Excentrochloris fraunhoferiana sp. nov. (Botrydiopsidaceae, Xanthophyceae), a new aerophytic species from the surfaces of modern buildings

Wolfgang Hofbauer[1]*, Georg Gärtner[2], Thorsten Rennebarth[1], Klaus Sedlbauer[1], Florian Mayer[1] & Klaus Breuer[1]

[1]*Fraunhofer–Institute for Building Physics Holzkirchen, Fraunhoferstraße 10, D–83626 Valley, Germany; * corresponding author e–mail: wolfgang.hofbauer@ibp.fraunhofer.de*
[2] *Innsbruck University, Institute of Botany, Sternwartestraße 15, A–6020 Innsbruck, Austria; Georg.Gaertner@uibk.ac.at*

Abstract: A new aerophytic species of the xanthophycean genus *Excentrochloris* – *Excentrochloris fraunhoferiana* Hofbauer, Gärtner, Rennebarth, Sedlbauer, Mayer et Breuer from a building surface is described. Light microscopically investigation and sequence analyses showed a clear relationship of the new alga to *Botrydiopsis constricta* Broady. Cytomorphology and reproduction of the new species was investigated on cultures. *E. fraunhoferiana* differs from the type species *E. gigas* by bigger size of adult vegetative cells with obligate thickening of wall, and none amoeboid zoospores with one chloroplast. Adult zoospores are peripherically arranged in the sporangia. The relation to *Botrydiopsis constricta* is discussed in comparison with characters of *E. fraunhoferiana*.

Key words: Biodiversity, Biogenic crusts, Building relevant microorganisms, *Excentrochloris*, Taxonomy, Xanthophyceae

Introduction

The biodiversity of algae appearing on buildings is composed of special adapted forms. Taxonomic investigations of the primary biological succession on the outer surface of buildings within the scope of a doctoral thesis documented an unexpected rich biodiversity (Hofbauer 2007). Many taxa could be assigned even to species level, some would need further investigation and some were found to be new species which have not been described yet. These investigations listed in total more than 75 different species of algae (cyanoprokaryota and eukaryotic algae), apart from fungi, bacteria, lichens and animal organisms (in total more than 180 species), which are represented mainly as cultures. More than 20 species of algae have been identified for the first time as components of the primary biological succession of biological crusts on buildings. Among them a very peculiar species of Xanthophyceae has been isolated which appeared as an unknown species of the genus *Excentrochloris* (Hofbauer 2007).
Within the last few years some new genera and species of the Xanthophyceae have been described from various habitats. However it is quite a surprise that on an only a few years old surface of a modern building coating a hitherto unknown species of the yellow–green algae (Xanthophyceae) was found. In the following the new species *Excentrochloris fraunhoferiana* is described and depicted.

Materials and Methods

For the investigations, already established cultures were used which are maintained in the culture collection of building relevant microorganisms at the Fraunhofer–Institute for Building Physics. Additional pure cultures of the new species were set up, outgoing from still existing enrichment and interim cultures of the original analysis.

The strains originate from the surface of one specimen representing a coating of a so called mineral coating (lime or cement bound) without overlying paint. The specimen has been exposed to the local climate for three years at the Fraunhofer–Institute for Building Physics, Holzkirchen. Samples of the coating were taken and treated aseptically according to the procedure described in Hofbauer (2007). In spite of isolation into unialgal cultures, some strains still are

Table 1. Used Primers for SSU, according to F. RIMET (personal communication).

Designation of DNA Segment	Sequence
1F	AAC CTG GTT GAT CCT GCC AGT A
528F	GCG GTA ATT CAA GCT CCA A
1055F	GGT GGT GCA TGG CCG TTC TT
1528R	CTT CTG CAG GTT CAC CTA C
536R	AAT TAC CGC CGC KGC TGG CA
1050R	ACG GCC ATG CAC CAC CAC CCA T

contaminated with bacteria. Only axenic algal strains or strains free from fungal contaminations were used. Strains are deposited in the culture collection of building relevant microorganisms established at the Fraunhofer–Institute for Building Physics, Holzkirchen) and labelled as: HOKI A 13 – A 20 and HOKI A 318 – A 321. Additionally, subcultures of the investigated strains were transferred to the algal culture collection at the botanical institute of the Innsbruck University (ASIB, GÄRTNER 2004).

The different strains were cultivated on solidified Bold´s Basal Medium (BBM), modified according to BISCHOFF & BOLD (1963), and also described in ETTL & GÄRTNER (1995). Cultures were maintained in a 12 to 12 h cycle (light–25°C; dark, 16°C) or according to long day conditions (16 h daylight, 25°C, 8 h darkness, 16°C) on culture slants or on agar plates in Petri–dishes sealed with Parafilm within culture–cabinets (Binder company) equipped with special culture fluorescent tubes (Osram company). Unialgal cultures were propagated by periodically inoculation onto new culture medium. For investigation of zoospore development additional clones were grown in liquid cultures, with BBM.

To prove the position of the new alga within Xanthophyceae also chloroplast pigment extracts were produced by use of acetone, according to DALES (1960). Absorption spectra of the acetone–extracts were taken by use of a standard photometer (Perkin–Elmer, Lambda 2 UV/VIS spectrophotometer).

Microscopic examination was done by light microscope (Axioscop 40, Zeiss Company) using magnifications up to x1000. Photographic documentation was performed with a Sony digital camera (MPEGMOVIEEX). Size measurements on living cells were made from young clones in the exponential phase, in the phase of zoospore development, and from older cultures in the stationary phase. Lugol's iodine was used for contrasting the cell contents and for checking the presence or absence of starch. With use of Indian ink and methylene blue the presence of mucilage was tested. Sudan 4 was used to stain for oil. Drawings of the new species were done with Indian ink with the use of a Camera Lucida.

