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# DESCRIPTIONS OF NEW EDAPHIC AND AQUATIC SPECIES OF CHLOROCOCCUM MENEGHINI (CHLOROCOCCALES)

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A detailed study of 32 algal cultures, tentatively identified as *Chlorococcum* at the time of initial isolation, resulted in the description of seven species of *Chlorococcum* new to science. Five of the new species are reported as edaphic and two as aquatic forms. Although previously described species were isolated from soil, aquatic isolates of *Chlorococcum rugosum* and *Chlorococcum minutum* were identified in this study.

Several months ago, the author received 32 cultures from the algal collection then maintained at The Laboratory of Algology in Třeboň, Czechoslovakia. A cursive, screening examination of all strains at the time of their isolation by workers at Třeboň resulted in their being labelled as possible species of Chlorococcum. Prolonged and detailed observation of the isolates, under conditions identical to those utilized by Archibald and Bold (1970), in monographing the genus, revealed only 10 of the 32 isolates to be valid species of the genus Chlorococcum. Starr (1955) defined the chlorococcalean, unicellular, zoosporic, microalga *Chlorococcum* as exhibiting the following attributes: vegetative cells with a parietal chloroplast, and with at least one pyrenoid; zoospores with a wall, two flagella of equal length, and a stigma. Archibald and Bold (1970) selected specific morphological traits for characterizing the genus to the level of species. Taxonomic characteristics based upon these morphological features are summarized for seven new species of *Chlorococcum*. Three of the isolates identified as Chlorococcum are species already known to science.

# MATERIALS AND METHODS

The organisms studied were isolated by Drs Hindák, Komárek and Marvan during algological studies on material from Czechoslovakia, Cuba, and Egypt during the period 1962 to 1966. Unialgal cultures of the strains were maintained at Třeboň for an extended period of time on L-C medium solidified with agar (Bourrelly, 1948). For study, the author transferred the unialgal isolates to  $1.5 \% 3 \times N$  BBM agar medium and maintained growth under standard conditions (3000 lx cool-white fluorescent light on a diurnal light cycle of 12 h light and 12 h darkness at 22<sup>-</sup>C). Descriptions of all materials and methods employed for the morphological observations are outlined in detail in Archibald & Bold (1970).

A culture remained in log-phase of growth for a period of 2 to 3 weeks on  $3 \times N$  BBM agar medium. Morphological features were observed both in log-phase and in stationary-phase of growth. Early stationary-phase observations were made after 6 weeks of growth. Final stationary-phase measurements were concluded after 3 months. Characteristic patterns of growth were described for each isolate. Change in the colour of cultures due to the development of secondary carotenoids was produced by growing the organisms on 0-1 N BBM agar. Photomicrographs were made with a Nikon microscope and 35 mm Nikon attachment.

Measurements of growth under standard conditions and photography were accomplished 305

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in the author's laboratory in Pennsylvania. Additional observations and measurements of growth were made at the laboratory in Třeboň where the author spent part of a sabbatical leave. Preparation of the manuscript was completed at Třeboň.

# **OBSERVATIONS**

#### CHLOROCOCCUM TATRENSE SP. NOV. (FIG. 1)

Cellulae vegetativae in incrementi statu logarithmico sphericae ellipsoideaeque, sphericae cellulae  $10-15 \ \mu m$  diam., membrana  $0.5 \ \mu m$  crass.; cellulae in culturis in statu immobili minus quam 20  $\mu m$ , membrana usque ad  $2.5 \ \mu m$  crass.; chloroplastus singulari pyrenoide non perspicua, per laminam amyli ut videtur continuam circumdata praeditus; cellulae multi-nucleatae.

Reproductio per zoosporas aplanosporasque effecta, zoosporis ovoideis,  $7 \,\mu m$  long.  $\times 3 \,\mu m$  lat., nucleum posteriorem atque stigma anterius manifestum habentibus.

Origo: species in detrito nevi in loco Belánska Tatra Mountains (Velky Zlab) Czechoslovakia dicto reporta; a Hindák isolata et in Collections Culturarum Algarum in Laboratorie Algologiae in Třeboň, Czechoslovakia deposita.

