

Morphological and taxonomic study of three terrestrial eustigmatophycean species

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with 29 figures

Abstract: The morphology, life cycle and taxonomy of three terrestrial eustigmatophycean species have been investigated. The ontogenetic sequences of morphological stages have been ascertained. One species has been determined as *Pseudocharaciopsis ovalis* (CHODAT) HIBBERD. The species designated as *Vischeria* sp. has probably close affinities to *V. punctata* VISCHER but exhibits the distinct elongated roll-like stage in its life cycle. One taxon – *Pseudellipsoidion edaphicum* is proposed as a new genus. In its life cycle *P. edaphicum* exhibits the ellipsoidal stage similar to some species of xanthophycean genus *Ellipsoidion* and the globular *Eustigmatos*-like stage. The alga possesses typical eustigmatophycean ultrastructural features – chloroplasts without girdle lamella and lamellate vesicles. The zoospores are uniflagellate, of irregular oval shape and with prominent extraplastidial stigma. The contribution of morphological approach and the importance of zoospores structure for eustigmatophycean taxonomy are discussed. For the proper identification of eustigmatophycean species the knowledge of the life cycle is considered to be essential.

Key words: Eustigmatophyceae, *Pseudellipsoidion*, *Pseudocharaciopsis*, *Vischeria*, morphology, taxonomy, soil algae.

Introduction

The members of the class Eustigmatophyceae compose an autochthonous part of terrestrial and aerial algal synusia (HIBBERD 1981, Ettl & GÄRTNER 1995). However, our knowledge of eustigmatophycean biodiversity is only fragmentary. NORTON et al. (1996) even estimate that probably only negligible 0,2 to 2% of eustigmatophycean species have been discovered so far. The ignorance of real biodiversity, of the extent of phenotypic plasticity as well as of suitable determination features retards the progress of knowledge of eustigmatophycean distribution and ecology.

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The algal class Eustigmatophyceae has been erected by HIBBERD & LEEDALE (1971, 1972) by detachment of several coccoid xanthophycean species. The Eustigmatophyceae are characterized by complex of some unique structural features of both vegetative cells and zoospores in which they are differing from Xanthophyceae as well as from other algal groups. In vegetative cells these are above all the absence of the thylakoidal girdle lamella in chloroplasts, the presence of polyhedral pyrenoid with homogeneous matrix and the presence of characteristic vesicles with lamellate content and globular reddish body both of unknown function. The typical features of the zoospores are the prominent extraplastidial stigma and the reduction or absence of second flagella (HIBBERD 1990).

The inner taxonomy of Eustigmatophyceae is based mainly on structural features of both vegetative cells and zoospores (HIBBERD 1981, 1990). The main features used in delimiting taxa are, e.g., the shape of vegetative cells (globular, drop-like, polyhedral, etc.), the presence/absence of zoospores, the number of flagella, the number of chloroplasts in vegetative cells, etc. As the Eustigmatophyceae are often small-sized organisms with relatively simple morphology, the usage of molecular species concept has been proposed, e.g., for the azoosporic genus *Nannochloropsis* (ANDERSEN et al. 1998, KRIENITZ et al. 2000). However, complete reduction to 18S rRNA information, howsoever powerful it might be, seems us as unnecessary and potentially misleading in case when the insight into the taxonomy of the group is feasible at the more complex organismal level. In this study the applicability of morphological approach for eustigmatophycean taxonomy is presented.

This paper deals with morphology, life cycle and taxonomy of three eustigmatophycean species isolated from the soil biotopes of the Czech Republic.

Material and methods

The strains of *Pseudellipsoidion edaphicum* and *Pseudocharaciopsis ovalis* (CHODAT) HIBBERD were isolated from the peat-bog soil sample of the horizon of 0–5 cm in the Nature reserve “V Bahnách” in the Central Bohemia (altitude 420 m a.s.l., geographical coordinates: latitude 50°10'32", longitude 13°52'02", pH_{H₂O} 5,01–5,69). The strain of *Vischeria* sp. was isolated from the soil sample of the horizon 0–5 cm in the marlstone-based beech forest in the Central Bohemia (altitude 450 m a.s.l., latitude 50°14'21", longitude 13°50'40", pH_{H₂O} 6,18–7,09) (for the species list of the locality see NEUSTUPA (2001)).

The morphology was observed both direct by populations growing under the coverslip on the surface of the soil sample (ETTL & GÄRTNER 1995) or in unialgal cultures. The strains were cultivated on both agar-solidified and liquid BBM (BISCHOFF & BOLD 1963) and in bifasic cultures (PRINGSHEIM 1954); in the temperatures 6°C, 13°C and 26°C; under the illumination of about 2500 lux (light source: TUNGSRAM 36W F33, cool white).

The liberation of zoospores was induced by transfer of aged cells into the fresh media both in the dark or in daylight illumination. For better visualisation of flagella the zoospores were fixed at 0,5% osmium tetroxide in phosphate buffer, dried on a coverslip and examined with use the of phase contrast in LM or dried on a formvar coated copper grid, shadowcast with chromium in a high vacuum evaporator Polaron and examined in TEM (PHILIPS T300).

The cells for TEM sections were fixed at 2% glutaraldehyde in 0,05M phosphate buffer, postfixed at 1% osmium tetroxide in phosphate buffer and at 1% uranyl acetate in methanol.

The cells were dehydrated in graded concentration of ethanol, than transferred to butanol and embedded in Spurr's low viscosity resin (SPURR 1969). Sections were cut with a diamond knife, poststained with uranyl acetate and bismuth oxynitrate and examined with a PHILIPS T300 TEM.

All the investigated strains are kept in The Collection of Algae of Charles University of Prague, Czech Republic (CAUP).

Results and discussion

Pseudellipsoidion NEUSTUPA et NĚMCOVÁ gen. nov.

Diagnosis: Eustigmatophyceae ellipsoidae quam sphaericae, membranis laevibus. In cellula sphaerica pyrenoide polyedrica et globulus rubens adsunt. Propagatio per zoosporas, hemiautosporas et autosporas effecta. Zoosporae irregulares vel ovatae, flagello singulo antico emergenti, stigmati prominenti extra chloroplasti teneo.

Typus: *Pseudellipsoidion edaphicum* NEUSTUPA et NĚMCOVÁ

Eustigmatophyceae with ellipsoidal and globular cell stages in the life cycle. The cell wall smooth. In globular cells the polyhedral pyrenoids and the reddish globule are developing. The reproduction takes place by means of zoospores, hemiautospores or autospores. The zoospores are of irregular or oval shape, with one prominent flagellum and anterior extraplastidial stigma.

Pseudellipsoidion edaphicum NEUSTUPA et NĚMCOVÁ sp. nov. Figs 1,5,8,9,12,13,18-21,24

Diagnosis: Cellulae ellipsoidae quam sphaericae, membranis laevibus. Cellulae ellipsoidae (5,7-)6,3-9,3(-10,2) x (3,2-)4,2-5,9(-7,1) µm diametro. Cellulae sphaericae (5,9-)7,1-10(-12,8) µm diametro. In cellula sphaerica pyrenoide polyedrica et globulus rubens adsunt. Chloroplasti parietales, 1 tenuis 4. Propagatio per zoosporas, hemiautosporas et autosporas effecta. Zoosporae irregulares vel ovatae, flagello singulo antico emergenti, stigmati prominenti extra chloroplasti teneo. Dimensiones 4,1-6,1 x 2,5-4,1(-5) µm.

