

# PHENOTYPIC PLASTICITY OF WALL ULTRASTRUCTURE IN THE GREEN ALGA *PEDIASTRUM* S.L. (CHLOROPHYTA, SPHAEROPLEALES)

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**Abstract.** This study examined wall ultrastructure variability in the microscopic green alga *Pediastrum* s.l. Its value as a diagnostic character is discussed. Field and cultured material of 21 taxa were compared using light and scanning electron microscopy. Nine ultrastructural elements occurring on the surface of *Pediastrum* are documented with LM and SEM micrographs. The highest number of taxa showed reticulate ornamentation composed of a trigonal mesh and granules situated on its corners. The paper considers the use of wall ultrastructure to reconcile traditional and modern taxonomical systems with regard to *Pediastrum* varieties, and addresses the phylogenetic relationships between strains representing different varieties.

**Key words:** cell wall patterns, green algae, *Pediastrum*, scanning electron microscopy, taxonomy, ultrastructure

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## INTRODUCTION

*Pediastrum* Meyen (Chlorophyceae, Sphaeropleales) is currently recognized as containing 46 taxa belonging to 27 species (Buchheim *et al.* 2005; Lenarczyk 2014). Although the genus itself is cosmopolitan, only a few species such as *Pediastrum boryanum* (Turpin) Menegh. and *P. duplex* Meyen occur worldwide (Komárek & Jankovská 2001). Other species such as *P. privum* (Printz) E. Hegewald (Kowalska & Wołowski 2010a; Lang *et al.* 2012) are less common. Taxa of the genus form distinctive flat aggregations of cells (coenobia) as a disc-like structure usually composed of 16, 32 or 64 individual cells. Species and infraspecific taxa are distinguished mainly by the shape of marginal cells, including their lobes and processes, incisions between the lobes, and wall surface ornamentation. The latter character usually requires observation by scanning electron microscopy.

A broad study of wall structure in *Pediastrum* was published by Parra (1979), who introduced terminology for ultrastructure and ornamentation

types. That work examined 11 species from field, cultured and herbarium material, originating mainly from Europe (e.g., Germany, France, Sweden) and also from other continents including Asia and North and South America. Parra concluded that cell wall ornamentation is an important character for distinguishing taxa on the infraspecific level. This opinion was supported by Wu (1987), who revised taxa from Taiwan and documented them with SEM micrographs. However, in a study of granule density on the cell wall surface in cultured *P. boryanum*, Nielsen (2000) concluded that those structures alone cannot be used as characters for diagnosing infraspecific taxa. Wall ultrastructure in *Pediastrum* has also been analyzed in taxonomic work by Couté and Tell (1979), Hegewald and Yamagishi (1994), An *et al.* (1999), Hegewald and Jeon (2000), McManus and Lewis (2011) and others.

Wall ultrastructure has been treated in studies of the formation of the coenobium and cell wall (Gawlik & Millington 1969; Millington & Gawlik 1970; Marchant 1974; Millington *et al.* 1981), and more recently in research on phylogenetic

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relationships (Buchheim *et al.* 2005; McManus & Lewis 2005, 2011; Jena *et al.* 2014).

The aim of the present study was to determine the variability of wall ultrastructure in *Pediastrum* taxa observed in field and cultured material from various waterbodies in Poland, and to consider these findings in the context of a practical taxonomical identification system.

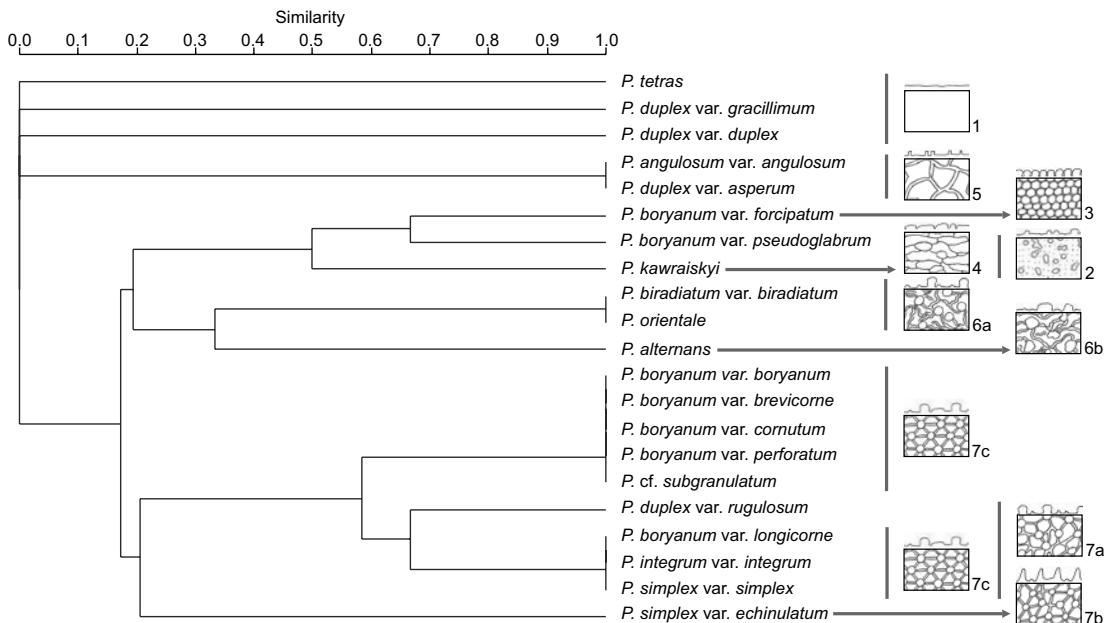
## MATERIAL AND METHODS

Inland water samples containing *Pediastrum* were collected in summer 2008 and 2009 from 48 water bodies in lowland and upland regions of Poland, and in summer 2011 from Wyżni Czerwony Stawek lake in the Tatra Mountains (Table 1). Additional water samples from Jezioro Warnowskie lake and Jezioro Zaterek lake used to initiate some of the *Pediastrum* cultures were obtained in 2008 (Table 2). The samples were taken with a plankton net from 1 or 2 localities of each waterbody. The material from each locality was preserved with 2–4% formaldehyde, except for three samples (3, 5, 8) collected in 2009 (Table 1), which were preserved with 1–2% glutaraldehyde in cacodylate buffer in order to apply the protocol for preparing samples for scanning

electron microscopy (SEM) with critical-point drying. Additionally, material from six localities (asterisked in Table 1) was kept alive for culturing.

