spiny strains including Spiny clade 1 and 2. 1 CCALA 365 ***Lagerheimia marssonii,*** 2 SAG 48.94 ***Lagerheimia gemenevensis,*** 3 SAG 11.92 ***Lagerheimia hindakii,*** 4 SAG 2083 ***Lagerheimia ciliata,*** 5 SAG 1194 ***Lagerheimia ciliata,*** 6 SAG 57.81 ***Lagerheimia longiseta,*** 7 SAG 2084 ***Lagerheimia subsalsa,*** 8 SAG 10.81***Franceia amphitricha.*** Scale bar indicates 20µm.

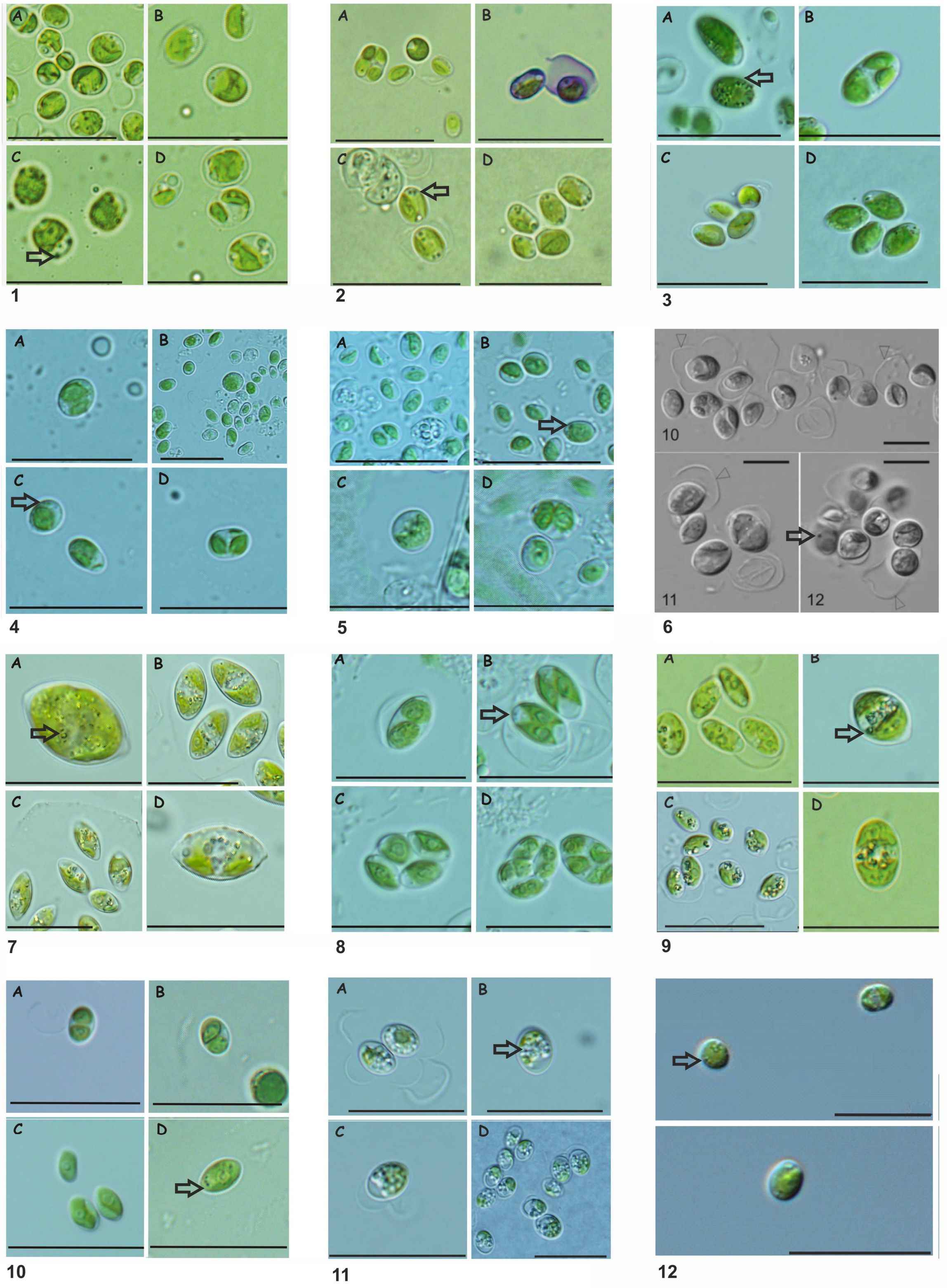
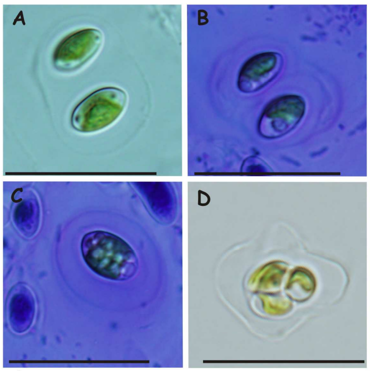


