

Ultrastructural examination of the pycnidia and conidia of the genus *Opegrapha* (Arthoniales, Ascomycota)

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The paper presents a summary of examinations on the variation of pycnidia and conidia of the following *Opegrapha* species occurring in Poland: *O. atra*, *O. calcarea*, *O. dolomitica*, *O. gyrocarpa*, *O. niveoatra*, *O. rupestris*, *O. varia*, *O. vermicellifera*, *O. viridis* and *O. vulgata*.

Key words: lichens, *Opegrapha*, conidiomata, conidial spores, Poland

INTRODUCTION

Pycnidia are the main type of anamorph structures and are pear-shaped or globose receptacles, within which conidia are formed on a special hyphal type, called conidiophores (Büdel, Scheidegger 2008). The taxonomic value of pycnidia and conidia has been discussed as far back as in the days of Nylander (1858–1860). This question was more broadly described by Steiner (1901), according to whom a degree of the taxonomic value of these structures can be shaped in a different way, depending on relation to taxonomy itself. This author, as the first one, paid attention to the fact that pycnidia and conidia should be carefully observed just as apothecia, perithecia, pseudothecia and ascospores. Despite a large diversity of forms, these structures have been given much lower rank than apothecia (Esslinger 1978). The genus *Opegrapha* is one of many pycnidia-producing genera. Although the genus itself was a subject of many treatments (Stizenberger 1865; Redinger 1940; Nowak 1983; Ertz et al. 2009) the structure of pycnidia have been described so far only by means of regular light microscopy and only superficially within the framework of other major research projects (Upadhyay 1964; Atienza 1992; Egea, Tibell 1993; Herrera-Campos, Lücking 2002; Ertz, Diederich 2003). Research works on the vari-

ation of species of that taxonomic group have been carried out by the author for six years. The present paper is a contribution to the better knowledge of the genus.

MATERIAL AND METHODS

In the study were used selected epiphytic and epilithic species of the genus *Opegrapha* occurring in Poland: *O. atra* Pers., *O. calcarea* Sm., *O. dolomitica* (Arnold) Clauzade & Cl. Roux, *O. gyrocarpa* Flot., *O. niveoatra* (Borrer) J. R. Laundon, *O. rupestris* Pers., *O. varia* Pers., *O. vermicellifera* (Kune) J. R. Laundon, *O. viridis* (Ach.) Behlen & Desberger and *O. vulgata* (Ach.) Ach.

Species nomenclature was adopted after Santesson et al. (2004) and Diederich et al. (2008). Out of each species, 30 specimens were studied coming from different locations in the country. In total, about 400 pycnidia were examined coming from different specimens deposited in 9 lichen herbaria in Poland and the author's private collection (GPN, KRAM, KTC, LBL, LOD, POZ, UGDA, WRSL and hb. Wieczorek). In order to analyse in detail subtle structures, such as conidiophores and conidia, well developed pycnidia were selected from the whole collection. They were cleaned, properly prepared and then cut into microtome sections, 10 to 25 µm thick, by means of rotary microtome. The whole analysed material was subject to examination using high quality optic-computer equipment, i.e. a QUANTA 200 scanning electron microscope and a light microscope of NIKON Eclipse E-600 type.

SELECTED SPECIMENS EXAMINED. POLAND. *O. atra* – Pojezierze Mazurskie lakeland, Pojezierze Olsztyńskie lakeland, ca 1.5 km NE of Smolajny village, on *Fraxinus excelsior*, 1993, leg. S. Cieśliński (KTC); *O. calcarea* – Western Carpathians, Tatry Zachodnie Mts., Dolina Olczyńska valley, on calcareous rock, 20 July 2007, leg. A. Wieczorek (hb. Wieczorek); *O. dolomitica* – Western Carpathians, Beskid Mały Mts., Żurawica, on calcareous rock, 26 Aug. 1961, leg. J. Nowak (KRAM-L 9661); *O. gyrocarpa* – Eastern Sudetes, Góry Białskie Mts., Nowy Gieraltów, Skały Trzy, on siliceous rock, 7 Aug. 2003, leg. K. Szczepańska (WRSL); *O. niveoatra* – Nizina Północnopodlaska lowland, Równina Bielska plain, Puszcza Białowieska forest, Hajnówka forest division, forest section no. 246, on *Alnus glutinosa*, 1983, leg. S. Cieśliński & Z. Tobolewski (KTC); Wysoczyzna Białostocka high plain, Puszcza Knyszyńska forest, on *Carpinus betulus*, 1987, leg. Z. Tobolewski & K. Glanc (KTC); *O. rupestris* – Western Carpathians, Małe Pieniny Mts., Wąwóz Homole gorge, on limestone, 9 June 1956, leg. Z. Tobolewski (POZ); Eastern Sudetes, Góry Białskie Mts., Dolina Kleśnicy valley, on calcareous rock, 27 June 2004, leg. K. Szczepańska (WRSL); *O. varia* – Western Carpathians, Beskid Żywiecki Mts., Pasma Policy range, Sidzińskie Pasionki, on *Fagus sylvatica*, 8 May 1964, leg. J. Nowak (KRAM-L 16204); Puszcza Białowieska forest, Zwierzyniec forest division, forest section no. 220, on *Fraxinus excelsior*, 25 Aug. 1965, leg. J. Rydzak (LBL); Pojezierze Kaszubskie lakeland, Staniszewskie Błoto nature reserve, on *Acer* sp., 23 Apr. 1977, leg. W. Fałtynowicz (UGDA-L 1861); *O. vermicellifera* – Puszcza Białowieska forest, Białowieża National Park, on *Carpinus betulus*, 12 Aug. 2002, leg. P. Czarnota (GPN 2947); Nizina Północnopodlaska lowland, Kotlina Biebrzańska basin, Puszcza Augustowska forest, on *Acer* sp., 18 Sept. 1986,

