



A study of newly recorded genera and species of aerial algae in the order Chlorococcales (Chlorophyta) from the Hongcheon-river, Korea

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Abstract

Aerial algae were sampled from 28 sites on rocks, tree barks, and mosses along the Hongcheon-river in Gangwon-do, Korea, from December 2011 to September 2012 and then cultivated. Seven genera and eight species of the order Chlorococcales were newly recorded in Korea. These were *Spongiococcum tetrasporum*, *Tetracystis aggregata*, *Myrmecia bisecta*, *Coenocystis inconstans*, *Lobosphaeropsis pyrenoidosa*, *Pseudococcomyxa simplex*, *Coelastrella oocystiformis*, and *C. vacuolata*. As a result, the known Korean flora of the order Chlorococcales now includes 12 families with 54 genera, 263 species, 76 varieties, and 27 forma, giving a total of 366 taxa.

Key words: aerial algae, Chlorococcales, Chlorophyta, Korean flora, newly recorded species

INTRODUCTION

The order Chlorococcales belongs to the division Chlorophyta and class Chlorophyceae, which widely inhabit freshwater and soil. It is composed of useful microorganisms with a short life cycle that are easily cultured (Richmond 1986, Haag 2007). Komárek and Fott (1983) reported that this order included 15 families, 148 genera, and 1,025 species, whereas Hindák (1977, 1980, 1984, 1988) reported that it included only 168 species, excluding the genus *Scenedesmus*. According to Algaebase (Guiry and Guiry 2014), the order Chlorococcales includes 20 families, 109 genera, and 292 species (Koreiviene and Kasperovičienė 2011). The order Chlorococcales flora was reported to include 12 families, 56 genera, 181 species, 64 varieties, and 20 forma, giving a total of 246 taxa in Korea (The Korean National Council for Conservation of Nature

1996). The two floristic studies on the order Chlorococcales in Korea were conducted by An and Chang (1990), Chang and Kim (1994), Kim (2013a), Kim (2013b), and Kim (2013c). The ecology and distribution of aerial algae found on Korean stoneworks have been studied (Lim and Lee 2008, Song et al. 2012). The composition of aerial algal was studied in the Czech Republic (Škaloud 2009) and in the Great Smoky Mountains National Park (Khaybullina et al. 2010). Studies on aerial algae in Korea are less advanced than those in other countries.

In this study, aerial algae were collected from tree barks, stones, soil, mosses, and lichen around the Hongcheon-River. We aimed to add genera and species of Korea flora to the order Chlorococcales and newly described these discoveries.

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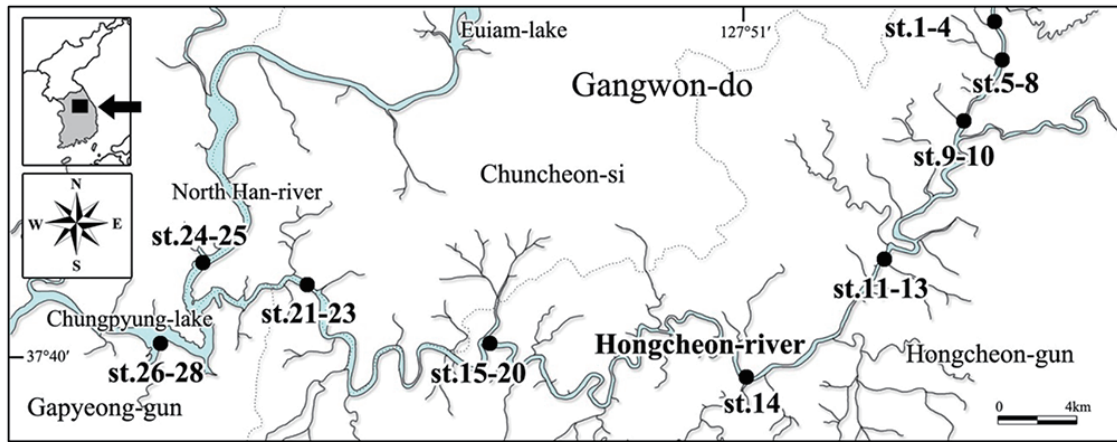


Fig. 1. The map showing the 28 sampling sites of Hongcheon-river at Gangwon-do from December 2011 to September 2012. st. 1-4 indicates 4 stations numbered from 1 to 4 (i.e., st.1, st.2, st. 3, st. 4); this is applied to other st. numbers.

MATERIALS AND METHODS

Aerial algae were sampled from 28 sites around the Hongcheon-river at Gangwon-do from December 2011 to September 2012 (Table 1 and Fig. 1). The samples were collected from tree barks, stones, soil, and mosses around the river by using a soft brush and a spatula. Each sample was sealed and refrigerated in a light-tight container with sterilized distilled water and transferred to the laboratory (Crispim et al. 2004). Some of the samples were fixed and stored in 1% formalin. Enriched cultures of aerial algae were made on Bold's basal medium (Stein 1973) and maintained in the algal culture collection of Kyonggi University (ACKU).

