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New and known taxa of *Chlorella* (*Chlorophyceae*): occurrence as lichen phycobionts and observations on living dictyosomes

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Key words: Lichens, fungi, *Ascomycetes, Deuteromycetes, Pseudocyphellaria, Woessia; Chlorophyceae, Chlorococcales* (s. l.), *Chlorella sphaerica,* spec. nova, *Chl. saccharophila* var. *ellipsoidea.* – Characters of phycobionts in situ and in culture, dictyosomes forming double platelets, fungal haustoria. – Lichen flora of New Zealand.

Abstract: The algal partner of the lichen *Pseudocyphellaria carpoloma* is a new *Chlorella* species, *Chl. sphaerica.* It has a saucer- or band-shaped parietal to subparietal chloroplast with a spherical pyrenoid surrounded by a shell of starch. In some of the fully grown cells two dictyosomes lying parallel to each other and changing their position can be observed in the living state. Reproduction occurs by up to 16 autospores. 8 other *Pseudocyphellaria* species are also lichenized with *Chlorella* phycobionts, apparently belonging to *Chl. sphaerica.*

The phycobiont of *Woessia fusarioides* belongs to *Chlorella saccharophila* var. *ellipsoidea,* and to a strain which is morphologically almost identical to one formerly isolated from the lichen *Trapelia coarctata.* Its ability to gather granules of india ink on the surface of young cells is one of the remarkable characters differentiating it from the latter. In the lichen thallus its cells are regularly penetrated by fungal haustoria.

As is well known, many species of free-living *Chlorella* are wide-spread, and some of them thrive under rather adverse conditions, e.g. in distilled water or under conditions of high salinity, acidity and temperature, or in the presence of toxic heavy metals (KESSLER 1980, 1985, 1986). *Chlorella* also participates in many symbioses with heterotroph organisms from the animal kingdom, but up to now only rarely has been found as a lichen phycobiont (cf. TSCHERMAK-WOESS, in press). This may in part be due to the fact that in some cases the characters of *Chlorella* are changed by lichenization, so that the generic position is obscured and only revealed by careful isolation and cultivation, as shown for the phycobiont of *Woessia fusarioides.* In other cases, to be reported here, phycobionts can be easily recognized in situ as members of the genus *Chlorella,* as e.g. the algal partners of several *Pseudocyphellaria* species[1]. From one of them, *Ps. carpoloma,* the *Chlorella*

[1] Based on the present author's verbal communication GALLOWAY (1985) mentions the phycobionts of *Pseudocyphellaria* to the partly "*Chlorella*-like".

phycobiont was isolated and studied during prolonged cultivation. It differs in several characters from the species of this genus known until now and is remarkable by the fact that under favourable conditions the dictyosomes can be observed in the living state and that the latter are arranged as double platelets.

Material and methods

The lichens investigated with respect to their *Chlorella* phycobionts with the exception of specimen 3 and 4 of *Pseudocyphellaria coriacea* and *Woessia* were collected by J. K. BARTLETT and revised or determined by D. J. GALLOWAY. They had the following provenances: ***Pseudocyphellaria carpoloma*** **(DELISE) VAINIO**, (1) specimen on *Rhopalostylus sapida*, Church Rd. Bush, Kaitaia, New Zealand (N.Z.), 800 feet, 35° 10′ S, 26. 10. 1981; (2) on *Rhopalostylus*, Mt Auckland near Glorit, N.Z., 700 feet, 36° 42′ S, 2. 10. 1982, herb. BARTLETT no. 19721; (3) on *Rhopalostylus sapida*, Waweira Scenic Reserve, N.Z., 17. 3. 1984; (4) on *Metrosideros excelsa*, Hatfields Beach, N.Z., sea level, 36° 50′ S, 30. 10. 1984; (5) in dense bush, Waitakere Range, Auckland, N.Z., 800 feet, 23. 9. 1982, herb. Bartlett 19570. ***Ps. chloroleuca*** **(T. D. HOOK. & TAYLOR) DU RIETZ**, on *Rhopalostylus*, near Glorit, Mt Auckland, 700 feet, 36° 42′ S, 2. 10. 1982, herb. BARTLETT 19720. ***Ps. coriacea*** **(J. D. HOOK. & TAYLOR) D. GALLOWAY & P. JAMES**, (1) on *Rhopalostylus sapida*, Church Rd. Bush, Kaitaia, N.Z., 800 feet, 35° 10′ S, 26. 10. 1981; (2) on *Rhopalostylus sapida*, Waweira Scenic Reserve, N.Z., 17. 3. 1984; (3) on *Beilschmiedia tarairi*, inland Taipa, Northland, N.Z., c. 80 m, grid ref. O 04/565 868, leg. det. B. W. HAYWARD 1. 3. 1987; (4) on *Podocarpus dacrydioides*, Orongoronga V., Wellington, N.Z., grid ref. R 27/757833, leg. det. B. W. HAYWARD 11. 4. 1987. ***Ps. episticta*** **(NYL.) VAINIO**, on *Rhopalostylus sapida*, Waweira Scenic Reserve, N.Z., 17. 3. 1984. ***Ps. lividofusca*** **(KREMPELH.) D. GALLOWAY & P. JAMES**, Waitakere Range, Auckland, N.Z., 800 feet, 36° 55′ S, 23. 9. 1982, herb. BARTLETT 19569. ***Ps. montagnei*** **(CHURCH. BAB.) D. GALLOWAY & P. JAMES**, (1) on *Rhopalostylus sapida*, Church Rd. Bush, Kaitaia, N.Z., 800 feet, 35° 10′ S, 26. 10. 1981; (2) on *Rhopalostylus*, Puketi State Forest, N.Z., 35° 14′ S, 173° 14′ E, 8. 9. 1982, herb. BARTLETT 19381; (3) on *Rhopalostylus sapida*, Waweira Scenic Reserve, N.Z., 17. 3. 1984. ***Ps. pickeringi*** **(TUCK.) D. GALLOWAY**, (1) on *Rhopalostylus sapida*, Church Rd. Bush, Kaitaia, N.Z., 800 feet, 35° 10′ S, 26. 10. 1981; (2) Waitakere Range, Auckland, N.Z., 800 feet, 36° 55′ S, 23. 9. 1982, herb. BARTLETT 19571; (3) on *Rhopalostylus*, Mt Auckland, N.Z., 700 feet, 36° 42′ S, 2. 10. 1982, herb. BARTLETT 19722. ***Ps. subvariabilis*** **(NYL.) VAINIO**, Waitakere Range, Auckland, N.Z., 800 feet, 23. 9. 1982, herb. BARTLETT 19572. ***Ps. wilkinsii*** **D. GALLOWAY**, on *Rhopalostylus sapida*, Church Rd. Bush, Kaitaia, N.Z., 800 feet, 35° 10′ S. – ***Woessia fusarioides*** **D. HAWKSW., POELT & TSCH.-WOESS**, on trunc of *Quercus*, near Bad Tatzmannsdorf, Burgenland, Austria, 350 m, isotypus, leg. J. POELT 26. 3. 1980 (comp. HAWKSWORTH and POELT 1987).