leg. S. Cieśliński (KTC); Wyżyna Przedborska upland, Niecka Włoszczowska basin, Dębowiec nature reserve, forest section no. 161, on *Ulmus* sp., 25 Aug. 1970, leg. K. Czyżewska (LOD-L 833); Puszcza Białowieża forest, Białowieża National Park, forest section no. 283, on *Tilia cordata*, 1982, leg. S. Cieśliński & Z. Tobolewski (KTC); Nizina Środkowomazowiecka lowland, Równina Kozienicka plain, Puszcza Kozienicka forest, Zagożdżon nature reserve, on *Carpinus betulus*, 2002, leg. S. Cieśliński (KTC); Równina Bielska plain, Białowieża National Park, forest section no. 256, on *Carpinus betulus*, 16 Sept. 1987, leg. K. Czyżewska (LOD-L 9039); Western Carpathians, Tatry Zachodnie Mts., Dolina Olczyńska valley, Polana Olczyńska glade, on *Acer pseudoplatanus*, 12 May 1998, leg. U. Bielczyk (KRAM-L 44575); Białowieża National Park, forest section no. 283, on *Tilia cordata*, 1982, leg. S. Cieśliński & Z. Tobolewski (KTC); Białowieża National Park, forest section no. 256, on *Acer platanoides*, 1990, leg. S. Cieśliński (KTC); *O. viridis* – Pojezierze Kaszubskie lakeland, Porzecze, forest section no 261, on *Acer* sp., 15 July 1985, leg. W. Fałtynowicz (UGDA-L 2868); *O. vulgata* – Białowieża National Park, forest section. no. 340A, on *Picea abies*, 12 Aug. 2002, leg. P. Czarnota (GPN 3006).

RESULTS AND DISCUSSION

Within the genus *Opegrapha*, pycnidia are known of different shape and size. Basic pycnidia shapes include: a **round form** found, among others, in *O. vermicellifera* (Fig.1), *O. dolomitica* or *O. vulgata*, an **oval form** observed in *O. rupestris* (Fig.2) or *O. niveoatra*, and a **pyriform** occurring in *O. varia* and also in *O. vermicellifera* (Fig.3). There is variation of these forms, also within single species, which consist first of all in a larger or smaller flattening of pycnidium or, like for example in *O. vermicellifera* (Fig.4a), in development of local thickenings of the pycnidial wall. The size of pycnidia is much varied; for example in *O. vermicellifera*, the height of these structures ranges from 130 μm to 260 μm , being measured from the thallus base, while their width from 105 to 270 μm . Considerable differences in the height of these structures have been observed, for instance, in *O. dolomitica*. Pycnidia in this species are structures once deeply immersed in the thallus but some other time they protrude above the thallus surface. The width of pycnidium in the examined specimens of *O. dolomitica* ranges from 106 μm to 380 μm .

Pycnidia are distributed on the thallus singly – such cases were observed in *O. atra* or *O. dolomitica*, or in smaller or larger agglomerations, like in *O. vermicellifera* (Fig.5). Pycnidia of the examined species have been divided into two groups: **sessile pycnidia** (protruding above the thallus) and **thallus-immersed pycnidia**. Majority of the examined species had more or less protruding pycnidia. These taxa included *O. atra*, *O. niveoatra* (Fig.6), *O. varia*, *O. vermicellifera*, *O. viridis* and *O. vulgata*. Pycnidia of the epilithic species, such as *O. calcarea*, *O. dolomitica* and *O. rupestris* (Fig.7), were more variable and it occurred frequently that both sessile pycnidia and those immersed in the thallus were found on one specimen of a given species.

In the genus *Opegrapha*, there are non-chambered (unilocular) pycnidia in all species (Figs 1, 2, 3, 4, 8, 9). From point of view of ontogenesis, a non-chambered

pycnidium can develop in a much diversified way. The lack of partition walls inside pycnidium is always typical. An advantage of such a construction is possibility to produce a large number of conidia in the pycnidium (Vobis 1980). When compared with the pycnidium volume, the layer producing conidia is rather narrow and, for example in *O. vermicellifera* (Fig.10), is barely 10 to 25 μm broad. The pycnidial walls are composed of basal conidiogenous cells which, as the paraplectenchymatic [= pseudoparenchymatic] layer, separate a pycnidium from the thallus (Vobis 1980) (Fig.11). The wall-building cells differ from proper conidiophores in this, among others, that they can not produce conidia. Until the complete development of pycnidia, they can proliferate by division so that pycnidium dimensions become enlarged this way. Determination of a clear boundary between the cells of conidiophores and those building the pycnidial wall is impossible in the greater part of cases, in particular when conidiogenous cells producing conidia are in the early stage of development. Therefore, it is hard to determine the thickness of pycnidial wall. In cases which are not questionable (mature pycnidia filled with conidia), the wall thickness is, for example in *O. vermicellifera* (Fig.12), from 13 μm to 30 μm (without pruina). A characteristic external element of pycnidium in *O. vermicellifera* is its pruina (Figs 4b, 13). The thickness of pycnidium pruina in specimens belonging to that species varies largely and is ca 4 μm but locally also up to 22 μm .

Apical cells of the pycnidial wall have a tendency to assume a black colour, so called "carbonisation", among others in *O. vermicellifera* (Fig.14). This phenomenon was earlier observed in many lichen species (Tibell 1978; Coppins, James 1979). As reported by Vobis (1980), a colour pigment is deposited in most cases in external layers of cellular walls but pigment penetration into the cell interior has been also observed, among others in *Lecanactis abietina*.

Majority of the examined specimens have only one ostiolum, with *O. atra* (Fig.9), *O. rupestris* (Fig.7), *O. calcarea* or *O. dolomitica* among others, although there are pycnidia, admittedly rarely, with two openings on the apex found in *O. vermicellifera* (Figs 15 and 16), *O. varia* or *O. viridis*. The ostiolum is situated most frequently on the apex of pycnidium, although it can also develop in any place on pycnidium; such cases were quite frequently observed for instance in *O. niveoatra* or *O. vermicellifera*. The shape of opening is circular in the greater part of cases, among others in *O. niveoatra* (Fig.17) or *O. rupestris* (Fig.18) but longitudinal and irregular openings were also observed, e.g., in *O. atra* or *O. calcarea*. The width of ostiolum ranges from 9.81 μm in *O. niveoatra* (Fig.17) and *O. vermicellifera* to 45.21 μm , among others in *O. rupestris* (Fig.18).

Development of the ostiolum may take place in a different ways. An additional cracking of the pycnidial wall was observed just after development of a proper ostiolum on the apex, among others in *O. vermicellifera* (Fig.4c) or *O. viridis*. In this connection, one can assume until more careful examinations with the use of transmission electron microscopy that the ostiolum of species from the genus *Opegrapha* develops by a simple disruption in a place formed earlier, like in *O. gyrocarpa* (Fig.19). Disruption of pycnidium takes place only in the wet state since only then the mucus flows into the pycnidium cavity and this way increases the internal pressure. The opening can crack and masses of conidia immersed in the mucous substance leave the ostiolum like a drop or cirrus, e.g., in *O. varia* (Fig.20). If lichens shrivel up again, then a thickened plug develops over the ostiolum, like for instance in *O. viridis* (Fig.21).