Analyses of physico-chemical environmental factors, such as the surface temperature of the substrates, light intensity, and humidity, were conducted on all the sampling dates (Song et al. 2012). The temperature was measured

with a stem thermometer, and the surface temperature was measured with a Testo 830-T1 infrared thermometer (Testo, Lenzkirch, Germany). Humidity was determined with a Testo 625 hygrometer (Testo), and light intensity was determined by using a LX-1108 light meter (Lutron, Taipei, Taiwan).

The taxonomic classification system of the order Chlorococcales was based on that of Komárek and Fott (1983), and the taxa were identified according to the system used by Hindák (1977, 1980, 1984, 1988), Chung (1993), Prescott (1973), Prescott et al. (1972, 1977, 1981, 1982), Hirose et al. (1977), John et al. (2002), and Wehr and Sheath (2003). The cultured samples were examined at $\times 400$ –1,000 magnification under a light microscope (BX41; Olympus, Tokyo, Japan), equipped with Nomarski differential interference optics. Species were illustrated in the drawing attachment, together with light microscope photographs.

Table 1. List of 28 sampling sites of Hongcheon-river at Gangwon-do from December 2011 to September 2012

Sites ^a	Location				
st.1-4	Jaeun-ri	Duchon-myeon	Hongcheon-gun	Gangwon-do	37°52'24.16" N; 128°01'27.36" E
st.5-8	Yeongnae-ri	Duchon-myeon	Hongcheon-gun	Gangwon-do	37°50'16.98" N; 128°01'03.67" E
st.9-10	Cheoljeong-ri	Duchon-myeon	Hongcheon-gun	Gangwon-do	37°48'23.82" N; 127°59'25.91" E
st.11-13	Gurun-ri	Hwachon-myeon	Hongcheon-gun	Gangwon-do	37°44'19.01" N; 127°56'30.22" E
st.14	Hahwagye-ri	Bukbang-myeon	Hongcheon-gun	Gangwon-do	37°40'46.51" N; 127°51'30.82" E
st.15-20	Eoyupo-ri	Seo-myeon	Hongcheon-gun	Gangwon-do	37°41'58.40" N; 127°41'56.95" E
st.21-23	Balsan-ri	Nam-myeon	Chuncheon-si	Gangwon-do	37°43'31.68" N; 127°35'19.27" E
st.24-25	Saryong-ri	Seorak-myeon	Gapyeong-gun	Gyeonggi-do	37°42'12.21" N; 127°29'32.77" E
st.26-28	Gwancheon-ri	Nam-myeon	Chuncheon-si	Gangwon-do	37°44'25.17" N; 127°30'42.93" E

^ast. 1-4 indicates station 1, 2, 3, 4 (from 1 to 4).

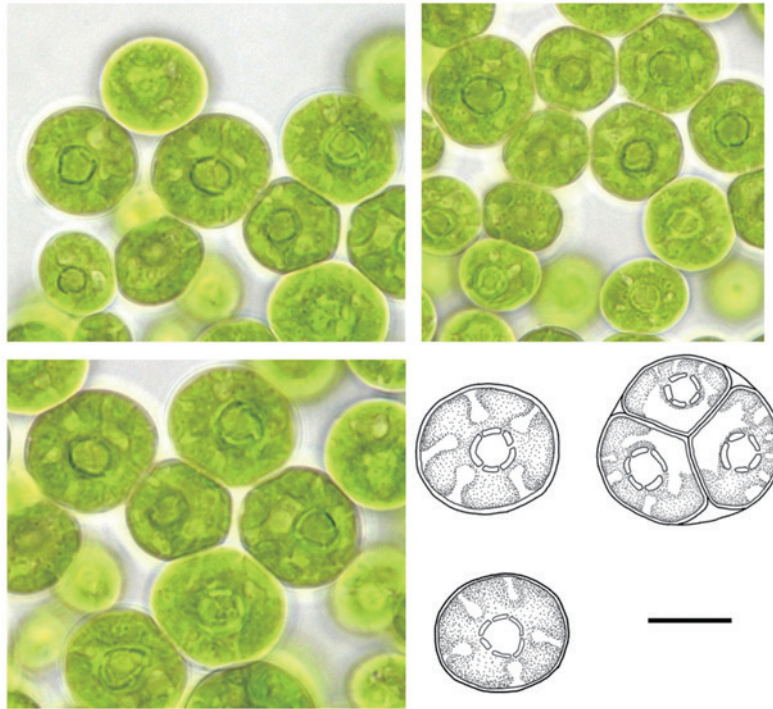


Fig. 2. Microscopic photographs and illustrations of *Spongiococcum tetrasporum* Deason found at Hongcheon-river, Gangwon-do, from December 2011 to September 2012. Scale bar, 10 μ m.

RESULTS AND DISCUSSION

In this study, seven genera belonging to the order Chlorococcales and eight species belonging to these seven genera were newly recorded in Korea. The newly recorded genera are *Spongiococcum*, *Tetracystis*, *Myrmecia*, *Coenocystis*, *Lobosphaeropsis*, *Pseudococcomyxa*, and *Coelastrella*. As a result, the known Korean flora of the order Chlorococcales now includes 12 families, 54 genera, 263 species, 76 varieties, and 27 forma, giving a total of 366 taxa. We described below the ecological and morphological characters of the seven genera and eight species previously unrecorded in Korea and provided microscopic photographs and illustrations.