Voucher specimens of all lichens listed above are deposited in WU, those for which a number in herb. BARTLETT is given, are dupla. BARTLETT's herbarium lately has been included in AK (Auckland Museum).

From the phycobionts of *Ps. carpoloma*, thallus 4, and from *Woessia fusarioides* clone cultures were established on Bristol- and 3 NBBM-agar by the method described by TSCHERMAK-WOESS (1981). They were kept in climatic chambers (one 10–12 °, the other 20–22 °C, at a light regime of 16 hours light, 8 hours dark) and in northern windows. From *Ps. carpoloma*, thallus 3, at first microscopic cuttings were put on agar for some weeks and later controlled parts of these were used for isolation of clones. Tiny granular parts of the *Woessia* thallus were kept as a living reserve on agar for some weeks too. In all lichens mentioned the phycobionts were investigated in the living state in situ. After herbarization they cannot be identified. Morphology and development were studied in detail in the clone cultures, also mainly in the living state. Besides, some conventional reactions mentioned below were applied. The haustoria of *Woessia* could be well documented by the old method

of GEITLER (1934) and his school by making burst algal cells freshly isolated from the lichen thallus and by colouring their walls by zinc chloride-jodine.

Herbarized voucher specimens of *Chl. sphaerica* (clone 1 from thallus 3) and of the two strains (*Woessia* phycobiont and *Trapelia* phycobiont) of *Chl. saccharophila* var. *ellipsoidea* are in WU and W. Cultures of *Chl. sphaerica* (clone 2, thallus 3) are deposited in IB, SAG and UTEX; *Chl. saccharophila* var. *ellipsoidea* isolated from *Woessia fusarioides* (clone 2) was sent to SAG, the latter and also the strain from *Treapelia coarctata* to IB.

Results

Microscopic cuttings of the thalli of ***Pseudocyphellaria*** *carpoloma* show that its **phycobiont** belongs to *Chlorella* (Fig. 1). In all thalli checked differential characters were identical and did not correspond with those of known species. Therefore, several clones were isolated from thallus 3 and 4, and 3 clones were kept for close investigation (2 from thallus 3, one from 4). No differences have been observed between the three clones.

Development was good at 20–22 °C, whereas at 10–12 °C during the first passages it was impaired (pale green, relative small chloroplast, rate of division low). During later passages, also at 10–12 °C, good growth was achieved. The typical cell form is spherical (diam. 3–9.5 µm), occasionally it can be slightly ellipsoidal, especially in just dividing and young cells. The percentage of ellipsoidal cells in the thalli is somewhat higher than in culture. This may be due to crowded conditions within the lichen. If not mentioned specifically, the following characters of cells refer to cultures.

The chloroplast is thickly saucer- or band-shaped (Fig. 2*a–d*). In fully grown cells (occasionally also in smaller ones) it may also be slightly and irregularly lobed (Fig. 2 *e, k*) or sometimes girdle-shaped (Fig. 2 *l*). Its position is subparietal to parietal. Under good growing conditions it fills about half of the cell volume, rarely more. The spherical, sometimes a little flattened pyrenoid is nearly always surrounded by a shell of starch, the contours of which are concentric with the contour of the pyrenoid proper and are not bulging as in other cases. The shell of starch mostly appears multipartite, occasionally bipartite (Fig. 2). In the stroma flat grains of starch can also be encountered. The small nucleus is regularly located eccentrically, but not in a fixed position. Tiny droplets, probably lipids, occurring particularly in old cultures, are distributed mainly in the periphery.

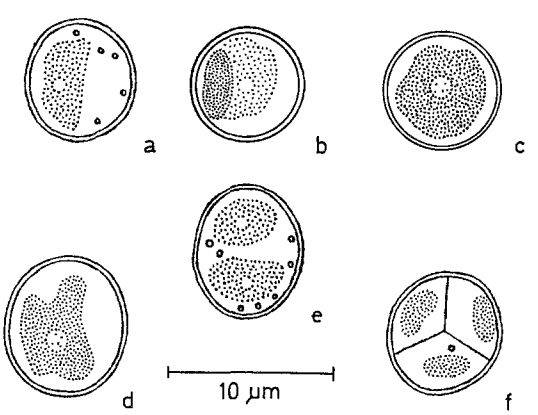

Fig. 1. *Chlorella sphaerica*, directly from living thallus (no. 3) of *Pseudocyphellaria carpoloma* (sheath of starch around pyenoids not drawn)

