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ORIGINAL ARTICLE

Euchlorocystis gen. nov. and *Densicystis* gen. nov., Two New Genera of Oocystaceae Algae from High-altitude Semi-saline Habitat (Trebouxiophyceae, Chlorophyta)

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ABSTRACT

The Oocystaceae family is generally considered to contain common freshwater eukaryotic microalgae, and few are reported living in semi-saline habitats. Our latest ecological survey in Qinghai Lake and Angzicuo Lake, both large, closed, high-altitude, semi-saline lakes located on the Qinghai-Tibet plateau in China, revealed Oocystaceae species as a dominant group among plankton. Since limited knowledge exists about semi-saline species in the Oocystaceae family, a taxonomical study was carried out using morphological and phylogenetic methods. Using this approach, four new strains of Oocystaceae were identified and successfully cultured in the lab. Molecular results correlated with morphological characters and resolved these species into at least three genera. A new genus, *Euchlorocystis*, with type species *Euchlorocystis subsalina*, is described here as having the distinctive morphology of multiple pyrenoids per chloroplast among Oocystaceae, and an independent phylogenetic position at the base of the Oocystaceae. Similarly, the genus *Densicystis*, with type species *Densicystis glomerata*, is newly proposed here as having a unique colony morphology of dozens or hundreds of little cells tightly embedded in ellipsoid to round mucilage masses. *Oocystis marina*, originally described from the Baltic Sea, was also identified in Qinghai Lake and Angzicuo Lake and phylogenetically positioned in the semi-saline clade of the Oocystaceae. The result that a marine species was detected in the closed inland lakes implies a further need to reevaluate the origins of these species.

QINGHAI Lake (36°32′–37°15′N and 99°36′–100°47′E, 3,200 m alt) is a closed, high-altitude, semi-saline lake located on the northeast Qinghai-Tibet plateau, with a surface area of approximately 4,500 km^2 and a catchment area of over 30,000 km^2 (Wu et al. 2014). The average water depth of the lake is 19 m, with a maximum depth of 29 m (Ao et al. 2014). Being the largest lake in China, Qinghai Lake has been studied previously for physical (Ji et al. 2005) and hydrochemical features (Li et al. 2007) as well as geographical and geological features (Kelts et al. 1989). Biological research of the lake was mainly focused on bacteria, especially in sediments (Dong et al. 2006; Jiang et al. 2008). However, knowledge of eukaryotic microorganism resources was limited. Our latest research, carried out in September 2015, revealed the Oocystaceae family algae a dominant plankton status in Qinghai Lake.

Similarly, our survey in May 2015 of the plankton of Angzicuo Lake, another semi-saline lake, was also revealed to be occupied by Oocystaceae family algae. Angzicuo Lake is located in the southwest of the Qinghai-Tibet plateau with a smaller area than Qinghai Lake at

840 km^2 but a higher altitude at 4,535 m. No research on algae has previously been conducted in this lake.

The Oocystaceae family, with the type genus *Oocystis*, is generally considered to be a kind of common freshwater coccal microalgae with the distinctive morphology of oval or fusiform cells usually persisting within several layers of a mother cell wall for a long time (Hepperle et al. 2000; Huang et al. 2013; Komárek and Fott 1983; Stoyneva et al. 2007). This family was first described in 1901 by Bohlin (Bohlin 1901) and was widely accepted to be a distinct family by later researchers according the characteristic cell wall substructure consisting of parallel cellulose fibrils that are arranged in layers with perpendicular orientations (Bourrelly 1966; Hindák 1977, 1980, 1984, 1988; Komárek & Fott 1983). Further phylogenetic studies proved the family to be an independent lineage in Trebouxiophyceae and closely related to the Chlorellaceae (Hepperle et al. 2000; Krienitz and Bock 2011). Currently, there are up to 268 species within the Oocystaceae listed in AlgaeBase (Guiry and Guiry 2016). However, few of these species have been recorded from saline water. *Oocystis marina* was originally described in the Baltic Sea and was probably also found in other salt lakes in the former USSR, but this result has not been confirmed by later studies. (Komárek and Fott 1983). *Oocystis submarina* was documented in light brackish water at first (Komárek and Fott 1983). However, it was later documented in both a saline lake (Ramírez-Olvera et al. 2009) and fresh water (Li and Bi 1998). Whether this species is taxonomically uniform remains a question (Komárek and Fott 1983). Additionally, *Oocystis lacustris* and *Oocystis parva* were always considered to be widely adapted and existed in some saline lakes (Campos et al. 1995; Chen et al. 2016; Ramírez-Olvera et al. 2009). Although the members of the Oocystaceae family had been recorded in saline or semi-saline conditions by some limnological studies, taxonomic research was scarce.

Here, we report a taxonomic study of semi-saline species in the Oocystaceae in Qinghai Lake and Angzicuo Lake. Four strains were collected and resolved into at least three genera, including two new genera using a combined approach of molecular and morphological methods.

MATERIALS AND METHODS

During September 2015, the dominant species in Qinghai Lake were members of the Oocystaceae family. The average cell density of Oocystaceae was 4.15×10^6 (cells/L), and they occupied 89.82% of the total plankton cell number. Among them, samples of *O. marina* (strain LXD-18, FACHB-2130) and *Densicystis glomerata* (strain LXD-56, FACHB-2132) were collected in same site from Qinghai Lake (36°55′N, 99°53′E, alt. 3,200 m, salinity 10.50‰, Qinghai Province, China, in September 2015). *Euchlorocystis subsalina* (strain LXD-42, FACHB-2131) was sampled at another site at Qinghai Lake (36°48′N, 100°21′E, alt. 3,200 m, salinity 11.22‰, Qinghai Province, China, in September 2015). Another strain of *O. marina* (strain LXD-34) was collected in Angzicuo Lake (31°2′N, 87°9′E, alt. 4,535 m, salinity 7.29‰, Tibet, China, in May 2015). Three additional Oocystaceae samples were identified and sequenced for phylogenetic analysis.