Spherical and ellipsoidal vegetative cells in log-stage, spherical cells  $10-15 \mu m$  with a wall 0.5  $\mu m$  thick; in stationary-phase cultures cells less than 20  $\mu m$  with wall to 2.5  $\mu m$  thick; chloroplast with a single indistinct pyrenoid surrounded by an apparently continuous starch sheath; cells multinucleate.

Reproduction by zoospores and aplanospores, zoospores 7  $\mu$ m long  $\times$  3  $\mu$ m wide, with a posterior nucleus and prominent anterior stigma.

Source: snow detritis, Belánska Tatra Mountains (Velky Zlab) Czechoslovakia. Isolated by Hindák and deposited in Culture Collection of Algae at Laboratory of Algology in Třeboň. Isolate HIN 62/29. Holotype: Fig. 1. Material deposited at The Culture Collection of Algae at The University of Texas-Austin, Texas.

### CHLOROCOCCUM NIVALE SP. NOV. (FIG. 2)

Cellulae vegetativae in increment statu logarithmico sphericae, 7–15  $\mu$ m diam., membrana 0·5–1  $\mu$ m crass.; multae cellulae ellipsoideae 5  $\mu$ m × 12  $\mu$ m, membrana 1  $\mu$ m crass.; cellulae in culturis in statu immobili usque ad 20–25  $\mu$ m diam.; membranis usque ad 2·5  $\mu$ m spissescentibus; chloroplastus pyrenoide (plerumque) singulari, per vaginam amyli ut videtur continuam circumdata praeditus. Cellulae multinucleatae.

Reproductio per zoosporas aplanosporasque effecta, zoosporis ovoideis,  $8-10 \,\mu\text{m}$  long.,  $\times 4-6 \,\mu\text{m}$  lat., nucleum medio-posteriorem atque stigma anterius perspicuum habentibus.

Origo: species in loco High Tatra Mountains (Dolina Bielych plies) Czechoslovakia dicto reporta; a Hindák isolata et in Collectione Culturarum Algarum in Laboratorie Algologiae in Třeboň, Czechoslovakia deposita.

Spherical vegetative cells in log-state 7–15  $\mu$ m with wall 0.5–1  $\mu$ m thick; many ellipsoidal cells 5  $\mu$ m × 12  $\mu$ m with wall 1  $\mu$ m thick; in stationary-phase cultures cells up to 20–25  $\mu$ m, their walls thickening to 2.5  $\mu$ m; chloroplast with a single (usually) pyrenoid surrounded by an apparently continuous starch sheath; cells multinucleate.

Reproduction by zoospores and aplanospores, zoospores ovoid,  $8-10 \ \mu m \ \log \times 4-6 \ \mu m$  wide, with a median-posterior nucleus and prominent anterior stigma (Fig. 11).

Source: snow detritis, High Tatra Mountains (Dolina Bielych plies) Czechoslovakia. Isolated by Hindák and deposited in The Culture Collection of Algae at Laboratory of Algology in Třeboň. Isolate HIN 63/166.

Holotype: Fig. 2. Material deposited at The Culture Collection of Algae at The University of Texas-Austin, Texas.

#### CHLOROCOCCUM AEGYPTIACUM SP. NOV. (FIGS 3, 14)

Cellulae in incrementi statu logarithmico sphericae ellipsoideaeque; cellulae sphericae usque ad 15  $\mu$ m diam., membranis tenuibus, 0·3  $\mu$ m crass.; cellulae ellipsoideae 7  $\mu$ m × 10  $\mu$ m, membranis tenuibus; cellulae in culturis in statu immobili usque ad 30  $\mu$ m crass., membranis 3-10  $\mu$ m crass.; chloroplastus una (pluribusve) pyrenoide, per vaginam amyli ut videtur continuam circumdata praeditus.

Reproductio per zoosporas aplanosporasque effecta, zoosporae pyriformes 10  $\mu$ m long.  $\times$  5-7  $\mu$ m lat., nucleum medio-anteriorem atque stigma bene definitum habentes.