Family Chlorococcaceae Subfamily Spongiococcoideae Genus *Spongiococcum* Deason

This genus was named by Deason in 1959, and newly recorded in this study in Korea. Deason (1959) isolated the genus *Spongiococcum* from a soil sample in Alabama, USA, and described it as a sponge-like plastid, with a pyrenoid and *Chlamydomonas*-type zoospore. In Guiry and

Guiry (2014), 6 species of the genus *Spongiococcum* were reported, but only *S. tetrasporum* has been accepted taxonomically in this genus.

Spongiococcum tetrasporum Deason (Fig. 2)

Distribution: Caribbean Islands, Alabama (Ettl and Gärtner 1995).

Spherical cells, 10–22 μ m in diameter. A sponge-like chloroplast, with a pyrenoid usually situated in the center. Large and fragmented pyrenoid. Cells mostly solitary, with four young cells, which are sometimes enclosed in the wall of a mother cell. Cells up to 30 μ m in diameter have been recorded (Deason 1971). Zoospores unobserved.

This species has been found among aerial algae (Deason 1959). In this study, this species was found in a wide range of habitats where the light intensity and humidity ranged from 3,915–48,000 lux and 26–65%, respectively (Table 2). However, it was found only in the moss samples, suggesting that the moss might protect it from environmental changes.

Sites of collection: 1, 5, 19, 21, 27; hereafter, site numbers correspond to Fig. 1.

Specimen: NIBRCL0000104610, ACKU HC1-96.

Family Chlorococcaceae
Subfamily Spongiococcoideae
Genus *Tetracystis* Brown & Bold

This genus was named by Brown et al. (1964) and recorded for the first time in Korea in this study. The genus *Tetracystis* is similar to the genus *Chlorococcum*, in having a single parietal chloroplast and many pyrenoids. In asexual reproduction, *Tetracystis* and *Chlorococcum* produce similar zoospores. However, unlike *Chlorococcum*, *Tetracystis* forms four or more daughter cells, with each daughter cell adjacent to the other and to the mother cell wall during cell division. This genus was reported to have 23 species globally (Brown et al. 1964).

***Tetracystis aggregata* Brown & Bold (Fig. 3)**

Distribution: in Europe, Italy (Ettl and Gärtner 1995); in North America, Mexico (Ettl and Gärtner 1995) and Texas (Ettl and Gärtner 1995); in Asia, Japan (Ettl and Gärtner 1995).

The cells are spherical, 15–30 µm in diameter. The species recorded in the present study was larger than 10 µm, which was larger than that observed by Nakano (1983). This species is distinguished from other species by a fragmented pyrenoid, a single nucleus, and an ellipsoidal zoospore. The cell wall is generally thin, but older cell walls may be thick. Sometimes, two or four young cells are enclosed in the mother cell wall. The zoospore is ellipsoidal, 9–12 µm long, and 3–6 µm wide. This species was found in soil samples and similar to that reported by Nakano (1983).

The genus *Tetracystis* has varied cell sizes within the same species. Therefore, we included this species under *Tetracystis aggregata* and decided that it was not suitable to classify it based on cell size because of the wide range of its cell sizes. This species, which is known to inhabit various environments (Nakano 1983), was sampled during all seasons.

Sites of collection: 5, 6, 7, 9, 11, 15, 22, 24, 25.

Specimen: NIBRCL0000104612, ACKU HC4-128.

Table 2. The environmental factors of 28 sites in Hongcheon-river from December 2011 to September 2012

Sites	Date	T (°C)	Hu (%)	L (lux)	ST (°C)	Habitats
1	Dec. 2011	2.9	61.4	3,915	0.0	rock, moss
2	Mar. 2012	10.8	50.6	11,010	7.5	rock, moss
3	Mar. 2012	10.8	50.6	11,010	7.5	tree bark, moss
4	Sep. 2012	27.4	55.4	2,500	26.0	tree bark, lichen, moss
5	Dec. 2011	2.5	65.4	6,600	0.0	soil, moss
6	Mar. 2012	14.7	41.0	26,950	15.0	soil, moss
7	Jun. 2012	29.0	47.9	3,530	17.5	moist soil
8	Sep. 2012	28.0	45.4	10,000	18.0	soil, rock, moss, subaerial
9	Mar. 2012	16.5	48.7	54,300	17.0	moist soil
10	Sep. 2012	28.2	56.7	1,034	25.0	tree bark, lichen
11	Mar. 2012	15.5	40.0	8,120	15.0	soil, moss
12	Jun. 2012	30.7	40.9	3,830	25.5	tree bark, lichen
13	Sep. 2012	28.3	60.0	2,100	15.0	rock, moss, subaerial
14	Sep. 2012	28.8	57.1	1,248	24.0	tree bark, lichen
15	Mar. 2012	14.6	28.9	4,400	8.0	soil, moss
16	Mar. 2012	14.6	28.9	4,400	8.0	tree bark, lichen
17	Jun. 2012	30.2	39.4	2,085	25.5	tree bark, moss
18	Jun. 2012	30.2	39.4	2,085	25.5	tree bark, lichen
19	Sep. 2012	28.7	49.4	5,000	24.0	rock, moss
20	Sep. 2012	28.0	55.4	5,520	24.0	tree bark, lichen, moss
21	Mar. 2012	17.0	25.8	6,600	11.0	rock, moss
22	Jun. 2012	30.0	37.9	1,523	25.0	soil, rock, moss
23	Sep. 2012	28.0	40.0	7,880	25.0	tree bark, lichen
24	Mar. 2012	16.9	23.4	9,900	9.0	soil, moss
25	Sep. 2012	27.7	50.0	4,055	24.0	soil, moss
26	Dec. 2011	3.2	55.9	4,400	1.0	tree bark
27	Mar. 2012	18.1	26.9	48,000	15.0	rock, moss
28	Sep. 2012	28.0	55.5	2,800	25.0	tree bark, lichen, moss

