Morphology, cytology and sexual reproduction in the aerophytic cave diatom *Luticola dismutica* (*Bacillariophyceae*)

Morfologie, cytologie a pohlavní rozmnožování aerofytické jeskynní rozsivky *Luticola dismutica* (*Bacillariophyceae*)

Aloisie P o u l í č k o v á

Department of Botany, Faculty of Science, Palacký University, Svobody 26, CZ-771 46, Olomouc, Czech Republic; e-mail: aloisie.poulickova@upol.cz

Poulíčková A. (2008): Morphology, cytology and sexual reproduction in the aerophytic cave diatom *Luticola dismutica* (*Bacillariophyceae*). – Preslia 80: 87–99.

Monoclonal cultures of the aerophytic cave diatom *Luticola dismutica* were studied and its frustule morphology, cytology and reproduction recorded. *Luticola dismutica* is a laterally asymmetrical, monoplastidic pennate diatom with imposed chloroplast division and nuclear behaviour of type 1.A sensu Mann & Stickle. Clones of *L. dismutica* decreased in cell size in culture until they have reached the sexual size range. Homothallic sexual reproduction and auxosporulation (type IB1a auxosporulation sensu Geitler) were induced in four sexualized clones. Gametangia paired via the girdle, two isogametes were formed per gametangium and hence two zygotes were produced per pair of gametangia. No surviving superfluous nuclei were observed in the gamete and zygote stages and no unfused haploid nuclei were seen in the auxospore stage; zygotes and expanded auxospores had only one nucleus. Auxospores expanded perpendicular to the apical axis of gametangia. Expanded auxospores and initial cells had a swollen central part, the linear-lanceolate outline shape of the vegetative valves was restored during the first divisions of the post-initial cells. Initial cells left the perizonium by a route unique to pennate diatoms, through a transverse rupture of the perizonium. The key cytological and reproductive characteristics reviewed in this paper indicate, that *Luticola* is more closely related to *Placoneis* and *Dickieia*, than to *Navicula* sensu stricto.

K e y w o r d s: auxosporulation, diatoms, homothally, meiosis, sexual reproduction

Introduction

The genus *Luticola*, containing 30 species, was separated from the genus *Navicula* by Round et al. (1990) on the basis of frustule ultrastructure. Morphological differences in the frustule are usually accompanied by cytoplasmic and reproductive differences (Cox 1998, Medlin & Kaczmarska 2004). In contrast to *Navicula* s.s., which has two chloroplasts, *Luticola* spp. contain a single chloroplast, similar to genera *Sellaphora, Dickieia, Anomoeoneis, Brachysira* and *Placoneis* (Cox 1980, 1996, 2003b, Mann 1996a, Cox & Williams 2006). Some naviculoid diatoms have been intensively studied (Cox 1982, 1985, 2003a, b, 2004, Cox & Reid 2004), however, morphological and cytological investigation of many species/genera are needed for better understanding of diatom diversity (Finlay et al. 2002, Vyverman et al. 2007). Although there are many species in the *Luticola* complex (Van de Vijver et al. 2006) nothing is known about their sexual reproduction.

In most pennate diatoms, mean cell size declines during the vegetative phase of the life cycle as a result of the rigidity of their silica frustules (Lewis 1984, Chepurnov et al. 2004). Original cell size is restored by means of auxospore formation, which is commonly connected with sexual reproduction (Chepurnov et al. 2004). The first references to diatom

sexual reproduction were published 150 years ago (Thwaites 1847, Griffith 1855, Smith 1856) and enough is now known to establish the main features of auxosporulation and show that the sexual process is highly variable. On the other hand, the vast majority of species have never been observed undergoing auxosporulation, although variation in size and shape of the vegetative cells indicate that it must occur.

The genus *Luticola* belongs to the raphid diatoms, which are predominantly allogamous (Geitler 1973, Chepurnov et al. 2004). Homothally is known in *Sellaphora* "elliptical" deme (Chepurnov et al. 2004) and in some species of *Nitzschia, Haslea, Eunotia, Seminavis* and *Amphora* (reviewed by Chepurnov et al. 2004). Homothally has also recently been observed in *Navicula cryptocephala* (Poulíčková & Mann 2006) and *Pinnularia* sp. (A. Poulíčková & D. G. Mann, unpublished data).

In this paper sexual reproduction in *Luticola* is reported for the first time. The key cytological and reproductive characteristics are documented and compared with those of closely related genera.

Materials and methods

Luticola dismutica (Hustedt) D. G. Mann was collected from the wall of the Podkova natural cave in the Mladeč karst, Czech Republic (coordinates 49°42'43" N; 17°00'45" E) on 19 April 2006 (Poulíčková & Hašler 2007). Algal crusts were scraped of the wall using a scalpel, placed in labelled plastic bags and used as inocula for cultures on agar plates. Clonal cultures were obtained by isolation of colonies from agar plates after ca 2 weeks and transfer to WC medium with silica (Guillard & Lorenzen 1972) in 25-well 100 mm square Petri dishes (Sterilin, Barloworld Scientific, Stone, UK). Subsequently, cultures were transferred to 50-mm Petri dishes. Cultures were maintained under a constant temperature of 17 °C with cool-white fluorescent lights providing 12h light per day and 30 $\mu mol \cdot m^{2} \cdot s^{-1}$.

