

Observations in *Hemichloris antarctica* Tschermak-Woess & Friedmann (Chlorophyceae) and the occurrence of a second *Hemichloris* species, *Hemichloris polyspora* n. sp.

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Abstract. The chlorococcalean genus *Hemichloris* is characterized by the possession of two chloroplasts per vegetative cell. The occurrence of a second species of the genus is reported (*H. polyspora* sp. nov.). Just as *H. antarctica* it grows cryptoendolithically in sandstone in Southern Victoria Land, Antarctica. In *H. antarctica* propagation by two autospores prevails over four, whilst in the new species *H. polyspora* in general four or eight (rarely 16 or 32) autospores are produced and *Borodinella*-stages do occur typically. Sexuality and zoosporulation do not exist in both species. Internal structures of chloroplasts can be observed by light microscopy more regularly in *H. polyspora* than in *H. antarctica* and under various conditions. Investigations of both *Hemichloris* species by transmission electron microscopy show them to go back to more or less extended assemblages of plastoglobuli. In both species the plastoglobuli are arranged around tubular inflations of thylakoids and apparently attached to the thylakoids. Keeping the cultures for three (even up to seven) months without light makes them survive and causes coming forth of the chloroplast structure throughout.

Key words: Algae, Chlorophyceae, *Hemichloris antarctica*, *Hemichloris polyspora* Tschermak-Woess, Hua, Gärtner et Hesse, structure, development, plastoglobuli assemblages.

Introduction

In the seventies and eighties of the last century *Hemichloris antarctica* Tschermak-Woess & Friedmann was found in an antarctic region high up in the mountains of Ross Desert of Southern Victoria Land. It lives cryptoendolithically in a certain layer of Beacon Sandstone (Friedmann and Ocampo-Friedmann 1984, Tschermak-Woess and Friedmann 1984). Rock samples kept frozen for some time and especially enrichment cultures allowed investigations of this chlorococcalean alga. During a later season a similar and apparently related alga was discovered. This caused a reinvestigation of *H. antarctica* which partially needs a slight correction of its diagnosis. On the other

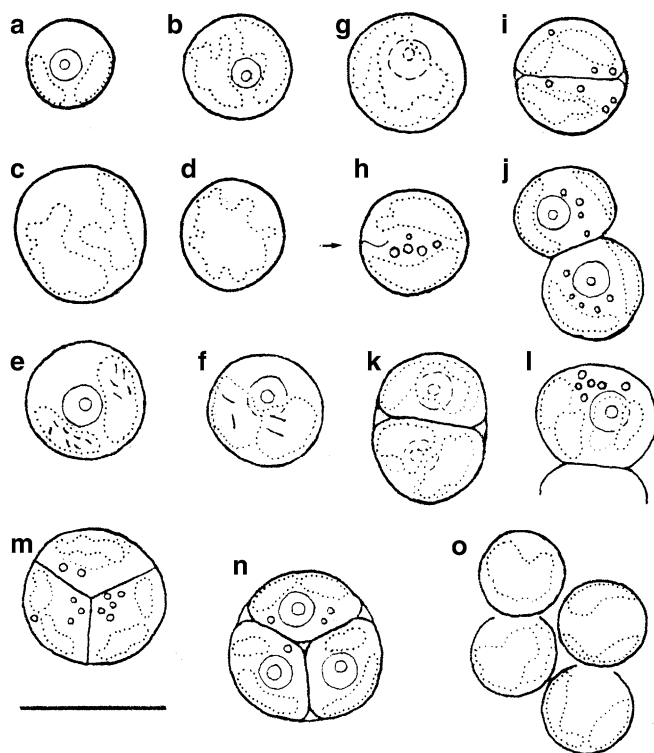


Fig. 1. a–o. *Hemichloris antarctica*. a–c Cells with 2 chloroplasts in different views, d chloroplast in face view, covering the lower chloroplast, e, f internal structure of chloroplasts (in f relative large), g after division of chloroplasts, h start of cytoplasmic division furrow from periphery marked by arrow (only 2 of the 4 chloroplasts represented), i–o stages of production of autospores: i, j with 2 chloroplasts, k, l already with 4 chloroplasts, m–o tetrads of different age. Drawings of living material grown in culture. Scale bar 10 μm

hand the characters of the new strain which separate it from *H. antarctica* are to be dealt with. In both strains changing structures of the chloroplasts may be of interest and TEM pictures allow their interpretation.

