

# A survey of the testate amoeba genus *Diffflugia* Leclerc, 1815 based on specimens in the E. Penard and C.G. Ogden collections of the Natural History Museum, London. Part 3: Species with shells that are spherical or ovoid

---

Yuri Mazei<sup>1</sup> and Alan Warren<sup>2</sup>

<sup>1</sup> Department of Zoology and Ecology, Penza State University, Penza, Russia

<sup>2</sup> Department of Life Sciences, Natural History Museum, London, UK

## Summary

We review the species of *Diffflugia* with shells that are spherical or ovoid, 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 testate amoebae taxa grouped into seven species complexes, namely *Diffflugia globulosa* Dujardin, 1837, *Diffflugia rotunda* (Chardez, 1956) Ogden, 1983, *Diffflugia minuta* Rampi, 1950, *Diffflugia viscidula* Penard, 1902, *Diffflugia pulex* Penard, 1902, *Diffflugia glans* Penard, 1902, and *Diffflugia molesta* Penard, 1902.

Within the *D. globulosa*-complex we: (i) distinguish as a separate taxon *D. globulosa* Dujardin, 1837, and (ii) synonymise *D. chardezi* Godeanu, 1972 with *D. globulosa* Dujardin, 1837. Within the *D. rotunda*-complex we: (i) distinguish as separate taxa *D. rotunda* (Chardez, 1956) Ogden, 1983, and *D. lebes* Penard, 1902; (ii) synonymise *D. lebes* var. *sphaerica* Gauthier-Lièvre et Thomas, 1958 with *D. lebes* Penard, 1902, and *D. lebes* var. *masurica* Schönborn, 1965 and *D. lebes* var. *bretschkoi* Laminger, 1971 with *D. viscidula* Penard, 1902. Within the *D. minuta*-complex we: (i) distinguish as a separate taxa *D. minuta* Rampi, 1950, *D. angulostoma* Gautier-Lièvre et Thomas, 1958, and *D. geosphaira* Ogden, 1991; (ii) synonymise *D. minuta* var. *grandis* Gautier-Lièvre et Thomas, 1958 with *D. difficilis* Thomas, 1954, and *D. minuta* var. *minor* Godeanu, 1972 with *D. pulex* Penard, 1902; and (iii) discuss the validity of *D. dujardini* Chardez, 1957. Within the *D. viscidula*-complex we: (i) distinguish as a separate taxon *D. viscidula* Penard, 1902; and (ii) synonymise *D. lemani* Blanc, 1892, *D. histrio* Penard, 1908, *D. finstertaliensis* Laminger, 1971, *D. lebes* var. *masurica* Schönborn, 1965, and *D. lebes* var. *bretschkoi* Laminger, 1971 with *D. viscidula* Penard, 1902. Within the *D. pulex*-complex we: (i) distinguish as separate taxa *D. pulex* Penard, 1902, *D. pristis* Penard, 1902, and *D. mica* Frentzel, 1892; (ii) synonymise *D. ovalisina* Beyens et Chardez, 1994 and *D. minuta* var. *minor* Godeanu, 1972 with *D. pulex* Penard, 1902, and (iii) discuss the validity of *D. richmondiae* Playfair, 1914, *D. stechlinensis* Schönborn, 1962, and *D. humilis*

Chardez, 1991. Within the *D. glans*-complex we: (i) distinguish as separate taxa *D. glans* Penard, 1902, *D. ampullula* Playfair, 1918, and *D. penardi* (Penard, 1890) Hopkinson, 1909; (ii) synonymise *D. manicata* Penard, 1902 and *D. tenuis* (Penard, 1890) Ogden, 1983 with *D. penardi* (Penard, 1890) Hopkinson, 1909, and (iii) discuss the validity of *D. masaruzii* van Oye, 1958 and *D. declotitrei* Godeanu, 1972. Within the *D. molesta*-complex we: (i) distinguish as separate taxa *D. molesta* Penard, 1902, *D. brevicolla* Cash et Hopkinson, 1909, and *D. difficilis* Thomas, 1954; (ii) synonymise *D. pyriformis* var. *atricolor* Penard, 1902 with *D. brevicolla* Cash et Hopkinson, 1909; *D. difficilis* var. *ecornis* Chardez, 1956, *D. microstoma* (Thomas, 1954) Ogden, 1983, and *D. minuta* var. *grandis* Gauthier-Lièvre et Thomas, 1958 with *D. difficilis* Thomas, 1954; *D. levanderi* Playfair, 1918 with *D. molesta* Penard, 1902. As in the first two parts of this series of papers, 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, scanning electron microscopic, molecular, and environmental data are needed in order to characterize these species complexes in more detail and thus resolve their systematics.

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

## Introduction

This is the third of a series of papers that aims to review the genus *Diffflugia* 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 and shell measurements, and also on published literature. In the first paper (Mazei and Warren, 2012) we reviewed those species of *Diffflugia* with a shell that is pointed aborally and/or having aboral protuberances. In the second paper (Mazei and Warren, 2014) we reviewed those species of *Diffflugia* with a shell that is pyriform or elongate. The aim of the present paper is to review those species of *Diffflugia* with a shell that is spherical or ovoid.

### REVIEW OF THE LITERATURE ON THE TAXONOMY OF SELECTED *DIFFFLUGIA* SPECIES.

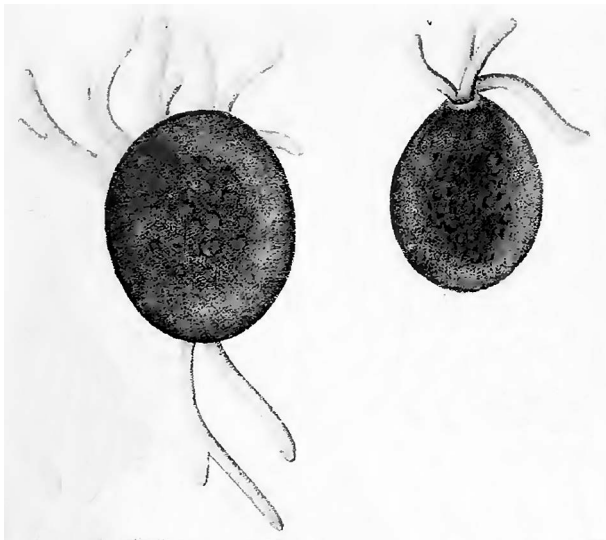
Dujardin (1837) described a species which he named *Diffflugia globulosa*, with a globular or corneous shell, ca. 100 µm long. His illustrations suggest that the shell is ovoid in shape with the aperture placed at the narrower end, and that the shell is slightly compressed laterally (Fig. 1). Ehrenberg (1838a, 1838b) described *D. proteiformis* and illustrated it as having an ovoid or sub-spherical shell that is laterally circular, ca. 100 µm long and

with a rough surface covered by sand grains (Fig. 2). These individuals do not correspond with any of the forms drawn by Leclerc (1815 – see fig. 1 in Mazei and Warren, 2014). Later Dujardin (1841) illustrated *D. globulosa* (Fig. 3) and noted that although both this species and *D. proteiformis* are globular to ovoid in shape, the former is 100–250 µm long and has a smooth surface whereas the latter is ca. 45–112 µm long and has a rough surface due to its covering of sand grains.

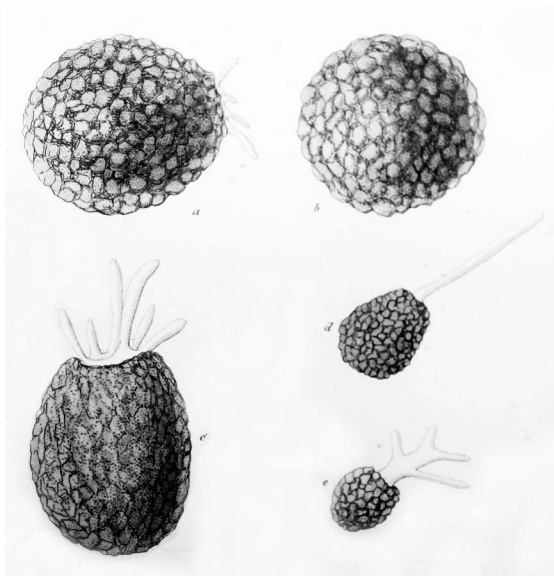
Wallich (1864) recognised only one valid species within the genus *Diffflugia*, namely *D. proteiformis* (Ehr.) with four subspecies. One of these subspecies, *D. globularis* (Duj.), consisted of three varieties: *D. tuberculata* (Wallich), *D. aculeata* (Her.), and *D. corona* (Wallich). He described *D. globularis* as having a more or less globular test the margin of which is circular in outline, but truncated at the aperture (Fig. 4). Most likely, the name 'globularis' used by Wallich (1864) and attributed to Dujardin's '*globulosa*' was a mistake (as it was noted by Leidy, 1879, p. 97 and Ogden, 1988, p. 367).

Carter (1864) described *D. bombaensis* with an ovoid-globose test, 127 µm long, dark-brown in color, truncated anteriorly, composed of grains of sand externally, which rest upon a structure formed by circles of large particles, scattered between which are numerous smaller particles (Fig. 5).

Leidy (1879, p. 97) described *D. globulosa* as varying in shape "...from oval to ovoid and



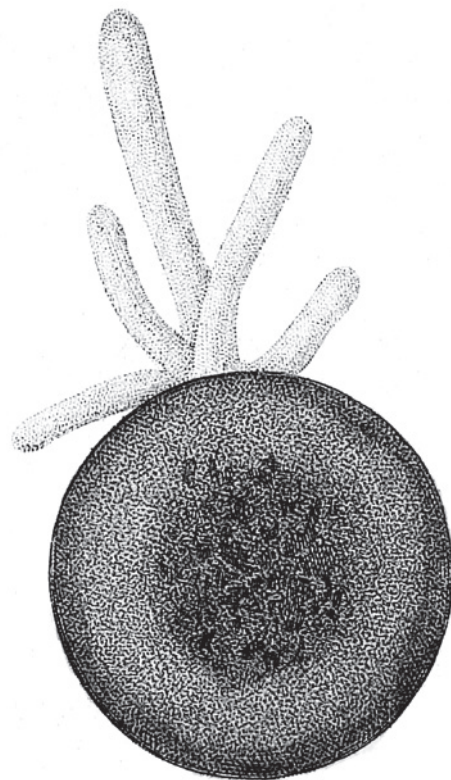
**Fig. 1.** *Diffflugia globulosa* after Dujardin (1837, plate 9, figs 1a, 1b).



**Fig. 2.** *Diffflugia proteiformis* after Ehrenberg (1838b, table IX, fig. I).

subpyriform, and to spheroidal and oblate spheroidal. The oral pole of the shell is more or less truncated, and the mouth is large, circular, entire, inferior, and commonly terminal". Leidy (1879) noted high variability in size (length 36–300  $\mu\text{m}$ ) and shell structure, from chitinous in small forms to those covered by diatom frustules and sand grains in larger forms (Fig. 6).

Penard (1890, 1902, 1908) described the following new taxa of *Diffflugia* with spherical or ovoid

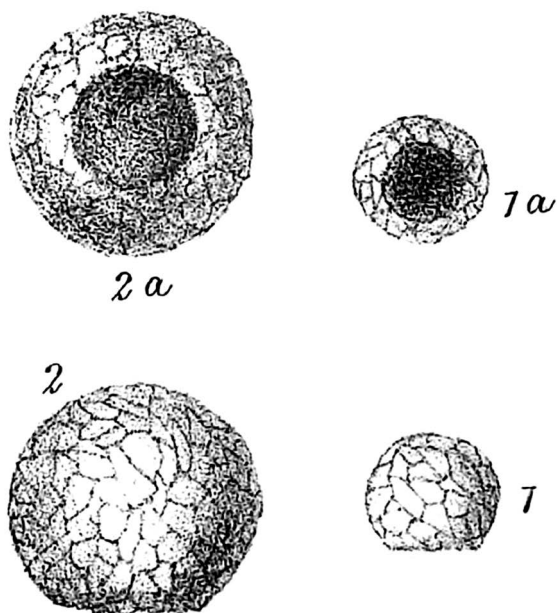


**Fig. 3.** *Diffflugia globulosa* after Dujardin (1841, plate 2, fig. 6).

shells (Figs 7–9): *D. pyriformis* var. *tenuis*, *D. saxicola*, *D. fallax* (Penard 1890), *D. pyriformis* var. *atricolor*, *D. manicata*, *D. pulex*, *D. glans*, *D. molesta*, *D. pristis*, *D. viscidula*, *D. globulosa* var. *globularis*, *D. lebes* (Penard 1902), and *D. histrio* (Penard 1908).

Cash and Hopkinson (1909) listed some of the existing species (*D. pulex* and *D. pristis*), changed the names of two (*D. penardi* for *D. fallax* and *D. globulus* for *D. globulosa*), and described one new species *D. brevicolla* (Fig. 10).

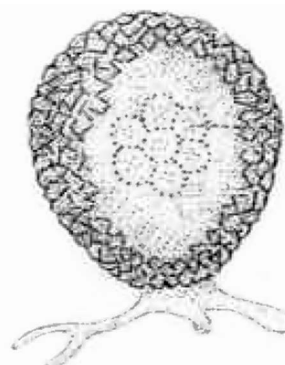
Several new taxa were established during the 20<sup>th</sup> century including: *D. richmondiae* (Playfair, 1914), *D. levanderi* (Playfair, 1918); *D. minuta* (Rampi, 1950), *D. globularis* var. *microstoma* (Thomas, 1954), *D. difficilis* (Thomas, 1954), *D. difficilis* var. *ecornis* (Chardez, 1956), *D. dujardini* (Chardez, 1957), *D. globularis* var. *sphaerica* (Chardez, 1957, 1962), *D. masaruzii* (van Oye, 1958), *D. stechlinensis* (Schönborn, 1962), *D. lebes* var. *masurica* (Schönborn, 1965), *D. finstertaliensis* (Laminger, 1971), *D. lebes* var. *bretschkoi* (Laminger, 1971), *D. minuta* var. *minor* (Godeanu, 1972), *D. chardezi* (Godeanu, 1972), *D. decloitrei* (Godeanu, 1972), *D. humilis* (Chardez, 1991), and *D. ovalisina* (Beyens and Chardez, 1994) (Figs 9, 11–13).



**Fig. 4.** *Diffflugia globularis* after Wallich (1864, plate XVI, figs 1-2); follow terminology of Wallich (1864): fig 1 – side view of a young specimen, fig. 1a – front view showing aperture, fig. 2 – side view of a more mature specimen, fig. 2a – front view.

In their report on testate amoebae of Africa, Gauthier-Lièvre and Thomas (1958) grouped the various *Diffflugia* spp. based on shell morphology. The following known species were included in the groups entitled “Globuleuses”, “Ovoides-globuleuses” (in part) “Colletees”, and “Piriformes”, i.e. those with shells that are spherical or ovoid, respectively: *D. brevicolla* Cash, 1909, *D. difficilis* Thomas, 1954, *D. globularis* (Wallich, 1864) Leidy, 1877, *D. globulosa* Dujardin, 1837, *D. levanderi* Playfair, 1918, *D. minuta* Rampi, 1950, *D. penardi* Hopkinson, 1909, *D. oblonga* var. *tenuis* Penard, 1890, *D. pristis* Penard, 1902, and *D. pulex* Penard, 1902. Furthermore, they described the following new taxa from northern Africa: *D. brevicolla* var. *major*, *D. lebes* var. *spherica*, and *D. minuta* var. *grandis* (Gauthier-Lièvre and Thomas, 1958) (Figs 9, d, e; 12, g, h). Although Leidy (1877) used the name *D. globularis*, he subsequently declared that *D. globularis* to be a junior synonym of *D. globulosa* – see above and Leidy (1879, p. 314). Gautier-Lièvre and Thomas (1958, p. 308) accepted both *D. globularis* and *D. globulosa* as being valid whereas Penard (1902, p. 256) also considered *D. globularis* Wallich, 1864 a junior synonym of *D. globulosa* Dujardin, 1837.

In a series of publications (Ogden, 1980, 1983, 1984, 1988, 1991; Ogden and Hedley, 1980; Ogden



**Fig. 5.** *Diffflugia bombaensis* after Carter (1864, plate II, fig. 16).

and Živković, 1983) Ogden redescribed, and in many cases changed the taxonomic status, of 21 taxa of *Diffflugia* with spherical or ovoid shells, including: *D. ampullula*, *D. angulostoma*, *D. brevicolla*, *D. decloitrei*, *D. difficilis*, *D. geosphaira*, *D. glans*, *D. globulosa*, *D. levanderi*, *D. manicata*, *D. masaruzii*, *D. mica*, *D. microstoma*, *D. minuta*, *D. molesta*, *D. penardi*, *D. pristis*, *D. pulex*, *D. rotunda*, *D. tenuis*, and *D. viscidula*. Unfortunately, Ogden never made a direct comparison of all of these species in a single publication.