Fourteen monoclonal cultures were started with single *Pediastrum* coenobia transferred with a Narishige MM 188 micromanipulator into sterile liquid medium (Table 2). The media used were Chu's medium (Parra 1979), a mixture of Chu's and Knop's media (Starmach 1963), and L-S<sub>2</sub>T<sub>2</sub>, for which a recipe was available at the website of the ACOI Coimbra Collection of Algae [<http://acoi.ci.uc.pt/>]. The strains were cultured in a Bolarus G-18-2 phytotron at ca 22°C under fluorescent tubes in the Phycological Laboratory of the W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków.

Wall patterns were observed in preserved field material (Table 1) and living isolates (Table 2) by both light (LM) and scanning electron microscopy (SEM). LM studies employed Nikon OPTIPHOT-2, Jenaval (Carl Zeiss) and Nikon Eclipse 600 microscopes. For SEM the material was prepared and analyzed in two ways. The material preserved with formaldehyde and the living isolates were first rinsed with distilled water, then placed on cover glasses and air-dried at ca 35°C. The cover glasses were affixed to aluminum stubs with double-sided carbon type. The material preserved with glutar-



**Fig. 1.** Similarities between wall patterns in *Pediastrum*, based on Jaccard's coefficient and UPGMA clustering. For numbers of types of wall patterns see Table 4. Drawings after Lenarczyk (2014).









aldehyde was first rinsed with 10% ethanol (3 × 20 min) and then dehydrated in a series of nine ethanol solutions increasing from 20% to 100% (20–30 min each) and finally in 100% acetone (2 × 30 min). The material was placed in micropore capsules, critical-point dried with CO<sub>2</sub> in an Anderson apparatus, and mounted on aluminum stubs with double-sided carbon tape. All the materials were sputter-coated with carbon or gold and viewed using Hitachi S-4700 or JEOL JSM 5410 scanning electron microscopes at the Institute of Geology and the Institute of Zoology of the Jagiellonian University, Kraków.

The nine structural elements observed on the surface of coenobia in 21 analyzed taxa and the states used in statistical analysis are given in Table 3. The wall patterns of *Pediastrum* taxa were compared by hierarchical clustering in Past ver. 2 (Hammer *et al.* 2001) and visualized as a classification dendrogram (Fig. 1). Clustering was based on Jaccard's coefficient and the UPGMA method. Morphological terminology follows Parra (1979) and Ziemińska-Tworzydło and Kohlman-Adamska (2003). The classification system used here for *Pediastrum* follows Komárek and Jankovská (2001).

## RESULTS

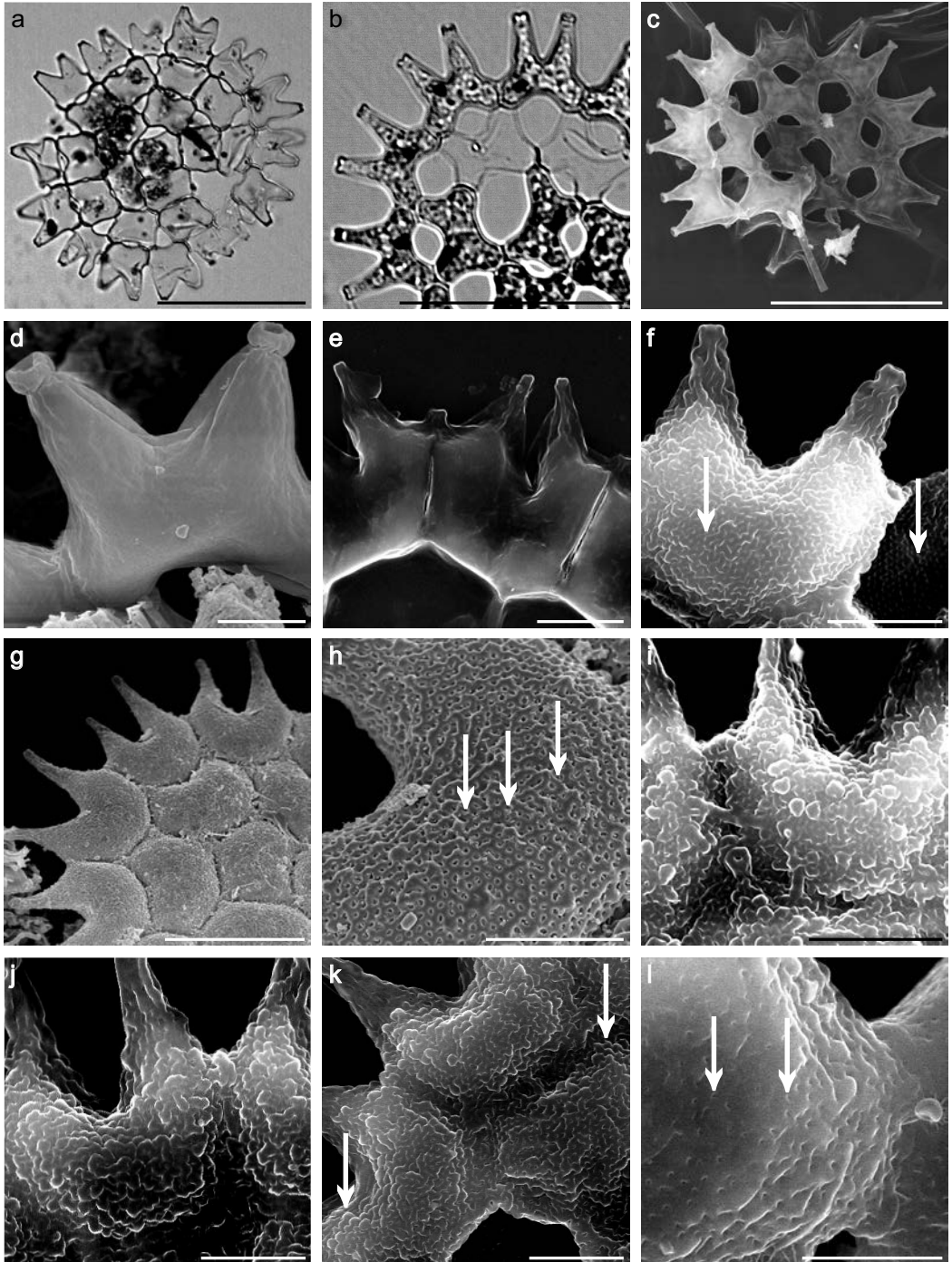
Depending on the occurrence or absence of ultrastructural elements, seven types of wall pattern were distinguished: (1) smooth, (2) scabrate, (3) granulate, (4) verrucate, (5) araneose, (6) rugulate, and (7) reticulate; they are described in Table 4. Rugulate ornamentation comprised two subtypes based on the elements situated on the wrinkles; reticulate ornamentation comprised three subtypes depending on the shape of mesh and the elements on it.