In case of renewed conidia production or at large internal pressure, a thickened plug can be thrown aside and conidia will flow out again through the ostiolum. If conidia have not been washed out by rain or splash water, the mucus shrivels up again and keeps a mass of conidia within the zone of ostiolum, e.g., in *O. calcarea* (Fig.22), or on the whole pycnidium, among others in *O. vermicellifera* (Figs 23 and 24).

The interior of pycnidium is filled with colourless mucous called matrix (Jones 1976). Under optical microscope, it is possible to detect its presence only in the dry state as a very thin boundary layer which keeps a mass of conidia. It is well-known that this is a substance different from the hyphae gel of apothecia since it does not have the amyloid character. The origin of mucous substance is not explained completely. According to Hammill (1974), the endoplasmatic reticulum is responsible for its production. On the other hand, Jones (1976) is of the opinion that this substance can be derived from the paramural bodies of conidial cells. Researchers are in accord as to the fact that this substance is a derivative of most external layers of the cellular wall, while precise separation between the matrix and cellular wall components is frequently impossible (Vobis 1980).

Conidia produced by species of the genus *Opegrapha* assume most frequently cylindrical form, e.g., in *O. varia* (Fig.25) or are bacilliform, like in *O. vermicellifera* (Fig.26). In the examined material, however, there was variation of those forms, consisting in smaller and larger flattening or twisting and development of asymmetric thickening and pointed tips, like in *O. varia* (Fig.27) or *O. vermicellifera* (Fig.28). The length of conidia in the examined material differs and depends on species; for example, it is 3 μm to 6 μm in *O. vermicellifera*, whereas 6 μm do 13 μm in *O. viridis*. Similar differences refer to the width of conidia; for example, the width of these structures ranges from 0.8 μm to 2.7 μm in *O. vermicellifera*, while from a 0.8 μm to 2.0 μm in *O. viridis*. The wall thickness in mature conidia varies; for example, it ranges from 210 nm to 244 nm in *O. vermicellifera*. For conidia, an existing pedicle is frequently distinguishable on the one end, which is seen among others in conidia of *O. vermicellifera* (Fig.29).

Under optical microscope, conidia appear like smooth and hyaline, among others in *O. niveoatra* (Fig.30) or *O. varia* (Fig.31). Under scanning electron microscope, a subtle granular ornamentation of their surface can be observed, among others in *O. vermicellifera* (Fig.32), which can be the external layer on conidia, with the same structure like the matrix of pycnidia.

CONCLUSIONS

Application of high quality light and scanning microscopy made the study of additional aspects of the pycnidia and conidia variation in the genus *Opegrapha* possible. Fluctuation of the forms of all pycnidium elements, as well as of the traits of conidia, confirms large diversity of these structures in the genus. Particular attention was paid to the formation of two ostiola in various places of pycnidium, which are frequently created in different periods of development. It was observed in *O. varia*, *O. vermicellifera* and *O. viridis*. In the examined material, no changes of that type

were observed in epilithic species. This phenomenon is perhaps connected with large internal pressure in the interior of pycnidium which causes additional disruption of the wall in species with a small ostiolum. Observation of the thickness of respective pycnidium structures was also made in different stages of development. The results of this examination are undoubtedly original contribution to the characteristic of particular species.

The analysis of scanning electron microscope images showed a considerable diversity in the shape of conidia. Attention was also paid to the surface of conidia which had been considered as smooth so far. The carried out observations showed the presence of subtle ornamentation in all examined species. This is perhaps a result of drying of a delicate hyaline layer covering conidia but more careful examinations are required here with the use of transmission electron microscopy.

The observations carried out in this study are one of many stages of the research on pycnidial ultrastructure in the genus *Opegrapha*. At present, examinations are under way with the use of transmission electron microscopy which certainly will be an important supplementation of the description presented here.

