

New and remarkable desmids (Zygnematophyceae, Streptophyta) from Europe: taxonomical notes based on LM and SEM observations

Jan ŠŤASTNÝ¹ & Frans A.C. KOUWETS²

¹ Department of Botany, Charles University of Prague, Benátská 2, CZ–128 01 Prague, Czech Republic; e-mail: stastny.jan@centrum.cz

² RWS Centre for Water Management, Department of Hydrobiology, Zuiderwagenplein 2, 8224 AD Lelystad, The Netherlands; e-mail: frans.kouwets@rws.nl

Abstract: In the present paper, the morphology and taxonomy of seven desmid taxa collected in various European habitats is discussed, mainly on the basis of scanning electron microscopic observations of cell wall sculpturing. Four taxa (*Actinotaenium riethii*, *Closterium pseudocostatum*, *Cosmarium discrepans* and *C. hostensiense*) are newly described and the name of one taxon (*Cosmarium lenzenwegeri*) is recombined. In addition, the morphology of *Cosmarium cataractarum* and *C. cinctutum* is described in greater detail, confirming their status as independent species.

Key words: *Actinotaenium*, cell wall sculpture, *Closterium*, *Cosmarium*, Desmidiaceae, ephemeral habitats, morphology, new species, scanning electron microscopy, taxonomy

Introduction

Traditional desmid taxonomy is mainly based on differences in cell morphology and wall sculpturing (e.g. BROOK 1981). However, since about the last two decades, molecular research has increasingly, and sometimes controversially been turning desmid taxonomy upside down, especially at the level of genus and higher (e.g. McCOURT et al. 2000; GONTCHAROV et al. 2003; NEUSTUPA & ŠKALOUD 2007; GONTCHAROV 2008; HALL et al. 2008; GONTCHAROV & MELKONIAN 2005, 2008, 2010). Only very recently molecular methods have also been used in the evaluation of taxonomic concepts of traditional, morphology-based taxa at the level of species and variety, resulting in remarkable taxonomic changes (NEUSTUPA et al. 2010, 2011; NEMJOVÁ et al. 2011).

However, assuming a careful and critical application, the traditional analysis of cell morphology may still provide a lot of important and useful taxonomical data. Some general morphological characters are prominent, well defined and clearly visible under the light microscope, and may even distinguish between genera. Other characters are very variable, or inconspicuous and less well visible, such as cell wall sculpturing and pore patterns. The

morphological variability ranging from apparently smooth to profusely sculptured makes the desmid cell wall perfectly suited for studies using scanning electron microscopy (SEM; see, e.g., COUTÉ & TELL 1981; COESEL 1984). Therefore, in addition to the regular line drawings after light microscopical observations in the present study we also used a scanning electron microscope to clearly illustrate the discriminative morphological characters of the individual taxa.

Extensive sampling of desmids by the first author in the Czech Republic (ŠŤASTNÝ 2010) and also in several other European countries revealed a number of remarkable forms that apparently needed a more detailed study to clear up their taxonomy. In the present paper we discuss seven of them.

Materials and Methods

The samples were microscopically analyzed using an Olympus BX51 light microscope, and line drawings were made using a drawing tube.

For scanning electron microscopy (SEM) acetone-washed glass coverslips (10 or 12 mm in diameter) were placed on a heating block, and coated three times with a poly-L-lysine solution (Sigma, 1:10 in distilled water) to ensure better adhesion of the desmid cells. After cooling, a drop of the formaldehyde-fixed

material was placed on the glass and when almost dry, it was transferred into 30% acetone and dehydrated by an acetone series (30, 50, 70, 90, 95, 99% and 2x in 100%, 10 minutes each). Finally, the cells were dried to critical-point with liquid CO₂, subsequently sputter coated with gold and examined using a Phenom Desktop or JEOL 6380 LV scanning electron microscope.

Environmental variables (pH, conductivity) were measured either with a Combo HI 98129 (HANNA, Germany) portable instrument or with a WTW 330 pH-meter and WTW LF 315 conductometer (WTW, Germany). The list of all sampling sites is given in Table 1.

Results and Discussion

Closterium pseudocostatum ŠŤASTNÝ et KOUWETS sp. nov. (Figs 1–9, 22–27)

Descriptio: cellulae moderate curvatae, media parte rectis, cingulis veris, ad utrumque polum sensim attenuatis, apicibus late tholiformibus, membrana ochroa costata striis circiter 2–6 / 10 µm. Cellularum longitudo 326–540 µm, latitudo 34–41 µm, apex 11–14 µm, long./lat. circiter 9–15.

Description: cells only slightly curved, in the midregion straight and cylindric, towards the ends gradually attenuated, with more or less conical, cowl- or dome-shaped apices. Cell wall with true girdle bands, brownish, more or less coarsely costate (2–6 str./10 µm).

Dimensions: length 326–540 µm, width 34–41 µm, length/width ratio approximately 9–15.

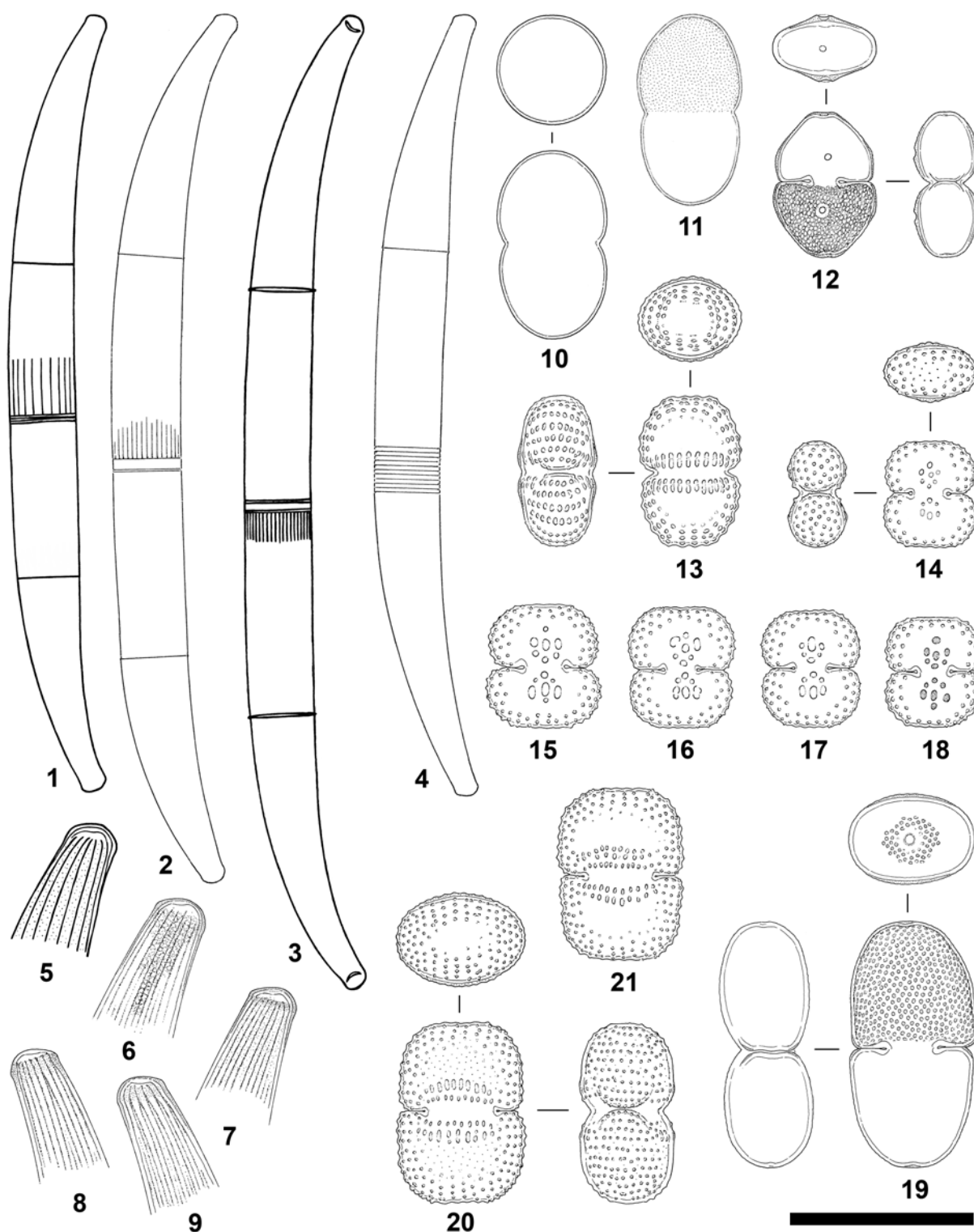
Type locality: site 2 (see Table 1), leg. J. Šťastný, 17.9. 2009. The holotype is deposited in PRC (Hic designatus).