Origo: Species in solo ex agro in loco Omo-Saber Providence, El Tahir, Egypt dicto reporta, a Hindák isolata et in Collectione Culturarum Algarum in Laboratorie Algologiae in Třeboň, Czechoslovakia deposita.



FIGS 1–6. New species of *Chlorococcum*. Fig. 1. *Chlorococcum tatrense* sp. nov. vegetative cells in log-phase. Fig. 2. *C. nivale*. sp. nov. vegetative cells in log-phase. Note that most cells are ellipsoidal. Fig. 3. *C. aegyptiacum* sp. nov. vegetative cells in log-phase. Note large zoosporangium and the two aplanosporangia in the centre. Fig. 4. *C. pyrenoidosum* sp. nov. vegetative cells in log-phase. Fig. 5. *C. granulosum* sp. nov. vegetative cells in log-phase. Note cells in log-phase. Fig. 5. *C. granulosum* sp. nov. vegetative cells in log-phase. Note zoosporangium and zoospores. Fig. 6. *C. granulosum* sp. nov. vegetative cells in log-phase. Note cells in log-phase. Note granular appearance of cells. Scale in all cases =  $15 \mu m$ .

Spherical and ellipsoidal vegetative cells in log-state, spherical cells to 15  $\mu$ m with thin walls 0.3  $\mu$ m thick, ellipsoidal cells 7  $\mu$ m × 10  $\mu$ m with thin walls; in stationary-phase cultures cells to 30  $\mu$ m with walls 3–10  $\mu$ m thick; chloroplast with one (or more) prominent pyrenoids surrounded by an apparently continuous starch sheath; cells multinucleate (Fig. 14).

Reproduction by zoospores and aplanospores. Pyriform zoospores 10  $\mu$ m long  $\times$  5–7  $\mu$ m wide with a median-anterior nucleus and well-defined stigma (Fig. 11).

Source: field soil, Omo-Saber Providence, El Tahir, Egypt. Isolated by Hindák and deposited in Culture Collection of Algae at Laboratory of Algology in Třeboň. Isolate HIN 63/191.

Holotype: Fig. 3. Material deposited at The Culture Collection of Algae at The University of Texas-Austin, Texas.

# CHLOROCOCCUM PYRENOIDOSUM SP. NOV. (FIG. 4, 12)

Cellulae vegetativae sphericae in incrementi statu logarithmico 5–15  $\mu$ m diam., membrana 0.5  $\mu$ m crass.; in culturia in statu immobili cellulae virides usque ad 20  $\mu$ m diam., membrana 1  $\mu$ m crass. cellulae luteae usque ad 55  $\mu$ m crass., membrana in stratis striatis 5–10  $\mu$ m crass., chloroplastus aliquot pyrenoidibus, omni per vaginam amyli e duabus laminis compositam circumdata praeditus, chloroplastus in cellulis iuvenibus parietalis., lumene cellulae, autem, mox implents; cellulae multinucleatae.

Reproductio per zoosporas aplanosporasque effecta, zoosporis fusiformibus,  $10-12 \,\mu m$  long.  $\times 3 \,\mu m$  lat., stigma medium habentibus.

Origio: species in solo ex agro in loco Finca Leneor Providence Oriente, Cuba dicto reporta; a Hindák isolata et in Collectione Culturarum Algarum in Laboratorie Algologiae in Třeboň, Czechoslovakia deposita.

Spherical vegetative cells in log-phase 5-15  $\mu$ m with cell wall 0.5  $\mu$ m thick; in stationaryphase cultures green coloured cells to 25  $\mu$ m with wall 1  $\mu$ m thick, orange coloured cells to 55  $\mu$ m with wall in striated layers 5-10  $\mu$ m thick; chloroplast with several pyrenoids, each surrounded by a starch sheath of 2 plates; chloroplast parietal in young cells, but the lumen of the cell soon fills; cells multinucleate.

Reproduction by zoospores and aplanospores, the former fusiform,  $10-12 \mu m \log \times 3 \mu m$  wide with a median stigma (Fig. 11).