Fig. 18: Granulated strains assigned to the Granulated clade 1 and 2. 1 SAG 30.96 ***Amphikrikos nanus,*** 2 SAG 2074 ***Amphikrikos nanus,*** 3 SAG 33.81 ***Granulocystopsis coronata,*** 4 MDL6-7 **Quadricoccus sp.,** 5 As7-C **Quadricoccus sp.,** 6 ***Quadricoccus ellipticus*** (Krienitz and Bock 2011), 7 SAG 56.81 ***Granulocystis verrucosa,*** 8 CCALA 396 ***Siderocystopsis* sp.,** 9 SAG 3.96 ***Oocystella oogama***, 10 CH 99 ***Oocystis bispora***, 11 SAG 28.81 ***Siderocystopsis punctifera***, 12 CCMP 245 ***Schizochlamydella capsulata.*** Scale bar indicates 20µm, except picture 6 (10µm). Arrow poin to granula.



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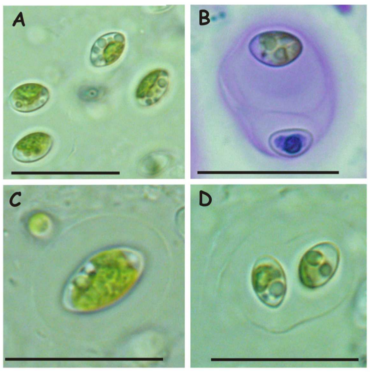

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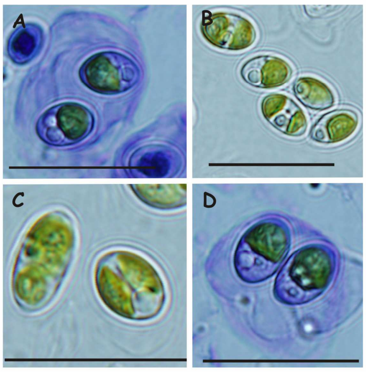

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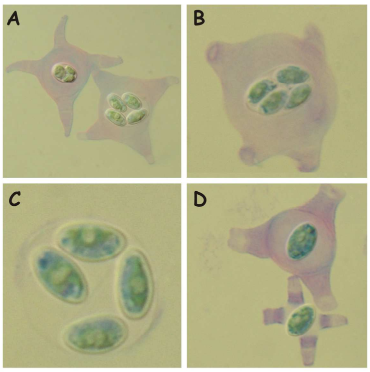

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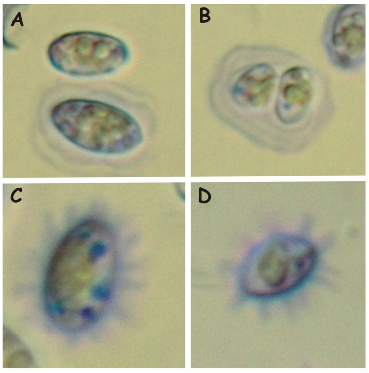

5

Fig. 19: Strains with **mucilage c**overs and projections including *Oocystidium* clade.1 AN9-1 ***Oocystidium polymammilatum,*** 2AN2/29-4 ***Oocystidium polymammilatum,*** 3 SAG 81.80 ***Oocystidium* sp.,** 4 SAG 37.93 ***Echinocoleum elegans,*** 5 CAUP H 5502 ***Ooplanctella planoconvexa.*** Scale bar indicates 20µm.