Taxonomy: *Closterium pseudocostatum* at first glance resembles *C. costatum* RALFS. Corresponding characters are especially the coarsely costate cell wall [although the rib-like striae are usually not so much pronounced as in typical *C. costatum* and in some populations cells with a denser striation, similar to *C. costatum* var. *borgei* (WILLI KRIEG.) RŮŽIČKA, may occur (see e.g. Figs. 2, 3, 25 and 27)] and the distinctive cowl-shaped apex. However, there are also some remarkable differences in comparison with *C. costatum*, namely the somewhat less regular and rather weak curvature and particularly the presence of true girdle bands in all cells of the populations presently studied. True girdle bands differ from false or pseudo girdle bands in that they are formed more orderly: they develop following a vegetative cell division as a straight extension of

the youngest semicell. In full-grown cells there is only one per semicell, each of approximately the same length (LÜTKEMÜLLER 1902). Pseudo girdle bands, on the contrary, vary in place, length and number, and may even be slightly curved. Not all *Closterium* species do form girdle bands, and there is a striking and fundamental difference in the way of cell-division in *Closterium* species with or without true girdle bands (RŮŽIČKA 1977). In the original description of *C. costatum* (RALFS 1848, p. 170, tab. 29, fig. 1) no mention is made of any girdle bands at all. However, in later desmid monographs (e.g. RŮŽIČKA 1977; FÖRSTER 1982; COESEL & MEESTERS 2007) the occasional presence of pseudo girdle bands was reported; most remarkably, KRIEGER (1937) even ranks *C. costatum* among the girdle band species!

Forms very similar to the present material have been reported from Arctic Norway by SCHARF (1981, Fig. 15) and from Austria by LENZENWEGER (1996, pl. 6:2), both as *C. costatum*. Other findings probably representing *C. pseudocostatum* come from France (DEFLANDRE 1927, fig. 3) and from south Germany (FÖRSTER 1964, tab. 2, figs 7–8), both labeled as *C. costatum* var. *westii*. Moreover, BROOK & WILLIAMSON (2010) present a semicell of a form with a (true?) girdle band that they consider similar to var. *westii*. This variety has been described by CUSHMAN (1905) referring to figures of W. & G.S. WEST (1896, pl. 13: 23–24) from North America, showing two cells of an unnamed forma of *C. costatum* that are only faintly curved, have truncate apices and clear pseudo girdle bands, which are not mentioned in the text. Later, CUSHMAN (1908, pl. 3: 14) provided an additional, somewhat poor and atypical figure of his new variety. Next, also from North America, a much more characteristic figure of a form of *C. costatum* with a pseudo girdle band is presented as var. *westii* by BORGE (1909). Referring to BORGE (*l.c.*), GRÖNBLAD (1920) reported *C. costatum* var. *westii* from central Finland, but presented a figure of a cell with a rather long, straight girdle band. However, a constant character of the forms mentioned above is that they only measure 230–300 × 24–32 µm. Later, the concept of var. *westii* has been further confused by KRIEGER (1937; compare RŮŽIČKA 1977) and therefore, we consider this variety very doubtful.

The last record of a form possibly representing *C. pseudocostatum*, is reported by JOHN & WILLIAMSON (2009, p. 32, Pl. 4A) from the west of Ireland. They labeled their specimen as



Figs 1–21. (1–9) *Closterium pseudocostatum*, (1–3) typically developed mature cells with two girdle bands and a various striation density, (4) young daughter cell with only one girdle band, (5–9) details of apices; (10–11) *Actinotaenium riethii*; (12) *Cosmarium cataractarum*; (13) *C. cinctutum*; (14–18) *C. discrepans*; (19) *C. hostensiense*; (20–21) *C. lenzenwegeri*. Scale bar 100 μm (Figs 1–4), 50 μm (Figs 5–21).

C. costatum var. *erectum* GRÖNBLAD. This variety has been described by KRIEGER (1937) after a drawing sent to him by GRÖNBLAD that represented a cell with a straight middle part and measured $350 \times 42 \mu\text{m}$. Unfortunately, KRIEGER (*l.c.*) did not reproduce this figure, no mention is made of girdle bands and this variety is also considered very doubtful.

On the basis of the observations mentioned above, we consider the description of *Closterium pseudocostatum* as a new species as fully justified since raising one of the doubtful varieties mentioned to species level seems not appropriate. Unfortunately, no details are available on the molecular background of the formation of girdle bands, and on the phylogenetic relation between the groups with and without these structures. It seems therefore a matter of speculation whether the present form has evolved from a typical *C. costatum*, or rather has evolved in parallel and independently, including the mutual morphological features (the coarsely costate cell wall and the typical cowl-shaped apex).

Cells of *C. pseudocostatum* with a denser striation are somewhat similar to *C. intermedium* RALFS, whose cells, however, are narrower and on average also shorter (approximately $200\text{--}400 \times 20\text{--}30 \mu\text{m}$) and lack the characteristic cowl-shaped apex.

Ecology and distribution: *Closterium pseudocostatum* prefers mesotrophic, slightly acidic habitats and judging from the nature of its Czech (Břehyně–Pecopala NR, Swamp NR) and Austrian (the very desmid-rich “Schwemm” near Walchsee peat bog, see LENZENWEGER 2000; ŠŤASTNÝ & LENZENWEGER 2008) sampling sites, its occurrence seems to be limited to well-preserved biotopes with a high desmid diversity.

***Actinotaenium riethii* ŠŤASTNÝ et KOUWETS sp. nov. (Figs 10–11, 28–30)**

Descriptio: cellulae cylindricae, a fronte visae late ellipticae, circiter $1\frac{3}{4}$ longiores quam latae, in medio parum constrictae, apicibus late rotundatis, a vertice visae circulares, membrana subtilissime poribus ornata. Cellularum longitudo $39\text{--}54 \mu\text{m}$, latitudo $25\text{--}31 \mu\text{m}$, long./lat. circiter 1.55–1.95.

Description: cells approximately cylindrical with a slight median constriction, sinus very shallow and widely open. Apices broadly rounded, lacking any apical indentation or a distinct pore. Chloroplast stelloid, with 10–11 longitudinal ridges (4–5 visible in frontal view). Apical view

circular. Cell wall densely set with fine pores.

Dimensions: length $39\text{--}54 \mu\text{m}$, breadth $25\text{--}31 \mu\text{m}$, length/breadth ratio approximately 1.55–1.95.

Type locality: site 4 (see Table 1), leg. J. Šťastný, 18.4. 2007. The holotype is deposited in PRC (Hic designatus).

Etymology: the new species is described in honour of Prof. Alfred Rieth who conducted an extensive study into diverse aspects of the biology of the form in question and presented arguments in favour of their description as a separate species.

Taxonomy: the *Actinotaenium* in question has been found on a number of periodically desiccating sites in the Czech Republic (the type locality and others) and could not be identified with any species described so far. The only paper reporting forms being probably identical with the newly described species is that by RIETH (1985). He described many aspects of the morphology and biology of two *Actinotaenium* forms he had found in a habitat very similar to the Czech sites (“von Regenwasser gespeisten, temporären Wegerandpfützen”). One of these taxa (“Form I” in RIETH *l.c.*) is obviously identical with our material. RIETH (*l.c.*) provisionally identified his material as *A. curtum* (RALFS) TEILING var. *globosum* WILLE, originally described as *Penium curtum* BRÉBISSEON var. *globosum* by WILLE (1879, tab. 13, fig. 72) from the Novaja Zemlja archipelago. However, at once he presented numerous arguments for raising this variety to the rank of species. Although the description presented by WILLE (*l.c.*) is rather poor and confusing (no apical view, no chloroplast details), our material as well as that described by RIETH (1985) obviously represent a taxon different from *A. curtum* var. *globosum*. As dimensions WILLE (1879) gave $31\text{--}38 \times 24\text{--}28 \mu\text{m}$, whereas RIETH’s ($39\text{--}54 \times 25\text{--}31 \mu\text{m}$) as well as our specimens ($41.5\text{--}51.5 \times 26\text{--}29 \mu\text{m}$) were much larger. The species in question has also nothing to do with the nominate variety of *A. curtum*, as the observations of RIETH (1985, under “Form II”) convincingly demonstrate. *A. curtum* clearly differs by distinctly fusiform, comparatively narrower cells with an apical indentation bearing a distinct pore, and by cell wall that is much less densely set with pores than in *A. riethii* (ŠŤASTNÝ, pers. observation). Therefore, in our opinion the description of *A. riethii* as a new species is fully justified.

Ecology and distribution: *Actinotaenium riethii* is well characterized by its striking ecology. It is typical for periodically desiccating, usually

Table 1. List of sampling sites.

