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Protistology

A survey of the testate amoeba genus *Difflugia* Leclerc, 1815 based on specimens in the E. Penard and C.G. Ogden collections of the Natural History Museum, London. Part 2: Species with shells that are pyriform or elongate

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Summary

We review the species of *Difflugia* with a shell that is pyriform or elongate, based primarily on examinations of two collections in the Natural History Museum, London, UK: (i) Penard's collection of balsam-mounted microscope slides, and (ii) Ogden's scanning electron micrographs and shell measurements. We discuss taxa grouped into seven species complexes, namely *Difflugia oblonga* Ehrenberg, 1838, *Difflugia pyriformis* Perty, 1849, *Difflugia bryophila* (Penard, 1902) Jung, 1942, *Difflugia linearis* (Penard, 1890) Gautier-Lièvre et Thomas, 1958, *Difflugia gigantea* (Chardez, 1967) Ogden et Fairman, 1979, *Difflugia petricola* Cash, 1909, and *Difflugia lanceolata* Penard, 1890.

Within the *D. oblonga*-complex we: (i) distinguish as a separate taxon the typical form of *D. oblonga* Ehrenberg, 1838; (ii) synonymise *D. parva* (Thomas, 1954) Ogden, 1983, *D. lacustris* (Penard, 1899) Ogden, 1983, *D. bacillifera* Penard, 1890, *D. oblong*a var. *incondita* Gauthier-Lièvre et Thomas, 1958, and *D. oblonga* f. *cyphodera* Jung, 1942 with *D. oblonga*. Within the *D. pyriformis*-complex we: (i) distinguish as separate taxa the typical form of *D. pyriformis* Perty, 1849, as well as *D. capreolata* Penard, 1902, and (ii) synonymise *D. cylindrus* (Thomas, 1953) Ogden, 1983 with *D. pyriformis* Perty, 1849. Within the *D. bryophila*-complex we: (i) distinguish as a separate taxon *D. bryophil*a (Penard, 1902) Jung, 1942, and (ii) synonymise *D. gassowskii* (Gassowsky, 1936) Ogden, 1983 with *D. bryophila*. Within the *D. linearis*-complex we: (i) distinguish as a separate taxon *D. linearis* (Penard, 1890) Gautier-Lièvre et Thomas, 1958, and (ii) synonymise *D. paulii* Ogden, 1983 and *D. nebelondes* Gauthier-Lièvre and Thomas, 1958 with *D. linearis*. Within the *D. gigantea*-complex we: (i) distinguish as a separate taxon the typical form of *D. gigantea* (Chardez, 1967) Ogden et Fairman, 1979, and (ii) discuss the validity of *D. oblonga* var. *angusticollis* Štěpánek, 1952, and *D. oblonga* var. *stepaneki* (Štěpánek, 1952) Decloitre. Within the *D. petricola*-complex we accept *D. petricola* Cash, 1909 as a valid species and suggest its possible relationships with other *Difflugia* species.

Within the *D. lanceolata*-complex we accept *D. lanceolata* Penard, 1890 as a valid species and illustrate its variability based on C.G. Ogden's SEM micrographs.
As in the first part of this series of papers in which we taxonomically revise the genus *Difflugia* we conclude that, based on current knowledge, it is unclear whether these species complexes represent single, highly polymorphic species, or groups of sibling species. Further studies based on a combination of morphometric, ultrastructural (SEM), molecular, and environmental data are needed in order to characterize these species complexes in more detail and thus resolve their systematics.

Key words: *Difflugia*, morphospecies, species complex, taxonomic revision, testate amoebae

Introduction

This is the second of a series of papers that aims to review the genus *Difflugia* based primarily on examinations of two collections in the Natural History Museum (NHM), London, UK, i.e. Penard's collection of balsam-mounted microscope slides, and Ogden's scanning electron micrographs (SEM) and shell measurements, and also on published literature. In the first paper of this series (Mazei and Warren, 2012) we reviewed those species of *Difflugia* with a shell that is pointed aborally and/or has aboral protuberances. The aim of the present paper is to review those species with a shell that is pyriform or elongate.

Review of the literature on the taxonomy of selected *Difflugia* species.

Leclerc (1815) erected the genus *Difflugia*, however he failed to use binary nomenclature and did not give specific names to any of the organisms he described (Fig. 1). A year later Lamarc (1816, p. 95) attributed the name *D. proteiformis* to the forms described by Leclerc in 1815 but did not provide any descriptions discriminating between them. Later Ehrenberg (1838a, p. 131; 1838b, table 9, fig. 1) applied the name *D. proteiformis* to those forms which "appear rather to apply to the *D. globulosa* of Dujardin" (Leidy, 1879, p. 105). Dujardin (1841, p. 248–249) distinguished both *D. globulosa* and *D. proteiformis* noting that although both species are globular to ovoid in shape, the former is 100–250 µm long and has a smooth surface whereas the latter has a length of 45–112 µm and is covered by sand grains. Dujardin did not illustrated the latter species. Perty (1852) applied name *D. proteiformis* to those specimens of Lecrec shown on his plate 17, fig. 1 (Fig. 1) which are currently known as *Lesquereusia modesta* (Cash and Hopkinson, 1909). Thus, as noted by Ogden and Ellison (1988), the taxonomy of *Difflugia proteiformis* remains questionable.

Among the specimens of *Difflugia* figured by Leclerc (1815; Fig. 1) two are currently recognized as valid: *D. pyriformis* (figs 2; 3 of Fig. 1) and *D. acuminata* (fig. 5 of Fig. 1). Leidy (1879, p. 98) and Penard (1902, p. 214), for example, both treated Leclerc's figs 2 and 3 as *D. pyriformis*.

Ehrenberg (1831, p. 90) listed three species of *Difflugia*, namely *D. proteiformis* Leclerc, *D. oblonga* Ehrenberg, and *D. acuminata* Ehrenberg, with a brief description of each. Later Ehrenberg (1838a, 1838b) described *Difflugia oblonga* in more detail as having a shell that is ovoid-elongate, laterally circular, with a smooth surface, generally transparent in appearance, and 110 µm long (Fig. 2).

Perty (1849, 1852) described *Difflugia pyriformis* (Fig. 3, a-c) the shell of which is 140–200 µm long, pyriform or sometimes irregular in shape, and with a rough surface (Fig. 3c). He also questioned the validity of *D. oblonga* (Fig. 3d) noting that he had found specimens of *D. pyriformis* that were similar shape to *D. oblonga* and had a surface structure that was similar to *D. proteiformis*, which he considered should be *Lesquereusia molesta* (Perty, 1852).

Wallich (1864) provided first hypothetical scheme showing relationships between different testate amoebae including notes on *D. oblonga* and *D. pyriformis* (Fig. 4). He mentioned "... four so-called species, namely, *D. proteiformis, D. oblonga, D. acuminata* (Ehr.), and *D. pyriformis* (Perty)" (Wallich, 1864, p. 222). He described *D. oblonga* as having an almost cylindrical shape (fig. 3t on his plate XV – Fig. 4a) and characterized *D. pyriformis* by its clearly pyriform shape (fig 3s on his plate XV – Fig. 4a; figs 9; 10 on his plate XVI – Fig. 4b). However, in his systematic scheme (p. 240) he recognized only one valid species within the genus *Difflugia*, namely *D. proteiformis* (Ehrenberg) with four subspecies. One of these subspecies, i.e. *D.*

mitriformis (Wall.), consisted of four varieties: *D. acuminata* (Ehr.), *D. spiralis* (Leclerc), *D. pyriformis* (Perty), and *D. lageniformis* (Wall.). He described the shell of *D. pyriformis* as "varying from the pear- to the ballon-shape" (Wallich, 1864, p. 240). This means that according to Wallich (1864), *D. oblonga* is a variety of *D. pyriformis*. Carter (1864) also used the name *D. pyriformis* and, according to his illustrations, included forms with shells that are elongate, pyriform, or acuminate (Fig. 5).

Leidy (1879) described *Difflugia pyriformis* as varying considerably in size and shape and distinguished following varieties within this species: '*pyriformis*', '*compressa*', '*nodosa*', '*cornuta*', and '*vas*'. Even the typical *D. pyriformis* appeared to be highly polymorphic (Fig. 6). Regarding *D. oblonga* Leidy (1879, p. 105) argued that "... in its shape it appears rather to be related with *D. acuminata* without its point" and thus did not consider *D. oblonga* and *D. pyriformis* as synonyms.

Penard (1890) followed Leidy's approach, recognizing within *D. pyriformis* the varieties '*nodosa*' and '*vas*' and describing the new taxa *D. pyriformis* var. *linearis* and *D. pyriformis* var. *tenuis* (Fig. 7). Later he added the varieties *D. pyriformis* var. *lacustris* (Penard, 1899) and *D. pyriformis* var. *bryophila* (Penard, 1902) and described three new species that are closely related to *D. pyriformis*, namely *D. lanceolata, D. bacillifera* (Penard, 1890), and *D. capreolata* (Penard, 1902). It is noteworthy that Penard (1890, p. 145; 1902, p. 250) considered *D. lanceolata* as closely related with *D. acuminata*, i.e. in terms of size and general appearance and the lack of an acuminated end of the shell. Given the similarity of *D. lanceolata* sensu Penard and *D. oblonga* sensu Ehrenberg (compare Fig. 2d and Fig. 7q, t, u, v), it is evident that Penard followed the principle established by Leidy (1879).

Cash and Hopkinson (1909) and Cash et al. (1919) revived the concept of *Difflugia oblonga*. They argued that "although this species is now almost universally called *Difflugia pyriformis*, Ehrenberg's name has the priority and must be adopted in accordance with the rule of zoological nomenclature" (Cash and Hopkinson, 1909, p. 8). Subsequently they listed some of the known taxa, such as typical *D. oblonga* (Fig. 8, a-c, f-g), *D. oblonga* var. *lacustris* (Fig. 8, d-e), *D. oblonga* var. *bryophila* (Fig. 8o), *D. lanceolata* (Fig. 8, k-m), and *D. bacillifera* (Fig. 8n), and described the new species *D. petricola* (Fig. 8, h-j). It is interesting that Wailes and Penard (1911) used the name *D. oblonga* var. *bryophila* despite the fact that Penard (1902) had previously referred to it as *D. pyriformis* var. *bryophila*. Jung (1942) subsequently raised this taxon to species rank as *D. bryophila*. Four additional varieties were established during the following decades: *D. oblonga* var. *longicollis* (Gassowsky, 1936), *D. oblonga* var. *cylindrus* (Thomas, 1953), *D. oblonga* var. *elongata* (van Oye, 1953), and *D. oblonga* var. *parva* (Thomas, 1954).

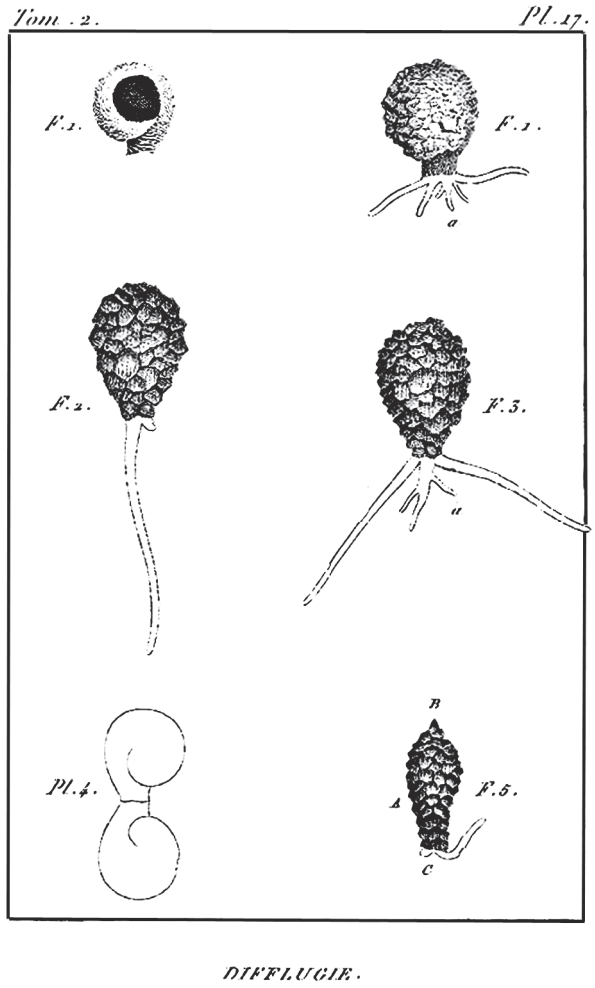


Fig. 1. Plate illustrating the first described *Difflugia* (Leclerc, 1815, plate 17).

Štěpánek (1952) proposed hypothetical schemes describing morphological relationships between pyriform and acuminate species of *Difflugia*. Štěpánek's entire scheme is reproduced in our previous publication (Mazei and Warren, 2012); here we present a reduced version, which is important for discussing taxa with pyriform and elongate shells (Fig. 9). In his exhaustive study of *Difflugia* in a single pond, Štěpánek (1952) recorded numerous transitional forms within the *D. oblonga* 'ultraspecies' and in many cases made decisions concerning synonymy. For example, he noted that "the surface of the test is covered according to the material provided by the environment and also according to

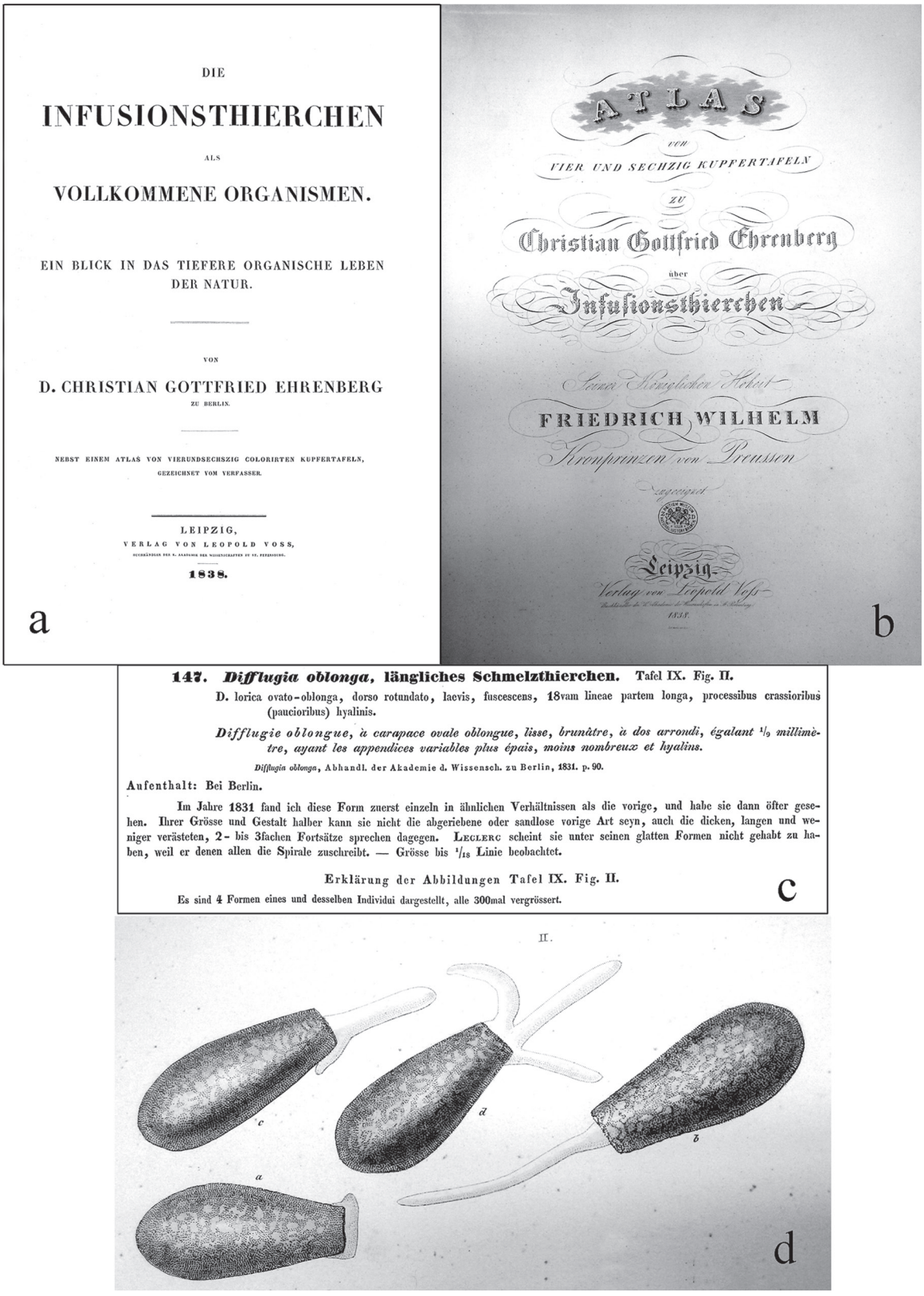


Fig. 2. Description of *Difflugia oblonga* from Ehrenberg's book (Ehrenberg, 1838a) and atlas (Ehrenberg, 1838b). a – front page of the book; b – front page of the atlas; c – description of *D. oblonga* (Ehrenberg, 1838a, p. 131); d – illustration of *D. oblonga* (Ehrenberg, 1838b, table 9, fig. 2).

a

Zur

KENNTNISS

kleinſter Lebensformen

nach Bau, Funktionen, Systematik,

mit Specialverzeichniss der in der

SCHWEIZ

beobachteten.

Von

Dr. MAXIMILIAN PERTY,

o. o. Professor an der Hochschule zu Bern, Mitglied gelehrter Gesellschaften.

Mit XVII lithochromischen Tafeln,

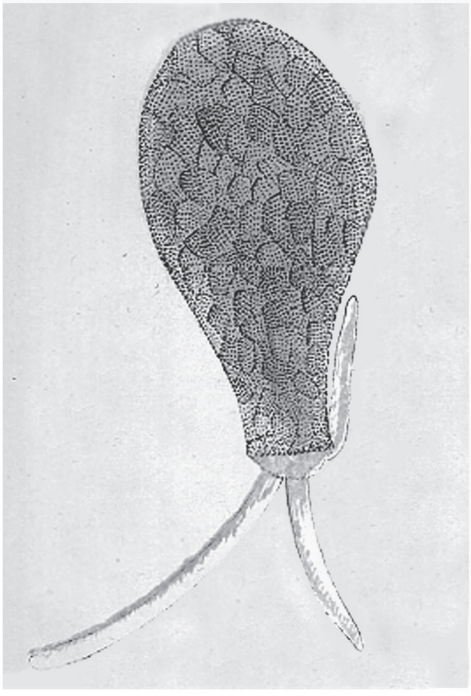
ausgeführt in der Lithographie von C. Dennin.

BERN, 1852.

Verlag von JENT & REINERT.

(Platzfirma: *Jent und Gassmann.*)

b



c

*Pyriformis** t. IX ob. Abth. f. 9. Schale birnförmig, manchmal unregelmässig; Textur grob. L. 1/7 — 1/5‴. Bern, 9 — 11. St Gotthard, Sanetsch, Lago d'Origlio und di Muzzano, 8. — Das dünnere Ende der Birne ist das nach der Mündung gewendete. Textur wie bei voriger; Schale in durchfall. Licht schwärzlich, von oben beleuchtet weissgrünlich.

d

? *Oblonga* E. p. 131, t. 9, f. 2. Bei Rosenlawi, 8, fanden sich Ex. einer D., welche in Form der oblonga, in Textur der Schale der proteiformis glich. Sollten sie eher zu meiner D. pyriformis gehört haben?