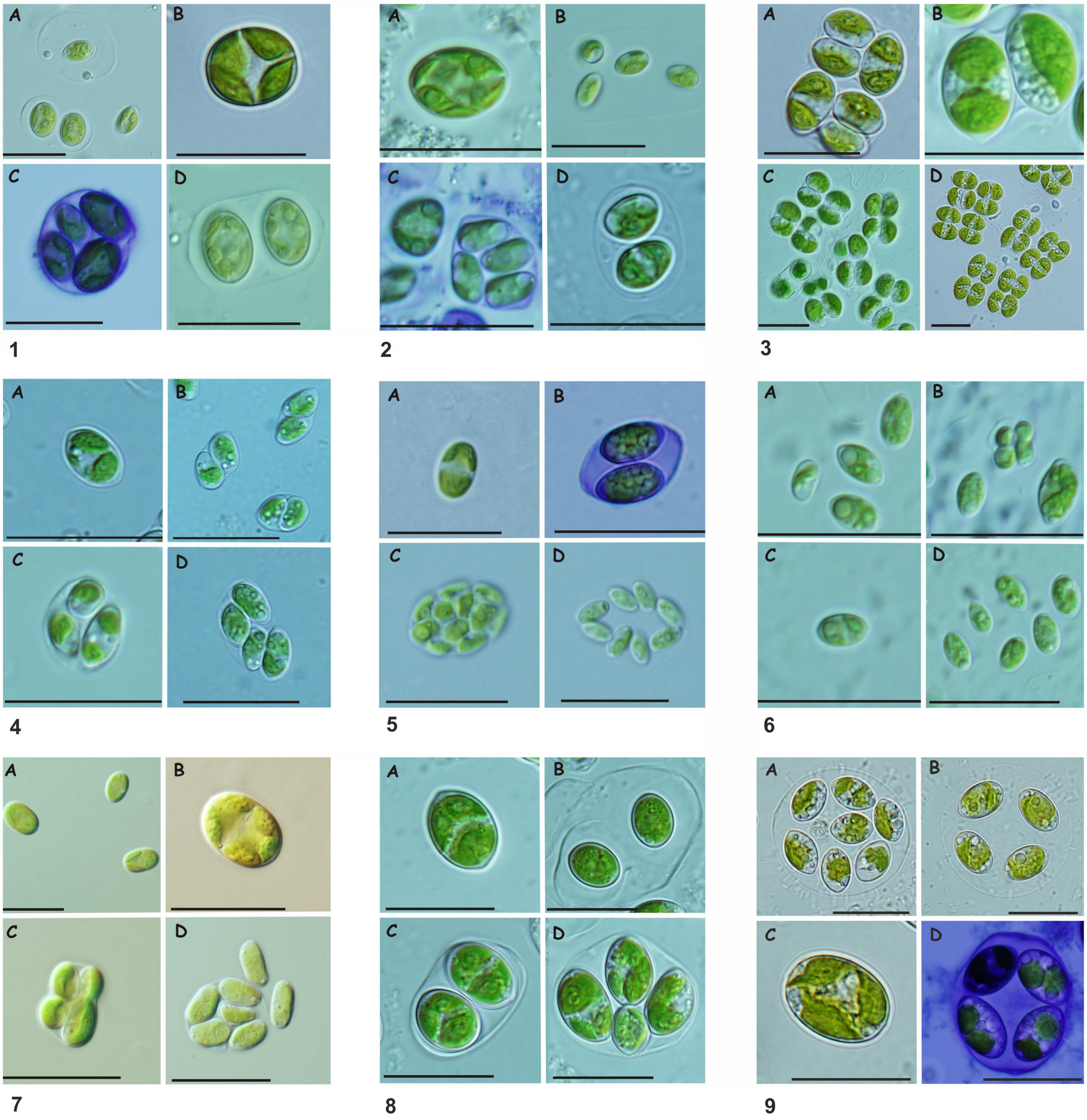

Figure 20: Strain assigned to the ***Oocystis sensu lato*** group. 1 SAG 1.99 ***Oocystis heteromucosa,*** 2 KR 210 ***Oocystis heteromucosa,*** 3 SAG B42.81 ***Tetrachlorella alternans,*** 4 Tow 6/3 P-1ou ***Oocystis* cf. *parva,*** 5 W Twin SlisT-7d ***Oocystis* cf. *rhomboidea***, 6 SAG 82.80 ***Oocystis parva*** , 7 CAUP H 1110 ***Oocystis* sp.**, 8 STE7 ***Oocystis* cf. *nägelii***, 9 SAG 2085 ***Oocystis* cf. *marssonii*.** Scale bar indicates 20µm.

7.5. Appendix 5: Table of analysed sequences

7.5.1. Oocystaceae

Table 6: Sequence of the SSU rRNA and *rbcl* genes used for molecular analysis of Oocystaceae. * HEG. 1995-26, P = present thesis. B = authors bachelory thesis.

STRAIN	**NAME**	**SSU rRNA**	***Rbcl***	**Concatenated**
SAG 30.96	*Amphikrikos nanus*	-	P	-
SAG 2074	*Amphikrikos nanus*	AF228690*	P	YES
SAG 2081	*Crucigeniella rectangularis*	AH012990	-	-
SAG 37.93	*Echinocoleum elegans*	FM881776	P	YES
CCAP 274/3	*Elongatocystis*	HQ008713	-	-
ACOI 1819	*Eremosphaera gigas*	P	P	YES
SAG 228-1	*Eremosphaera viridis*	P	P	YES
SAG 39.92	*Eremosphaera viridis*	P	P	YES
SAG 10.81	*Franceia amphitricha*	P	P	YES
SAG 56.81	*Granulocystis verrucosa*	P	P	YES
SAG 33.81	*Granulocystopsis coronata*	-	P	-