Living cells were observed using light microscopy (LM) following the methods described by Poulíčková & Mann (2006) and Poulíčková et al. (2007). The most sensitive gamete stages were observed directly in Petri dishes using an inverted microscope (Zeiss Axiovert), or by LM using a Zeiss Axioimager with a ×63 water immersion (WI) objective. Samples for fluorescence microscopy (FM) were fixed with PGA solution (2.5% glutaraldehyde in 0.1M phosphate buffer, pH 7.0; Karnovsky 1965). Cells were stained with DAPI (4,6-diamino-2-phenylindole.2HCl; Sigma, St. Louis, MO, USA) as described in detail by Poulíčková et al. 2007. To test for the presence of mucilage around gametangia and auxospores, cover-slips were mounted on suitably diluted Indian ink and excess ink drawn off with tissue. Cleaned valves were prepared by boiling in a mixture of concentrated sulphuric and nitric acids, followed by washing with distilled water. Valves were mounted in Naphrax (currently available from Brunel Microscopes, Chippenham, Wiltshire, SN14 6NQ, UK: http://www.brunelmicroscopes.co.uk/). Photomicrography (LM, FM) was carried out using a Zeiss Axioimager with a Zeiss Axiocam HRc digital camera (Carl Zeiss, Jena) capable of 1388 × 1040 pixel resolution. Images were captured and managed using Zeiss Axiovision Version 4.5 imaging software. Bright field (BF) or differential interference contrast (DIC) optics were used at ×100, ×40 or ×63 (planapochromat lenses, nominal numerical aperture 1.32, 1.4 and 0.95). For FM we used a Zeiss DAPI filter set 001 and 49 with the same objectives.

For scanning electron microscopy (SEM), cover-slips with material were treated with fuming nitric acid (> 95% HNO3; below boiling point), attached to aluminium stubs (for details see Poulíčková et al. 2007) and coated with platinum for 2 min in an Emitech K575X sputter coater and examined using a LEO Supra 55VP Field Emission SEM operated at 5kV (6 mm working distance; aperture 20 μm). Images were captured as 3 Mb TIFF files.

Voucher material of clones and source populations are kept in the diatom herbarium of the Department of Botany, Palacký University Olomouc, Czech Republic.

Results

Morphological and cytological characteristics

Isolated clones were identified as *Luticola dismutica* (Hustedt) D. G. Mann; formerly *Navicula dismutica* Hustedt or *Navicula suecorum* var. *dismutica* (Hustedt) Lange-Bertalot (cf. Krammer & Lange-Bertalot 1986, Round et al. 1990). Length and outline shape changes during the vegetative phase of the life cycle are illustrated in Fig. 1 and Table 1. Initial cells (Fig. 1: 5) are centrally swollen, each half in the form of a tapering cylinder, contrasting strongly with the lanceolate shape with clearly differentiated valve face and mantle of normal cells (cf. Fig. 1: 1). Although post-initial cells are lanceolate with slightly subcapitate ends, small vegetative cells within the sexual size range were almost elliptical (cf. Fig l: 7–11 and Fig 1: 1, 12–13). Cell dimensions and stria densities are given in Table l. Valves are bilaterally asymmetrical with a small isolated stigma on one side of the central area (Fig.1: 7–13). Although terminal raphe fissures are not usually well developed, central raphe ends are always curved to the opposite side (without stigma), the primary side of the valve. As a result of valve morphogenesis pathway, faults in stria pattern, previously named Voigt discontinuities (Mann 1983, 2006) are present (Fig. 1: 8–9) on the secondary side of the valve (with the stigma).

Live cells have a single, complex chloroplast, H-shaped in girdle view, lying with its centre along the secondary side of the girdle (Fig. 2: 16). One pyrenoid, flattened in girdle view (Fig. 2: 16), oval (layered internally) in valve view (Fig. 2: 14), lies in the centre of the chloroplast near the girdle (cell side with stigma = secondary valve side), slightly eccentric with respect to the apical axis of the cell (Fig. 2: 14). Two lobes of the chloroplast extend beneath each valve face, forming a K-shape in valve view (Fig. 2: 14). Interphase cells usually seen in valve view but dividing cells, being deeper, were often seen in girdle view (Fig. 2: 17–21). No mucilage was secreted by vegetative cells. Only "cis" cells, with primary sides of both valves on the same side of the cell (Fig. 2: 27–29), were observed during the study. The interphase nucleus is displaced towards the primary cell side (that opposite the pyrenoid), displaced eccentrically with respect to the apical axis in the opposite direction to the pyrenoid (Fig. 2: 14). The chloroplast and nucleus did not move during mitosis. The chloroplast division was imposed; the cleavage furrow divides the chloroplast during cytokinesis (Fig. 2: 18–19). Nuclear division is illustrated in Fig. 2: 25–26.