For a first information on the phylogenetic position of the new alga and to check consistency with morphologic data overview genetic analyses were performed. Deoxyribonucleic acid (DNA) was extracted by use of the QIAamp® DNA Mini Kit (QIAGEN). The small subunit of the ribosomal DNA (18S rDNA; SSU) genes and of the large subunit of the ribulose–1,5–bisphosphate carboxylase/oxygenase (*rbc*L) genes were amplified from DNA–extracts via the polymerase–chain–reaction (PCR) using the Silver Star Polymerase (Eurogentec) with the provided buffer and 2mM Magnesium Chloride ($MgCl_2$) For SSU primers according to F. RIMET (personal communication) were used (table 1) whereas for *rbc*L primers according to DAUGBERG & ANDERSEN (1997) were taken. PCR products were cleaned by using the QIAquick® PCR Purifikation Kit (QIAGEN). Sequencing of PCR products was done by an external contract partner. The sequences were compared with those of other heterokont algae obtained from GeneBank; table 2 shows which were used for *rbc*L–sequence phylogenetics. Sequences were aligned using Geneious (Biomatters). The complete alignments are available from the corresponding author on request. Parsimony analysis was conducted using PAUP (Sinauer Associates; SWOFFORD 2003). Phylogenetic bootstrapping was implemented in PAUP to assess relative support for branches in the most parsimonious trees (100x replicates for a first overview for each data set; SWOFFORD 2003; SUNDBERG et al. 2008). Bayesian Analysis was conducted using the Mr Bayes–Plugin of Geneious. The applied Hasegawa–Kishono–Yano model (HKY85) nucleotide substitution model is provided by Mr Bayes (HUELSENBECK & RONQUIST, 2001). Maximum likelihood analysis was not performed because this method does not have an evolutionary background.

Results

The modern approach in algal classification is to use the so called „polyphasic approach" (ASLAM et al. 2007; PRÖSCHOLD & LELIAERT 2007; NEUSTUPA et al. 2009). This usually means the establishment of simultaneous and complementary consideration of morphological, biochemical and genetic characters.

First step to elucidate the position of the investigated alga within the class Xanthophyceae was investigation with light microscope and pigment analysis.

Table 2. Accession numbers of the *rbc*L sequences.

Accession No.	Organism	Strain–No.
AF476927	*Asterosiphon dichotomus*	not specified
AB280609	*Botrydiopsis alpina*	not specified
AJ579569	*Botrydiopsis callosa*	not specified
AJ579566	*Botrydiopsis constricta*	not specified
EF589158	*Botrydiopsis constricta*	LCR–C
EF589159	*Botrydiopsis constricta*	LCR–P
AF015587	*Botrydiopsis intercedens*	not specified
AJ579570	*Botrydiopsis intercedens*	not specified
AJ579568	*Botrydiopsis pyrenoidosa*	not specified
AF465706	*Botrydium becharianum*	not specified
AF465708	*Botrydium cystosum*	not specified
AF064743	*Botrydium stoloniferum*	not specified
AJ579564	*Botryochloris* sp. ‚Southern Victoria Land‘	not specified
AJ874707	*Bumilleria sicula*	not specified
AJ874703	*Bumilleriopsis filiformis*	not specified
AJ579572	*Bumilleriopsis petersenia*	not specified
AJ874706	*Bumilleriopsis* sp.	SAG 22.93
AB280604	*Chattonella antiqua*	not specified
DQ273989	*Chattonella ovata*	not specified
DQ273994	*Chattonella ovata*	not specified
AJ579565	*Chlorellidium pyrenoidosum*	not specified
AJ580948	*Chlorellidium* sp.	SAG 811–1
AJ580947	*Chlorellidium tetrabotrys*	SAG 5.90
AJ580947	*Chlorellidium tetrabotrys*	SAG 5.90
AJ287862	*Choristocarpus tenellus*	SGAD–103
DQ273999	*Haramonas dimorpha*	not specified
AB280608	*Haramonas dimorpha*	not specified
AF084610	*Heterococcus caespitosus*	not specified
AM421003	*Heterococcus chodatii*	SAG 835–3
AJ580926	*Heterococcus pleurococcoides*	not specified
AJ579575	*Heterococcus protonematoides*	not specified
AJ580925	*Heterococcus* sp. ANT	not specified
AF064744	*Mischococcus sphaerocephalus*	not specified
AJ874700	*Ophiocytium capitatum*	not specified
AJ874699	*Ophiocytium majus*	not specified
AJ874701	*Ophiocytium parvulum*	not specified
AM421005	*Phaeobotrys solitaria*	SAG 15.95
AF064746	*Phaeothamnion confervicola*	not specified

Table 2 Cont.

AF069499	*Pleurochloridella botrydiopsis*	not specified
AJ579567	*Pleurochloris meiringensis*	not specified
AJ579573	*Bumilleriopsis pyrenoidosa*	not specified
AJ580924	*Pseudopleurochloris antarctica*	not specified
AJ579574	*Sphaerosorus composita*	not specified
AF155585	*Tetrasporopsis fuscescens*	not specified
AF084611	*Tribonema aequale*	not specified
AY682399	*Tribonema elegens*	not specified
AF465709	*Tribonema intermixtum*	not specified
AJ874340	*Tribonema minux*	not specified
AJ874338	*Tribonema ulotrichoides*	not specified
AY682445	*Tribonema viride*	not specified
AJ874336	*Tribonema vulgare*	not specified
AJ874331	*Xanthonema bristolianum*	not specified
AY682454	*Xanthonema debile*	not specified
AY682398	*Xanthonema hormidioides*	not specified
AY682455	*Xanthonema solidum*	not specified
AJ874334	*Xanthonema tribonematoides*	not specified

According to current knowledge the pigment profile in our investigation was typical for heterokont algae (WILHELM et al. 1987).