Samples were isolated into single cells or colonies using the serial dilution pipetting technique (Hoshaw and Rosowski 1973) and maintained in liquid BG11 medium (Stanier et al. 1971) mixed with sterile water from Qinghai Lake (salinity 5.00‰) under constant light of 30–50 μmol/m^2/s and constant temperature of 25 °C. Photomicrographs were taken on an Olympus BX53 light microscope (Olympus Corp., Tokyo, Japan) equipped with an Olympus BX53 camera. For transmission electron microscopy (TEM), cells were fixed with 3% glutaraldehyde in 0.1 M cacodylate buffer, fixed with 1% aqueous OsO_4 in 0.1 M cacodylate buffer, and then dehydrated in acetone and embedded in Spurr's resin. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds 1963). The induction of zoospores and gametes was performed by flooding and light starvation (Fučíková et al. 2013).

Algal cells were broken with mini beads in a bead beater (3110BX, Biospec Products, Bartlesville, OK). Total DNA was extracted using a Universal DNA Isolation Kit (AxyPrep, Shuzhou, China). Primers and PCR conditions for the 18S rDNA and *rbc*L cpDNA genes were previously described in Xia et al. (2013).

The four strains were aligned with gene sequences downloaded from GenBank of 57 representative species of the Trebouxiophyceae. The sequences were initially aligned using ClustalX v 2.0 (Larkin et al. 2007). Phylogenies were estimated using maximum likelihood (ML) in RAxML v.8.0 (Stamatakis et al.) and Bayesian inference (BI) in MrBayes 3.1.2 (Huelsenbeck and Ronquist 2001). For ML analysis, GTRGAMMA was selected as best-fit model. The different substitution models of each partition used in the BI analyses were selected by MrModeltest 2.3 (Nylander 2004). The best-fit models applied to MrBayes were GTR + I + G for both the 18S rDNA dataset and *rbc*L cpDNA. All Markov Chain Monte Carlo (MCMC) analyses were performed with seven Markov chains (six heated chains, one cold) for $3{\cdot}10^6$ generations, where one tree was kept every 1,000 generations. Each analysis reached stationarity (an average standard deviation of split frequencies between runs < 0.01) well before the end of the run. A burn-in sample of 750 trees was removed before calculating the majority rule consensus trees in MrBayes.

RESULTS

Morphological observations

Oocystis marina L. MOEWUS 1951 (Strains LXD-18 and LXD-34)

Compound colonies of 2–32 cells were microscopic with 2–3 generations enclosed in mother cell walls and seldomly found solitary (Fig. 1A, B). Mother cell walls extended to an elongated and usually irregular elliptical shape with a gelled and obscured edge (Fig. 1C). The

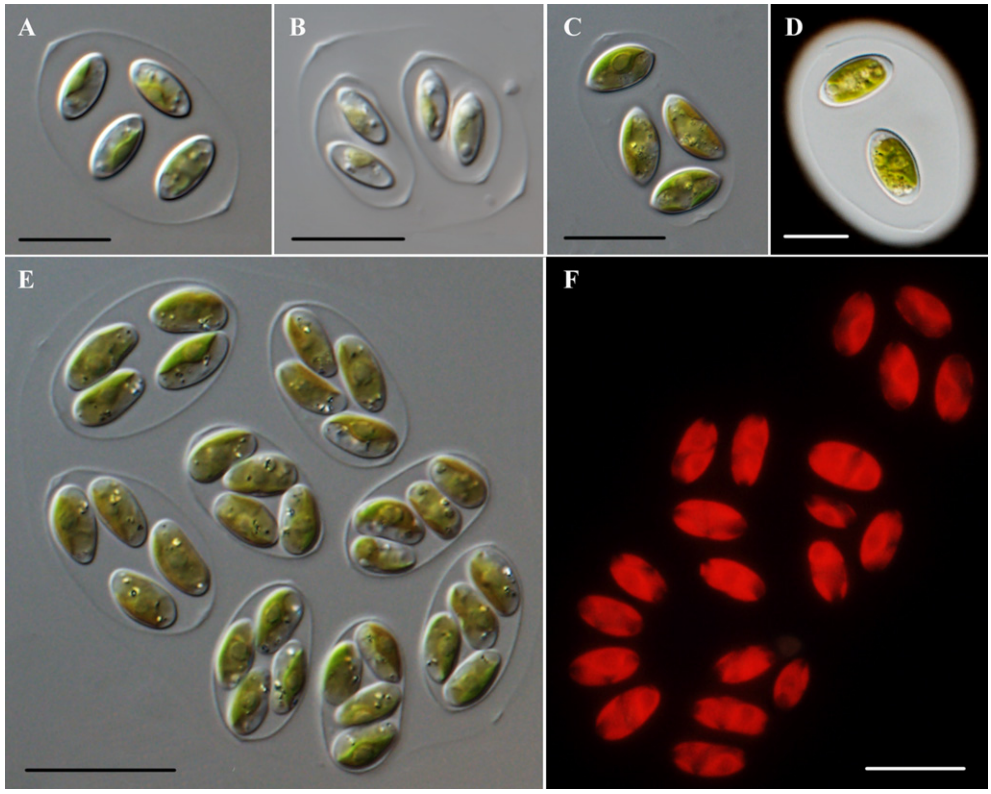

Figure 1 Light microscopy of *Oocystis marina*. (**A**) Colony from the field. (**B**) Colony with two generations in the field. (**C**) Colony cultured in the laboratory. (**D**) Colony was negatively stained by ink indicating the mucilage envelope. (**E**) Colony with multiple generations when cultured in the laboratory. (**F**) Autofluorescence shows the shape of chloroplasts. Scale bar 10 μm.

thick ends of mother cell walls were relatively clearer than daughter cells (Fig. 1A, B, 2E). The colony mucilage that surrounds mother cell walls was fine, hyaline and homogeneous (Fig. 1D). Cells were elliptical to cylindrical, with round ends and sometimes tapered thickenings, 6.1–13.8 μm long and 3.0–6.3 μm wide. When cultured, cells occasionally became a little curved (Fig. 1C). Lobed chloroplasts were parietal, occurring either singly or as a pair, and centrally located within the cell leaving the ends free where numerous assimilate particles and oil droplets were present (Fig. 1F). Each chloroplast contained a large central pyrenoid. Propagation was by 2-4-8 autospores, which were released by gelatinization or rupturing of the extended mother cell wall. Sexual reproduction and flagellated stages were not observed.