Most of the patterns were shared by at least two taxa. Three ornamentation types were represented by single taxa: granulate (3) by *P. boryanum* var. *forcipatum* (Corda) Chodat (Fig. 3a–h), verrucate (4) by *P. kawraiskyi* Schmidle (Figs 2 & 3i–l), and rugulate with verrucae (6b) by *P. alternans* Nygaard (Fig. 5a–c) (Table 4, Fig. 1). There were some irregularities in the granulate wall pattern of *P. boryanum* var. *forcipatum* which made the pattern resemble the scabrate type (2) in *P. boryanum* var. *pseudoglabrum* Parra (Fig. 2f–k). The surface of *P. boryanum* var. *pseudoglabrum* was sometimes covered with short rows of granules, which

made the pattern resemble the granulate type (3) in *P. boryanum* var. *forcipatum*. Besides the verrucate pattern (type 4) composed of large verrucae and punctae, an old coenobium of *P. kawraiskyi* (Fig. 2l) exhibited scabrate-type (2) ornamentation composed of tiny verrucae and punctae, making the species most similar to *P. boryanum* var. *pseudoglabrum* and *P. boryanum* var. *forcipatum* in the classification diagram (Fig. 1). In contrast to *P. kawraiskyi*, *P. alternans* (Fig. 5a–c) had only one wall pattern, composed of wrinkles and verrucae (type 6b), which was not shared with other species. *Pediastrum alternans* formed one clade with *P. biradiatum* var. *biradiatum* (Fig. 4k–l) and *P. orientale* (Fig. 4g–j), because the three taxa were covered with wrinkles (Table 3, Fig. 1).