No.	Locality	Geographic coordinates	Details concerning habitats
1	Pond on the margin of the Schwemm Nature Reserve (NR), Austria	47°39'16.50"N, 12°17'24.69"E	meso-eutrophic; pH = 6.8, cond. = 184 µS. cm ⁻¹
2	Pool in the Břehyně-Pecopala NR, Czech Republic	50°35'1.10"N, 14°42'11.85"E	mesotrophic; pH = 6.0, cond. = 166 µS. cm ⁻¹
3	Pool in the Břehyně-Pecopala NR, Czech Republic	50°35'1.47"N, 14°43'0.05"E	oligo-mesotrophic; pH = 5.5, cond. = 113 µS. cm ⁻¹
4	Periodically desiccating concrete drainage gutter in the town of Roztoky u Prahy, Czech Republic	50°9'9.57"N, 14°23'50.49"E	pH = 7.9, cond. = 175 µS. cm ⁻¹
5	Mucilaginous growths on granite rocks in the Vlašim chateau park, Czech Republic	49°42'29.57"N, 14°52'58.78"E	n.d.
6	Boggy pool in the northern part of the Swamp NR, Czech Republic	50°35'37.93"N, 14°38'37.70"E	oligo-mesotrophic; pH = 5.2, cond. = 40 µS. cm ⁻¹
7	Držník pond, part of the Hradčanské rybníky NR, Czech Republic	50°36'40.19"N, 14°43'15.90"E	mesotrophic; pH = 5.5–6.0, cond. = 120–285 µS. cm ⁻¹
8	Bohdanečský pond, Czech Republic	50°5'18.44"N, 15°39'54.38"E	slightly eutrophic; pH = 7.4
9	Ephemeral pool near the Mlýnský pond, Novohradské hory Mts, Czech Republic	48°42'33.09"N, 14°42'47.09"E	oligo-mesotrophic; pH = 5.6, cond. = 13 µS. cm ⁻¹
10	Periodically desiccating pool near the town of Hostens, France	44°29'55.18"N, 0°38'22.51"W	oligo-mesotrophic
11	Moorland pool north of Hostens, France	44°32'42.37"N, 0°40'04.49"W	oligo-mesotrophic
12	Rivulet in Marais du Cla, near "Le Gat Mort", northeast of Hostens, France	44°30'36.72"N, 0°36'45.05"W	oligo-mesotrophic
13	L'Étang Bleu, France	48°01'55.15"N, 2°10'44.25"W	mesotrophic
14	Lac de Lamoura, France	46°23'40.20"N, 5°58'47.25"E	mesotrophic
15	Mucilaginous growths on a concrete platform near the Vidrenjak river, Serbia	43°02'42.17"N, 20°16'27.24"E	n.d.
16	Ephemeral pool near the way from the Remetské Hámre village to the Morské oko lake, Slovakia	48°52'30.84"N, 22°12'27.23"E	oligo-mesotrophic

man-made substrata like, e.g., concrete drainage gutters, concrete platforms, etc. where it may co-occur in particular with *A. curtum*, *Cosmarium pericymatium* var. *pericymaticum* and var. *corrugatum* and *Staurostrum habeebense*, a drought-resistant desmid community typical for this type of habitats (ŠŤASTNÝ 2008). Here, it can be easily confused with *C. pericymatium* whose cells, however, are not omniradiate and therefore have different frontal and lateral views (compare, e.g., WILLIAMSON 2000, tab. 2, fig. 7). Most likely also the reports of *A. cucurbita* by BROOK (2001, found together with *C. pericymatium* and *S. habeebense*) from a sun-dial and by WILLIAMSON (2002, in a community with *C. pericymatium* var. *pericymatium* and var. *corrugatum*, *S. habeebense* and *A. curtum*) from a stone birdbath actually represent *A. riethii*.

***Cosmarium cataractarum* (RACIBORSKI) EICHLER 1895 (Figs 12, 33–37)**

Basionym: *Cosmarium variolatum* LUNDELL var. *cataractarum* RACIBORSKI [1889, pl. 5(1), fig. 3]

Morphology and taxonomy: the cells are longer than broad with a deep, linear and closed sinus. Semicells are in outline pyramidal with rounded angles, straight to slightly convex sides and a slightly concave apex. In apical and lateral view they are elliptical, in apical view a slight median inflation is present. Cell wall coarsely scrobiculated and with a large scrobicula in the semicell centre.

Dimensions: length 37–42 µm, breadth 26–29 µm, thickness 17–19 µm, isthmus 7.5–9 µm; length/breadth ratio approximately 1.30–1.40.

Cosmarium cataractarum, found in sample from sites 7, 8 and 14, had originally been described as *C. variolatum* var. *cataractarum* by RACIBORSKI (1889, pl. 5, fig. 3) from southern Poland. RACIBORSKI (*l.c.*) obviously related his new form with *C. variolatum* LUNDELL on the basis of the similarity of the cell wall sculpture. The nominate variety of *C. variolatum* is also characterized by a coarsely scrobiculated cell wall and, according to the original description, var. *cataractarum* should only differ in the presence of a large scrobicula in the centre of each semicell (which, however, may rarely be reduced or even lacking; see Figs 36–37).

Under the light microscope the cell wall sculpture of both taxa indeed appears roughly the same (compare figs 264–5 and 266 in ŠŤASTNÝ

2010 or figs. 2 and 3 on pl. 49 in LENZENWEGER 1999). However, the use of SEM revealed remarkable differences. In *C. variolatum*, the cell wall sculpture consists of equally large and evenly distributed scrobiculae, each of them bearing a distinct pore (see Figs 31–32 after material from site 3). On the other hand, in var. *cataractarum*, the scrobiculae are generally more shallow, unevenly large (the larger being usually situated in the apical and lateral parts of the semicells), more densely distributed over the cell wall and the pores are located between them (see Figs 33–37). Generally, the cell wall sculpture in *C. variolatum* var. *cataractarum* more resembles that in *C. pseudovariolatum* GRÖNBLAD (see COUTÉ & TELL 1981, pl. 14, figs 3–6) than that in *C. variolatum*.

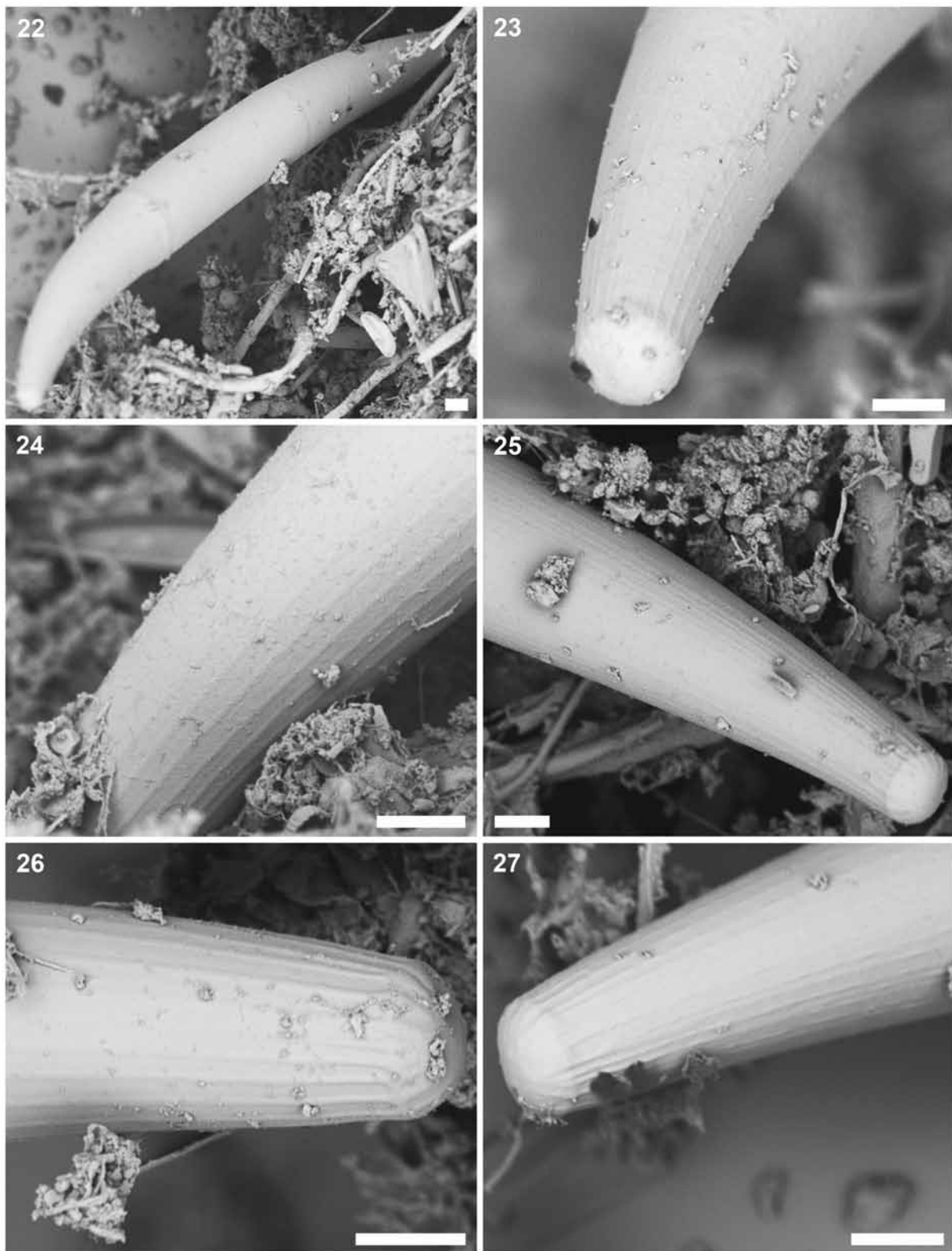
In addition, the ecological demands of the nominate variety of *C. variolatum* and var. *cataractarum* are quite different. The nominate variety is a rare species of mesotrophic, slightly acidic bogs, while var. *cataractarum* is a characteristic form from the tychoplankton of greater, meso- to slightly eutrophic and neutral to slightly alkaline water bodies (see e.g. ŠŤASTNÝ 2010).

Despite all the above-mentioned morphological and ecological differences, in desmidiological literature *C. cataractarum* is still generally considered a variety of *C. variolatum*. Only EICHLER (1895) treated it as a separate species, but his view has not been generally accepted. However, our observations fully support EICHLER's (*l.c.*) opinion and confirm the status of *C. cataractarum* as a separate species.