Homothallic reproduction in monoclonal cultures

Clones LUTM45–48 were isolated on 17 May 2006 and clones LUTM 52–96 on 31 July 2006, four of which (clones LUTM47, 48, 59, 67) exhibited sexual reproduction in

Table 1. – Valve dimensions and stria densities of clones of *Luticola dismutica*. Values are mean ± standard deviation (range)

Clone	Date of culture	Cell type	n	Length (μm)	Breadth (μm)	Stria density (10 μm^{-1})
LUTM45	29.6.06	vegetative	10	21.2±0.9 (20–23)	6.3±0.4 (6–7)	16.0±1.3 (14–18)
	4.10.06	vegetative	10	19.7±0.8 (18.5–21.0)	6.0±0.3 (5.5–6.5)	17.0±1.3 (14–18)
LUTM47	1.8.06	vegetative[1]	10	15.3±2.5 (13–20)	5.9±0.2 (5.5–6.0)	16.6±1.3 (14–18)
	1.8.06	initial	10	32.4±1.0 (31–35)	7.7±0.6 (6.5–9.0)	16.2±1.4 (14–18)
LUTM48	7.6.06	vegetative	10	16.3±0.8 (15–18)	6.1±0.2 (6.0–6.5)	17.0±1.0 (16–18)
	29.6.06	vegetative[1]	30	15.0±0.8 (13–17)	5.9±0.3 (5.0–6.5)	17.1±1.5 (14–20)
	29.6.06	initial	10	33.4±1.7 (29–35)	7.4±0.4 (6.5–8.0)	16.0±1.3 (14–18)
LUTM52	4.10.06	vegetative	10	21.2±0.9 (20–22)	6.4±0.4 (6–7)	17.4±1.3 (16–20)
LUTM53	4.10.06	vegetative	10	22.0±1.0 (20–23)	6.3±0.3 (6.0–6.5)	17.0±1.0 (16–18)
LUTM59	4.10.06	vegetative	10	20.7±0.9 (19–22)	6.3±0.4 (6–7)	18.0±1.6 (16–20)
	19.4.07	vegetative[1]	10	16.6±0.9 (15–18)	6.0±0.3 (5.5–6.5)	17.0±1.3 (16–20)
	19.4.07	initial	10	35.8±2.3 (33–41)	8.2±0.5 (7.5–9.0)	16.8±1.6 (14–20)
LUTM60	4.10.06	vegetative	10	22.9±0.9 (22–25)	6.5±0.3 (6–7)	17.6±2.0 (14–20)
LUTM61	4.10.06	vegetative	10	24.9±1.1 (23–27)	6.6±0.3 (6–7)	16.6±1.6 (14–18)
LUTM67	4.10.06	vegetative[1]	10	14.1±0.8 (13–15)	5.9±0.4 (5.0–6.5)	17.6±1.5 (16–20)
	4.10.06	initial	10	32.8±0.4 (32–33)	7.6±0.5 (7–8.5)	17.6±1.7 (14–20)
LUTM71	4.10.06	vegetative	10	23.0±1.6 (21–25)	6.8±0.6 (6–8)	17.0±2.2 (14–20)
LUTM86	4.10.06	post-initial	10	31.4±1.4 (29–34)	7.4±0.3 (7–8)	17.2±1.6 (16–20)
LUTM89	4.10.06	vegetative	10	22.9±0.5 (22–24)	6.8±0.3 (6.5–7.5)	17.6±2.0 (14–20)
LUTM95	4.10.06	vegetative	10	22.3±1.0 (20–24)	6.4±0.3 (6–7)	17.6±1.7 (16–20)
LUTM99	4.10.06	post-initial	10	32.7±1.6 (31–35)	7.5±0.2 (7–8)	17.6±1.5 (16–20)