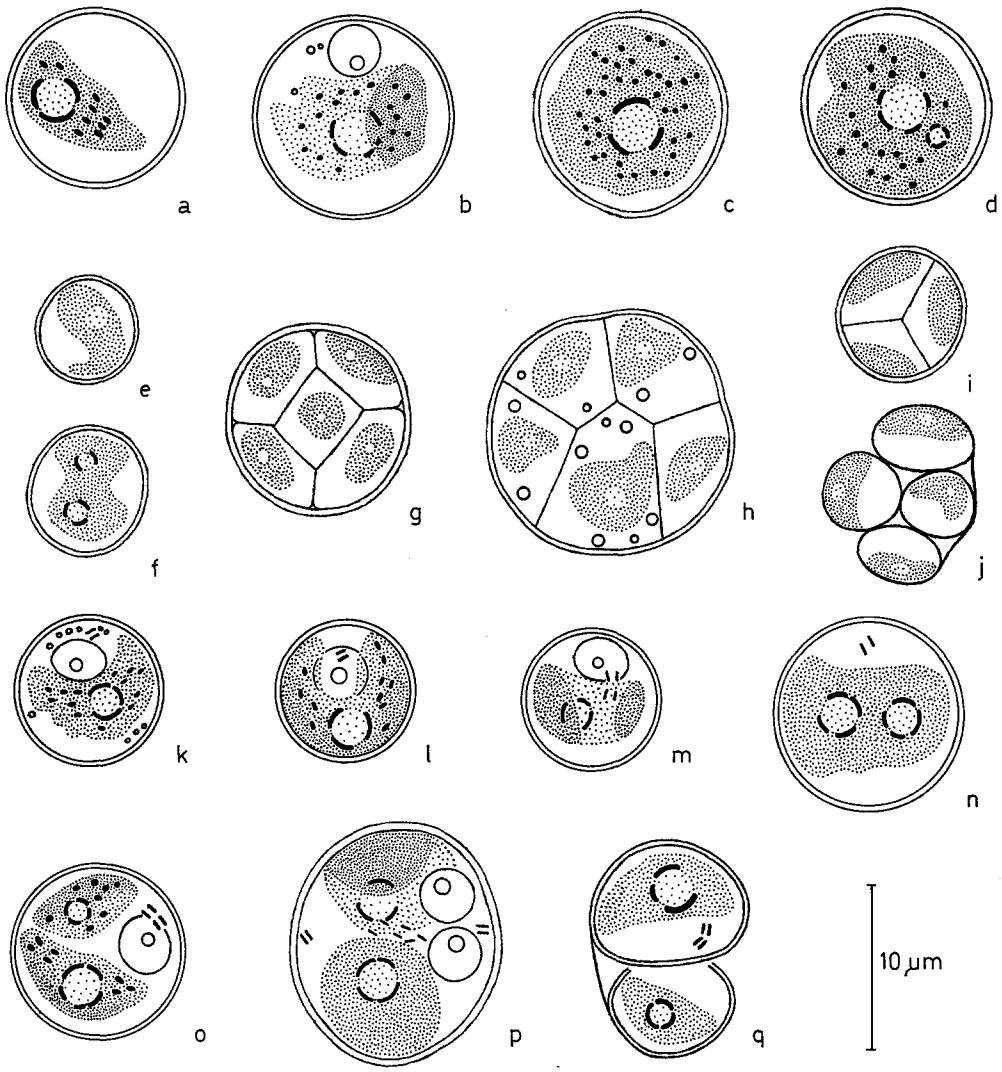

Fig. 2. *Chlorella sphaerica*, living cells from clone cultures with saucer-shaped (*a, c, d, k, o–q*), band-shaped (*b, m, n*) and girdle-shaped (*l*) chloroplasts; reproduction of pyrenoids before division of chloroplasts (*d, f, n*); dictyosomes in the form of double platelets (*k, l, n*) and 4 platelets after duplication (*m, o, q*), in *p* besides two pairs of dictyosomes a group of single dictyosomes near the future septum. – Starch drawn only in part of the cells; all bright field

In some of the fully (or nearly fully) grown cells of relatively good growing young cultures, in which not many droplets mask the cell contents, two **dictyosomes** can be discerned in the living state (Figs. 2 *k, l, n* and 3 *a, b*)[1]. They lie regularly at small distances from each other and are oriented in a parallel fashion, with their

[1] It must be stressed that it is difficult to observe the dictyosomes. They were first discerned by bright field microscopy. Later it turned out that by DIC microscopy (with best optics) they can be shown more distinctly. Because of difficulties with fixation preliminary investigation by electron microscopy did not produce good results, but it was shown unequivocally that they are dictyosomes.

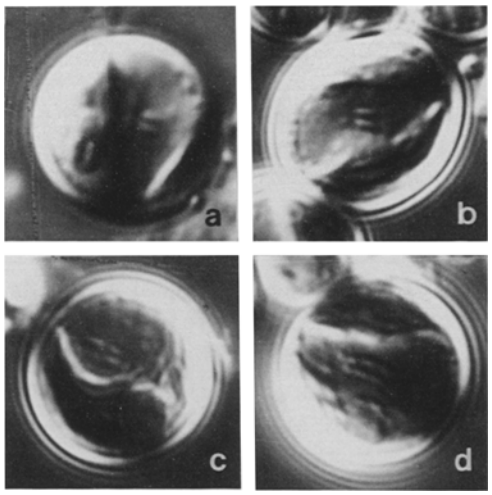

Fig. 3. *Chlorella sphaerica*, cells with dictyosomes. *a, b* dictyosomes forming double platelets; *c, d* dictyosomes after their reproduction (the cell in *c* has one girdle-shaped chloroplast, that in *d* has two chloroplasts similar to Fig. 2 *o*). – Living, from clone cultures, DIC, × 3 300

flat sides facing each other, which means that they form double platelets. There is a relation between them and the nucleus in the way that they always lie at a distance from the latter and mostly obliquely to its envelope. They can be discovered only when seen in profile, and in spite of their small size (diam. c. 0.6–0.8 µm) one can follow their extension over different levels by careful focussing. As they usually turn around and slightly change their position one sees them disappear (while they turn their flat sides towards the observer) and reappear in a new position. Apparently the parallel position is retained during the process. The difference in light-diffraction between cytoplasm and dictoyosomes is low and equal to that of the dictyosomes of diatoms and of algae of the genus *Dictyochloropsis* (Gschöpf 1952, Tschermak-Woess 1980, 1984). Colour reactions as e.g. recommended for the larger dictyosomes of *Micrasterias* (Drawert & Mix 1961/62) did not give positive results, probably because the dictyosomes in *Chlorella* are much smaller.

In the course of cytokinesis the dictyosomes are often the first organelles that duplicate (Figs. 2 *m, q* and 3 *c*). After (or sometimes before) that a second pyrenoid is formed de novo at the side of the old one or a little farther off from it (Fig. 2 *n*); dumb-bell shaped pyrenoids, indicating cleavage never occur. Then the division of the chloroplast comes about (Fig. 2 *o*), and at last the nucleus divides. From a single observation one can conclude that in the meantime the dictyosomes have further multiplied and have in part become distributed separately on both sides of the future septum (Fig. 2 *p*).

As observed repeatedly, the **division processes** leading to the formation of (2) 4, 8 or 16 autospores are strictly successive. Often one of 8 or 16 autospores is larger than the others (Fig. 2 *h*). In daughter cells still kept together by the wall of the mother cell occasionally new autospores may already form. During growth of the autospores the sporangial wall is extended and probably dissolves at last. Empty sporangial walls, characteristic of other *Chlorella* species, in general are not found in the preparations. Cell walls intensively react with ruthenium red and turn violet with cinc chloride-iodine. Preparations in diluted india ink do not show the existence of mucilaginous envelopes. Attachment of particles of india ink to the cell wall, as observed in the phycobiont of *Woessia*, do not occur.

Old cultures change their colour into yellow-orange, and thus demonstrate the

production of secondary carotinoids, but only rarely one finds droplets in the cytoplasm which are large enough to show their orange colour. Usually droplets are too small to detect their putative colour.

About $1^1/_2$ weeks after collection of the thalli the *Chlorella* species in *Ps. carpoloma* and the other *Pseudocyphellaria* species mentioned below still appeared to thrive well. It produced autospores and consisted of cells similar in dimensions to those in the cultures; only their chloroplasts in general were thicker and more diversified than those in cultured cells, and they filled a greater part of the cell volume. The pyrenoids were regularly surrounded by a thin shell of starch. In the chloroplast stroma grains of starch were present only sporadically. Flattened or even irregularly formed pyrenoids occurred within the thalli more often than in the cultured phycobiont. In the lichens the algal cells are not very tightly encircled by fungal hyphae and no intracellular haustoria are present, but it could not be checked, whether at times intraparietal haustoria occur.