Material and methods

From *H. antarctica* strain A778-50 (126) isolated and investigated before was used again (Tschermak-Woess and Friedmann 1984). The new strain of *H. polyspora* A778-26 (187) was collected by E.I. Friedmann from a sandstone rock sample colonized by cryptoendolithic microorganisms at University Valley, Southern Victoria Land, Antarctica. The algal strain was isolated by Roseli Ocampo-Friedmann. Enrichment cultures were established mainly on BBM, 3NBBM, Bristol, 1/103NTM (=3NBBM + 0,1% peptone, 0,2% glucose) with 1% agar, exceptionally also in liquid media; all

with vitamins and trace elements added. The temperature was kept at 10°C during a 16:8 hour light:dark cycle with a photon flux density of 25 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (in part also less). Best development was visible on 3NBBM and 1/10 3NTM and at pH 5; pH 8 was detrimental. *H. polyspora* drastically showed more intensive and faster growth than *H. antarctica*. Investigation with light microscope was performed mainly in the living state; also some colourizations and reactions were applied. TEM procedure: the material was fixed in glutaraldehyde, postfixed in osmium tetroxide, embedded in Spurr's medium (Spurr 1969), stained with uranyl acetate and lead citrate (Reynolds 1963).

Results

Hemichloris polyspora Tschermak-Woess, Hua, Gärtner et Hesse spec. nova. Diagnosis: Cellulae sphaericae, 5–18,5 μm diametro. Chloro-

plasti integri vel diverse modo articulate, vel lobati, vel perforati. Paries firma, tenuis; in cultura sine involucro. Propagatio 4 aut 8 (raro 16 vel 32) autosporis, saepe stadium *Borodinellae* exhibens, longe coherentibus; cellulae vegetativae singulares raro. Paries cellulae maternalis diffluens aut rumpens. Reproductio per zoospores et reproductio sexualis non occurrunt. Alga cryptoendolithica. In saxis, Vallis Universitatis, Antarctica.

Holotypus: a cultura A 778-26 (187) in herbario Musei Britannici (Historiae Naturalis) (BM); isotypi in herbariis Universitatis Vindobonensis (WU) et Musei Historiae Naturalis Vindobonensis (W) depositi; item culturae in collectione Algarum Universitatis St. Gottingae (SAG, Germania) et in collectione Algarum Universitatis Oenipontis (ASIB, Austria) depositi.

Cells spherical, 5–18,5 μm in diameter. Chloroplasts entire, articulated, often lobed. Cell wall thin, compact, in culture without sheath. Multiplication by 4 or 8 (16–32) autospores, often producing *Borodinella*-stages and cohering for a relatively long time. Single, isolated trophical cells occur rarely. Cell wall of mother cell dissolves or is ruptured. Reproduction by zoospores and sexual reproduction do not occur. Growth cryptoendolithic. On sandstone rock, University Valley, Southern Victoria Land, Antarctica.

Holotype: from culture no. A778-26 (187) deposited in the herbarium of the British Museum (Natural History) (BM), isotypes in the herbaria of the University of Vienna (WU) and the Museum of Natural History, Vienna (W). Living material: deposited in the Sammlung von Algenkulturen Göttingen (SAG), Germany, and Culture Collection of Algae at the Botanical Institute of the University at Innsbruck (ASIB), Austria.