T, temperature (°C); Hu, humidity (%); L, light intensity (lux); ST, surface temperature (°C).

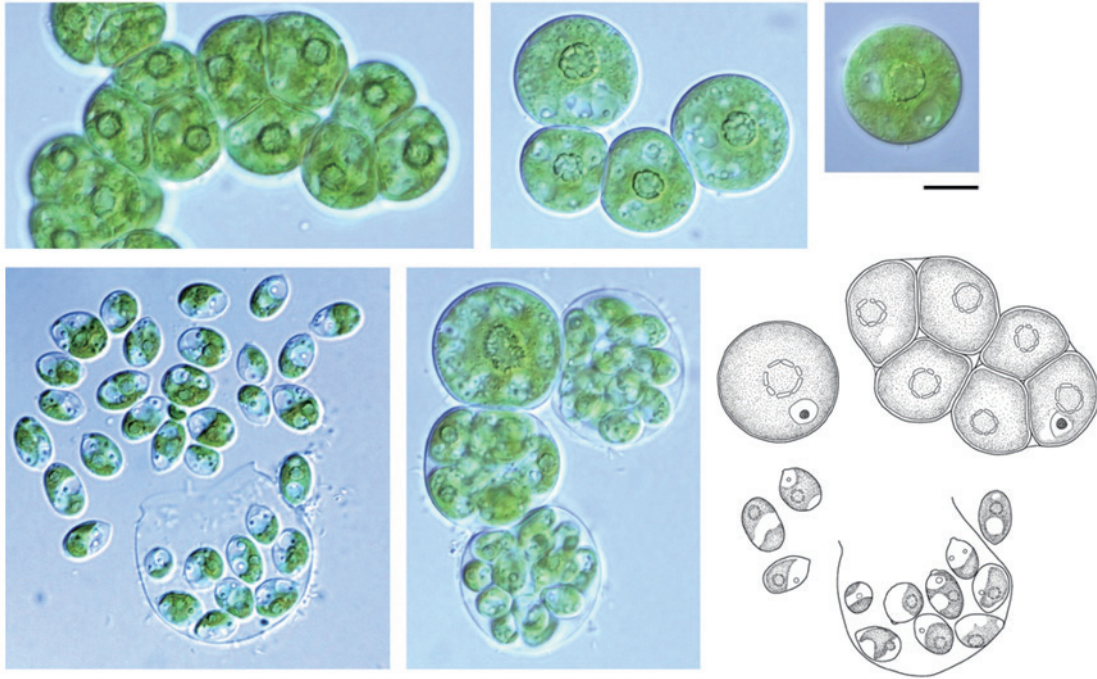


Fig. 3. Microscopic photographs and Illustrations of *Tetracystis aggregata* Brown & Bold found at Hongcheon-river, Gangwon-do, from December 2011 to September 2012. Scale bar, 10 μ m.

Family Palmellaceae
Subfamily Neochloridoideae
Genus *Myrmecia* Printz

This genus was named by Printz (1921), and recorded for the first time in Korea in this study. The morphological characteristics of this genus are similar to those of the genus *Chlorella*, but *Chlorella* possesses a pyrenoid; *Myrmecia* does not (Komárek and Fott 1983). Globally, this genus has been reported to have 11 species (Guiry and Guiry 2014).

***Myrmecia bisecta* Reisiigl (Fig. 4)**

Distribution: in Europe, Austria (Ettl and Gärtner 1995), Czech Republic and/or Slovakia (Ettl and Gärtner 1995), Romania (Caraus 2002), and Russia (Black Sea) (Ettl and Gärtner 1995); in Australia and New Zealand, Victoria (Day et al. 1995); in Antarctic and the subantarctic islands, Signy Island (Ettl and Gärtner 1995).

The cells are ellipsoidal or spherical, 8–15 μ m wide, with a parietal cup-shaped chloroplast. This species is an aerial algae (Gomez et al. 2003). We isolated this species from the aerial environment of soils and rocks.