The new form has solitary living cells combined with a considerable growth in size connected with multiple nuclei in vegetative cells. Firstly addressed as a new species within the genus *Botrydiopsis* BORZI (HOFBAUER 2007) it became clear after detailed investigation of cultures with light microscope that it is an unknown species of the genus *Excentrochloris* PASCHER.

***Excentrochloris fraunhoferiana* HOFBAUER, GÄRTNER, RENNEBARTH, SEDLBAUER, MAYER et BREUER sp. nov.**

Diagnosis: Cellulae vegetativae singulae et multinucleatae. Cellulae maturae plerumque pyriformae, ellipsoideae, lagenariae vel fusiformae, rarae sphericae. Cellulae ad 91 (– 96) µm longae, 68 (– 87) µm latae. Membrana cellulae laevis firma, frequentis incrassatis inaequalis. Cellulae chloroplastibus numerosis, lentiformis, sine pyrenoide. Propagatio autosporis vel zoosporis multis (4.4 – 6 µm longae, 2.3 – 3.8 µm latae), flagellis binis inaequalis, cum stigmate, in sporangis parietalis aggregatis.

Habitatio: Species aerophytica de superficie politionis apud institutionem fraunhoferianum ad viciniam Holzkirchen, Germania.

Iconotypus: figura nostra 1, ex culturam Hoki A 14. Cultura in collectione Algarum Universitatis Oeniponti (ASIB, Austria) deposita.

Vegetative cells are usually single, multinucleate, pyriform, ellipsoidal, lageniform or fusiform, rarely spherical, with size up to 91 (– 96) µm in length and 68 (– 87) µm in width. Chloroplasts are numerous and elongated–flattened, lens–shaped without pyrenoids. The cell wall is smooth (light microscope), always firm and often with unequal thickenings which become stratified in adult cells. Propagation is by zoospores and autospores. Zoospores are 4.4 – 6 µm in length and 2.3 – 3.8 µm in width. They are metabolic and possess two flagella of different length which are inserted slightly lateral from the cell pole. They always contain only one chloroplast and a red stigma.The are arranged peripherically within the sporangia.

Habitat: Aerophytic species collected from the surface of plaster at the Fraunhofer–Institute near Holzkirchen, Germany.

Holotypus: fixated (preserved) material from culture Hoki A 14 is deposited at the herbarium of the University at Innsbruck under designation (hic designatus) Fix-Hoki A 14.

Iconotypus: our figure 1, from culture Hoki A 14. Cultures (ex-holotypes) deposited in the algal collection of the University at Innsbruck (ASIB, Austria) designated as Hoki A 14.
Species epithet „*fraunhoferiana*“: The name „*fraunhoferiana*“ of the new species is derived from the Fraunhofer society (named after Josef Ritter von Fraunhofer) and the “Fraunhofer–Institute for Building Physics”, in Holzkirchen, Germany.

For *Excentrochloris* the pear–shaped, irregular elongated, even fusiform or lemon shaped adult cells are very characteristic (Fig. 1). Whereas young cells possess one or few disc shaped chloroplasts, adult ones have numerous elongated and flattened lens shaped chloroplasts, never spindle shaped. There are no pyrenoids visible (light microscopic investigation). In vegetative cells usually some of the chloroplasts are in parietal position but also many are scattered within the cell lumen (Fig. 2). As described by Tschermak–Woess (1979) for *Botrydiopsis alpina* Vischer also *Excentrochloris fraunhoferiana* shows often stacks of chloroplasts in vegetative cells. Up to now this arrangement of plastids is unique within xanthophyceae. Oil droplets within the cell plasma were identified using Sudan 4. Whereas young cells often contain several colourless oil droplets, old cells may contain a considerable large yellow or orange oil–vacuole.

Soon after beginning of cell growth the cell wall develops local thickenings; only few cells remain with a regular firm membrane. This can be shown in a typical form at the frequent pyriform cells, in which the stalk like part shows a more or less thickened cell wall with a distinct stratification. In other cells the thickenings appear at both cell poles (Fig. 1).

Older adult cells with particular growth in size regularly show a partial casting of the membrane (Fig. 3). This is very unique. Often there remains a cap like part of the burst cell wall attached to the cell which allows estimation of the original size and form of the cell.

A further important diagnostic character is a special kind of protoplast fragmentation which is connected to the development of the cell wall. By means of aperture like increase of local cell wall thickenings at the „stem base“ or at a section of an ellipsoidal or fusiform cell a part of the protoplast may be segregated (Fig. 4). Even three portions can be formed. The parts of the protoplast are of undefined proportions, but mostly of distinctly different size. In contrast with Broady (1976) a complete division of the cells never happens; the daughter protoplasts always remain enclosed in the mother cell wall. The further development of the daughter protoplasts often differs. Whereas the bigger portion soon may evolve into a zoo– or autosporangium the smaller remains in the vegetative phase, in which it usually gains further size. However sometimes the smaller protoplast degenerates and dies.