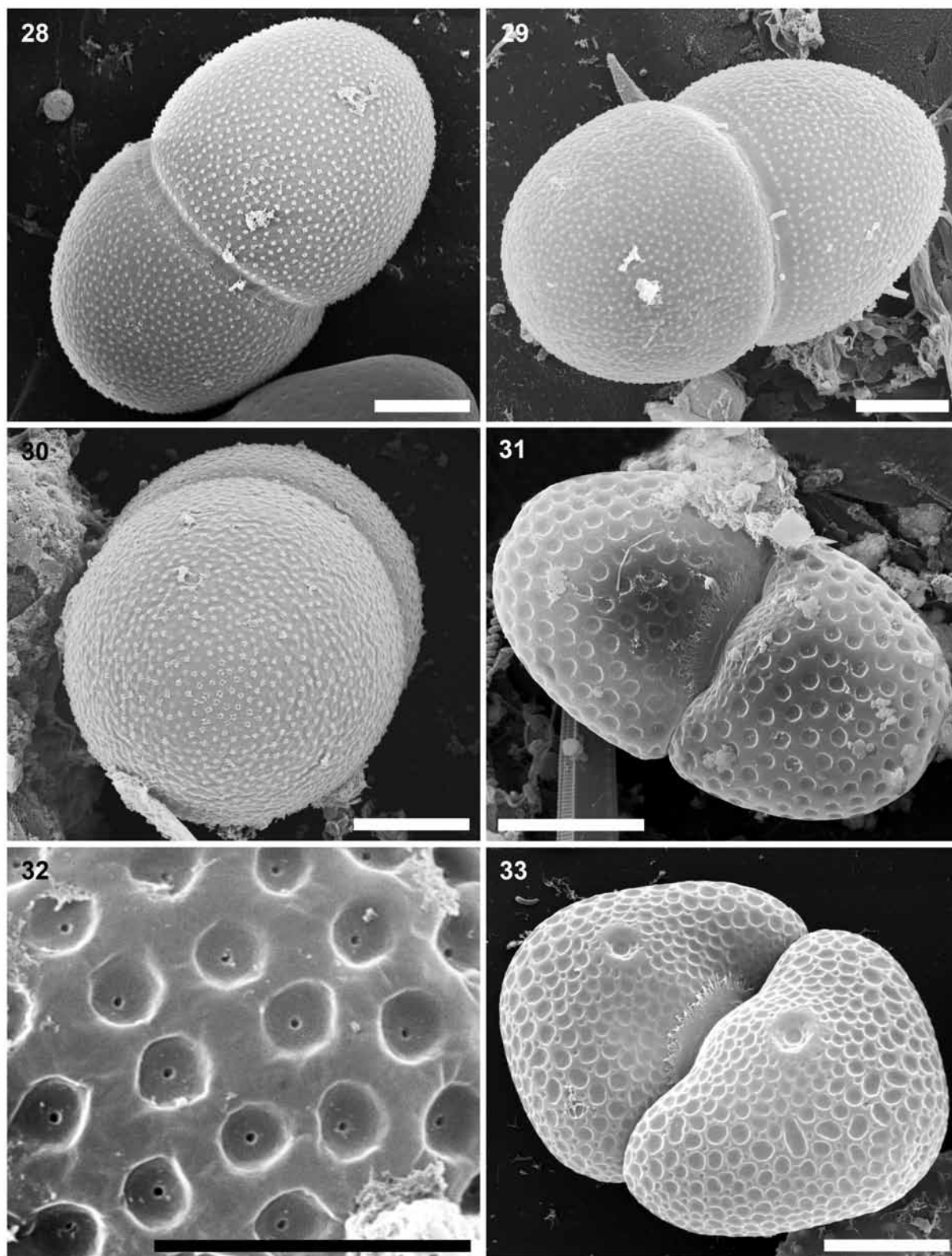
***Cosmarium cinctutum* NORDSTEDT 1875 (Figs 13, 42–49)**

Morphology and taxonomy: the cells in our material, coming from site 5, are longer than broad with a very shallow, open sinus. Semicells are in outline trapeziform with convex lateral sides and undulate-crenate margins. In both apical and lateral view they are elliptical. Cell wall ornamentation is composed of radiating series of intramarginal and marginal, flat granulae and in addition of a horizontal band of 8–12 (mostly 10) elongate, irregular verrucae just above the isthmus (Figs 13, 42–47). The cells are 36–45 µm long, 25–29 µm wide and 20–23 µm thick. The isthmus is 18.5–21.5 µm wide. The length/width ratio is approximately 1.40–1.55.

Our material showed some similarities with *C. basiornatum* (GRÖNBLAD) COESEL. This species



Figs 22–27. *Closterium pseudocostatum*: (22) whole cell, (23) detail of the same cell, (24–25) parts of cells with a less dense (24) and more dense (25) striation, (26–27) details of apices. Scale bar 10 µm.



Figs 28–33. (28–30) *Actinotaenium riethii*, (30) apical view; (31–32) *Cosmarium variolatum*, (32) detail of cell wall sculpture; (33) *C. cataractarum*. Scale bar 10 μm , 5 μm (Fig. 32).

was originally described by GRÖNBLAD (1926) as a variety of *C. davidsonii* J. ROY & BISSET and rightly made a separate species by COESEL (1998). However, a closer look revealed several subtle differences between *C. basiornatum* and our specimen. *C. basiornatum* is characterized by truncate–pyramidal semicells with straight or only faintly convex sides, a very moderate, largely closed sinus, and an ornamentation of short, radiating series of comparatively large granules leaving the semicell centre smooth. In addition a supra-isthmial ornamentation of 6–8 longitudinal series of mostly two (seldom fusing) elongate warts is present. Also between this horizontal band and the radiating series near the basis a smooth zone is present (see Figs 38–39 after material from site 9; compare also COESEL 1998, pl. 11: 39–40; ŠTASTNÝ 2010, figs 116–117). Dimensions are $37\text{--}41.5 \times 27\text{--}31 \mu\text{m}$; isthmus $15\text{--}17 \mu\text{m}$. The length/width ratio is approximately 1.28–1.37.

Our material, on the other hand, has slightly more rounded semicells, an even more shallow, gaping sinus, and a different supraisthmial ornamentation that merges with the radiating series of granules near the basis. Finally, it has a higher length/breadth ratio. These characters rather match the diagnosis of *C. cinctutum*, given by NORDSTEDT (1875). Unfortunately, NORDSTEDT only presented the figures of two semicells, one agreeing with our material, one significantly broader which possibly is an artifact (NORDSTEDT *l.c.* pl. 7: 20a and 20a', respectively). As dimensions he gave $40 \times 28\text{--}33 \mu\text{m}$, the larger width agreeing with his broader form.

Most remarkably, despite the above mentioned differences COESEL & MEESTERS (2007) without argumentation consider *C. basiornatum* a synonym of *C. cinctutum*, which name accordingly would have priority. However, in our opinion, both species should be kept apart.

C. cinctutum should also be compared with a few other species. *C. tumens* NORDSTEDT clearly differs by its characteristic supraisthmial ornamentation composed of 3–4 transversal rows of small granulae (Figs 40–41). In addition it has somewhat greater dimensions (in the Serbian population from site 15 cell dimensions were: length $43\text{--}52 \mu\text{m}$, width $30\text{--}35 \mu\text{m}$, thickness $24.5\text{--}26.5 \mu\text{m}$, isthmus width $19\text{--}21 \mu\text{m}$).

A second rather similar form is *C. speciosum* var. *rostaftinskii* (GUTWIŃSKI) W. et G.S. WEST, originally described as *C. rostaftinskii* ["Rostaftinskii"] by GUTWIŃSKI (1890; see also

GUTWIŃSKI 1892). However, general morphology and ornamentation of this form shows more affinity with *C. basiornatum* (compare also SKUJA 1928). CROASDALE (1962) described the slightly smaller *C. cinctutum* var. *reductum*, but this variety does not seem very closely related although the figures are somewhat confusing (CROASDALE *l.c.* pl. 5: 80–82). Finally, RACIBORSKI (1889) reported a form of *C. cinctutum* from a locality near Dresden, unfortunately without figures, measuring $36\text{--}47 \times 26\text{--}30 \mu\text{m}$, thickness $21\text{--}22 \mu\text{m}$ and isthmus $21\text{--}25 \mu\text{m}$.

Ecology and distribution: as far as could be traced, up till now only one other more or less reliable record of *C. cinctutum* has been published. KOSSINSKAJA (1936) mentioned the species presenting a detailed drawing after material collected near the Gulf of Jenisej, in Arctic Russia (KOSSINSKAJA *l.c.* pl. 3: 6). However, her – single – figure seems slightly different from the present material concerning the shape of the isthmus and the development of the lateral crenations. The dimensions of her material are near the lower end of the range given above. *C. cinctutum* apparently is a rare, predominantly Arctic–Alpine species, presumably preferring hemi–atmophytic habitats.