When observed with TEM, the cell wall structure was revealed to be multilayered (Fig. 2B, C). The *Oocystis*-like ultrastructure of the cell wall, composed of several cellulose layers with perpendicular fibril orientations, was more obvious in the mother cell walls (Fig. 2B). One global pyrenoid with a homogenous matrix was situated in the chloroplast and surrounded by a thick starch sheath that contained three to four starch plates (Fig. 2A, D). Thylakoids extended the length of the chloroplast and occurred in stacks of four to eight (Fig. 2C, D). Some of the tubular thylakoids penetrated the pyrenoid matrix (Fig. 2D). Starch grains were numerous inside the chloroplast (Fig. 2A, D).

Euchlorocystis subsalina Liu, Zhu, Song, Wang, Liu et Hu sp. nov. (Strain LXD-42)

Colonies contained 2–16 cells though a solitary cell could be observed sometimes when cultured (Fig. 3A, E). Mother cell walls usually extended to a primitively lemma-shape and then round- to square-shape with 2–3 generations of cells enclosed inside (Fig. 3B, C). Cells were oval to elongated elliptical with round ends and no thickenings, ranging in size from 11.3–16.6 μm long and 6.3–10.3 μm wide. A single parietal chloroplast was a wide trough-shape and occupied most of the cell volume (Fig. 3D), which made its colony mass a darker green than other Oocystaceae when cultured in the solid medium. Chloroplasts commonly contained multiple pyrenoids of different sizes with up to 6 per chloroplast observed (Fig. 3B, C). Propagation was by 2–4 autospores. Sexual reproduction and flagellated stages were not observed.

When observed with TEM, a multilayer cell wall with perpendicular fibril orientations was obvious (Fig. 4A, C). Multiple pyrenoids were seen in the chloroplast and surrounded by a thick starch sheath (Fig. 4A, B). Some of the tubular thylakoids penetrated the pyrenoid matrix (Fig. 4B).

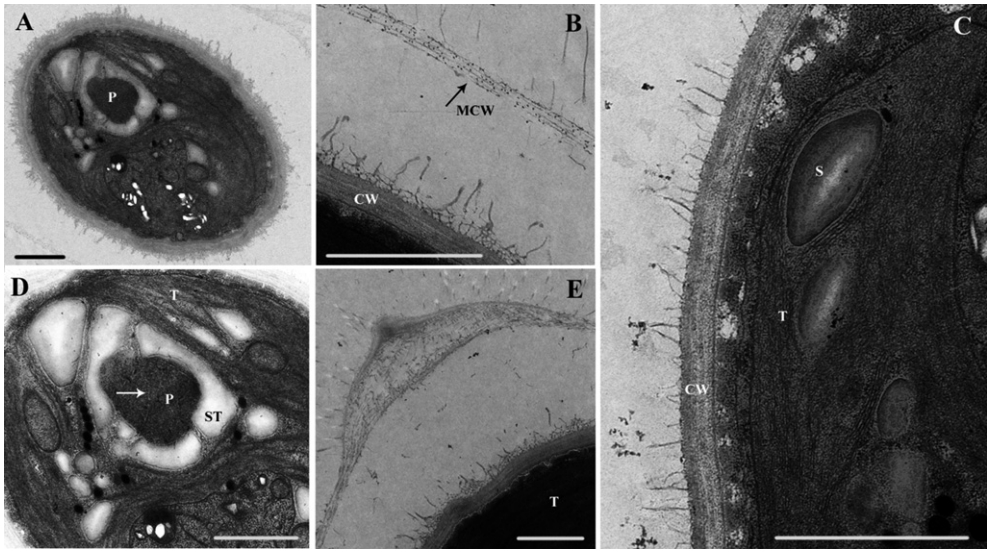

Figure 2 Transmission electron microscopy of *Oocystis marina*. (**A**) Longitudinal section of a cell. (**B**) Details of the mother cell wall. (**C**) Details of a cell wall. (**D**) Details of a pyrenoid showing the tubular thylakoids penetrating the pyrenoid matrix. (**E**) Details of a thickened end of the mother cell wall. (CW = cell wall; MCW = mother cell wall; P = pyrenoid; ST = starch sheath; S = starch grains; T = thylakoids; N = nucleus). The white arrow shows tubular thylakoids penetrating the pyrenoid matrix. The black arrow shows the mother cell wall. Scale bar 1 μm.