Sites of collection: 5, 8, 11, 19, 21, 22, 27.

Specimen : NIBRCL0000107672, ACKU HC1-65.

Family Radiococcaceae
Subfamily Radiococcoideae
Genus *Coenocystis* Korshikov

This genus was named by Korshikov (1953). The genus *Coenocystis* is mostly cosmopolitan (John et al. 2002), but newly recorded in Korea. Species are assigned to the genus *Coenocystis* based on chloroplast structure and cell size. Globally, 14 species and 3 varieties of this genus have been reported (Guiry and Guiry 2014).

***Coenocystis inconstans* Hanagata & Chihara (Fig. 5)**

Cells 7–15 μ m in diameter, surrounded by a mucilage layer. Parietal chloroplast, with a single pyrenoid. Four ellipsoidal cells surrounded by a mucilage layer when young, and a single spherical mature cell surrounded only by a mucilage layer.

We isolated this species from aerial environments of soils and mosses. In this study, this species was sampled at low light intensity (2,100–3,915 lux) and high humidity (60–61%) conditions (Table 2). We expected this species to inhabit areas with high temperatures but low surface temperatures.

Sites of collection : 1, 13.

Specimen : NIBRCL0000104596, ACKU HC1-74.

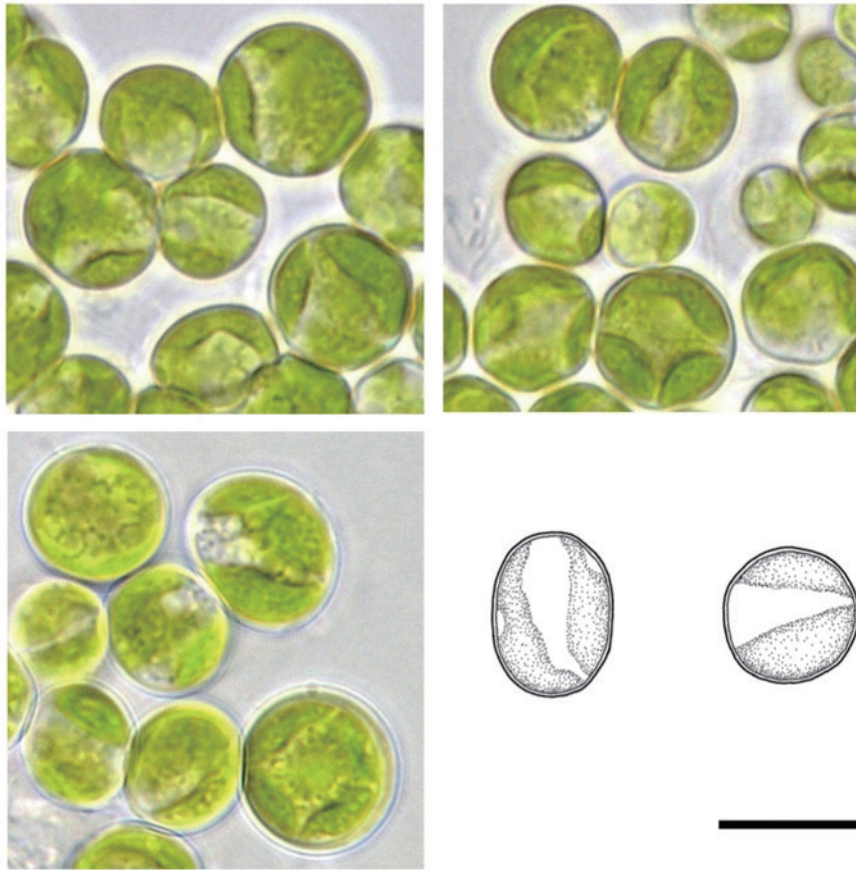


Fig. 4. Microscopic photographs and illustrations of *Myrmecia bisecta* Reisigl found at Hongcheon-river, Gangwon-do, from December 2011 to September 2012. Scale bar, 10 μ m.