In view of the observation of dictyosomes by light microscopy in the above described *Chlorella* species it was tried to find these organelles in other species and strains of *Chlorella* as well. This gave positive results in the *Chlorella* strain 211-9 b from the SAG collection. In this case two dictyosomes are also present in vegetative cells; they can be found in some of the larger cells [1]. They are also located in a certain distance from the nucleus and near each other, but they do not lie parallel (Fig. 4). Slight changes in position, as mentioned above, occur as well.

At last the taxonomic position of the *Chlorella* phycobiont of *Ps. carpoloma* must be discussed. It exhibits certain similarities in the form of the chloroplast with *Chl. saccharophila* var. *ellipsoidea* (GERNECK) FOTT & NOVÁKOVÁ (= *Chl. saccharophila* GERNECK). However, in addition to cell form the pyrenoid characters are quite different: spherical vs. often flattened and with vs. without shell of starch. The position of the dictyosomes differs too (as far as seen and as far as 211-9 b actually belongs to *Chl. saccharophila*). From other *Chlorella* species with spherical cells the present species differs by the form, position and relative dimensions of the chloroplast, by characteristics of the pyrenoid, the lack of vacuoles, the behaviour of the cell wall of autospore mother cells and other characters (cf. FOTT & NOVÁKOVÁ 1969, ANDREEVA 1975). It is therefore thought to be **a new species.** Formal description under the designation ***Chlorella sphaerica*** follows on p. 136.

In *Pseudocyphellaria chloroleuca, Ps. coriacea, Ps. episticta, Ps. lividofusca, Ps. montagnei, Ps. pickeringi, Ps. subvariabilis* and *Ps. wilkinsii* the phycobiont was investigated only in situ, but clearly belongs to *Chl. sphaerica.* As example, Fig. 5 shows the phycobiont of *Ps. coriacea* as observed in the lichen thallus.

The green **phycobiont of *Woessia fusarioides*** on the basis of its characters could not be classified safely in situ, even with respect to genus. Usually it has broadly

[1] In some cells only one dictyosome was seen during prolonged observation. It is possible that a second one was present which permanently turned its flat side towards the observer. Only investigation of serial sections by TEM could solve this problem. Besides, it may be mentioned that the strain 211-9 b in the SAG-lists is given as *Chlorella saccharophila.* This species is characterized by the possession of a pyrenoid. In strain 211-9 b the present author could not detect a pyrenoid by light microscopy. DEASON & FLOYD (1987), and other authors quoted by them, think that environmental factors may determine presence or absence of pyrenoids. The present author found pyrenoid characters in all algae investigated to be very stable.

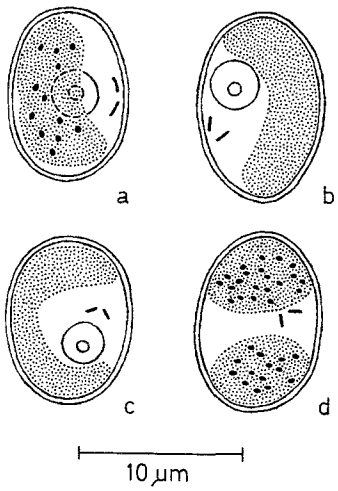

Fig. 4. *Chlorella "saccharophila"* strain SAG 211-9 b; dictyosomes near each other, but not forming double platelets. – Living, bright field

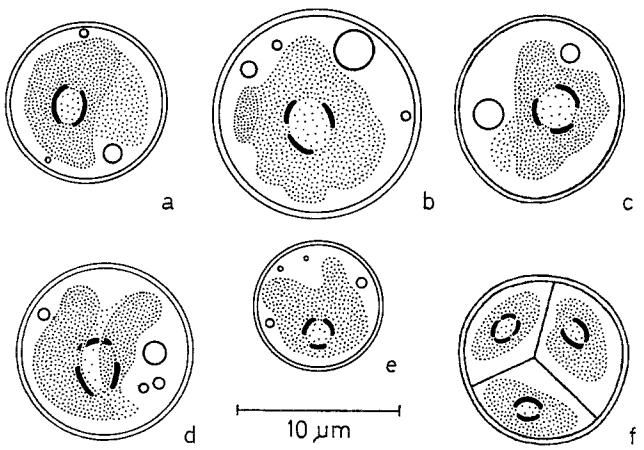

Fig. 5. *Chlorella sphaerica,* cells directly from living thallus (no. 2) of *Pseudocyphellaria coriacea*

ellipsoidal, sometimes also spherical cells with diameters from 4.5 × 6.5 to 16 × 17, 19 µm (Fig. 6 *a–f*) which are tightly encircled by fungal hyphae. The chloroplasts (one per cell) nearly extend over the whole interior, especially in relatively large cells (Fig. 6 *a, b*). They are massive, irregularly lobed, and contain one, occasionally two pyrenoids without a shell of starch. Tiny grains of starch occur scattered in the stroma (not drawn in the figures). The pyrenoids are mostly oblong and located centrally or eccentrally. In small cells they often cannot be observed in the living state and even after treatment with Lugol's solution they remain rather indistinct. This also refers to the cultivated material discussed below. In the living state one to five fungal haustoria can be found in many algal cells (Fig. 6 *a, b*). The health of the alga appears not to be drastically impaired by penetration of haustoria. In preparations in which the algal cells have been isolated and in which their protoplasts have been extruded, the haustoria are observed regularly after coloration with zinc chloride-jodine (Fig. 6 *c–f*); their number may reach up to 7 and only sporadically small (young) cells have none as they probably have not yet been