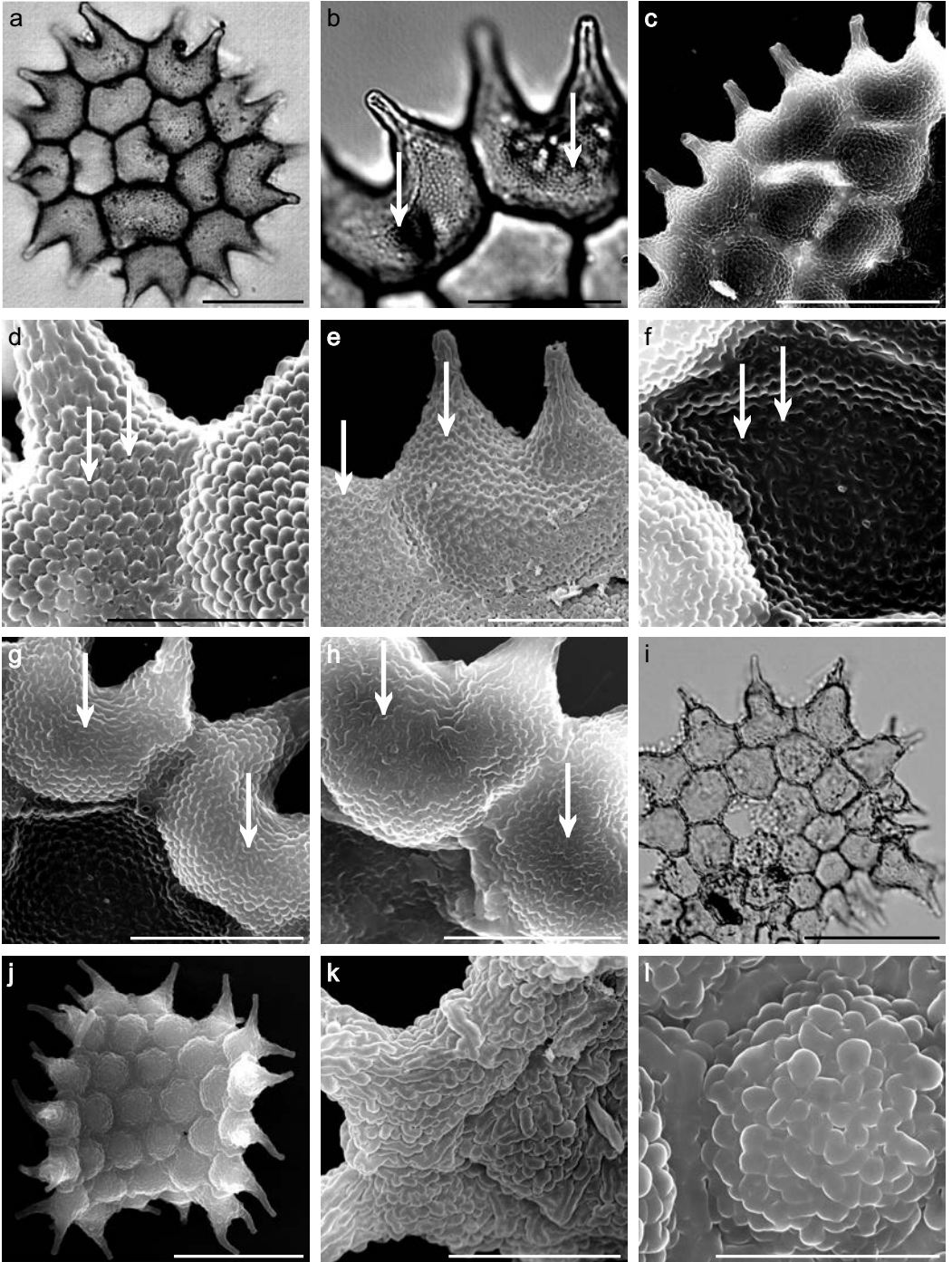
The reticulate pattern with a trigonal mesh and granules (type 7c) was shared by the highest number of taxa (8), mostly *P. boryanum* varieties. The density and size of granules, as well as the shape of the mesh, differed between and within some *P. boryanum* varieties. The densest granulation, up to 18–20 granules per cell width, was observed in *P. boryanum* var. *boryanum* (Fig. 6b) and *P. boryanum* var. *brevicorne* A. Braun (Fig. 6c), and the least dense (6–7 granules per cell width) in *P. boryanum* var. *boryanum*, *P. boryanum* var. *longicorne* Reinsch (Fig. 6d–f) and *P. boryanum* var. *perforatum* (Racib.) Nitardy (Fig. 6i). Granule density was a stable feature in *P. boryanum* var. *longicorne*, but SEM revealed differences in the shape of granules and mesh between cultures from different localities. The granules were delicate and the mesh trigonal in coenobia from lowland waters (Fig. 6d, e), but the granules were prominent and rounded, and the mesh polygonal or trigonal, in a strain from Wyżni Czerwony Stawek lake in the Tatra Mountains (Fig. 6f).

Similarly to *P. boryanum*, *P. simplex* Meyen varieties were also covered with mesh and additional elements. *Pediastrum simplex* var. *echinulatum* Wittr. was covered with spines disposed on a trigonal or tetragonal mesh (type 7b; Figs 1 & 5h–k). However, the differences between the spines of this variety and the granules of *P. simplex* var. *simplex* (Fig. 6l) were so slight in some cells that it was difficult to distinguish the two taxa.