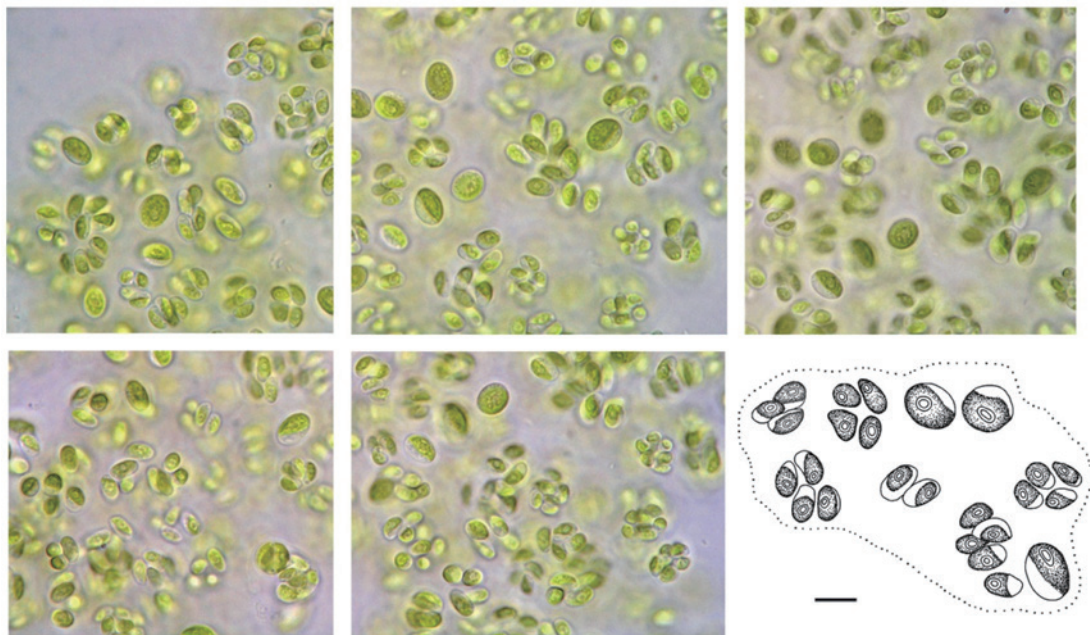


Fig. 5. Microscopic photographs and illustrations of *Coenocystis inconstans* Hanagata & Chihara found at Hongcheon-river, Gangwon-do, from December 2011 to September 2012. Scale bar, 10 μ m.

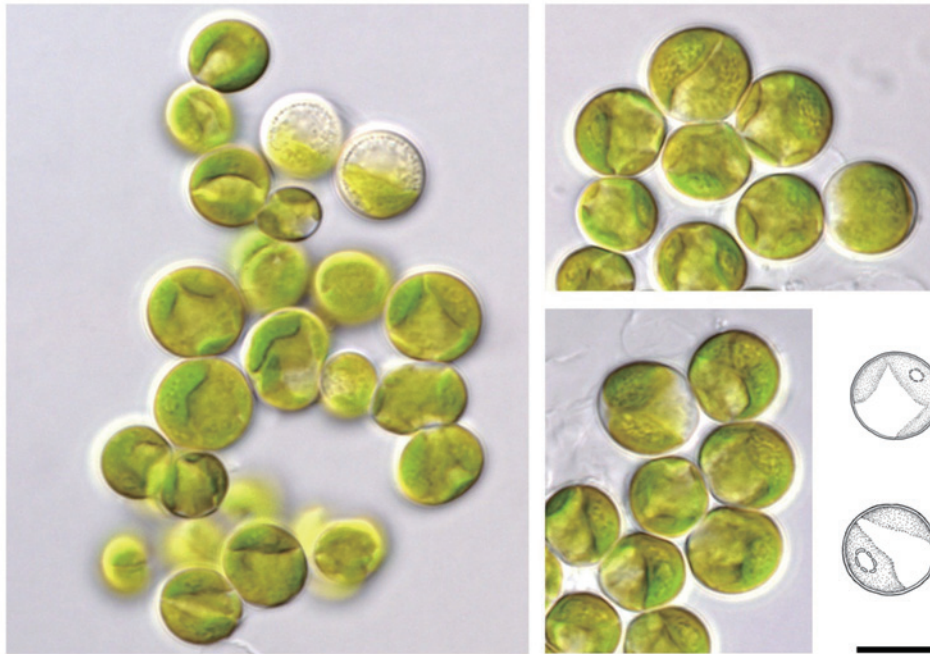


Fig. 6. Microscopic photographs and illustrations of *Lobosphaeropsis pyrenoidosa* Reisigl found at Hongcheon-river, Gangwon-do, from December 2011 to September 2012. Scale bar, 10 μm .

Family Chlorellaceae
Subfamily Chlorelloideae
Genus *Lobosphaeropsis* Reisigl

This genus was named by Reisigl (1969), and first recorded in Korea. Komárek and Fott (1983) suggested that this could be transferred to *Chlorolunula* Dangeard, but *Lobosphaeropsis* has been accepted to include this species taxonomically (Guiry and Guiry 2014). The genus *Lobosphaeropsis* is similar to the genus *Lobosphaera*, but it is classified according to the presence of a pyrenoid. Globally, this genus has been reported to contain 2 species.

***Lobosphaeropsis pyrenoidosa* Reisigl (Fig. 6)**

Distribution: in Europe, Austria (Ettl and Gärtner 1995) and Rumania (Carauș 2002).

Spherical cells, 5–13 μm in diameter. Parietal chloroplast parietal, 2 fragments, with a single fragmented pyrenoid.

Škaloud (2009) recorded this species as aerial algae isolated from soil, but we sampled this species from tree bark lichens in this study.

Sites of collection : 3, 4, 14, 16, 26.

Specimen : NIBRCL0000104604, ACKU HC3-6.

Family Chlorellaceae
Subfamily Ankistrodesmoideae
Genus *Pseudococcomyxa* Korshikov

This genus was named by Korshikov (1953), and recorded for the first time in Korea in the present study. The genus *Pseudococcomyxa* is similar to the genus *Coccomyxa*, but the *Pseudococcomyxa* genus is solitary and has a mucilage cap (Khaybullina et al. 2010). Globally, this genus has been reported to contain 4 species.