Fig. 3. *Difflugia pyriformis* and *D. oblonga* from Perty (1852). a – front page of the book (Perty, 1852); b – illustration of *D. pyriformis* (table 9, fig. 9); c – description of *D. pyriformis* (p. 187); d – description of *D. oblonga* (p. 187).

the quantity present in the environment" (Štěpánek, 1952; p. 22). He found a continuum of forms between those species with shells covered by sand grains and those covered by diatom frustules, concluding that coverage by diatoms cannot be regarded as a good feature for species (or even sub-species or variety) separation. Consequently, he synonymised *D. bacillifera* with *D. oblonga*. Similarly he synonymized *D. oblonga* var. *lacustris* with *D. oblonga* noting that: "In some cases I observed fission of this [*Difflugia oblonga* var. *lacustris*] species. It is interesting that on a new specimen the building material settles in most cases first at the aperture, and in case of a scarcity of material in shards do not cover the remaining surface of the test at all, or they cover it only very scantily" (Štěpánek, 1952; p. 22). But such uneven distribution of xenosomes is one of the accepted diagnostic features of *D. oblonga* var. *lacustris*. Štěpánek also concluded that *D. oblonga* f. *cyphodera* described by Jung (1942) and *D. pyriformis* var. *bryophila* described by Penard (1902) should be synonymized with *D. oblonga*. Nevertheless, he erected two new varieties, namely *D. oblonga* var. *angusticollis* and *D. oblonga* var. *vas*. Finally, he suggested lumping

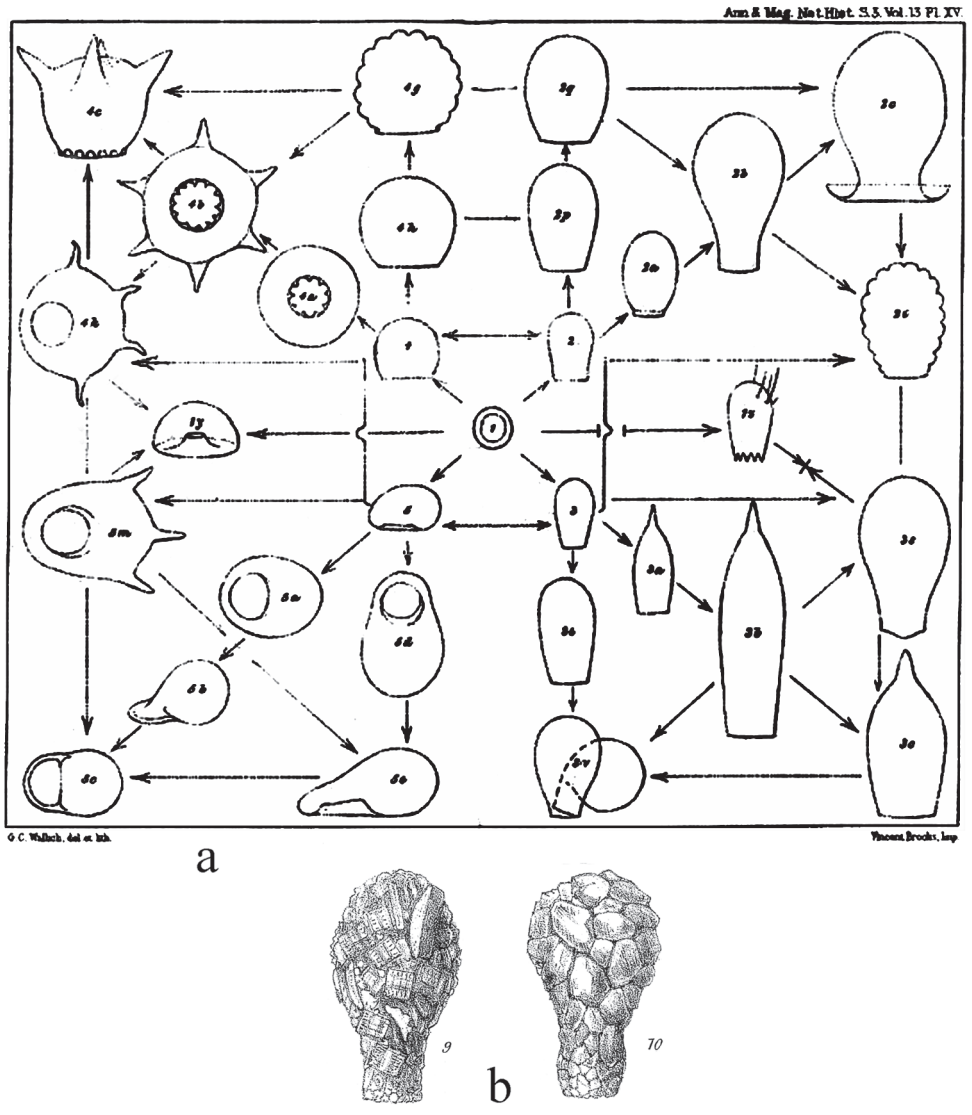


Fig. 4. *Difflugia pyriformis* and *D. oblonga* from Wallich (1864). a – plate XV of Wallich's paper showing "...the order in which the four subspecies of *Difflugia proteiformis* arrange themselves around a common archetypal ... centre..." (Wallich, 1864, p. 243); b – illustration of *D. pyriformis* from plate XVI of Wallich's paper showing on fig. 9 "... how completely the test is made up of frustules and valves of diatoms..." and on fig. 10 "common form ... [with] moderately large sandy granules" (Wallich, 1864, p. 244).

D. lanceolata with *D. oblonga* based primarily on the resemblance of the former with the first description of *D. oblonga* made by Ehrenberg (1838a, 1838b).

In their report on the testate amoebae of Africa, Gauthier-Lièvre and Thomas (1958) grouped the various *Difflugia* spp. based on shell morphology. The following known species were included in the groups entitled "Allongees", and (in part) "Pyriformes", i.e. those with shells that are elongated or pyriform, respectively: *D. lanceolata* Penard, 1890, *D. oblonga* var. *cylindrus* Thomas, 1953, *D. oblonga* var. *elongata* van Oye, 1953, *D. bacillifera* Penard, 1890, *D. bryophila* (Penard, 1902) Jung, 1942, *D. capreolata* Penard, 1902, *D. oblonga* Ehrenberg, 1838 (note that Gauthier-Lièvre and Thomas treated *D. pyriformis* as a synonym of *D. oblonga*), *D. oblonga* var. *parva* Thomas, 1954, *D. oblonga* var. *lacustris* Penard, 1902, and *D. oblonga* var. *longicollis* Gassowsky, 1936. Furthermore, they raised *D. oblonga* (pyriformis) var. *linearis* Penard, 1890 to species rank as *D. linearis* and described two new taxa from Africa: *D. nebeloïdes* and *D. oblonga* var. *incondita* (Gauthier-Lièvre and Thomas, 1958).

Following a review of the diagnostic features of *Difflugia oblonga*, Chardez (1967) recognized 12 infraspecific forms excluding those with shells

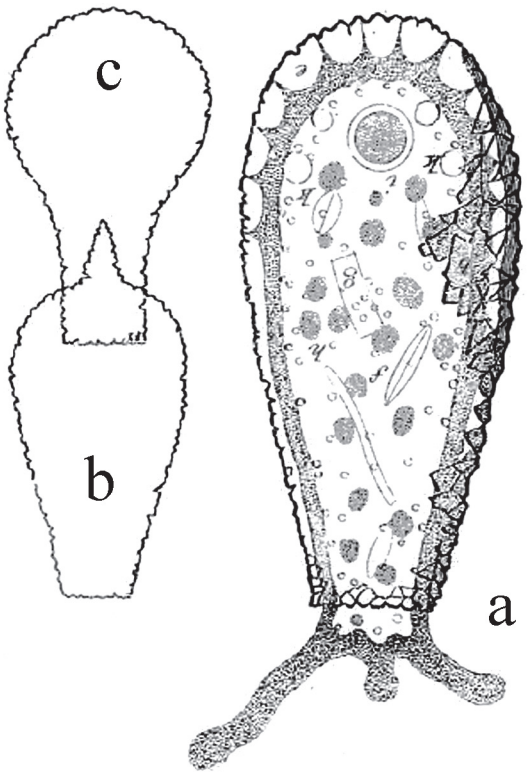


Fig. 5. *Difflugia pyriformis* from Carter (1864). a – typical form; b – acuminate variety of the test; c – pyriform variety.

that are neither elongate nor pyriform (Fig. 10): *D. oblonga* Ehrenberg, 1838 (note that Chardez (1967) treated *D. pyriformis* as a synonym of *D. oblonga*), *D. oblonga* var. *gigantea* Chardez, 1967 (this taxon was erected by Chardez (1967) based on some illustrations from Leidy, 1879), *D. oblonga* var. *lacustris* Penard, 1902, *D. oblonga* var. *cylindrus* Thomas, 1953, *D. oblonga* var. *elongata* van Oye, 1953, *D. oblonga* var. *parva* Thomas, 1954, *D. oblonga* var. *incondita* Gauthier-Lièvre and Thomas, 1958, *D. oblonga* f. *cyphodera* Jung, 1942, D. *oblonga* var. *angusticollis* Štěpánek, 1952 (note that Chardez (1967) used the name '*angusticaulis*', however in the original description by Štěpánek (1952) it is '*angusticollis*'), *D. oblonga* var. *stepaneki* (Štěpánek, 1952) Decloitre (known previously as *D. oblonga* var. *vas* Štěpánek, 1952), *D. oblonga* var. *longicollis* Gassowsky, 1936, and *D. oblonga* var. *rocki* Štěpánek, 1963).

Subsequently, Chardez and Decloitre (1973) concluded that *Difflugia oblonga* Ehrenberg, 1838 and *Difflugia pyriformis* Perty, 1849 are distinct species. Although both have a great range in size (length 80–300 μm in *D. oblonga* and 90–400 μm in *D. pyriformis*), *D. oblonga* could be separated from *D. pyriformis* by its elongate-ovoid shell with smooth surface and its more conspicous neck, however it did not correspond with the figures they provided. They also supplied a list of varieties and forms attributed to *D. pyriformis* which was identical to those listed by Chardez (1967).

In a series of publications C.G. Ogden redescribed, and in many cases changed the taxonomic status, of 14 taxa of *Difflugia* with pyriform or elongate shells: *D. oblonga, D. pyriformis, D. gigantea, D. bacillifera, D. lacustris, D. bryophila, D. capreolata, D. lanceolata, D. parva, D. paulii, D. petricola, D. gassowskii, D. linearis,* and *D. cylindrus* (Ogden, 1980, 1983, 1984; Ogden and Fairman, 1979; Ogden and Hedley, 1980; Ogden and Živković, 1983). Unfortunately, a direct comparison of all of the species listed above was never made in a single publication. It is noteworthy that Ogden (1980) supported the suggestion of Chardez and Decloitre (1973) and clearly distinguished *D. oblonga* from *D. pyriformis* based on morphometric and SEM data. We discuss this in more detail below.

We have applied Ogden's morphometric data (both published and unpublished) to compare the 14 species mentioned above, 11 of which are morphologically similar, the other three (*D. gigantea, D. capreolata*, and *D. lanceolata*) being easily distinguished by their size and/or other morphological features (Fig. 11). Scanning electron micrographs of each of these 14 species are reproduced here to the same scale in order to faciliate comparisons of both size and external morphology of 'typical' individuals (Fig. 12).

According to the scatter plot (Fig. 11) we can distinguish five main size classes: (1) large and broad with a shell length of 150–265 μm and shell width 95–145 μm, comprising *D. pyriformis, D. cylindrus*, and *D. capreolata* (not shown in the figure), and some individuals identified by C. Ogden as *D. oblonga*; (2) relatively large and narrow, with a shell length of 130–230 μm and shell width 55–95 μm, comprising *D. oblonga, D. lacustris, D. parva*, and *D. bacillifera*; (3) medium-size and broad, with a shell length of 100–150 μm and shell width 60–100 μm, comprising *D. petricola*; (4) relatively small and broad, with a shell length of 90–130 μm and shell width 45–70 μm, comprising *D. bryophila* and *D. gassowskii*; (5) small and narrow, with a shell length of 85–120 μm and shell width 30–45 μm, comprising *D. linearis* and *D. paulii*. Thus, according to these size classes we can distinguish five species complexes (sensu Foissner and Korganova, 2000): *D. pyriformis*-complex, *D. oblonga*-complex, *D.*

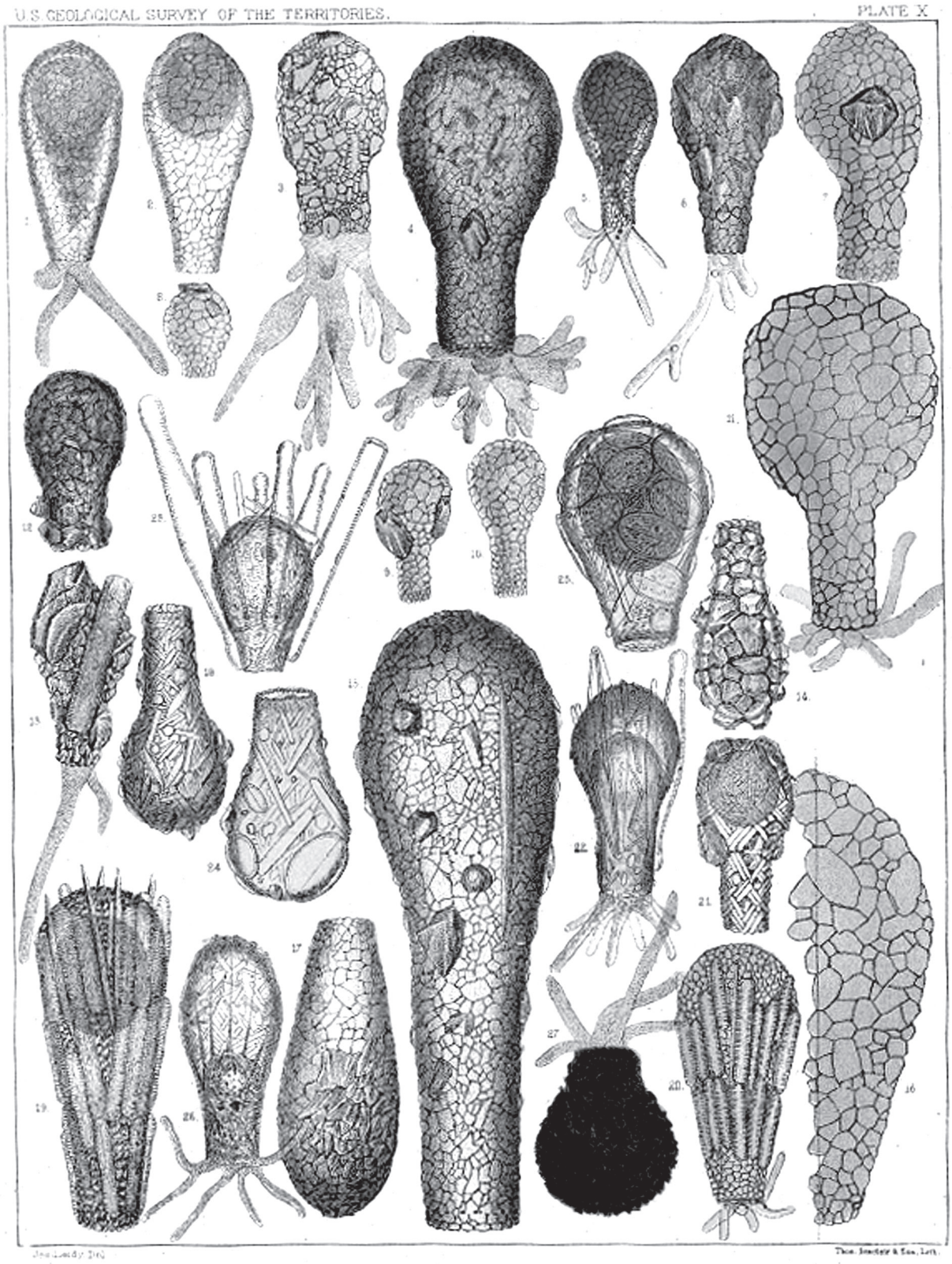


Fig. 6. *Difflugia pyriformis* from Leidy (1879).

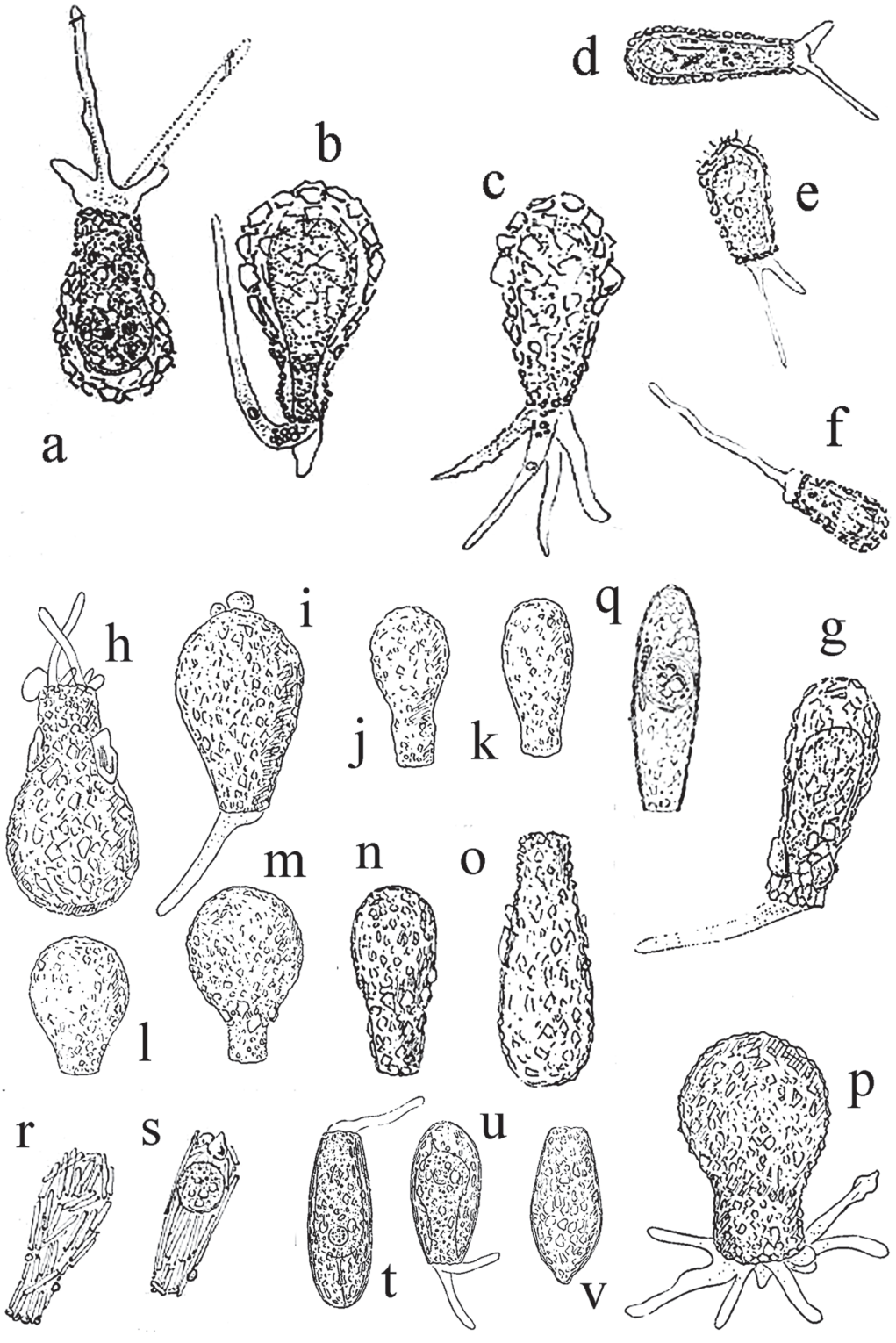


Fig. 7. *Difflugia pyriformis* and related species from Penard (1890, 1899, 1902). a-c – typical form of *D. pyriformis* (Penard, 1890, plate III, figs 30, 31, 33); d – *D. pyriformis* var. *linearis* (Penard, 1890, plate III, fig. 42); e – *D. pyriformis* var. *tenuis* (Penard, 1890, plate III, figs 47, 48); g – *D. pyriformis* var. *lacustris* (Penard, 1899, plate 2, fig. 11); h-m – variability of typical form of *D. pyriformis* (Penard, 1902, p. 216); n – *D. pyriformis* var. *bryophila* (Penard, 1902, p. 218); o – *D. pyriformis* var. *lacustris* (Penard, 1902, p. 218); p – *D. capreolata* (Penard, 1902, p. 223); q – *D. lanceolata* (Penard, 1890, plate IV, fig. 59); r-s – *D. bacillifera* (Penard, 1890, plate IV, figs 61–63); t-v – *D. lanceolata* (Penard, 1902, p. 251).

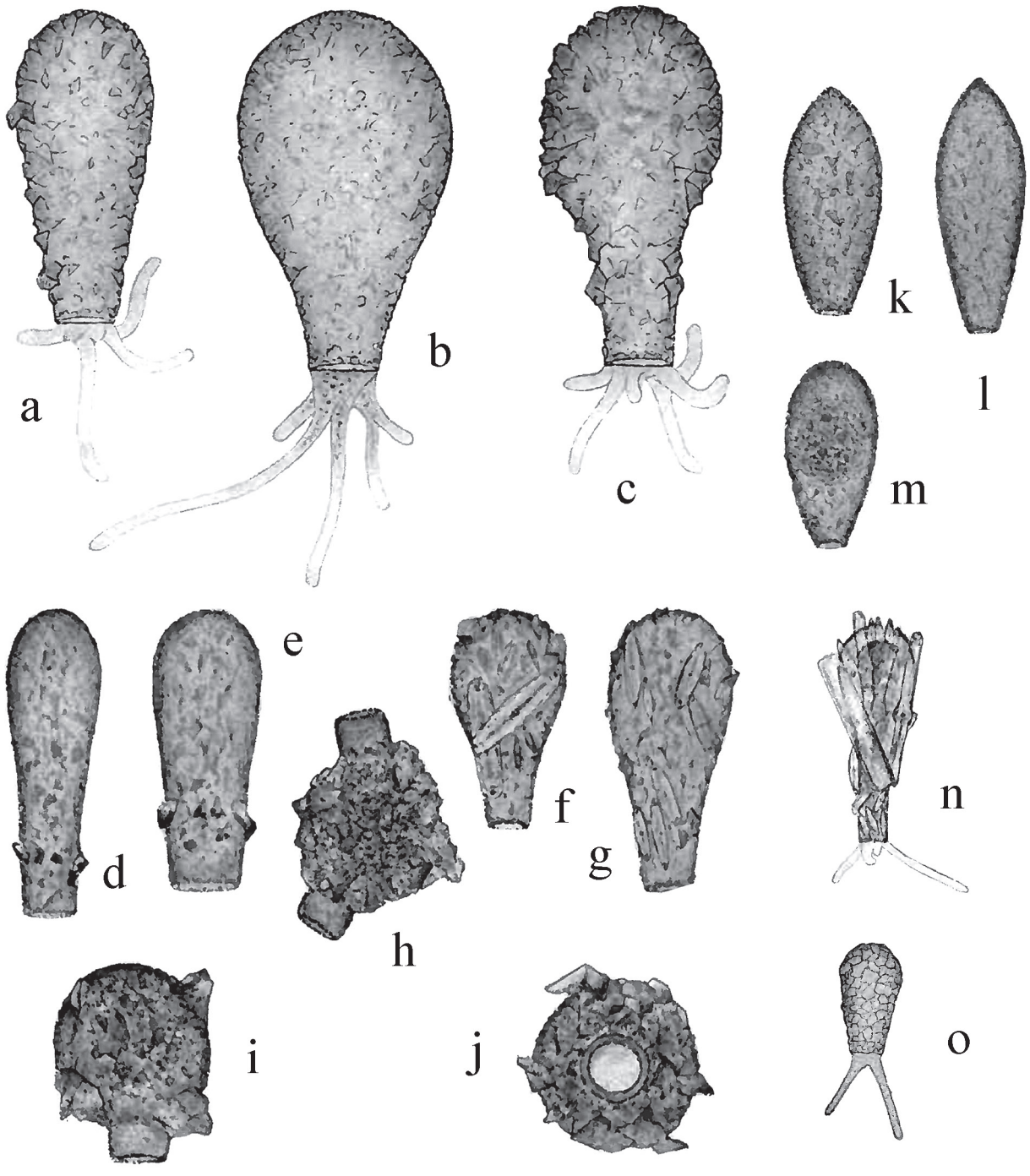


Fig. 8. *Difflugia oblonga* and related species from Cash and Hopkinson (1909, 1919). a-c – variation in typical form of *D. oblonga* (tableXVII, figs 1–3); d-e – *D. oblonga* var. l*acustris* (table XIX, figs 1–2); f-g – *D. oblonga* from *Sphagnum* habitats (table XIX, figs 3–4); h-j – *D. petricola* (table XIX, figs 5–7); k-m – *D. lanceolata* (table XIX, figs 9–11); n – *D. bacillifera* (table XX, fig. 1); o – *D. oblonga* var. *bryophila* (table LXI, fig. 9).

petricola-complex, *D. bryophila*-complex, and *D. linearis*-complex, as well as two other species complexes which seem to be well defined in terms of both size and morphology, namely *D. lanceolata*-complex and *D. gigantea*-complex.