Iconotypus: Figura nostra 1.

The ellipsoidal and globular cells occurring in the life cycle. The cell wall smooth. The dimensions of ellipsoidal cells are (5,7-)6,3-9,3(-10,2) x (3,2-)4,2-5,9(-7,1) µm. The dimensions of globular cells are (5,9-)7,1-10(-12,8) µm. In globular cells the polyhedral pyrenoid and the reddish globule are developing. One to four chloroplasts are in the cells. The reproduction takes place by means of zoospores, hemiautospores and autospores. The zoospores are of irregular or oval shape, with one anterior mastigonemes-bearing flagellum and prominent extraplastidial stigma. The dimension of zoospores are 4,1-6,1 x 2,5-4,1(-5) µm.

This species is capable to produce free-living elliptical to globular or irregular cells. The strain was isolated and originally determined as a member of traditional delimited xanthophycean genus *Ellipsoidion* PASCHER with typical regular elliptical cells (Figs 1a, 21). However, in the course of further investigation it became apparent that the elliptical shape is only one stage in the ontogeny of the cells after which the globular cells are developing (Figs 1b, 24). The cells possess mostly one to two, rarely to four chloroplasts without thylakoidal girdle lamella (Figs 1, 8, 9, 12). In elliptical cells one of the chloroplasts is often bigger than the

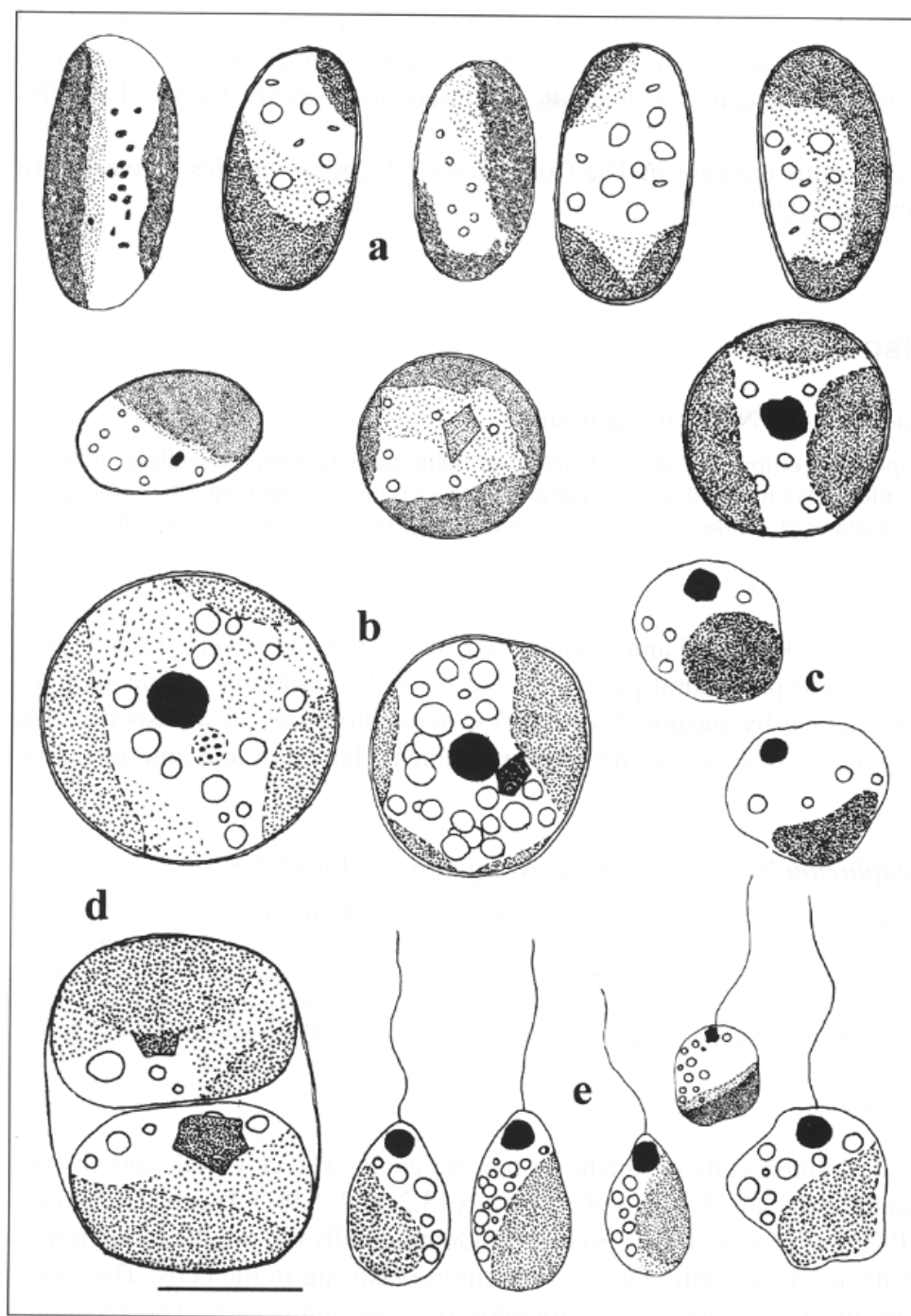


Fig. 1. *Pseudellipsoidion edaphicum*, **a** – ellipsoidal-stage cells, **b** – globular-stage cells, **c** – hemiautospores, **d** – autospores, **e** – zoospores. [Bar = 5 μ m]

other one. The multiplication of chloroplasts during the cell ontogeny needs not to be necessarily the rule. The young cells with three chloroplasts, as well as the aged cells with one chloroplast are normally present. The typical eustigmatophycean polyhedral pyrenoid with dimensions 1.5–3 μ m was observed in the cells of globular stage. The single reddish globule is also developing in globular cells. The vesicles with lamellate content typical for members of Eustigmatophyceae were observed on TEM sections (Fig. 13).

The width : long ratio in elliptical cell-stage cultures is 1 : 1.3–2.2.