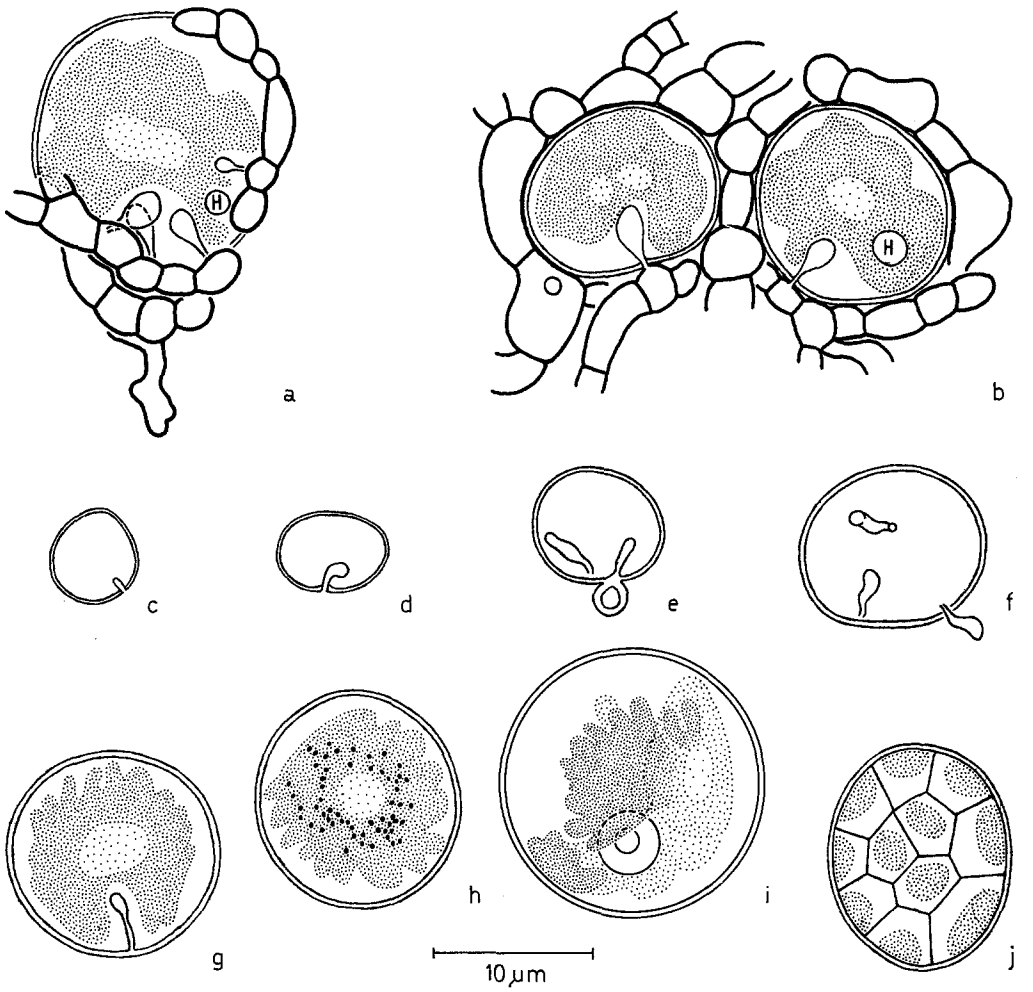

Fig. 6. *a–f Woessia fusarioides. a, b* Parts of the lichen (H haustoria in cross section), *c–f* algal cells with fungal haustoria (after coloration with zinc chloride-jodine, protoplasts squeezed out, in *f* one haustorium inverted), *g–j* algal cells after 17 days of culture. – *a, b, g–j* Living specimens

attacked. A reaction of the algal cells against the haustoria by covering them eventually with cellulosic wall material, as found in certain other lichens (HONEGGER 1986, TSCHERMAK 1941), was not observed. The haustoria are firmly fixed to the algal cell wall and occasionally are tilt over (Fig. 6*f*).

In thallus parts kept on agar for 17 days some algal cells started to divide and showed formation of 8 or 16 autospores. 1–4 of these in a polar or lateral position frequently were larger than the others, a character often found in *Chlorella* (Fig. 6*j*). Others were still undivided and partly showed chloroplasts of very extraordinary form (Fig. 6*i*), partly with irregular, narrow lobes and ribs (Fig. 6*g, h*). A subparietal location of the main body of the chloroplasts could often be observed (Fig. 6*i*).

As soon as visible growth had occurred in the clone cultures, maximal cell dimensions were reduced (e.g., 6×6.5, 5×7, 5×8, 7×8 µm) and the cell form became generally ellipsoidal. During the first passages the chloroplasts in some of