We have applied Ogden’s morphometric data (both published and unpublished) to compare 19 of these 21 morphologically similar taxa (Fig. 14), *D. molesta* and *D. glans* having been omitted since we could not find Ogden’s raw data for the four individuals of the former and the three individuals of the latter that he reported in Ogden (1983). Three major groups can be clearly distinguished according to their size distribution. Group 1, which comprises *D. rotunda* and *D. viscidula*, is well-defined and clearly separated from the other two groups (Fig. 14). Group 3, comprising primarily of *D. pristis* and *D. pulex*, overlaps with the much larger Group 2. A more detailed analysis of Group 2 shows that it can be subdivided into four subgroups (Fig. 15, see 2, a–d). Although there is a degree of overlap among these groups, further analysis allows their separation into two clear morphologically distinguished groups: (1) those in subgroups 2a and 2d, which are essentially spherical, i.e. width/length ratio not less than 0.88 on average (Fig. 16); and (2) those in subgroups 2b and 2c, which are essentially ovoid, i.e. width/length ratio not less than 0.82 on average (Fig. 17). Fig. 18 shows 5 species of *Diffflugia* (*D. angulostoma* presented in its two distinct size morphs) with spherical shells as defined by C.G. Ogden.

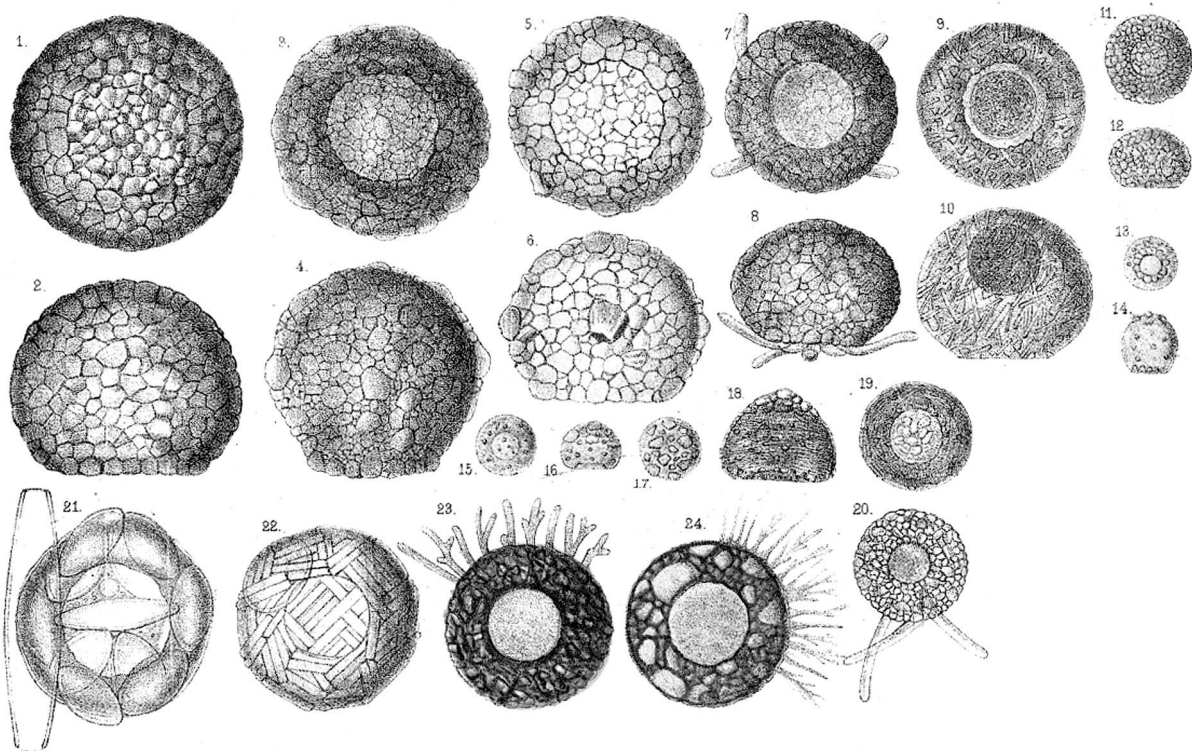


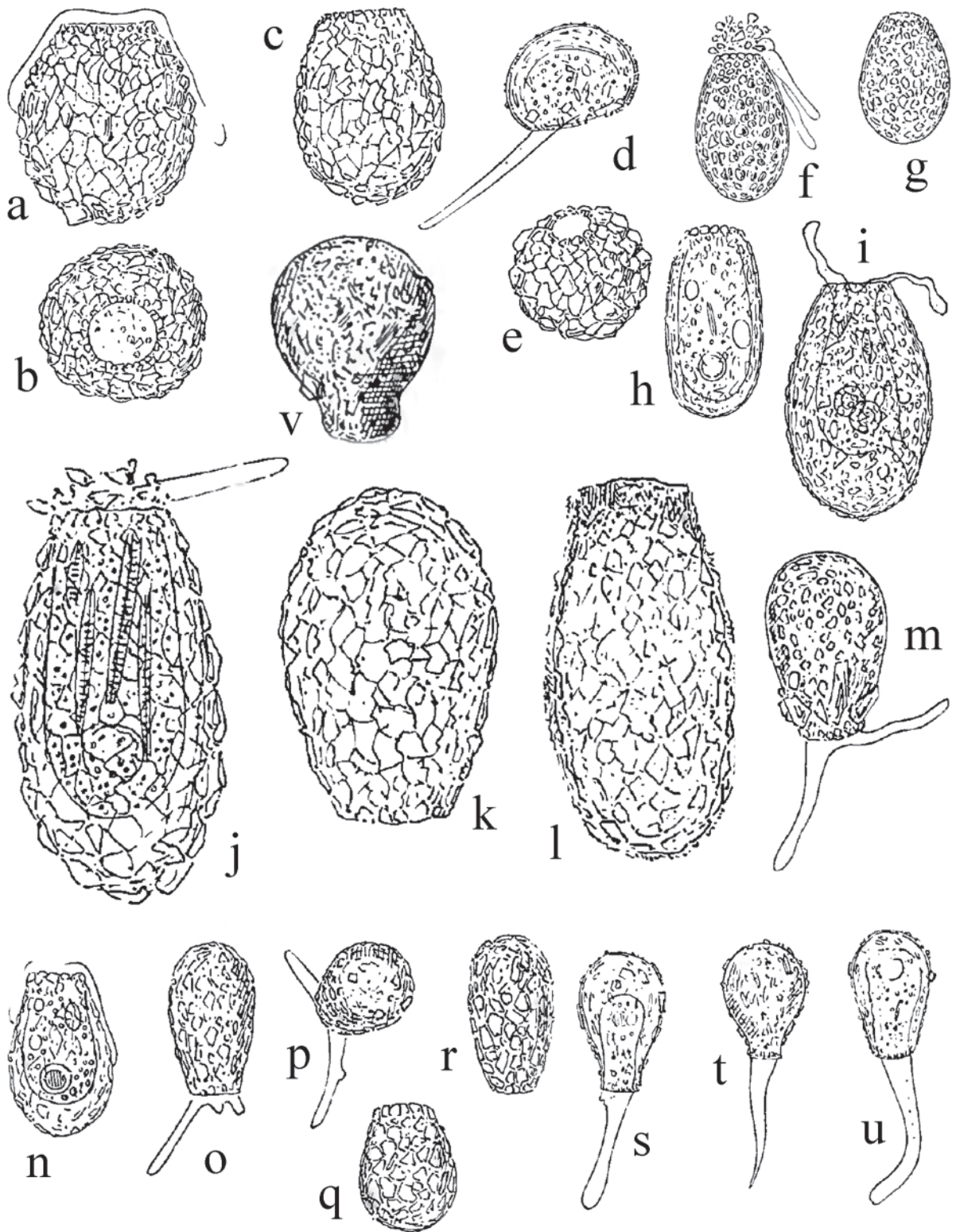
Fig. 6. *Diffflugia globulosa* after Leidy (1879, table XVI, figs 1–24).

Fig. 19 shows 14 species of *Diffflugia* (*D. viscidula* is not presented due to its huge size compared with other taxa; *D. molesta* also not presented since it is likely that Ogden misidentified it – see below) with ovoid shells as defined by Ogden. The SEM images in both figures are shown to the same scale in order to facilitate comparisons of both size and external morphology of ‘typical’ individuals.

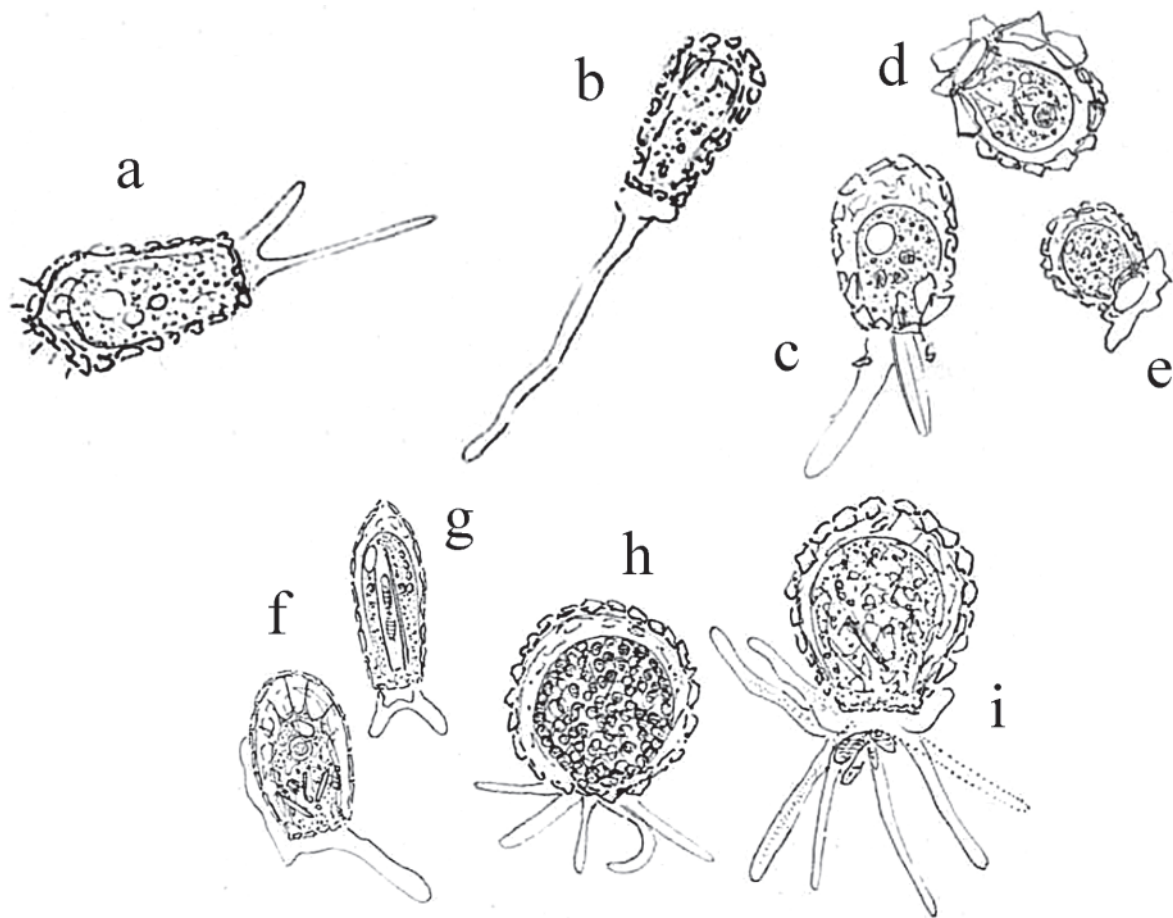
According to the scatter plots (Figs 14–17) we can distinguish seven main size classes, three of which include species with spherical shells, and four with ovoid shells: (1) **spherical large** [group 1 (in part) in Fig. 14; group 1 in Fig. 16; Fig. 18, a] with a shell length of 132–200  $\mu\text{m}$  and shell width 138–192  $\mu\text{m}$ , including *D. rotunda*; (2) **spherical medium** [subgroup 2a in Fig. 16; Figs 18, b, c] with a shell length of 70–118  $\mu\text{m}$  and shell width 54–112  $\mu\text{m}$ , including *D. globulosa* and the large form of *D. angulostoma*; (3) **spherical small** [subgroup 2d in Fig. 16; Figs 18, d–f] with a shell length of 40–62  $\mu\text{m}$  and shell width 54–112  $\mu\text{m}$ , including *D. geosphaira*, *D. minuta*, and the small form of *D. angulostoma*; (4) **ovoid large** [group 1 (in part) in Fig. 14] with a shell length of 163–382  $\mu\text{m}$  and shell width 114–253  $\mu\text{m}$ , including *D. viscidula*; (5) **ovoid medium and broad** [subgroup 2b in Fig. 17; Figs 19, a–c, e] with a shell

length of 66–119  $\mu\text{m}$  and shell width 48–100  $\mu\text{m}$ , including *D. brevicolla*, *D. microstoma*, *D. difficilis*, *D. molesta* (not shown on the scatter plot), and *D. levanderi*; (6) **ovoid medium and narrow** [subgroup 2c in Fig. 17; Figs 19, d, f–k] with a shell length of 55–103  $\mu\text{m}$  and shell width 34–54  $\mu\text{m}$ , including *D. penardi*, *D. decloitrei*, *D. manicata*, *D. tenuis*, *D. masaruzii*, *D. glans* (not shown on the scatter plot), and *D. ampullula*; (7) **ovoid small** [group 3 in Fig. 14; Figs 19, l–n] with a shell length of 30–66  $\mu\text{m}$  and shell width 21–56  $\mu\text{m}$ , including *D. pulex*, *D. pristis*, and *D. mica*.

Thus, according to the size classes described, we can distinguish seven species complexes (sensu Foissner and Korganova, 2000): *Diffflugia globulosa*-complex, *Diffflugia rotunda*-complex, *Diffflugia minuta*-complex, *Diffflugia viscidula*-complex, *Diffflugia glans*-complex, *Diffflugia manicata*-complex, and *Diffflugia pulex*-complex. Each species complex is discussed in detail based on data from the E. Penard and C.G. Ogden collections in the NHM, London. We do not aim 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.



**Fig. 7.** Different species of *Diffugia* with spherical or ovoid shells, after Penard (1902): a-c – ‘typical’ form of *D. globulosa* (p. 258, figs 1-3), d-e – *D. globulosa* var. *globularis* (p. 258, figs 5-6), f-g – *D. pristis* (p. 254, figs 1-2), h – *D. glans* (p. 247, fig. 1), i – *D. molesta* (p. 248), j-l – *D. viscidula* (p. 260, figs 1-3), m – *D. manicata* (p. 226, fig. 1), n-r – *D. fallax* (p. 246, figs 1-5), s-u – *D. pulex* (p. 230, figs 1, 2, 4), v – *D. pyriformis* var. *atricolor* (p. 218, fig. 6).



**Fig. 8.** Different species of *Diffflugia* with spherical or ovoid shells, after Penard (1890): a-b – *D. pyriformis* var. *tenuis* (plate III, figs 47, 48), c-e – *D. saxicola* (plate III, figs 50-53), f-g – *D. fallax* (plate IV, figs 41, 44), h-i – *D. globulosa* (plate IV, figs 21, 25).