Fig. 2. *Pseudocharaciopsis ovalis*, **a** – *Characiopsis*-like sessile cells, **b** – *Monodus*-like free-living cells, **c** – autosporangium, **d** – zoospores. [Bar = 5 μ m]

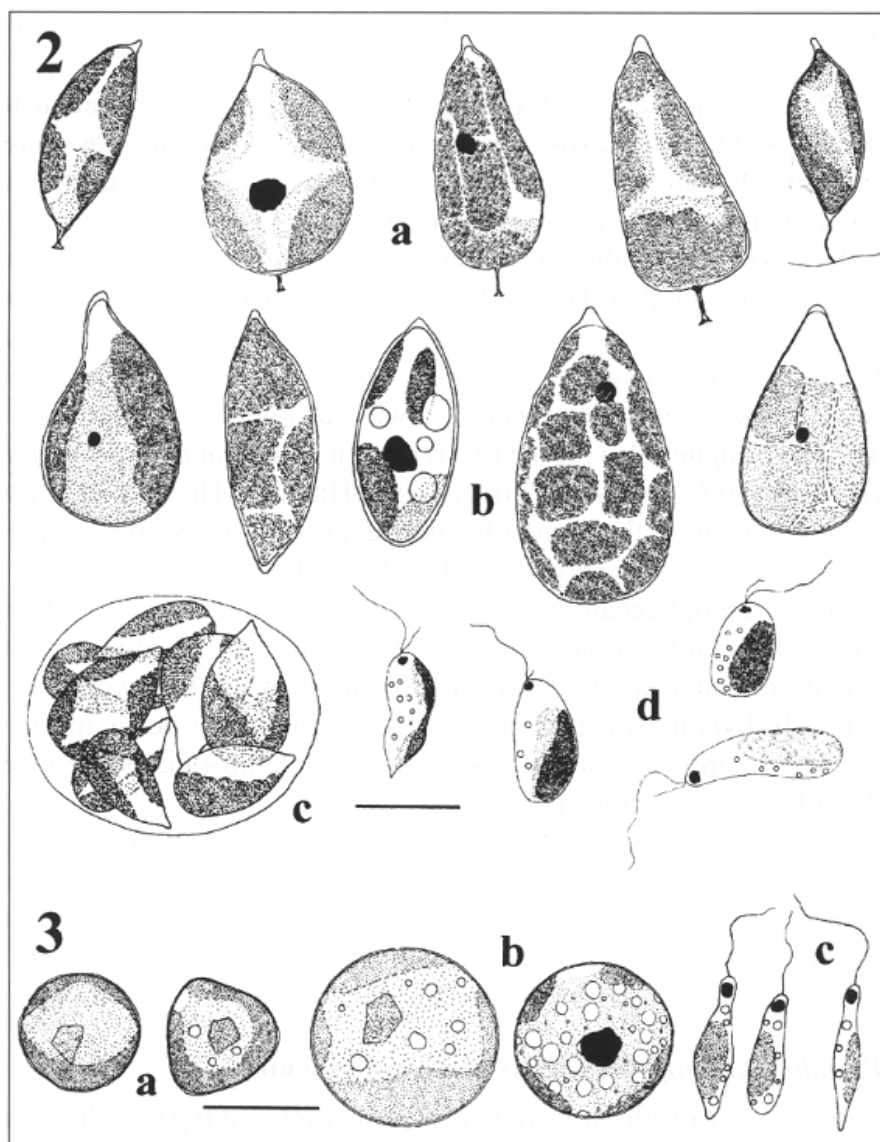


Fig. 3. *Eustigmatos vischeri*, **a** – young cells released from autosporangium, **b** – adult cells, **c** – zoospores. [Bar = 5 μ m]

The reproduction takes place by means of autospores, hemiautospores and zoospores. The autospores are produced by two to eight and are of elliptical or irregular shape. The mass production of hemiautospores was only rarely met. The hemiautospores lack the cell wall as well as the flagellum, but they possess the extraplastidial stigma (Fig. 1c). The dimensions of the hemiautospores were in general identical with those of zoospores.

The naked zoospores are of irregular or oval shape (Fig. 1e). They possess one or rarely two parietal chloroplasts. Near the cell apex the prominent yellow-red extraplastidial stigma of irregularly globular shape is clearly visible. The zoospores possess one apical flagellum (Figs 19, 20) with mastigonemes typical for members of the division Heterokontophyta (Figs 16, 18).

The life cycle with all observed developmental possibilities is pictured on Figure 5. The ontogenetic sequence of ellipsoidal and globular cells was retained in cultures cultivated either on BBM or on biphasic soil-water media as well as in natural populations observed as growths under the coverslip placed on the soil surface and in all temperatures used for cultivation.

The development of globular cells seems to be necessary before the production of the autospores.

Due to the presence of typical features there is no doubt that the investigated species should belong to the class Eustigmatophyceae. The uniflagellate zoospores and globular stage with smooth cell wall in the life cycle could indicate the relationship with *Eustigmatos*. The morphology and the life cycle of the original strain of *Eustigmatos vischeri* HIBBERD (CAUP Q 101 = SAG 860-1), the type species of the genus *Eustigmatos*, was investigated for the comparison with our alga. The marked differences between our species and *E. vischeri* were stated as the findings in *E. vischeri* agree with HIBBERD (1981). In *E. vischeri* the zoospores are of lageniform flask-like shape (Fig. 3c). The ellipsoidal cells are never developing in the life cycle of the organism and there is always a single chloroplast in the vegetative cells (Fig. 3). The same applies also for the two remaining *Eustigmatos* species – *E. magnus* (J.B. PETERSEN) HIBBERD and *E. polyphem* (PITSCHMANN) HIBBERD (HIBBERD & LEEDALE 1972, HIBBERD 1981).

Consequently, the typical features expressed in the life cycle of our investigated species suggest that the alga should be placed into the separate genus.

The xanthophycean genus *Ellipsoidion* PASCHER has been delimited for species with ellipsoidal shape of the vegetative cells (PASCHER 1939). As the majority of species in this genus was described without careful investigation of the life cycle and phenotypic plasticity, we can only hypothesise that globular cells could also occur in the ontogeny of *Ellipsoidion* species. In any case, we cannot interpret the ellipsoidal stage of our alga as any of already described *Ellipsoidion* species.

The species *E. annulatum* PASCHER has been established as a type species of the genus by LOEBLICH (1967). Whether this species belongs to Xanthophyceae or Eustigmatophyceae is not known. However, studies on *E. regulare* PASCHER demonstrate that organisms of truly xanthophycean nature can occur within this genus (PRASAD 1979). That's why we propose a new generic name for the accommodation of our species within the class Eustigmatophyceae.

***Pseudocharaciopsis ovalis* (CHODAT) HIBBERD**

Figs 2,6,22,23,26

This alga is capable to form two principal morphotypes. While on solid media the irregular drop-like *Monodus*-like cells are developing (Figs 2b, 22, 23), in liquid conditions the sessile *Characiopsis*-like forms are also present (Fig. 2a). Aged *Monodus*-like cells are sometimes becoming more globular with somewhat thickened cell wall. The stalk of *Characiopsis*-like forms is developing as the derivate of the cell wall. The base of the stalk is circular extended. While the stalk of young cells developed from zoospores is short and thick, in adult cells it becomes longer and thinner. On the apical end of the cells the cell wall derivated papilla is developing. The cells possess several parietal chloroplasts. The typical eustigmatophycean reddish globule is present in most of the adult cells (Fig. 2). Pyrenoid is not visible in LM.

The dimensions of the vegetative cells are (10,6-)11,4–14,5(-16) x (3-)4–10,2(-11) µm, the stalk is up to 3 µm long.

The reproduction takes place by means of autospores and zoospores. The number of autospores in sporangium varies from two to 16 with eight being the most usual number. The autospores are of irregular or *Monodus*-like shape (Fig. 2c).