***Cosmarium discrepans* ŠTASTNÝ et KOUWETS sp. nov. (Figs 14–18, 52–57)**

Descriptio: cellulae subparvae, paullo longiores quam latae, medio sinu anguste lineato profunde constrictae; semicellulae subtrapezi–subreniformes, angulis basalibus late rotundatis, superioribus obtuso–rotundatis, dorso truncatae, a vertice visae ellipticae, a latere visae subrotundatae; membrana intra margine granulis seribus irregulariter dispositis ornatis, supra isthmum granulo paullo majore ornatae, apicem versus granulis 2 minoris, granulis verruciformis plerumque geminatis 3, interdum granulo singulo ornatae. Cellularum longitudo $29\text{--}34 \mu\text{m}$, latitudo $24\text{--}29 \mu\text{m}$, crassitudo $16.5\text{--}18.5 \mu\text{m}$, long./lat. circiter 1.10–1.25.

Description: the cells are slightly longer than broad, with a deep, linear and closed sinus. The semicells are approximately oval in outline with rounded basal angles and convex lateral sides that rather merge with the truncate apex. The cell wall ornamentation is composed of radiating series of small intramarginal/marginal granulae and a characteristic central ornamentation, consisting of a uniquely arranged rosette of wart–like granules. Between central and marginal ornamentation a smooth zone is present. The central ornamentation is rather variable, generally showing a prominent

supraisthmial granule with, more apically, on either side a somewhat smaller granule. Above this a more or less horizontal series of three elongate warts is situated. These warts, and particularly the central one, show a strong tendency to subdivide, so that the total number of granulae may vary between six and nine and usually comprises seven. The variability of the central sculpture is particularly well visible in Figs 52–57. The apical view is ellipsoid with a slight median inflation, the lateral view is (sub)circular (see Fig. 14). **Dimensions:** length 29–34 µm, breadth 24–29 µm, thickness 16.5–18.5 µm, isthmus 9–11 µm; length/breadth ratio approximately 1.10–1.25.

Type locality: site 6 (see Table 1), leg. J. Šťastný, 22.9. 2006. The holotype is deposited in PRC (Hic designatus).

Etymology: the epith “*discrepans*” means “different” pointing to the fact that this new species obviously has been considered a form of *C. punctulatum*.

Taxonomy: *Cosmarium discrepans* rather closely resembles *C. punctulatum* BRÉB. var. *subpunctulatum* (NORDST.) BØRGESSEN, originally described as *C. subpunctulatum* by NORDSTEDT (1887, p. 160) from New Zealand. Searching the literature several reports of this taxon were found that are almost certainly identical with *C. discrepans*. Compare in particular the forms in COMPÈRE (1980, fig. 48) from the Belgian part of the Ardennes highlands, in WILLIAMSON (1992, figs 19, 4) from the Shetland Islands and also the interesting find of WILLIAMSON (2004, pl. 3, fig. 7) from the Indonesian Island Sulawesi. Most probably also the findings of W. & G.S. WEST (1897, pl. 6, fig. 19, as *C. subpunctulatum* NORDSTEDT) from South England and DUBOIS-TYLSKI (1969, pl. 1, fig. 20) from the French part of the Ardennes highlands represent the taxon in question.

The above-mentioned records are generally specified to originate from acidic and oligotrophic environments. On the other hand, *C. punctulatum* var. *subpunctulatum* is a common species of meso-eutrophic, slightly acidic to slightly alkaline waters (e.g. COESEL & MEESTERS 2007, ŠŤASTNÝ 2010). In addition to this striking difference in ecology of both taxa there is also a difference in cell wall ornamentation. The granulae in *C. punctulatum* var. *subpunctulatum* are generally somewhat more delicate than those in *C. discrepans* and in particular the central ornamentation of the semicells is very different. In *C. punctulatum*

var. *subpunctulatum* this central ornamentation is composed of a horizontal row of 4 or 5, rather small supraisthmial granulae with more apically a group of 6–9, variably arranged and sometimes geminate granulae (compare NORDSTEDT 1888, pl. 5, fig. 8, see also Figs 50–51 after material from site 1).

Ecology and distribution: *Cosmarium discrepans* was found by the first author in several localities in northern Bohemia (the type locality and some others) and northern Belgium (De Teut NR). The second author encountered it almost simultaneously on several sites in the west of France (Bretagne, Aquitaine, sites 11, 12, 13). All sampling sites were generally acidic and oligo(–meso)trophic and the species in question often occurred in high numbers. Most likely it is a rather rare, but widely distributed species of oligo–mesotrophic, acidic, well preserved habitats.

***Cosmarium hostensiense* ŠŤASTNÝ et KOUWETS sp. nov. (Figs 19, 58–61)**

Descriptio: cellulae a fronte visae ellipticae, profunde constrictae, sinu anguste lineari, apicibus subtruncatis–rotundatis in medio insectis, a vertice visae late ovatae, a latera visae compresse ovatae; membrana scrobiculata, scrobiculis poris ornatis. Cellularum longitudo 53–67 µm, latitudo 30–35 µm, crassitudo 23–25 µm, long./lat. circiter 1.75–2.00

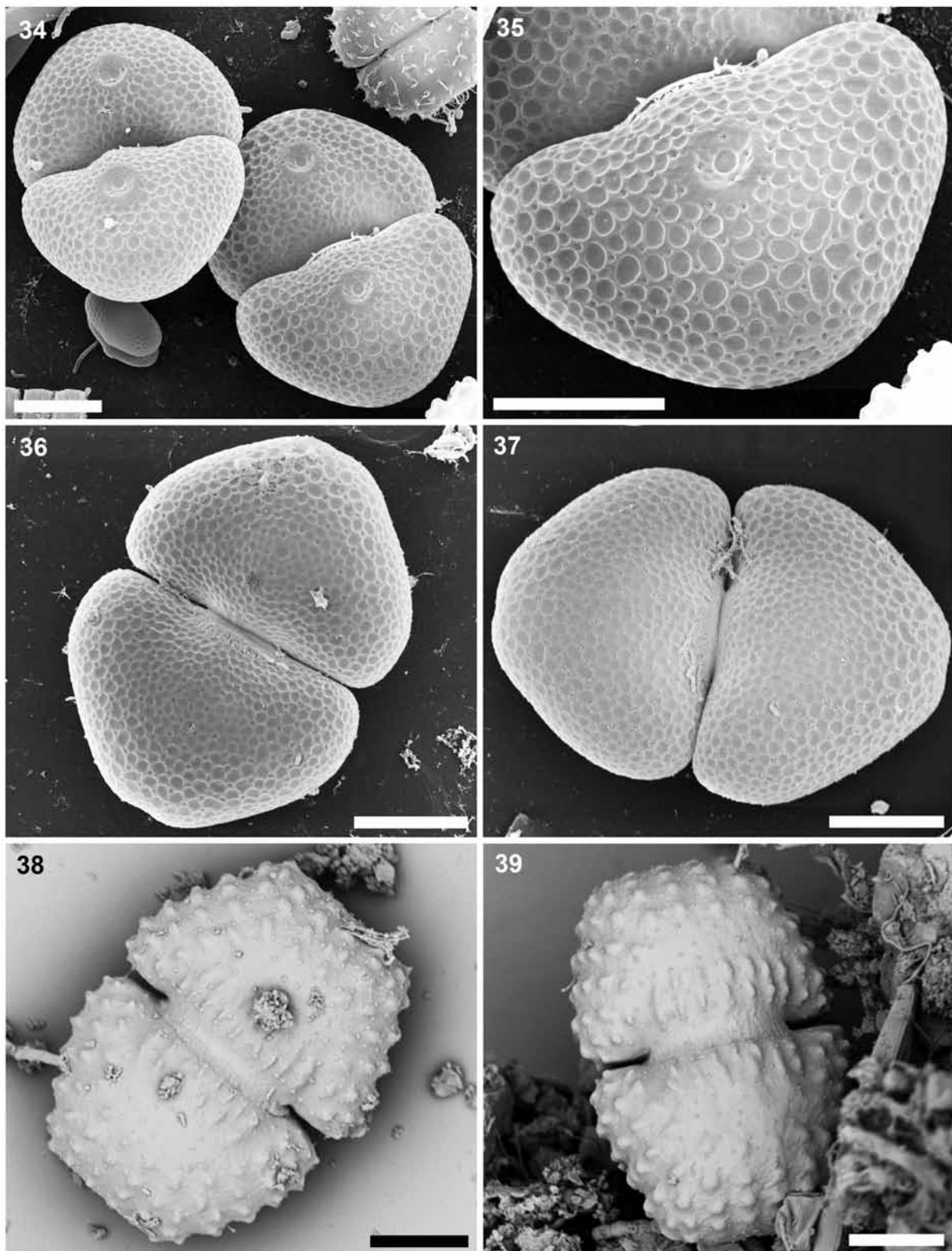
Description: cells in outline truncately elliptic, about twice as long as broad, deeply constricted; basal angles rather acutely rounded, sides convex, straight to occasionally slightly concave and gradually tapering towards the truncately rounded apex. Cell wall evenly and densely covered with marked scrobiculae. Dimensions: length 53–67 µm, breadth 30–35 µm, thickness 23–25 µm, isthmus 12.5–14 µm; length/breadth ratio approximately 1.75–2.00.

Type locality: site 10 (see Table 1), leg. J. Šťastný, 12.3. 2009. The holotype is deposited in PRC (Hic designatus).