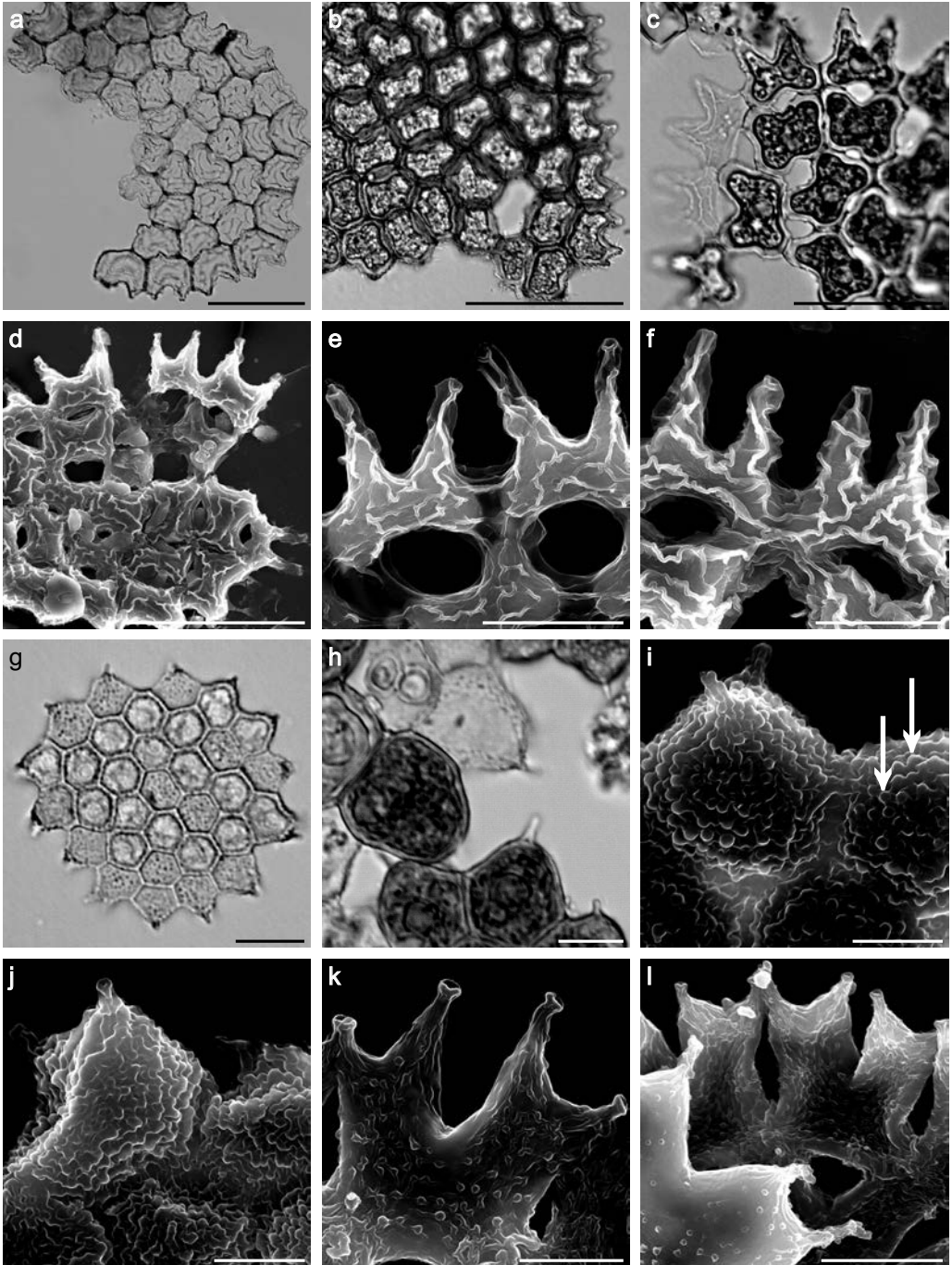


**Fig. 2.** Cell wall surface in *Pediastrum* Meyen: smooth (a–e) and scabrate (f–l). a, c, d – *P. duplex* Meyen var. *duplex*, b – *P. duplex* cf. var. *duplex*, e – *P. tetras* (Ehrens.) Ralfs, f–k – *P. boryanum* var. *pseudoglabrum* (Turpin) Menegh., l – *P. kawraiskyi* Schmidle. Arrows – punctae (f, h, l) or rows of granules (k). Scale bars: a–c – 40  $\mu$ m; g – 20  $\mu$ m; d–f, h–l – 5  $\mu$ m.