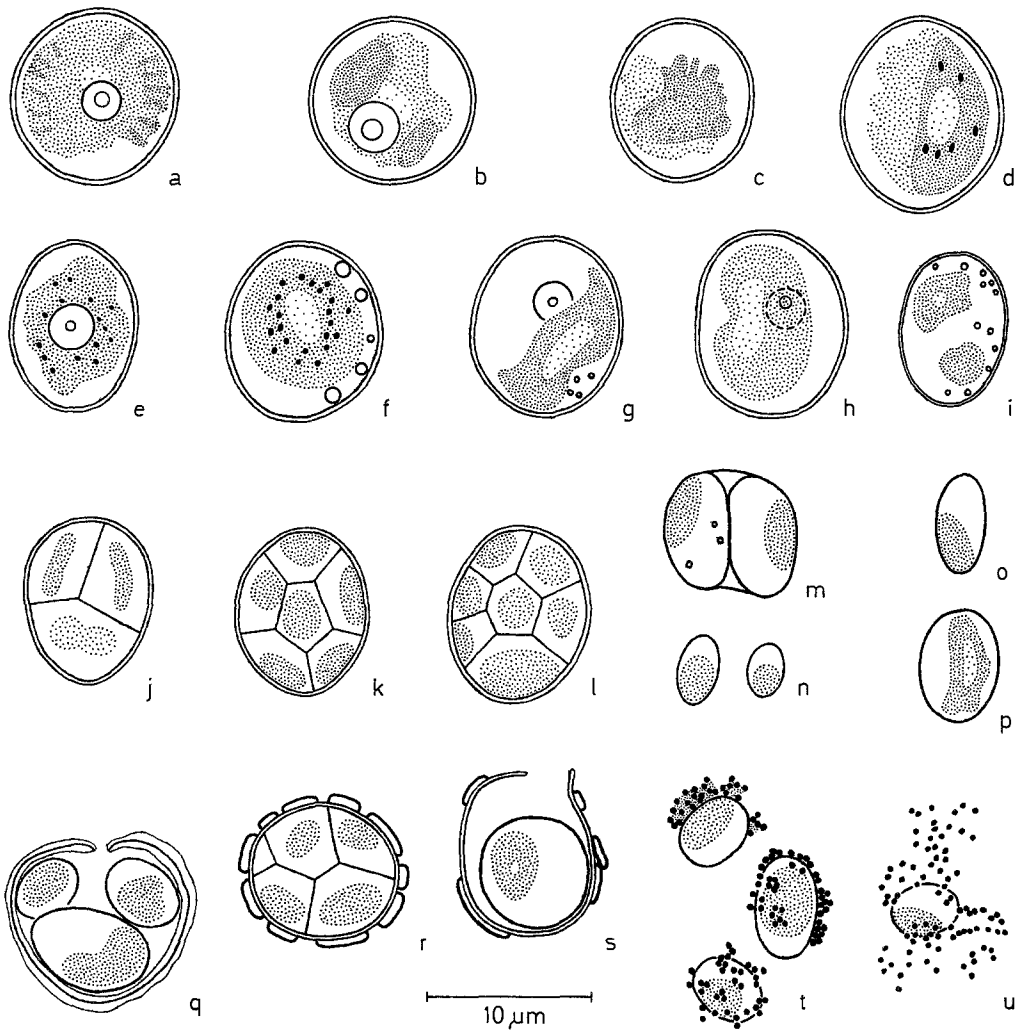

Fig. 7. *Chlorella saccharophila* var. *ellipsoidea*, strain of *Woessia fusarioides*, after some weeks (*a–c, g, h*) respectively 6 years of culture (remainder); *a–f* different forms of chloroplasts, starch forming no sheath around pyrenoid; *g, h* beginning, *i* accomplished first division of chloroplast, *h* also indentation of pyrenoid; *j* successive division during development of autospores (chloroplast in lower cell dividing); *k–m* autosporangia, (*k* with 8, *l* with 16 autospores); *n–p* young cells; *q* gradual dissolution of sporangial wall; *r, s* scales on sporangial walls; *t* particles of india ink adhering to the cell walls of young cells; *u* immobilized particles of india ink forming rows and irregular aggregations on the surface of young cell. – *a–f, i–u* Living; *g, h* in Lugol's solution; *q, t, u* in india ink (not adhering particles of india ink not shown)

the larger cells still displayed bizarre shapes (Fig. 7 *c*). In smaller cells, and more generally after prolonged cultivation, chloroplast form was simplified to saucer- or occasionally to band-shape (Fig. 7 *b, d–n*). However, even after 6 years of cultivation a certain tendency to form lobes and ribs can be noticed in relatively large chloroplasts (Fig. 7 *d*). The position of the chloroplasts is subparietal to parietal. Division, as in *Chlorella sphaerica*, is successive (Fig. 7 *j*), but in contrast, the py-

renoid always is without a shell of starch. It probably is reproduced during bipartition of the chloroplast after elongation and adoption of dumb-bells shaped contours (Fig. 7 *g, h*). The number of autospores varies between 2 and 16 and especially, but not exclusively in the window one from 8 or 16 is larger than the others (Fig. 7 *k–m*). Occasionally mother cells can be found, in which a difference in size can be observed already after the first division of the chloroplast (Fig. 7 *i*). The sporangial wall expands during growth of autospores, it also may be torn, and decomposes. By embedding in india ink, occasionally it can be shown that the decomposition comes about by transformation of an inner and an outer layer into mucilaginous material, while the middle layer is sharply delimitated (Fig. 7 *q*). In one culture (Bristol, 1 month old, window) scales were observed on the cell walls of sporangia which showed a stronger light-diffraction than the walls (Fig. 7 *r, s*). On some of the younger cells a thin uniform coating was seen apparently of the same material. Similar coatings and scales were observed in *Dictyochloropsis* species (GEITLER 1966; TSCHERMAK-WOESS 1980, 1984). In all cases they could not be stained. Probably they develop if a mucilaginous cover changes its consistency under gradually drier conditions. Old cultures change their colour to orange. Their cells contain orange droplets which lie free within the chloroplast or around the pyrenoid, or may develop a confluent cover around the pyrenoid, or may sporadically occur in the cytoplasm.

From the morphological characters of the alga (*Woessia* phycobiont = *W.* ph.) in culture one must conclude that it belongs to ***Chlorella saccharophila*** var. *ellipsoidea* (GERNECK) FOTT & NOVÁKOVÁ (= *Chlorella ellipsoidea* GERNECK) and to a strain which is near to that isolated from *Trapelia coarctata* (TURN. ex SM. & SOW.) CHOISY (TSCHERMAK-WOESS 1978 b; ***Trapelia* phycobiont** = *Tr.* ph.). Dimensions and length: breadth ratio of cells of the two strains are practically identical, as shown by comparison of two series of joint cultures on Bristol and 3 NBBM medium[1]. Nevertheless differences between the two strains do exist and can be observed when both are cultivated on the same agar plates over several passages and under different regimes of light, temperature and medium: one to three months old colonies of the *Tr.* ph. are regularly shiny, those of the *W.* ph. matt (in younger cultures the latter also appear shiny). This apparently depends on a stronger tendency of the *Tr.* ph. to produce mucilaginous covers round the cell walls or confluent assemblages of mucilage. Thus, in one month old culture of *Tr.* ph. the covers around small cells are about $^1/_2$ µm, around larger cells 1 µm thick; besides, confluent masses of mucus exist as shown by embedding in diluted india ink. The *W.* ph. exhibits no covers or confluent masses in its matt colonies, and only now and then thin covers in younger cultures. The *Tr.* ph. changes to the stationary phase growth earlier than the *W.* ph., as observed, e.g., in 40 d old cultures on 3 NBBM, climatic chamber 20–22 °C or on Bristol, window; both 3rd joint passage. In the *Tr.* ph., under these circumstances, there is a strong prevalence of small cells (diam. 3 × 3.5–

[1] In connection with reproduction by autospores cell-dimensions of *Chlorella* vary considerably as is well known. The comparison was restricted to cells with a length above 6 µm, that means to potential autospore mother cells; in all cases n was 20. In the Bristol samples: *Tr.* ph. mean length ± s. d. of cells 6.6 ± 0.7 µm, mean breadth 5.6 ± 0.9 µm, mean quotient 1.2 ± 0.1µm; *W.* ph. 7.0 ± 0.6 µm, 6.0 ± 0.8 µm, 1.1 ± 0.1. 3 NBBM samples: *Tr.* ph. 6.4 ± 0.5 µm, 5.3 ± 0.6 µm, 1.2 ± 0.1; *W.* ph. 6.8 ± 0.7 µm, 5.6 ± 0.7 µm, 1.2 ± 0.1; probability of identity in all cases 99%.