Further observations

Observations by light microscopy. With respect to *Hemichloris antarctica* a correction concerning morphology and life history is necessary. Young and fully developed cells do not possess

one chloroplast, as was thought formerly (Tschermak-Woess and Friedmann 1984); they regularly possess two of them. This can best be stated, when they are observed in profile (Fig. 1a-c, e, i, j, n, o). They roughly have the form of flat saucers and may develop some lobes or lobes all over their surface (Fig. 1b-d). Lobes of one chloroplast may reach into a recess of the other which often makes discrimination difficult (Fig. 1c). When seen in face view one chloroplast in part or totally may hide the other one (Fig. 1c, d).

Propagation in general comes about by production of two autospores (Fig. 1i-k); besides also four per mother cell occur in low rate (Fig. 1m-o) and also eight very seldom come about, 16 are a rare exception. As a first step in propagation the chloroplasts divide. This comes about in isolated cells (Fig. 1g), but also in autospores still surrounded by the mother cell wall. Nuclear division starts later and the cytoplasmic division furrow starts to grow inwards from the periphery (Fig. 1h). The 4-chloroplast stage may last for a relative long time. The relation of the number of autospores per mother cell in a 14 days culture on 3NBBM, e.g. was: 60 dyads, 10 genuine tetrads, 1 *Borodinella*-stage (4 daughter cells, clearly resulting from a first dyad, the members of which had produced dyads once more, while still surrounded by the cell wall of their mother cell); in a 1/10 3NTM culture the percentage of tetrads was somewhat enlarged (50:26). Sporulation is not bound to the size of the cells and production of *Borodinella*-stages may lead also to the occurrence of 6 small cells within a mother cell in which after a first dyad production a second round of sporulation has resulted in a dyad plus a tetrad.

In the course of the former investigation in some cells the chloroplasts showed an accumulation of tiny structural elements (Tschermak-Woess and Friedmann 1984, Fig. 1i-k); their form was longitudinal, delimitation mostly not sharp. Even in some living cells from the natural habitat they were discerned. The chloroplasts appeared quite homogenous. In old cultures (age 3 to 5 or even 7 months),