#### TAXONOMIC REVISION OF SELECTED *DIFFFLUGIA* SPECIES

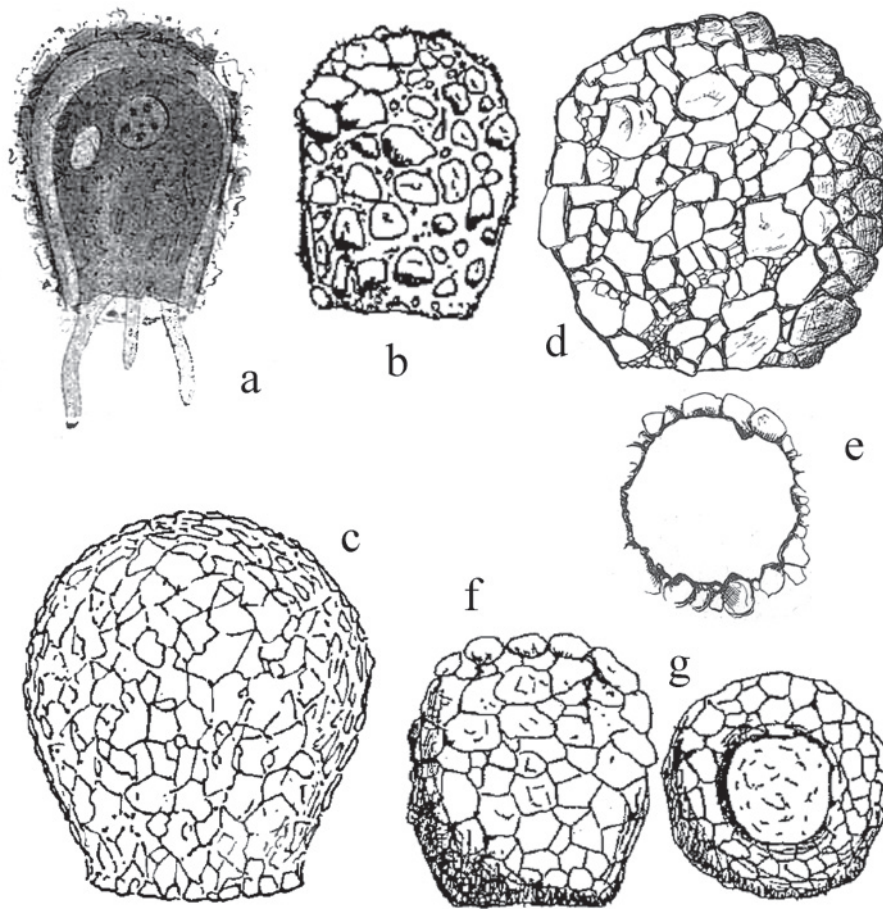
All the species discussed below have spherical or ovoid shells. In addition we include here some species with pyriform shells (*D. pulex*, *D. brevicolla*, *D. penardi*, *D. manicata*, *D. tenuis*, and *D. molesta*) and shells with small collars around the aperture (*D. difficilis*, *D. microstoma*, *D. ampullula*, and *D. mica*) which might be confused with the 'typical' ovoid shells because of their small size (usually less than 100 µm). Illustrations comprise LM photomicrographs, scanning electron micrographs and line diagrams. All LM photomicrographs 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 legends.

#### *Diffflugia globulosa* Dujardin, 1837 species complex

The members of this group include *D. globulosa* (Fig. 16, subgroup '2a'; Fig. 18, b) and *D. chardezi*.

##### *Diffflugia globulosa* Dujardin, 1837

According to Ogden and Hedley (1980) the shell of *D. globulosa* is spherical or hemispherical, usually composed of large quartz particles but may also include diatom frustules (Fig. 20). The general appearance is a rough shell although some smoother forms have been seen. The aperture is circular and is surrounded by smaller particles which often appear smooth due to the overlying cement. Ogden and Hedley (1980, p. 134) noted that variation in this species is prolific, both in the composition of the shell and the size of the aperture in relation to the diameter of the shell. Ogden measured 22 specimens of *D. globulosa* (Fig. 14), 13 of which were published



**Fig. 9.** Different species of *Diffflugia* with spherical or ovoid shells: a – *D. histrio* (after Penard, 1908, plate XVII, fig. 4), b – *D. histrio* (after Schönborn, 1965, fig. 8a), c – *D. lebes* (after Penard, 1902, p. 271, fig. 1), d-e – *D. lebes* var. *sphaerica* (after Gauthier-Lièvre and Thomas, 1958, fig. 37d), f-g – *D. lebes* var. *masurica* (after Schönborn, 1965, figs 7 b, c).

(Ogden and Hedley, 1980) and nine unpublished. The ranges of the shell dimensions are as follows: length 88–119  $\mu\text{m}$ , width 72–113  $\mu\text{m}$ , aperture diameter 33–58  $\mu\text{m}$ .

#### ***Diffflugia chardezi* Godeanu, 1972**

According to Godeanu (1972) the shell of *D. chardezi* is almost spherical, colorless, and covered by sand grains of various sizes (Fig. 13, a). As a diagnostic feature Godeanu (1972) mentioned 3–4 extra large particles incorporated into the shell wall. The aperture is circular. The shell dimensions are: length 100–110  $\mu\text{m}$ ; width 100–107  $\mu\text{m}$ ; aperture diameter 55–58  $\mu\text{m}$ . We believe that the differences stated by Godeanu (1972) are not sufficient for species separation. Thus we consider *D. chardezi* Godeanu, 1972 a junior synonym of *D. globulosa* Dujardin, 1837.

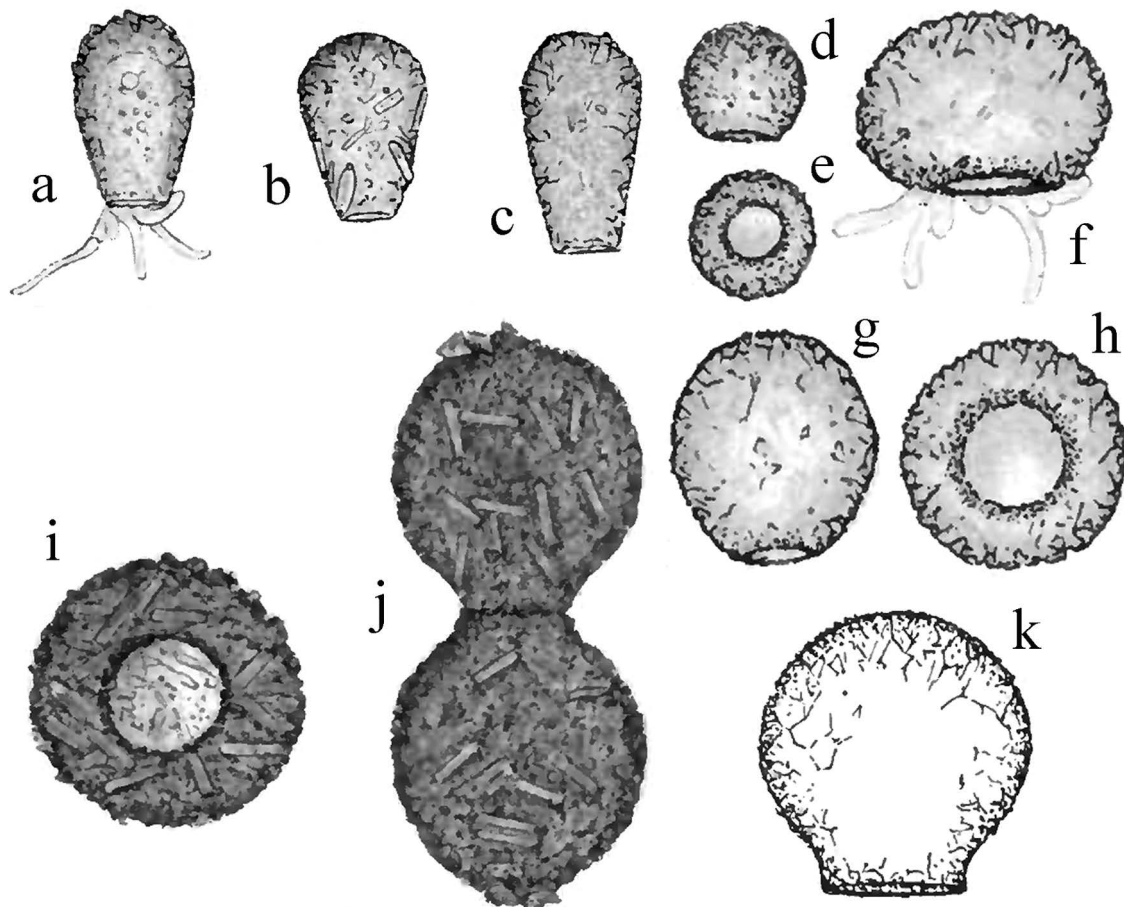
#### ***Diffflugia rotunda* (Chardez, 1956) Ogden, 1983 species complex**

This group is composed of *D. rotunda* (Fig. 16, group '1'; Fig. 18, a) and *D. lebes*.

#### ***Diffflugia rotunda* (Chardez, 1956) Ogden, 1983**

According to Ogden (1983) the shell of *D. rotunda* is spherical or hemispherical, with the outline frequently distorted by the addition of large diatom frustules (Fig. 21). The basic structure is made mainly of quartz but diatom frustules, or fragments of frustules, are often mixed with this in different proportions. The aperture is circular, sometimes slightly irregular, but usually surrounded by a shallow rim of small particles. Ogden measured 17 specimens of *D. rotunda* (Fig. 14), one of which is unpublished and 16 are published (Ogden, 1983).





**Fig. 10.** Different species of *Diffflugia* with spherical or ovoid shells, after Cash and Hopkinson (1909): a-c – *D. penardi* (plate XVIII, figs 4-6), d-h – *D. globulus* (plate XXI, figs 5-9), i-k – *D. brevicolla* (plate XIX, figs 12, 13; p. 38, fig. 55).

Based on these data, the shell dimensions are: length 133 to 204  $\mu\text{m}$ ; width 129–193  $\mu\text{m}$ ; aperture diameter 72–113  $\mu\text{m}$ .

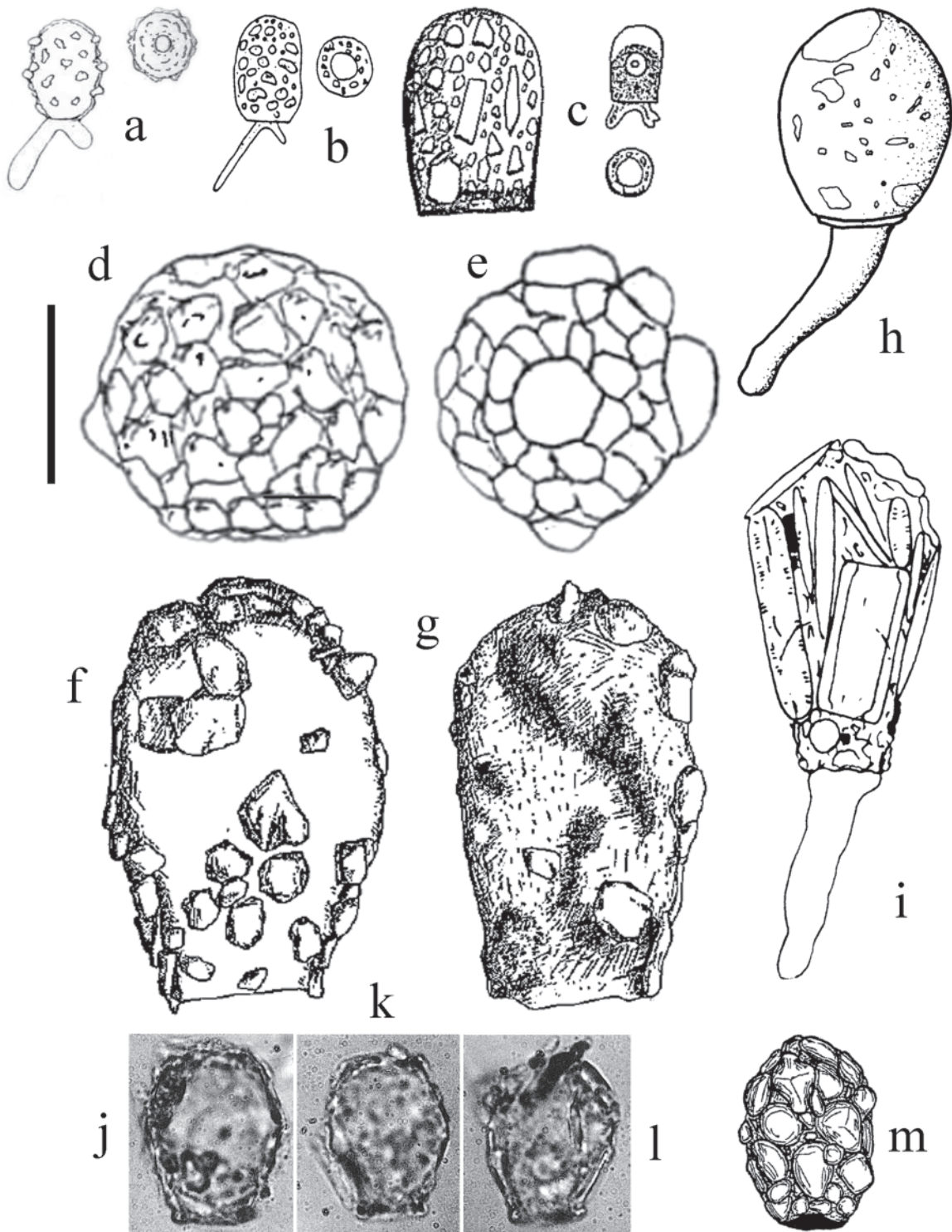
In the original description of this variety, *D. globularis* var. *sphaerica* (Fig. 12, d), Chardez (1956) noted that it differed from *D. globulosa* in both size and diameter of aperture, the shell width of the latter being about half that of the former. Assuming that the name ‘globularis’ was used in error by Wallich in 1864 for *D. globulosa* (see above), Ogden (1983) raised this taxon to the species rank as *D. rotunda*, a decision that we accept.

#### *Diffflugia lebes* Penard, 1902

According to Penard (1902), *D. lebes* (Figs 9, c; 22) closely resembles *D. urceolata* in terms of its large, almost spherical shell. However, the former differs from the latter by the appearance of the aperture, i.e. large, circular, surrounded by sand grains and

without a large everted collar. The shell length is 360–400  $\mu\text{m}$ . Gauthier-Lièvre and Thomas (1958) described the shell of *Diffflugia lebes* var. *sphaerica* as being circular in outline, 270–290  $\mu\text{m}$  in diameter, and with an aperture 130–180  $\mu\text{m}$  wide (Figs 9, d, e). Its validity is questionable; the size range 270–400  $\mu\text{m}$  is consistent with other large species of *Diffflugia*, see for example *D. acuminata* which is 350–550  $\mu\text{m}$  long (Mazei and Warren, 2012) and *D. gigantea* which is 340–480  $\mu\text{m}$  long (Mazei and Warren, 2014). Therefore we consider *D. lebes* var. *sphaerica* Gauthier-Lièvre and Thomas, 1958 a junior synonym of *D. lebes* Penard, 1902.

Schönborn (1965) established *Diffflugia lebes* var. *masurica*, for a population that is smaller than ‘typical’ specimens of *D. lebes* and having a more elongated shell with the following dimensions: length 150–200  $\mu\text{m}$ , width 150–180  $\mu\text{m}$  (Figs 9, f, g). Laminger (1971) erected *Diffflugia lebes* var.



**Fig. 11.** Different species of *Diffugia* with spherical or ovoid shells: a – *D. richmondiae* (after Playfair, 1914, plate VIII, fig. 12), b – *D. richmondiae* (after Schönborn, 1965, figs 12a, b), c – *D. stechlinensis* (after Schönborn, 1962, fig. 4), d–e – *D. minuta* (after Rampi, 1950, figs 23–24), f–g – *D. masaruzii* (after van Oye, 1958, plate I, figs 6–7), h – *D. dujardini* (after Jax, 1985), i – *D. humilis* (after Chardez, 1991, fig. 9), j–l – *D. ovalisina* (after Beyens and Chardez, 1994, fig. 1), m – *D. minuta* var. *minor* (after Godeanu, 1972, fig. 6). Scale bar: 25  $\mu$ m.