The naked zoospores are of oval to pear-like or irregular shape (Fig. 2d). They possess one or two parietal chloroplasts in the posterior part of the cell. Near the cell apex the prominent extraplastidial stigma of irregular shape is clearly visible. The zoospores possess two

flagella. The second one, however, is rudimentary and not visible on wet preparations in LM. The dimensions of the zoospores are 2,5–3,5 x 4,7–5 x 5–6 µm.

The life cycle of the species is pictured on the Figure 6. The development of *Characiopsis*-like stage depends on environmental conditions. In liquid conditions the young cells frequently develop the stalk to catch themselves on the available substrate. However, probably only free-living *Monodus*-like cells are capable of autospore production.

This species was originally described by CHODAT (1913) as *Monodus ovalis*. Later, however, CHODAT transferred the species into the genus *Characiopsis* because of the occurrence of stalked sessile form (POULTON 1925). During the Second World War the CHODAT's strain was lost. HIBBERD & LEEDALE (1972) studied strain CCAP 822/1 originally determined as *Ellipsoidion acuminatum* PASCHER and transferred it to the class Eustigmatophyceae. HIBBERD (1981) redetermined this strain as identical with the description of *Ch. ovalis* (CHODAT) CHODAT and established the generic name *Pseudocharaciopsis* and the family Pseudocharaciopsidaceae within the Eustigmatophyceae. The members of the family Pseudocharaciopsidaceae are characterised by presence of sessile stalked vegetative cells and biflagellate zoospores.

PIZZARO (1995) described *Characiopsis mammelonata* from temporary ponds of northern Argentina as species identical with *Characiopsis ovalis* (CHODAT) CHODAT. The name *Ch. ovalis* in her view is the synonym of her species because the description of the former was based only on culture observations. However, the CHODAT's description was made according to the code and the relevance of his observations was confirmed by HIBBERD & LEEDALE (1972). On the contrary, we cannot consider the nature of PIZZARO's species because she did not mention any data on cell structure, reproduction or the life cycle of the organism.

Despite the considerable phenotypic plasticity the organism investigated in this study fits well in both CHODAT's and HIBBERD's descriptions and is readily interpretable as *P. ovalis* (CHODAT) HIBBERD.

For the present there have been four reports of the occurrence of *P. ovalis* (CHODAT) HIBBERD. The original finding of CHODAT from unspecified aerophytic biotope in Switzerland; the WESTLAKE's isolation of strain CCAP 822/1 from the freshwater pond in Hertfordshire, England (TOMPKINS et al. 1995); the unpublished finding of LUKEŠOVÁ from the forest soil in South Bohemia and the report in this study. Freshwater *Characiopsis* specimens from Argentina (TELL & PIZARRO 1984, PIZARRO 1995) determined as identical with CHODAT's description cannot be considered with certainty because there is no evidence of their eustigmatophycean nature.

We can summarise that *Pseudocharaciopsis ovalis* (CHODAT) HIBBERD is an aero-terrestrial alga, which can occur also in the freshwaters, perhaps as the result of inoculation from wind-blown aerial biotopes.

Vischeria sp.

Figs 4,7,10,11,14,15,17,25,27-29

This species has free-living globular, irregular-polyhedral and elongate-elliptical cells. Young cells developing from either autospores or zoospores are prevalently globular or they possess more or less irregularly disposed cell projections shaping them often somewhat polyhedral (Figs 4a, 17). The angular cells with regularly distributed conical projections are rare, but sometimes present in the population. The angular cells are generally smaller than the globular

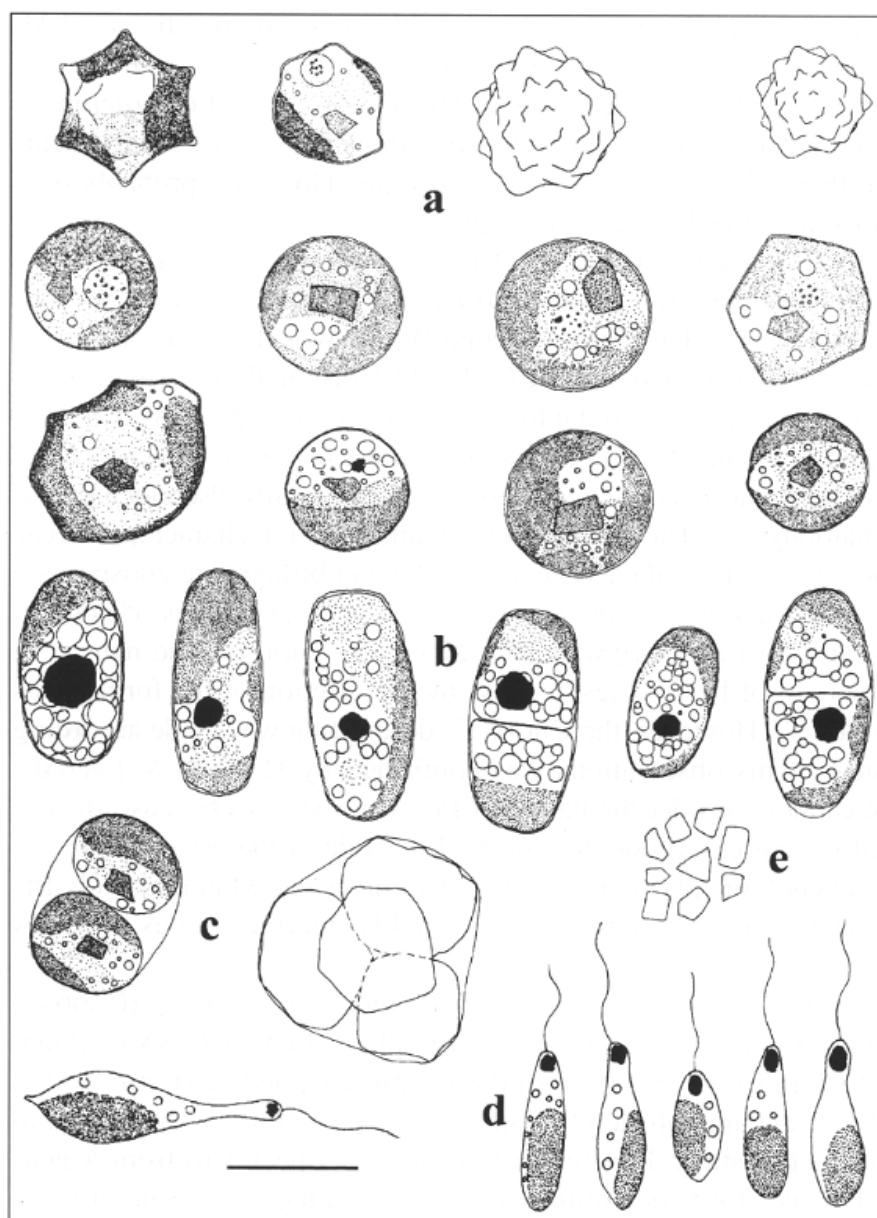


Fig. 4. *Vischeria* sp., **a** – globular and angular-stage cells, **b** – elongated-stage cells, **c** – autospores, **d** – zoospores, **e** – different shapes of pyrenoids in vegetative cells. [Bar = 5 μ m]

cells. The adult cells are becoming more and more elongated with cells in aged culture possessing prevalently roll-like ellipsoidal shaped cells (Figs 4b, 25, 27 – 29). The cells possess distinct eustigmatophycean polyhedral pyrenoid (Figs 4, 11) connected by stalk with chloroplast matrix (Fig. 14). The dimensions of the pyrenoid are (2,5–)3,4–5,1–(6) μ m. The prominent single reddish globule is developing in adult cells. The globule consists of several smaller globules of homogenous content (Fig. 10). During the reproduction the reddish globule is inherited by one of the autospores (Figs. 4b, 29) so that some cells possess the reddish globule for their whole life cycle. The single chloroplast of complicated shape is parietal, with numerous lobes (Fig. 4), without the thylakoidal girdle lamella (Fig. 15).