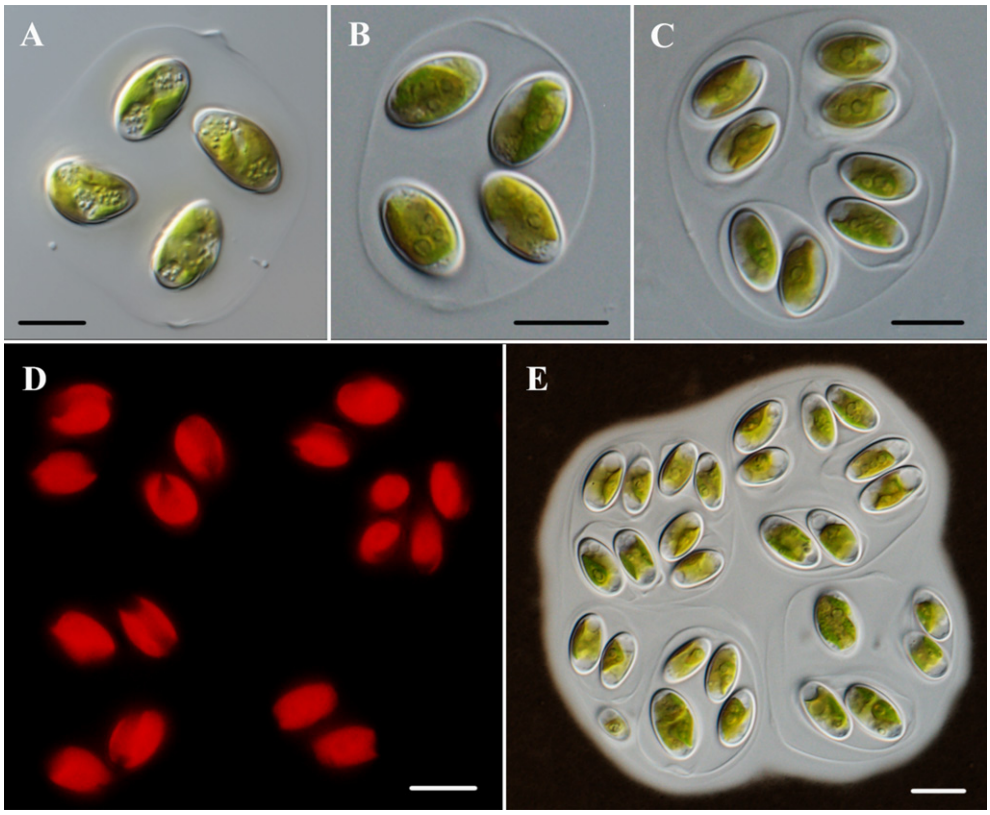

Figure 3 Light microscopy of *Euchlorocystis subsalina* sp. nov. (**A**) Colony from the field. (**B**) Colony cultured in the laboratory. (**C**) Colony with multiple generations when cultured in the laboratory. (**D**) Autofluorescence shows the shape of the chloroplasts. (**E**) Colony was negatively stained by ink indicating the mucilage envelope. Scale bar 10 μm.

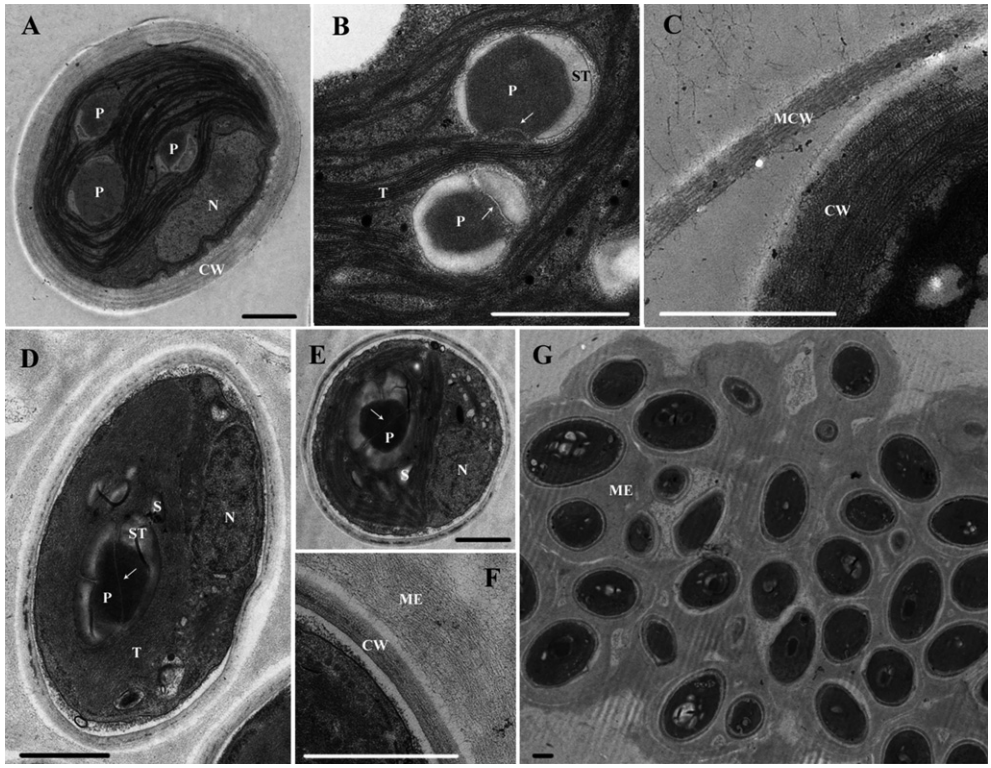

Figure 4 Transmission electron microscopy of *Euchlorocystis subsalina* sp. nov. (**A–C**) and *Densicystis glomerata* sp. nov. (**D–G**). (A) Lateral section of a cell. (B) Details of a pyrenoid showing tubular thylakoids penetrating the pyrenoid matrix. (C) Details of a cell wall and a mother cell wall. (D) Longitudinal section of a cell. (E) Lateral section of a cell. (F) Details of a cell wall and the mucilage envelop. (G) Colony with mucilage. (CW = cell wall; MCW = mother cell wall; P = pyrenoid; ST = starch sheath; S = starch grains; T = thylakoids; ME = mucilage envelope; N = nucleus). White arrows show the tubular thylakoids penetrating the pyrenoid matrix. Scale bar 1 μm.

Densicystis glomerata Liu, Zhu, Song, Wang, Liu et Hu sp. nov. (Strain LXD-56)

Mucilage colonies were microscopic with dozens or hundreds of cells tightly embedded and forming spherical to pyramidal to amorphous masses approximately 40–50 μm diameter in the field (Fig. 5A). When cultured, bigger colonies were observed (Fig. 5B, F). Mother cell wall was not extended and close against to young cells at the beginning. Then, with the growth of daughter cells, the mother cell walls gradually gelled and enclosed daughter cells, which formed new daughter mucilage groups embedded in the old colony. The mucilage was irregular with uneven margins and differentiated in density between daughter groups and the old colony (Fig. 5C). Cells were oval-shaped with round ends and no polar thickenings. Cells ranged in size from 6.0 to 8.0 μm long and 3.0–5.4 μm wide (Fig. 5D). The parietal chloroplast occurred singly with one pyrenoid (Fig. 5D, E). Propagation was by four autospores. Sexual reproduction and flagellated stages were not observed.

When observed with TEM, mucilage envelopes were thick and filled the regions between cells in the colony (Fig. 4G). The mucilage showed a similar though less compact ultrastructure with the cell wall, which was *Oocystis*-like being multilayered with perpendicular fibril orientations (Fig. 4F). Pyrenoids within the chloroplast occurred singly with tubular thylakoids penetrating (Fig. 4D, E). Starch grains were numerous inside the chloroplast (Fig. 4D, E).