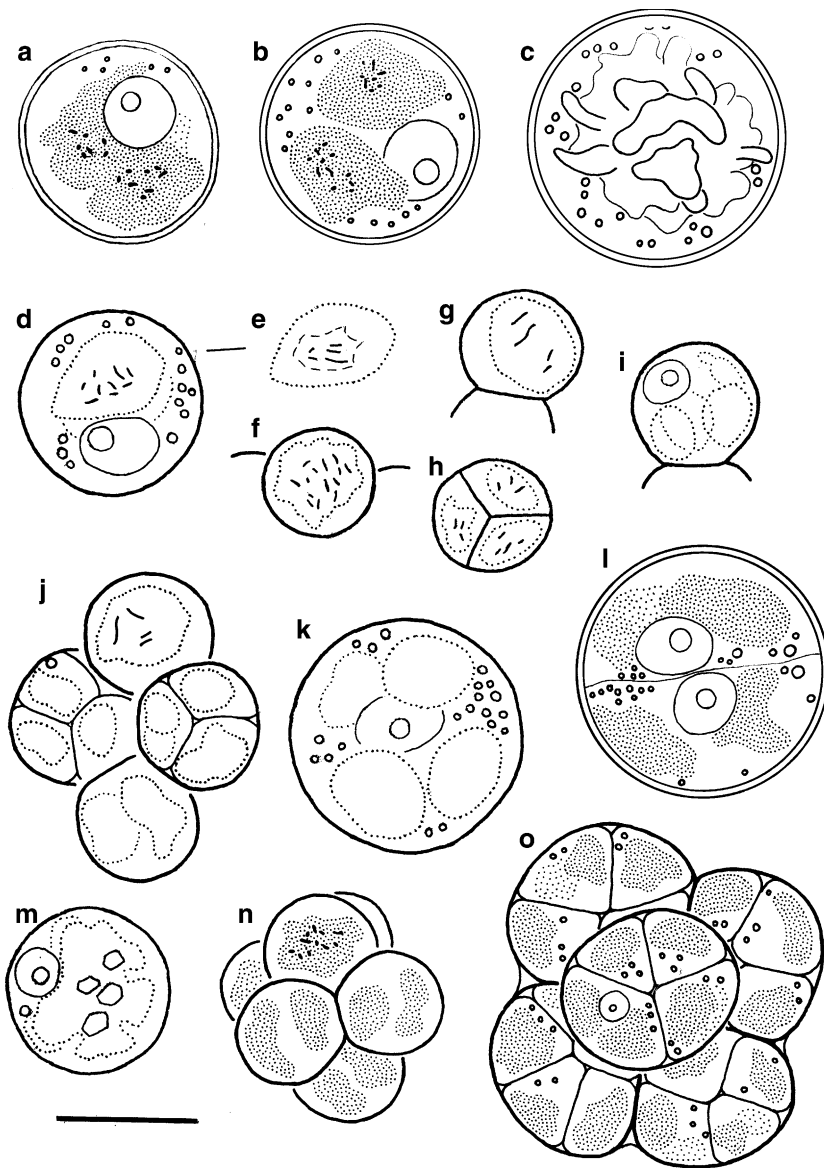


Fig. 2. a–o. *Hemichloris polyspora*. a, b, d–i Cells from autosporangia, lines within chloroplasts representing assemblages of plastoglobuli (in part only one of 2 chloroplasts visible), c intense lobation of upper chloroplast, d cell with distinct structure in upper chloroplast, e low level of this chloroplast (border of area of high pellucidity roughly marked by interrupted line), i preparation for autosporeulation, division of chloroplast into 4 parts, k 4 chloroplasts in the mother cell, nucleus still not divided, l first division furrow, m cell with polygonal grains of starch, n group of autospores, o *Borodinella*-stage. Drawings of living material grown in culture. Scale bar 10 μm

however, all of the cells or parts of them show the internal structure of the chloroplasts. Keeping the cultures all this time without a transfer to fresh medium and totally or in part without light apparently promotes increase and emergence of this structure. In comparison

to *H. polyspora* (compare the following details) its development per cell is reduced. The same also holds true for the cultures as a whole and in general. The nature and derivation of the internal structure in chloroplasts is treated in the chapter on TEM investigations (see below).

Here it may suffice to state that its elements besides being centrally located, as depicted formerly, often are more widely and irregularly distributed (Fig. 1e, f). Their dimensions differ from tiny corpuscles to lines reaching $1\ \mu\text{m}$ in length; the latter rather rare. Further an inner part of the chloroplast containing several of the structural elements is distinguished by its pellucidity in a number of cases.

The new strain on the basis of the most obvious characters separating it from *H. antarctica* was denominated *Hemichloris polyspora*. Its trophical cells reach slightly higher dimensions than in *H. antarctica*: maximum in *H. antarctica* in general $11\ \mu\text{m}$, in *H. polyspora* $18,5\ \mu\text{m}$. Their structure is nearly identical with that of *H. antarctica*. Their chloroplasts occupy a slightly larger part of the cell lumen and in relative large cells just as in *H. antarctica* they often are spectacularly lobed – often with finger-like extensions which may intercalate in an indentation of the second chloroplast. The nuclei are often situated in an excentric position.

At their side a group of about 4 tiny dictyosomes now and then may be made out, their position against each other often slightly changing. In accordance with *H. antarctica* the chloroplasts do not contain a pyrenoid.

When cultured under standard (cold) conditions of the present laboratory (see Material and methods), in a relative high number of living trophical cells the chloroplasts exhibited

an assemblage of internal particles; they appeared dark, mostly of longitudinal form – even of relative long lines – and mostly without sharp delimitation (Fig. 2a, b, d–j); only occasionally they had clear contours and even then discerned in different levels of a plastid (Fig. 2d, e). They often occupied a large part of a chloroplast and this area often differed by its pellucidity from the more peripheral parts; but a clear delimitation of this area was never given. By JJK no change came about; fumigation by OsO_4 made the structural elements vanish and so did treatment by alcohol-acetic acid. Altogether this differentiation in *H. polyspora* occurs much more regularly than in *H. antarctica* and under various cultural conditions. Another important feature of *H. polyspora* different from *H. antarctica* is its way of reproduction: one mother cell produces more than two, that is (4) 8 (16, 32) autospores (Figs. 3, 4). They remain united for a relative long time and in well growing cultures soon go through a next round of autospore. So *Borodinella*-stages (compare Gärtner 1985 a, b) occur regularly and in relative high proportion (Fig. 2o). Free spherical cells can be found but rarely. Besides two also three generations of autospores may be united in a *Borodinella*-stage.