SAG 2082	*Komarekia rotundata*	P	P	NO
SAG 1194	*Lagerheimia ciliata*	P	P	YES
SAG 2083	*Lagerheimia ciliata*	P	P	YES
SAG 48.94	*Lagerheimia genevensis*	AY122336	P	YES
SAG 11.92	*Lagerheimia hindakii*	-	P	-
SAG 57.81	*Lagerheimia longiseta*	P	P	YES
CCALA 365	*Lagerheimia marssonii*	B	P	YES
SAG 2084	*Lagerheimia subsalsa*	P	P	YES
SAG 28.97	*Makinoella tosaensis*	P	P	YES
CCALA 961	*Makinoella tosaensis*	AF228691	P	YES
SAG 37.96	*Neglectella peisonis*	P	P	YES
SAG 34.81	*Nephrocytium agardhianum*	P	P	NO
SAG 3.96	*Oocystella oogama*	P	-	-
AN9-1	*Oocystidium polymammilatum*	P	P	YES
AN2/29-4	*Oocystidium polymammilatum*	AY195966	P	YES
SAG 81.80	*Oocystidium* sp.	P	P	YES
CH 99	*Oocystis bispora*	B	-	-
SAG 1.99	*Oocystis heteromucosa*	AF228689	P	YES
KR 210	*Oocystis heteromucosa*	B	P	YES
SAG 2085	*Oocystis* cf. *marssonii*	P	P	YES
MP STE7	*Oocystis* cf. *naegelii*	P	P	YES
CCALA 397	*Oocystis cf. nephrocytioides*	-	P	-
SAG 82.80	*Oocystis parva*	P	P	YES
Tow 6/3 P-1ou	*Oocystis* cf. *parva*	AY197635	P	YES
W Twin SlisT.	*Oocystis* cf. *rhomboidea*	P	P	YES
SAG 83.80	*Oocystis solitaria*	AF228686	P	YES
CAUP H 1106	*Oocystis solitaria* var. *major*	P	P	YES
CAUP H 1110	*Oocystis* sp.	P	P	YES
CAUP H 5502	*Ooplanctella planoconvexa*	FM881777	P	YES