The dimensions of the globular cells are (6,9–)7,3–10,4(–13) μ m. The angular cells 7,2–9 μ m in diameter. The width of elongated cells (5,5–)5,5–7,7(–8), the length (8,3–)10,2–14,5(–19,5) μ m. The width: long ratio is 1:1,6–2,8.

The reproduction takes place by means of autospores and zoospores. The autospores are produced mainly by two to eight (Figs 4b, c) The elongated cells produce almost entirely only two autospores (Fig. 29).

The naked zoospores are of elongated sideward obliterated lageniform and flask-like shape (Fig. 4d). They possess one parietal chloroplast. Near the cell apex the prominent yellow-red extraplastidial stigma of irregular shape is clearly visible. The zoospores possess one apical flagellum. The dimensions of the zoospores are $7-15 \times 3-4 \times 1,5-3 \mu\text{m}$.

The life cycle with all observed developmental possibilities is pictured on Figure 7. The developmental sequence of globular/angular and elongated cells was retained in cultures cultivated both on BBM and in biphasic soil-water media as well as in the populations grown under the coverslip on the soil surface. However, in more diluted media – e.g. BBM/5 the

Fig. 5. The life cycle of *Pseudellipsoidion edaphicum*, VC-E – ellipsoidal-stage cells, VC-G – globular-stage cell, HAS – hemiautospores, ZS – zoospores, AS – autospores. The not observed, but expected developmental pathways, are marked with the question mark.

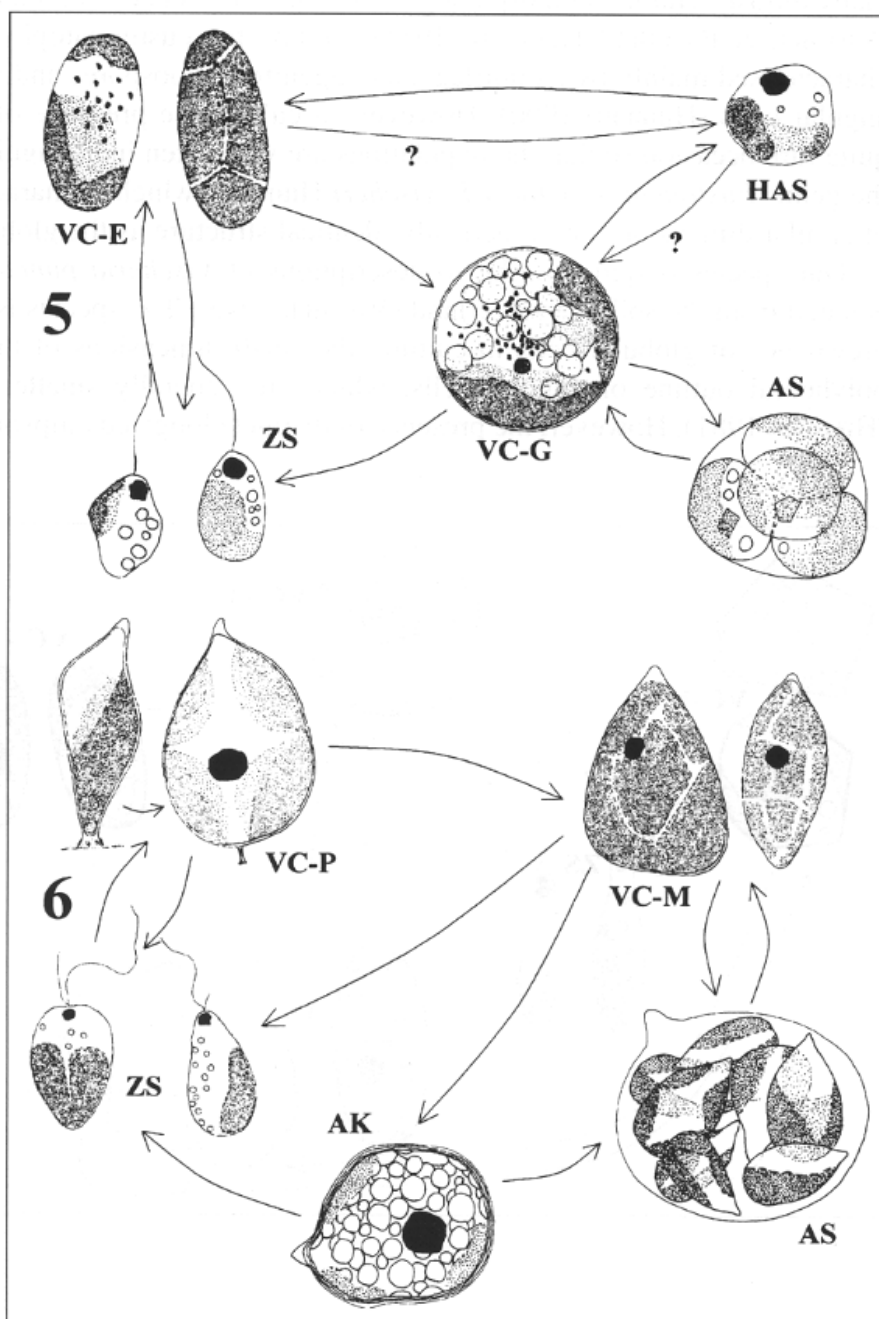


Fig. 6. The life cycle of *Pseudocharaciopsis ovalis*, VC-P – sessile-stage cells, VC-M – free-living-stage cells, AK – akinete-like cells, AS – autospores, ZS – zoospores.

elongated cells were produced more quickly than in nutrient rich solutions. There has been no observed pattern in frequency and amount of angular cells production. They have occurred seldom and irregularly both on agar and in liquid media, both in BBM and biphasic soil-water media and in all temperatures used for cultivation. However, the angular cells occurred more often in natural samples grown under the coverslip on the soil surface than in the cultures.