Source: field soil Finca Leneor Providence Oriente, Cuba. Isolated by Hindák and deposited in Culture Collection of Algae at Laboratory of Algology in Třeboň. Isolate HIN 65/121.

Holotype: Fig. 4. Material deposited at The Culture Collection of Algae at The University of Texas-Austin, Texas.

# CHLOROCOCCUM GRANULOSUM SP. NOV. (FIG. 5, 6, 13)

Cellulae vegetativae ellipsoideae sphericaeque in incrementi statu logarithmico; ellipsoideae cellulae 5-7  $\mu$ m × 10-12  $\mu$ m, membrana 0.5  $\mu$ m crassa; sphericae cellulae 10-15  $\mu$ m diam., membrana 0.5  $\mu$ m crass; cellulae in culturis in statu immobili usque ad 30  $\mu$ m, membrana, autem, non spissescente; chloroplastus pyrenoide conspicua per vaginam amyli ut videtur continuam circumdata praeditus; chloroplastus aetate aspectu granulosus factus; cellulae multinucleatae.

Reproductio in culturis iuvenibus per zoosporas, in culturis vetusieribus per aplanosporas effecta; zoosporae 7  $\mu$ m long.  $\times$  3  $\mu$ m lat., nucleum anterio-medium atque stigma anterius habentes.

Origio: species in detrito nivis in loco Belánska Tatra Mountains (Velky Zlab) Czechoslovakia dicto reporta; a Hindák isolata et in Collectione Culturarum Algarum in Laboratorie algologiae in Třeboň, Czechoslovakia deposita.

Ellipsoidal and spherical vegetative cells in log-stage of growth; ellipsoidal cells 5–7  $\mu$ m × 10–12  $\mu$ m with wall 0.5  $\mu$ m thick; spherical cells 10–15  $\mu$ m with wall 0.5  $\mu$ m thick; in stationary-phase cultures up to 30  $\mu$ m but wall not thickening; chloroplast with a prominent pyrenoid surrounded by an apparently continuous starch sheath; chloroplast becoming granular in appearance with age, cells multinucleate.

Reproduction by zoospores in young cultures and aplanospores in older cultures; zoospores 7 μm long × 2 μm wide, with an anterior-median nucleus and anterior stigma. Source: snow detritis, Belanska Tatra Mountains (Velky Zlab) Czechoslovakia. Isolated by

Source: snow detritis, Belánska Tatra Mountains (Velky Zlab) Czechoslovakia. Isolated by Hindák and deposited in Culture Collection of Algae at Laboratory of Algology in Třeboň. Isolate HIN 65/167.

Holotype: Fig. 5, 6. Material deposited at The Culture Collection of Algae at The University of Texas-Austin, Texas.



FIGS 7-10. Species of *Chlorococcum*. Fig. 7. *Chlorococcum aquaticum* sp. nov. vegetative cells in stationary-phase. Fig. 8. *C. elbense* sp. nov. vegetative cells in stationary-phase. Note that zoospores are still being formed in stationary-phase. Fig. 9. *C. rugosum* vegetative cells in log-phase. Fig. 10. *C. minutum* vegetative cells in log-phase. Scale in all cases =  $15 \mu m$ .

# CHLOROCOCCUM AQUATICUM SP. NOV. (FIG. 7)

Cellulae sphericae ellipsoideaeque in incrementi statu logarithmico; pleraeque cellulae ellipsoideae per peruidyn tenoirus extensum manentes,  $5-7 \,\mu m \times 10-12 \,\mu m$ , membrana  $0.5 \,\mu m$  crass., cellulae sphericae  $10-12 \,\mu m$  diam., membrana  $0.5 \,\mu m$  crass., cellula in culturis in statu immobili usque ad  $20 \,\mu m$ , membrana usque ad  $2.5 \,\mu m$  spissescente; chloroplastus pyrenoide conspicua per vaginam amyli ut videtur continuam circumdata praeditus; cellulae multinucleatae.

Reproductio per zoosporas aplanosporasque, zoosporis 7  $\mu$ m long.  $\times$  3  $\mu$ m lat., nucleum medium atque stigma anterius habentibus.