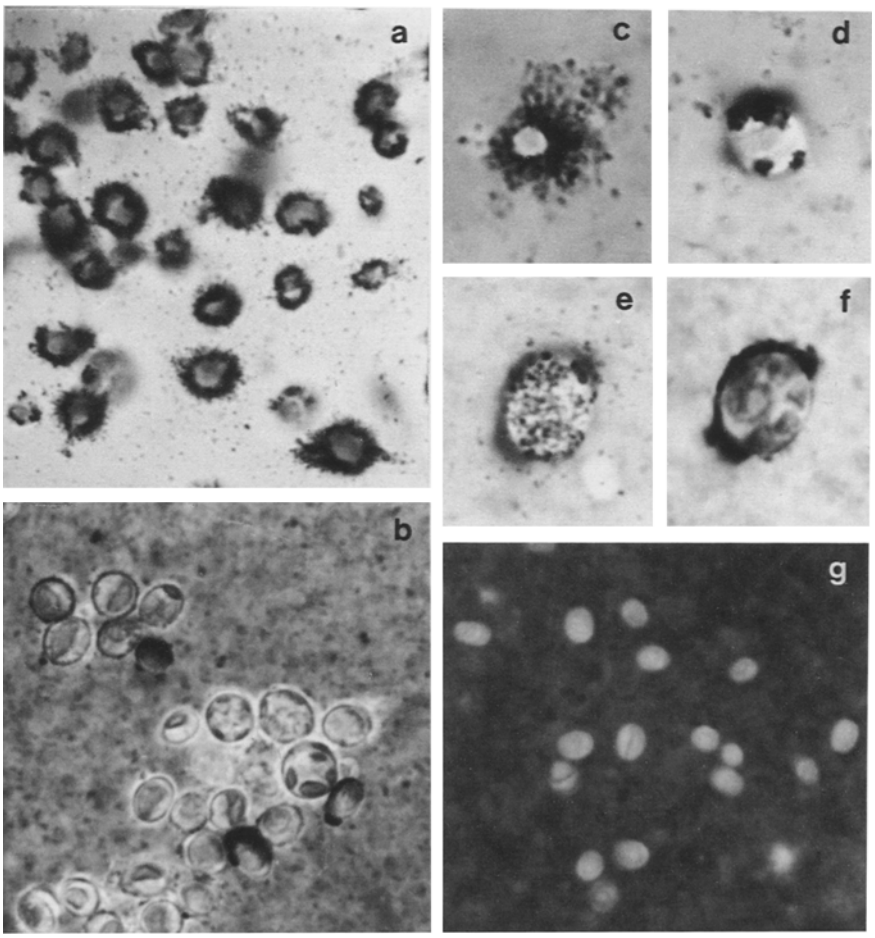

Fig. 8. *Chlorella saccharophila* var. *ellipsoidea*. Living cells after treatment with india ink; *a–f* of *Woessia* phycobiont, *g* of *Trapelia*; *a* high, *b* relatively low number of cells loaded with immobilized particles, *c* strong accumulation of grains which radiate in rows, *d* patchy arrangement of groups of granules, *e,f* autosporangium, *g* no accumulation of particles on cell surface. – *a,b,f,g* Optical section, *c–e* surface view; *a,b,g* × 1 090, *c–f* × 1 790

4 × 5 µm), forming now and then two autospores only, with singular large cells not producing autospores. In the *W.* ph. all categories of cell sizes are richly represented, and there is abundant formation of 4 or 8 autospores. In many cells of the *W.* ph., especially in small ones, the pyrenoids are indistinct, even after treatment with Lugol's solution, whilst in the *Tr.* ph. this treatment makes them very conspicuous.

The cell walls of the two strains also react differently to treatment with diffeent agents: *Tr.* ph. exhibitis no colour (c) with aniline blue, with ruthenium red and toluidine blue strong c (mucilaginous covers no c); *W.* ph. gives no c with aniline blue , with ruthenium red and toluidine blue weak c in vegetative cells, stronger c in sporangia. One remarkable difference concerns the behaviour in india ink. In the *Tr.* ph., kept under diverse conditions, immediately after preparation and also after prolonged observation, the particles of the india ink are evenly distributed and show the habitual motion in connection with molecular motion (Fig. 8 *g*). On the contrary in the *W.* ph., kept for 14–40 d on 3 NBBM- or Bristol-agar at 10–

12 °C or 20–22 °C or in the window during cold and warm periods, shortly after preparation, the particles gather on the walls of many small cells and form groups of few to many granules (Figs. 7 *t, u* and 8 *a–f*), but never cover the cell walls completely. In general, large cells and sporangia are free from granules. However, in older cultures (3–5 months under the above conditions), granules (mostly in low number) become attached mainly to the sporangia. The granules firmly adhere to small and large cells, usually in 1–3 layers, but sometimes radiating even farther (Fig. 8 *c*). In contrast to free particles, these attached particles do not show any molecular movements[1]. The cause for this peculiar reaction is not known. Mucilage production was not encountered in these cultures and neither colouring nor DIC investigations gave any evidence for differentiations in or on the cell walls that might be responsible for these aggregations.

Comparison of the *Woessia* phycobiont with four other strains of *Chl. saccharophila*, kept jointly under diverse conditions, gave the following results: CCAP 211-1 a length breadth ratio similar, but maximum cell dimensions larger, maximum number of autospores 32 vs. 16 in the *W.* ph; in CCAP 211-1 f, SAG 211-9 a, SAG 211-9 b the length breadth ratio is greater and the maximum numbers of autospores differ, in SAG 211-9 a with only 8, in SAG 211-9 b with 32. Only in large cells of SAG 211-9 b dictyosomes sometimes were observed (see above).

Discussion

The present communication demonstrates again that our knowledge with respect to the taxonomic position of phycobionts of lichens is still quite incomplete. This refers not only to more or less inconspicuous crustose lichens, as *Woessia fusarioides*, but also to foliose lichens which reach considerable dimensions and have been collected by many lichenologists, as *Pseudocyphellaria*, and very probably *Sticta*. Certainly, new algal species will be found in the future among the numerous not yet identified phycobionts.

Until now *Chlorella* phycobionts have only been reported in a few cases, and some of these reports are dubious (cf. TSCHERMAK-WOESS in press). The cases reported in this study clearly show that *Chlorella* is not so rare as an algal partner of lichens, and that further instances of its occurrence in the lichenized state can be anticipated.

It is remarkable that in *Chlorella sphaerica* (the phycobiont of several species of *Pseudocyphellaria*), a species with relatively small cells (diam. not exceeding 9.5 µm), two dictyosomes may be detected by light microscopy, at least under favourable conditions and in its largest cells. Formerly dictyosomes have been observed by light microscopy mainly in algae with relatively large cells, i.e., in diatoms (lit. see below) and in *Micrasterias* species (e.g., DRAWERT & MIX 1961/62, UEDA & NOGUCHI 1979). Still more noteworthy is the parallel position of these dictyosomes, as double platelets of this kind have been reported elsewhere only in

[1] The intensity of the reaction differs in different cultures with respect to relative number of cells affected and with respect to loading with particles. It comes about only when the material at first is prepared in tap water and when diluted india ink (about 1 : 8) is added under the cover glass afterwards. Replacement of the india ink by tap water does not change the adherence of the particles. When the cells are put directly into india ink the reaction fails even if subsequently more india ink is sucked through the preparation.