Old cultures are coloured orange by accumulation of oil droplets in the cytoplasm containing secondary carotenoids. Cultures appear dim and their surface granular. Growth

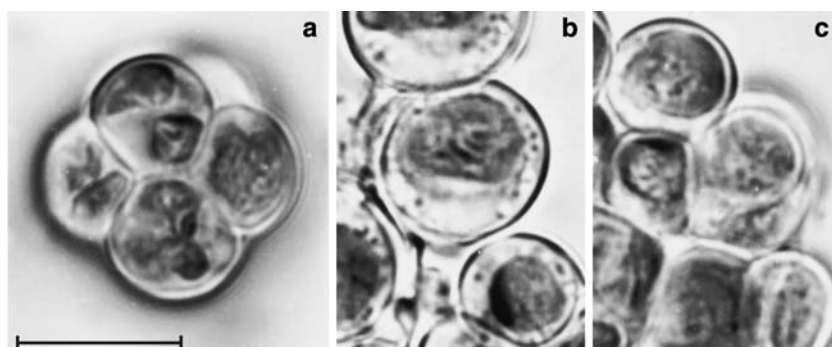


Fig. 3. a–c. *Hemichloris polyspora*. Autosporangium; autospores show internal structure of chloroplasts (only in b with sharp delimitation). Photomicrograph, living material from cultures. Scale bar $10\ \mu\text{m}$

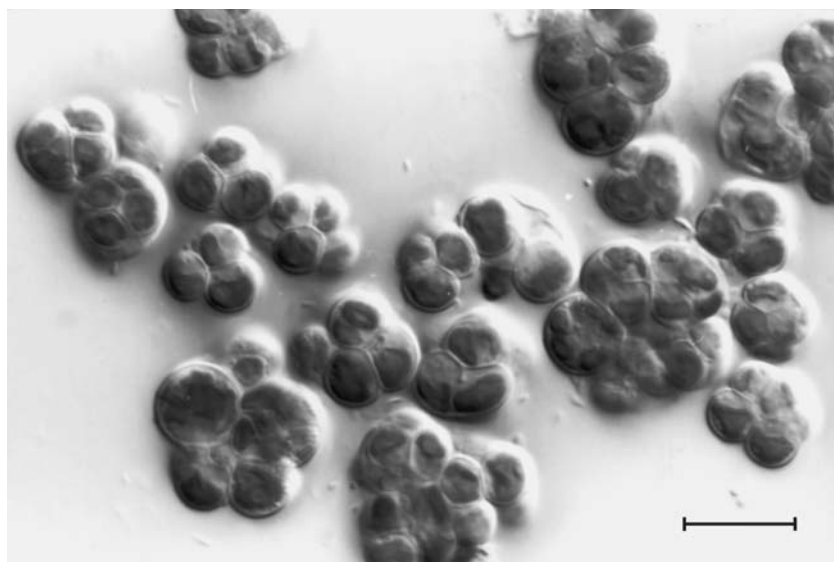


Fig. 4. *Hemichloris polyspora*. Cells grown in culture, groups of autosporangia.- Photomicrograph (DIK), living material grown in cultures. Scale bar 10 μm



Fig. 5. *Hemichloris polyspora*. Part of a cell in an autosporangium, showing chloroplast with thylakoids tightly arranged at the periphery and more separated from each other in the interior; internal thylakoids surrounded by rows of plastoglobuli (arrows). TEM. Scale bar 200 nm