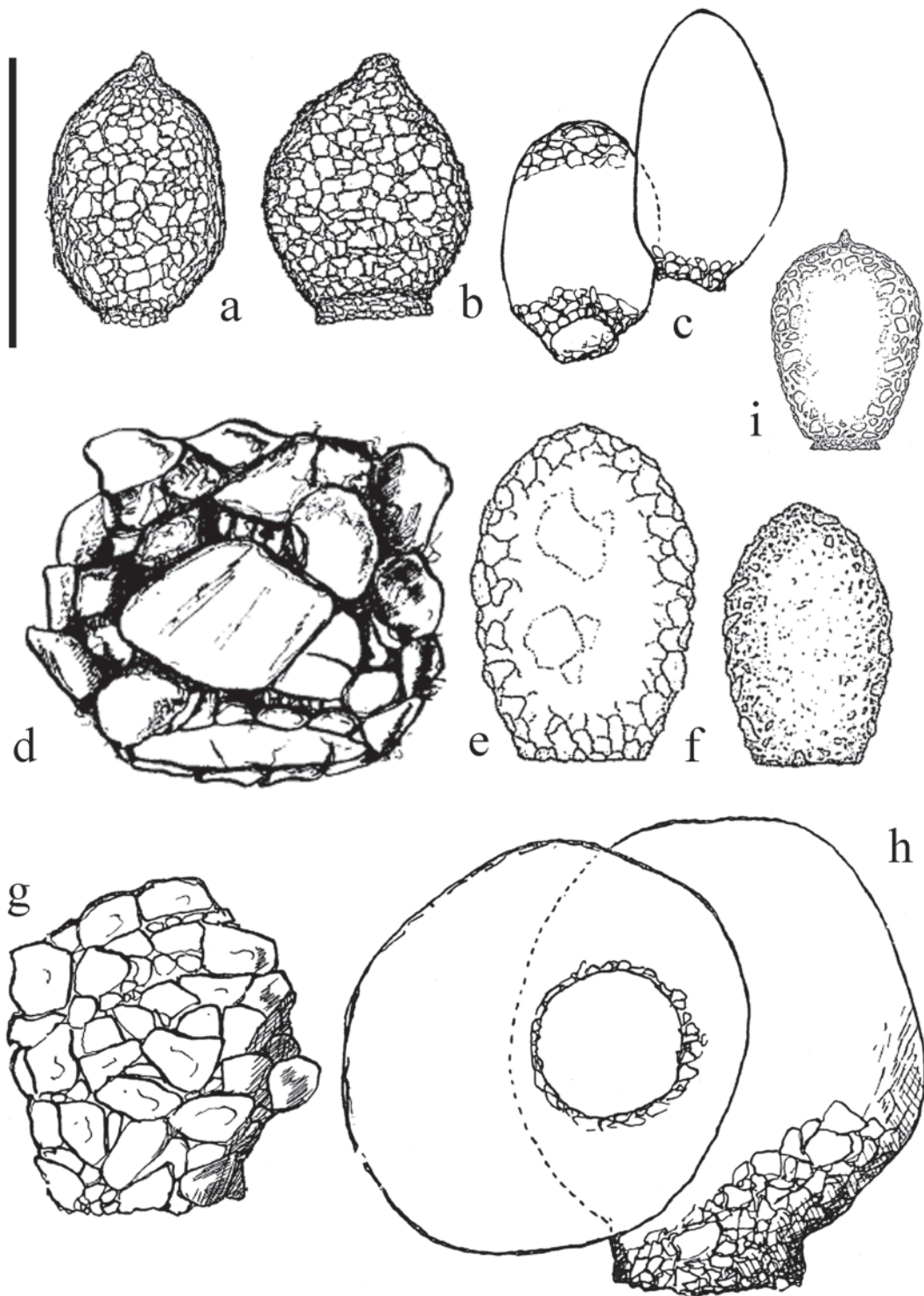
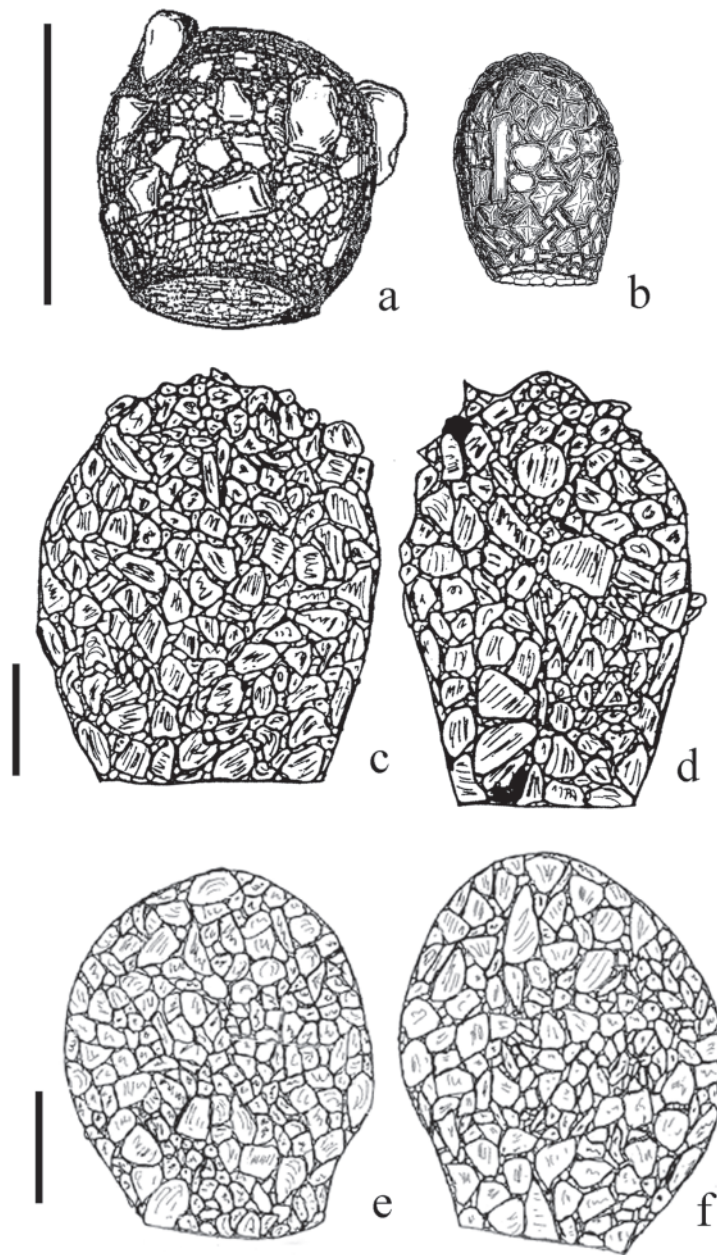


Fig. 12. Different species of *Diffugia* with spherical or ovoid shells: a-b – *D. difficilis* (after Thomas, 1954, plate I, figs 4-5), c – *D. difficilis* var. *ecornis* (after Gauthier-Lièvre and Thomas, 1958, fig. 16 e), d – *D. globularis* var. *sphaerica* (after Chardez, 1958, fig. 2), e-f – *D. levanderi* (after Playfair, 1918, plate XXXVII, figs 8-9; e – larger form with xenosomes, f – smaller form with chitinous shell), g – *D. minutav* var. *grandis* (after Gauthier-Lièvre and Thomas, 1958, fig. 37 d), h – *D. brevicolla* var. *major* (after Gauthier-Lièvre and Thomas, 1958, fig. 15 c), i – *D. ampullula* (after Playfair, 1918, plate XXXVII, fig. 10). Scale bar: 100  $\mu$ m.



**Fig. 13.** Different species of *Diffugia* with spherical or ovoid shells: a – *D. chardezi* (after Godeanu, 1972, fig. 3), b – *D. decloitrei* (after Godeanu, 1972, fig. 4), c – *D. finstertaliensis* (after Laminger, 1971, fig. 11), d – *D. lebes* var. *bretschkoi* (after Laminger, 1971, fig. 13). Scale bars: 100  $\mu$ m.

*bretschkoi*, (Figs 13 e, f) also for specimens with a shell that is more elongated than the original but whose dimensions are within that of the original, i.e. length 313–362  $\mu$ m, width 251–297  $\mu$ m, aperture diameter 140–176  $\mu$ m. Both these varieties closely resemble *D. viscidula* in terms of shell size and general shape (compare Figs 9, f; 13, e-f; 27, a-c; 28) thus we consider them to be junior synonyms of the latter.

***Diffugia minuta* Rampi, 1950 species complex**

The members of this group include *D. minuta*, *D. geosphaira*, *D. dujardini*, and *D. angulostoma* (Fig. 16, group ‘2d’ and in part group ‘2a’; Figs 18, c–f; Fig. 11, h).

***Diffugia minuta* Rampi, 1950**

According to Ogden (1983) the shell of *D. minuta*

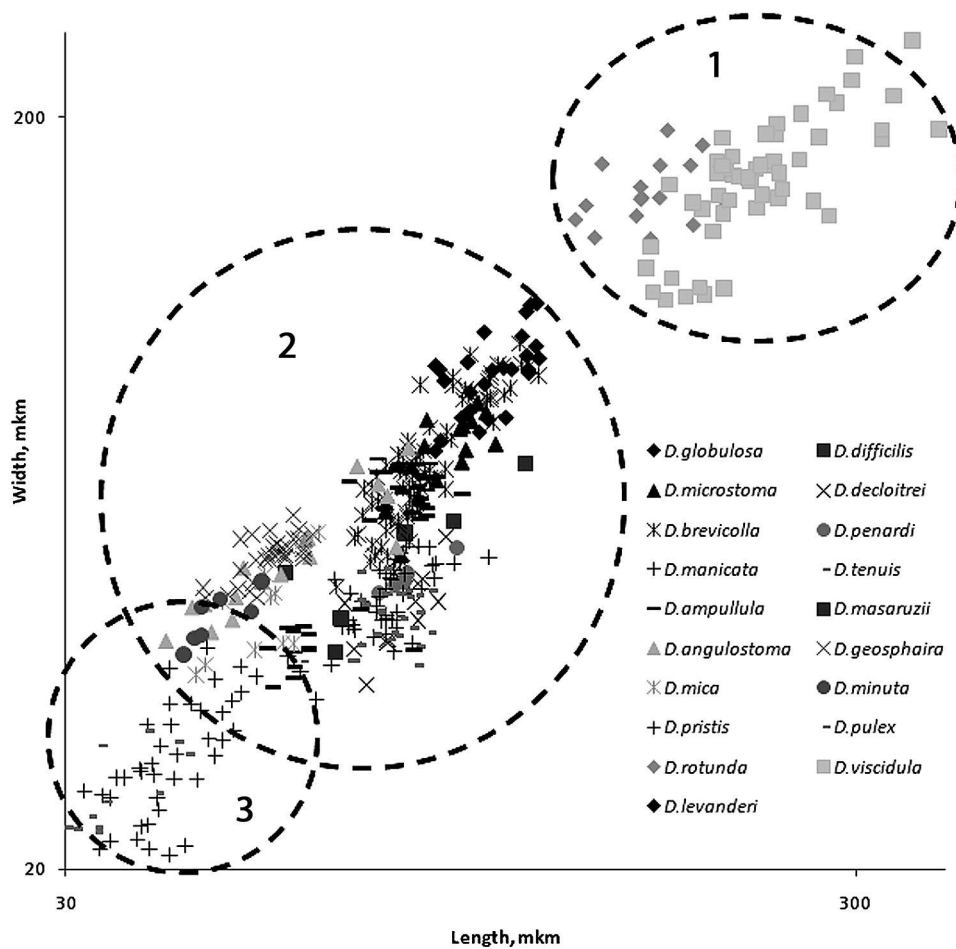


Fig. 14. Length-width bivariate scatter plot in logarithmic scale of *Diffflugia* with spherical and ovoid shells, based on C.G. Ogden's measurements (range of shell length 30–381  $\mu\text{m}$ ): 1-3 – size groups.

is spherical or ovoid, composed mainly of flattish quartz and the occasional fragments of diatom frustules (Fig. 23). The particles are packed closely together to give a robust structure. The aperture is small and often surrounded by a narrow lip of organic cement. The lip is not apparent in side view but gives the aperture opening a distinctive appearance when viewed 'en face'. Ogden measured 7 specimens of *D. minuta* (Fig. 14), 6 of which were published (Ogden, 1983), one unpublished: shell length 42–53  $\mu\text{m}$ , shell breadth 39–48  $\mu\text{m}$ , aperture diameter 9–13  $\mu\text{m}$ . According to Rampi (1950) the shell of *D. minuta* is spherical and completely covered by sand quartz (Figs 11, d, e). The aperture is small and surrounded by a series of regularly arranged plates. Rampi (1950) noted that this species cannot be confused with *D. globulosa* from which it differs by its minute dimensions. Shell length 53  $\mu\text{m}$ , shell width 48  $\mu\text{m}$ . We accept here the validity of *D. minuta*. This species is easily

distinguished by its small shell size and small aperture.

Later Gauthier-Lièvre and Thomas (1958) described *D. minuta* var. *grandis* with shell length 100–130  $\mu\text{m}$ , shell width 120–125  $\mu\text{m}$ , aperture diameter 20–28  $\mu\text{m}$  (Fig. 12, g), and Godeanu (1972) described *D. minuta* var. *minor* with a smaller, more elongated shell: shell length 28–35  $\mu\text{m}$ , shell width 17–20  $\mu\text{m}$ , aperture diameter 8–10  $\mu\text{m}$  (Fig. 11, m). *Diffflugia minuta* var. *grandis* closely resembles *D. microstoma* regarding the small aperture and shell dimensions, the specimens described by Gauthier-Lièvre and Thomas (1958) having a slightly greater shell length compared with those described by Ogden (1983). However, since *D. microstoma* is a junior synonym of *D. difficilis* (see below), we consider *D. minuta* var. *grandis* Gauthier-Lièvre et Thomas, 1958 to be a junior synonym of *D. difficilis* Thomas, 1954. *Diffflugia minuta* var. *minor* resembles *D. pulex* so closely (compare Figs 11, m and 19, n; see

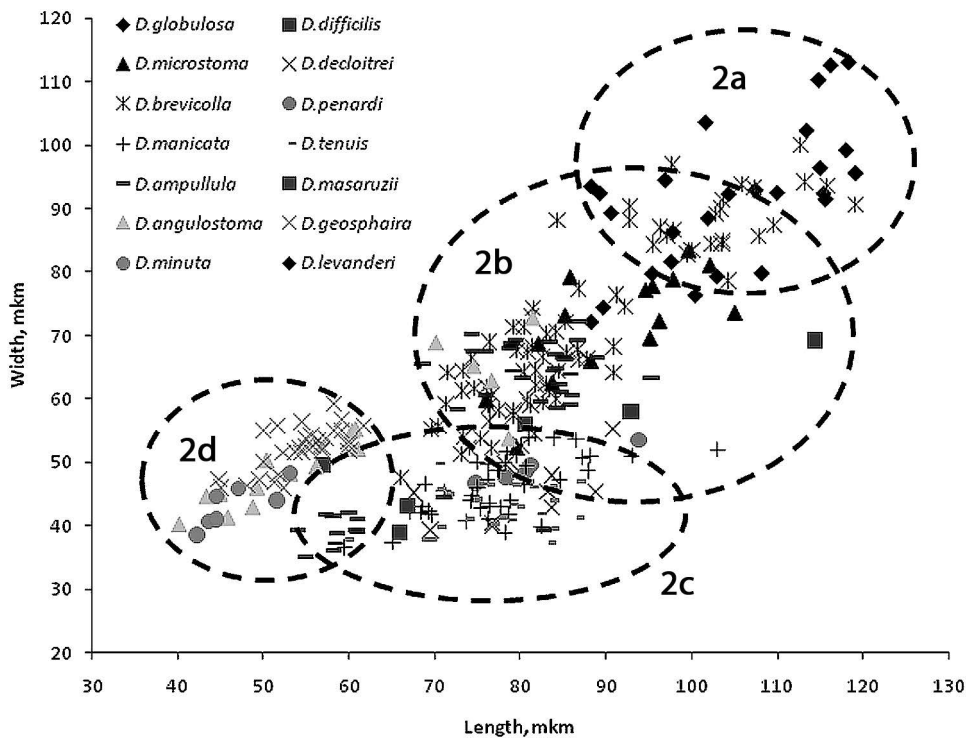


Fig. 15. Length-width bivariate scatter plot of *Diffugia* with spherical and ovoid shells, based on C.G. Ogden's measurements (range of shell length 40–119  $\mu\text{m}$ ): 2a–2d – size sub-groups.

also Ogden 1983, p. 22) that we consider it a junior synonym of the latter.

#### *Diffugia geosphaira* Ogden, 1991

According to Ogden (1991) the shell of *D. geosphaira* is ovoid, circular in cross section, with a well defined aperture rim (Fig. 24). The shell wall is constructed of an organic matrix with siliceous particles added. The aperture is large and circular. Ogden measured 39 specimens of *D. geosphaira* (Fig. 14) all of which are published (Ogden, 1988): shell length 45–62  $\mu\text{m}$ , shell breadth 46–59  $\mu\text{m}$ , aperture diameter 19–28  $\mu\text{m}$ . Ogden (1988) studied the variability of the shell wall composition in accordance with the material available in the culture and concluded that the shell can be constructed entirely of organic building units in the absence of suitable mineral particles in the environment (Figs 24, a, b). When sterilized soil was introduced into fresh cultures that previously lacked mineral particles, the testate amoebae promptly included mineral grains into the fabric of the shell (Fig. 24, c). Occasionally diatom frustules are also incorporated (Ogden, 1988, see his fig. 13). In addition to the differences in the structure of the nucleus (Ogden, 1991) this taxon is easily distinguished from other species with spherical shells, even within *D. minuta*-

complex, by having a large aperture bordered by a prominent lip.

#### *Diffugia angulostoma* Gauthier-Lièvre et Thomas, 1958

According to Ogden (1983) the shell of *D. angulostoma* is spherical and composed mainly of diatom frustules (Fig. 25). The particles are packed close together with many overlapping, to give a rough surface. The aperture is circular, but it may have irregularities depending on the arrangement of surrounding diatom frustules. Ogden (1983) measured two size classes of *D. angulostoma* (Figs 14; 18, c, d): 'a' (nine individuals, eight of which were published, one unpublished) – with shell length 40–56  $\mu\text{m}$ , shell breadth 40–48  $\mu\text{m}$ , aperture diameter 18–23  $\mu\text{m}$ ; 'b' (eight individuals all of which were published) – with shell length 60–82  $\mu\text{m}$ , shell breadth 50–73  $\mu\text{m}$ , aperture diameter 28–51  $\mu\text{m}$ .