CCALA 398	*Oonephris obesa*	P	P	NO
SAG 11.95	*Planctonema lauterbornii*	-	-	-
SAG 68.94	*Planctonema lauterbornii*	P	P	YES
MDL6-7	*Quadricoccus* sp.	AY197626	P	YES
As7-C	*Quadricoccus* sp.	P	P	YES
CCMP 245	*Schizochlamydella capsulata*	P	P	YES
SAG 28.81	*Siderocystopsis punctifera*	P	P	YES
CCALA 396	*Siderocystopsis sp.*	-	P	-
SAG 24.81	*Tetrastrum heteracantum*	JQ356709	P	YES
SAG 45.81	*Tetrastrum staurogeniiforme*	JQ356703	P	YES
KR 1996/3	*Tetrastrum staurogeniiforme*	JQ356702	P	YES
SAG 42.81	*Tetrachlorella alternans*	AF228687	P	YES
CCALA 515	*Willea vilhelmii*	B	P	YES
	GENBANK			
-	*Ecballocystis hubeiensis*	JX018185	JX018187	YES
-	*Ecballocystopsis dichotomus*	JX018184	JX018186	YES
KMMCC 1544	*Lagerheimia longiseta*	JQ315525	-	-
CCAP 222/49	*Oocystidium* sp.	HQ008711	-	-
KRI. 96/10	*Oocystis marssonii*	AF228688	-	-
KMMCC 443	*Oocystis parva*	JQ315649	-	-
KMMCC 356	*Oocystis* sp.	JQ315800	-	-
GR35	*Planctonema lauterbornii*	-	EF113462	-
M110-1	*Planctonema* sp.	AF387148	EF113463	YES
CCAP 286/1	*Quadricoccus ellipticus*	HQ008712	-	-
	OUTGROUP (GENBANK)			
-	*Auxenochlorella protothecoides*	FN29893	EU038285	YES
-	*Chlorella variabilis*	AB206549	AB260903	YES
-	*Chlorella vulgaris*	FR865658	AB260909	YES
-	*Micractinium pusillum*	AF364101	EF113451	YES

7.5.2. Other

Table 7: Sequence of the SSU rRNA gene used for molecular analysis of Chlorellaceae. 1 added sequence is marked by bold.

CHLORELLACEAE	
Actinastrum hantzschii AF288365	Hindakia tetrachotoma GQ867590
Catena viridis AH012205	Kalenjinia gelatinosa HG322129
Chlorella chlorelloides HQ111432	**Komarekia rotundata SAG 2082**
Chlorella pituita GQ176853	Marasphaerium gattermannii GQ477057
Chlorella rotunda HQ111433	Marvania geminata AF124336
Chlorella singularis HQ111435	Masaia oloidia HQ322128
Chlorella sorokiniana AB731602	Meyerella planktonica AY543042
Chlorella variabilis AB260893	Micractinium belenophorum AY323837
Chlorella volutis HQ111434	Micractinium pusillum AF364101
Chlorella vulgaris FR865683	Micractinium pusillum AF364102
Coronastrum ellipsoideum GQ507370	Mucidosphaerium palustre GQ487217
Closteriopsis acicularis FM205847	Mucidosphaerium sphagnale GQ487218
Compactochlorella dohrmannii GQ477058	Parachlorella hussii HM126551
Compactochlorella kochii HQ322126	Parachlorella kessleri JQ797561
Crucigenia´ lauterbornii JQ356710	**OUTGROUP**
Dicloster acuatus FM205848	Eremosphaera viridis SAG 39.92
Dictyosphaerium ehrenbergianum GQ487213	Lagerheimia genevensis AY122336
Dictyosphaerium libertatis GQ487211	Oocystis heteromucosa KR 210
Didymogenes sphaerica AB731603	Planctonema sp. AF387148
Gloeotila contorta AY422074	Tetrastrum staurogeniaeforme JQ356702
Heynigia dictyosphaerioides GQ487221	

Table 8: Sequence of the SSU rRNA gene used for molecular analysis of Sphaeropleales. 1 added sequence is marked by bold.