Fig. 6. *Hemichloris polyspora*. Part of an autospore in an autosporangium, near the outer surface of chloroplast a group of microtubule-like elements (arrow) in cross section and arranged in regular distance, possibly parallel to each other. TEM. Scale bar 200 nm

is much faster and spectacularly more intensive than in *H. antarctica*. In *H. antarctica* in the course of the former investigation rare cases of starch production had been observed (see Fig. 6a, b in Tschermak-Woess and Friedmann 1984). To induce it again by cultivation on different media and under different light conditions gave no positive results likewise in *H. polyspora* also production of starch was observed only exceptionally; its shape was exceptional too: when reaching appreciable dimensions it turned out to consist of angular particles (Fig. 2m). The cell wall in both species contains a cellulosic component. This can not be shown by reaction of living cells with chlorzinc-iodine, but is displayed by cell

walls which are disrupted or more homogeneously by material that has been fixed in alcohol-acetic acid. Chlorzinc-iodine causes the cell wall to swell spectacularly in *H. polyspora*, whilst in *H. antarctica* it does not so distinctly. Whether under natural conditions in *H. polyspora* a gelatinous sheath in the way of *H. antarctica* may be produced, could not be checked.

TEM observations. Fine structure in both species is in general agreement, but its details are more conspicuous in *H. polyspora* than in *H. antarctica* mainly concerning the chloroplasts. In *H. polyspora* their more deep green colour in LM comes from the tight package of thylakoids all over their periphery; even the



Fig. 7. *Hemichloris polyspora*. Part of a cell with dividing chloroplast; on both sides of the indentation an assemblage of microtubule-like elements which are arranged criss-cross (arrows). TEM. Scale bar 200 nm

central area of pellucidity is traversed by stacks of thylakoids (Fig. 5). Grana are not present (on the structure of chloroplasts in general see Sarafis 1998). The most spectacular structure is represented by mostly linear assemblages of plastoglobuli; these are arranged along the surface of tubular-inflated thylakoids and apparently are attached to the latter. Clearly these assemblages represent the basis of the corpuscles and lines visible by light microscope. Plastoglobuli positioned free in the matrix of chloroplasts very probably do not exist. Often one can conclude that above and below a plastoglobuli surface a tubule goes on which is not met by the plane of section.

A structure found only rarely in *H. polyspora* is an assemblage of microtubule-like

elements in dividing chloroplasts. They are located near the indentation; at the outside of the chloroplast in this region regularly a mitochondrion (or part of a mitochondrion) can be found (Figs. 6, 7). This structures probably take part in the cell division process. They need further investigation, e. g., comparison with the striated microtubule-like structures in chloroplasts of *Oedogonium*, of *Chara* and of higher plants (e.g. Pickett-Heaps 1968, Oross and Possingham 1991). In *H. antarctica*-chloroplasts the thylakoids are less densely arranged, giving them a slightly light-green colour and often stacks of two or three pass through the total length of a cross section (Figs. 8, 9). Assemblages of plastoglobuli in general are smaller and less frequent than in