Each species complex is here discussed in detail based on data from the E. Penard and C.G. Ogden collections in the NHM, London. We do not aim

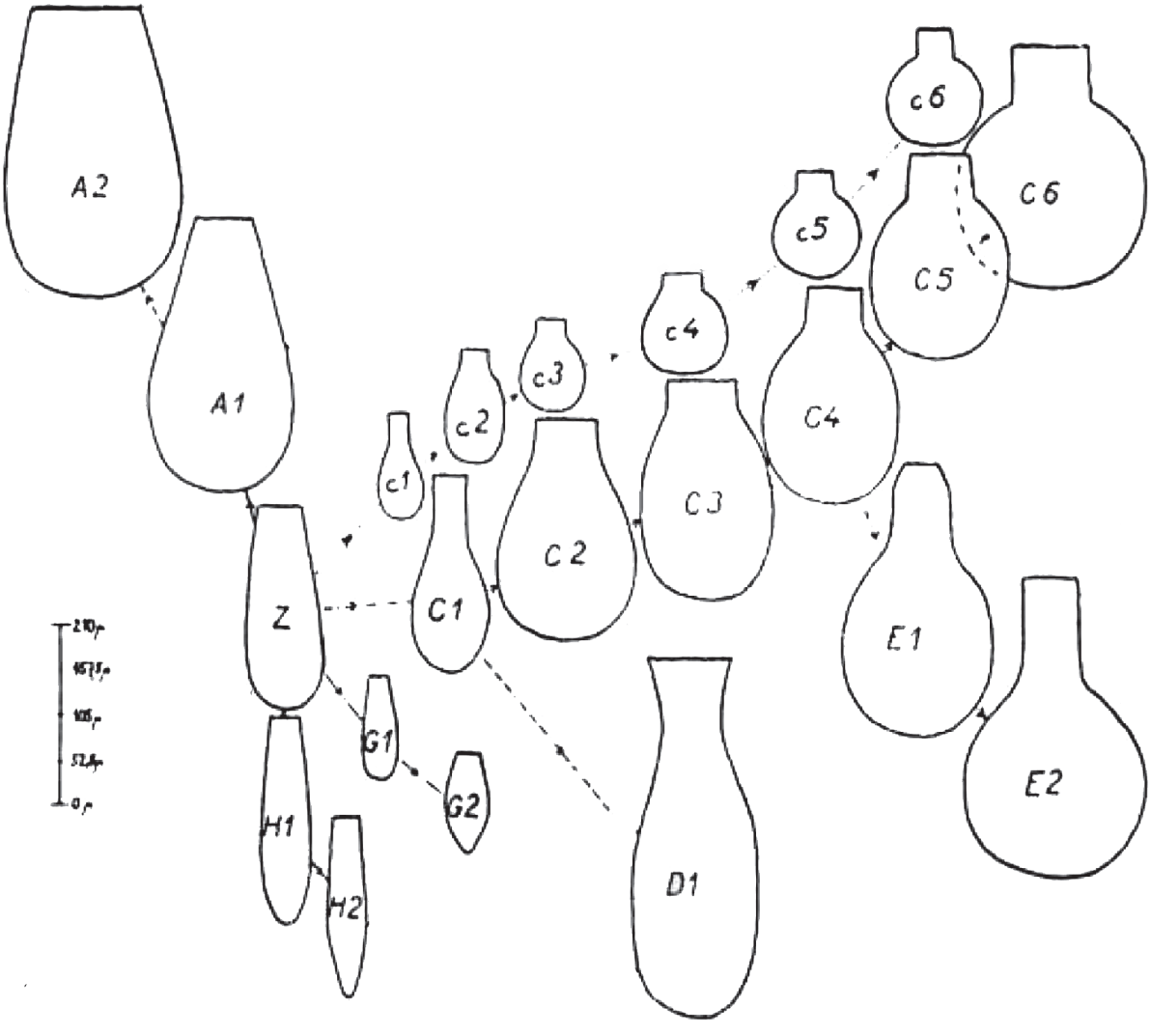


Fig. 9. Hypothetical phylogenetic scheme of *Difflugia oblonga* ultraspecies (after Štěpánek, 1952 – modified): abbreviations show different forms distinguished by Štěpánek (1952) in a single pond.

to make comprehensive revision of all published taxa related to each species complex. However, in many cases we discuss taxa not represented in the NHM collections but based instead on data from the literature.

Taxonomic revision of selected *Difflugia* species

All the species discussed below have a pyriform or elongate shell that is usually more than 100 μm in length (with the exception of few specimens of *D. linearis* and *D. gassowskii*). Illustrations comprise light micrographs (LM), scanning electron micrographs and line diagrams. All light micrographs are originals of specimens from the Penard microscope slide collection held at the NHM, London. All scanning electron micrographs are from the Ogden SEM collection held at the NHM, some of which are unpublished. Line diagrams are from different sources cited in the corresponding figure legends.

Difflugia oblonga Ehrenberg, 1838 species complex

The members of this group include *D. oblonga, D. lacustris, D. parva*, and *D. bacillifera* and all of which have a similar size distribution and general appearance (Fig. 11, group 2; Fig. 12, e-g, i).

Difflugia oblonga Ehrenberg, 1838

Typical individuals are shown in Fig. 13. The

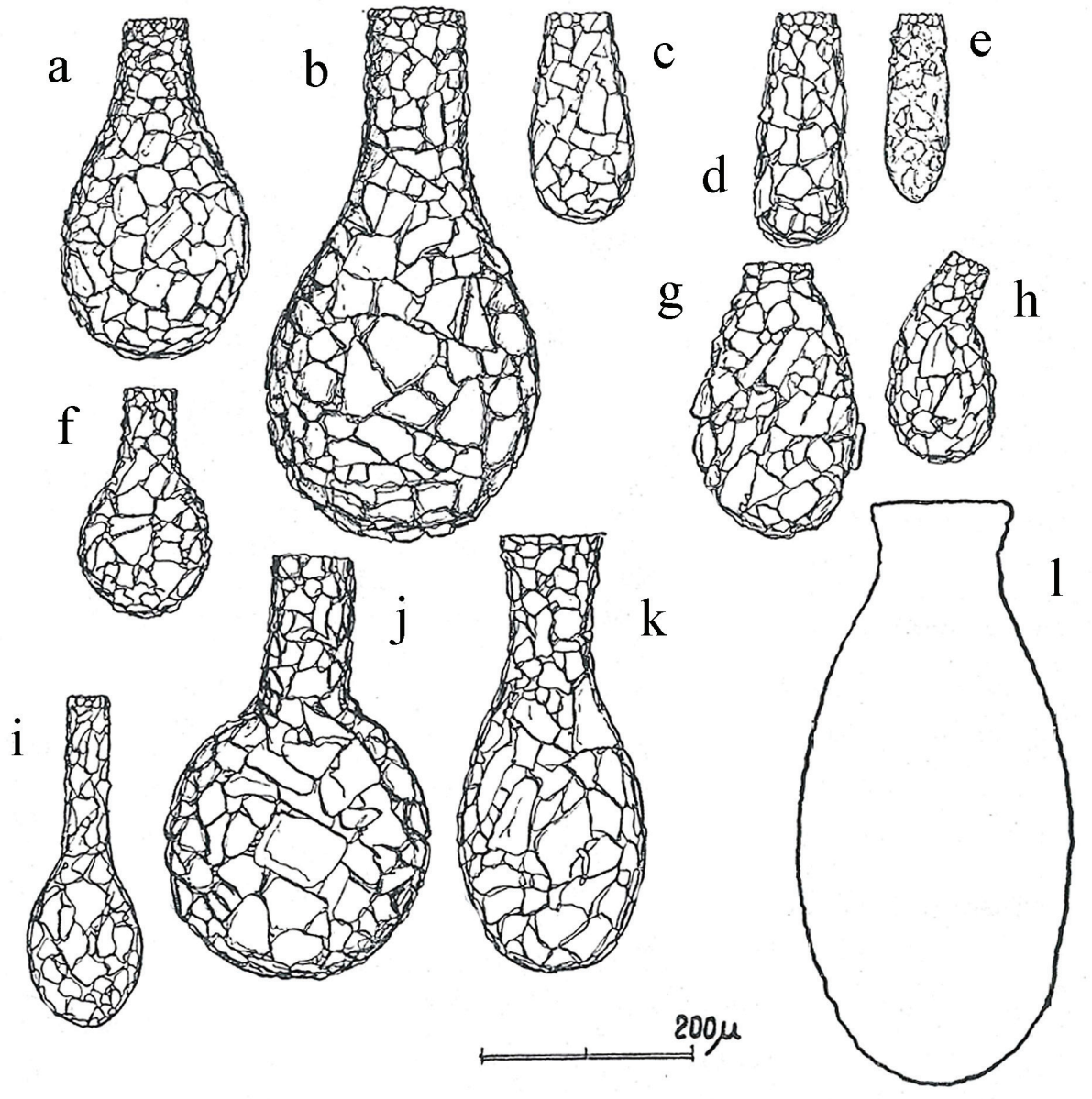


Fig. 10. Selected *Difflugia oblonga* and its varieties from Chardez (1967, planche II). a – typical form of *D. oblonga*; b – *D. oblonga* var. *gigantea*; c – *D. oblonga* var. *lacustris*; d – *D. oblonga* var. *cylindrus*; e – *D. oblonga* var. *elongata*; f – *D. oblonga* var. *parva*; g – *D. oblonga* var. *incondita*; h – *D. oblonga* f. *cyphodera*; i – *D. oblonga* var. *longicollis*; j – *D. oblonga* var. *angusticollis*; k – *D. oblonga* var. *stepaneki*; l – *D. oblonga* var. *rocki.*

shell of *Difflugia oblonga* is elongate-pyriform with a long neck that is usually up to 1/3 of the shell length, sometimes clearly defined (Fig. 13b), sometimes not (Fig. 13a). The surface is rough to moderately smooth and covered with quartz particles of different sizes. The aperture is circular and surrounded by sand grains. Ogden measured 68 specimens of *D. oblonga* (Fig. 11), 67 of which were published (Ogden and Fairman, 1979; Ogden and Hedley, 1980; Ogden and Živković, 1983), one unpublished. The shell dimensions are as follows: length 128–263 μm, width 60–147 μm, aperture diameter 19–46 μm.

***Difflugia parva* (Thomas, 1954) Ogden, 1983**

Variability of *D. parva* according to C.G. Ogden is shown in Fig. 14. Ogden (1983) described this species as having a shell that is pyriform, tapering

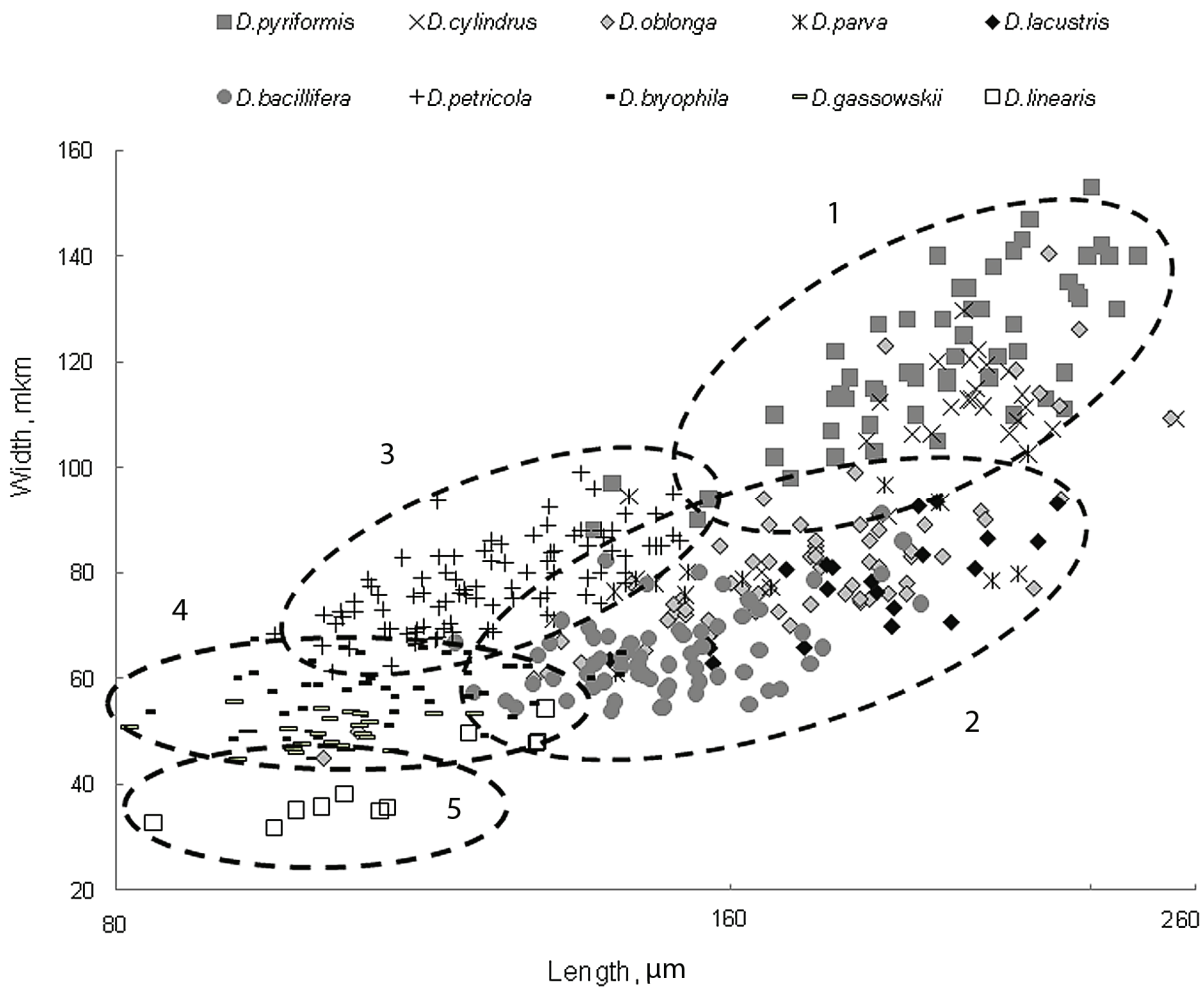


Fig. 11. Length-width measurements scatter plot on logarithmic scale of *Difflugia* with pyriform and elongate shells, based on C.G. Ogden's measurements.1-5 – size groups.
Note: *D. linearis* group includes three specimens of *D. paulii*; both species are shown in detail in Fig. 32.

evenly from the swollen and rounded aboral third to the aperture. Ogden measured 15 specimens of *D. parva* (Fig. 11), 5 are published (Ogden, 1983; Ogden and Živković, 1983) and 9 unpublished. Based on these data, the shell dimensions are: length 131 to 224 μm, width 61–103 μm, aperture diameter 19–40 μm.

Ogden (1983) separated *D. parva* from *D. oblonga* by its clean outline, relatively smooth surface and detailed cement pattern. The first two characters seem to be inappropriate given the high variability of the shell covering which depends on the material available in the environment (Štěpánek, 1952). The importance of the cement pattern is also questionable (see: Mazei and Warren, 2012). Thomas (1954) described a new variety, *D. oblonga* var. *parva* (Fig. 10f), which is separated from the typical form only by its size. However, he assumed that typical *D. oblonga* is 300–400 μm long compared to a size range of 169-256 μm for the new variety. Furthermore, neither Penard (1902) nor Cash and Hopkinson (1909) gave the length of *D. oblonga* (or *D. pyriformis*) as being greater than 300 μm. Based on these data we conclude that *D. parva* (Thomas, 1954) Ogden, 1983 is a junior synonym of *D. oblonga* Ehrenberg, 1831.

***Difflugia lacustris* (Penard, 1899) Ogden, 1983**

According to Ogden (1983) the shell of *D. lacustris* is transparent or hyaline, elongate, and cylindrical or slightly pyriform (Fig. 15). It is composed of small to medium-size pieces of quartz, diatom frustules and small siliceous flagellate cysts that together form a thin structure that is intermediate between smooth and rough. The aperture is usually circular and surrounded by

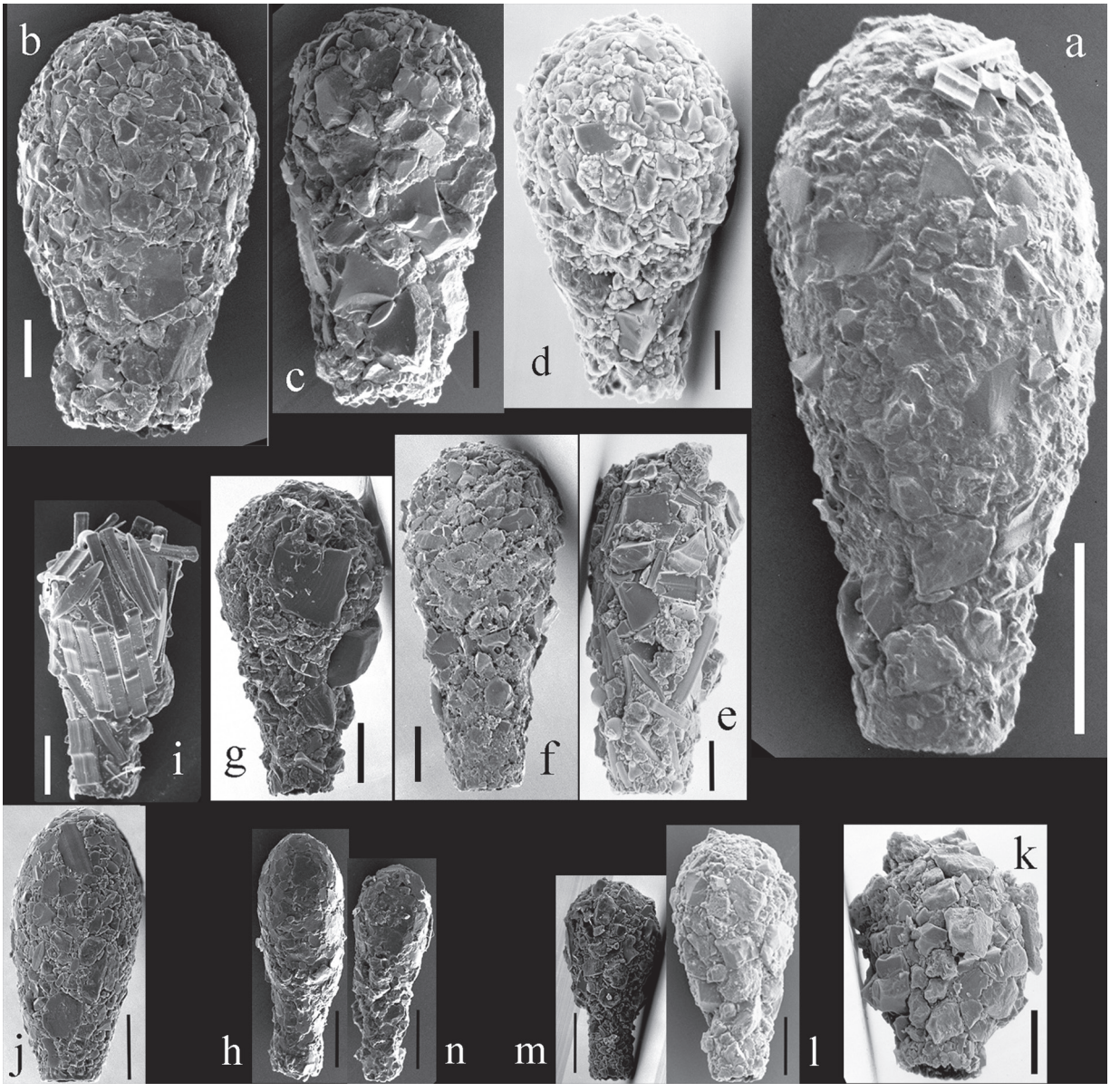


Fig. 12. Comparative morphology of "typical" pyriform and elongate *Difflugia* ssp. (length 100-450 µm) from C.G. Ogden's SEM collection. a – *D. gigantea* (SEM EM-12-587); b – *D. capreolata* (SEM CZ-03.181); c – *D.cylindrus* (SEM CZ-04.811); d – *D. pyriformis* (SEM CZ-01.155); e – *D. lacustris* (SEM CZ-04.630); f – *D. oblonga* (SEM CZ-03.198); g – *D. parva* (SEM CZ-04.365); h – *D. paulii* (SEM CZ-06.678); i – *D. bacillifera* (SEM EM-06-731); j – *D. lanceolata* (SEM CZ-04.665); k – *D. petricola* (SEM EM-12-457); l – *D. bryophila* (SEM CZ-04.041); m – *D. gassowskii* (SEM CZ-05.036); n – *D. linearis* (SEM CZ-06.677). Numbers after taxon names are NHM index numbers of SEM negatives. Scale bars: a – 100 µm, b-n – 30 µm.

small particles so that the margin is smooth. Ogden measured 23 specimens of *D. lacustris* (Fig. 11) all of which are published (Ogden, 1983): shell length 140–231 µm, shell breadth 63–94 µm, aperture diameter 26–42 µm.

Penard (1902) described *D. lacustris* as having an elongate shell, sometimes almost cylindrical, or broadened at the posterior end and tapering to the aperture forming a neck (Figs. 7g; 16). The shell is usually covered by small sand grains sometimes with large sand grains near the aperture. The length of the shell is 160–180 µm. As discussed above, the nature of the shell surface is usually dependent on environmental factors. Here we follow Štěpánek (1952) and regard *D. lacustris* as a junior synonym of *D. oblonga*.