SPHAEROPLEALES	
Ankistrodesmus bibraianus Y16938	Neochloris vigenis M74496
Ankistrodesmus fusiformis X97352	**Nephrocytium agardhianum SAG 34.81**
Ankistrodesmus gracilis Y16937	Pediastrum duplex JQ315560
Asterarcys-quadricellulare AF388375	Planktosphaeria gelatinosa AY044648
Bracteacoccus aerius JQ259915	Polyedriopsis spinulosa AY780667
Bracteacoccus minor JQ259944	Pseudomuriella aurantiaca AB005748
Bracteacoccus pseudominor JQ259953	Pseudomuriella cubensis HQ292770
Bracteacoccus ruber JQ259919	Pseudomuriella engadinensis HM852442
Bracteacoccus sp. JQ259940	Pseudomuriella sp. AY195974
Chlorella zofingiensis X74004	Pseudoschroederia antillarum AF277649
Chlorotetraedron bitridens AY663043	Radiococcus polycoccus AF388378
Coelastrum astroideum var. rugosum AF388377	Selenastrum bibraianum HM483514
Coelastrum morus AF388374	Scenedesmus bajacalifornicus HQ246321
Coelastrum sphaericum AF388376	Scenedesmus obliquus X56103
Dictyococcus schumacherensis HM852439	Scenedesmus regularis FR865732

Dictyococcus schumacherensis HQ292769	Scenedesmus rubescens X74002
Enallax acutiformis AB037089	Schizochlamys gelatinosa AY781662
Follicularia texensis JN630516	Sorastrum spinulosum AY663041
Graesiella emersonii FR865687	Schizochlamys gelatinosa AY781662
Graesiella vacuolata FR865685	Sorastrum spinulosum AY663041
Hydrodictyon reticulatum HE610123	**OUTGROUP**
Kirchneriella obesa HM483513	Characium vacuolatum M63001
Monoraphidium contortum AY846382	Chlamydomonas reinhardtii JX888472
Monoraphidium saxatile AY846385	Chlamydomonas monadina JN903976
Mychonastes zofingiensis GU827478	Dunaliella salina EU589200

Table 9: Sequence of the SSU rRNA gene used for molecular analysis of Volvocales. 1 added sequence is marked by bold.

VOLVOCALES	
Carteria crucifera D86501	*Hafniomonas reticulata* AB248250
Carteria eugametos U70595	*Lobocharacium coloradoense* AF395436
Carteria lunzensis JN904001	*Lobochlamys culleus* AJ410461
Carteria radiosa D86500	*Lobochlamys segnis* AB701525
Characiochloris sasae AB360741	*Oogamochlamys ettlii* AJ410469
Characiosiphon rivularis AF395437	*Oogamochlamys gigantea* AJ410466
Chlamydomonas culleus U70594	*Oogamochlamys zimbabwiensis* AJ410472
Chlamydomonas fimbriata U70784	***Oonephris obesa* CCALA 398**
Chlamydomonas monadina JN903976	*Phacotus lenticularis* AY009897
Chlamydomonas noctigama AB701503	*Polytoma uvella* U22943
Chlamydomonas reinhardtii N903984	*Tetracystis aeria* U41175
Chlorogonium euchlorum AB278610	*Tetracystis pampae* JN903997
Chloromonas brevispina AF517092	*Tetracystis vinatzeri* JN903998
Chloromonas reticulata GU117583	*Treubaria schmidlei* U73474
Chlorosarcinopsis arenicola AB218701	*Treubaria setigera* U73475
Cylindrocapsa geminella U73471	*Trochiscia hystrix* AF277651
Cylindrocapsa geminella AF387159	**OUTGROUP**
Dunaliella lateralis DQ009762	*Bracteacoccus minor* JQ259943
Dunaliella salina EU589200	*Bracteacoccus ruber* JQ259919
Elakatothrix viridis AY008844	*Scenedesmus obliquus* AJ249515
Golenkinia longispicula JN968588	*Pediastrum duplex* JQ315560
Hafniomonas conica AB248251	