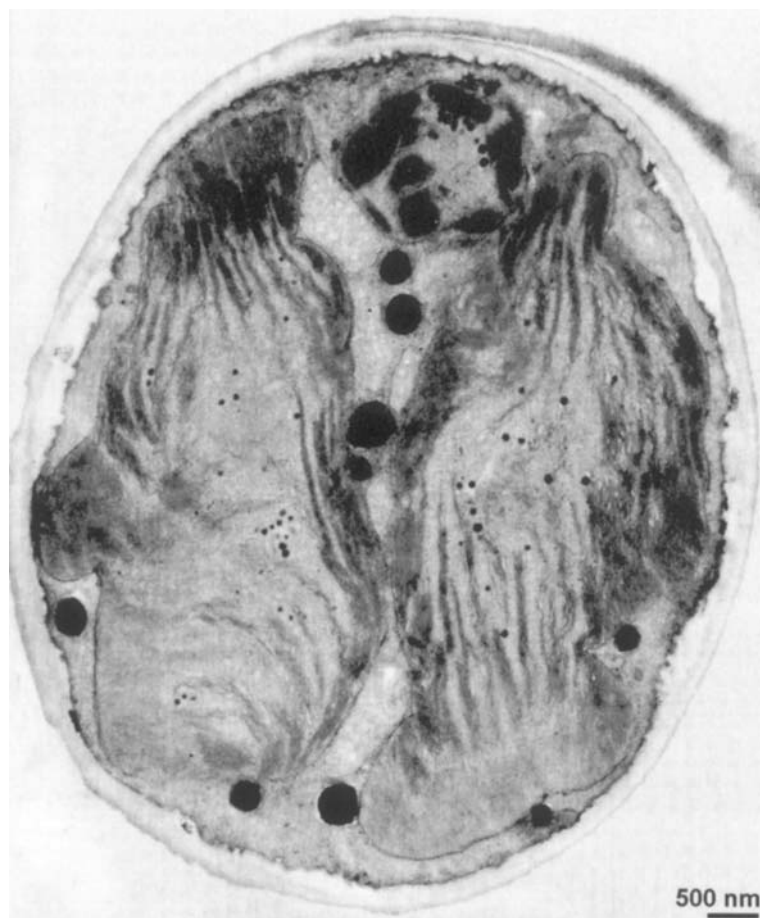


Fig. 8. *Hemichloris antarctica*. Autospore in an autosporangium with 2 chloroplasts showing internal area of pellucidity; plastoglobuli in low number, partially arranged along thylakoids. TEM. Scale bar 200 nm

H. polyspora. The arrangement on tubular inflated thylakoids agrees with that in *H. polyspora*, but the length of these formations as well as the number of plastoglobuli participating in general is low. Our observations support the view that plastoglobuli represent a stock of material which under certain circumstances takes part in the composition of thylakoids and which is set free in relative high amount during senescence (compare Lichtenthaler 1970).

Discussion

The two *Hemichloris* species reported until now show the peculiarity of growing high up in the mountains of Antarctica and within a certain

type of rock, that is Beacon Sand stone. They inhabit a certain layer of this rock (compare description and discussion in Tschermak-Woess and Friedmann 1984) and have in common the construction of trophic cells and the mode of reproduction – only by autospores. The designation *Hemichloris* is well adapted to the species *H. antarctica* found at first, but not to *H. polyspora* in which the chloroplasts quite in general fill more than half of the cell lumen. Another difference concerns the mode of reproduction: in general only two autospores in *H. antarctica*, universally more in *H. polyspora* (the etymology of the species name refers to this fact), and *Borodinella*-stages much more frequent in the latter. In addition the genus diagnosis of *Hemichloris* was to modify as it is