Phylogenetic analyses

18S rDNA and *rbc*L cpDNA sequences were obtained for these four strains. Sequencing of the 18S rDNA PCR product of *Oocystis marina* produced a 2,221 bp sequence containing one intron (LXD-18) and a 1,657 bp sequence without an intron (LXD-34). The 18S rDNA sequence from *E. subsalina* was 2,446 bp in length and had two introns. The same gene from *D. glomerata* was 2,999 bp in length with three introns. Introns were not found in *rbc*L cpDNA sequences. Sequences obtained herein were submitted to GenBank under accession numbers MF100784–MF100794.

The final alignment of the 18S rDNA exon regions included the main taxa in Trebouxiophyceae. *Ankistrodesmus fusiformis* (Chlorophyceae) was chosen as the outgroup. The aligned *rbc*L cpDNA sequences included the most closely related members of the taxa Oocystaceae and Chlorellaceae. *Ankistrodesmus falcatus* (Chlorophyceae) were chosen for the outgroup.

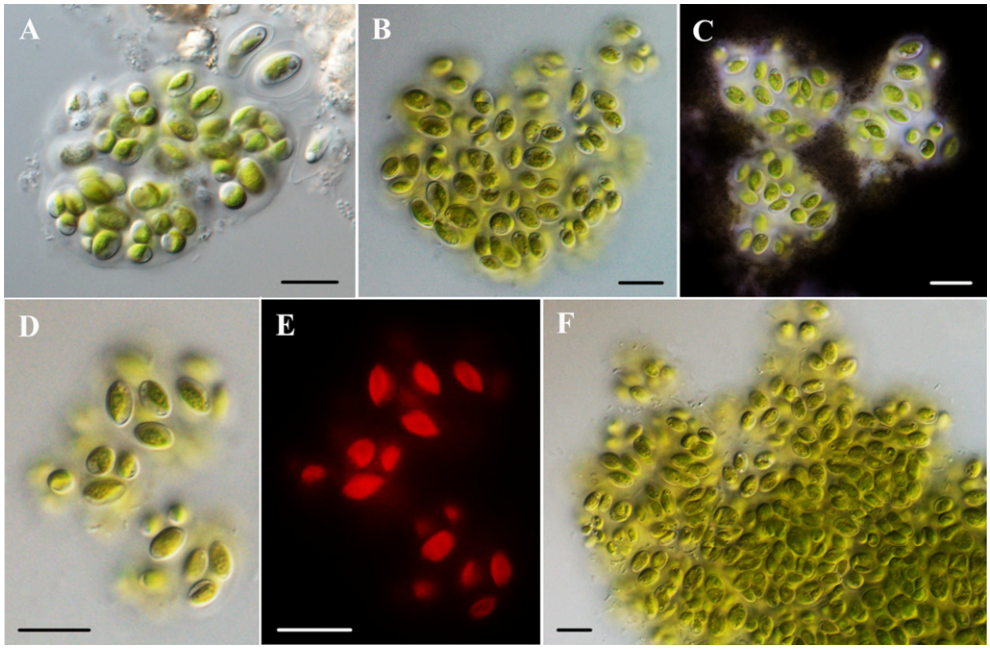

Figure 5 Light microscopy of *Densicystis glomerata* sp. nov. (**A**) Colony from the field. (**B**) Colony cultured in the laboratory. (**C**) Colony was negatively stained by ink indicating the mucilage envelope. (**D**) Detail of cells in colony. (**E**) Autofluorescence shows the shape of the chloroplasts. (**F**) A large colony when cultured in the laboratory. Scale bar 10 μm.

The ML and Bayesian analyses yielded similar topologies, which were consistent with previous phylogenetic studies (Bock et al. 2013). The 18S rDNA phylogenetic trees and the *rbc*L cpDNA trees both revealed that the three semi-saline species from this study were included in the Oocystaceae (Fig. 6, 7). *Oocystis marina* was positioned in one of the *Oocystis* clusters and formed a well-supported saline or semi-saline clade with the other three marine strains (FN690734, JQ315649, and JQ315800). Genus *Euchlorocystis* formed an independent clade at the base of Oocystaceae with good support. Genus *Densicystis* had a close relationship with the genus *Echinocoleum* and the spiny clade in Oocystaceae (Fig. 6).

DISCUSSION

Members of the Oocystaceae family are common but, as far as we know, rarely dominant in large bodies of water. However, a surprising dominance was recorded in two semi-saline lakes, Qinghai Lake, which is the largest lake in China, and Angzicuo Lake. Eukaryotic microalgae have not been researched in these two lakes, and little was known about Oocystaceae species in semi-saline habitats. Therefore, a taxonomic approach to study these semi-saline species of Oocystaceae was conducted by combining morphological and phylogenetic methods. The results revealed species diversity and resolved these specimens into at least three genera of Oocystaceae, including two novel genera, *Euchlorocystis* and *Densicystis,* described here and *O. marina* which was also identified.

Although morphologically similar with some *Oocystis* species, such as *O. marina* and *O. submarina*, genus *Euchlorocystis* was easy to distinguish from them and other Oocystaceae members by having multiple pyrenoids per chloroplast. Among the Oocystaceae family, only the genera *Fusola*, *Oonephris,* and *Eremosphaera* were described as having more than one pyrenoid according to Komárek and Fott (1983). *Fusola*, with fusiform-shaped cells and tip ends, could be morphologically differentiated from *Euchlorocystis*. Tsarenko (2011) classified *Fusola* in Cylindrocapsaceae, whereas some other scholars considered it as *Elakatothrix* or *Ankistrodesmus* (Guiry and Guiry 2016). The taxonomic position of *Fusola* needs further molecular research. *Oonephris* typically has a large, central pyrenoid with many smaller ones widespread in the chloroplast. The chloroplast shape ranges from stellate to spongiform. *Oonephris* had been positioned in the Volvocales and formed a clade with *Cylindrocapsa* (Stenclova 2013). *Euchlorocystis* morphological differed from *Oonephris* by its trough-shaped chloroplasts and its presence of layered cell wall. Some species, described as multi-pyrenoids and originally placed in the genus *Oocystis*, were suggested to belong to *Oonephris* by Komárek and Fott in 1983, including *Oocystis mucosa*, *Oocystis* cf. *apiculata,* and *Oocystis arctica*. *Euchlorocystis* was morphologically distinct from *Oocystis mucosa* by absence of a radially stratified mucilage envelop and from *Oocystis arctica* by different colony arrangement (cells of *Oocystis arctica* were arranged in rows and connected with remnants of the ruptured mother cell wall). *Oocystis apiculata* was always taxonomically confused, and one of the possible species described by Smith and Bold (1966) showed many pyrenoids. However, a reticular and perforated chloroplast and bigger cell size (28 × 18 μm) could morphologically