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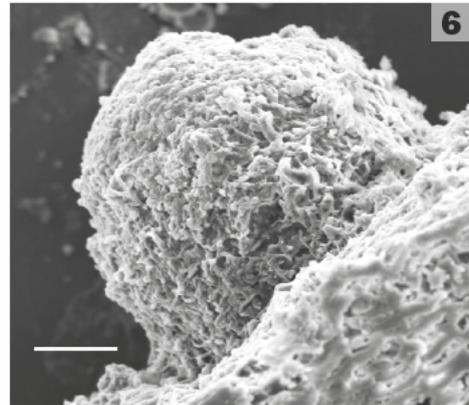
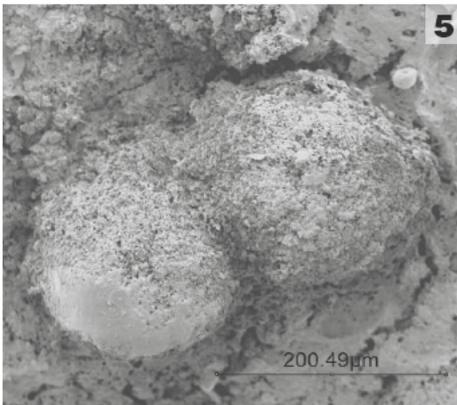
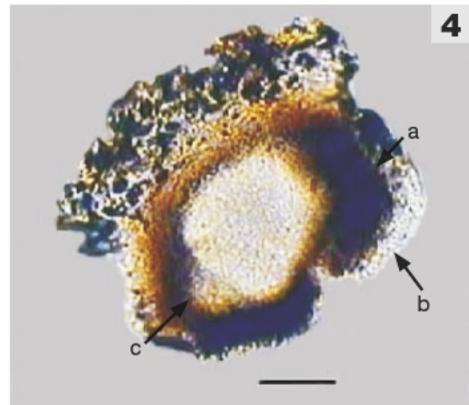
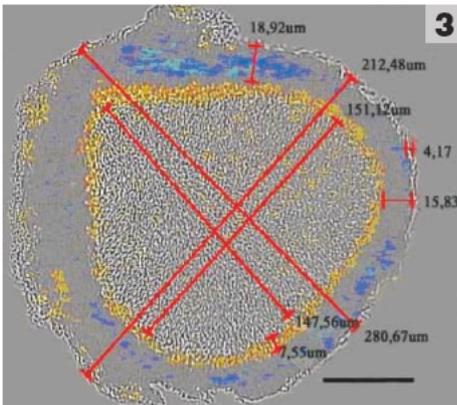
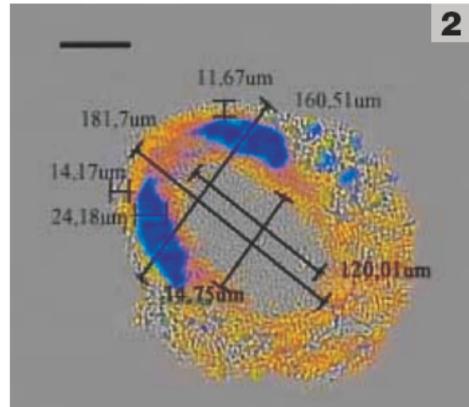
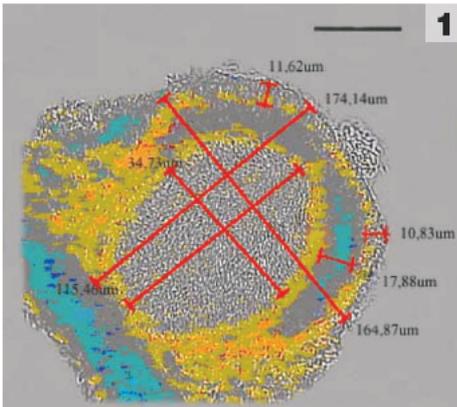
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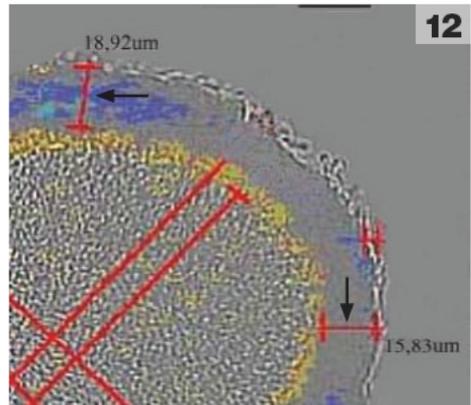
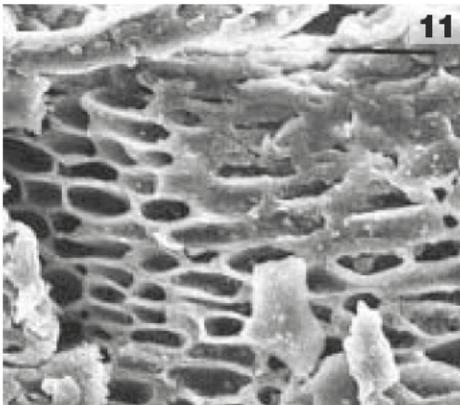
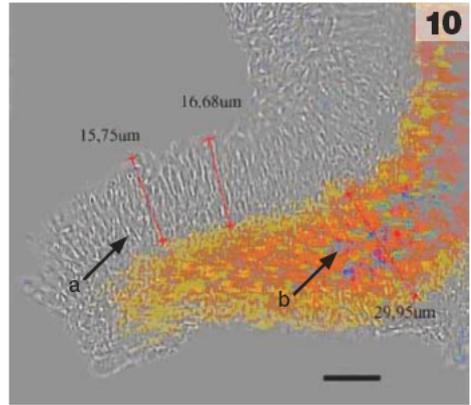
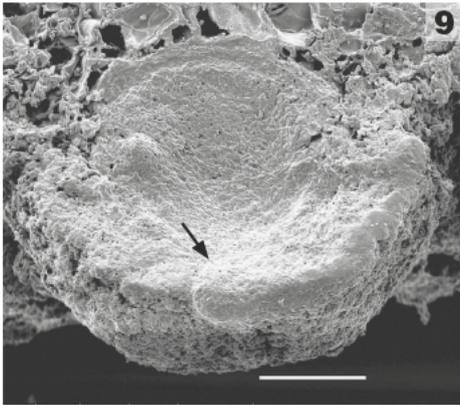
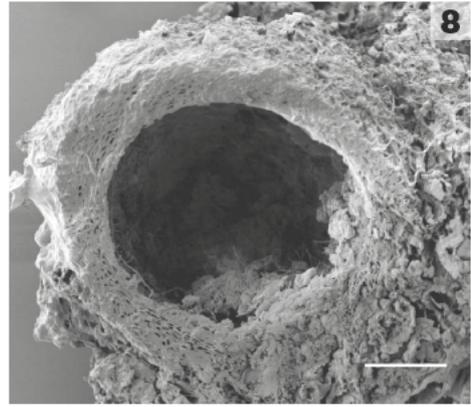
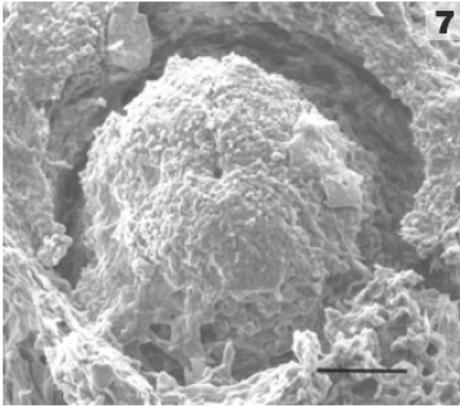
Badania ultrastrukturalne pyknidiów i zarodników konidialnych porostów z rodzaju *Opegrapha* (Arthoniales, Ascomycota)