This species was described by Gauthier-Lièvre and Thomas (1958), who considered that it differed from *D. minuta* by the large size of the aperture, the diameter of which was quoted as being about one-third the maximum shell width (Fig. 26). They also described high variability of the shell covering including both sand grains and diatom

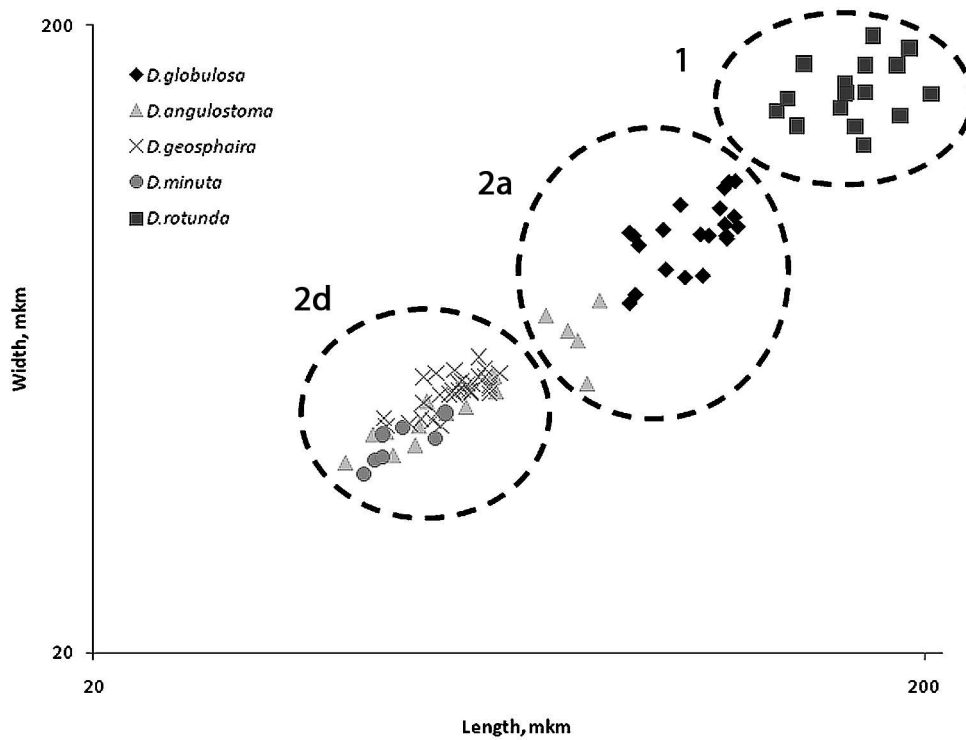


Fig. 16. Length-width bivariant scatter plot in logarithmic scale of *Diffugia* with spherical shells, based on C.G. Ogden's measurements (range of shell length 40–119 μm): numbers allocated to groups and subgroups are as in Figs 14 and 15.

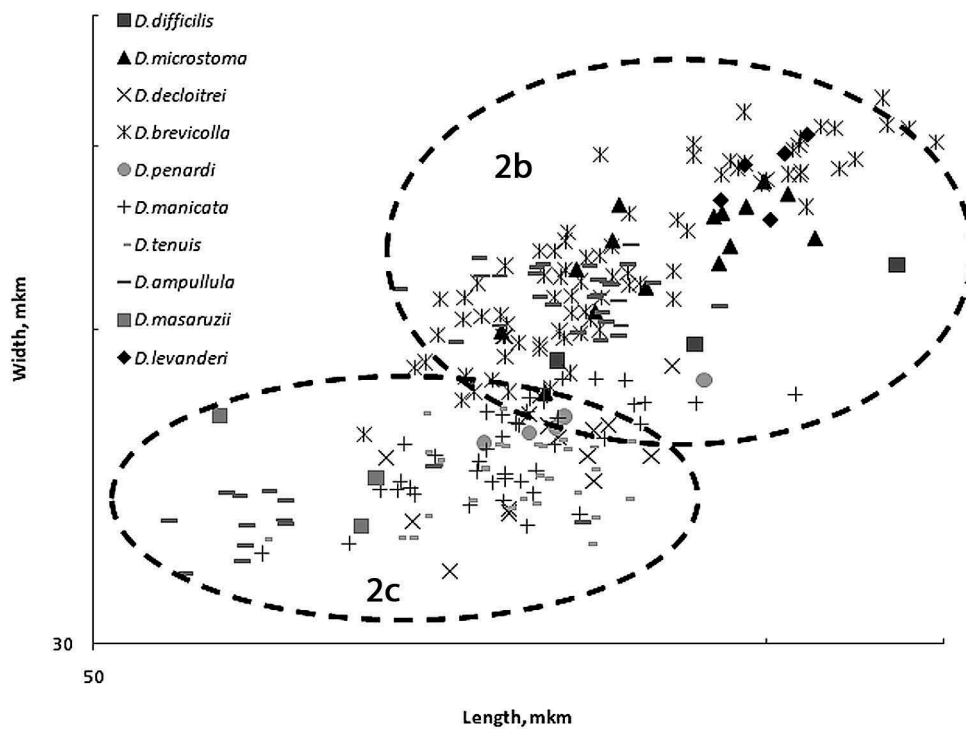
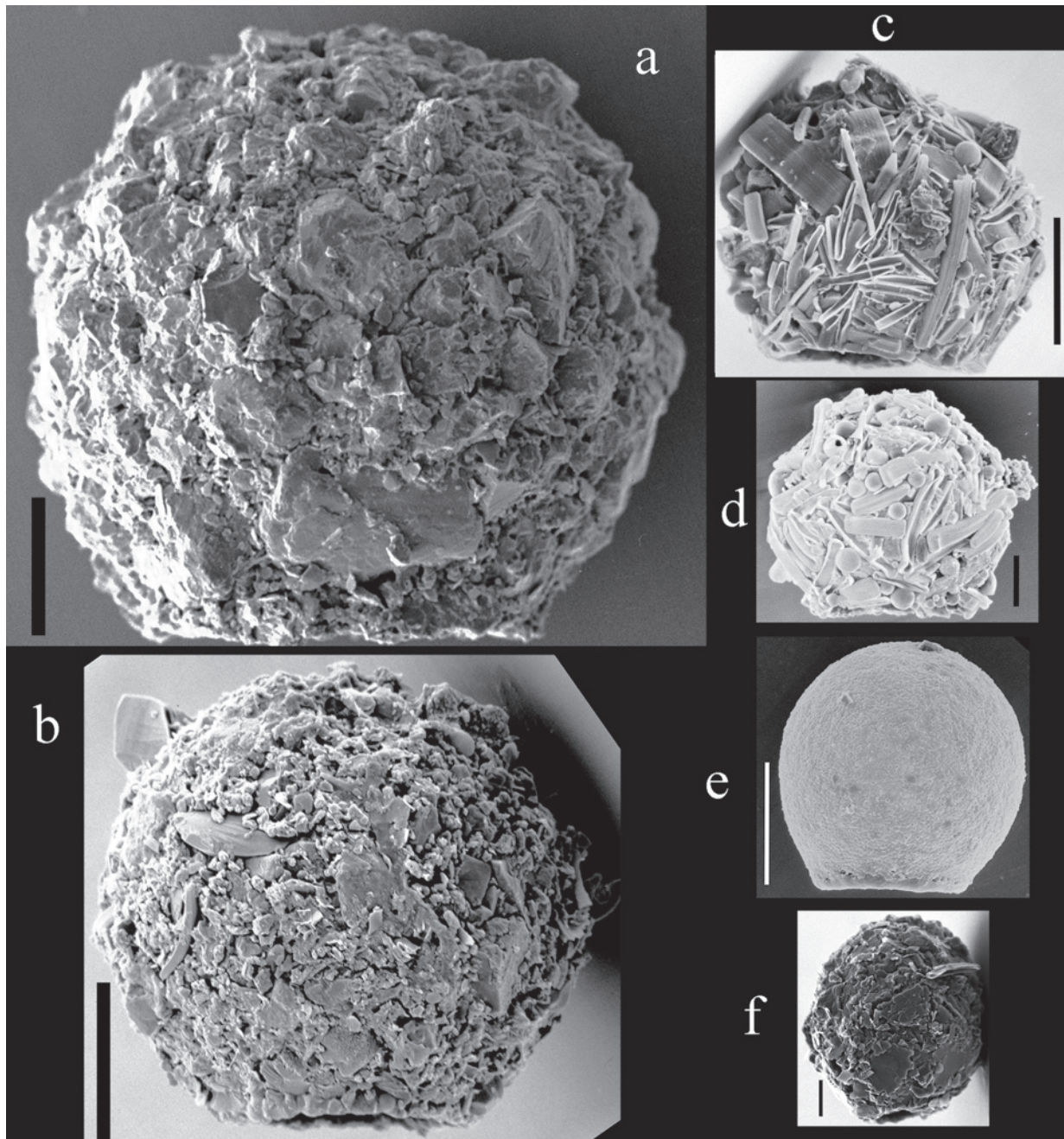


Fig. 17. Length-width bivariant scatter plot in logarithmic scale of *Diffugia* with ovoid shells, based on C.G. Ogden's measurements (range of shell length 54–119 μm): numbers allocated to groups and subgroups are as in Figs 14 and 15.

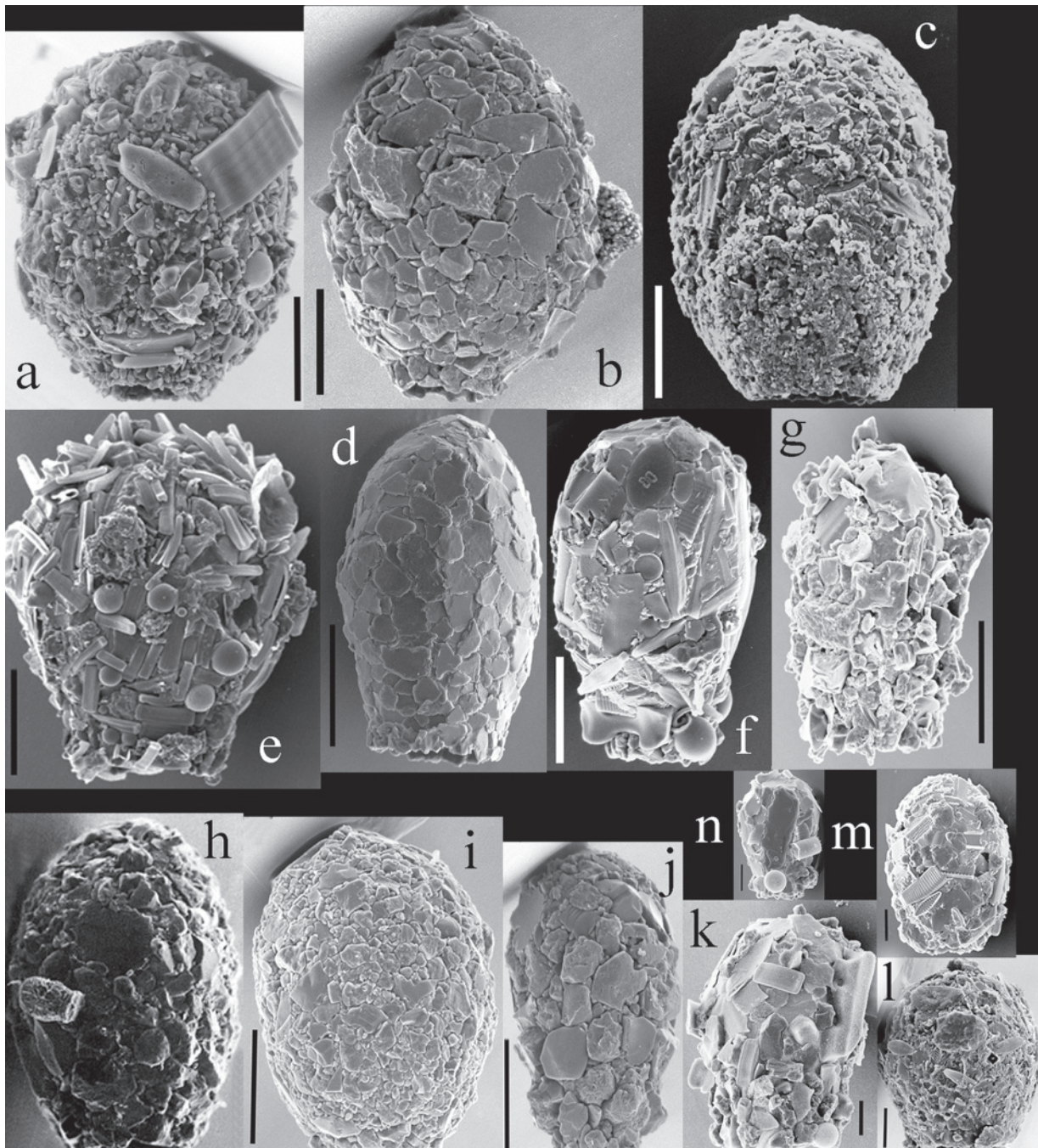


**Fig. 18.** Comparative morphology of “typical” spherical *Diffugia* spp. from C.G. Ogden’s SEM collection: a – *D. rotunda* (SEM CZ-07.182), b – *D. globulosa* (SEM EM-11-844), c – *D. angulostoma* ‘large’ form (SEM CZ-01.640), d – *D. angulostoma* ‘small’ form (SEM CZ-01.799), e – *D. geosphaira* (SEM 015709), f – *D. minuta* (SEM CZ-06.649). Numbers after taxon names are NHM index numbers of SEM negatives. Scale bars: a-c – 30  $\mu$ m; d, f – 10  $\mu$ m; e – 25  $\mu$ m.

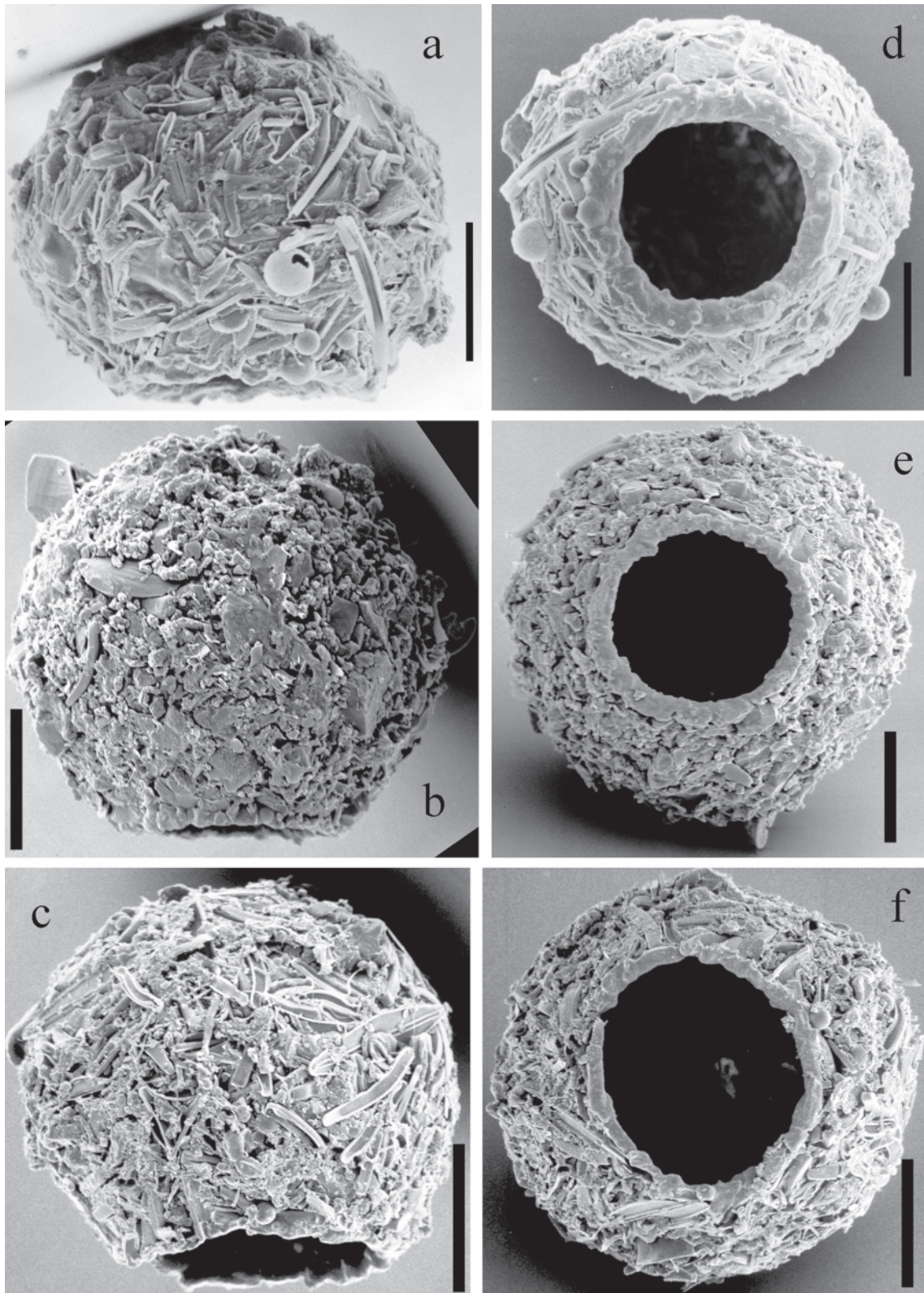
frustules. Moreover, they drew an attention to the presence of a small but easily distinguishable organic collar surrounding the aperture. We consider *D. angulostoma* a valid species that is rather variable in both size and shell cover. It is distinguished

from *D. geosphaira* by the presence of an organic lip surrounding the aperture in latter, and from *D. minuta* by its greater aperture diameter, and from *D. globulosa* by its smaller shell size.