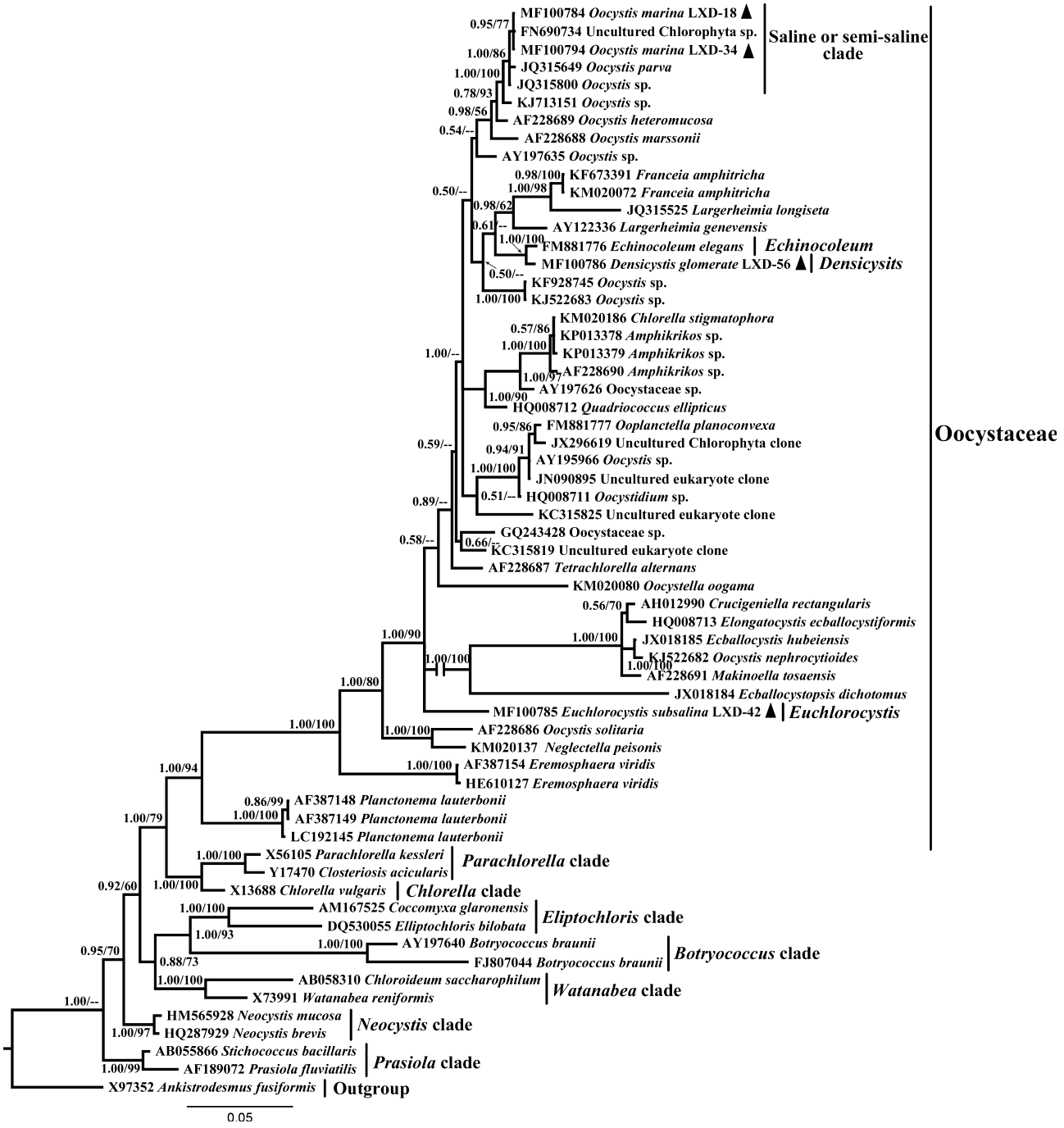

Figure 6 Phylogenetic tree of 18S rDNA sequences from Trebouxiophyceae species. Bootstrap support from Bayesian inference (BI) posterior probabilities and maximum likelihood (ML, constructed by RAxML) are presented on the nodes in order. Values above 0.5 for BI and 50 for ML are shown.

distinguish it from *Euchlorocystis*. *Eremosphaera*, with a bigger cell size and more chloroplasts per cell than *Euchlorocystis*, was positioned at the base of the Oocystaceae family tree (Krienitz and Bock 2011; Stenclova 2013) and closely related to *Euchlorocystis*. This result might imply that a basal origin of multiple pyrenoids in the Oocystaceae although there are big morphological differences between these two genera. Furthermore, genera at the base of phylogenetic trees of Oocystaceae were found with bigger cell size (e.g. *Eremosphaera*, *Neglectella*, *Makinoella*), more chloroplast (e.g. *Eremosphaera*, *Neglectella*, *Ecballocystis*, *Ecballocystopsis*, *Makinoella*) and more pyrenoid (*Euchlorocystis*). If there existed an evolution path deserves further study.