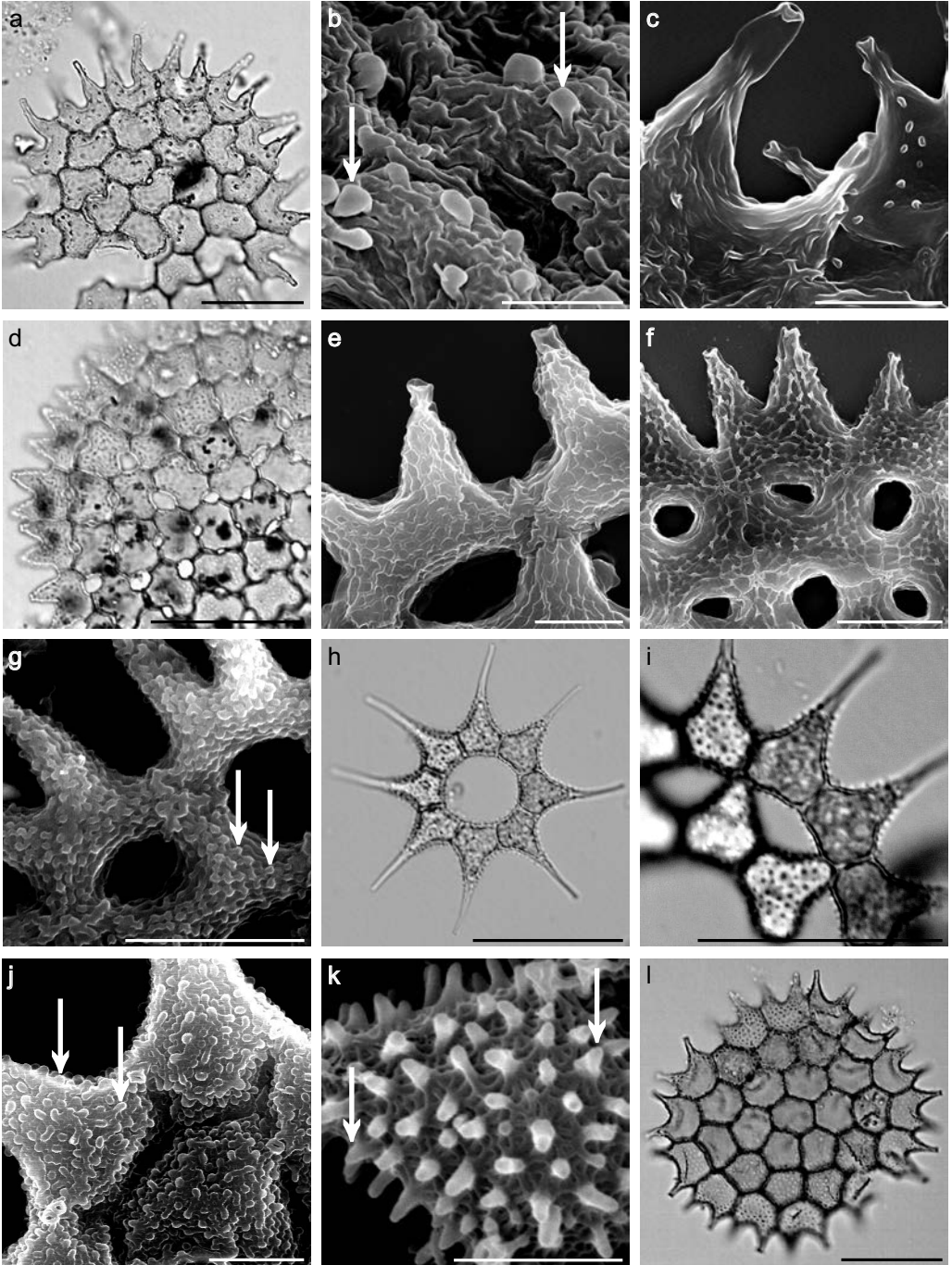




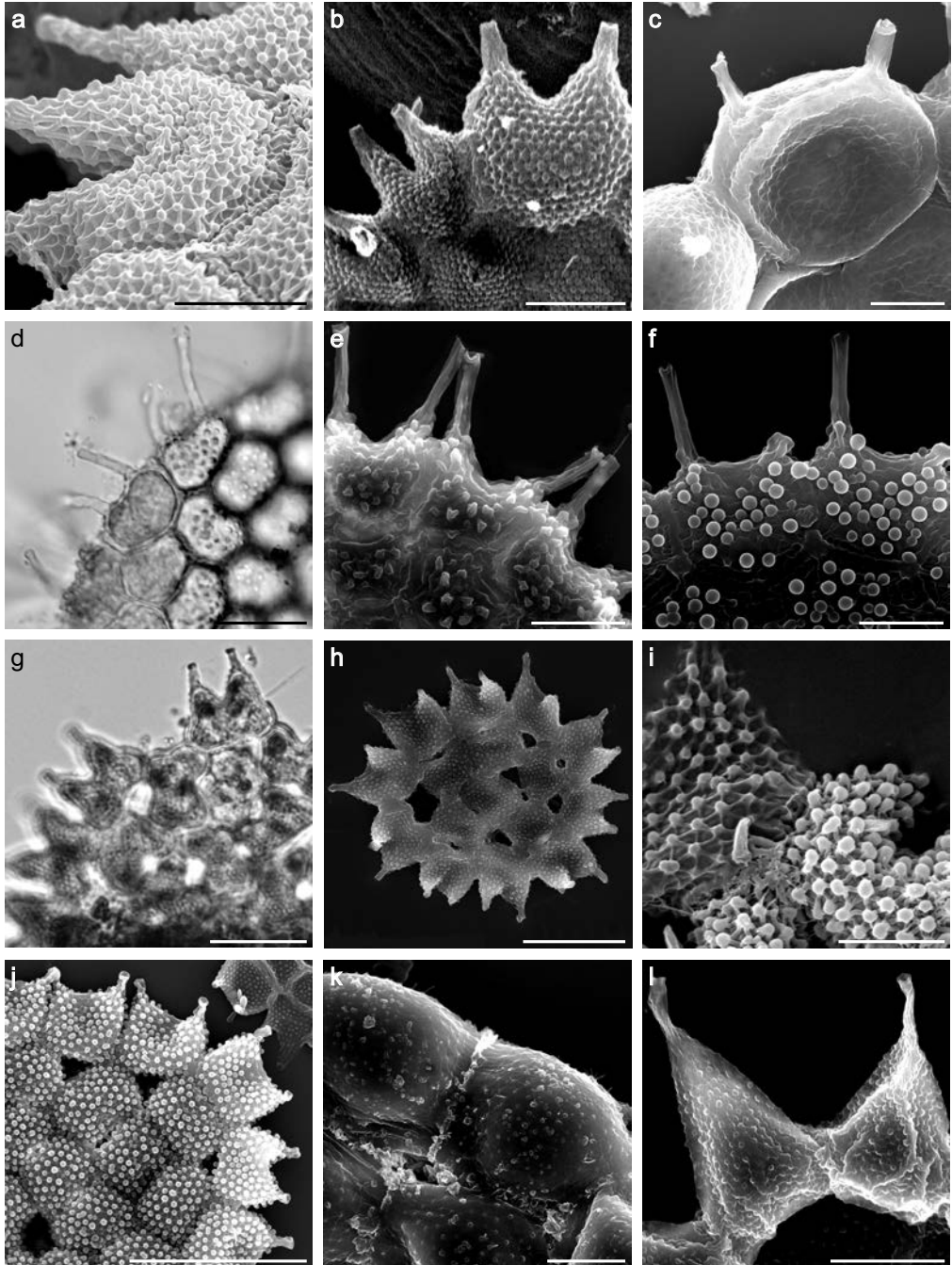
**Fig. 3.** Cell wall surface in *Pediastrum* Meyen: granulate (a–h) and verrucate (i–l). a–h – *P. boryanum* var. *forcipatum* (Corda) Chodat, i–l – *P. kawraiskyi* Schmidle. Arrows – granules (b), punctae (d–f) or deviations of cell wall (g, h). Scale bars: a, i – 40  $\mu$ m; b, c, j – 20  $\mu$ m; e, g, h, k – 10  $\mu$ m; d, f, l – 5  $\mu$ m.



**Fig. 4.** Cell wall surface in *Pediastrum* Meyen: araneose (a–f) and rugulate with granules (g–l). a & b – *P. angulosum* (Ehrenb.) Menegh. var. *angulosum*, c–f – *P. duplex* var. *asperum* (A. Braun) Hansg., g–j – *P. orientale* (Skuja) Jankovská & Komárek, k & l – *P. biradiatum* Meyen var. *biradiatum*. Arrows – granules (i). Scale bars: a, b – 60  $\mu\text{m}$ ; c, d – 40  $\mu\text{m}$ , e–h, l – 10  $\mu\text{m}$ ; i–k – 5  $\mu\text{m}$ .



**Fig. 5.** Cell wall surface in *Pediastrum* Meyen: rugulate with verrucae (a–c), reticulate with granules on polygonal mesh (d–g), reticulate with spines on trigonal or tetragonal mesh (h–k), and reticulate with granules on trigonal mesh (l). a–c – *P. alternans* Nygaard, d–g – *P. duplex* var. *rugulosum* Racib., h–k – *P. simplex* var. *echinulatum* Wittr., l – *P. boryanum* (Turpin) Menegh. var. *boryanum*. Arrows – verrucae (b), granules (g) or spines (j, k). Scale bars: a, d, h, i, l – 40  $\mu$ m; f, g – 10  $\mu$ m; b, c, e, j, k – 5  $\mu$ m.