Streszczenie

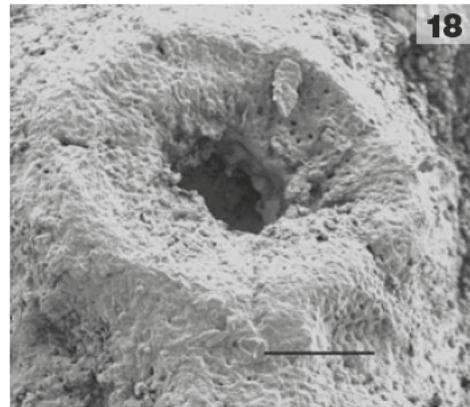
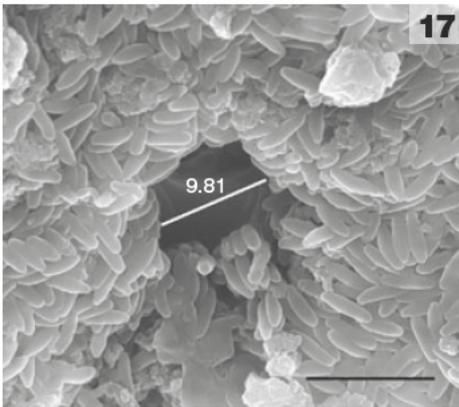
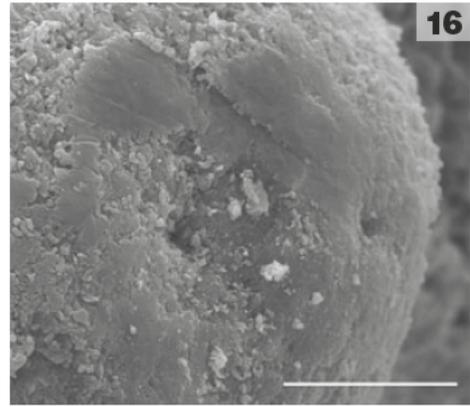
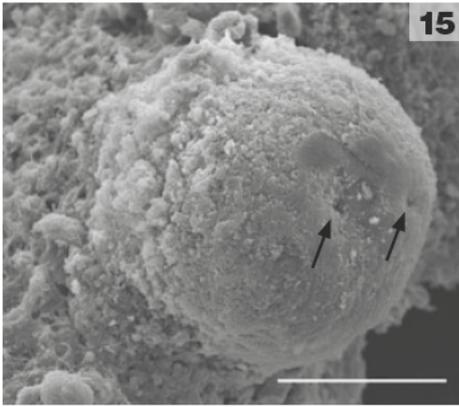
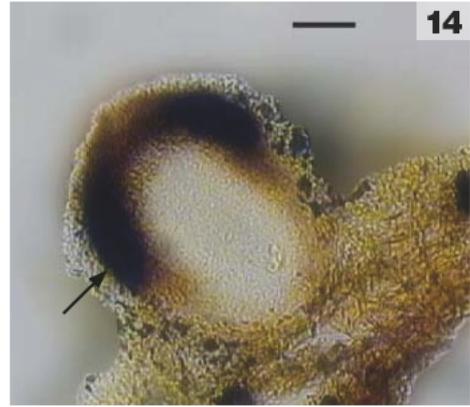
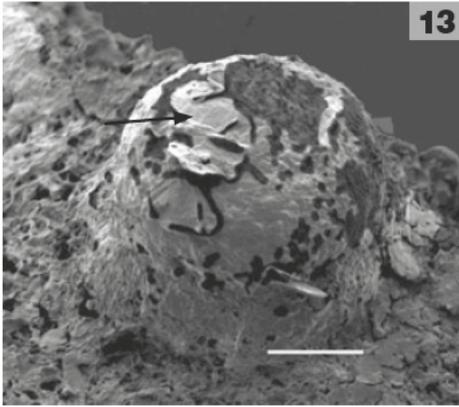
W pracy przedstawiono wyniki badań nad zmiennością pyknidiów należących do 10 gatunków porostów z rodzaju *Opegrapha* występujących w Polsce. (*O. atra* Pers., *O. calcarea* Sm., *O. dolomitica* (Arnold) Clauzade & Cl. Roux, *O. gyrocarpa* Flot., *O. niveoatra* (Borrer) J. R. Laundon, *O. rupestris* Pers., *O. varia* Pers., *O. vermicellifera* (Kune) J. R. Laundon, *O. viridis* (Ach.) Behlen & Desberger, *O. vulgata* (Ach.) Ach.). W badaniach wykorzystano mikroskop elektronowy skaningowy QUANTA 200 oraz mikroskop świetlny typu NIKON Eclipse E-600. Praca jest podsumowaniem dotychczasowych badań nad zróżnicowaniem wszystkich struktur pyknidium. Dzięki przeprowadzonym obserwacjom dokładnie poznano między innymi grubość ściany pyknidium oraz warstwy konidiotwórczej, grubość ściany zarodników konidialnych, szerokość ostiolum w różnych stadiach rozwojowych pyknidium. Zastosowanie mikroskopii skaningowej ujawniło występowanie delikatnej ornamentacji na powierzchni zarodników konidialnych do tej pory uważanych za gładkie. Obserwacja pyknidiów pozwoliła na ujawnienie zjawiska tworzenia się dwóch ostioli u takich gatunków jak *O. varia*, *O. vermicellifera* i *O. viridis*. Przeprowadzone badania pokazały również jak duże jest zróżnicowanie form pyknidiów oraz produkowanych przez nie zarodników konidialnych.