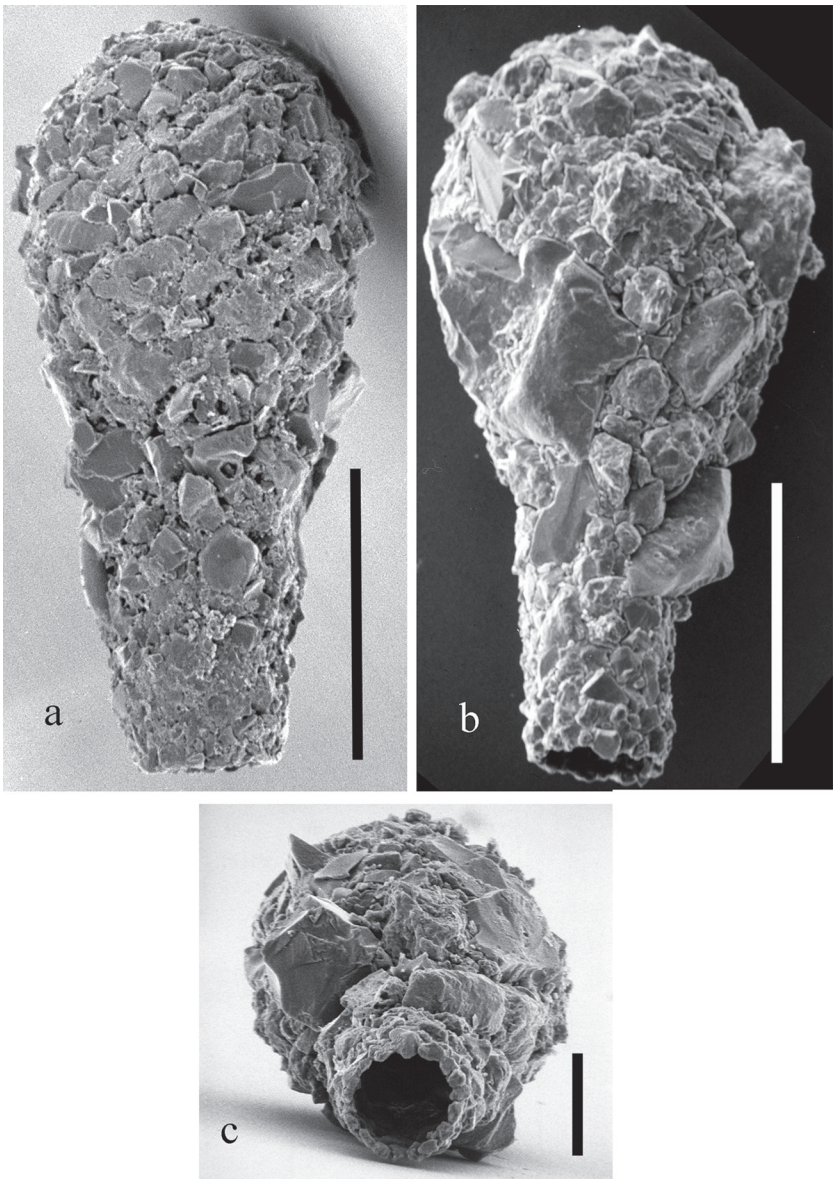


Fig. 13. Different specimens of *Difflugia oblonga* from C.G. Ogden's SEM collection: a-b – lateral view (a – SEM CZ-03.198, b – SEM EM-06-717), c – apertural view (c – SEM Z-15/951). Scale bars: a-b – 100 μm, c – 30 μm.

***Difflugia bacillifera* Penard, 1890**

Ogden described *D. bacillifera* as having an elongate shell, the outline of which is often concealed by adhered diatom frustules (Figs 17; 18). However, the number of frustules varies considerably among different specimens (Fig. 17). The aperture is circular and surrounded by small quartz particles. Ogden measured 66 specimens of *D. bacillifera* (Fig. 11), 57 of which are published (Ogden, 1980; Ogden and Hedley, 1980) and 9 unpublished. The shell dimensions are as follows: length 117–198 μm, width 54–91 μm, aperture diameter 17–36 μm.

Penard (1890) described the shell of this species as being 150–170 μm long, elongate-pyriform in shape, usually transparent and covered with large diatom frustules (Figs 7, r-s; 19). Following Štěpánek (1952), we consider *D. bacillifera* to be synonymous with *D. oblonga*.

***Difflugia oblonga* var. *incondita* Gauthier-Lièvre and Thomas, 1958**

This variety is characterized by its robust, ovoid-pyriform shell that is densely covered by angular sand grains to form rough surface (Figs 10g; 20).

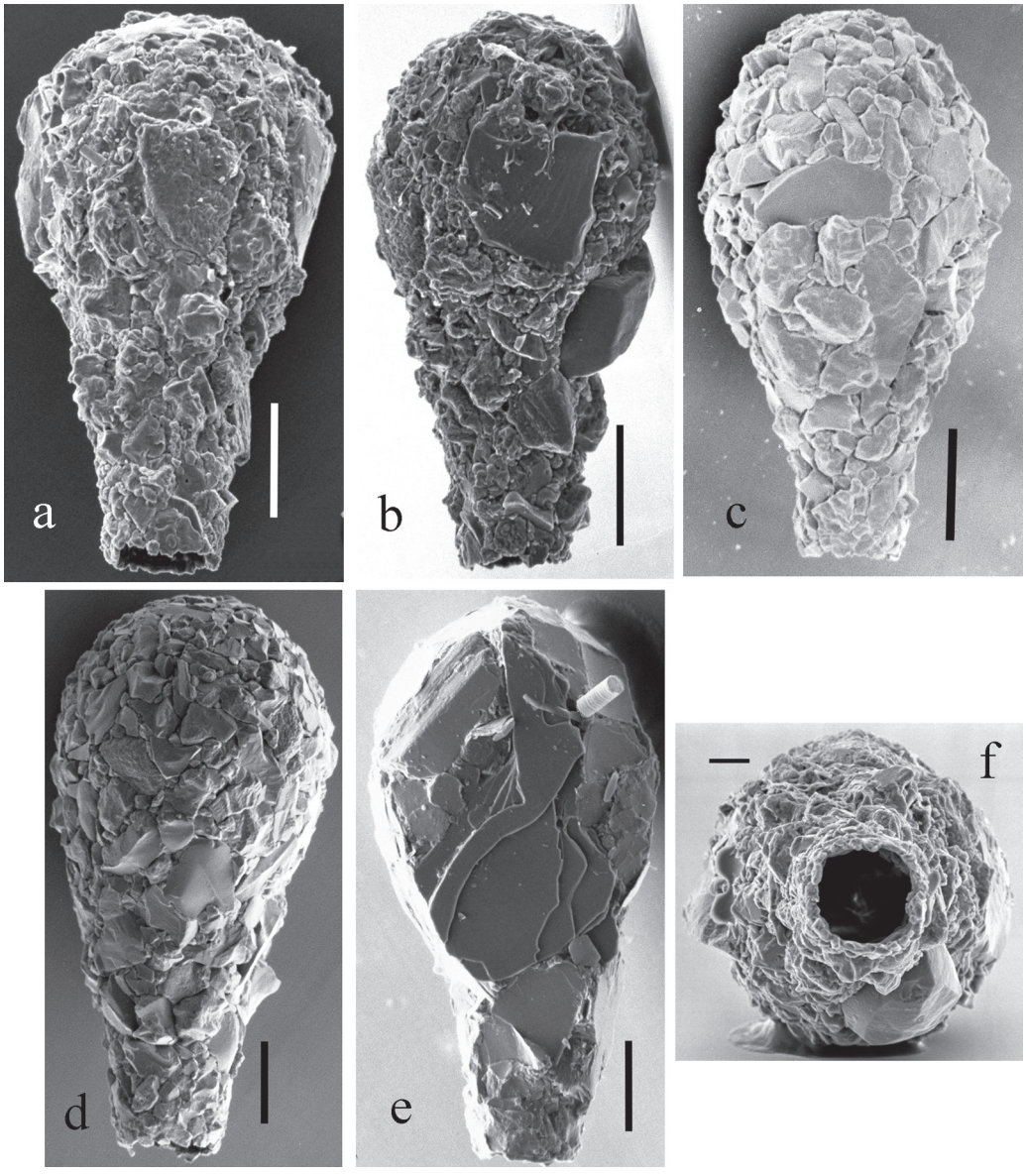


Fig. 14. Different specimens of *Difflugia parva* from C.G. Ogden's SEM collection. a-e – lateral view (a – SEM CZ-04.356, b – SEM CZ-04.365, c – SEM CZ-03.329, d – SEM CZ-03.192, e – SEM CZ-10.975); f – apertural view (SEM CZ-04.357). Scale bars: a-e – 30 μm, f – 10 μm.

The maximum width is in the mid-region of the shell, which tapers conspicuously towards the aperture and slightly towards the posterior end. Measurements given by Gauthier-Lièvre and Thomas (1958) are: length 195–245 μm, width 80–83 μm, aperture diameter 35–40 μm.

Difflugia oblonga var. *incondita* has never been studied in detail using morphometrical and/or SEM investigations. The size range matches closely with *Difflugia oblonga* senu Ogden. Differences in shape are not sufficiently significant for this taxon to be valid. Thus, we here synonymise *D. oblonga* var. *incondita* with *D. oblonga*.

***Difflugia oblonga* f. *cyphodera* Jung, 1942**

This form represents a slight deviation from the basic type (Fig. 10h). In his study of the variability of *Difflugia* in a single pond, Štěpánek (1952) found a series of transitions from specimens with a slight indication of a bending of the test to the specimen figured by Jung (1942). Štěpánek (1952) noted that a possible cause of the bending could be "...minute

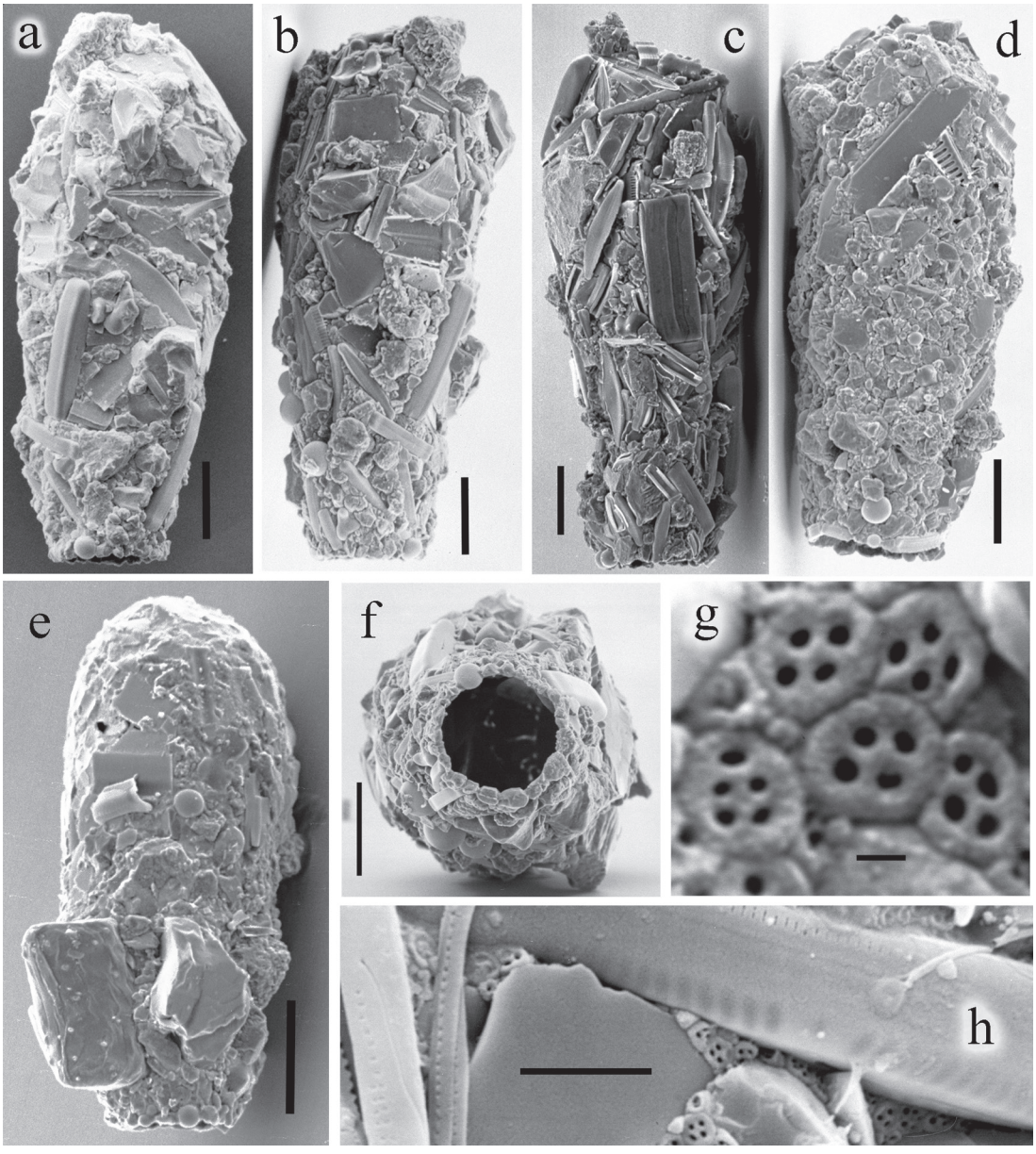


Fig. 15. Different specimens of *Difflugia lacustris* from C.G. Ogden's SEM collection. a-e – lateral view (a – SEM CZ-04.637, b – SEM CZ-04.630, c – SEM CZ-04.798, d – SEM CZ-05.718, e – SEM CZ-07.381); f – apertural view (SEM CZ-04.626); g-h – structure of organic cement (g – SEM CZ-04.631, h – SEM CZ-04.655). Scale bars: a-f – 30 μm, g – 0.3 μm, h – 3 μm.

water currents at the time of the development of the young specimens, which was then moderately bent" (Štěpánek, 1952; p. 24). Regardless, we believe that such charactersitics cannot be treated as taxonomically valid. Thus, we consider *D. oblonga* f. *cyphodera* to be a junior synonym of *D. oblonga*.

Difflugia pyriformis Perty, 1849 species complex

The members of this group include *D. pyriformis*, *D. capreolata*, and *D. cylindrus* all of which have a similar size distribution and general appearance (Fig. 11, group 1; Fig. 12, b-d).

Difflugia pyriformis Perty, 1849

Ogden (1980) described the shell of *D. pyriformis* as opaque, tapering evenly from the aperture to about the mid-body position and curving in the aboral region (Fig. 21). The shell wall is composed of an assortment of quartz particles, arranged in

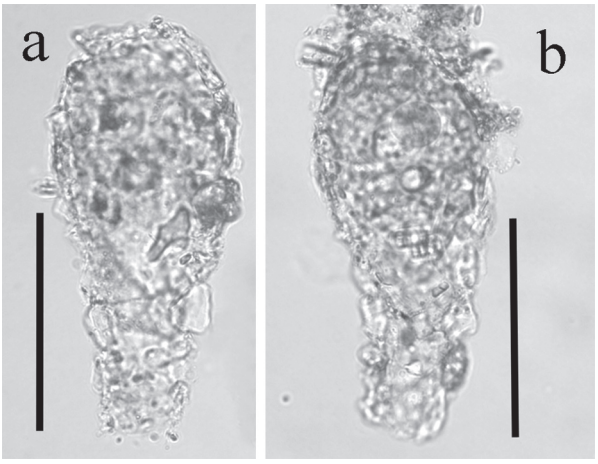


Fig. 16. Different specimens of *Difflugia lacustris* from E. Penard's slides. a-b – lateral view (slide 04.5.9.125). Scale bars: 100 μm.

such a way that the outline is usually regular and the surface is intermediate between rough and smooth. The anterior region is usually covered with small pieces of quartz but occasionally has large, irregular particles. The apetrure is circular and surrounded by a regular arrangement of small quartz particles. Ogden measured 57 individuals of *D. pyriformis*, 43 of which were published (Ogden, 1980) and 14 unpublished, with the shell length ranging from 137 to 253 μm, shell breadth 88–153 μm, and aperture diameter 33–56 μm (Fig. 11).

***Difflugia cylindrus* (Thomas, 1953) Ogden, 1983**

Ogden (1983) described the shell of *D. cylindrus* as usually opaque, almost cylindrical but tapering evenly from the aboral region to the aperture (Fig. 22, a, c). However, in addition to this shape we found in the Ogden SEM collection some specimens with a lanceolate shape (e.g. the width in the middle of the long axis of the shell being greater than that of the rest of the shell – see Fig. 22, b, d). The shell is composed mainly of medium to large pieces of quartz with the occasional diatom frustule on the rough surface, but the latter are seldom incorporated into the thick wall-structure. The aperture is irregular in both outline and composition, being roughly circular and usually surrounded by small particles but often incorporating medium particles that produce a jagged margin (Fig. 22e). Ogden measured 24 specimens of *D. cylindrus* (Fig. 11), 22 of which were published (Ogden, 1983), two unpublished. The shell dimensions are as follows: length 166–264 μm, width 80–130 μm, aperture diameter 34–50 μm.

This species was initially described by Thomas (1953) as a new variety of *D. oblonga* (Fig. 10d). It is distinguished from the typical form by its elongate-cylindrical shape and covering of large sand grains. The shell dimensions are: length 220–260 μm, width 70–90 μm, aperture diameter 40–44 μm.

Both Ogden (1983) and Thomas (1953) highlighted the rough surface of the shell caused by the large particles incorporated in the shell wall. This could be a reason for the apparent difference in shape between *D. cylindrus* and *D. oblonga* (compare Figs 12c and 12d). Taking into the account that both taxa have the same size range (Fig. 11) and lack any clearly described permanent differentiating characteristics, we consider *D. cylindrus* a junior synonym of *D. pyriformis.*

***Difflugia capreolata* Penard, 1902**

Ogden and Živković (1983) described *D. capreolata* as having an opaque, thick, pyriform shell with a restriction of the neck in the anterior third before it expands in the posterior two-thirds (Fig. 23). The shell wall is composed of small to medium-size pieces of angular quartz. The aperture is circular and surrounded by a regular distribution of medium-size particles. Ogden measured two individuals of *D. capreolata*, both published (Ogden and Živković 1983; Ogden, 1984) with the shell length 225–237 μm, shell width 128–164 μm, aperture diameter 58–65 μm (Fig. 11).

In the original species description of *D. capreolata*, Penard (1902) underlined three main characteristics that distinguish it from *D. pyriformis*. Two are cell structures, i.e. the peculiarities of the nucleus and the pseudopodia. The third is shell shape, namely the constriction in the anterior third which was also described by Ogden and Živković (1983). Having such a good morphological marker we retain this as a valid species until more data on its morphological variability are available. Photomicrogrphs of specimens from Penard's slides are shown in Fig. 24.

Notes on *D. oblonga* Ehrenberg, 1838 and *D. pyriformis* Perty, 1849

As discussed above, the validity of both *D. oblonga* and *D. pyriformis* is questionable due to the lack of good original descriptions and the high variability of elongate and pyriform taxa of *Difflugia*. Until 1973 both names were widely used without any redescription of either species. Some authors (Leidy, 1879; Penard, 1890, 1902) preferred to use name '*pyriformis*', whereas others (Cash and Hopkinson,

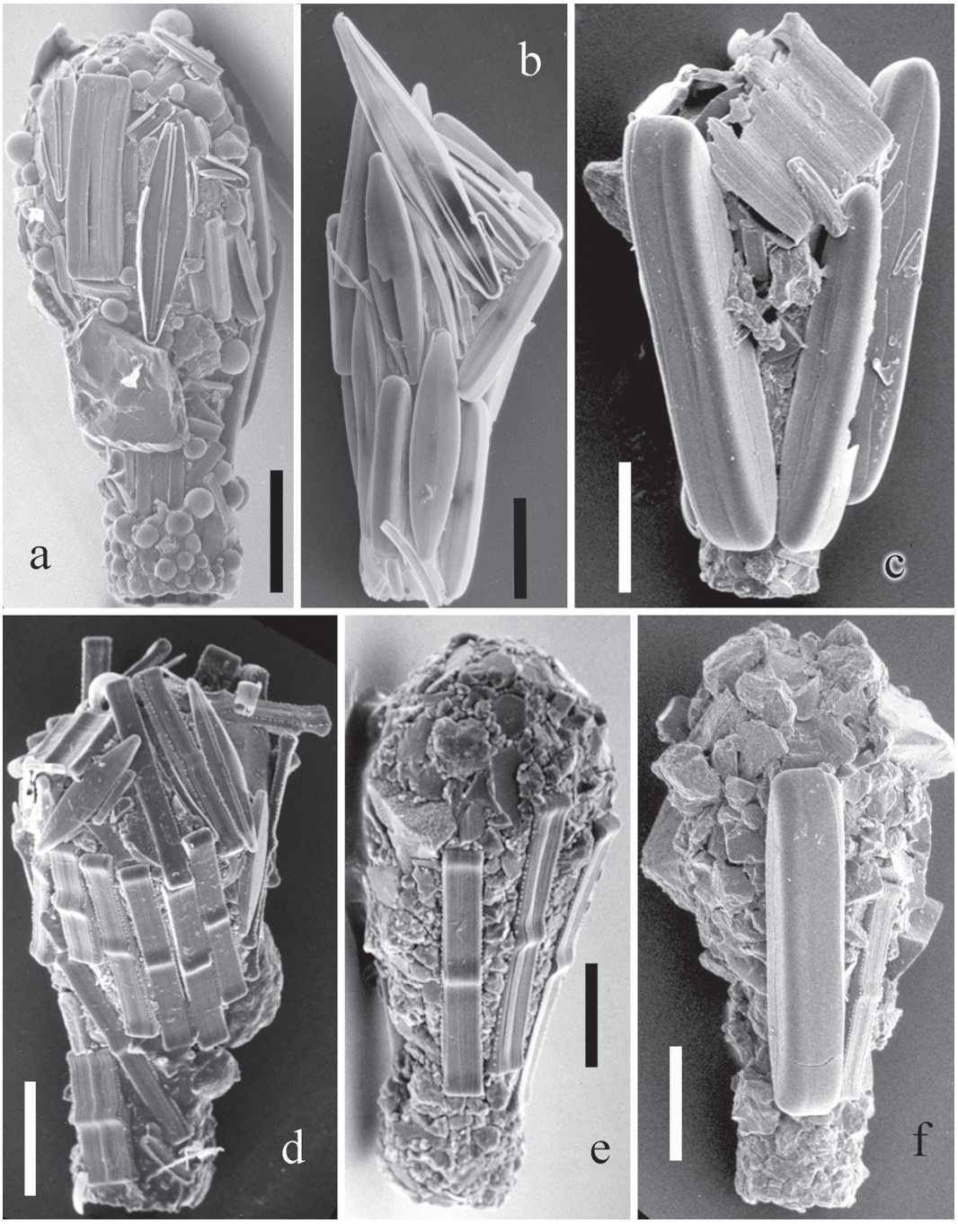


Fig. 17. Different specimens of *Difflugia bacillifera* from C.G. Ogden's SEM collection. a-f – lateral view (a – SEM CZ-02.608, b – SEM CZ-01.324, c – SEM EM-08-990, d – SEM EM-06-731, e – SEM EM-06-726, f – SEM EM-10-493). Scale bars: 30 μm.