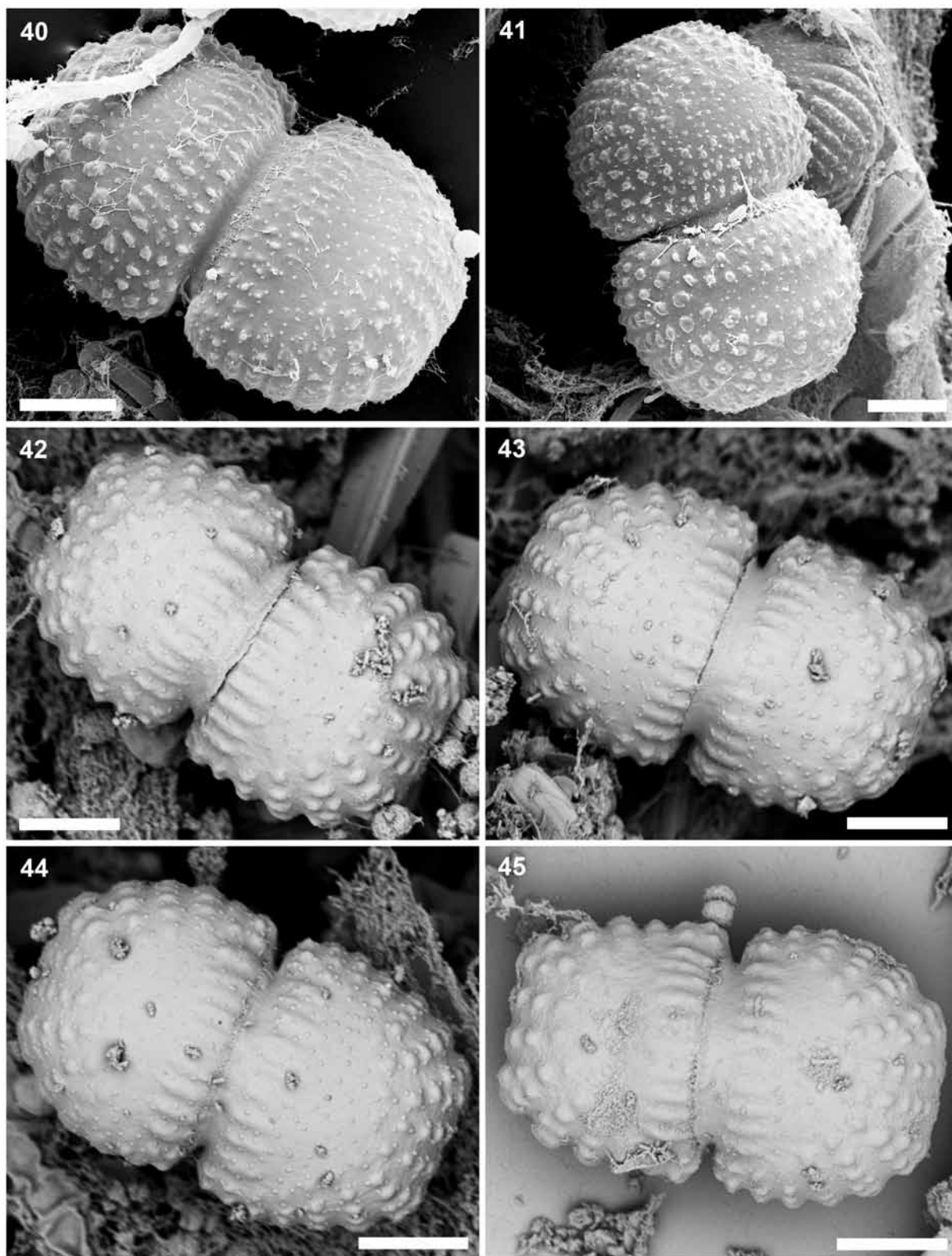
Etymology: the new species is named after Hostens, the French district where the type locality is situated.

Taxonomy: in a sample from locality 10, the first author found yet another desmid that is generally associated with *C. variolatum*, i.e. a form currently known as *C. variolatum* var. *skujae*.

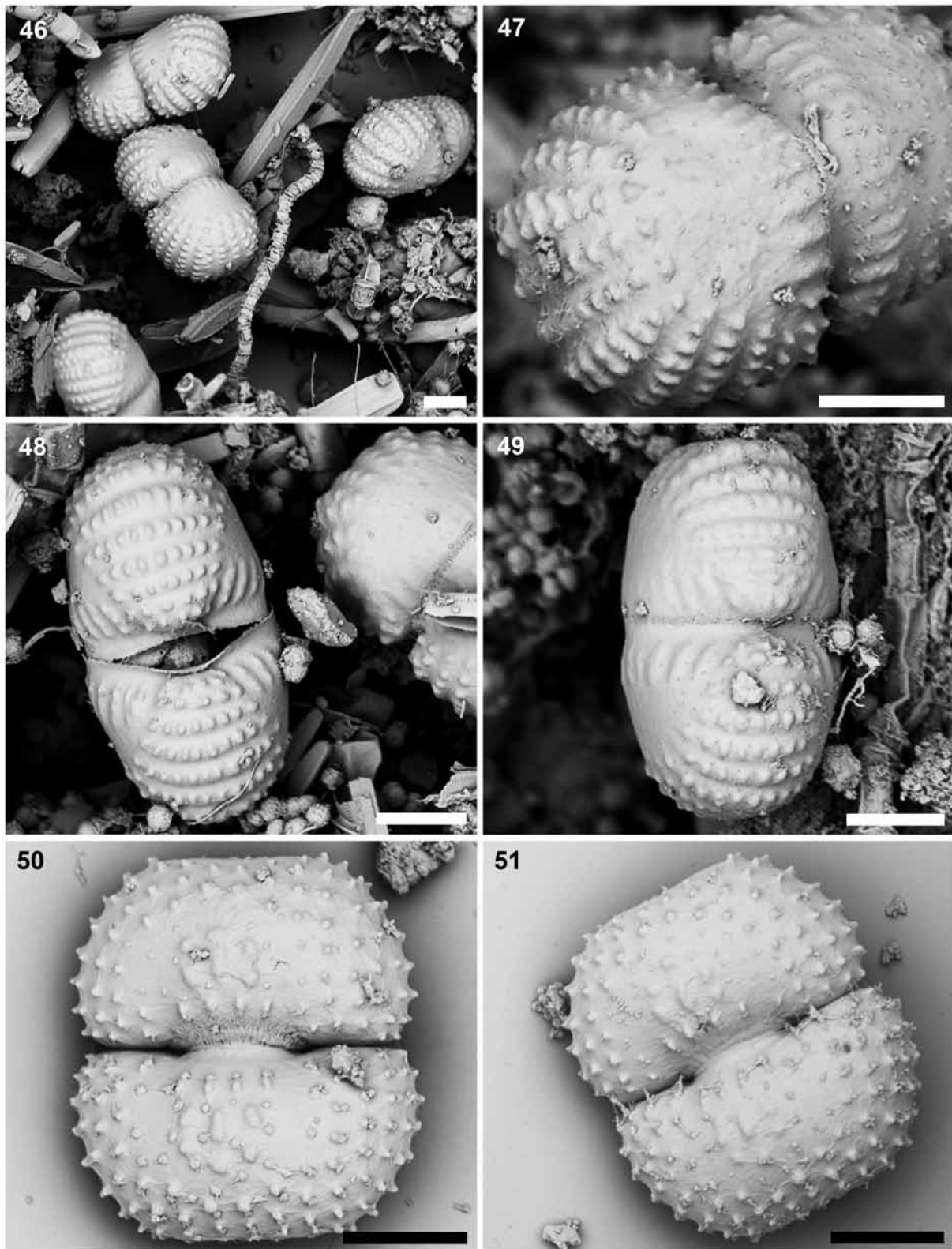
This rather conspicuous variety had originally been described by CROASDALE in CROASDALE & FLINT (1988, p. 112, pl. 36, fig. 13), after a figure published by SKUJA (1976)