**Fig. 6.** Wall surface: reticulate with granules on trigonal mesh, in *Pediatrum* Meyen. a – *P. boryanum* (Turpin) Menegh. cf. var. *boryanum*, b – *P. boryanum* var. *boryanum*, c – *P. boryanum* var. *brevicorne* A. Braun, d–f – *P. boryanum* var. *longicorne* Reinsch, g & h – *P. boryanum* var. *cornutum* (Racib.) Sulek, i – *P. boryanum* var. *perforatum* (Racib.) Nitardy, j – *P. cf. subgranulatum* (Racib.) Jankovská & Komárek, k – *P. integrum* Nägeli var. *integrum*, l – *P. simplex* Meyen var. *simplex*. Scale bars: d, g, h, j – 20  $\mu$ m; e, k, l – 10  $\mu$ m; a–c, f, i – 5  $\mu$ m.

## DISCUSSION

Among the 21 taxa investigated, only *Pediastrum alternans* (Fig. 5a–c) is characterized by a single wall pattern – rugulate with verrucae (type 6b) – not shared with other taxa (Table 4, Fig. 1). It follows that this pattern is a reliable diagnostic character for the species. Kowalska and Wołowski (2010b) proposed verrucae as a new taxonomical character for *P. alternans*, since Nygaard (1949) did not mention it in the original description.

In our analysis the wall pattern of *P. alternans* proved to be most similar to that of *P. biradiatum* var. *biradiatum* (Fig. 4k–l) and *P. orientale* (Fig. 4g–j) in the classification dendrogram (Fig. 1). These three taxa have wrinkles on their surface, but with different additional elements: verrucae in *P. alternans* and granules in the other two. The three taxa are not morphologically similar (Lenarczyk 2014). No molecular data on *P. orientale* have been published so far, but some of our preliminary data indicate that *P. alternans* forms a clade with *P. boryanum* strains. This means that the similarities in wall ultrastructure in *P. alternans*, *P. biradiatum* var. *biradiatum* and *P. orientale* could have arisen independently in their evolution.

Our description of the wall surface in *P. orientale* differs from those given by other authors. Komárek and Jankovská (2001) described its ornamentation as a mesh with granules, Sulek (1969) as rugulate with tiny depressions, and Nygaard (1977) as covered with pores in oblique decussate series. The differences between the descriptions

may be connected with a taxonomical problem in *P. orientale* pointed out by Kowalska and Wołowski (2010b). Based on the variability of wall surface and cell shape, Kowalska and Wołowski (2010b) suggested that *P. orientale* is a complex taxon composed of several similar species. Further studies comparing the morphology and phylogenetic relations of its monoclonal strains are needed to address the taxonomical problem; until it is resolved, wall ornamentation cannot be regarded as a diagnostic character in *P. orientale*.

The variability of the reticulate pattern with granules disposed regularly on a trigonal mesh (type 7c) is so great that this pattern has little diagnostic value for *Pediastrum* taxa. *Pediastrum boryanum* var. *boryanum* (Fig. 6b), with its wide range of granule density, is a good example of that variability. Lenarczyk (2015) stated that granule density within a single *P. boryanum* var. *boryanum* strain does not change significantly when cultured at different nutrient concentrations, but in preliminary studies, McManus and Lewis (2005) found that the nutrient status of the culture medium influences phenotypic variability in a strain with *P. boryanum*-like morphology, including granule density. In our present study we found that granule density in *P. boryanum* var. *brevicornis* (Fig. 6c) was within the range of *P. boryanum* var. *boryanum* (Fig. 6b). According to Komárek and Jankovská (2001), these varieties differ from each other in the length of processes and the depth of marginal cell incisions, but Nielsen (2000) stated that the variety *brevicornis* should be rejected and

**Table 4.** Types of wall surface in *Pediastrum* taxa noted in the present study.

Type	Wall pattern	Description
1	smooth	without any concave or convex elements (Fig. 2a–e)
2	scabrate	tiny verrucae and punctae, exceptionally short rows of granules (Fig. 2f–l)
3	granulate	granules in regular rows and punctae (Fig. 3a–h)
4	verrucate	verrucae or verrucae and punctae (Fig. 3i–l)
5	araneose	ridges lying irregularly (Fig. 4a–f)
6	rugulate	<b>a</b> wrinkles with granules (Fig. 4g–l) <b>b</b> wrinkles with verrucae (Fig. 5a–c)
7	reticulate	<b>a</b> granules on a polygonal mesh (Fig. 5d–g) <b>b</b> spines on a trigonal or tetragonal mesh (Fig. 5h–k) <b>c</b> granules on a trigonal mesh (Figs 5l, 6a–l)

included into the nominate variety, as their cell shape varies depending on ontogeny and there is no difference in granule density. Therefore density should not be regarded as a diagnostic character for the variety *brevicornis*. We also found that the coenobia of *P. boryanum* var. *cornutum* (Racib.) Sulek (Fig. 6g, h) and *P. boryanum* var. *perforatum* (Fig. 6i) are similar in granule density and in coenobium shape and cell shape. This supports Nielsen's (2000) doubts about the distinction between these varieties.

Some preliminary data (Lenarczyk & Saługa 2013) indicate that in the phylogenetic tree the strain *P. boryanum* var. *longicornis* isolated from mountains (Table 2, no. 6) is not closely related to two other strains of *P. boryanum* var. *longicornis* isolated from lowland (Table 2, nos 7 and 8). That suggestion is supported by morphological differences between the lowland and mountain strains we observed in the present study. The variety *longicornis* is a complex taxon.