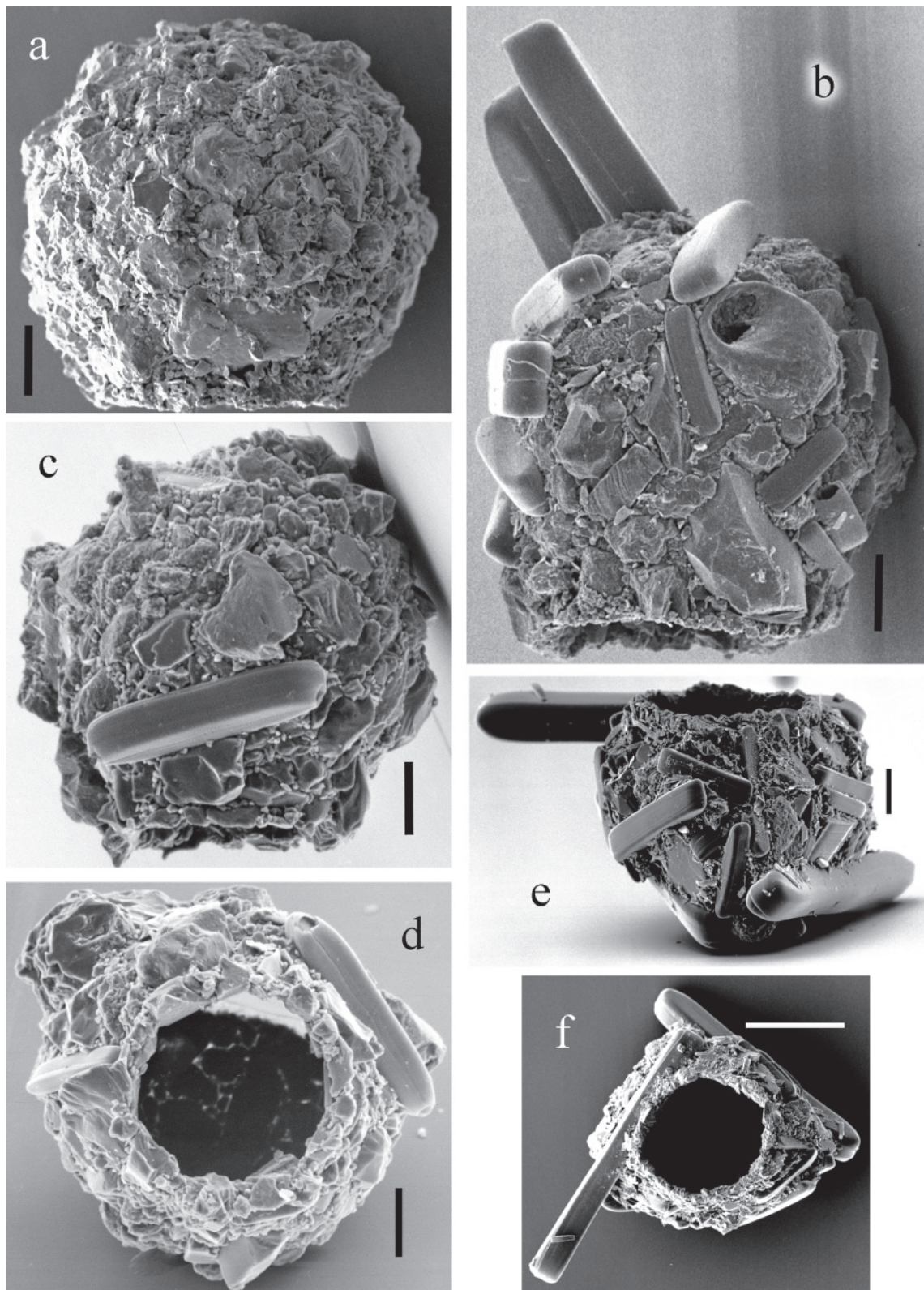




**Fig. 19.** Comparative morphology of “typical” ovoid *Diffugia* spp. (several forms with pyriform shells and shells with small apertural collars are also presented; their shell length does usually not exceed 100 µm and they were not reported in Mazei and Warren, 2014) from C.G. Ogden’s SEM collection: a – *D. microstoma* (SEM CZ-01.223), b – *D. difficilis* (SEM CZ-03.087), c – *D. levanderi* (SEM CZ-03.691), d – *D. declotrei* (SEM CZ-06.144), e – *D. brevicolla* (SEM CZ-02.319), f – *D. penardi* (SEM EM-11-624), g – *D. tenuis* (SEM CZ-06.721), h – *D. glans* (from Ogden, 1983, p. 8, fig 4a), i – *D. ampullula* (SEM CZ-04.507), j – *D. manicata* (SEM CZ-02.594), k – *D. masaruzii* (SEM CZ-05.762), l – *D. mica* (SEM CZ-05.484), m – *D. pristis* (SEM CZ-09.402), n – *D. pulex* (SEM CZ-10.242). Scale bars: a-j – 30 µm, k-n – 10 µm.



**Fig. 20.** Different specimens of *Diffugia globulosa* from C.G. Ogden's SEM collection: a-c – lateral view (a – SEM CZ-01.616, b – SEM EM-11-844, c – SEM EM-09-896), d-f – apertural view (d – SEM CZ-01.615, e – SEM EM-11-843, f – SEM EM-09-867). Scale bars: a-c – 30  $\mu$ m, d-f – 10  $\mu$ m.



**Fig. 21.** Different specimens of *Diffugia rotunda* from C.G. Ogden's SEM collection: a-d – lateral view (a – SEM CZ-07.182, b – SEM CZ-06.510, c – SEM CZ-01.220, d – SEM CZ-06.885), e-f – apertural view (e – SEM CZ-01.222, f – SEM CZ-06.880). Scale bars: a-d – 30  $\mu$ m; f – 100  $\mu$ m.

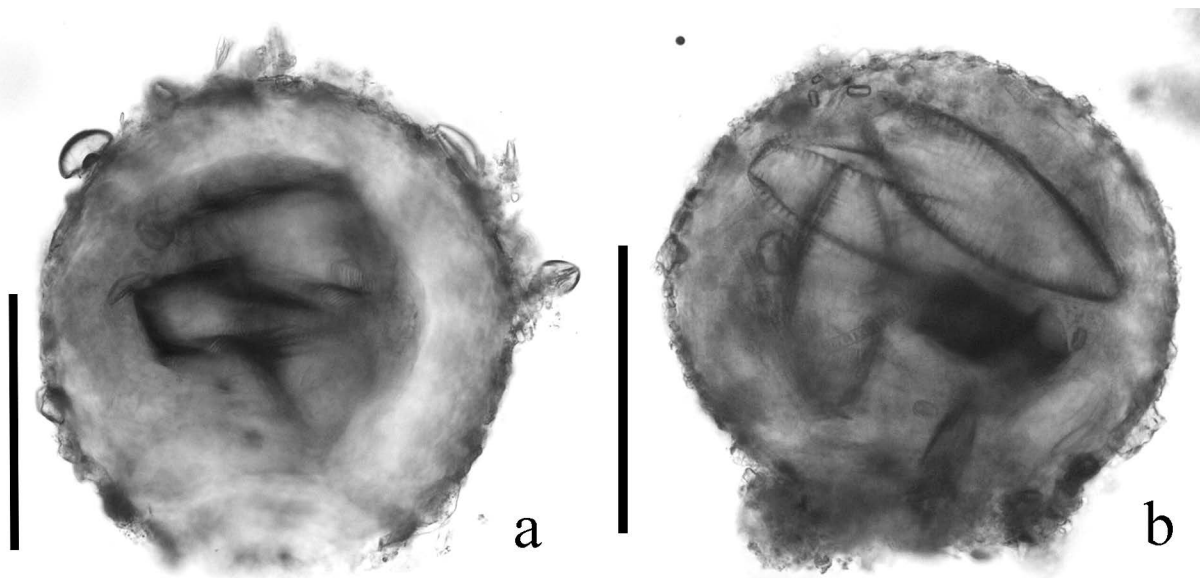


Fig. 22. Different specimens of *Diffflugia lebes* from E. Penard's slides: a-b – lateral view (a – slide 04.5.9.107, b – slide 20.12.8.217). Scale bars: 200  $\mu\text{m}$ .

#### *Diffflugia dujardini* Chardez, 1957

According to Chardez (1957) the shell of *D. dujardini* is transparent and circular to broadly ovoid in outline (Fig. 11, h). Sand grains are rarely scattered on the shell surface. The aperture is circular and surrounded by a small collar. The shell dimensions are: length 45  $\mu\text{m}$ , width 42–45  $\mu\text{m}$ , aperture diameter 19  $\mu\text{m}$ . In terms of its general shape and the presence of an aperture collar, this species resembles *D. minuta*. However, its aperture diameter matches more closely with *D. geosphaira* and *D. angulostoma*. We consider this species as questionable until additional data are available concerning its morphological variability.

#### *Diffflugia viscidula* Penard, 1902 species complex

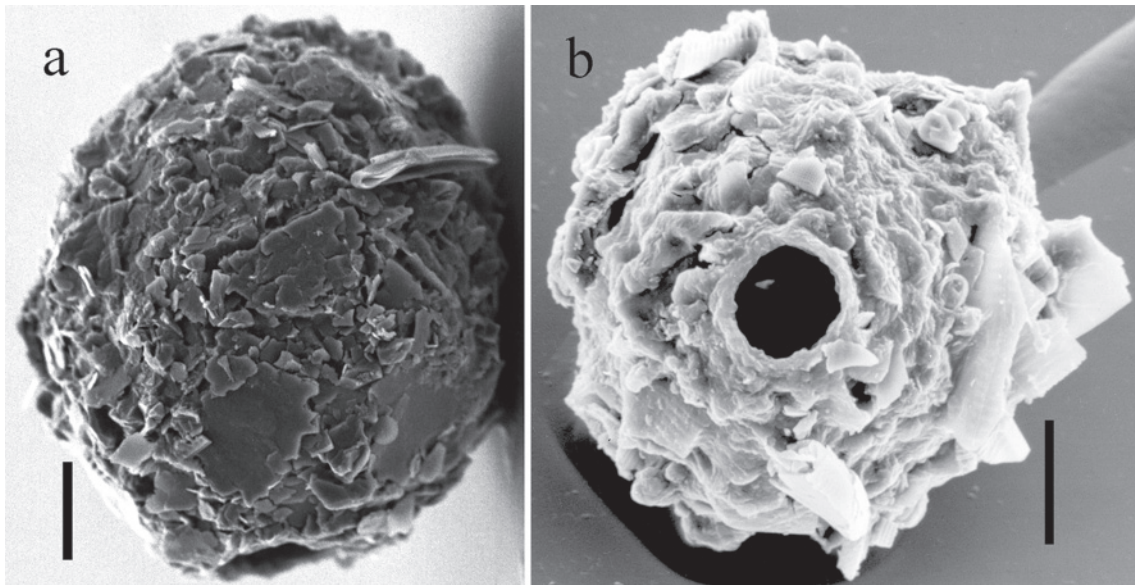
The members of this group include *D. viscidula*, *D. lebes*, *D. finstertaliensis* and *D. histrio* (Fig. 14, group '1' in part).

#### *Diffflugia viscidula* Penard, 1902

According to Ogden and Hedley (1980) and Ogden (1983), the shell of *D. viscidula* is opaque, ovoid, elongate or pyriform in shape, aborally it is usually rounded but may occasionally be pointed (Fig. 27). It is composed of angular quartz particles of different sizes. The aperture is circular and usually surrounded by small particles which give it a characteristic, well-defined outline. Ogden measured 54 specimens of *D. viscidula* (Fig. 14), 49

of which were published (Ogden and Hedley, 1980; Ogden, 1983), five unpublished. The ranges of the shell dimensions are as follows: length 163–382  $\mu\text{m}$ , width 114–253  $\mu\text{m}$ , aperture diameter 46–107  $\mu\text{m}$ . It is noteworthy that five unpublished individuals are much larger compared with published specimens.

Ogden (1983, p. 28) also discussed the nomenclatural problems concerning the priority between the names *D. lemani* Blanc, 1892 and *D. viscidula* Penard, 1902, concluding the latter name as valid for two reasons: (i) in contradiction with Article 8 of the International Code of Zoological Nomenclature, the work of Blanc (1892) was not available by purchase or freely distributed, and (ii) the confusion made by Penard (1902, 1905), who separated these two species mainly based on size (Penard, 1902), but later noted that he had used incorrect measurements of *D. lemani* (Penard, 1905). The slides of Penard deposited in NHM support the view that these taxa are identical (Fig. 28). On his slides dated 1920 (Figs 28, a-c, e-f) Penard used the name *D. lemani*, having previously concluded that *D. viscidula* is a junior synonym of *D. lemani* (Penard, 1905), whereas in the slides dated 1904 (Fig. 28, d) he applied the name *D. viscidula*, these specimens closely resembling the illustrations in his earlier description (Figs 7, j-l). We here follow the logic of Ogden (1983) and consider the name *D. viscidula* as valid, whereas *D. lemani* is invalid.



**Fig. 23.** Different specimens of *Diffflugia minuta* from C.G. Ogden's SEM collection: a – lateral view (SEM CZ-06.649), b – apertural view (SEM CZ-01.153). Scale bars: a-b – 10  $\mu$ m.

#### ***Diffflugia histrio* Penard, 1908**

According to Penard (1908) the shell of *D. histrio* is colorless or slightly yellowish, roughly ovoid, with a shape that resembles a shower-cap (Figs 9, a, b; 29). The surface is covered by siliceous fragments of all kinds, silt particles, siliceous flakes and large sand grains. The aperture is circular. The shell dimensions are: length 170–220  $\mu$ m, width 120–135  $\mu$ m. Penard (1908, p. 453) also noted that this species could easily be confused with *D. lemani* (i.e. *D. viscidula* – see Ogden, 1983, p. 26 for explanation). Although there are some differences in shell shape between these two taxa (compare Figs 28 and 29) and in the size and shape of the aperture (e.g. relatively large in *D. histrio*, more elongated in *D. viscidula*), we consider *D. histrio* Penard, 1908 to be a junior synonym of *D. viscidula* Penard, 1902.

#### ***Diffflugia finstertaliensis* Laminger, 1971**

According to Laminger (1971) the shell of *D. finstertaliensis* is broad-ovate to (more commonly) long-oval in shape (Figs, 13 c, d). The aperture is usually circular, rarely broad oval. The shell is covered with large quartz particles, sometimes mixed with smaller ones. The shell dimensions are: length 326–368  $\mu$ m, width 235–284  $\mu$ m, aperture diameter 147–189  $\mu$ m. Given the close similarity between this species and *D. viscidula*, we consider *D. finstertaliensis* Laminger, 1971 to be a junior synonym of *D. viscidula* Penard, 1902.