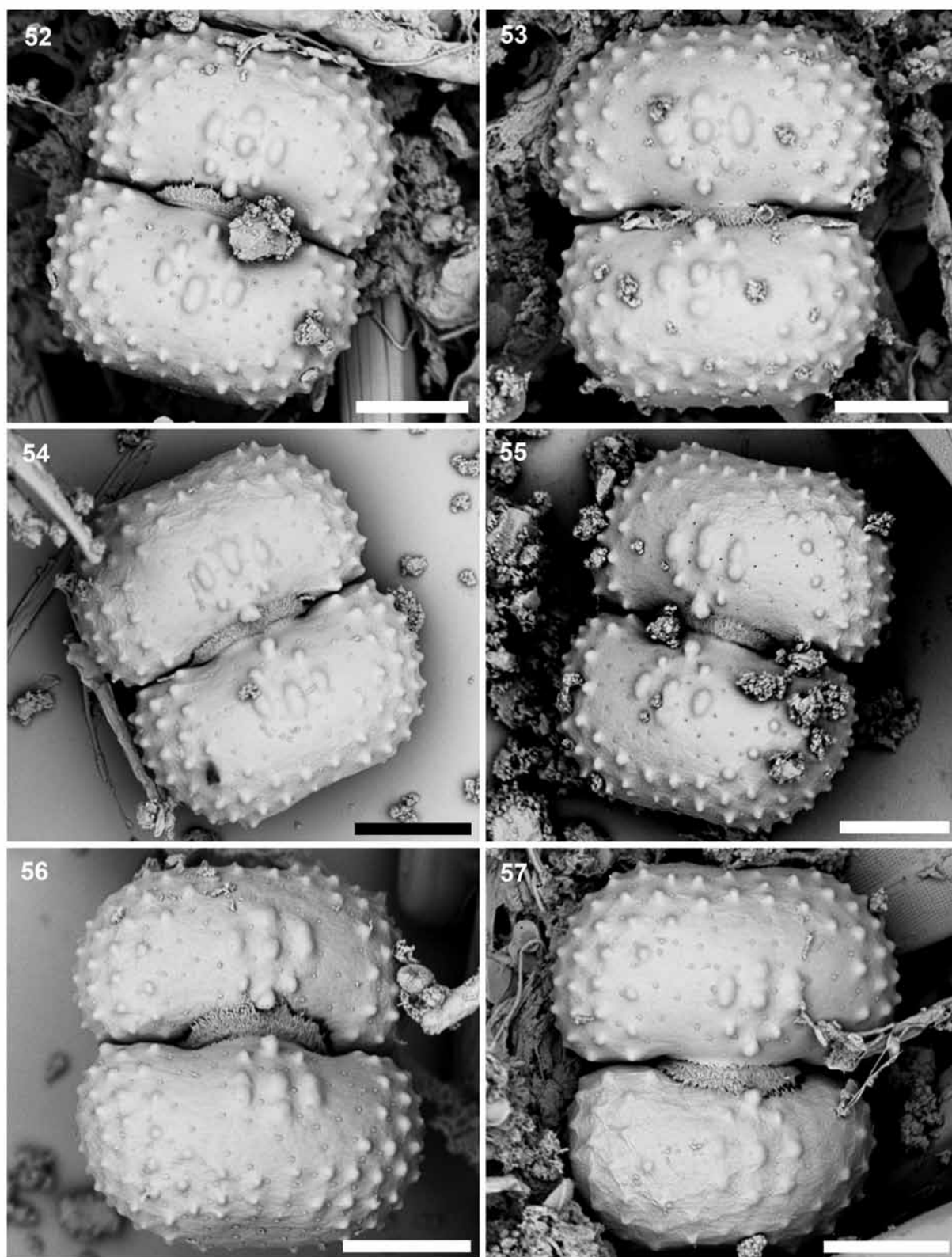
Figs 34–39. (34–37) *Cosmarium catarractarum*, (34) typically developed cells, (35) detail of cell wall sculpture, (36–37) anomalous cells with very weakly developed (upper semicell in 36) or completely lacking central scrobiculae; (38–39) *C. basiornatum*. Scale bar 10 μm .



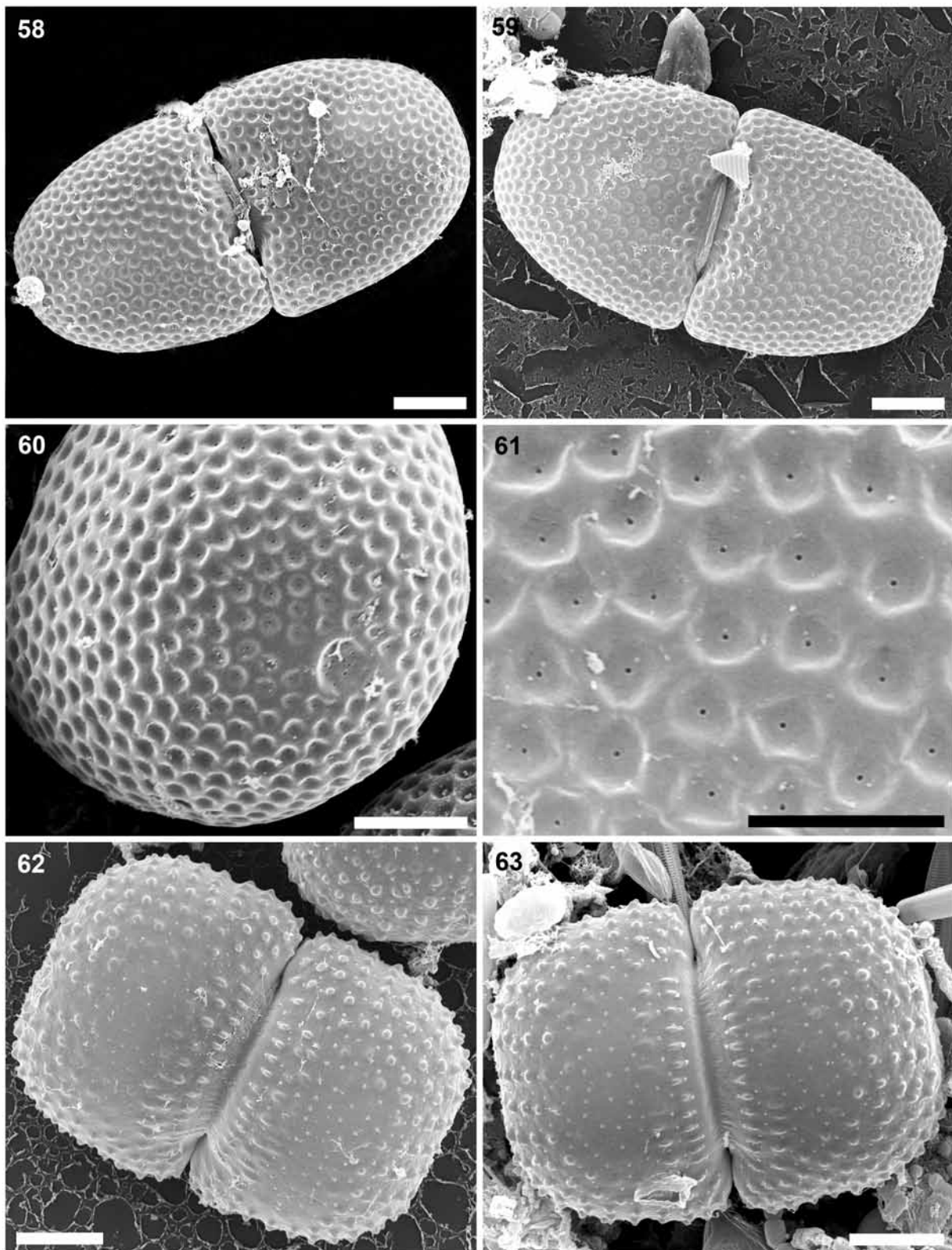
Figs 40–45. (40–41) *Cosmarium tumens*; (42–45) *C. cinctutum*. Scale bar 10 µm.



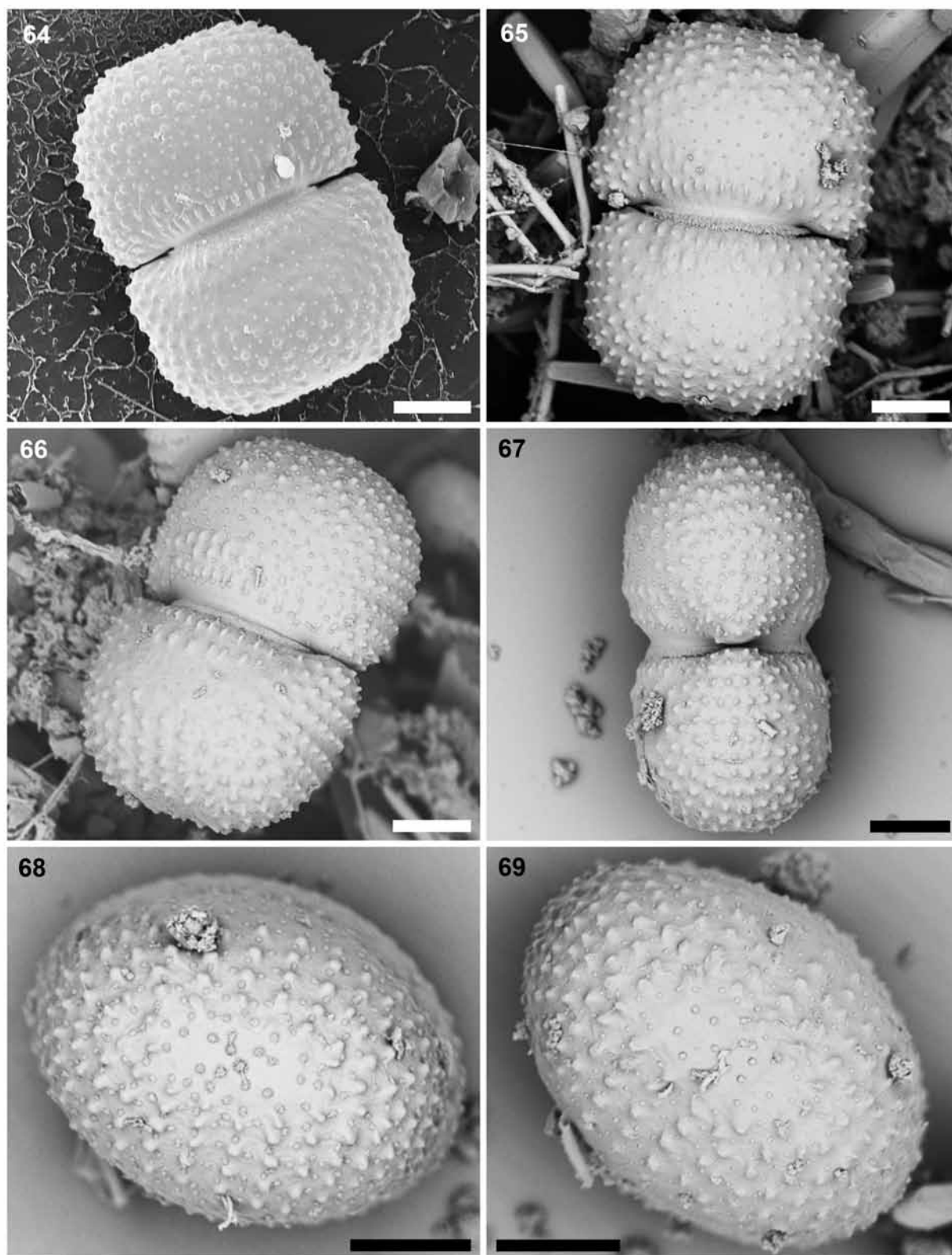
Figs 46–51. (46–49) *Cosmarium cinctum*, (47) detail of the apical part of a cell, (48–49) lateral view; (50–51) *C. punctulatum* var. *subpunctulatum*. Scale bar 10 µm.