***Pseudococcomyxa simplex* (Mainx) Fott (Fig. 7)**

Synonym: *Coccomyxa simplex* Mainx.

Distribution: in Europe, Britain (John et al. 2002), Czech Republic (Gross et al. 2002), Czech Republic and/or Slovakia (Ettl and Gärtner 1995), Italy (Ettl and Gärtner 1995), Romania (Carauș 2002), and Russia (Black Sea) (Ettl and Gärtner 1995); in North America, Mac Robertson Land (Ettl and Gärtner 1995); in Asia, Japan (Ettl and Gärtner 1995); in Australia and New Zealand, New Zealand (Broady et al. 2012) and Victoria (Day et al. 1995).

Ellipsoidal or oval cells, with the anterior end slightly pointed, 8–14 μm long, 3–5 μm wide. Parietal chloroplast, with a smooth margin, filling two-thirds of the cell, with a single nucleus, without a pyrenoid. The cells are attached at one or two ends by a cap (small mucilage pad).

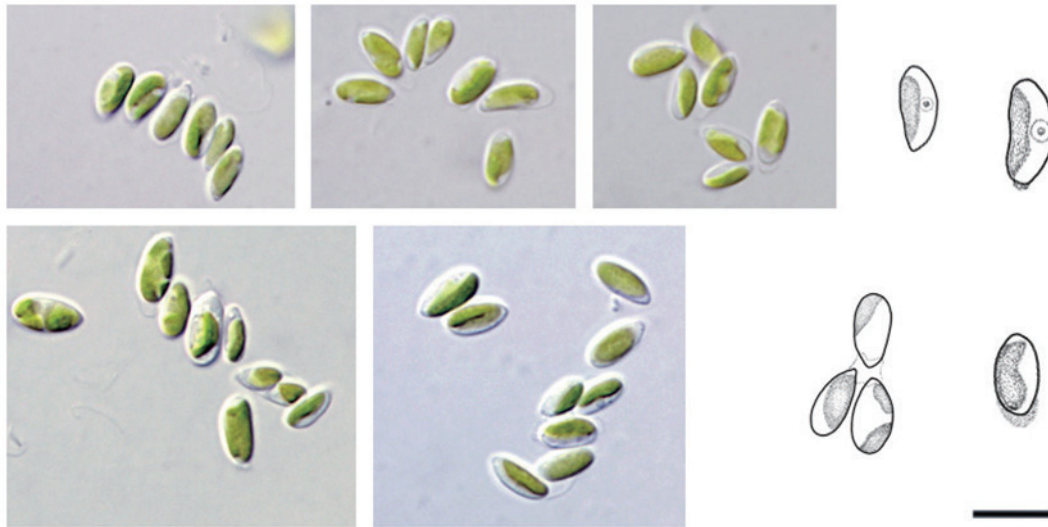


Fig. 7. Microscopic photographs and illustrations of *Pseudococcomyxa simplex* (Mainx) Fott found at Hongcheon-river, Gangwon-do, from December 2011 to September 2012. Scale bar, 10 μ m.

This species is known to be aerial algae, which attaches to rocks. In this study, this species was found at low light intensities and in humid aerial environments of mosses.

Sites of collection: 2, 13, 22.

Specimen : NIBRCL0000104607, ACKU HC3-30.

Family Scenedesmaceae
Subfamily Coelastroideae
Genus Coelastrella Chodat

This genus was named by Chodat (1922), and it belongs to the subfamily Scotiello cystoideae according to Kalina and Punčochářová (1987). Hanagata (1998) and Hegewald and Hanagata (2000, 2002) suggested that the subfamily Scotiello cystoideae should be removed and that *Coelastrella* should be included into the subfamily Scenedesmoideae based on 18S ribosomal RNA gene sequence data. *Coelastrella* was moved to the subfamily Coelastroideae as a result of an ITS2 sequence-structure study by Hegewald et al. (2010), and it was accepted in Guiry and Guiry (2014). Globally, this genus has been reported to contain 10 species and 2 varieties. It is newly reported in Korea.

***Coelastrella oocystiformis* (Lund) Hegewald & Hanagata (Fig. 8)**

Synonym: *Scotiella oocystiformis* Lund.

Distribution: in Europe, Britain (Ettl and Gärtner 1995, John et al. 2002), Czech Republic and/or Slovakia (Ettl and Gärtner 1995), and Spain (Rifón-Lastra and Noguero-

Seoane 1999).

Spindle-shaped, asymmetric cells, with both ends pointed. Parietal chloroplast, with several fragments and a single pyrenoid. The cell wall has 8–12 ribs.

According to John et al. (2011), this species mostly inhabits damp or wet terrestrial surfaces, including rocks, sand, and soil. In this study, *C. oocystiformis* was sampled from tree barks and mosses, but not in areas of high humidity (Table 2).

Sites of collection: 3, 12, 16, 17, 18.

Specimen: NIBRCL0000104595, ACKU HC3-59.