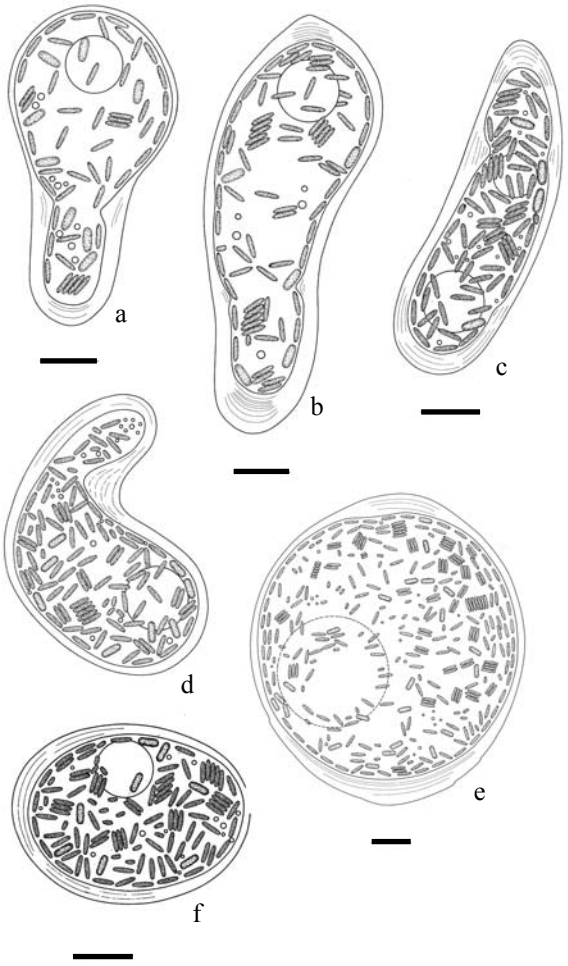


Fig. 1. Different typical forms of solitary adult cells. One or two small vacuoles within the cells are visible. Often stacks of chloroplasts can be recognized. The chloroplasts are shown in front view, side view and from above. Additionally small oil droplets are visible in vegetative cells. The largest cell (f) shows signs of a former partial casting of the membrane. Scale bar 10 µm.

The normal propagation runs by zoospores and autospores. Zoospores are formed when mature sporangia from agar cultures are inoculated in fresh solidified or liquid medium. Even if adult sporangia are suspended in tap water zoospores may hatch. During different stages of the devel-

opment of the coenoblasts they may convert to a zoosporangium, so not only fully sized cells but also rather small cells may release spores. In the first stage of zoospore formation the chloroplasts become transversally orientated in the periphery of the cell and a red stigma appears in each chloroplast (Fig. 5).

After the liberation of the zoospores there always remains a reticular residue of sterile plasma in the sporangia (Fig. 6). The zoospores which are produced in different numbers, according to the size of the zoosporangium are metabolic but do not form pseudopodia (Fig. 7a). They always contain only one chloroplast with a red stigma. The two flagella of the metabolic zoospores are inserted slightly laterally and at the apex of the cell are two hardly visible contractile vacuoles. After a short time as swarmers (often just a few minutes) the zoospores loose their flagella and develop into globular or ellipsoidal form and start to grow. The stigma is withdrawn soon and the chloroplasts become multiplied. Autospores are also supplied with a stigma in the beginning (Fig. 7b). Apart from small autospores which resemble rounded zoospores and possess only one cup shaped chloroplast with a stigma, bigger ones can also be seen with up to three disc–shaped chloroplasts without stigma. Often autospores form a loose aggregate outside the empty sporangium, but they are neither united by the cell walls nor by secreted gelatinous substance, and therefore separate easily. During growth most cells (spores) soon lose their globular form, become ellipsoidic or asymmetric to fusiform (Fig. 7c) and soon multinucleated (Fig. 7d). Only once the development of much bigger daughter cells with thickened cell walls was observed. They were interpreted as resting cells.

Since the alga presented no exceptional features within the detailed light microscopic investigation with magnitudes up to 1,000–fold, that could influence the accurate diagnosis, there was no necessity to provide different views of the ultra–structural characters by electron micrographs. These data shall be presented in a different work.

Apart from the morphological findings, the genetic investigations show clearly that the new taxon is integrated in a special position within the system of the Xanthophyceae. Different mathematical and statistical analyses of the *rbc*L gene in the first overview do not differ in the main results (Fig. 8, Fig. 9, see also for accession numbers): *Excentrochloris fraunhoferiana* is genetically different from a close group of *Botrydiopsis* species

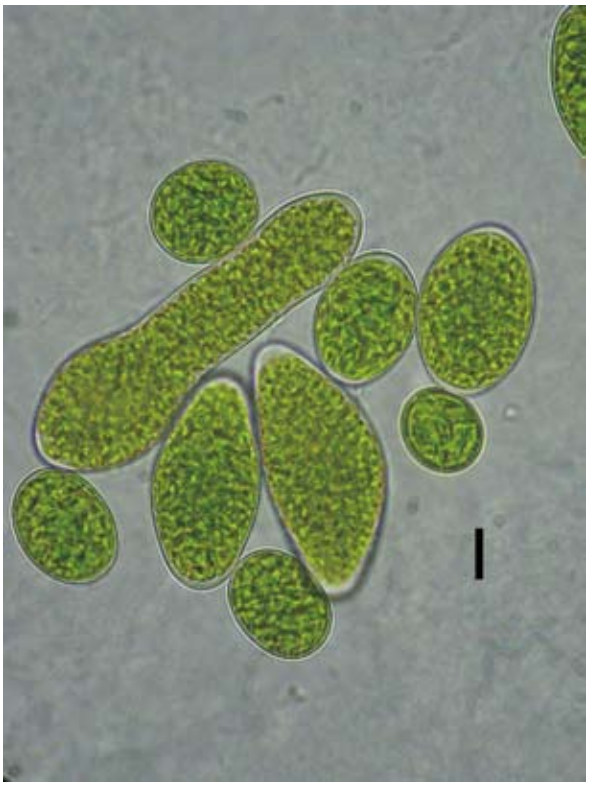


Fig. 2. Young coenoblasts with partly visible stacks of chloroplasts. Scale bar 10 µm.