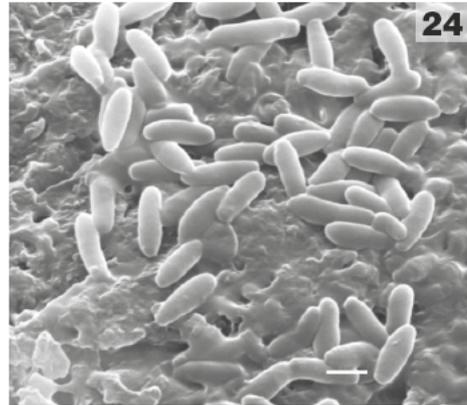
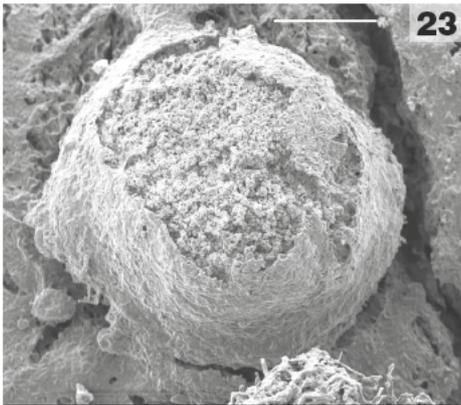
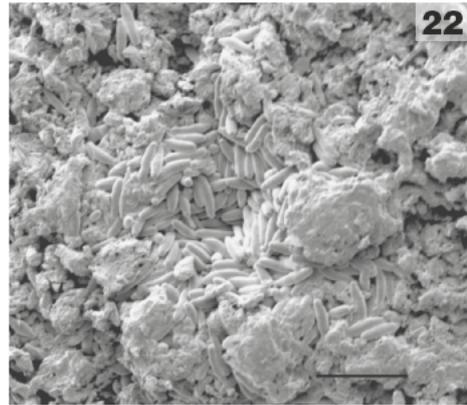
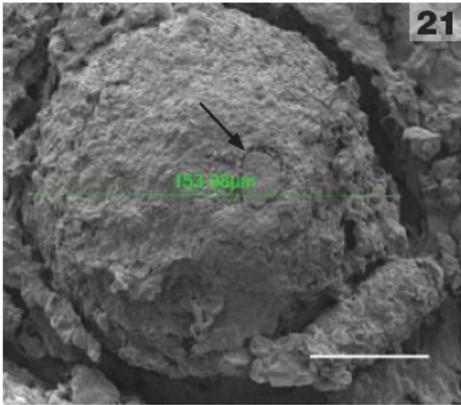
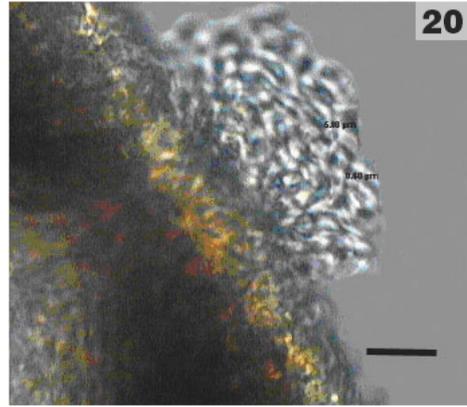
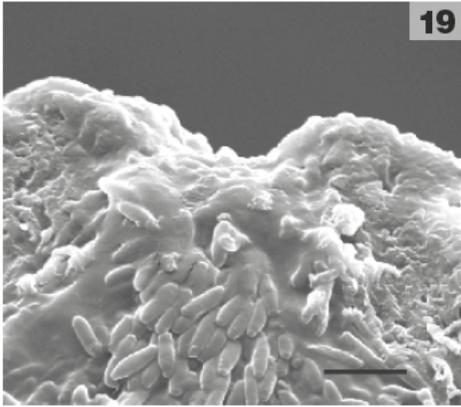
Figs 1-6. 1-4. Cross-section through a pycnidium: 1. *Opegrapha vermicellifera* (2002, Czarnota, GPN 2947); 2. *O. rupestris* (1956, Tobolewski, POZ); 3. *O. vermicellifera* (1986, Cieśliński, KTC); 4. *O. vermicellifera* (1970, Czyżewska, LOD-L 833): a – wall thickening, b – pruina, c – place of development of the second ostiolum. 5. *O. vermicellifera* – pycnidia agglomeration [SEM] (1982, Cieśliński & Tobolewski, KTC). 6. *O. niveoatra* – pycnidium, external appearance [SEM] (1983, Cieśliński & Tobolewski, KTC). Scale bars: Figs 1-4 = 50 µm, Fig. 5 = 100µm, Fig. 6 = 30 µm.



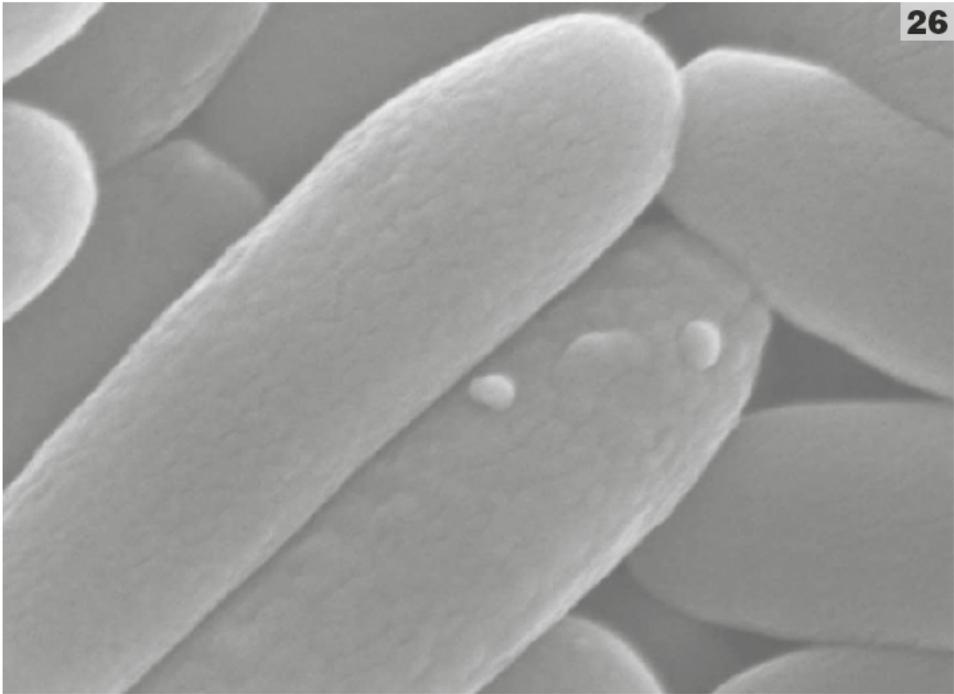
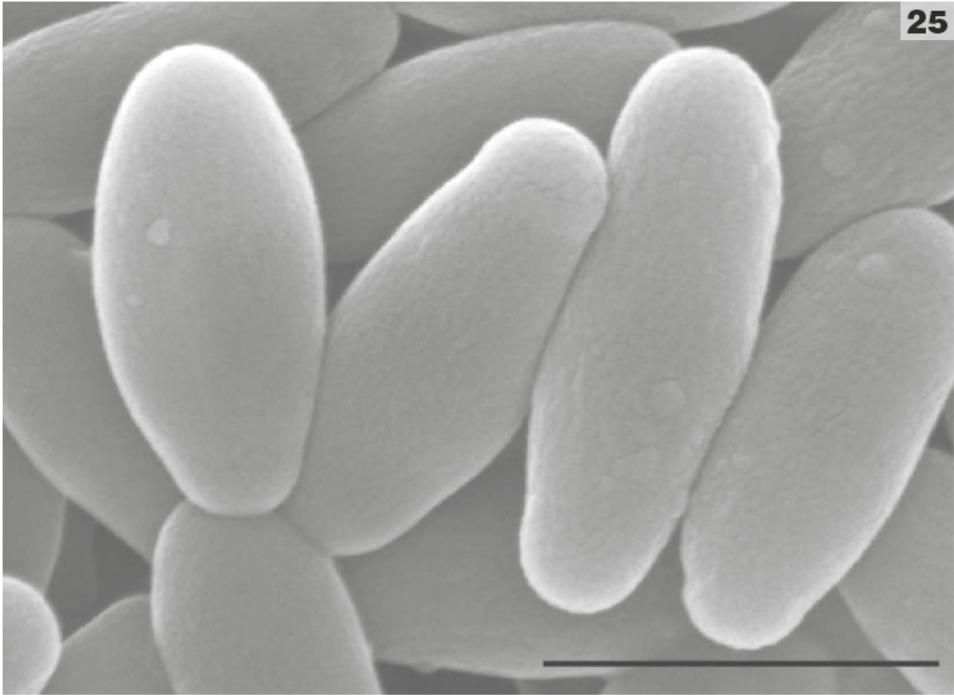
Figs 7-12. 7. *O. rupestris* – ostiolum [SEM] (2004, Szczepańska, WRSL). 8. *O. vulgata* [SEM] (2002, Czarnota, GPN 3006). 9. *O. atra* – ostiolum [SEM] (1993, Cieśliński, KTC). 10. *O. vermicellifera* (2002, Czarnota, GPN 2947): a – the height of conidiogenous filaments, b – pycnidial wall thickness. 11. *O. dolomitica* – cross-section through the paraplectenchymatic layer of the pycnidial wall (1961, Nowak, KRAM-L 9661). 12. *O. vermicellifera* – pycnidial wall thickness (2002, Czarnota, GPN 2947). Scale bars: Fig. 7 = 25µm, Figs 8-9 = 50µm, Fig. 10 = 10 µm, Figs 11-12 = 20 µm.