#### ***Diffflugia pulex* Penard, 1902 species complex**

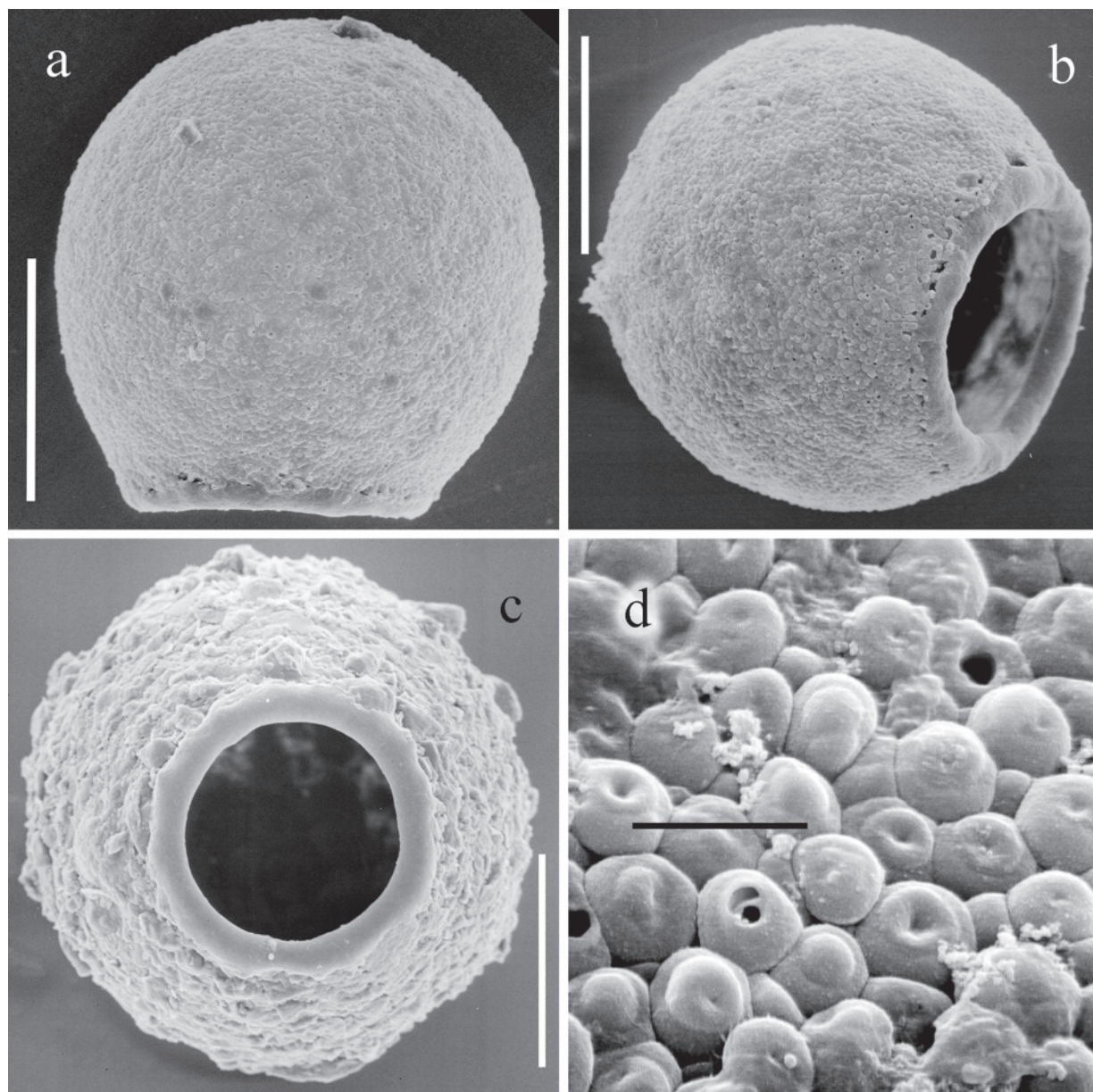
The members of this group include *D. pulex*, *D. pristis*, *D. mica*, *D. richmondiae*, *D. stechlinensis*, *D. humilis*, and *D. ovalisina* (Fig. 14, group '3'; Figs 19, 1–n; Figs 11, a–c, i–l).

#### ***Diffflugia pulex* Penard, 1902**

According to Ogden (1983) the shell of *D. pulex* is transparent and ovoid or elongate ovoid in shape (Fig. 30). It is composed of a mixture of small thin pieces of flat quartz and pieces of diatom frustules, often with whole frustules or round flagellate cysts adhering to the surface. The aperture is circular but may vary due to the arrangement of the surrounding particles. Ogden measured 18 specimens of *D. pulex* (Fig. 14), all of which were published (Ogden, 1983, 1984; Ogden and Živković, 1983). Based on these data, the shell dimensions are: length 28 to 43  $\mu$ m, width 21–31  $\mu$ m, aperture diameter 7–14  $\mu$ m. In his original description, Penard (1902) stated that the shell is pyriform, with or without narrowing of the aperture, chitinous, slightly yellow, covered with small scales or particles of amorphous silica, plates, transparent; shell length 22–30  $\mu$ m (Figs 7, s–u; 31, f–g).

#### ***Diffflugia pristis* Penard, 1902**

According to Ogden (1983) the shell of *D. pristis* is brown or opaque, ovoid, tapering from the mid-



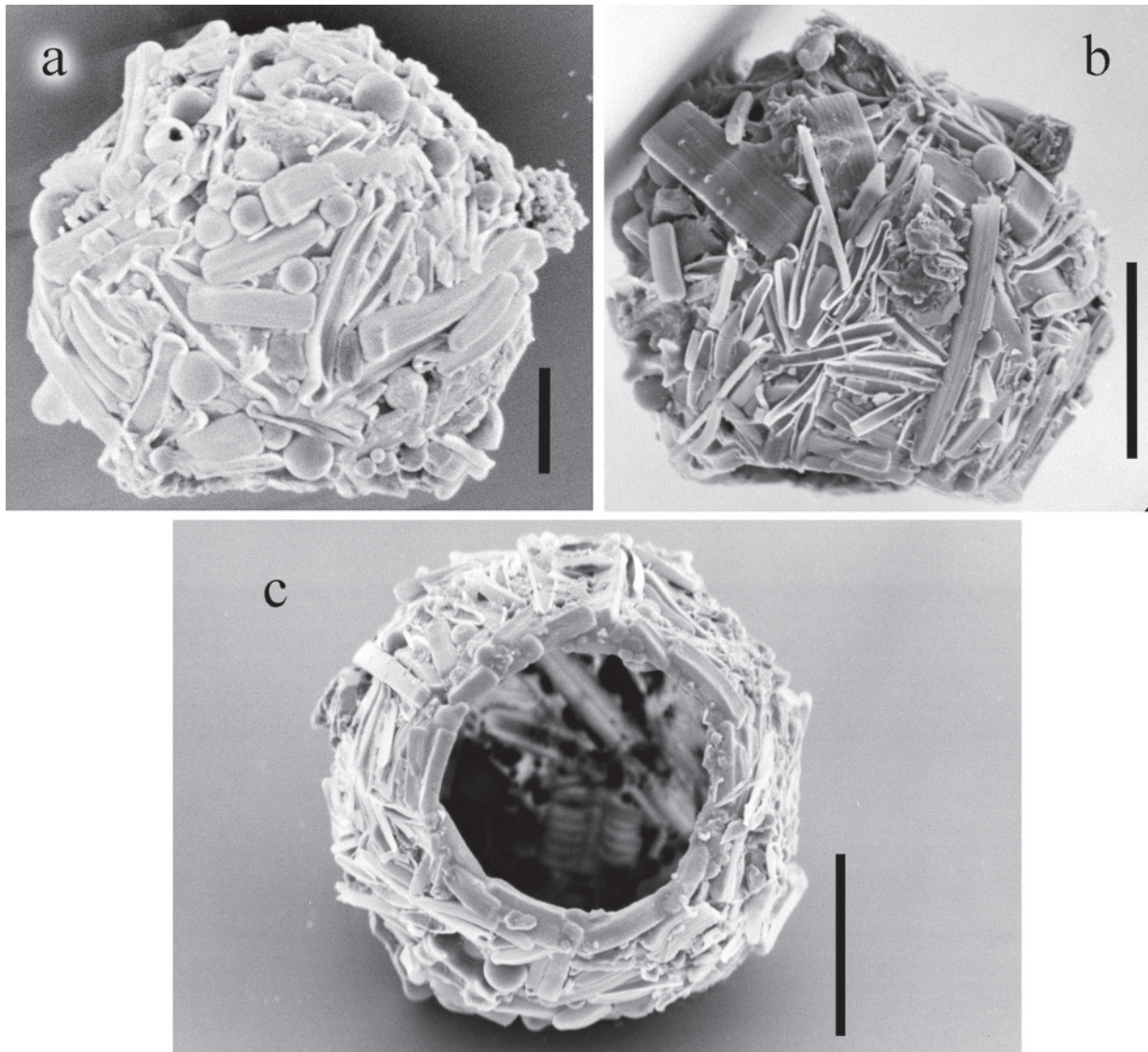
**Fig. 24.** Different specimens of *Diffugia geosphaira* from C.G. Ogden's SEM collection: a – lateral view (SEM 015709), b – ventro-lateral (SEM 015704), c – apertural view (SEM 026249), d – portion of shell surface (SEM 015705). Scale bars: a-c – 25  $\mu\text{m}$ ; d – 1.5  $\mu\text{m}$ .

body towards the aperture and curved aborally (Fig. 32). The shell is thin, smooth and composed of flattish pieces of quartz, occasionally with flat pieces of diatom frustules. The aperture is circular with a regular margin. Ogden measured 43 specimens of *D. pristis* (Fig. 14), 41 of which were published (Ogden, 1983, 1984; Ogden and Živković, 1983), two unpublished. Based on these data, the shell dimensions are: length 32 to 66  $\mu\text{m}$ , width 21–49  $\mu\text{m}$ , and aperture diameter 6–16  $\mu\text{m}$ . Ogden's (1983)

description matches well with the original by Penard (1902) in terms of shell shape although the size is slightly smaller (Figs 7, f-g; 31, d).

#### *Diffugia mica* Frenzel, 1892

According to Ogden (1983) and Ogden and Živković (1983), the shell of *D. mica* is brownish, spherical or ovoid in shape, sometimes with a shallow aperture collar (Fig. 33). It is composed of flattish pieces of quartz arranged to give a relatively smooth



**Fig. 25.** Different specimens of *Diffflugia angulostoma* from C.G. Ogden's SEM collection: a-b – lateral view (a – SEM CZ-01.799, b – SEM CZ-01.640), c – apertural view (SEM CZ-01.639). Scale bars: a – 10  $\mu\text{m}$ , b, c – 30  $\mu\text{m}$ .

surface. The aperture is circular, well-defined, usually with an organic margin and sometimes with a collar. Ogden measured 11 specimens of *D. mica* (Fig. 14), 9 of which were published (Ogden, 1983; Ogden and Živković, 1983), two unpublished. Based on these data, the shell dimensions are: length 44 to 63  $\mu\text{m}$ , width 36–56  $\mu\text{m}$ , aperture diameter 12–20  $\mu\text{m}$ .

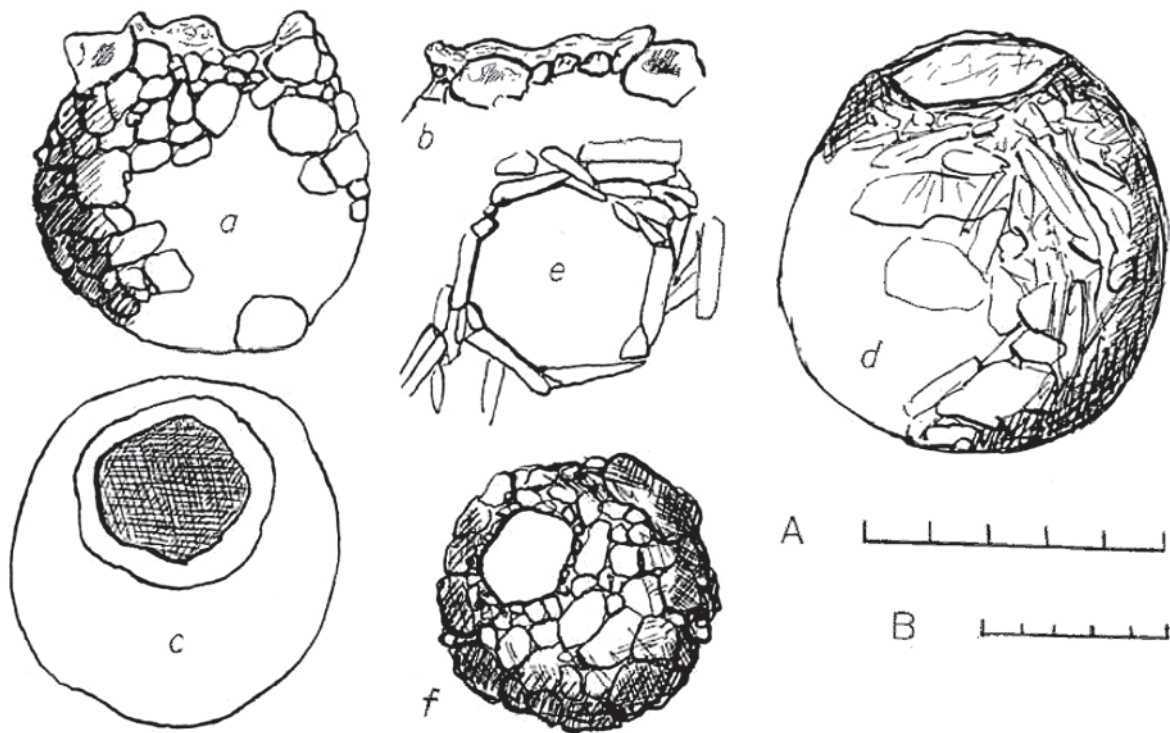
#### *Diffflugia richmondiae* Playfair, 1914

According to Playfair (1914) the shell of *D. richmondiae* is ovoid, curved aborally, aperture is circular, shell length 14  $\mu\text{m}$ , shell width 12  $\mu\text{m}$ ,

aperture diameter 3  $\mu\text{m}$  (Fig. 11, a). Schönborn (1965) found this species in Masurian Lakes and described it as almost cylindrical, hyaline with shell length 16–25  $\mu\text{m}$ , shell width 10–13  $\mu\text{m}$ , aperture diameter 3–4  $\mu\text{m}$  (Fig. 11, b). This is the smallest member of the species complex. Its relationship with *D. pulex* awaits more detailed investigations.

#### *Diffflugia stechlinensis* Schönborn, 1962

The shell is hyaline and with small sand grains scattered on the surface. The aperture is circular. The shell dimensions are: length 29–48  $\mu\text{m}$ , width 22–39  $\mu\text{m}$ , aperture diameter 10–15  $\mu\text{m}$  (Fig. 11, c).



**Fig. 26.** Variability of *Diffflugia angulostoma*, after Gauthier-Lièvre and Thomas (1958, p. 254, fig. 1): a – lateral view, c, d, f – semi-lateral view showing aperture, b – details of the aperture in lateral view (collar is visible), e – detail of the aperture of a specimen covered by diatom frustules; a, f – individuals covered by sand grains, c, d – individuals covered by diatom frustules. Scale bars 50  $\mu\text{m}$  (A – for figs d, e; B – for figs a-c, f).

Schönborn (1962) noted that the shell size range of *D. stechlinensis* overlaps with *D. pulex*, however the shell length of *D. pulex* rarely exceeds 30  $\mu\text{m}$  whereas *D. stechlinensis* has a mean length of about 40  $\mu\text{m}$ . Furthermore, the shell shape differs significantly in these two species, *D. pulex* being pyriform whereas *D. stechlinensis* has parallel sides. It is not clear in this case if the differences in the shell shape can be considered as taxonomically significant. Further studies are needed in order to resolve the systematics of these two taxa.