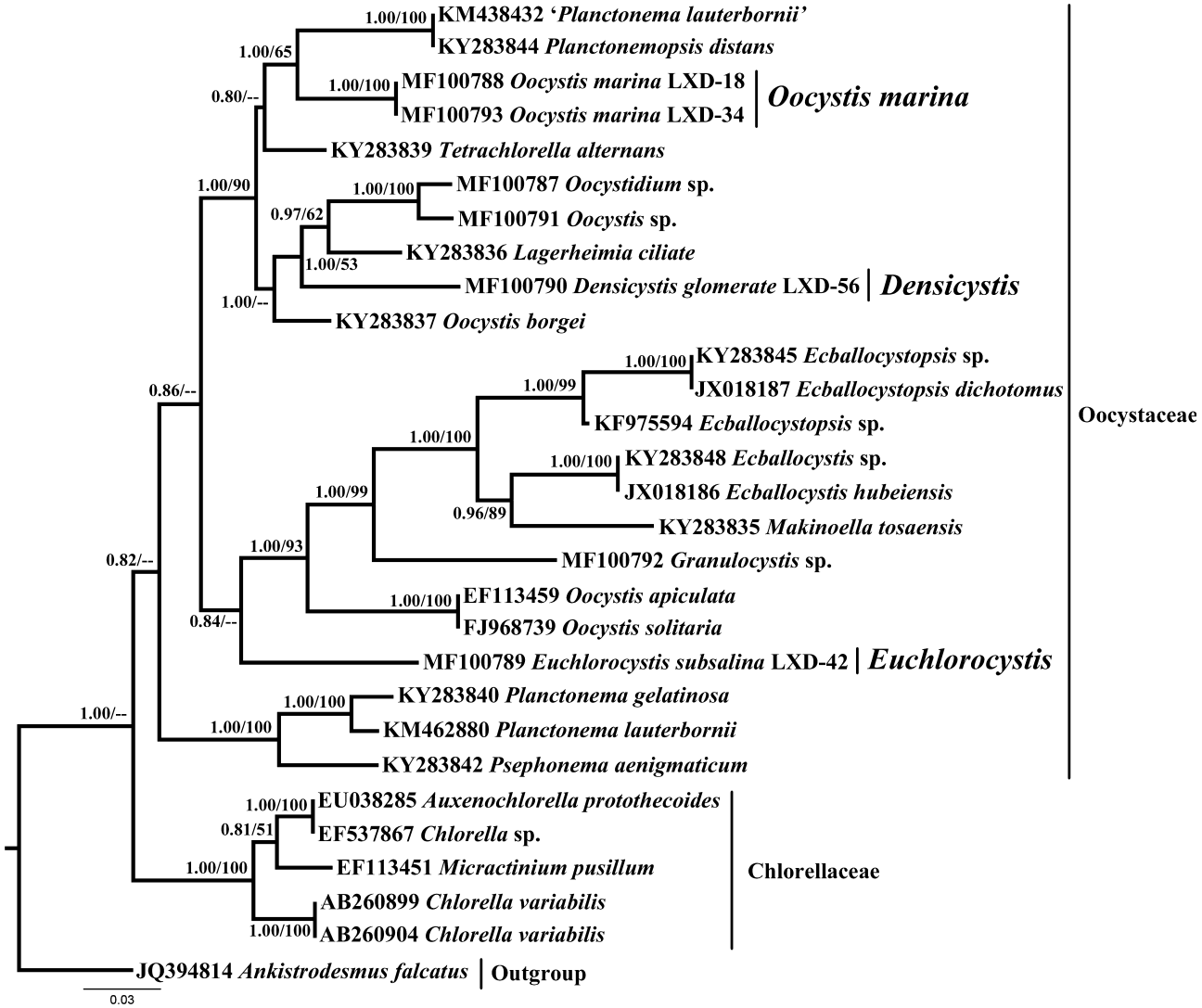

Figure 7 Phylogenetic tree of *rbc*L cpDNA sequences from Oocystaceae and Chlorellaceae species. Bootstrap support from Bayesian inference (BI) posterior probabilities and maximum likelihood (ML, constructed by RAxML) are presented on the nodes in order. Values above 0.5 for BI and 50 for ML are shown.

The genus *Densicystis* distinguished itself as having dozens or hundreds of little cells tightly embedded in an ellipsoid or round mucilage colony. Among Oocystaceae, cell masses embedded in common mucilage have been described for some *Oocystis* species, such as morphologically similar species *O. submarina* var. *variabilis* and *O. bispora*. The colony of *Densicystis* differed from the common mucilage colony by more dense mucilage, bigger cell density and more cell numbers. The mucilage in colony of genus *Oocystis* was uniform with smooth edge and tenuous which was invisible by TEM (Fig. 2A). On the contrary, mucilage in *Densicystis* was irregular with uneven margins and differentiated in density between daughter groups and the overall colony. The tough mucilage of daughter cell was obviously visible by TEM (Fig. 4F, G). On the other hand, when form mucilage colony, the *Oocystis* usually produced wide mucilage and, therefore, adjacent cells showed relative far distance. However, mucilage in *Densicystis* was usually narrow and cells even seemed directly clingy by edge. Therefore, colony of *Densicystis* was tightly embedded by cells and owned an obviously bigger cell density. Furthermore, the cell number in mucilage colony of *Oocystis* was usually low at fixed number as 8-16-32 in field conditions. As contrast, *Densicystis* usually owned a big colony with unfixed cell number as dozens or hundreds, which was even visible by eye sometimes no matter in field or cultured conditions. Apart from colony morphology, *Densicystis* could also distinguish from the similar species of *Oocystis* by cell morphology, propagation, and habitat. The *O. submarina* var. *variabilis* showed a bigger cell size (4–20 × 1.8–12 μm), widely expanded mother cell wall and

freshwater habitat which was different from *Densicystis*. The *O. bispora* characterized itself by two autospores during propagation, which was also different from *Densicystis* (4 autospores). In the Oocystaceae, colonies with large numbers of cells, some numbering over one hundred, was not rare. For example, the genus *Lobocystis*, which shared a similar colony formation, cell shape, and chloroplast morphology with *Densicystis*, often formed a *Dictyospaerium*-like multicells dichotomically branched colony (Komárek and Fott 1983). But cells in colonies of *Lobocystis* were characteristically connected by mucilaginous stalks, which were not observed in *Densicystis* (Fig. 5A–F). The genera *Ecballocystis* and *Ecballocystopsis* were characterized having hundreds of cells and forming a dendroid colony (Xia et al. 2013). However, the colony formation methods of them were by cells enclosed in expanded mother cells, which was different from the mucilage-connection of *Densicystis*. In addition, *Ecballocystis* and *Ecballocystopsis* were revealed a bigger cell size (12–35 × 6–15 μm) and more chloroplasts (2–6) than *Densicystis*. Phylogenetic analysis positioned the genus *Densicystis* within the Oocystaceae and revealed it to be away from any *Oocystis* clades and closely related to *Echinocoleum*. Though *Echinocoleum* shared a small cell size (4.0–8.0 μm long and 3.5–7.0 μm wide) and irregular mucilage with *Densicystis*, these two genera differ in colony shape and cell number.