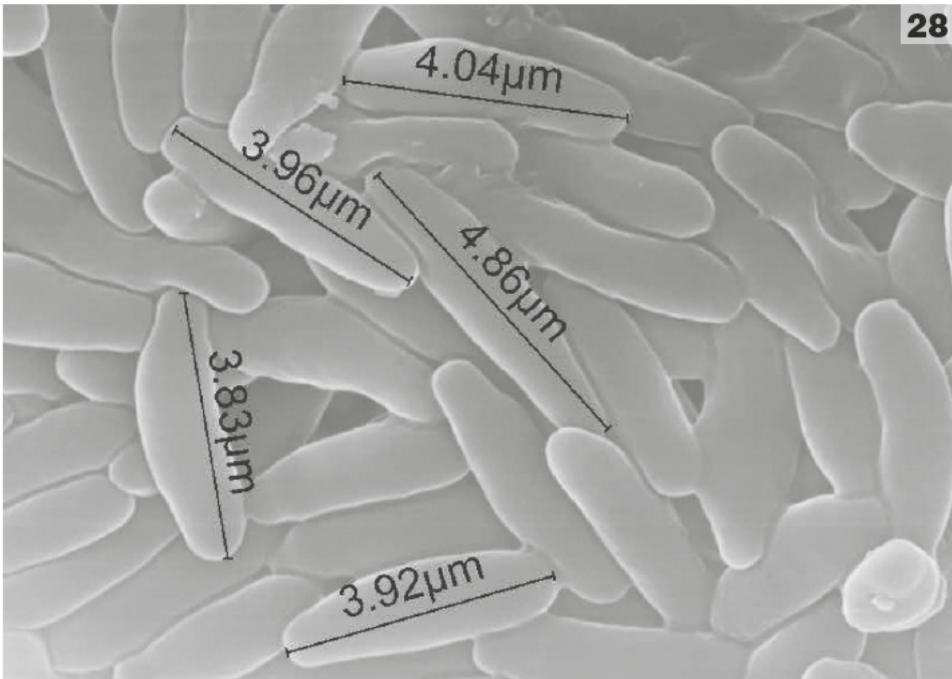
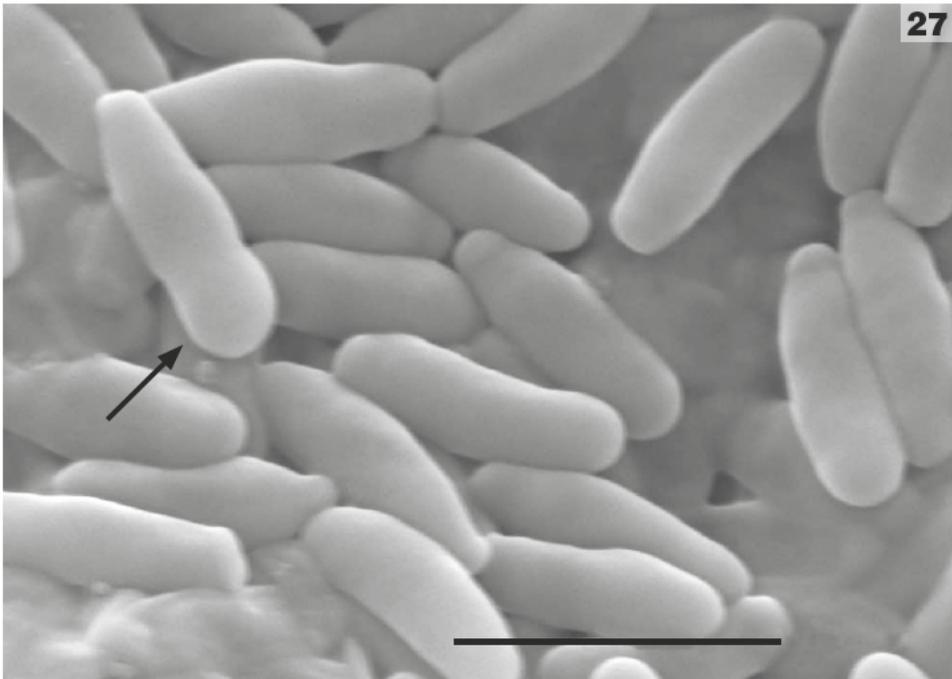
Figs 13-18. 13-16. *O. vermicellifera* – pycnidium (2002, Czarnota, GPN 2947; 2002, Cieśliński, KTC; 1990, Cieśliński, KTC): 13. Pruina [SEM], 14. Carbonisation, 15. Ostiolum [SEM], 16. Ostiolum [SEM]. 17-18. The width of ostiolum: 17. *O. niveoatra* [SEM] (1987, Tobolewski & Glanc, KTC), 18. *O. rupestris* [SEM] (2004, Szczepańska, WRSL). Scale bars: Figs 13-14, 18 = 50 μm , Fig. 15 = 100 μm , Fig. 16 = 40 μm , Fig. 17 = 10 μm .