The genus *Vischeria* PASCHER has been originally delimited for Xanthophyceae in which the in the principle globular cells are raised into projections grading from a small number of relatively large rounded swellings to a great number of conical projections (PASCHER 1939). The relative genus *Polyedriella* PASCHER has been created for species with rather polyhedral cell outline. After the eustigmatophycean nature of the type species *Vischeria stellata* (CHODAT ex POULTON) PASCHER had been stated (HIBBERD & LEEDALE 1971, 1972), the genus was transferred into the class Eustigmatophyceae (HIBBERD 1981). As the species *Polyedriella helvetica* VISCHER et PASCHER in PASCHER is closely related to known *Vischeria* species in many ultrastructural and morphological features it has been combined as *Vischeria helvetica* (VISCHER et PASCHER) HIBBERD (1981). Today, the eustigmatophycean genus *Vischeria* is characterised mainly by its uniflagellate lageniform zoospores and the capability of forming angular cells (HIBBERD 1990). However, in culture the presence of angular cells may be a quite rare occasion so that the populations are very often indistinguishable from members of the genus *Eustigmatos*, namely *E. vischeri* HIBBERD, which is characterised by globular cells of similar dimensions and practically identical structure as the globular cells of our species.

Our species is quite similar to descriptions of *Vischeria punctata* VISCHER, the species isolated from the soil in Switzerland (VISCHER 1945). The species is characterised by a great prevalence of globular cells in culture, the small dimensions of the cells and the irregular polyhedral outline of angular cells, which are generally smaller than the globular cells (HIBBERD 1981). However, the presence of distinct elongated ellipsoidal stage in the life cycle

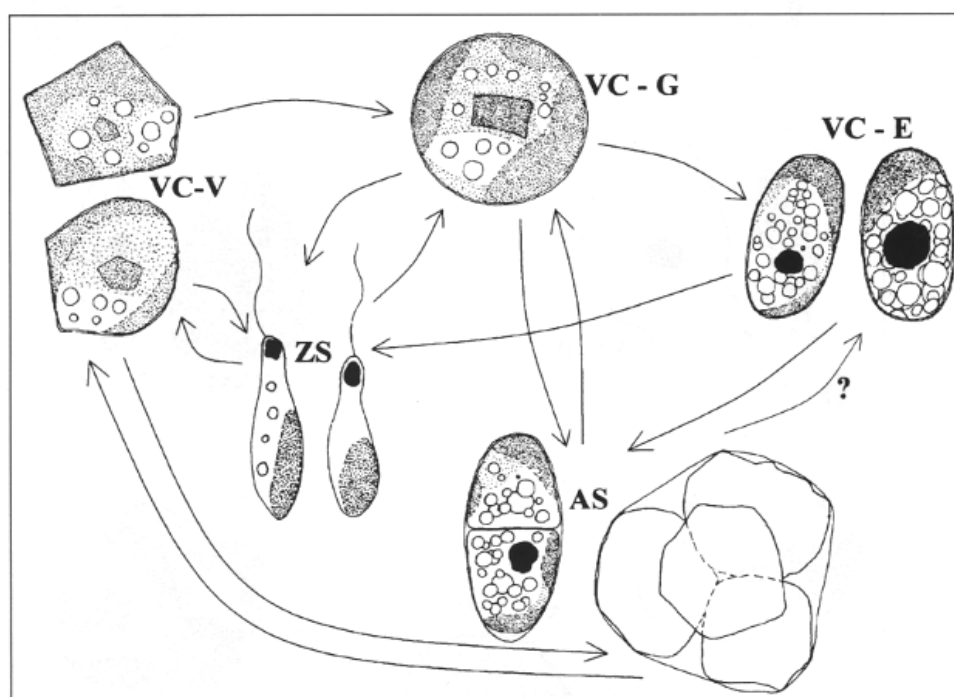


Fig. 7. The life cycle of *Vischeria* sp., **VC-V** – angular cells, **VC-G** – globular cells, **VC-E** – elongated-stage cells, **AS** – autospores, **ZS** – zoospores. The not observed, but expected developmental pathway, is marked with the question mark.

has not yet been reported in the genus *Vischeria*. Until the cultures of *Vischeria punctata* will be carefully investigated, we cannot state whether our alga should be interpreted as *V. punctata* or as separate species.

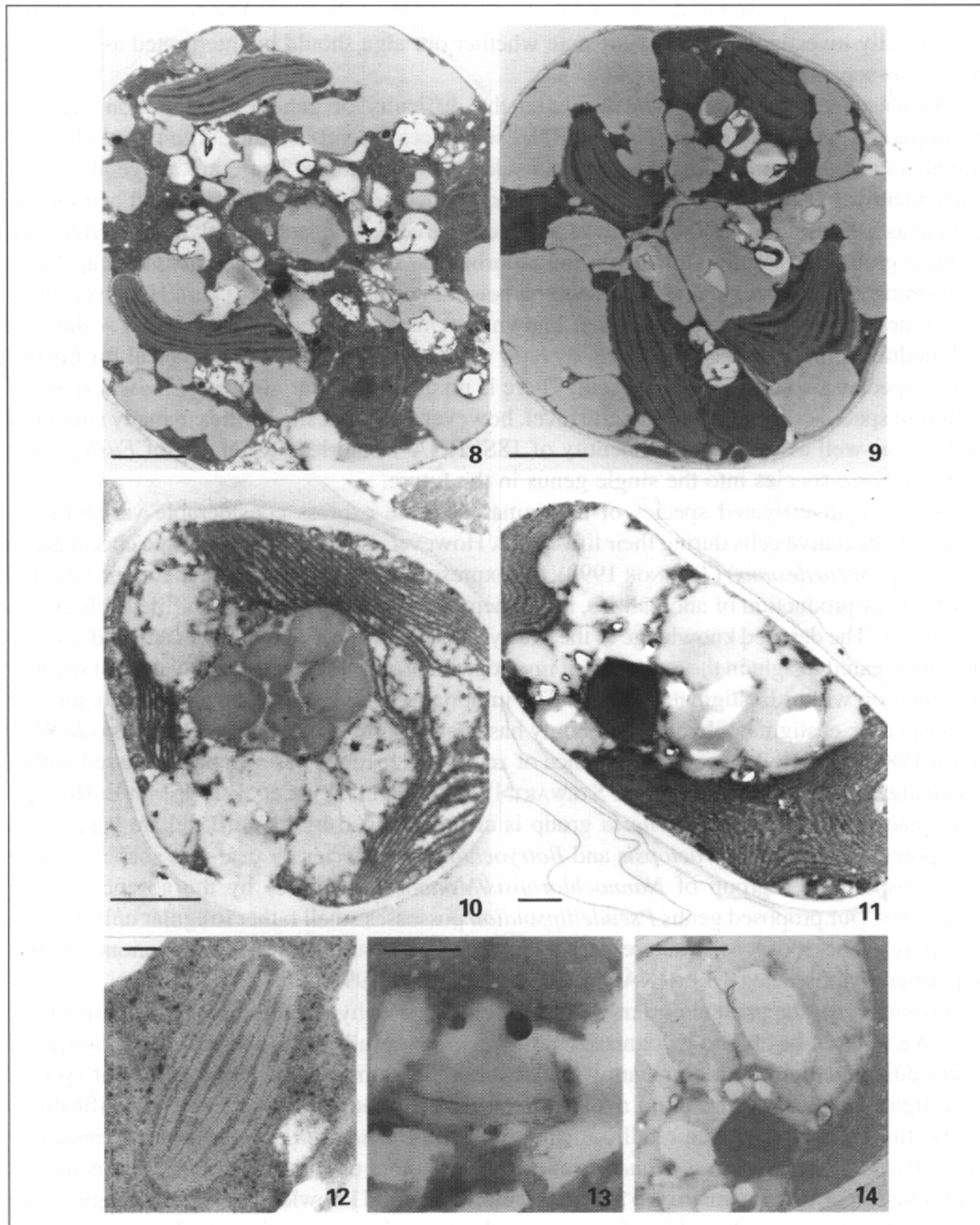
ANDERSEN et al. (1998) have discussed the difficulty of generic delimitation between *Eustigmatos* and *Vischeria* as the 18S rRNA sequences are very similar. The morphology of members of these genera also displays considerable similarities. These are above all practically identical shape and structure of zoospores, which are elongated lageniform and uniflagellate as well as quite similar structure of vegetative cells with stalked polyhedral pyrenoids, reddish globule and single lobed parietal chloroplast. Moreover, there is also the capability of forming slightly irregular autospores by type strain of *E. vischeri* (Fig. 3a). However, there are some marked differences between known *Vischeria* species and *E. vischeri* – the true polyhedral cells are never developing by later species and the “giant cell” typical for *Eustigmatos* species are occasionally present. There is no doubt that our alga and *E. vischeri* are of different specific origin. At the generic level, however, the great similarities in many structural features as well as the practical identity of 18S rRNA would lead to joining of *Eustigmatos* and *Vischeria* species into the single genus in the future.