Family Scenedesmaceae
Subfamily Coelastroideae

***Coelastrella vacuolata* (Shihira & Krauss) Hegewald & Hanagata (Fig. 9)**

Synonym: *Chlorella fusca* var. *vacuolata* Shihira & Krauss.

Spherical or ellipsoidal cells, 5–14 μ m in diameter. Young cells are spindle-shaped or ellipsoidal with both ends pointed. Mature cells are mostly spherical. Parietal chloroplast, with several fragments and a single distinct pyrenoid. The cell wall has several ribs.

The ribs of *Coelastrella* are morphological evidence of thermotolerance (Hu et al. 2013), and they are a major classification key. However, they are not easily observed in an algal culture. Dead or divided cells can be used to observe ribs. *C. vacuolata* is similar to *C. oocystiformis* with regard to the spindle shape of its young cells, but it is

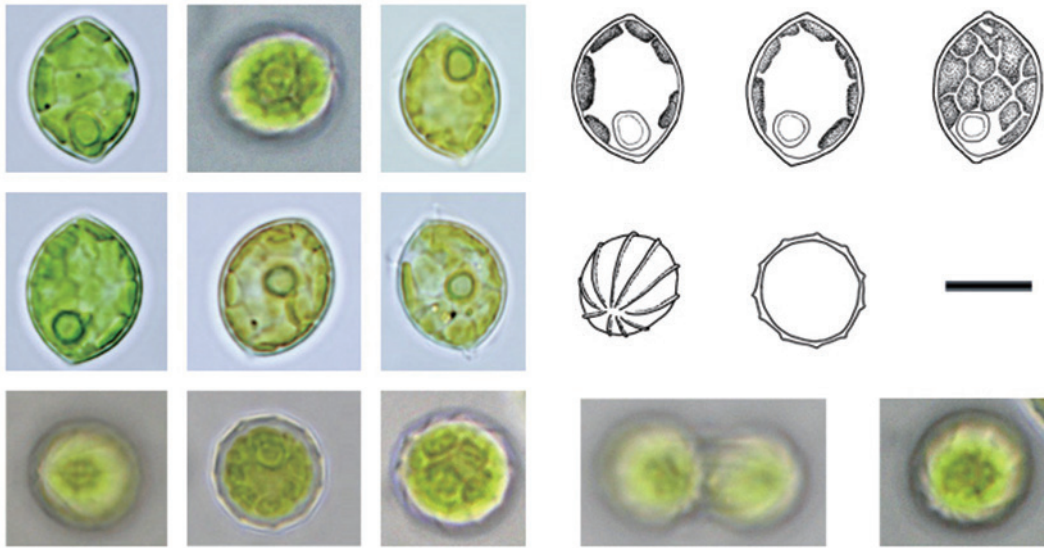


Fig. 8. Microscopic photographs and illustrations of *Coelastrella oocystiformis* (Lund) Hegewald & Hanagata found at Hongcheon-river, Gangwon-do, from December 2011 to September 2012. Scale bar, 10 μ m.

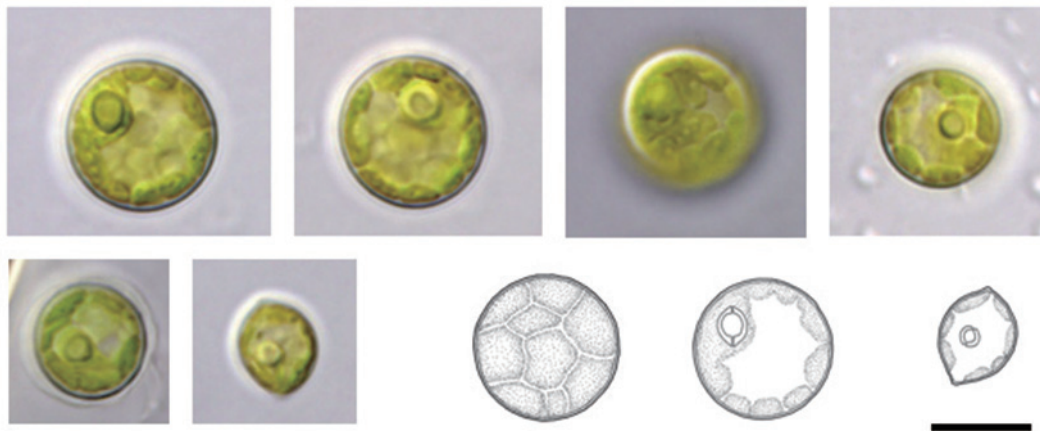


Fig. 9. Microscopic photographs and illustrations of *Coelastrella vacuolata* (Shihira & Krauss) Hegewald & Hanagata found at Hongcheon-river, Gangwon-do, from December 2011 to September 2012. Scale bar, 10 μ m.

separated from *C. oocystiformis* because of the ellipsoidal shape of the mature cells. This species was sampled from tree barks and in areas with humidity above 40% (Table 2).

Sites of collection: 10, 12, 20, 23, 28.

Specimen: NIBRCL0000107673, ACKU HC3-20.

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