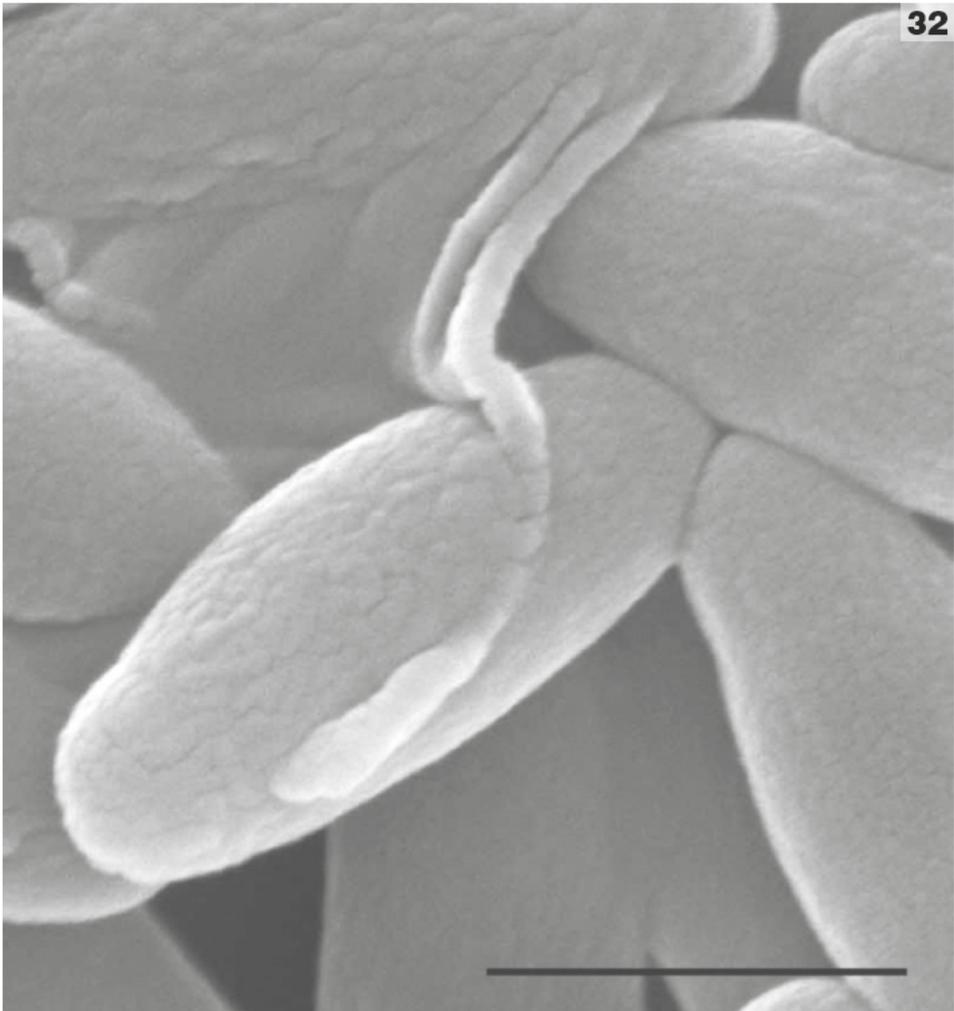
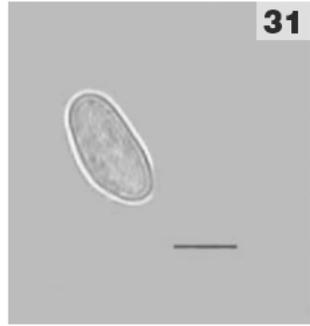
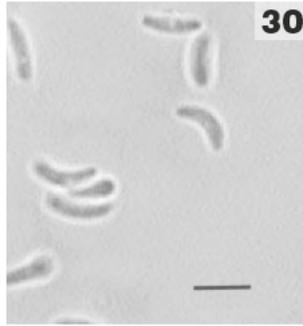
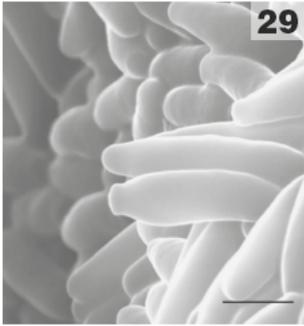
Figs 19-24. 19. *O. gyrocarpa* – cross-section through the ostiolum [SEM] (2003, *Szczepańska, WRSL*). 20. *O. varia* – mass of conidia immersed in the mucous substance (1964, *Nowak, KRAM-L 16204*). 21. *O. viridis* – dry masses of conidia blocking the ostiolum [SEM] (1985, *Fałynowicz, UGDA-L 2868*). 22-24. Conidia [SEM]: 22. *O. calcarea* – at the pycnidium apex (2007, *Wieczorek, hb. Wieczorek*), 23. *O. vermicellifera* – dry masses sticking around pycnidium (2002, *Czarnota, GPN 2947*), 24. *O. vermicellifera* – on the thallus surface (1987, *Czyżewska, LOD-L 9039*). Scale bars: Figs 19, 22 = 10 μm, Fig. 20 = 5 μm, Figs 21, 23 = 50 μm, Fig. 24 = 2 μm.



Figs 25-26. Conidia [SEM]: 25. *O. varia* (1965, Rydzak, LBL), 26. *O. vermicellifera* – asymmetric elongation (1982, Cieśliński & Tobolewski, KTC). Scale bars: Fig. 25 = 3 μm , Fig. 26 = 2 μm .



Figs 27-28. Conidia [SEM] : 27. *O. varia* – terminal asymmetric thickening (1965, Rydzak, LBL), 28. *O. vermicellifera* – asymmetric elongation (1982, Cieśliński & Tobolewski, KTC). Scale bars: 5 μm .



Figs 29-32. Conidia: [SEM]: 29. *O. vermicellifera* – tapered tip of conidium (1998, Bielczyk, KRAM-L 44575), 30. *O. niveoatra* (1987, Tobolewski & Glanc, KTC), 31. *O. varia* (1977, Fałtynowicz, UGDA-L 1861), 32. *O. vermicellifera* – surface (1987, Czyżewska, LOD-L 9039). Scale bars: 2 μ m.