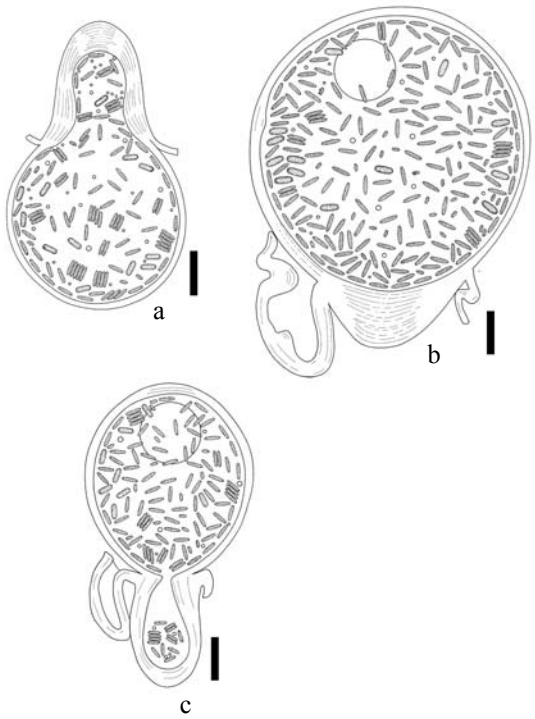
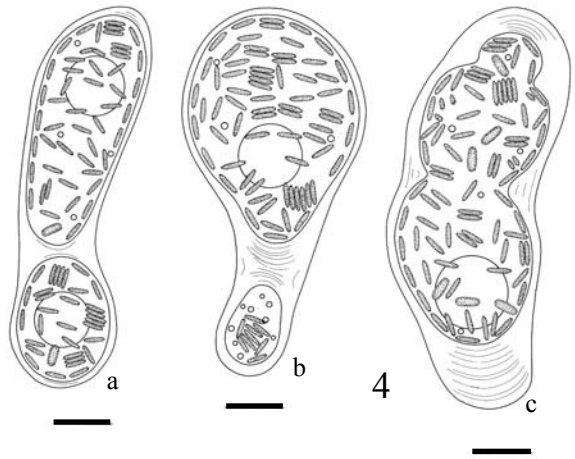
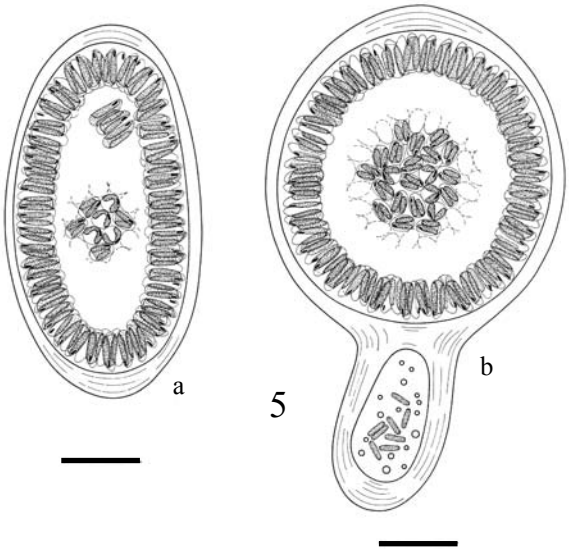
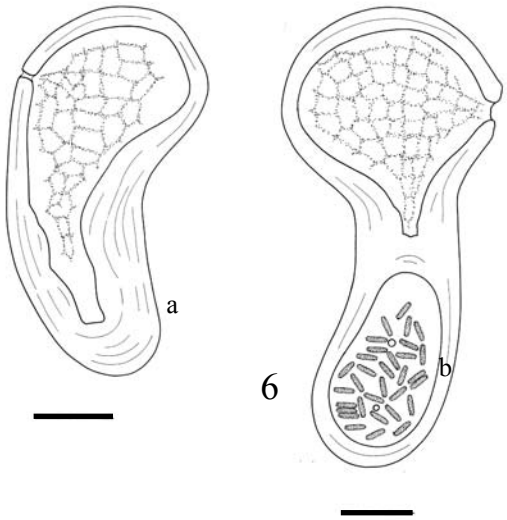


Fig. 3. Different mature coenoblasts which show a partial casting of the membrane. In (c) the different size of the chloroplasts in both parts of the cell indicates that there might be already two separated protoplasts, but in the light microscope no clear borderline could be seen. Scale bar 10 µm.







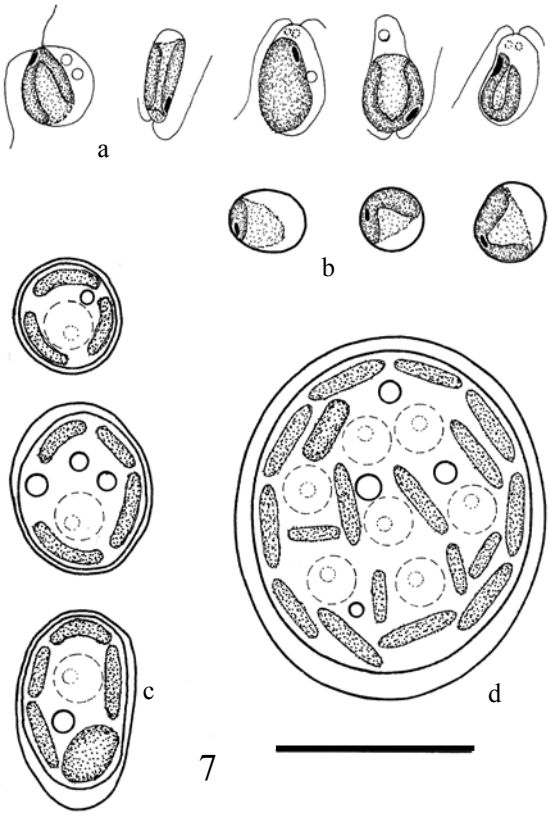


Fig. 4. Several cells that show atypical protoplast fragmentation. In (a) and (b) the cell wall has already separated into two daughter protoplasts of unequal size. The daughter protoplasts often develop into different stages, sometimes the smaller also degenerates. In (c) beginning of fragmentation into even three different protoplast portions is visible. Scale bar 10 µm.