[1] Homothallic auxosporulation observed

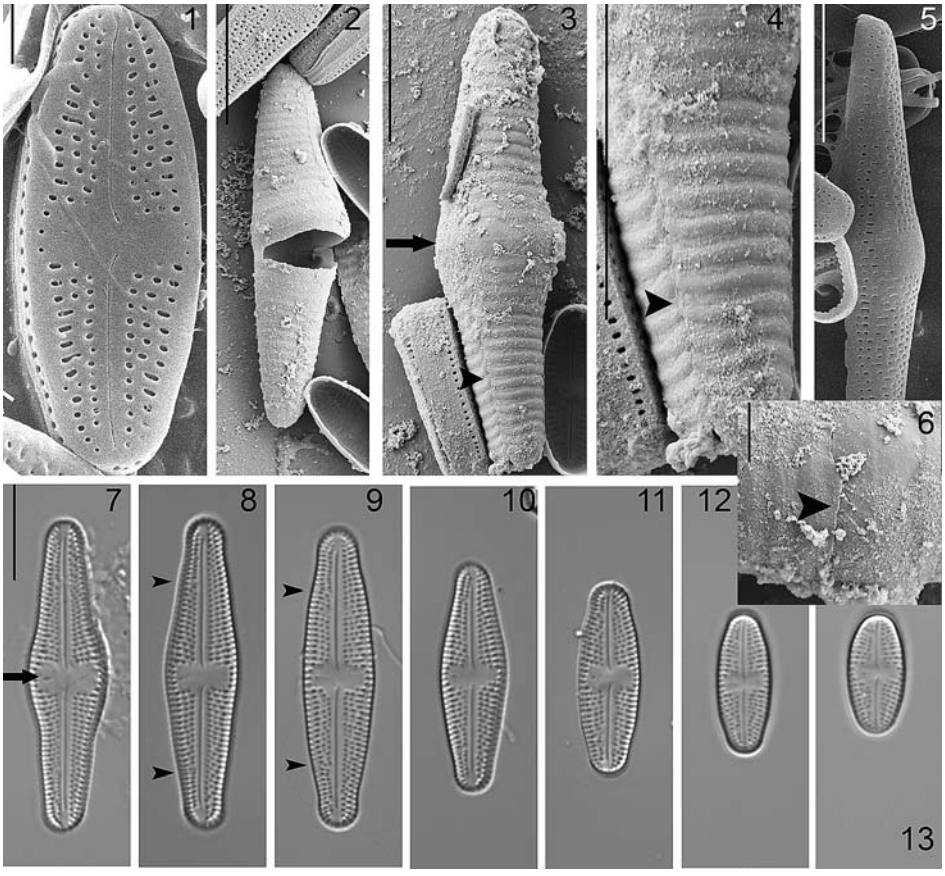
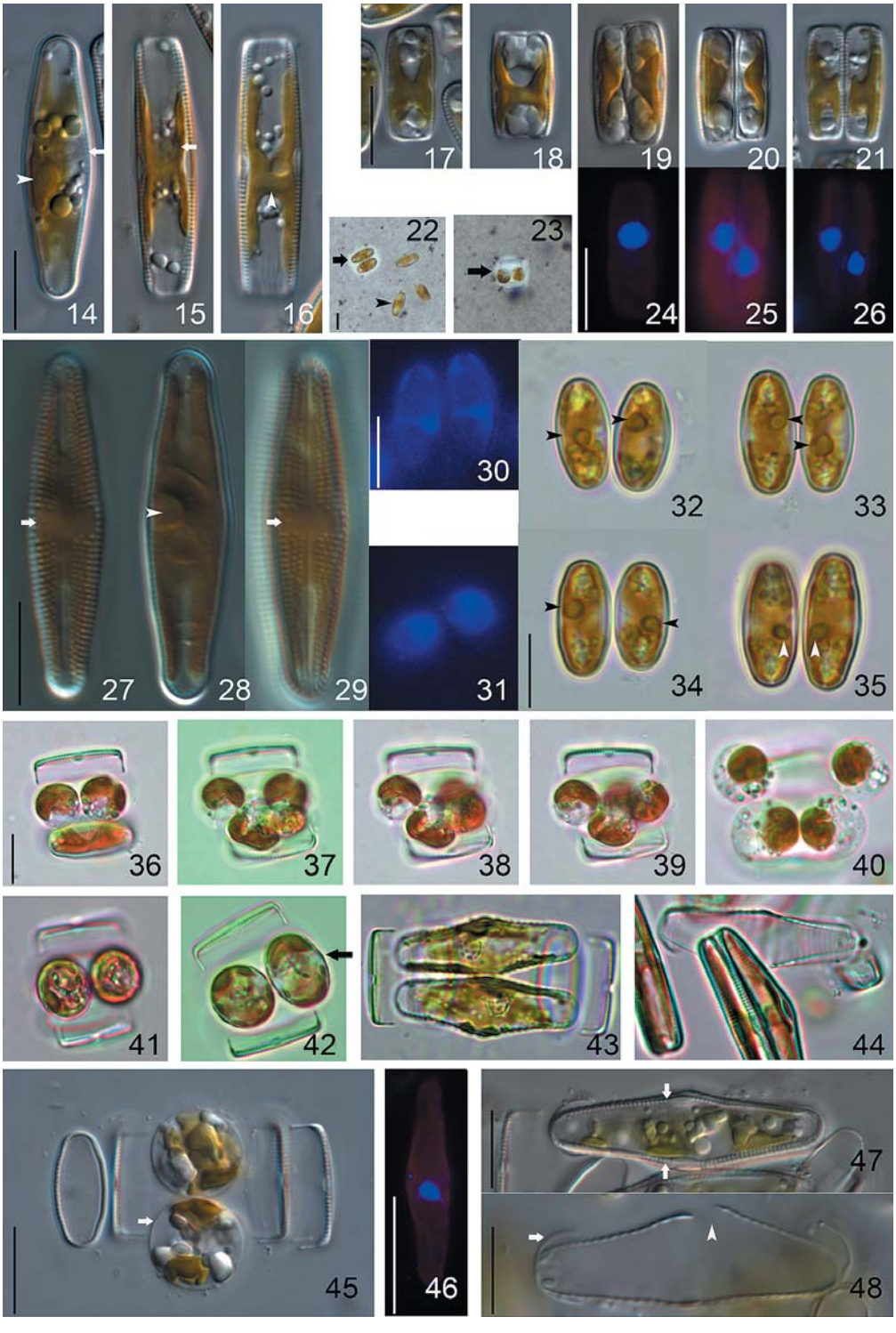