Figs 52–57. (52–57) *Cosmarium discrepans*. Scale bar 10 µm.



Figs 58–63. (58–61) *Cosmarium hostensiense*, (60) detail of the apical part of a semicell, (61) detail of cell wall sculpture; (62–63) *C. lenzenwegeri*. Scale bar 10 µm, 5 µm (Fig. 61).



Figs 64–69. (64–69) *Cosmarium lenzenwegeri*, (67) lateral view, (68–69) apical views. Scale bar 10 μ m.

under *C. variolatum* var. *extensum* NORDSTEDT (SKUJA *l.c.*, Pl. 9, Figs. 6–7). Last mentioned variety had previously been transferred to *C. pseudopyramidatum* P. LUNDELL by KRIEGER & GERLOFF (1965, p. 127), apparently on the basis of similarities in general cell wall sculpture and morphology (compare NORDSTEDT 1887, p. 161, 1888, p. 55, pl. 6:3a). However, SKUJA's form obviously is not related to NORDSTEDT's var. *extensum*, inducing CROASDALE to retain it in *C. variolatum*, giving it the new varietal name *skujae*. By doing so, she in fact described it as a new taxon, unfortunately without providing a Latin diagnosis and indicating a type, so that her description is invalid according to ICBN Articles 36.2 and 37.1 (McNEILL et al. 2006).

The present material provided the opportunity to study this taxon with SEM, in order to reveal its cell wall sculpture and clarify its systematic position. Contrary to *C. cataractarum*, the cell wall of the present form has a pattern very similar to the nominate variety of *C. variolatum*, consisting of rather coarse scrobiculae with a central pore (see Fig. 61). However, in our opinion the general cell morphology – including dimensions – of the present form is too much different from that of *C. variolatum* to merely consider it a variety of the latter (compare also KOUWETS 2008). Moreover, as with *C. cataractarum* there is a striking difference in the ecology between both taxa and, therefore, it is proposed to classify it as a separate species. Since a *C. skujae* has already been described by KRIEGER & GERLOFF (1965), a new name must be chosen, and we propose the name *Cosmarium hostensiense*.

Ecology and distribution: *Cosmarium hostensiense* obviously prefers acidic, more or less oligotrophic, often periodically desiccating water bodies. For instance in the west of Ireland it is one of the most common and often dominant desmids in this type of habitats (ŠTASTNÝ, personal observations; see also JOHN & WILLIAMSON 2009).

***Cosmarium lenzenwegeri* ŠTASTNÝ et KOUWETS
nom. nov. et stat. nov. (Figs 20–21, 62–69)**

Synonym: *Cosmarium subbroomei* SCHMIDLE var. *taylorii* CROASDALE (1956, pl. 16, figs 12–13)

Etymology: the new name of the present form is given in honour of Prof. Rupert Lenzenweger who conducted extensive investigations into desmids of higher altitudes, particularly in the Austrian Alps.

Morphology and taxonomy: the cells are distinctly longer than broad, in frontal view rather oblong–rectangular with a relatively shallow, linear, closed sinus and broadly rounded apical angles (Figs 20–21, 62–65). The semicells are somewhat subrectangular with rounded basal angles and parallel to slightly convergent, straight to slightly concave sides and a straight apex. In apical view they are elliptical; in lateral view subelliptic. The cell wall ornamentation is composed of radiating series of small granules running across the sides and by 2–3 transversal rows of elongate granules just above the isthmus (Figs 20–21, 62–66). The cells are 45–52.5 µm long, 33–37.5 µm wide and 24–26 µm thick. The isthmus is 16.5–20 µm wide. The length/width ratio is approximately 1.3–1.4.

The present, conspicuous, but unfamiliar *Cosmarium* form has been found in great abundance in an ephemeral pool in eastern Slovakia (site 16). Scrutinizing the literature for figures of this form only the report of *Cosmarium subbroomei* SCHMIDLE var. *taylorii* [“*Taylorii*”], described by CROASDALE (1956) from Alaska, came into consideration for the identification. This variety is morphologically almost identical with our material, differing basically only in its slightly greater dimensions, measuring 57–58 × 47–48 µm. Moreover, although CROASDALE (*l.c.*) does not mention any explicit details about the habitat her variety was found (“On stones, branches, etc., in brooks and rivers”), some of the accompanying species (e.g. *Cosmarium holmiense* var. *integrum*, *C. pokornyanum*, *C. speciosum* var. *simplex*) point to a (hemi-)atmophytic habitat type, similar to our sampling site. A superficially related form seems to be *C. diplodesmium*, described from northern Sweden by SKUJA (1964, p. 214, pl. 36, fig. 20), but this form is slightly smaller and more coarsely granulated.

However, in our opinion CROASDALE's (*l.c.*) form has no relationship with *C. subbroomei* SCHMIDLE. Moreover, *C. subbroomei* is generally considered a very dubious taxon. When examining SCHMIDLE's original figures of this species (SCHMIDLE 1893, pl. 5, figs 22–24), it seems obvious that he included two markedly different taxa, one represented by Fig. 22, the other by Figs. 23–24. In their “British Desmidiaceae”, W. & G.S. WEST (1912, pl. 100, fig. 10) only gave a copy of SCHMIDLE's fig. 22, at the same time stating that they had never seen a form exactly like it (W. & G.S. WEST *l.c.*, p. 24). Therefore, to

preclude any taxonomic confusion, we propose to raise *C. subbroomei* var. *taylorii* to the rank of a separate species. Since a *Cosmarium taylorii* has already been described by CARTER [1935, as “Taylori”, synonym *Actinotaenium taylorii* (CARTER) TEILING], a new name has to be chosen as to avoid the creation of a later homonym.

Ecology and distribution: *Cosmarium lenzenwegeri* obviously is a very rare (hemi-) atmophytic species, most probably with an arctic–alpine distribution.

Conclusions

The present study confirms the usefulness of SEM revealed morphological cell wall characteristics for the description and delimitation of desmid species. Although molecular research suggests a much more complicated taxonomy (e.g. GONTCHAROV & MELKONIAN 2008, 2011), cell morphology still is an important character for routine identification and SEM apparently is a welcome additional tool. This has also been concluded by other authors (e.g. COESEL 1984; GONTCHAROV et al. 2002; NEUSTUPA et al. 2010) and its full potential obviously hasn’t been realized yet. This especially concerns smaller forms with few morphological characteristics where it may be used for the delimitation of (pseudo)cryptic species. As already stressed by COESEL (1984) differences in cell wall sculpturing should be of equal importance in the identification of taxa as, e.g., differences in form and arrangement of cell wall ornamentation such as granules or spines. The significance of cell wall sculpture for the taxonomy of desmids should be further investigated.

The present results also draw attention to the actual biodiversity question, trying to assess the overall global number of desmid taxa. On one hand they illustrate the often desultory description of infraspecific desmid taxa by many authors (*Cosmarium variolatum* var. *cataractarum*, *C. subbroomei* var. *taylorii*; compare KOUWETS 2008), paying no attention to conspicuous distinctive morphological characters. On the other hand they show that in cases where a description of a new species would be fully justified (*Closterium pseudocostatum*, *Cosmarium discrepans*) such distinctive characters are frequently overlooked or simply ignored and obviously considered to be part of the variability of the species in question.

It is clear that the cases discussed in the

present paper only represent the “tip of the iceberg” and that many of the infraspecific desmid taxa described so far should be better considered separate species. This trend has been convincingly demonstrated in several recent papers (e.g. NEUSTUPA et al. 2010, 2011; NEMJOVÁ et al. 2011). In our opinion, it can be assumed that the estimation of the overall global desmid species number by HOSHAW et al. (1990, 15000 spp.) is much more realistic than for instance that by CRANWELL et al. (1990, 1500 spp.) and probably much higher than the recent number of desmid taxa described (ca. 4000; GERRATH 1993).

Finally, the present results also demonstrate that ephemeral pools, where several taxa discussed above have been found, certainly still include a number of little known or undescribed desmid species and should not be ignored in investigations of desmid diversity.

Acknowledgements

The study was supported by grant 206/09/0906 of Czech Science Foundation and by Charles University Science Foundation B BIO 432311/2011 grant.

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Received December 16, 2011

Accepted February 28, 2012