Fig. 5. Zoosporangia shortly before liberation of the zoospores. The developed zoospores are already differentiated and arranged in the periphery of the cell, in the center plasma substance without chloroplasts. a) Sometimes a second partial group is visible In the centre of each sporangium a part of the regular arrangement of zoospores is drawn from top view. Each zoospore has only one chloroplast which bears a small red stigma apically. The flagella are scarcely visible with light microscope or not yet differentiated. For the second two–celled plant (b) the smaller protoplast is still in a vegetative phase. Scale bar 10 µm.

Fig. 6. Empty zoosporangia of a single cell (a) and a two celled aggregate (b). The zoosporangia open usually by a fissure, not at a formation in the wall. After the depletion of the zoospores a reticulate plasma residue is left. Soon after the burst of the sporangium the cell wall partially begins to swell (a). Scale bar 10 µm.

Fig. 7. (a) Zoospores and initial stages of cell development. (b) Juvenile cells developed from zoospores. They usually also show stigmata in an early stage of development. (c) In an early stage of the vegetative development the cells already contain several chloroplasts but still one nucleus. (d) Young vegetative and multinucleate cell. Scale bar 10 µm.

Table 3. Comparison of different characteristics of *E. fraunhoferiana* sp. nov. and *Botrydiopsis constricta* BROADY (according to BROADY, 1976).

Characteristic	*Botrydiopsis constricta*	*Excentrochloris fraunhoferiana*
Size of adult cells	25 – 42 µm	various, up to 68 – 91 – 110 µm
Shape of adult cells	spherical, sometimes ellipsoidal or irregular	pyriform to lageniform to fusiform or irregular, rarely spherical
Cell wall of adult cells	Smooth and thin, occasionally with thickenings	Smooth and firm, often local lamellate thickenings
Stacks of plastids	unknown	Regularly observed in vegetative cells
Partial casting of the membrane	unknown	Sometimes observed in older vegetative cells
Unusuall vegetative division	Division of whole coenoblasts by transverse wall forming and constriction or by a kind of budding, finally giving rise to two liberated daughter cells	Division of the protoplast in often different sized daughter portions with further different development by formation of a transverse wall by unequal thickening of the cell wall. Never liberation of the daughter cells, daughter protoplast always remaining connected by the mother cell wall. Sometimes even indication of a further division.
Attributes of zoospores	Naked, unequally biflagellate, one chloroplast with stigma; metabolic to amoeboid (pseudopodia)	Naked, unequally biflagellate, one chloroplast with stigma; metabolic but no formation of pseudopodia
Plasma residue after spore release	unknown	Reticulate residue always present

around the type species *B. arhiza* BORZI. The investigated strains of the new species are grouped in a clade directly beside *B. constricta* BROADY but the separation between these two forms is well supported, both after Bayesian Analysis (fifure 8) and Parimony analysis (figure 9). Both forms are within a major phyletic line with *Mischococcus* NAEGELI and *Heterococcus* CHODAT according to Bayesian Analysis. *B. pyrenoidosa* H. TRENKWALDER is always grouped at the base of the Xanthophyceae and can even be used as outgroup. Because the phylogenetic analysis of SSU–sequences provides similar results these are not shown here.

Discussion

The algal class Xanthophyceae, in sense of FRITSCH (1935), HIBBERD & LEEDALE (1971a+b) and HIBBERD (1981) forms a well defined group within the Heterokontophyta. According to ADL (2005) the class Xanthophyceae is divided into two oders: Tribonematales PASCHER und Vaucheriales BOHLIN. Recent more detailed insight in the phylogeny of the algal class Xanthophyceae is given by MAISTRO et al. (2009). Taxa with a coenocytic main life form are distributed within the Xanthophyceae in different families respectively orders (ETTL 1978; RIETH 1980; ANDERSEN & BAILEY 2002). Different works with a genetic emphasis indicate that there are different lines of evolution