certain diatoms (GSCHÖPF 1952, JAROSCH 1962 b, DRUM 1966). The functional role of this arrangement is unknown. SCHNEPF & al. (1966) make the point that the position of dictyosomes is a character of taxonomical significance. The present findings and former observations on the dictyosomes of *Dictyochloropsis* underline this fact (TSCHERMAK-WOESS 1980, 1984). Whereas the dictyosomes regularly occur as a group lying between nucleus and chloroplast in *Dictyochloropsis,* their position in species of *Chlorella* is not uniform. BISALPUTRA & al. (1966) and ASHTON & al. (1966) found perinuclear dictyosomes (possibly also two per fully developed cell) in *Chl. vulgaris*, whereas in *Chl. sphaerica* and strain 211-9 b their position is of the disperse type. MURAKAMI & al. (1963) report the presence of one or two dictyosomes in *Chl. ellipsoidea* (strain not given) and place them in a disperse position in their schematic drawing. However, in the opinion of the present author, several of their electronmicroscopical micrographs rather suggest a perinuclear arrangement. The marine species *Chl. ovalis* has two perinuclear dictyosomes (RASCIO & al. 1980). The authors think them to originate from the nuclear envelope. According to ATKINSON & al. (1974) the location of the dictyosomes in *Chl. fusca* var. *vacuolata* 211-8p always is perinuclear (number not given, 3 in their Fig. 10). In *Chl. minutissima* only one perinuclear dictyosome is present (DEMPSEY & al. 1980). In *Dictyochloropsis* and *Asterochloris* the distribution of the dictyosomes during cell division changes from disperse to perinuclear (TSCHERMAK-WOESS 1980 a, b, 1984).

How dictyosomes reproduce is still a matter of controversy (see e.g., MORRÉ & al. 1971, WHALEY 1975). In the present case a number of observations suggest that this is achieved by bipartition perpendicular to the ± flat side. This corresponds with observations, e.g., of GSCHÖPF (1952) and KIERMAYER (1967, 1970, summarizing 1981). Dictyosomes are not static but slightly change their position, as shown before in *Asterochloris phycobiontica,* all species of *Dictyochloropsis* (TSCHERMAK-WOESS 1980 a and unpubl.), *Micrasterias rotata* (DRAWERT & MIX 1961/62) and in other plants (e.g., JAROSCH 1962 a).

In a number of *Chlorella* species several morphologically, physiologically and biochemically different strains are known (cf. KESSLER 1980, 1985, 1986, etc.). Therefore, it is not surprising that in *Woessia fusarioides* a strain of *Chl. saccharophila* var. *ellipsoidea* occurs which exhibits similarities and differences with a strain isolated formerly from *Trapelia coarctata.* One remarkable character of the *Woessia* strain is its trend to accumulate and immobilize grains of india ink on the cell surface after a certain regime of preparation. As no visible agent responsible for the attachment of these granules could be found, perhaps, electrostatic forces are involved. According to HUSS & al. (1987) *Chl. saccharophila* includes several groups of strains which differ in their DNA characters. Future investigations must show to which of them the *Woessia* phycobiont belongs.

The cells of *Chl. sphaerica,* when lichenized with *Pseudocyphellaria,* are not markedly enlarged compared to the free-living state in cultures. On the contrary, those of *Chl. saccharophila* var. *ellipsoidea* reach dimensions in the lichen thallus of *Woessia* which are not encountered in cultures (ratio of diameters of largest cells about 2 : 1). This may be due to differences in the parasitic influence of the fungal partner. As far as one can conclude from the present structural observations, this influence in *Pseudocyphellaria* is not very strong: The hyphae pass through the algal layer rather loosely, and no intracellular haustoria are present. In *Woessia,*

however, the cells of the alga are covered by hyphae more tightly, and are regularly intruded by fungal haustoria. In contrast to COLLINS & FARRER (1978) the present author thinks that haustoria do play a role in the transfer of photosynthetic products from alga to fungus[1]. Thus, strong transfer, as suggested by the presence of several haustoria per fully grown algal cell, might interfere with the balance between reproduction and enlargement, and could lead to abnormal increase of dimensions. Such an increase of phycobiont cells in comparison with free-living cells is typical for a number of lichens, especially in older parts of thalli. This is particularly characteristic of cyanophilous lichens (DEGELIUS 1954, GEITLER summarizing 1960, TSCHERMAK-WOESS 1983, TSCHERMAK-WOESS & al. 1983), but also for lichens with green algae (ZEITLER 1954, TSCHERMAK-WOESS 1981).

Finally it should be mentioned that some representatives of *Pseudocyphellaria* have been found to be lichenized with *Dictyochloropsis* (TSCHERMAK-WOESS 1984). This diversity of green algal partners (leaving out of account the blue-greens) is not astonishing, since even for smaller genera of mycobionts an increasing number of different green algae become known as phycobionts. An example is *Chaenotheca* with *Dictyochloropsis, Pseudotrebouxia, Stichococcus* and *Trentepohlia* (cf. POELT 1969, TSCHERMAK-WOESS 1978 a as *Trebouxia*).

Diagnoses

***Chlorella sphaerica* TSCH.-WOESS, spec. nova.** Cellulae sphaericae aliquando ellipsoideae, 3–9.5 µm diam. Paries tenuis, firmus. Chloroplastus subparietalis vel parietalis, plerumque pateriformis vel vittiformis. Pyrenoides globosa, duabus vel plurimis testis amylaceis tecta. Nucleus excentricus. Interdum duo dictyosomata parallela visibilia. Vacuola desunt. Propagatio 4 aut 8 aut 16 autosporis divisionibus succedaneis ortis, ruptura vel diffluentia parietis matricalis liberatis.

Cells spherical, occasionally ellipsoidal, diam. 3–9.5 µm. Cell wall thin, firm. Chloroplast subparietal or parietal, mostly saucer- or band-shaped. Pyrenoids spherical, surrounded by a multipartite or bipartite shell of starch. Nucleus eccentrically located. Occasionally two parallel lying dictyosomes visible. No vacuoles. Reproduction by 4, 8 or 16 autospores which arise by successive divisions and are set free by decomposition or rupture of the wall of the mother cell.

Holotype from cultured material, clone 1 from thallus 3: WU, illustrated in Figs. 1–3; isotype W. Habitat as phycobiont of *Pseudocyphellaria carpoloma* (DELISE) VAINIO on *Rhopalostylus sapida,* Waweira Scenic Reserve, New Zealand, leg. det. J. K. BARTLETT 17. 3. 1984, rev. D. J. GALLOWAY, herb. WU.

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[1] If one accepts with COLLINS & FARRAR that carbohydrates are transferred from the algal to the fungal partner by diffusion and membrane carriers, the relatively thick walls of algal cells and regular hyphae certainly cause more resistance than the generally extremly thin walls of fungal haustoria. The putative role of haustoria should be clarified by special experiments.

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