The taxonomical position of *P. boryanum* var. *pseudoglabrum* (Fig. 2i–h) and *P. boryanum* var. *forcipatum* (Fig. 3a–h) is questionable as well, in view of their similar cell shape and deviations from the scabrate (type 2) and granulate (type 3) wall patterns. According to Lenarczyk (2014), *P. boryanum* var. *pseudoglabrum* probably is *P. boryanum* var. *forcipatum* with not well developed granulate ornamentation.

To distinguish between *P. simplex* var. *simplex* (Fig. 6l) covered with granules and *P. simplex* var. *echinulatum* (Fig. 5h–k) covered with spines, and to identify the range of spine length in the latter variety from field material, analysis of these features in monoclonal cultures is necessary. By that method the length of structural elements, spines and granules can be measured in specimens unambiguously representing individual *P. simplex* genotypes. Parra (1979) and Wu (1987) did not distinguish the variety *echinulatum*, but determined *P. simplex* coenobia with spines as *P. simplex* var. *simplex*. McManus and Lewis (2011) genetically analyzed single strains of *P. simplex* var. *simplex* [as *Monactinus simplex* var. *simplex* (Meyen) Corda] and *P. simplex* var. *echinulatum* [as *M. simplex* var. *echinulatum* (Witttr.) H. McManus & L. A.

Lewis]; the two strains differed only slightly in their 26S rDNA and *rbcL* sequences.

We also observed some wall features differing from those given in the literature as characteristic for *P. simplex* var. *simplex*, *P. duplex* var. *rugulosum* Racib., *P. tetras* (Ehrenb.) Ralfs and *P. duplex* var. *duplex*. In *P. simplex* var. *simplex* (Fig. 6l) the granules were disposed regularly on a trigonal mesh (type 7c), irregularly on a polygonal mesh (type 7a), but not on wrinkles as stated by Komárek and Jankovská (2001). According to Parra (1979), trigonal mesh occurs in younger specimens and then turns into an irregular pattern whose elements are similar to wrinkles building Y-shaped connections. Besides the reticulate ornamentation in *P. simplex* var. *simplex*, Parra (1979) observed fossulate ornamentation in his newly described *P. simplex* var. *pseudoglabrum*.

In *P. duplex* var. *rugulosum* the granules were of different sizes (Fig. 5d–g). The bigger ones covered the mesh or wrinkles to which they were affixed (Fig. 5g). Hindák and Hindáková (2008) may also have observed coenobia with such granules, as they stated that the surface of this taxon is covered with granules, not reticulate with granules (type 7a).

The single coenobium of *P. tetras* observed by SEM (Fig. 2e) was smooth with sparse sites of roughness resembling granules or verrucae. Parra (1979) noted various ornamentation types in this species: irregularly granulate, reticulate, and rugulate with granules. Shubert and Wilk-Woźniak (2003) found a rugulate coenobium in a waterbody in Puławy, central Poland. Wu (1987) distinguished *P. tetras* var. *tetras* with a reticulate pattern and *P. tetras* var. *tetraodon* (Corda) Hansg. with a granulate one. McManus and Lewis (2005) observed a rugulate/reticulate surface in different strains of *P. tetras* from Europe and North America, and thick wrinkles in another strain from North America. Komárek and Jankovská (2001) and McManus and Lewis (2005) indicated that strains with *P. tetras*-like morphology may represent separate taxa.

A smooth surface (type 1) was observed in two *P. duplex* varieties: *P. duplex* var. *duplex* (Fig. 2a, c, d) and *P. duplex* var. *gracillimum*. Due

to the shared surface type, Sulek (1969) and Parra (1979) proposed that the latter variety should be included into the nominate one. However, McManus *et al.* (2011) used molecular and morphometric methods to describe a new genus *Lacunastrum* H. McManus and a new combination *L. gracillimum* (W. & G. S. West) H. McManus (formerly *P. duplex* var. *gracillimum*).

We observed the last type of wall surface – araneose (type 5) – in two varieties from different species: *P. angulosum* (Ehrenb.) Menegh. var. *angulosum* (Fig. 4a, b) and *P. duplex* var. *asperum* (A. Braun) Hansg. (Fig. 4c–f). McManus and Lewis (2011) reported phylogenetic results based on a single strain of *P. duplex* var. *asperum* and seven strains of *P. angulosum*, which were grouped in two different clades consisting mainly of *P. duplex*, not assigned names to variety level. Thus, it seems likely that araneose ornamentation evolved independently in various *Pediastrum* lineages.

In addition to differences in wall patterns between taxa, our SEM study of cultured material disclosed changes in wall patterns during coenobium growth and aging. In older specimens the reticulum and wrinkles were flatter, poorly visible and delicate, and the granules and verrucae were disposed more sparsely (Figs 4l & 6k). Such changes, resulting from wall stretching, were also reported by Hegewald and Yamagishi (1994). We also showed that the verrucate pattern can become scabrate as a result of wall stretching and flattening of verrucae during coenobium growth. According to Sulek (1969) and Parra (1979), each ornamentation type in *Pediastrum* is variable within a certain range, depending on the ontogenetic cycle and cell size. It should be stressed that the changes in wall patterns connected with the ontogenetic cycle or cell size are poorly recognized so far.

We found great phenotypic variability in the examined *Pediastrum* taxa from Polish waterbodies, especially within *P. boryanum*. We conclude that ultrastructural features cannot be used as a diagnostic tool for most taxa of the genus, but that those characters can be helpful in identifying certain taxa when they are found in similar environmental conditions, and therefore are of some value. The challenge for the future is to discover

the full range of wall variability in individual taxa throughout their life cycle, and to determine the impact of particular environmental factors more precisely.

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