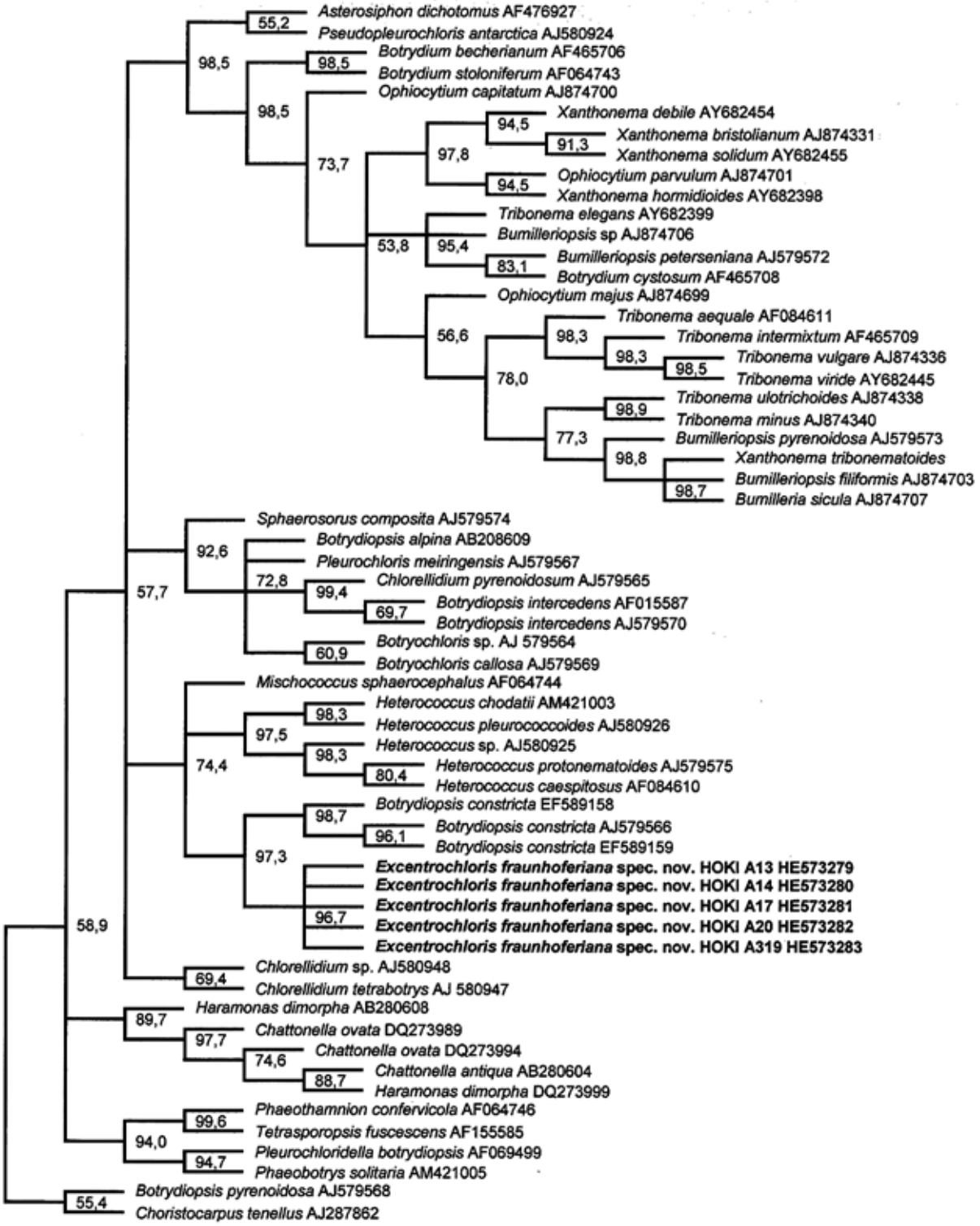


Fig. 8. *rbc*L–sequence phylogenetics of the Xanthophyceae, with *Excentrochloris fraunhoferiana* sp. nov. included. Tree topology reconstructed using Bayesian Analysis. Support values at nodes are given as bootstrap percentage.

within the Xanthophyceae, each with convergent developmental stages comparable to the chlorophyceae (Potter et al. 1997; Maistro et al. 2009). According to these genetic data a complete rearrangement would be needed, but the data base is still insufficient or inconsistent (Andersen & Bailey 2002; Maistro et al. 2009).

For the genus *Excentrochloris* Pascher, with the type species *E. gigas* Pascher, description and figures in Pascher (1939) are valid as