Fig. 1. – *Luticola dismutica* clone LUTM48 Poulíčková 2006: all cells in valve view with primary side to the right; SEM (1–6); LM, DIC optics (7–13). Scale bar = 10 µm except for 1, 4 = 2 µm. **1** cell within sexual size range; **2** empty perizonium left by initial cell via a transverse rupture of the perizonium; **3** fully expanded auxospore (perizonium), note swollen central part, wider primary perizonial band (arrow), narrower secondary bands with ends creating suture (arrowhead); **4** the same auxospore, detail of suture; **5** initial valve; **6** detail of primary perizonial band with rupture (early stage); **7** post-initial cell with swollen central part, **8–10** lanceolate vegetative cells, **11–13** small cells within sexual size range, note stigma (arrow) in central area and Voigt discontinuities (arrowheads) on secondary side; central raphe endings are curved to the right (primary side).

monoclonal culture. Auxospore formation was first observed on 7 June 2006 in clone LUTM48, when cells had an average length of 15.0 µm. Other clones auxosporulated later, when their average cell lengths had declined to 15.00–16.55 µm (e.g. LUTM59 on 19 April 2007). The frequency of reproduction increased 3–5 days after each subculturing. The proportion of small, sexually competent cells declined in cultures during the two months following clone isolation, due to the increase in enlarged (initial and post-initial) cells, which eventually made up reached approximately 50–90% of the culture. Sexual reproduction was vigorous in monoclonal cultures and produced viable initial cells that divided rapidly. This population of *L. dismutica* is therefore homothallic. No interclonal crosses were made, as the clones were not sexualized at the same time. Clones LUTM45, 52, 53, 60, 61, 71, 86, 89, 95, 99, with cell lengths > 19 µm, did not reproduce during the study period.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During reproduction, cells paired actively and became aligned side-by-side (girdle-girdle pairing; Fig. 2: 30, 32–35). Sexualized cells came together in pairs 3–4 days after subculturing; no triplets or larger groups of gametangia, as described in other diatoms, were found (e.g., Mann & Stickle 1995). No mucilage envelope was apparent without staining. Indian ink staining revealed a thin layer of watery mucilage around pairing cells, gametangia and zygotes; no mucilage was secreted by vegetative cells (Fig. 2: 22, 23).

After pairing, cells began meiosis and became gametangia. This process was not accompanied by chloroplast or nuclei movements. If the side of the cell occupied by the nucleus is termed the primary side (Mann 1983) three configurations were observed during pairing. The configurations were primary-primary (2 cases observed), primary-secondary (15 cases) and secondary-secondary (12 cases; Fig. 2: 30, 32–35). Cytokinesis occurred after the meiotic division, 1–2 days later, so that two gametes were produced per gametangium (Fig. 2: 36–40). Almost simultaneously, the gametangial frustules dehisced and the naked gametes rounded up. Gamete rearrangement took approximately 20–30 minutes (Fig. 2: 36–40). As these stages were sensitive to disturbance and temperature change, they were mostly only observed directly in Petri dishes at low magnification. Each gamete appeared to be naked, and contained one chloroplast (Fig. 2: 40) and one nucleus (not illustrated). Some of the gametes were lost, being extremely sensitive during all stages of sexual reproduction. After plasmogamy, one day later, the zygotes contracted, became spherical (Fig. 2: 41, 45), and secreted a thin wall. Zygotes contained two chloroplasts. Immediately after gamete fusion zygotes contained two nuclei, but fully rounded zygotes had only one diploid nucleus (Fig. 2: 31).