Oocystis marina was originally described as a marine species from the Baltic Sea and then probably also found in other salt lakes in the former USSR (Komárek and Fott 1983), but this fact was not further confirmed by later studies. Now, our strain LXD-18 (same with LXD-34) was considered to be a reference strain of *O. marina* having similar morphology and the same semi-saline habitat. Phylogenetic analysis resolved strains LXD-18 and LXD-34 into a clade with another uncultured clone FN690734 (Fig. 6). FN690734 was obtained from material collected in the Baltic Sea (Majaneva et al. 2012), which was the same as the type strain of *O. marina*. Another two strains were collected in saline habitats in the estuary of Nakdong River near Busan (JQ315649) and along the coast near Jeonbuk in South Korea (JQ315800) (Hur et al. 2015). The JQ315649, documented as *O. parva*, showed a similar morphology with *O. marina* (Komárek and Fott 1983). These five strains, with little molecular difference (less than 4 bp) and similar morphology and habitat, formed a saline or semi-saline clade in the Oocystaceae phylogenetic tree (Fig. 6).

It was very interesting that a marine species was detected in the inland lakes. Qinghai Lake was geographically far from any marine environment and evolved from the evaporation of freshwater. Therefore, contamination from any marine influence was not a reasonable conclusion. On the other hand, these three Oocystaceae species were not found in terrestrial environments, excluding their possible spread from terrestrial sources and air current. In fact, we were not the first to detect marine species in Qinghai Lake. A *Cladophora* sp., collected in Qinghai Lake, demonstrated a close relationship with marine species by ITS rDNA phylogenetic analysis (Zhu, unpubl. data). Apart from algae, marine benthic *Archaea* were also found to be dominant in Qinghai Lake (Jiang et al. 2008). A possible approach was spread by carriage of migratory birds, such as gulls, which migrated between the Qinghai Lake and the bay of Bengal. Or perhaps the saline or semi-saline strains were actually belong to closely relate but different cryptic species, which could not been determined by present available data. A reevaluate of the origin for these species was needed in the further.

TAXONOMIC IMPLICATIONS

Chlorophyta Reichenbach 1834
Trebouxiophyceae Friedl 1995
Chlorellales Bold & M.J.Wynne 1985
Oocystaceae Bohlin 1901

Euchlorocystis Liu, Zhu, Song, Wang, Xiong, Wu, Liu et Hu gen. nov.

Diagnosis. Cells oval to elliptical with round ends and no thickenings, usually 2–16 cells forming a colony in the extended mother cell wall. Single chloroplast, parietal, with 2–6 pyrenoids. Propagation by 2–4 autospores. In the Oocystaceae, this genus differs from other genera except *Eremosphaera* by multiple pyrenoids per chloroplast and differs from *Eremosphaera* by a smaller cell size and fewer chloroplasts.
Etymology. The genus is named for its darker green colony color (Euchlor-) and *Oocystis*-like cell shape (-Oocystis).
Type species. *Euchlorocystis subsalina*
Phycobank Registration. http://phycobank.org/100104

Euchlorocystis subsalina Liu, Zhu, Song, Wang, Xiong, Wu, Liu et Hu sp. nov.

Diagnosis. Cells oval to elongated elliptical with round ends round and no thickenings, 11.3–16.6 μm long and 6.3–10.3 μm wide. From 2 to 3 generations of cells are enclosed in an extended mother cell wall with a lemma- to square-shape. Single chloroplast with a wide trough-shape, parietal, with 2–6 pyrenoids. Propagation by 2–4 autospores.
Holotype. Formaldehyde-fixed material was stored at the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China, as specimen No. LXD42.
Reference strain. A living culture was deposited in the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China (FACHB) as strain FACHB-2131.
Type locality. Qinghai Lake (36°48′N, 100°21′E, alt. 3,200 m), Qinghai Province, China. Water samples were collected in September 2015.
Etymology. The species was named for its habitat of semi-saline water.
Phycobank Registration. http://phycobank.org/100105

Densicystis Liu, Zhu, Song, Wang, Xiong, Wu, Liu et Hu gen. nov.

Diagnosis. Colony microscopic with dozens or hundreds of cells tightly embedded in mucilage forming spherical, pyramidal, or amorphous masses. Cells oval, with round ends and no polar thickenings. Single chloroplast parietal, with one pyrenoid. Propagation by four autospores. Genus differs from other members of the Oocystaceae by colony size of dozens or hundreds of cells tightly embedded in mucilage with an ellipsoid to round colony shape.
Etymology. The genus is named for its dense colony of cells within mucilage (densi-) and *Oocystis*-like cell shape (-Oocystis).
Type species. *Densicystis glomerata*
Phycobank Registration. http://phycobank.org/100106

Densicystis glomerata Liu, Zhu, Song, Wang, Xiong, Wu, Liu et Hu sp. nov.

Diagnosis. Dozens or hundreds of oval cells tightly embedded in mucilage with an ellipsoid or round colony shape. Cells 6.0–8.0 μm long and 3.0–5.4 μm wide. Single chloroplast, parietal, with one pyrenoid. Propagation by four autospores.
Holotype. Formaldehyde-fixed material was stored at the Freshwater Algal Herbarium (HBI), Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China, as specimen No. LXD56.
Reference strain. A living culture was deposited in the Freshwater Algae Culture Collection, Institute of Hydrobiology, Chinese Academy of Science, Wuhan, China (FACHB) as strain FACHB-2132.
Type locality. Qinghai Lake (36°55′N, 99°53′E, alt. 3,200 m), Qinghai Province, China. Water samples were collected in September 2015.
Etymology. The species was named for its tightly clustered cells within a colony.
Phycobank Registration. http://phycobank.org/100107

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