1909; Štěpánek, 1952; Gauthier-Lièvre and Thomas, 1958; Chardez, 1967) applied the name '*oblonga*' to these morphologically similar species. Chardez and Decloitre (1973) were first who proposed the idea to fix both names and characterize their typical features. Based on SEM observations and morphometric analysis Ogden and Fairman (1979), and Ogden (1980) supported the findings of Chardez and Decloitre (1973), pointing out that although the shells of *D. pyriformis* and *D. oblonga* are similar in length, they differ significantly in both breadth and diameter of aperture (Fig. 11). Based on these

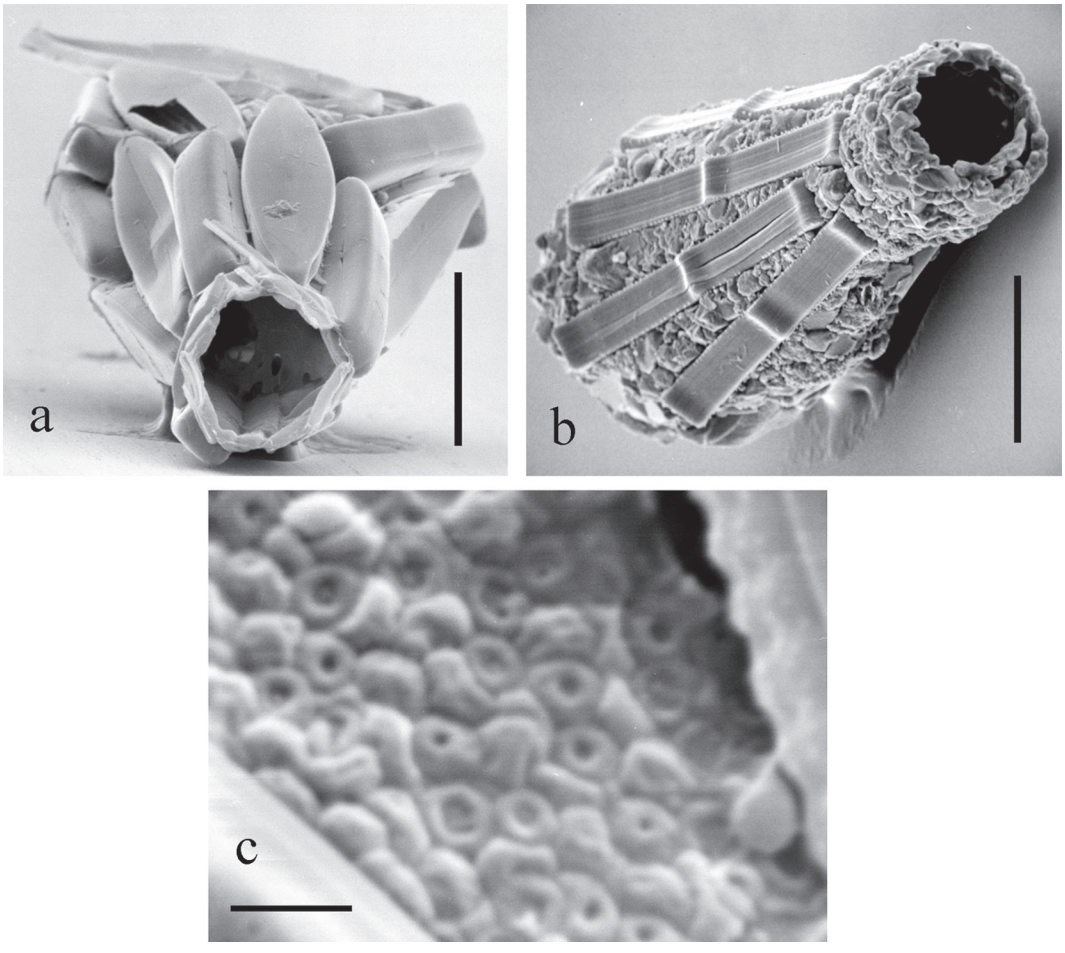


Fig. 18. Different specimens of *Difflugia bacillifera* from C.G. Ogden's SEM collection. a-b – apertural view (a – SEM CZ-01.327, b –EM-06-728); c – structure of organic cement (SEM CZ-01.322). Scale bars: a, b – 30 μm, c – 1 μm.

findings Ogden (1980) proposed an elegant solution to the taxonomic problem which we accepted here. He wrote: "Cash and Hopkinson (1909) suggested that according to the rules of zoological nomenclature *D. oblonga* was the correct name to use for specimens of *D. pyriformis* under the law of priority. The problem of accepting this synonymy has been caused by subsequent reports under either specific name has resulted in a heterogeneous description. Several authors ... recognized numerous varieties within this complex... The problem now is that we have clearly identified two species [in Ogden and Fairman, 1979; Ogden, 1980] that fall within the parameters of the descriptions of *D. oblonga* and *D. pyriformis*. It is almost certain that the type specimens of these species were never preserved..., so that a comparison with these is out of question. Furthermore the synonymy proposed by earlier workers emphasizes that original descriptions are inadequate. In resolving this situation... it seems unnecessary to create a new name when an accurate description of one of these species exists. I refer to Penard's (1902) description of *D. pyriformis* [Fig. 25 shows the Penard's vision of *D. pyriformis*]. ... This description is in such good agreement with the present description of *D. pyriformis* [Ogden, 1980] that they are considered to be conspecific. With regards to the other ... species [*D. oblonga* in Ogden and Fairman, 1979], it would appear that the simplest way to avoid confusion is to retain the name that has already been used to describe it, that is *D. oblonga*, but emphasise that these two species are distinct and not synonyms" (Ogden, 1980; p. 464–466). We already accepted such an approach in our previous publications which showed both morphological (Bobrov and Mazei, 2004) and ecological (Mazei and Tsyganov, 2006) differences between these two species (Mazei and Warren, 2012).

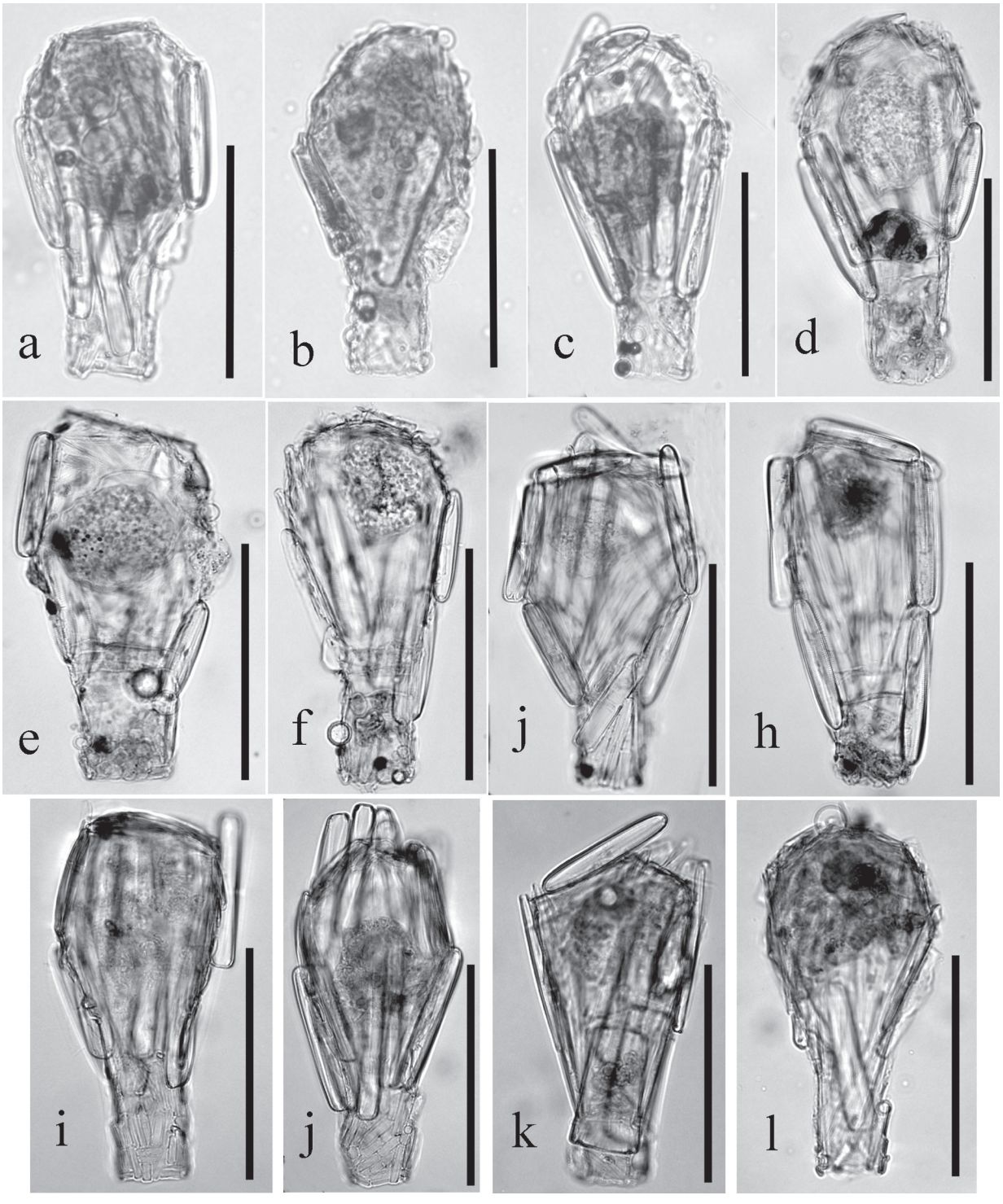


Fig. 19. Different specimens of *Difflugia bacillifera* from E. Penard's slides. a-l – lateral view (a-c – slide 04.5.9.82, d-f – slide 20.12.8.172, g-h – slide 20.12.8.173, i-j – slide 20.12.8.174, k-l – slide 20.12.8.175). Scale bars: 100 μm.

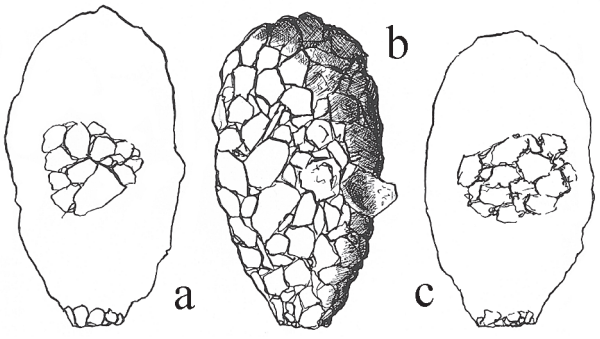


Fig. 20. Different specimens of *Difflugia oblonga* var. *inconditaa* after Gauthier-Lièvre and Thomas (1958). a-c – lateral view.

Difflugia bryophila (Penard, 1902) Jung, 1942 species complex

The members of this group include *D. bryophila* and *D. gassowskii* which have a similar size distribution and general appearance (Fig. 11, group 4; Fig. 12, l-m).

Difflugia bryophila (Penard, 1902) Jung, 1942

According to Ogden (1983), the shell of *D. bryophila* is brown, pyriform, with the sides usually tapering evenly to the aperture (Fig. 26), although the occasional specimen may be slightly miss-aligned, have a large particle obscuring the even-tapering (Fig. 26b) or be almost cylindrical (Fig. 26c). The shell wall is composed mainly of a mixture of small to medium-size pieces of quartz and the occasional diatom frustule or siliceous flagellate cysts. The aperture is circular and surrounded by small particles. Ogden measured 46 specimens of *D. bryophila*, 45 of which were published (Ogden and Živković, 1983; Ogden, 1983, 1984), one unpublished (Fig. 11). The shell dimensions are as follows: length 83–141 μm, width 45–67 μm, aperture diameter 15–23 μm.

Difflugia bryophila differs from *D. oblonga* and *D. pyriformis* by its size (Penard, 1902 gave the length as 100 μm) and more transparent shell. The general shape is similar to both *D. oblonga* and *D. pyriformis* and varies according to the building material available in the environment.

Difflugia gassowskii (Gassowsky, 1936) Ogden, 1983

Ogden (1983) described this species as having a pyriform shell with a distinctly short neck about one-third of the body length, and a rounded aboral region (Fig. 27). The surface is rough and composed of small to medium angular pieces of quartz. The aperture is circular. Ogden measured 23 specimens of *D. gassowskii* (Fig. 11) 21 of which are published (Ogden and Hedley, 1980; Ogden, 1983), two unpublished. Their dimensions are: shell length 81–120 μm, shell breadth 45–56 μm, aperture diameter 15–22 μm.

Ogden and Hedley (1980) referred to this species as *D. longicollis* since they raised the original *D. piryformis* var. *longicollis* of Gassowsky (1936) to the rank of species. Later, Ogden (1983) changed his decision because the name *D. longicollis* was used initially by Ehrenberg (1854) to describe specimens which now are not considered to belong to the genus *Difflugia*. Nevertheless, according to the International Code of Zoological Nomenclature the name is preoccupied.

Gassowsky (1936) originally described this variety under the name *Difflugia oblonga* var. *longicollis* as differing from the typical form by its very prominent neck and shell dimensions (length 72–116 μm, width 48–78 μm, aperture diameter 21–34.5 μm), which correspond well with the description of Ogden (1983). However, we do not believe that differences between *D. gassowskii* and *D. bryophila* are taxonomically significant because the appearance of the neck is determined by the material available for its construction. Even among Ogden's scanning electron micrographs, shells with or without a prominent neck can be observed (compare figures 26a and 26b). Thus, we treat *D. gassowskii* as a junior synonym of *D. bryophila,* based on the priority of the first description of *D. pyriformis* var. *bryophila* Penard, 1902 and *D. pyriformis* var. *longicollis* Gassowskii, 1936 as well as their redescriptions as *D. bryophila* Jung, 1942 and *D. gassowskii* Ogden, 1983, respectively.

Difflugia linearis (Penard, 1890) Gautier-Lièvre et Thomas, 1958 species complex

The members of this group include *D. linearis, D. paulii*, and *D. nebeloïdes*, all of which have a similar size distribution and general appearance (Fig. 11, group 5; Fig. 12, h, n).

Difflugia linearis (Penard, 1890) Gautier-Lièvre et Thomas, 1958

Ogden (1983) characterized this species as having a shell that is transparent, flask shaped or elongate-pyriform, with a long, thin neck with parallel sides and a slightly swollen, rounded aboral region (Fig. 28). The surface is sometimes slightly uneven because of projecting particles, but generally it has a regular outline. It is composed of a mixture of

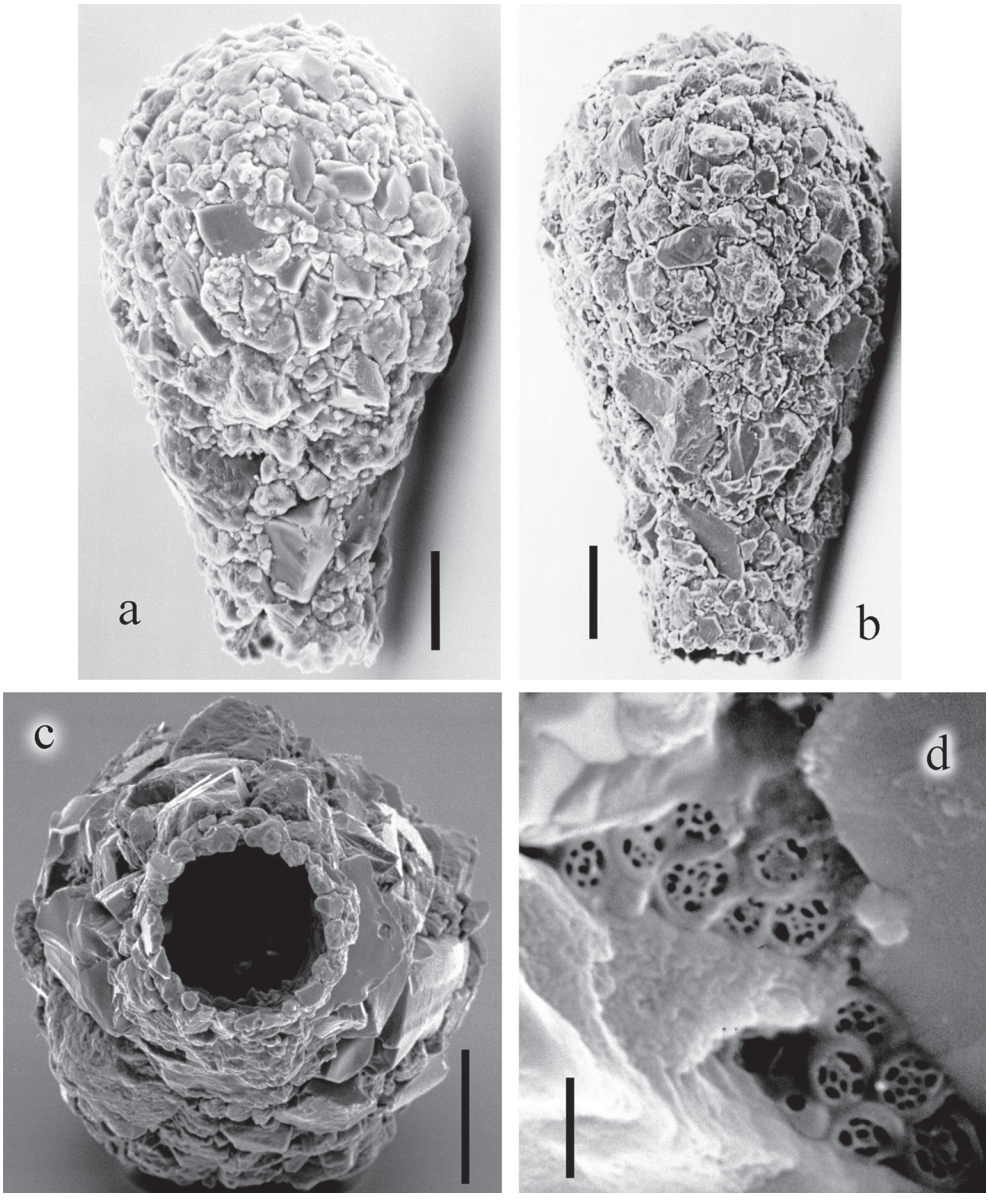


Fig. 21. Different specimens of *Difflugia pyriformis* from C.G. Ogden's SEM collection. a-b – lateral view (a – SEM CZ-01.155, b – SEM CZ-08.142); c – apertural view (SEM CZ-04.893); d – structure of organic cement (SEM CZ-05.680). Scale bars: a-c – 30 μm, d – 1 μm.

flattened pieces of quartz, small whole, flat diatom frustules, fragments of flattish frustules, small siliceous shell plates and globular flagellate cysts. The aperture is circular and usually surrounded by smal particles. Ogden measured 7 specimens of *D. linearis* (Fig. 11), all of which were published (Ogden, 1980 – as *D. lacustris*; Ogden, 1983). The shell dimensions are as follows: length 96–108 μm, width 32–38 μm, aperture diameter 12–13 μm.

In his original description, Penard (1890) referred to this taxon as *D. pyriformis* var. *linearis* and, based on his figures, the shell length varied from 60 to 100 μm (Fig. 7d). Gautier-Lièvre and Thomas (1958) raised this taxon to species level and gave the shell dimensions as 90–105 μm long and 30–35 μm wide (Fig. 29, a, b). Although neither of these earlier descriptions emphasized the prominence of the neck, they are consistent with Ogden's description in other regards, i.e. the transparent and elongated nature of the shell (Ogden, 1983).