Trikaryotic auxospores were observed in clone LUTM59 on 19th April 2007. They were uncommon compared to diploid auxospores (in only 10 cases out of 97 pairs). These sexual configurations included one larger zygote with three chloroplasts (the result of the fusion of three gametes) and the remnant of an unfused aborting gamete. Trikaryotic auxospores developed like dikaryotic ones, but had three chloroplasts and were broader

Fig. 2. – *Luticola dismutica* clones LUTM48 and 59 Poulíčková 2006: live vegetative cells and sexual stages, LM with DIC optics (14–21, 27–29, 45, 47–48), FM after DAPI staining (24–26, 30–31, 46), BF after Indian ink staining (22–23), LM with WI optics 32–35, 36–44. Scale bar = 10 µm. **14** post-initial cell, valve view with K-shaped chloroplast, nucleus on primary side (arrow; one nucleoli visible) and pyrenoid on secondary side (arrowhead) arranged diagonally relative to one another; **15–16** post-initial cell in girdle view, two foci; note H-shaped chloroplast, nucleus (arrow) and bar-like pyrenoid (arrowhead); **17–21** vegetative cells in girdle view, stages of cell and chloroplast division, note that chloroplast division is imposed (18); **22–23** thin layer of mucilage (arrow) around pairing cells (22) and zygotes (23), note that the vegetative cells have no mucilage (arrowhead); **24** nucleus in vegetative cell, **25–26** nucleus in dividing cell (girdle view); **27–29** three foci of the same post-initial cell – upper valve with stigma – arrow (27), protoplast with pyrenoid – arrowhead (28) and lower valve with stigma – arrow (29) showing the "cis" type of the cell = both valves have primary valve part without stigma on the same cell side (rigt hand side); **30, 32–35** pairing cells, pyrenoid (arrowheads) lies on secondary side: primary-secondary (30, 32), primary-primary (34), secondary-secondary (33,35); **31** two zygotes with one nucleus in each; **36–40** gametogenesis, the series of images of the same configuration from 11:05 to 11:35 a.m., note the asynchronous gamete rearrangement (36) with lower gametes developing later, each gamete contains one chloroplast; **41, 45** rounded zygotes within wall (arrow), **42** zygotes start to expand, note that right hand zygote has already a primary band (arrow), **43** auxospores expanding perpendicular to the apical gametangial axes; **44** dividing initial cell escaping from perizonium via rupture; **46** expanded auxospore with one nucleus; **47** mature auxospore with both initial valves (arrows), note swollen central part (no protoplast contraction); 48 empty perizonium (native preparation, no treatment) with rupture (arrowhead) and caps (arrow).

(not illustrated). One configuration with one large rounded zygote containing four chloroplasts was also observed. No information is available about the further development of these polyploids.

Auxospore expansion is bipolar (Fig. 2: 42, 43), due to the creation of transverse perisonial bands (Fig. 1: 3, 4) and usually took place more or less transverse to the apical axes of the gametangia (Fig. 2: 43), within 2 days of gamete formation. As the auxospore lengthened, secondary transverse bands were added on either side of the primary band (Fig. 1: 3, 4). The transverse perizonial bands were not equal in width, the primary band being by far the widest (Fig. 1: 3). A suture is present along one side of the auxospore (Fig. 1: 4), marking the open, incurved ends of the secondary perizonial bands. The primary band, however, seems to be a complete hoop. As the auxospore expanded, its tips decreased in width, so that the final shape of the cell was lanceolate, with a swollen centre (Figs 1–2: 3, 47). Torn caps of organic material were present during and after expansion (Fig. 2: 47, 48), covering the tips of the auxospore; these were the remnants of the first organic wall formed by the zygote (Fig. 2: 45), ruptured when the auxospore began to expand.

No auxospore contraction was observed after completion of the transverse perizonium and cessation of auxospore growth. Thus, the initial cell had a swollen centre (Fig. 2: 47). Initial valve formation was accompanied by two acytokinetic mitoses (not illustrated) as described previously (Mann 1984). The initial cell left the perizonium by a transverse rupture in the centre of the perizonium, most likely between the primary and adjacent bands (Figs 1–2: 2, 6, 44, 48.). Initial cells contain one nucleus (Fig. 2: 46) and two chloroplasts until the first division. When the initial cell divided, one chloroplast was segregated into each daughter cell and assumed the shape typical of vegetative cells.

The whole process of auxosporulation, from pairing to the completion of the initial cell, took 8 days. The dimensions and stria densities of postinitial cells are given in Table 1. Initial cells are 32–35 μm long.

Discussion

Luticola dismutica is a raphid, pennate diatom with lateral cell asymmetry. All frustules exhibit "cis" symmetry, as in *Placoneis,* whereas in *Navicula* there is a 1:2 ratio of "cis" to "trans" frustules (Mann & Stickle 1995). *Luticola dismutica* belongs to the monoplastidic diatoms. It has a complex chloroplast with an isthmus and the pyrenoid lying beneath the girdle on the secondary cell side, resemble the chloroplast of the marine naviculoid diatom *Dickieia ulvacea* (Mann 1994, 1996a). A similar type of chloroplast is reported in naviculoid, cymbelloid and gomphonemoid genera such as *Brebissonia*, *Anomoeoneis*, *Placoneis*, *Cymbella*, *Encyonema* and *Gomphonema*, unlike in some other monoplastidic genera (*Sellaphora*), which have a central isthmus beneath the valve (Mann 1996a).