Origio: species o loco Lake El Tesero Pl. Providence Las Villas, Zapata, Cuba dicto reporta; Komárek isolata et in Collectione Culturarum Algarum in Laboratorio Algologiae in Třeboň, Czechoslovakia deposita.

Spherical and ellipsoidal vegetative cells in log-stage of growth; most cells remaining ellipsoidal for an extended period of time, ellipsoidal cells  $5-7 \,\mu\text{m} \times 10-12 \,\mu\text{m}$  with wall 0.5  $\mu\text{m}$  thick; spherical cells  $10-12 \,\mu\text{m}$  with wall 0.5  $\mu\text{m}$  thick; in stationary-phase cultures cells



FIGS 11-14. Species of *Chlorococcum*. Fig. 11. Characteristic shapes of zoospores of *Chlorococcum*. From left to right they represent fusiform, ovoid, pyriform. Fig. 12. *Chlorococcum pyrenoidosum* old cell in stationary-phase with stratified cell wall, multiple pyrenoids with the starch sheath composed of two plates. Fig. 13. *C. granulosum* granular appearance of older vegetative cell. Magnification scale the same as the scale for Fig. 12. Fig. 14. *C. aegyptiacum* appearance of vegetative cells in log-phase and stationary-phase of growth. Scale bars = 10  $\mu$ m.

to 20  $\mu$ m with wall thickening to 2.5  $\mu$ m; chloroplast with a prominent pyrenoid surrounded by an apparently continuous starch sheath; cells multinucleate.

Reproduction by zoospores and aplanospores, the former 7  $\mu$ m long  $\times$  3  $\mu$ m wide with a median nucleus and anterior stigma.

Source: Lake El Tersero Pl. Providence Las Villas, Zapata, Cuba. Isolated by Komárek and deposited in Culture Collection of Algae at Laboratory of Algology in Třeboň. Isolate KOM 64/88.

Holotype: Fig. 7. Material deposited at The Culture Collection of Algae at The University of Texas-Austin, Texas.

#### CHLOROCOCCUM ELBENSE SP. NOV. (FIG. 8)

Cellulae in incrementi atu logarithmico sphericae ellipsoideaeque, sphericae cellulae in statu iuveni logarithmico 7–10  $\mu$ m diam., membrana 1  $\mu$ m crass., cellulae in statu sero logarithmico 10–12  $\mu$ m crass., membrana 2  $\mu$ m crass.; ellipsoideae cellulae in statu iuveni logarithmico 5  $\mu$ m  $\times$  7  $\mu$ m, in state stato soro logarithmico usque ad 12  $\mu$ m  $\times$  18  $\mu$ m crescentes; cellulae in statu immobili cellulae usque ad 35  $\mu$ m, membrana 4  $\mu$ m crass. crescentes; chloroplastus aliquot pyrenoidibus per vaginam amyli ut videtur continuam circumdatis; cellulae multinucleatae.

Reproductio praecipue per aplanosporas effecta. Zoosporae  $7 \,\mu\text{m}$  long.  $\times 3 \,\mu\text{m}$  lat., nucleum medio-posteriorem atque stigma anterius habentes.

Origo: species in ramo fluminis Elbe (Alte Süder-Elbe) dicti, Hamburg, Germany reporta; a Marvin isolata et in Collectione Culturarum Algarum in Laboratoria Algologiae in Třeboň, Czechoslovakia deposita.

Spherical and ellipsoidal vegetative cells in log-stage, spherical cells 7–10  $\mu$ m in early log stage with a wall 1  $\mu$ m thick, by late log-stage cells 10–12  $\mu$ m with a wall 2  $\mu$ m thick; ellipsoidal cells in early log-stage 5  $\mu$ m × 7  $\mu$ m increasing to 12  $\mu$ m × 18  $\mu$ m in late log-stage; in stationary-phase cells increase to 35  $\mu$ m with wall 4  $\mu$ m thick; chloroplast with several pyrenoids surrounded by an apparently continuous starch sheath; cells multinucleate.

Reproduction mainly by aplanospores. Zoospores 7  $\mu$ m long  $\times$  3  $\mu$ m wide, with a medianposterior nucleus and an anterior stigma.