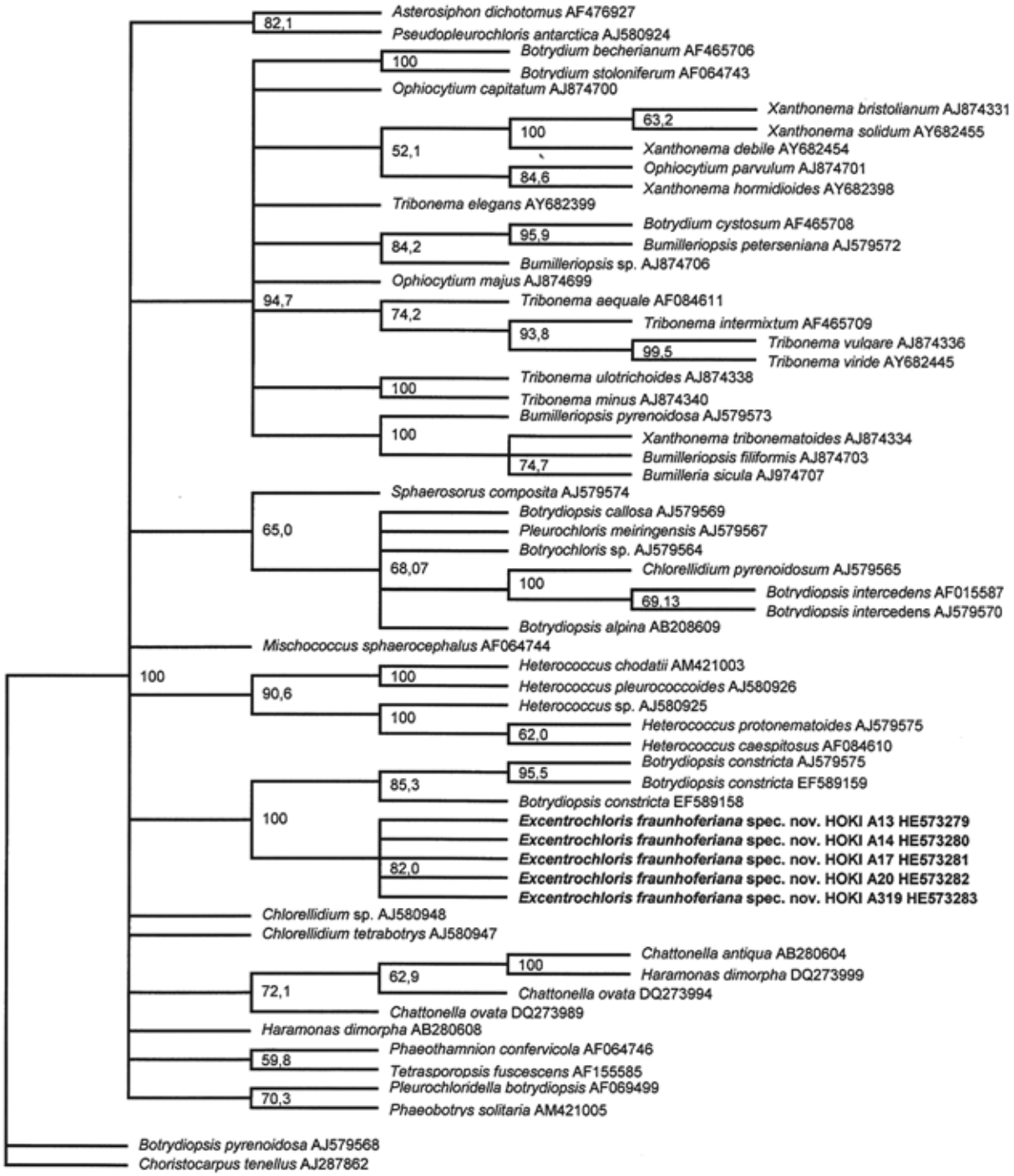


Fig. 9. *rbc*L–sequence phylogenetics of the Xanthophyceae, with *Excentrochloris fraunhoferiana* sp. nov. included. Tree topology reconstructed using Parsimony Analysis. Support values at nodes are given as bootstrap percentage.

lecto– and iconotypus; cultures of the typus do not exist. The coenocytic genus is characterized above all by an irregular shape of the cells, often from a young stage on and by partially, often one–sided, layered membrane thickenings. Adult cells possess numerous chloroplasts and the propagation is facilitated by metabolic zoospores or autospores. Resting cells may also occur. Furthermore, PASCHER (1939) declares that in old cells bleached chloroplasts may form a net like connection. This is interpreted in ETTL (1968) as an indication of cell degeneration.

Obviously, it must be a rare taxon. PASCHER (1939) mentions similar forms from algae coats on reed stalks and from Nile mud without addressing them closer. ETTL (1968) documented under the name *E. gigas* a smaller but also aquatic form. The first isolate of an *Excentrochloris*– like alga

from soil was done by Vinatzer as *E.* sp. and cultivated in ASIB (Vinatzer 1975; Gärtner 1976, 1985). A subculture of this strain in the Culture Collection of Algae at Göttingen/Germany (SAG) is kept there as *Botrydiopsis intercedens* Pascher. This strain occasionally forms ellipsoidal to lenticular coenoblasts, but apart from that it has more in common with the genus *Botrydiopsis* than with *Excentrochloris*.

Morphologically relations of *E. fraunhoferianum* with *B. constricta* can be established (shape of cells and membrane thickenings). In table 3 the different characters of *E. fraunhoferiana* and *B. constricta* are summarized. The newly found *E. fraunhoferiana* shows, in contrast with *B. constricta,* nearly **always** irregular shape and local layered membrane thickenings, whereby the affiliation to *Excentrochloris* becomes evident. *B. constricta* was obviously included in the genus *Botrydiopsis* by Broady (1976), because it shows mainly spherical coenoblasts and other cell shapes are rare. But in other species of the genus *Botrydiopsis* occasional divergence from the spherical cell shape is known (Ettl 1978; Ettl & Gärtner 1995). *B. constricta* was only documented from the Antarctic (Broady 1976), but recently was again recorded from mountainous regions of New Zealand (Novis et al. 2008).

Recent genetic investigations indicate that the genus *Botrydiopsis* is polyphyletic and therefore should be divided (Negrisolo et al. 2004; Maistro et al. 2009). For *B. pyrenoidosa* it is even uncertain if it should be placed at the basis of the Xanthophyceae or if it belongs to a different group of algae because it is genetically so different (Fig. 8, 9). In this investigation it therefore could be used as an outgroup. In current investigations already a new name is proposed: *Polykaryon pyrenoidosum* (Misner 2004; J.C. Bailey, personal comment, in press). For *B. constricta* it was shown that it certainly belongs to the Xanthophyceae but it groups at a distinct position far from the other species of the genus *Botrydiopsis* (Negrisolo et al. 2004; Maistro et al. 2009). According to our investigations *B. constricta* together with the new isolates from New Zealand (in concordance with Novis et al. 2008) and *E. fraunhoferiana* lay close together. If this means that they are two species of a single genus or if they resemble species from two different but closely related genera remains to be shown in future investigations. Because of the fact that the presented phylogenetic data are of an overview character further detailed investigations shall be done.

Within the artificial family Botrydiopsidaceae some more genera exist, but many of them need further revision and investigation (Bourrelly 1968; Ettl, 1978). Recently it has been shown (Juárez et al. 1998), that young plants from a *Botrydium* sp. also may develop stages that are strongly suggestive of *Botrydiopsis* (coccale stage with spherical cells) and *Excentrochloris* (irregular cellform and local membrane thickenings). In cultures these stages immediately begin to grow into typical thalli.

As a further step it is planned to assess the ecophysiological capacity of *Excentrochloris fraunhoferiana* (e.g. temperature range, light requirement, tolerance of different humidity's and/or solute concentrations). Since the alga produces oil substance it might also be an interesting strain for biotechnology. It has already been shown that Xanthophyceae might be grown under chemo–organic conditions (Casselton 1966).

Acknowledgements
The work for this paper was done with the support of the Fraunhofer–Institute for Building Physics (Holzkirchen, Germany). For graphical aid the authors want to thank Werner Conrad (Holzkirchen, Germany) and Georg Höck (Kufstein, Austria). For the English correction of the manuscript great thank is due to Prof. James Dickson (Glasgow University, Scotland). Many thanks also for scientific input to J. Craig Bailey (University of North Carolina at Wilmington, USA).

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Received May 15, 2010
Accepted December 9, 2010