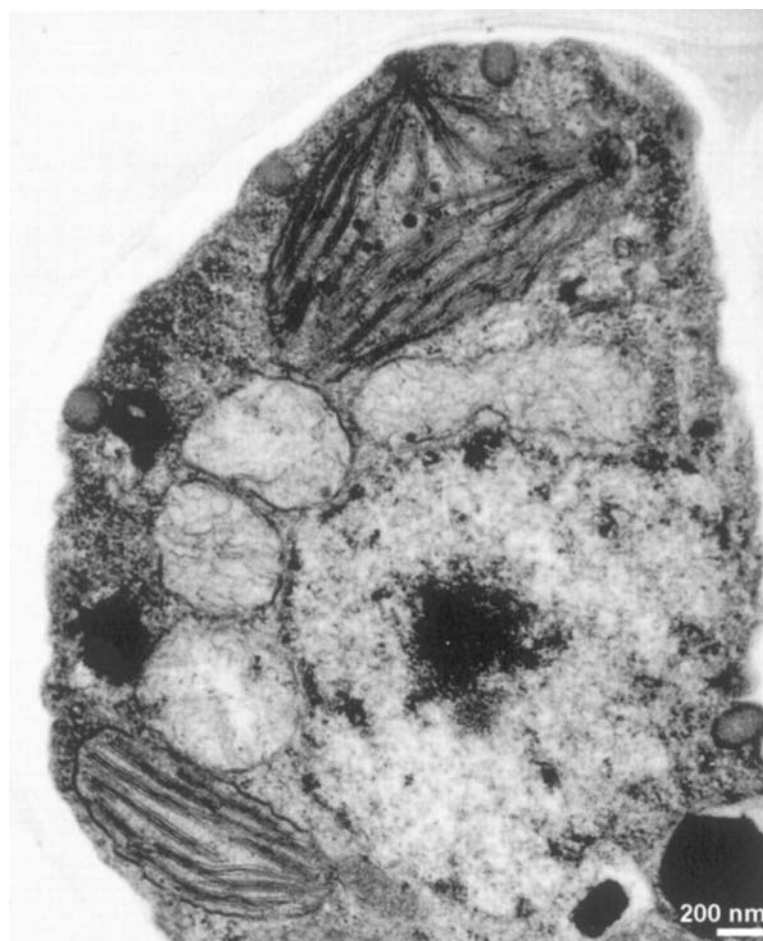


Fig. 9. *Hemichloris antarctica*. Autospore showing lobed chloroplasts, with low numbers of plastoglobuli and regular stacks of two thylakoids. TEM. Scale bar 200 m

shown below. Characters of cultures are conspicuously different between the two taxa and so is the speed of development (relative high in *H. polyspora* against low in *H. antarctica*). So the data reported justify separating the new strain as a species of its own.

A peculiarity of both species is the presence of an internal structure of the chloroplasts which can be realized already by light microscope; details of these structures, which go back to assemblages of plastoglobuli around and along tubular inflations of membranes, however, can be revealed only by TEM studies. In adjoining parts of chloroplasts the latter feature is joined by two membranes giving typical thylakoids. Plastoglobuli have been shown to

occur in many higher plants, but mostly are said to be positioned free, even to “swim” in the chloroplast stroma (Berkaloff et al. 1990). Lipid droplets corresponding to plastoglobuli are the pyrenoglobuli found in the pyrenoids of many algae (comprehensive description concerning the genus *Trebouxia* by Friedl 1989). Within pyrenoids they are arranged in a species-specific way along thylakoids which enter the pyrenoid matrix and are characteristically transformed- in most cases inflated. So it may be assumed that plastoglobuli too – and not only in *Hemichloris* - regularly adhere to membranes. In case this assumption turns not to be correct, as a next step a functional interpretation may be considered.

Modified diagnosis of the genus *Hemichloris*

Hemichloris Tschermak-Woess et Friedmann, gen. nov. ...

Chloroplasti bini, cellulam ad dimidium vel paulum plus complentes.

(The original diagnosis in: Tschermak-Woess and Friedmann 1984, p. 453)

Comments and acknowledgements

Emer. Prof. E. Tschermak-Woess passed away on April 26th, 2001. She carried out scientific research in an undiminished manner up to her last year of life. She published during her lifetime 108 papers, the last one in *Pl. Syst. Evol.* 225, 214–218 (2000). (An obituary with references to her publication record was published by M. Hesse in *Verh. Zool. Bot. Ges. Österreich* 138: 275–278, 2001). After the decease of Prof. E. T.-W. the present manuscript was found in a widely completed version, because E. T.-W. had devoted her mind and her forces to the subject of the rare cryptoendolithic Antarctic algae almost to her final days. The additional authors felt their duty to complete and to publish it as her 109th paper. They are deeply indebted to emer. Prof. Dr. E. I. Friedmann not only for extensive discussions with the late emer. Prof. Dr. E. Tschermak-Woess to prepare an earlier manuscript version but also encouraging during his Viennese stay in December 2002 to finish the manuscript. One of the authors (G.G.) reinvestigated the strains of *Hemichloris polyspora* which were deposited in the Culture Collection of Algae at the Botanical Institute of the University at Innsbruck and remembered vividly the intense discussions with Prof. E. T.-W. based on her very careful examinations with the microscope, as it is documented here in her last paper.

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