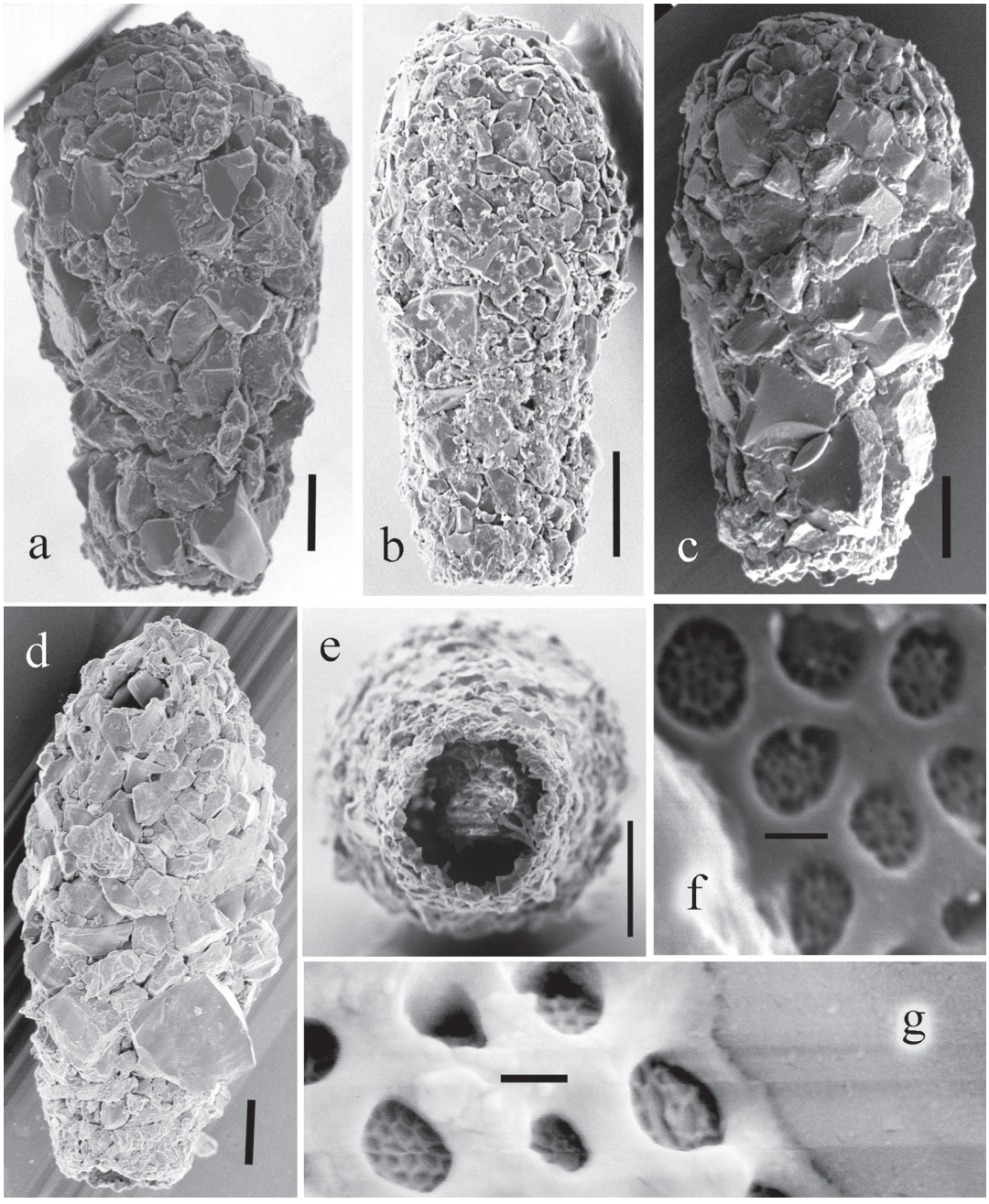


Fig. 22. Different specimens of *Difflugia cylindrus* from C.G. Ogden's SEM collection. a-d – lateral view (a – SEM CZ-04.411, b – SEM CZ-11.360, c – SEM CZ-04.811, d – SEM 008711); e – apertural view (SEM CZ-11.356); f-g – structure of organic cement (f – SEM CZ-04.415, g – SEM CZ-008710). Scale bars: a-e – 30 μm, f-g – 0.3 μm.

***Difflugia paulii* Ogden, 1983**

According to Ogden (1983), *D. paulii* is characterised by its transparent, slender, elongate shell that tapers evenly from just anterior of the mid-body region towards the aperture (Fig. 30). It is composed of flattish pieces of quartz to give a smooth appearance. The aperture is circular and surrounded by small pieces of quartz. Ogden measured 4 specimens of *D. paulii* (Fig. 11) all of which were published (Ogden, 1983). The shell dimensions are as follows: length 119–130 μm, width 48–54 μm, aperture diameter 19–23 μm.

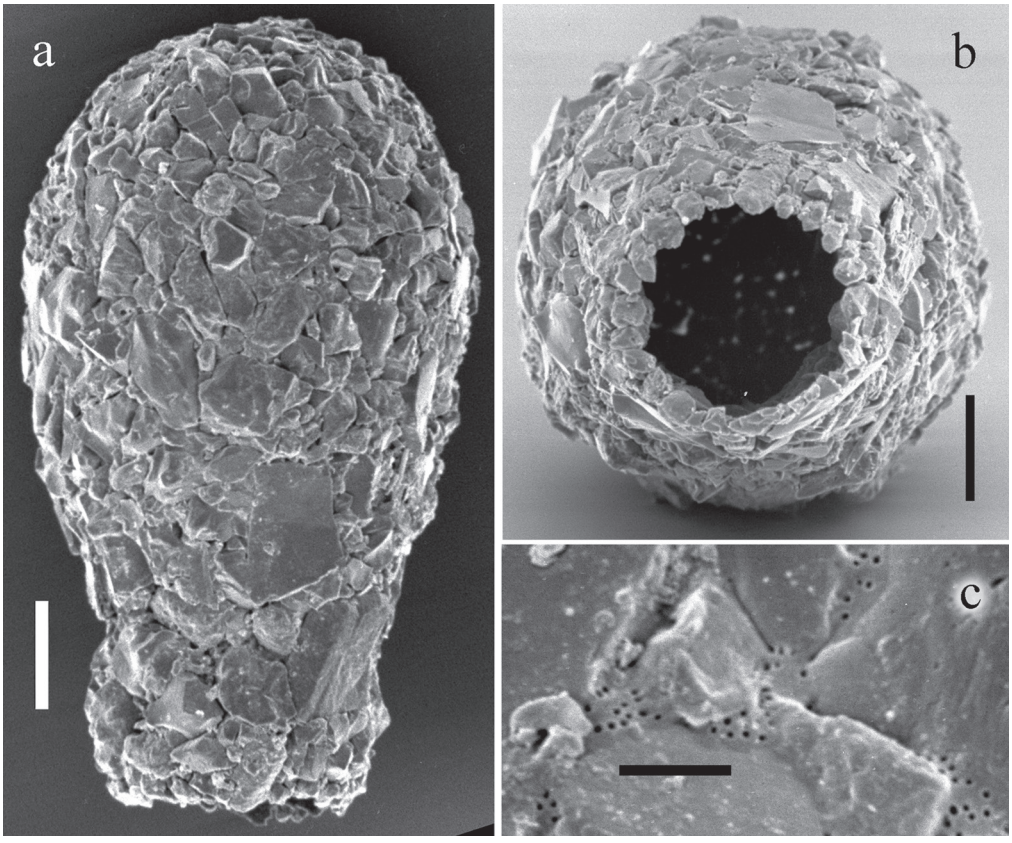


Fig. 23. Different specimens of *Difflugia capreolata* from C.G. Ogden's SEM collection. a – lateral view (SEM CZ-03.181); b – apertural view (SEM CZ-03.190); c – structure of organic cement (SEM CZ-03.182). Scale bars: a-b – 30 μm, c – 3 μm.

This species was described previously as *D. oblonga* var. *elongata* by van Oye (1953) and Gauthier-Lièvre and Thomas (1958) (Fig. 29, c, d). The dimensions given by van Oye (length 140 μm, width 43 μm, aperture diameter 30 μm) and Gauthier-Lièvre and Thomas (length 130–142 μm, width 38–40 μm, aperture diameter 28–37 μm) are in good agreement and correspond well with Ogden's data. Ogden (1983) also noted that within a group of more elongated pyriform species, *D. paulii* appears to occupy a position mid-way between *D. linearis* and *D. lacustris* in terms of its shell length.

Davidova (2012) investigated the morphometry of *D. paulii* based on 20 specimens (all previous findings were more limited in terms of the number of specimens examined, e.g. van Oye (1953) examined one specimen, Ogden (1983) measured four, and Gauthier-Lièvre and Thomas (1958) did not provided such information). The shell morphology and dimensions of the specimens examined by Davidova (2012) were consistent with Ogden's (1983) description: shell length 110–125 μm, shell length 44–51 μm, aperture diameter 18–24 μm (Fig. 31). Davidova (2012) also made morphometric comparisons with *D. linearis* and *D. lacustris* and concluded that the three are separate species.

Following a review of the available data, we do not consider the differences between *D. paulii* and *D. linearis* to be sufficiently significant for their separation at species level (compare figures 12h and 12n). Even in the figures of Gauthier-Lièvre and Thomas (1958) the differences are not clear (compare figures 29, a-b and 29, c-d). We believe that the data of Davidova (2012) are also insufficient since she compared her 20 specimens of *D. paulii* with only three individuals of *D. linearis*. We applied the measurements of R. Davidova captured from her fig. 5 (Davidova, 2012; p. 43) and analyzed them with C.G. Ogden's measurements. The resulting scatterplot is shown in Fig. 32 which reveals a continuum between the two species. Thus we conclude that there is insufficient evidence to support for the validity of *D. paulii* which we consider a junior synonym of *D. linearis*.

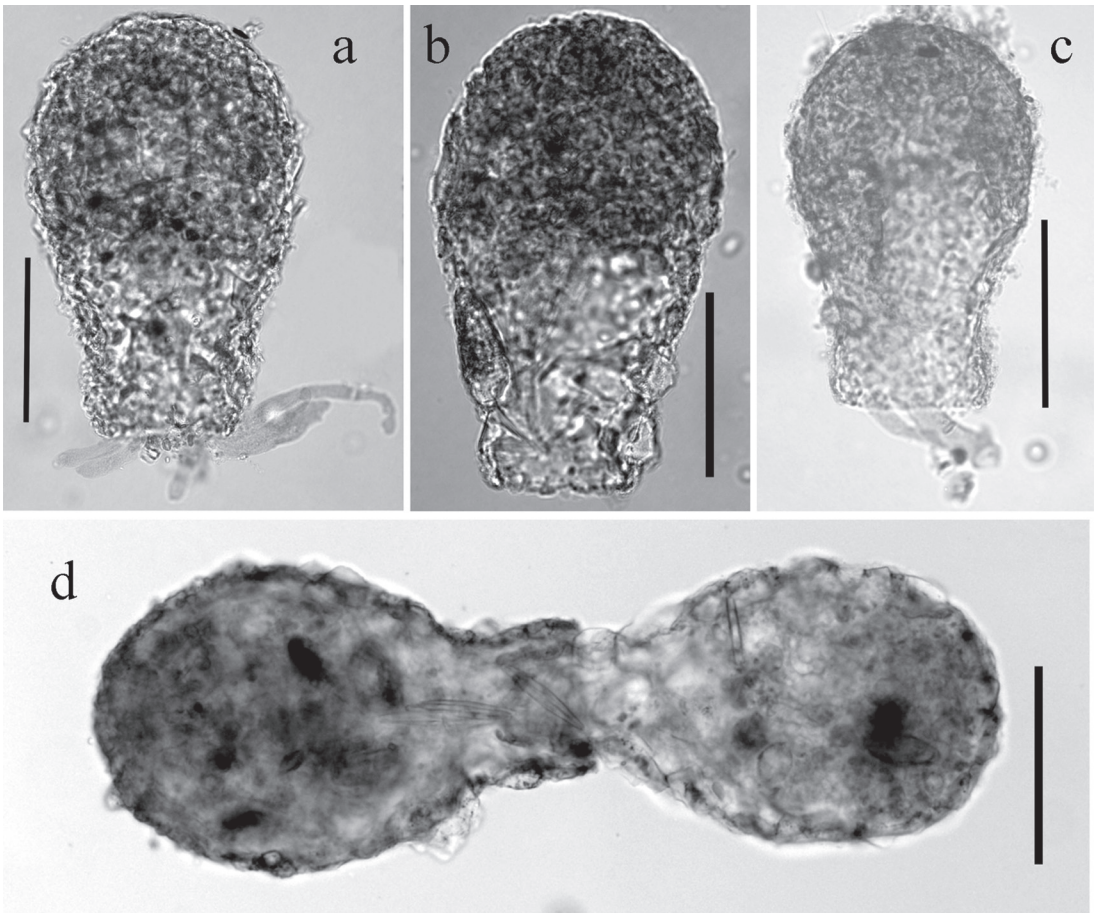


Fig. 24. Different specimens of *Difflugia capreolata* from E. Penard's slides. a-c – lateral view (a – slide 04.5.9.88, b – slide 20.12.8.186, c – slide 04.5.9.90); d – dividing cells (slide 20.12.8.188). Scale bars: 100 μm.

***Difflugia nebeloïdes* Gauthier-Lièvre and Thomas, 1958**

This species has not been redescribed since it was originally reported by Gauthier-Lièvre and Thomas (1958) who described its shell as being transparent elongate-pyriform, circular aperture, shell length 118–140 μm, shell width 35–45 μm, aperture diameter 17–20 μm (Fig. 29, e, f) . Based in its general appearance, this species closely resembles *D. linearis* and *D. paulii* (Fig. 29), its shell dimensions being precisely between these two. Thus we consider *D. nebeloïdes* a junior synonym of *D. linearis*.

***Difflugia gigantea* Chardez, 1967) Ogden et Fairman, 1979 species complex**

This species complex includes extra-large specimens with a pyriform or elongate shell and comprises three taxa: *D. gigantea* (Fig. 12a), *D. oblonga* var. *angusticollis* and *D. oblonga* var. *stepaneki*.

***Difflugia gigantea* Chardez, 1967) Ogden et Fairman, 1979**

Ogden and Fairman (1979) described this species as typically having a pyriform shell and a spherical fundus that tapers towards the aperture for about half the shell length (Fig. 33, c, d). The shell surface is smooth and constructed of medium-size pieces of flattened quartz. The aperture is circular or oval and surrounded by small particles of quartz. Other specimens from Ogden's collection allow us to broaden the range of characters for this species to include those with a lanceolate shape and those that incorporate large sand-grains into the shell wall (Fig. 33, a, b). Ogden measured 10 specimens of *D. gigantea*, seven of which were published (Ogden and Fairman, 1979), three unpublished. The shell dimensions are as follows: length 341–480 μm,

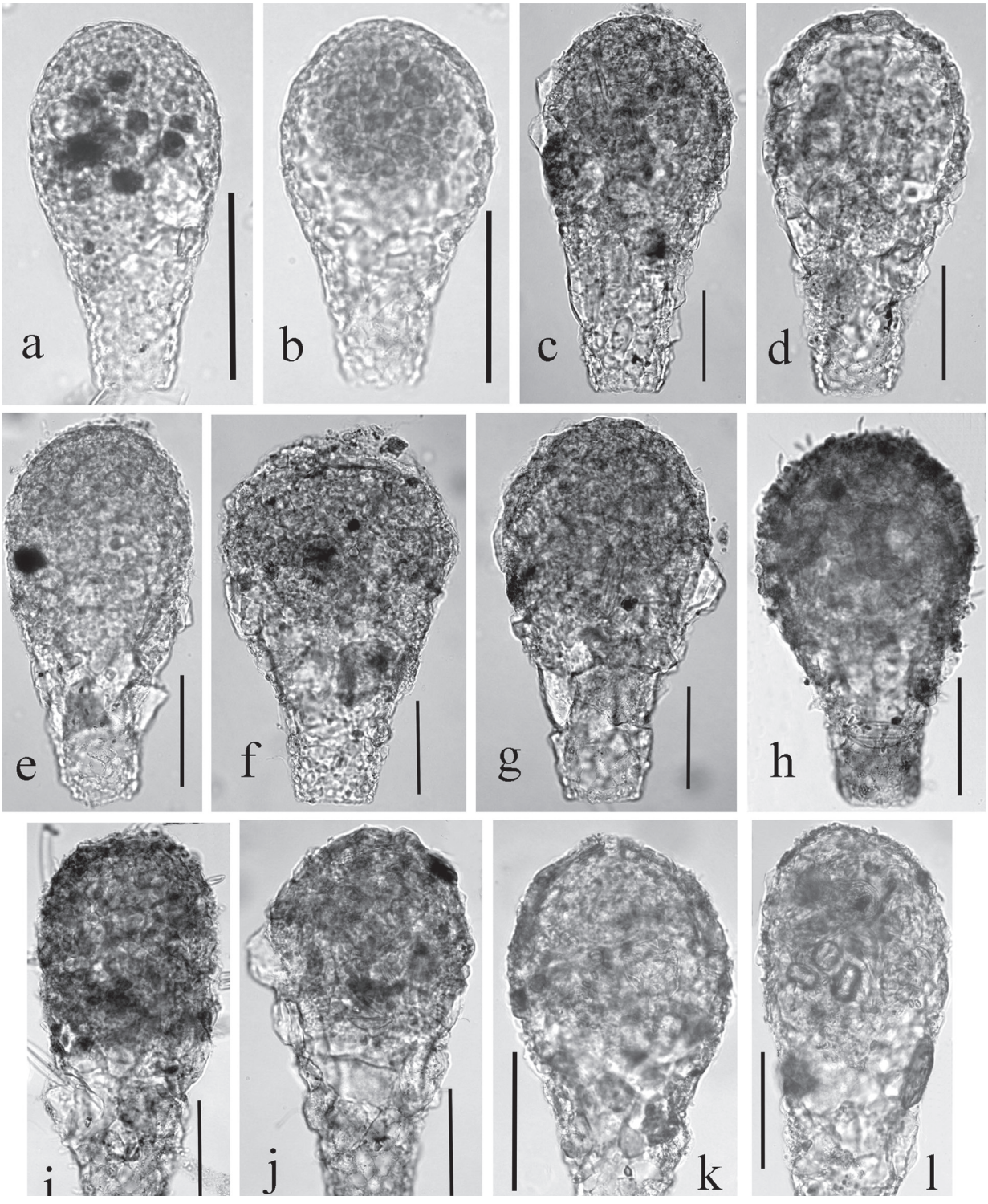


Fig. 25. Different specimens of *Difflugia pyriformis* from E. Penard's slides. a-h – lateral view (a – slide 04.5.9.138, b – slide 04.5.9.124, c-g – slide 4.5.9.132, h-j – slide 20.12.8.242, k-l – slide 20.12.8.268). Scale bars: 100 μm.

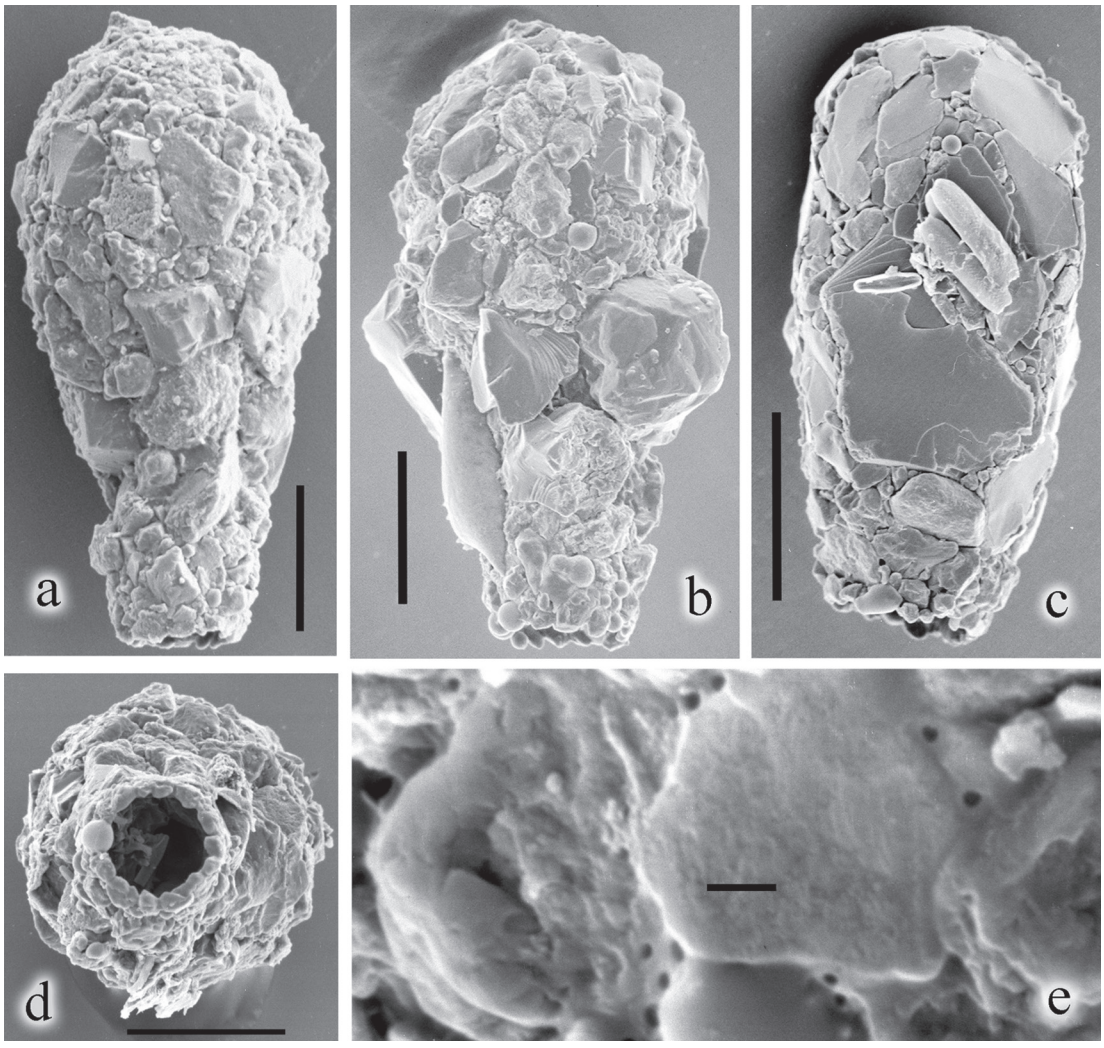


Fig. 26. Different specimens of *Difflugia bryophila* from C.G. Ogden's SEM collection. a-c – lateral view (a – SEM CZ-04.041, b – SEM 030054, c – SEM 003824); c – apertural view (SEM CZ-04.047); e – structure of organic cement (SEM CZ-04.045). Scale bars: a-d – 30 μm, e – 1 μm.

width 168–231 μm, aperture diameter 55–84 μm.

***Difflugia oblonga* var. *angusticollis* Štěpánek, 1952**

Štěpánek (1952) characterized this variety by its long, thin neck, and spherical test (Fig. 10j). The shell dimensions are as follows: length 315–367.5 μm, width 175–210 μm, aperture diameter 42–70 μm. In the collection of C.G. Ogden's SEM micrographs we found images of specimens that have a similar shape to *D. oblonga* var. *angusticollis* (Fig. 34). However, these specimens also had some notable peculiarities such as a conspicuous constriction between the neck and the fundus and an organic lip surrounding the aperture, which exclude them from being members of the genus *Difflugia*. Most likely they belong to *Lagenodifflugia, Pontigulasia* or *Zivkovicia*. Consequently, we now have no conclusive evidence concerning the validity of *D. oblonga* var. *angusticollis* which must await further investigation by SEM and morphometric methods.