All three investigated species of Eustigmatophyceae express considerable variability in shape of vegetative cells during their life cycles. However, similar to polymorphic coccal green algae (e.g. *Scenedesmus*) (TRAINOR 1998), the expressed variability is **not** a random phenotypic plasticity or production of anomalies, but rather the ontogenetic sequence of life cycle stages or morphs. The detailed knowledge of the life cycle peculiarities acquired from detailed, careful and open-eared insight in the ontogeny of investigated organisms can be readily used in specific delimitation within Eustigmatophyceae. The importance of zoospores shape and structure for supraspecific eustigmatophycean taxonomy has been reported (HIBBERD 1981, PREISIG & WILHELM 1989). The phylogenetic importance of zoospores features has also been stressed within green algae (ETTL 1981, MATTOX & STEWART 1984, VAN DEN HOEK et al. 1995). In Eustigmatophyceae the *Eustigmatos/Vischeria* group is clearly defined by its uniflagellate lageniform zoospores, the *Pseudocharaciopsis* and *Botryochloropsis* species by pear-like shape biflagellate zoospores, the group of *Nannochloropsis/Monodopsis* species by the absence of any zoospores. Our proposed genus *Pseudellipsoidion* possesses small rather irregular uniflagellate zoospores. The zoospore-structure based supraspecific eustigmatophycean taxonomy is also supported by published 18S rRNA data (ANDERSEN et al. 1998).

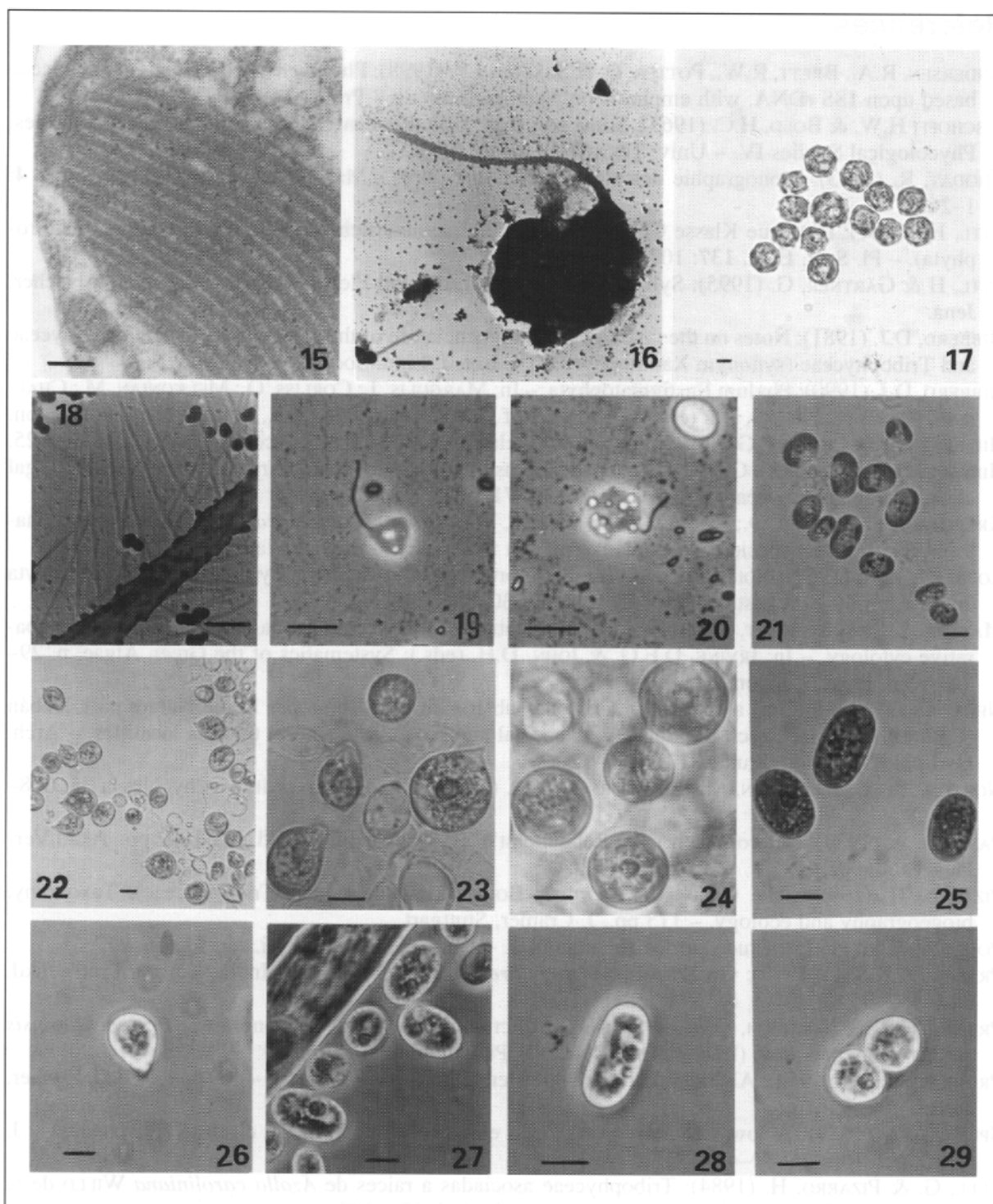
Probably, for the present our knowledge of eustigmatophycean biodiversity is in its infant age. We will not be able to recognise it until we will carefully investigate the peculiarities of every eustigmatophycean organism we encounter. The morphological variability of species investigated in this study should avoid us from hurried and careless specific identifications in the floristic lists. We can readily misinterpret some *Vischeria* species as *Eustigmatos* or some *Pseudocharaciopsis* as *Monodus* until we learn more on the morphology, ontogeny and reproduction of the organism. On the other side, with good knowledge of morphological and structural features we should be able to properly determine the eustigmatophycean species.