Chloroplast division in *L. dismutica* is not autonomous as in the majority of diatoms (*Navicula*, *Neidium*), but is imposed, as in *Dickieia ulvacea* (Mann 1994), *Placoneis gastrum* (Mann & Stickle 1995) and a few *Achnanthes*, *Nitzschia* and *Hantzschia* species (Mann 1996a). Both *Luticola dismutica* and *Dickieia ulvacea* share the same 1.A type of nuclear behaviour desribed by Mann & Stickle (1988), with the interphase nucleus adjacent to girdle and exhibiting no movement before or during mitosis. Different from *Dickieia*, the nucleus and pyrenoid in *Luticola* are eccentric with respect to the apical axis

and are arranged diagonally with respect to one another. As in *Placoneis* there are no chloroplast or nuclear movements during the cell cycle, whereas in *Navicula* both types of movement occur (Mann & Stickle 1995). Although the general features of its morphology and cytology are known, some of the new details may be important, since *Luticola*'s phylogenetic position remains unclear (Cox & Williams 2006).

The pattern of reproductive behaviour in *L. dismutica* can be classified as type IB1a auxosporulation in Geitler's (1973) scheme, because the gametangia pair via the girdle, two isogametes are formed per gametangium and after their fusion two zygotes (auxospores) are produced per pair of copulating cells. The zygotes elongate more or less transversely to the apical axes of the gametangia. However, the spatial relationships between them can be different in 40% of cases, due to abnormalities during reproduction (abortion of some gametes, zygotes, or young auxospores) or later when initial cells are leaving the perizonia. Although, this type of reproductive behaviour (IB1a auxosporulation) is recorded in only a few diatoms: *Amphora ovalis* (Geitler 1973), *Amphora copulata* (Mann & Poulíčková, unpublished data), some aspects are shared by other pennate diatoms (Table 2).

Luticola dismutica lacks a dense mucilaginous capsule, but an extremely thin layer of watery mucilage is revealed after staining (Indian ink preparation) cells around the time of pairing and gametes/zygotes, as recorded previously in *Navicula* s.s. (Mann & Stickle 1989). It is surprising that in the aerophytic diatom *L. dismutica*, which lives on more or less wet cave walls, there is little protection for the gametes and zygotes (Edlund & Spaulding 2006, Poulíčková & Hašler 2007).

The perizonium structure in *Luticola* is similar to that in *Navicula cryptocephala* (Poulíčková & Mann 2006) and *Dickieia ulvacea* (Mann 1994), particularly the swollen central part, but only in *Luticola* do the initial cells have a swollen centre. In *Dickieia* and *Navicula* the protoplast contracts markedly during the formation of the initial valves, so that these are more linear. Such contraction is more common in marine than freshwater diatoms (Mann 1994). The initial cells in *Luticola* have twice the number of chloroplasts of a normal vegetative cell, as in *Navicula cryptocephala* (Poulíčková & Mann 2006) and *Dickieia* (Mann 1994). When the initial cell divides, chloroplasts segregate into each daughter cell and assume the shape and arrangement typical of vegetative cells. Initial cells usually leave the perizonium at one end, as in *Pinnularia* (Poulíčková et al. 2007) and *Navicula* (Poulíčková & Mann 2006). The method of leaving observed in *Luticola* (via a transverse rupture of the perizonium) is new and unique for pennate diatoms.

Navicula s.l., originally an extremely large genus (Smith 1856, van Heurck 1885, Cleve 1894), has had a complicated history (reviewed by Mann & Stickle 1995) and been split into many smaller genera. Although some of the generic names are very old, some were accepted quite recently (*Placoneis* – Cox 1987, *Sellaphora* – Mann 1989, *Dickieia* – Mann 1994). The majority of "naviculoid" genera are separated primarily on the basis of clean frustule morphology, but also differ significantly in their cytology (number, shape, arrangement of chloroplasts, pyrenoids, nuclei position and behaviour during the cell cycle) and method of reproduction (Mann 1993, 1996a, Mann & Stickle 1988). Some of these genera are closely related, but cytological and life cycle characteristics are poorly documented and hence can rarely be interpreted in a phylogenetic context. Kociolek & Stoermer (1988) introduced *Placoneis* as a sister group of cymbelloid and gomphonemoid diatoms. Mann & Stickle (1995) agree with this suggestion and provide evidence that it is

Table 2. – Comparison of sexual reproduction characteristics found in *Luticola dismutica* with that known in other pennate diatoms.