Source: branch of the Elbe River (Alte Süder-Elbe), Hamburg, Germany. Isolated by Marvin and deposited in Culture Collection of Algae at Laboratory of Algology in Třeboň, Czechoslovakia. Isolate MAR 66/19.

Holotype: Fig. 8. Material deposited at The Culture Collection of Algae at The University of Texas-Austin, Texas.

# DISCUSSION

Chlorococcum tatrense (Fig. 1) most nearly resembles C. pulchrum. The walls of old vegetative cells are  $2.5 \times$  thicker in C. tatrense and the pyrenoid is indistinct. The chloroplast lacks a superficial opening and is less massive than that of C. pulchrum. Fissuring of the chloroplast does not occur in C. tatrense as it does in C. pulchrum.

There is little similarity between *Chlorococcum nivale* and any other species of *Chlorococcum*. It is nearest in morphology to *C. echinozygotum* and *C. minutum*. Stationary-phase cells of both *C. echinozygotum* and *C. minutum* have walls twice the thickness of the walls of *C. nivale*. The ovoid zoospores (Fig. 11) of *C. nivale* are  $2 \times$  the length of the zoospores of the other two species. Unlike *C. minutum* no evidence of sexual reproduction was noted in *C. nivale*. Cells of *C. nivale* in log-phase tend to remain ellipsoidal. Those of *C. echinozygotum* do not.

Chlorococcum aegyptiacum (Fig. 3) is more like C. lacustre than other species of Chlorococcum. Log-phase cultures of C. aegyptiacum contain both spherical and ellipsoidal cells, and the cells do not cohere as do the cells of C. lacustre. Zoospores of C. lacustre are fusiform (Fig. 11) and those of C. aegyptiacum are pyriform (Fig. 11). As much as two-thirds of the size of an old vegetative cell of C. aegyptiacum may be wall (Fig. 14). Older cells of C. aegyptiacum may frequently lose their pigment.

Both Chlorococcum hypnosporum and C. novae-angliae resemble C. pyrenoidosum (Fig. 4, 12) in minor degrees. Zoospores are of different shapes in each of the three species. Log-phase cells of C. novae-angliae have thicker walls than those of C. pyrenoidosum. The cell walls of older cells of C. hypnosporum are thicker than those of C. pyrenoidosum. The starch plate arrangement, a sheath composed of two plates, on the pyrenoid of C. pyrenoidosum is unlike that on the other two species. On the other species the plate is composed of discontinuous plates.

The aquatic isolate Chlorococcum aquaticum (Fig. 7) resembles C. oleofaciens and C. pulchrum more than any other species of Chlorococcum. Cell size differences in stationary-phase of growth exist between C. aquaticum and C. oleofaciens. Those of C. oleofaciens are larger and the cell wall is thicker. Vegetative cells of C. pulchrum do not remain ellipsoidal in older cultures, and the cell wall is not as thick. The chloroplast of C. aquaticum does not fissure as that of C. pulchrum does.

Chlorococcum elbense (Fig. 8) exhibits some resemblance to C. perforatum. Chlorococcum perforatum soon becomes spherical and the cell walls of the older cells are thicker than those in C. elbense. Zoospores are formed in both log-phase and stationary-phase in C. perforatum. General morphology of C. granulosum (Fig. 5, 6) is unlike any previously described species.

Two aquatic isolates from Cuba, Komárek 64/117 from the Zapata Swamps located in Las Villas Providence and Komárek 64/119 from a pond near the lagoon in San Juan Place, Pinar del Rio Providence, are strains of the species *Chlorococcum rugosum* (Fig. 9). This species is distinct from any other species of *Chlorococcum*. Material originally studied to characterize this species was isolated from a sample of coastal sand at Port Isabel, Texas, U.S.A.

Soil collected in Bombay, India, was the source of the species described as *Chlorococcum minutum* (Fig. 10). Hindák's culture 65/122 obtained from water at the Jardin Botanico de la University Havana exhibits the same taxonomic characteristics. Thus, two former edaphic species are now known to be able to inhabit aquatic environments as well.

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