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Figs 8, 9, 12, 13. *Pseudellipsoidion edaphicum*, **8** – the section through the vegetative cell, **9** – the section through the developing autosporangium, **12** – the section of the chloroplast without the thylakoidal girdle lamella, **13** – the vesicle with lamellate content. **Figs 10, 11, 14.** *Vischeria* sp., **10** – the section through the vegetative cell with reddish globule, **11** – the section through the vegetative cell with polyhedral pyrenoid, **14** – polyhedral pyrenoid connected with chloroplast matrix by plasmatic membrane. [8 – 11: bar = 1 μ m, 12, 13: bar = 0,1 μ m, 14: bar = 2 μ m]



Figs 15, 17, 25, 27–29. *Vischeria* sp., 15 – the section through the chloroplast without the thylakoidal girdle lamella, 17 – the angular vegetative cells, 25, 27, 28 – the elongated-stage vegetative cells, 29 – two autospores with one bearing the parental reddish globule.

Figs 16, 18 – 21, 24. *Pseudellipsoidion edaphicum*, 16 – the zoospore with heterokont flagella, 18 – the detail of flagella with mastigonemes, 19, 20 – the uniflagellate zoospores, 21 – the ellipsoidal-stage vegetative cells, 24 – the globular-stage vegetative cells.

Figs 22, 23, 26. *Pseudocharaciopsis ovalis*, the free-living-stage *Monodus*-like vegetative cells. [15: bar = 0,1 μ m, 16: bar = 1 μ m, 18: bar = 0,3 μ m, 17, 19 – 29: bar = 5 μ m]

References

- ANDERSEN, R.A., BRETT, R.W., POTTER, D. & SEXTON, J.P. (1998): Phylogeny of the Eustigmatophyceae based upon 18S rDNA, with emphasis on *Nannochloropsis*. – *Protist* **149**: 61–74.
- BISCHOFF, H.W. & BOLD, H.C. (1963): Some soil algae from Enchanted Rock and related algal species. *Phycological Studies* IV. – Univ. Texas Publ. **6318**: 1–95.
- CHODAT, R. (1913): Monographie des algues en culture pure. – *Mat. pour la flore Cryptog. Suiss* **4**: 1–266.
- ETTL, H. (1981): Die neue Klasse Chlamydomphyceae, eine natürliche Gruppe der Grünalgen (Chlorophyta). – *Pl. Syst. Evol.* **137**: 107–126.
- ETTL, H. & GÄRTNER, G. (1995): *Syllabus der Boden-, Luft- und Flechtenalgen*. – 721 pp., G. Fischer, Jena.
- HIBBERD, D.J. (1981): Notes on the taxonomy and nomenclature of the algal classes Eustigmatophyceae and Tribophyceae (synonym Xanthophyceae). – *Bot. J. Linn. Soc.* **82**: 93–119.
- HIBBERD, D.J. (1990): Phylum Eustigmatophyta. – In: MARGULIS, J.; CORLISS, O.; MELKONIAN, M.; CHAPMAN, D.J. & MCKHANN, H.I. (eds.): *Handbook of Protocista*, p. 326–333., Jones & Bartlett, London.
- HIBBERD, D.J. & LEEDALE, G.F. (1971): A new algal class - the Eustigmatophyceae. – *Taxon* **20**: 523–525.
- HIBBERD, D.J. & LEEDALE, G.F. (1972): Observations on the cytology and ultrastructure of the new algal class, Eustigmatophyceae. – *Ann. Bot.* **36**: 49–71.
- KRIENITZ, L.; HEPPERLE, D.; STICH, H.B. & WEILER, W. (2000): *Nannochloropsis limnetica* (Eustigmatophyceae), a new species of picoplankton from freshwater. – *Phycologia* **39**: 219–227.
- LOEBLICH, A.R. (1967): Notes on the divisions Chlorophyta, Chrysophyta, Pyrrophyta and Xanthophyta and the family Paramastigaceae. – *Taxon* **16**: 230–236.
- MATTOX, K.R. & STEWART, K.D. (1984): Classification of the green algae: a concept based on comparative cytology. – In: IRVINE, D.E.G. & JOHN, D.H. (eds.): *Systematics of the Green Algae*, p. 29–72, Acad. Press, London.
- NEUSTUPA, J. (2001): Soil algae from marlstone-substratum based biotopes in the Nature park Džbán (Central Bohemia, Czech Republic) with special attention to the natural treeless localities. – *Arch. Hydrobiol./Algolog. Studies* **101**: 109–120.
- NORTON, T.A.; MELKONIAN, M. & ANDERSEN, R.A. (1996): Algal biodiversity. – *Phycologia* **35**: 308–326.
- PASCHER, A. (1939): Heterokonten. – In: Rabenhorst's Kryptogamenflora, Bd. 11., 1092 pp., Akad. Verlagsges., Leipzig.
- PIZARRO, H. (1995): The Genus *Characiopsis* BORZI (Mischococcales, Tribophyceae). Taxonomy, biogeography and ecology. – 113 pp., J. Cramer, Stuttgart.
- POULTON, E.M. (1925): Etude sur les Heterocontes. – Univ. Genev. Fac. Sci. sér. **11**, Th. 777.
- PRASAD, A.K.S.K. (1979): On *Ellipsoidion regulare* PASCHER from South India. – *Arch. Protistenkd.* **122**: 352–359.
- PREISIG, H.R. & WILHELM, C.H. (1989): Ultrastructure, pigments and taxonomy of *Botryochloropsis similis* gen. et sp. nov. (Eustigmatophyceae). – *Phycologia* **28**: 61–69.
- PRINGSHEIM, E.G. (1954): *Algenreinkulturen, ihre Herstellung und Erhaltung*. – 109 pp., VEB G. Fischer, Jena.
- SPURR, A.R. (1969): A low viscosity epoxy resin embedding medium for electron microscopy. – *J. Ultrastruc. Res.* **26**: 31–43.
- TELL, G. & PIZARRO, H. (1984): Tribophyceae asociadas a raíces de *Azolla caroliniana* WILLD de la provincia De Corrientes (Argentina). – *Crypt. Algal.* **4**: 171–188.
- TOMPKINS, J.; DE VILLE, M.M.; DAY, J.G. & TURNER, M.F. (1995): Culture Collection of Algae and Protozoa. Catalogue of Strains. – 204 pp., Tit. Wilson & Son Ltd., Kendal, U.K.
- TRAINOR, F.R. (1998): Biological aspects of *Scenedesmus* (Chlorophyceae) phenotypic plasticity. – *Nova Hedwigia Beihefte* **117**: 367 pp., J. Cramer, Stuttgart.
- VAN DEN HOEK, C., MANN, D.G. & JAHNS, H.M. (1995): *Algae. An introduction to phycology*. – 627 pp., Cambridge Univ. Press, Cambridge.
- VISCHER, W. (1945): Heterokonten aus alpinen Böden, speziell dem schweizerischen Nationalpark. – *Ergebn. wiss. Unters. Schweiz. Nationalpark, N. F.* **1**: 477–512.