Mucilage during sexual reproduction	Wattery, inconspicuous	Dense/apparent capsule	None/almost none
	Luticola	*Placoneis*[e]	*Navicula cryptocephala*[j]
	Pinnularia[a]	*Dickieia*[f]	*Pseudo-nitzschia*[k]
	Navicula[b]	*Gomphonema*[g]	*Sellaphora*[l]
	Amphora[c]	*Cymbella*[h]	
	Neidium[d]	*Stauroneis*[i]	
Number of gametes per gametangium	2	1	
	Luticola	*Navicula cryptocephala*[j]	
	Dickieia[f]	*Sellaphora*[l]	
	Placoneis[e]		
	Pinnularia[a]		
	Navicula[b]		
	Stauroneis[i]		
	Neidium[d]		
	Pseudo-nitzschia[k]		
	Amphora[c]		
Zygote position	Between gametangial thecae (behavioral isogamy/anisogamy)	Within a gametangial theca (behavioral anisogamy)	
	Luticola	*Neidium*[d]	
	Dickieia[f]	*Placoneis*[e]	
	Pseudo-nitzschia[k]	*Sellaphora*[l]	
	Navicula[bj]	*Gomphonema*[g]	
	Stauroneis[i]	*Cymbella*[h]	
	Pinnularia[a]		
Superfluous mitotic nuclei	Quickly degenerate	Surviving up to late zygote stage (4–8 nuclei per zygote)	
	Luticola	*Placoneis*[e]	
	Dickieia[f]	*Pinnularia*[a]	
	Stauroneis phoenicenteron[i]	*Navicula*[b]	
	Sellaphora[l]	*Navicula cryptocephala*[j]	
		Stauroneis anceps[i]	
Number of auxospores per pair copulating cells	2	1	
	Luticola	*Sellaphora*[l]	
	Placoneis[e]	*Navicula cryptocephala*[j]	
	Dickieia[f]		
	Stauroneis[i]		
	Neidium[d]		
	Pinnularia[a]		
	Navicula[b]		
	Pseudo-nitzschia[k]		
	Cymbella[h]		
	Gomphonema[g]		
Expansion of auxospores	Perpendicular to apical axis of gametangia	Parallel to apical axis of gametangia	No fixed relationship
	Luticola	*Placoneis*[e]	*Pinnularia*[a]
	Diploneis didyma[m]	*Navicula cryptocephala*[j]	*Pseudo-nitzschia*[k]
	Amphora[c]	*Neidium*[d]	*Stauroneis phoenicenteron*[i]
	Pseudo-nitzschia[k]	*Stauroneis anceps*[i]	*Navicula*[b]
	Epithemia[n]	*Sellaphora*[l]	*Dickieia*[f]

References: [a] Poulíčková et al. 2007; [b] Mann 1988, Mann & Stickle 1989; [c] Geitler 1928, A. Poulíčková & D. G. Mann, unpublished; [d] Mann 1984, Mann & Chepurnov 2005; [e] Mann & Stickle 1995; [f] Mann 1994; [g] Geitler 1932; [h] Geitler 1927; [i] Mann & Stickle 1996, Mann 1996b; [j] Poulíčková & Mann 2006; [k] Davidovich & Bates 1998; Chepurnov et al. 2005; [l] Mann 1989; [m] Karsten 1899; [n] Geitler 1932, 1973

more distantly related to *Dickieia,* but has almost nothing in common with *Navicula* s.s. A recent molecular study (Bruder & Medlin 2007) confirms that *Placoneis* is a monophyletic sister clade to *Cymbella*. *Navicula* s.s. is distinct from this group (Bruder & Medlin 2007). *Luticola* shares many characteristics with *Placoneis* and *Dickieia*, and the reproductive characteristics found in this study support the classification cited above, since the only characteristic shared exclusively by *Luticola* and *Navicula* is the absence of robust mucilaginous capsule.

Acknowledgements

This research was funded by project GACR 206/07/0115 from the Grant agency of the Czech Republic and an EU Framework 6 SYNTHESYS GB TAF 643. Frieda Christie (Royal Botanic Garden Edinburgh) and Jana Veselá (Charles University Prague) provided invaluable help with scanning electron microscopy. I am very grateful to D. G. Mann (Royal Botanic Garden Edinburgh) for sharing culture techniques, expertise and literature, to B. van de Vijver (Univ. Antwerpen) for a second opinion on species identification and three anonymous reviewers for comments. English was corrected by D. A. Morris (Southampton), final version by Tony Dixon (Norwich).

Souhrn

Studie se zabývá morfologií, cytologií a rozmnožováním aerofytické rozsivky *Luticola dismutica*, která patří mezi laterálně asymetrické penátní rozsivky s jedním chloroplastem. Sexuální reprodukce probíhá intraklonálně, každý pár gametangií dává vznik čtyřem izogametám které splývají za tvorby dvou zygot (auxospor). Auxospory, obsahující dva chloroplasty a jedno jádro, expandují kolmo na podélnou osu gametangií. Zatímco popsané charakteristiky sdílí *Luticola* s jinými penátními rozsivkami, způsob úniku iniciálních buněk z perizonia (příčnou trhlinou uprostřed auxospory) je unikátní. Práce shrnuje dosud publikované cytologické a reprodukční charakteristiky penátních rozsivek, jejichž porovnání potvrzuje blízkou příbuznost rodu *Luticola* s rody *Placoneis* a *Dickieia* a oprávněnost jejich vyčlenění z rodu *Navicula.*

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Received 3 September 2007
Revision received 19 February 2008
Accepted 20 February 2008