***Difflugia oblonga* var. *stepaneki* (Štěpánek, 1952) Decloitre**

Štěpánek (1952) described this variety under the name *D. oblonga* var. *vas* (Fig. 10k) on account of its vase shape. It has an elongate test, slightly narrowed at the neck and widened toward the aperture. The covering on the shell surface is composed predominantly of medium-sized sand grains. Shell length 420 μm, shell width 160 μm, aperture diameter 88 μm. Chardez (1967) and Char-

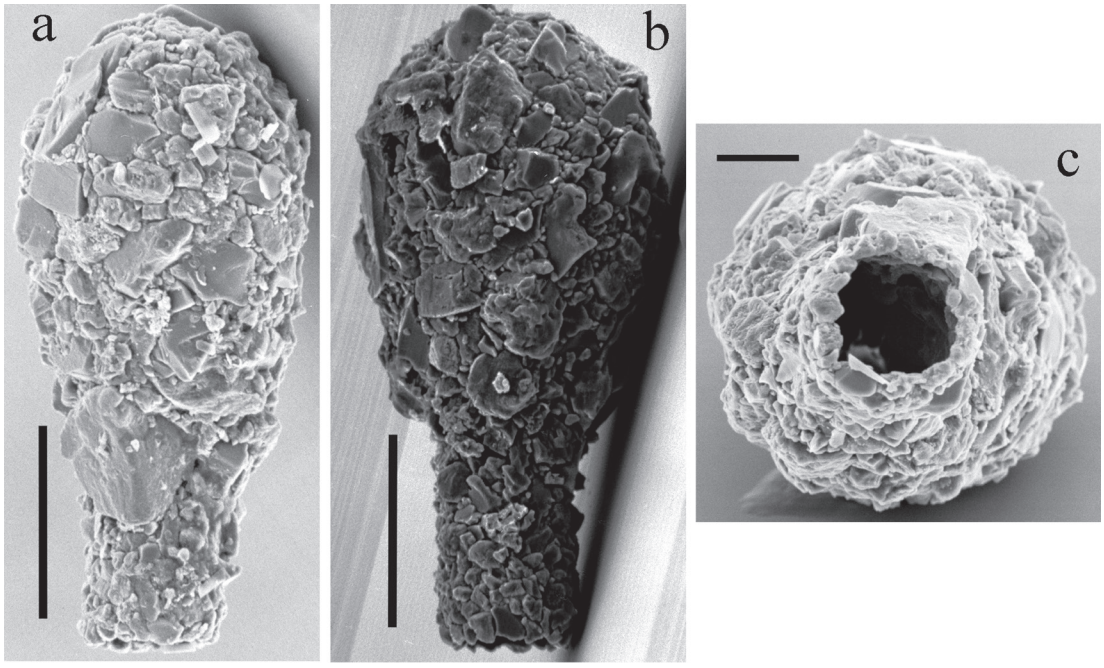


Fig. 27. Different specimens of *Difflugia gassowskï* from C.G. Ogden's SEM collection. a-b – lateral view (a – SEM CZ-04.623, b – SEM CZ-05.036); c – apertural view (SEM CZ-04.619). Scale bars: a-b – 30 μm, c – 10 μm.

dez and Decloitre (1973) used another name for this taxon, *D. oblonga* var. *stepaneki*, without giving an explanation or citing Decloitre's work. Thus, its validity is highly questionable.

Difflugia petricola Cash, 1909 species complex

This species complex includes broad-pyriform species belonging to group 3 of Fig. 11. It contains one species, *D. petricola* (Fig. 12k).

Difflugia petricola Cash, 1909

According to the description of Ogden and Hedley (1979), the shell of *D. petricola* is pyriform with a short neck that is about one-sixth of the shell length (Fig. 35). The aperture is circular and usually surrounded by a regular assortment of small quartz particles. The remainder of the shell is composed of randomly arranged sand grains and a few diatom frustules. The surface has a rough appearance. Ogden measured 89 specimens of *D. petricola*, all of which were published (Ogden and Fairman, 1979; Ogden, 1983, 1984). The shell dimensions are as follows: length 96–151 μm, width 58–99 μm, aperture diameter 20–36 μm.

Difflugia petricola is clearly distinguished from other species discussed in this paper by its broad-pyriform shell. However, it has some transitions with other broad-ovoid species which will be discussed in the next article of this series.

Difflugia lanceolata Penard, 1890 species complex

This species complex is characterized by its lanceolate shape and contains one rather polymorphic species, *D. lanceolata* (Fig. 12j).

Difflugia lanceolata Penard, 1890

According to Ogden (1983) the shell of *D. lanceolata* is yellow or hyaline, lanceolate, being widest about two-thirds of the body-length from the aperture, rounded aborally and tapering evenly towards the aperture (Figs 36; 37). It is composed of small to medium-size, flattish pieces of quartz and some flat diatom frustules that are arranged in such a way as to make the shell wall appear characteristically thin and smooth. An angular piece of quartz may occasionally protrude from the surface but these are uncommon and limited to one or two in any shell. The aperture is circular and well defined because the rim has a thin covering of organic cement. Ogden measured 50 specimens of *D. lanceolata*, 42 of which were published (Ogden and Hedley, 1980; Ogden, 1983), eight unpublished:

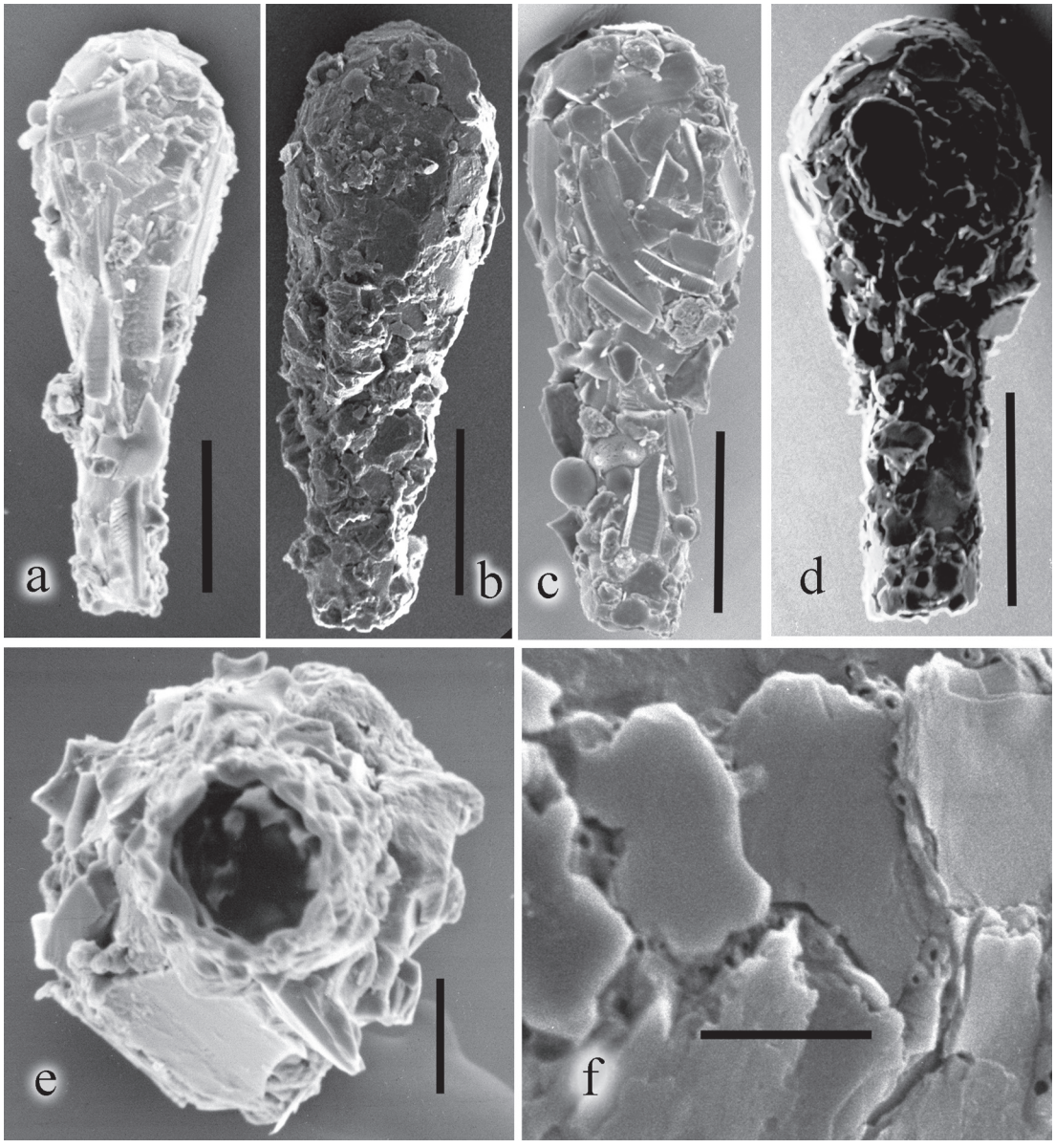


Fig. 28. Different specimens of *Difflugia linearis* from C.G. Ogden's SEM collection. a-d – lateral view (a – SEM CZ-01.161, b – SEM CZ-06.677, c – SEM CZ-07.828, d – SEM CZ-09.115); e – apertural view (SEM CZ-01.241); f – structure of organic cement (SEM CZ-06.664). Scale bars: a-d – 30 μm, e – 10 μm, f – 3 μm.

shell length 85–163 μm, shell breadth 29–92 μm, aperture diameter 14–32 μm. There are some variations in shape and size found in C.G. Ogden's SEM collection (Fig. 38, plate 59 in Ogden and Hedley, 1980).

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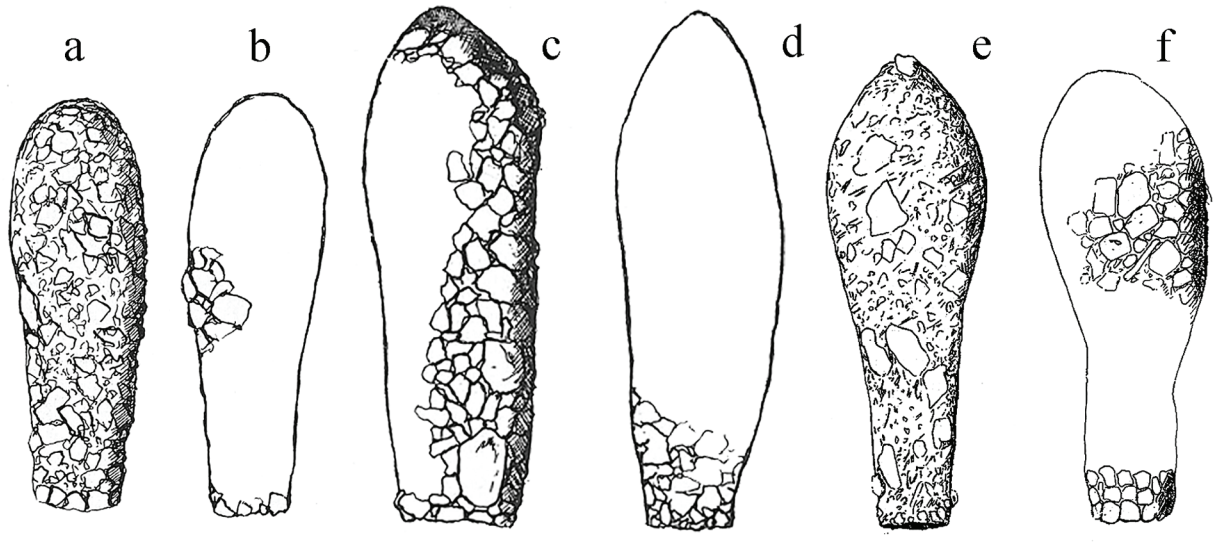

Fig. 29. *Difflugia linearis* (a, b), *Difflugia oblonga* var. *elongata* (c, d), and *Difflugia nebelondes* (e, f) from Gauthier-Lièvre and Thomas (1958). a, b – fig. 38, c, d – fig. 39, e, f – fig. 54.

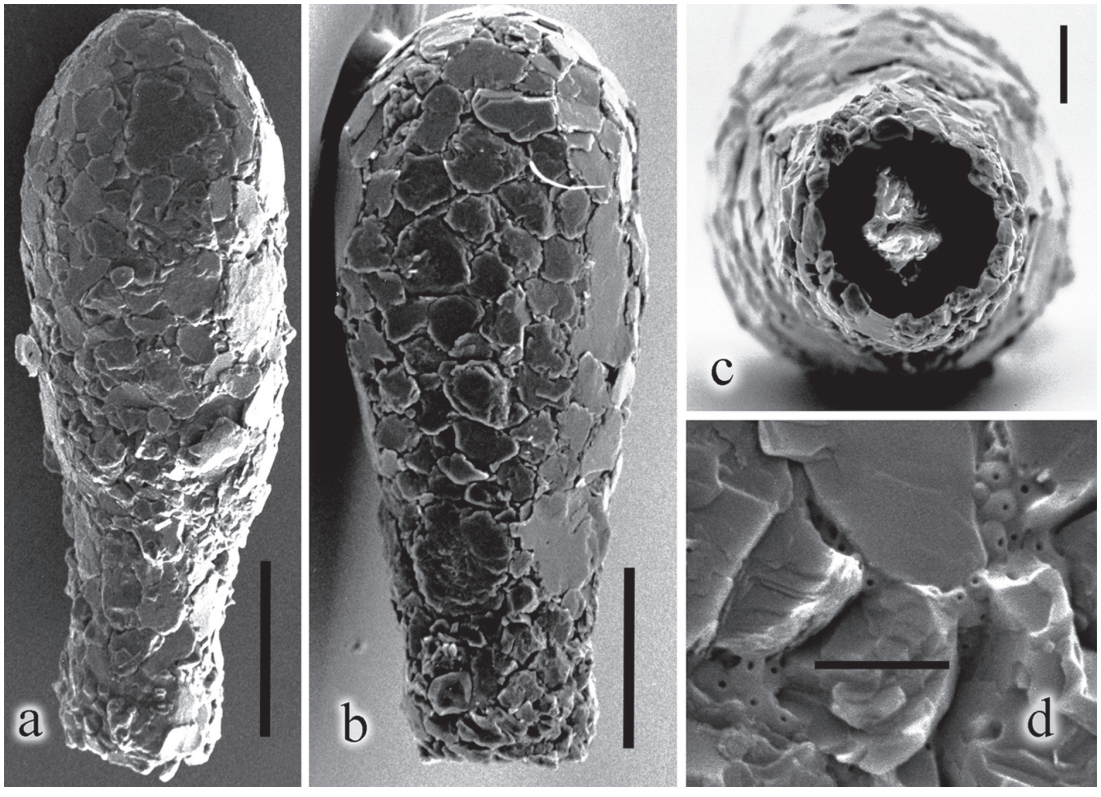

Fig. 30. Different specimens of *Difflugia paulii* from C.G. Ogden's SEM collection. a-b – lateral view (a – SEM CZ-06.678, b – SEM CZ-08.778); c – apertural view (SEM CZ-08.780); d – structure of organic cement (SEM CZ-06.668). Scale bars: a-d – 30 μm.

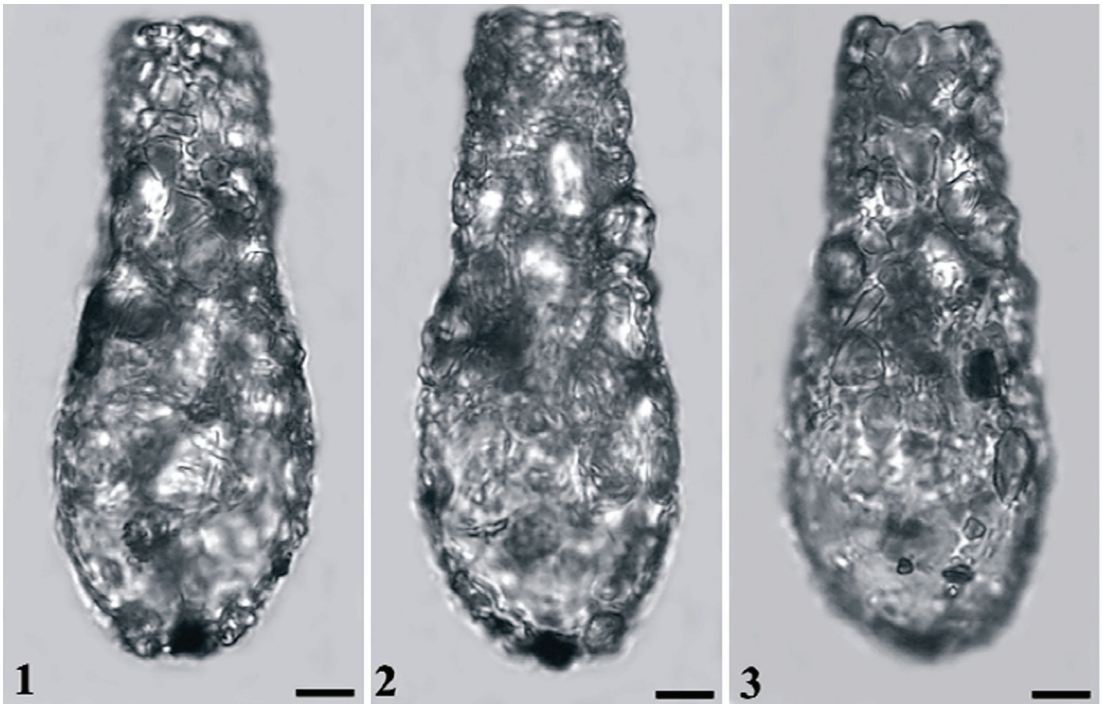

Fig. 31. Different specimens of *Difflugia paulii* from Davidova (2012).1-3 – lateral view. Scale bars: 1-3 – 10 μm.

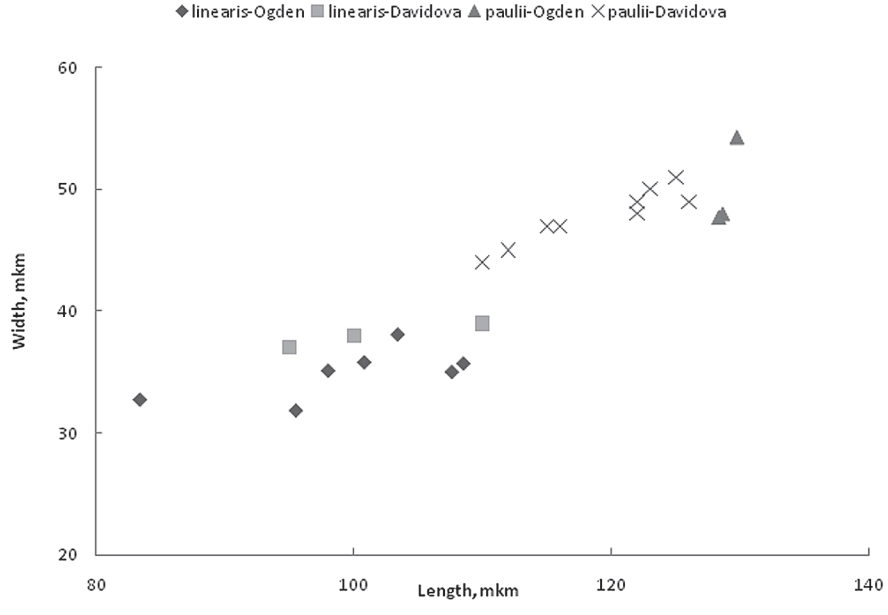

Fig. 32. Length-width measurements scatter plot of *D. paulii* and *D. linearis* based on C.G. Ogden's and R. Davidova's (2012) measurements.

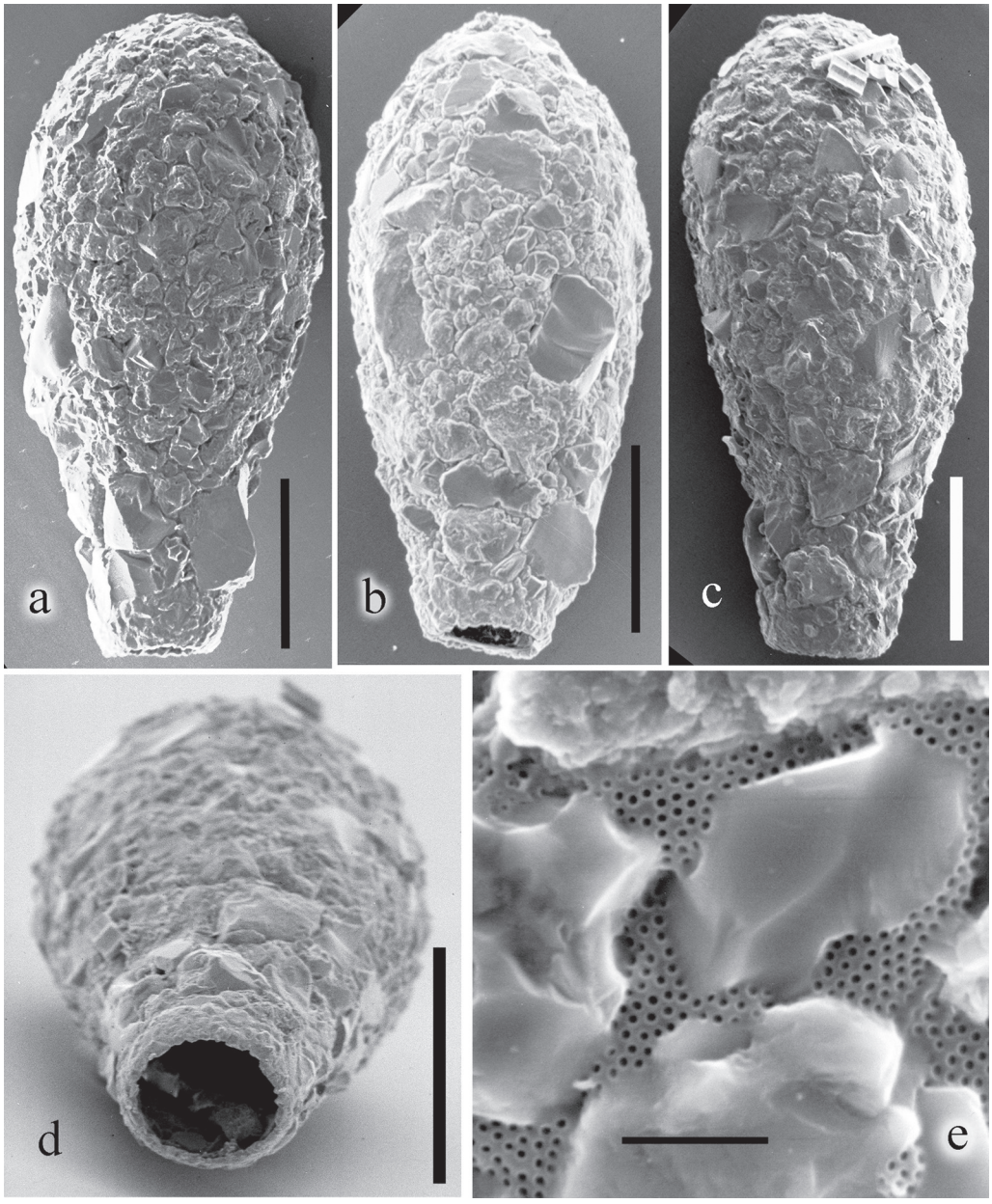

Fig. 33. Different specimens of *Difflugia gigantea* from C.G. Ogden's SEM collection. a-c – lateral view (a – SEM CZ-07.401, b – SEM CZ-01.103, c – SEM EM-12-587); d – apertural view (SEM EM-12-579); e – structure of organic cement (SEM CZ-01.104). Scale bars: a-d – 100 μm, e – 3 μm.

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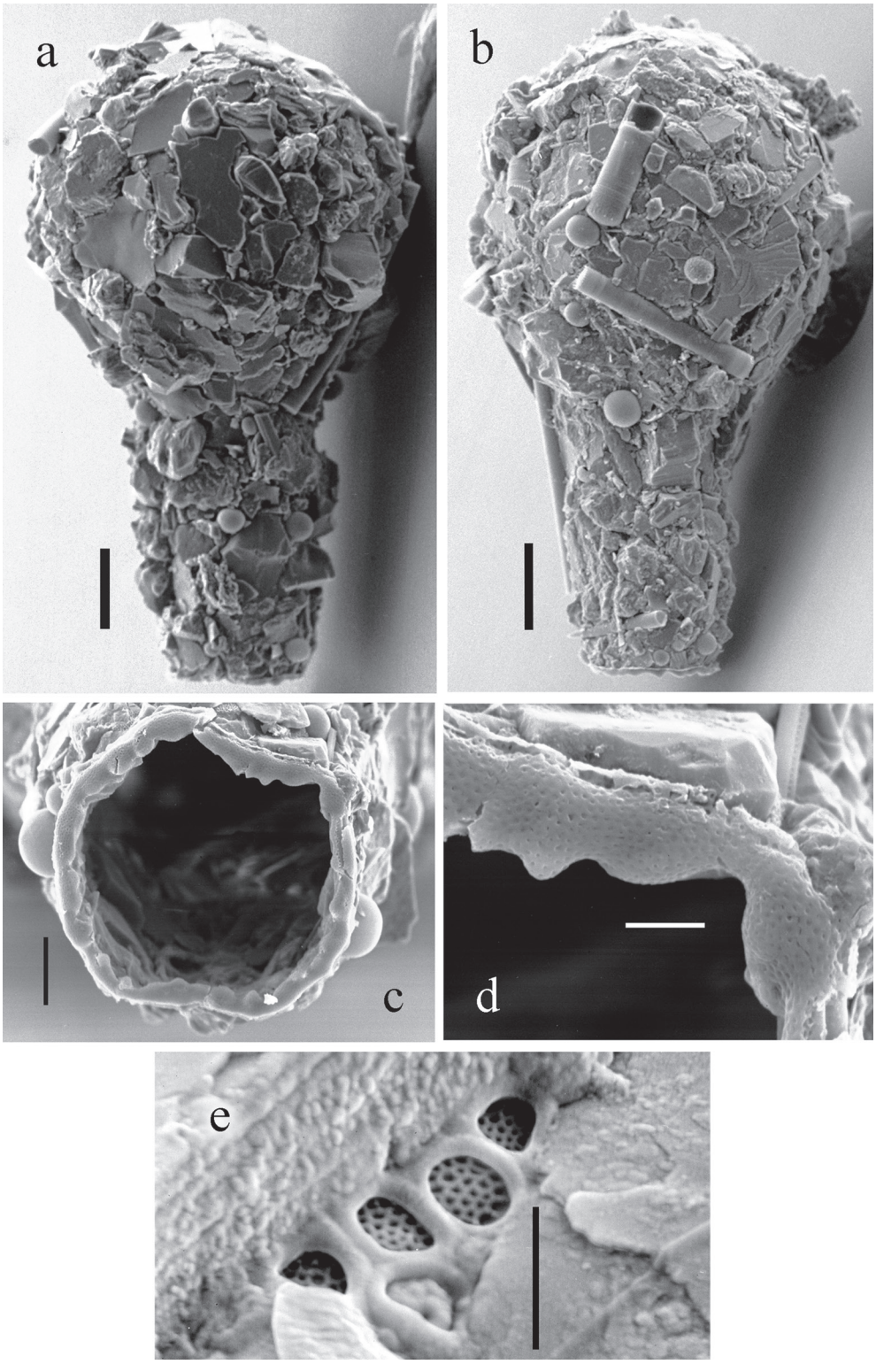

Fig. 34. Different specimens of the taxon similar to *Difflugia angusticollis* from C.G. Ogden's SEM collection. a-b – lateral view (a – SEM CZ-08.450, b – SEM CZ-08.454); c – apertural view (SEM CZ-08-463); d – structure of organic lip (SEM CZ-08.464); e – structure of organic cement (SEM 047303). Scale bars: a-b – 30 μm, c – 10 μm, d – 3 μm, e – 1 μm.

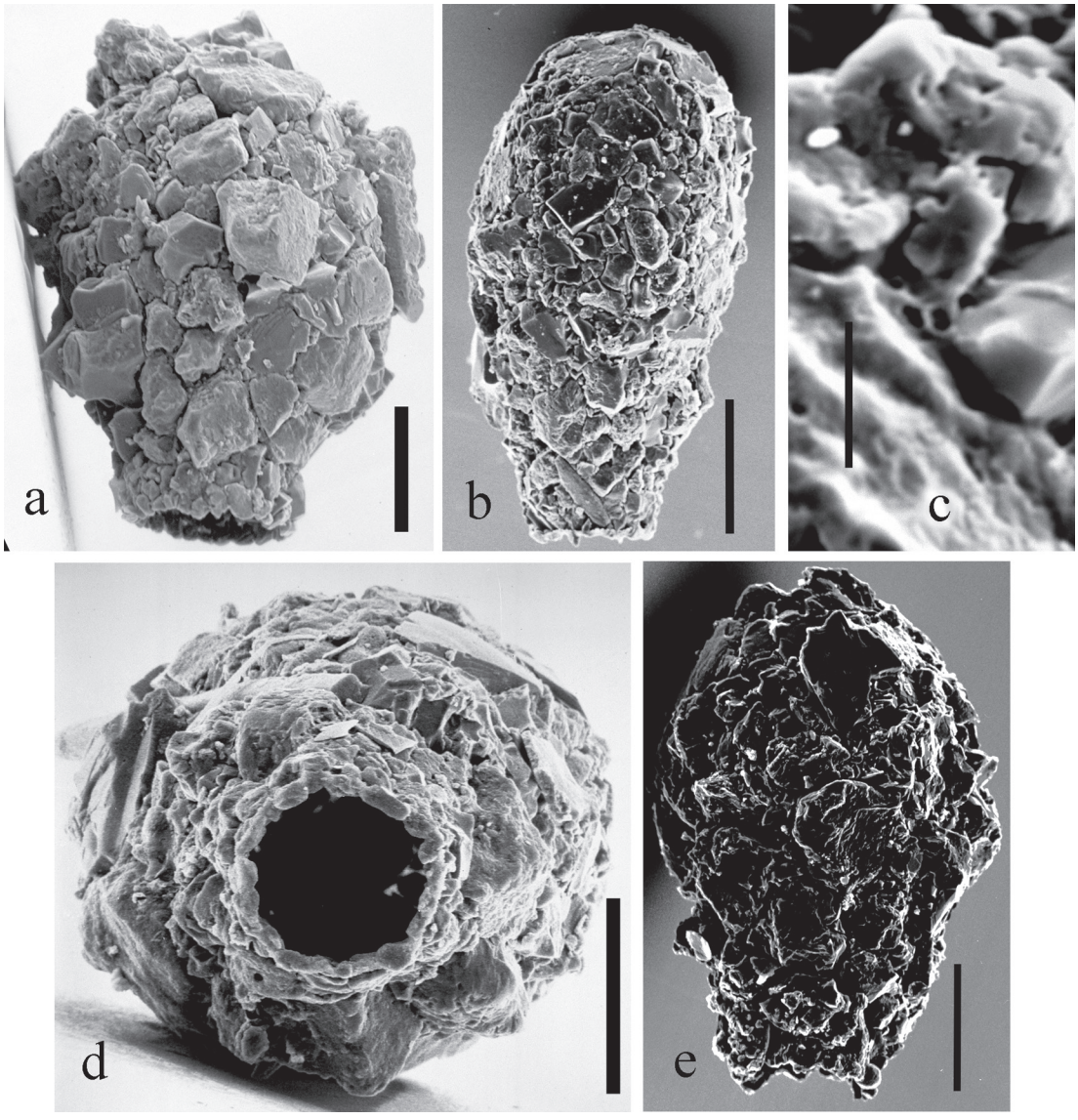

Fig. 35. Different specimens of *Difflugia petricola* from C.G. Ogden's SEM collection. a, b, e – lateral view (a – SEM EM-12-457, b – SEM CZ-06.817, e – SEM CZ-06.900); c – structure of organic cement (SEM CZ-06.705); d – apertural view (SEM Z-16/123). Scale bars: a, b, d, e – 30 μm, c – 3 μm.

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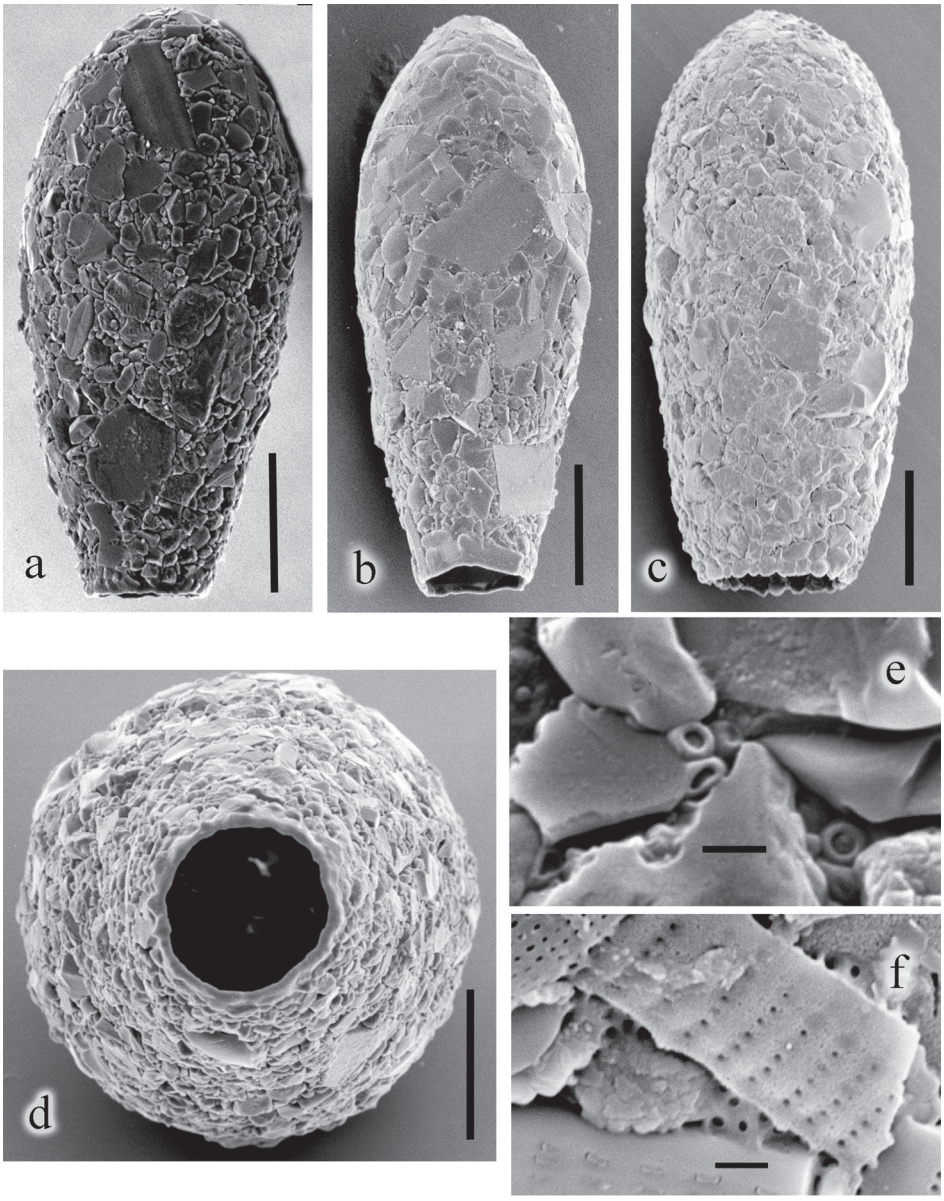

Fig. 36. Different specimens of *Difflugia lanceolata* from C.G. Ogden's SEM collection. a-c – lateral view (a – SEM CZ-04.665, b – SEM CZ-03.824, c – SEM 096683); d – apertural view (SEM CZ-04.617); e-f – structure of organic cement (e ¬– SEM CZ-04.662, f – SEM CZ-03.822). Scale bars: a-d – 30 μm, e-f – 1μm.

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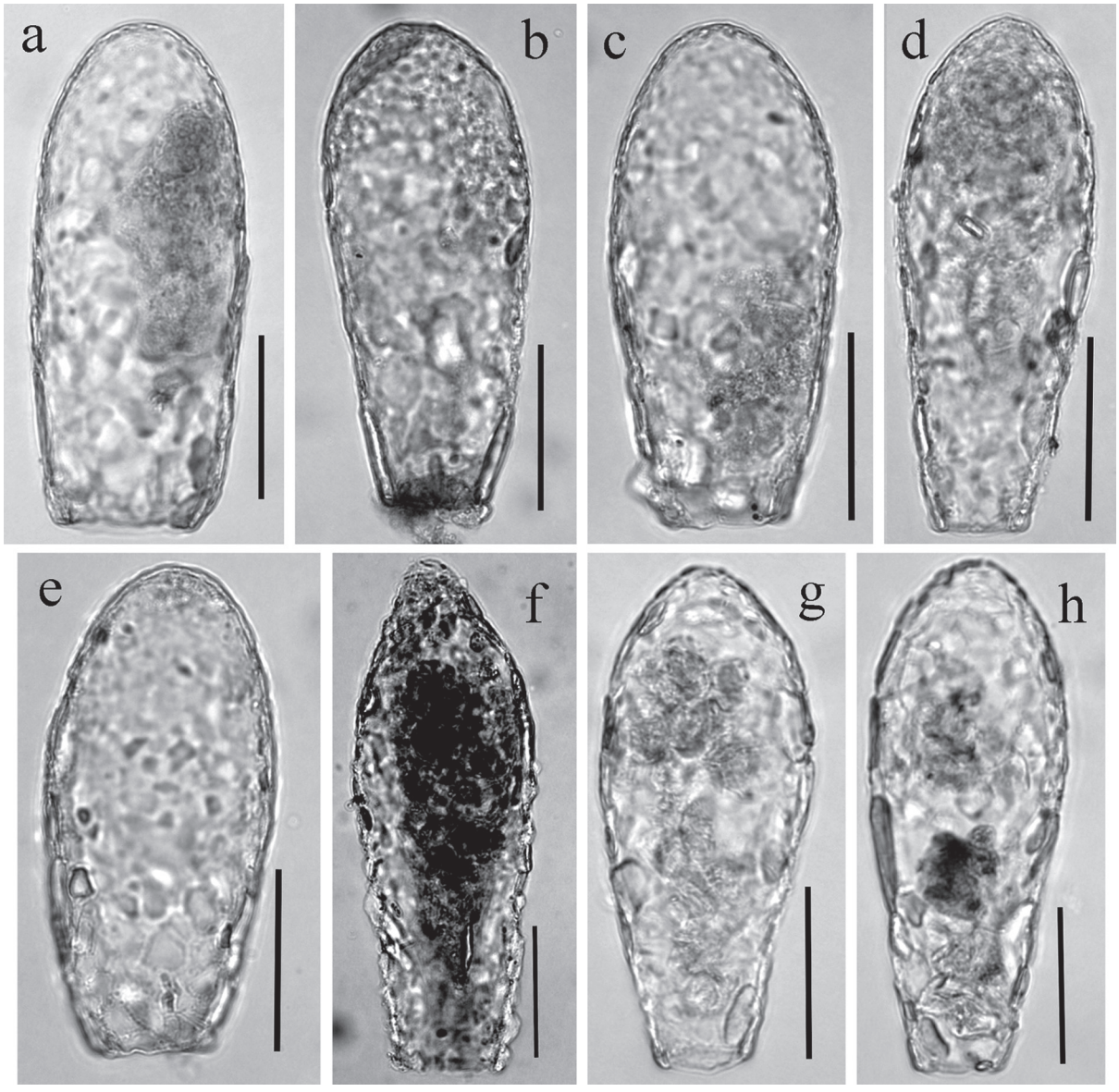

Fig. 37. Different specimens of *Difflugia lanceolata* from E. Penard's slides. a-h – lateral view (a – slide 04.5.9.102, b-e – slide 20.12.8.214, f – slide 20.12.8.215, g-h – slide 20.12.8.216). Scale bars: 50 μm.

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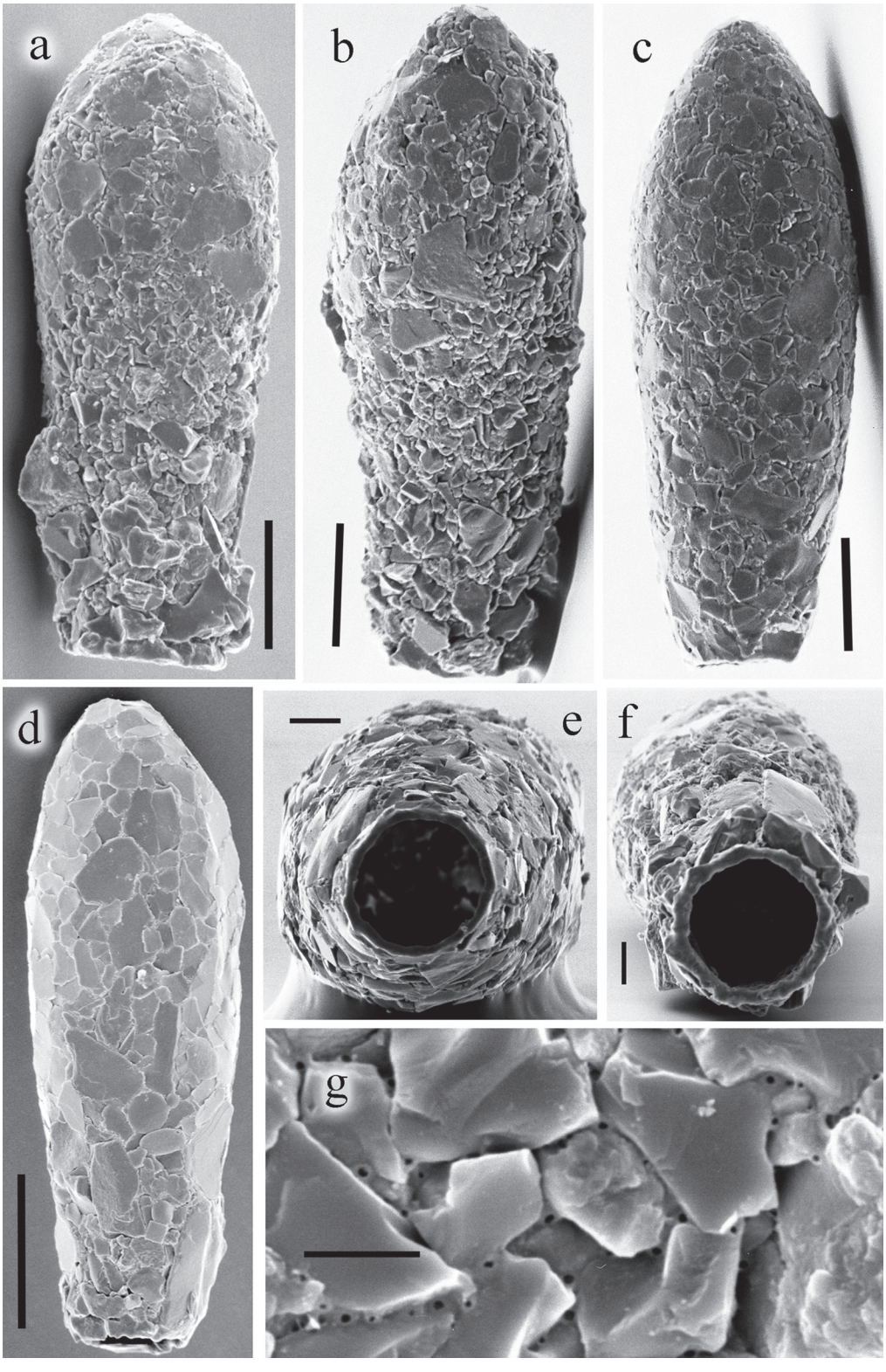

Fig. 38. Different specimens of *Difflugia lanceolata* from C.G. Ogden's SEM collection. a-d – lateral view (a – SEM CZ-11.373, b – SEM CZ-11.403, c – SEM CZ-11.411, d – SEM 003830); e-f – apertural view (e – SEM CZ-11.394, f – SEM CZ-11.383); g – structure of organic cement (SEM CZ-11.374). Scale bars: a-d – 30 μm, e-f – 10 μm, g – 3 μm.

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