#### ***Diffflugia humilis* Chardez, 1991**

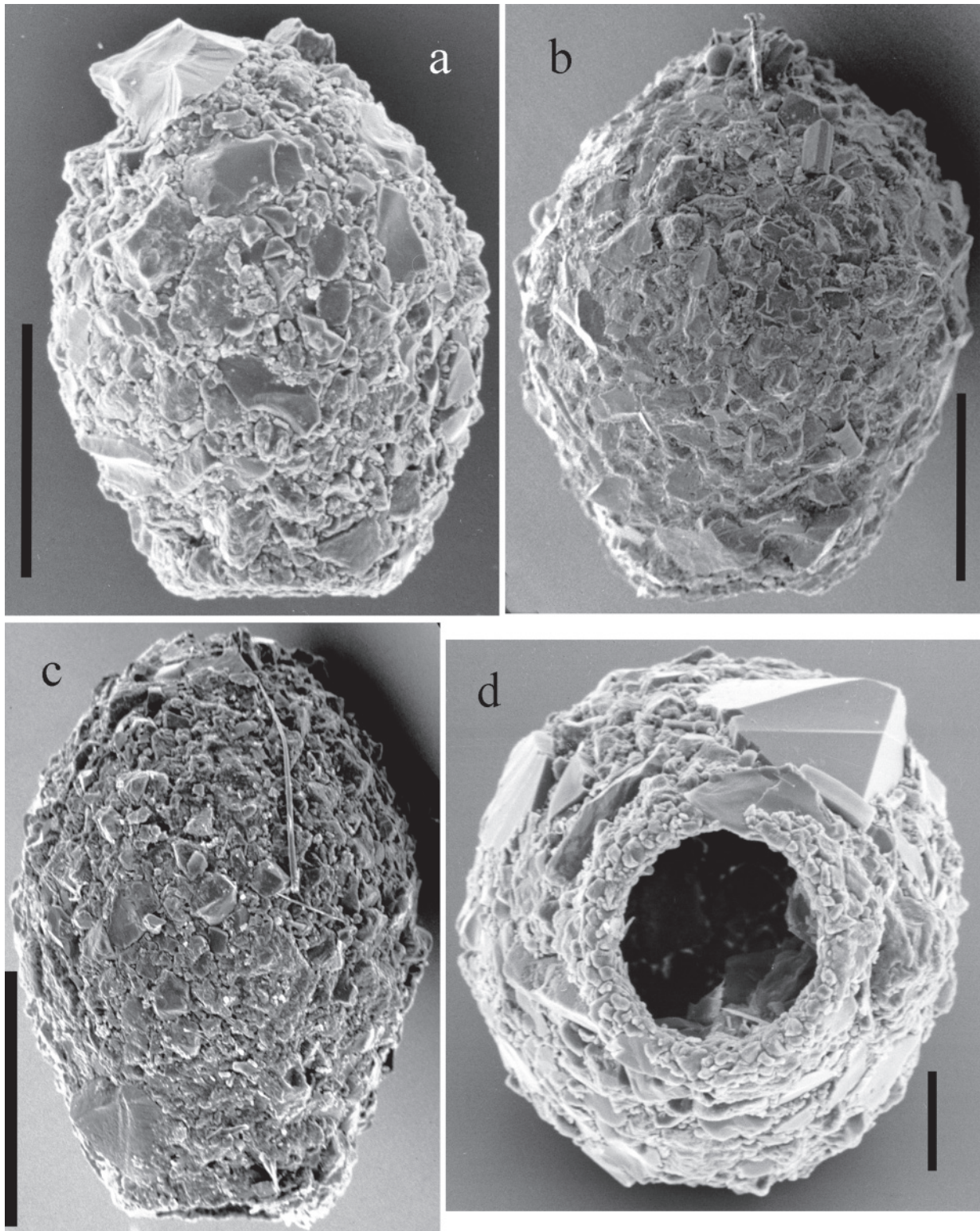
According to Chardez (1991) the shell of *D. humilis* is oval or elongate-oval, roughly circular, transparent, circular in cross-section, both sides converging towards the aperture sometimes forming a more or less distinct neck, other times with no apparent neck (Figs 11, I; 34). The shell is covered by diatom frustules resulting in an irregular shape. The aperture is circular and narrow, often irregular in contour. The shell dimensions are: length 40–60

$\mu\text{m}$ , width 22–36  $\mu\text{m}$ , aperture diameter 8–15  $\mu\text{m}$ . Unfortunately, Chardez (1991) did not compare this species with similar species, even though it matches well in size and shape with *D. pristis* (Figs 34, d-e). However, because of the high variability of the shape (Figs 34, a-c) it is not possible to make any final decision concerning the validity of this species, which must await further investigations.

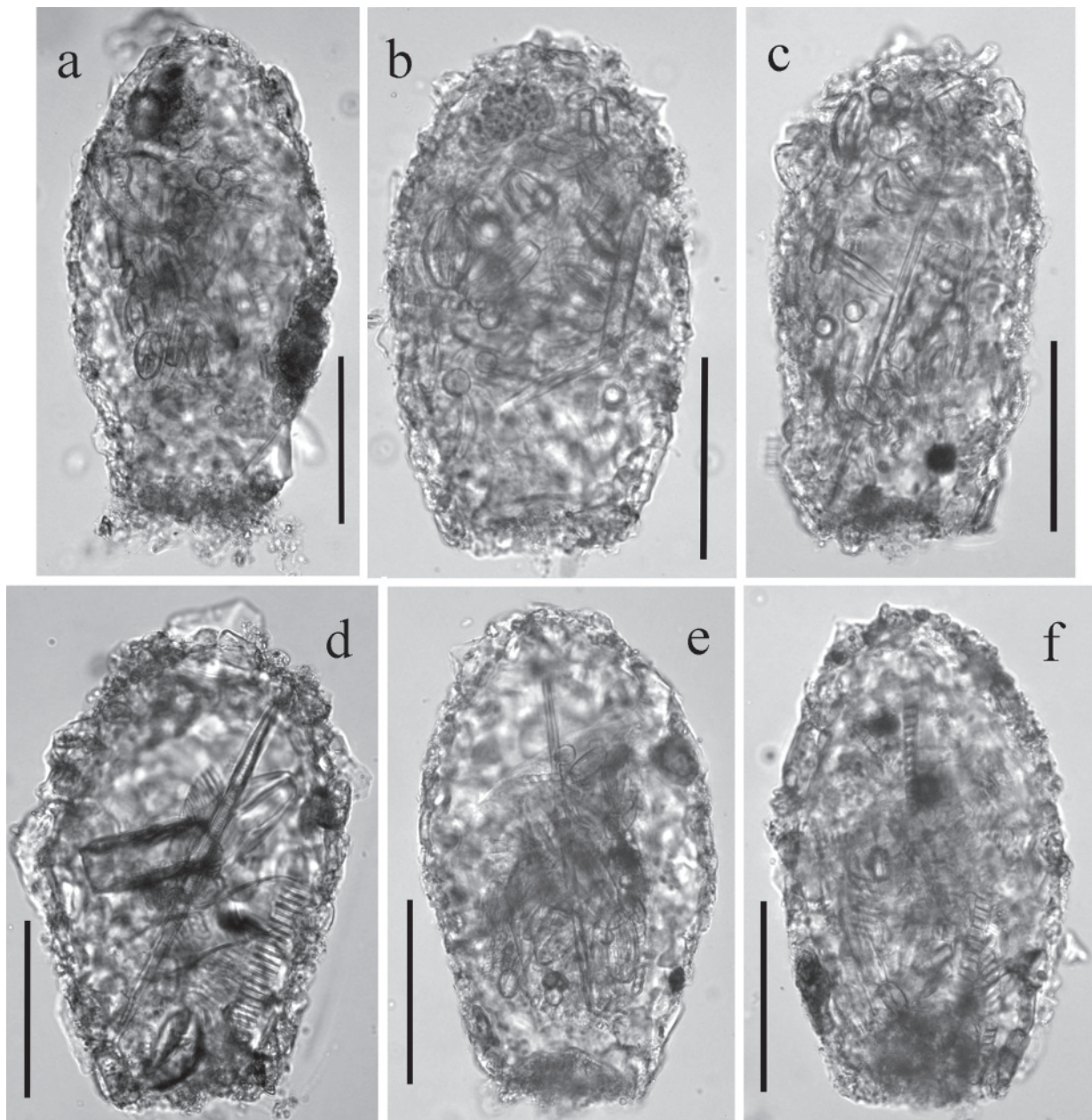
#### ***Diffflugia ovalisina* Beyens et Chardez, 1994**

According to Beyens and Chardez (1994) the shell of *D. ovalisina* is transparent, oval, and circular in cross-section (Figs 11, j-l). The shell is truncated at the aperture the border of which has a slightly swollen rim, sometimes slightly recurved to the interior. The surface is covered by small, flattish particles and diatom frustules. The shell dimensions are: length 25–29  $\mu\text{m}$ , width 18–28  $\mu\text{m}$ , aperture diameter 9–15  $\mu\text{m}$ . This taxon closely resembles *D. pulex* (compare Figs 11, k; 31 g) of which we consider it to be a junior synonym.





**Fig. 27.** Different specimens of *Diffugia viscidula* from C.G. Ogden's SEM collection: a-c – lateral view (a – SEM CZ-01.289, b – SEM CZ-08.282, c – SEM EM-12-298), d – apertural view (SEM CZ-01.290). Scale bars: a-c – 100  $\mu\text{m}$ , d – 30  $\mu\text{m}$ .



**Fig. 28.** Different specimens of *Diffflugia lemani* (a-c, e-f) and *Diffflugia viscidula* (d) from E. Penard's slides: a-f – lateral view (a-c – slide 20.12.8.222, d – slide 04.5.9.153, e-f – slide 20.12.8.223). Scale bars: 100  $\mu\text{m}$ .

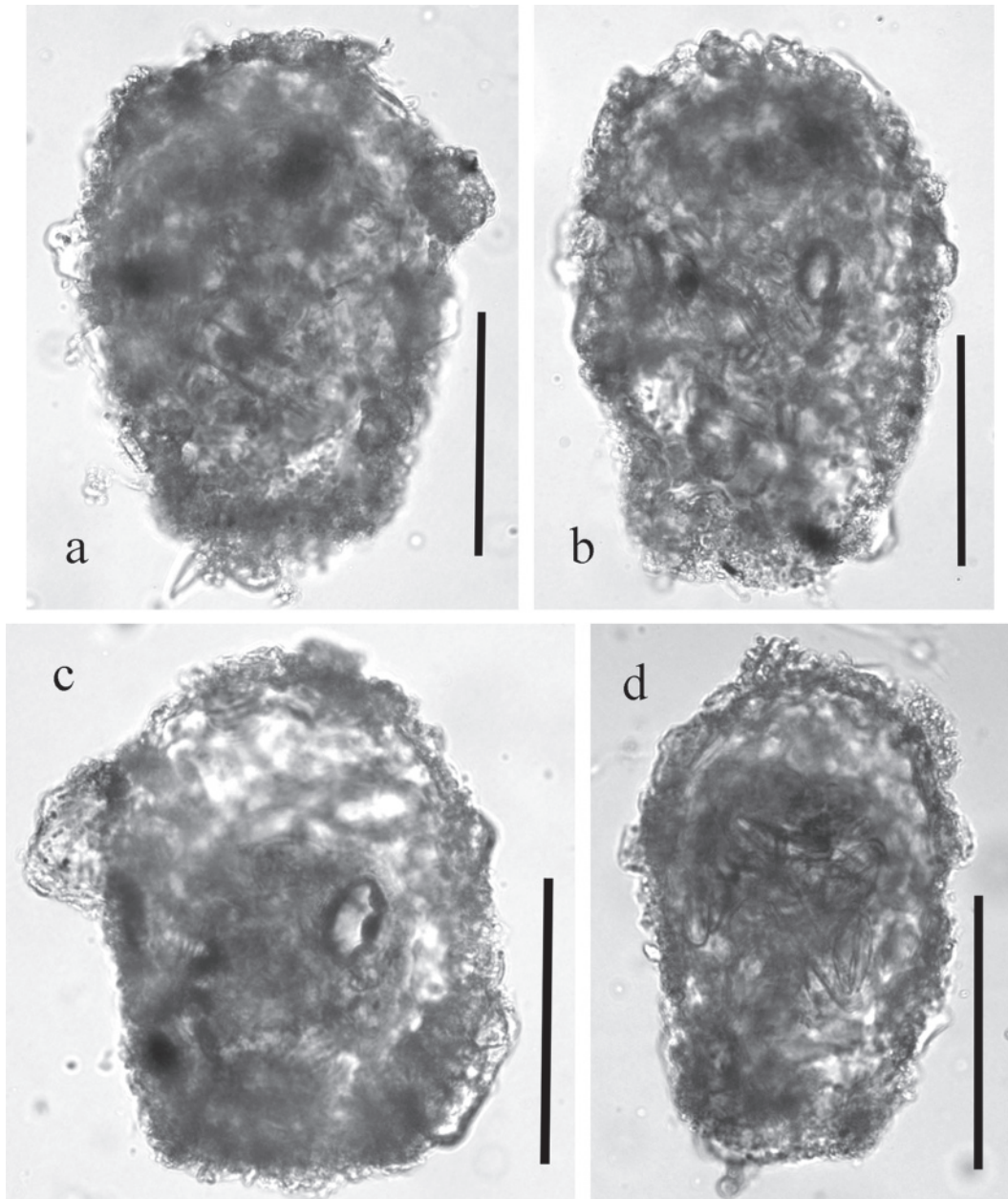
***Diffflugia glans* Penard, 1902 species complex**

The members of this group include *D. glans*, *D. ampullula*, *D. penardi*, *D. manicata*, *D. masaruzii*, and *D. tenuis* (Fig. 17, group '2c'; Figs 19, f–k).

***Diffflugia glans* Penard, 1902**

According to Ogden (1983) the shell of *D.*

*glans* is dark, elongate-ovoid, tapering towards the aperture and evenly rounded aborally (Fig. 35). It is composed mainly of small to medium-size pieces of quartz packed together rather densely. The aperture is circular and surrounded by both small and medium-size particles. Ogden (1983) measured three specimens of *D. glans* with the shell length 67 to 74  $\mu\text{m}$ , shell width 44–50  $\mu\text{m}$ , and aperture diameter



**Fig. 29.** Different specimens of *Diffugia histrio* from E. Penard's slides: a-d – lateral view (a-c – slide 20.12.8.211, d – slide 20.12.8.212). Scale bars: 100  $\mu\text{m}$ .

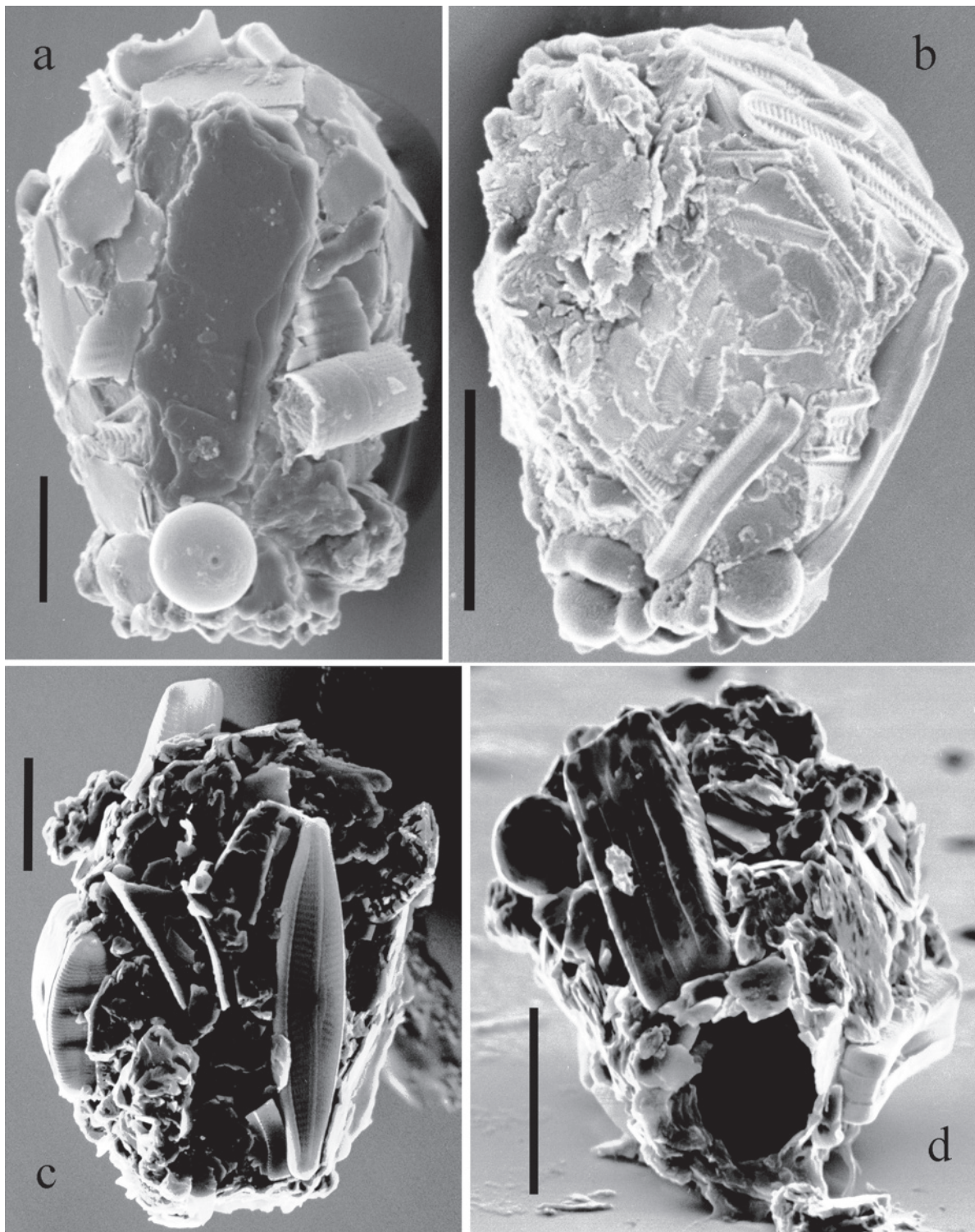
19–22  $\mu\text{m}$ . In his original description, Penard (1902) noted that the shell is thin and fragile, 66–77  $\mu\text{m}$  long (Figs 7, h; 31, d).

#### *Diffugia ampullula* Playfair, 1918

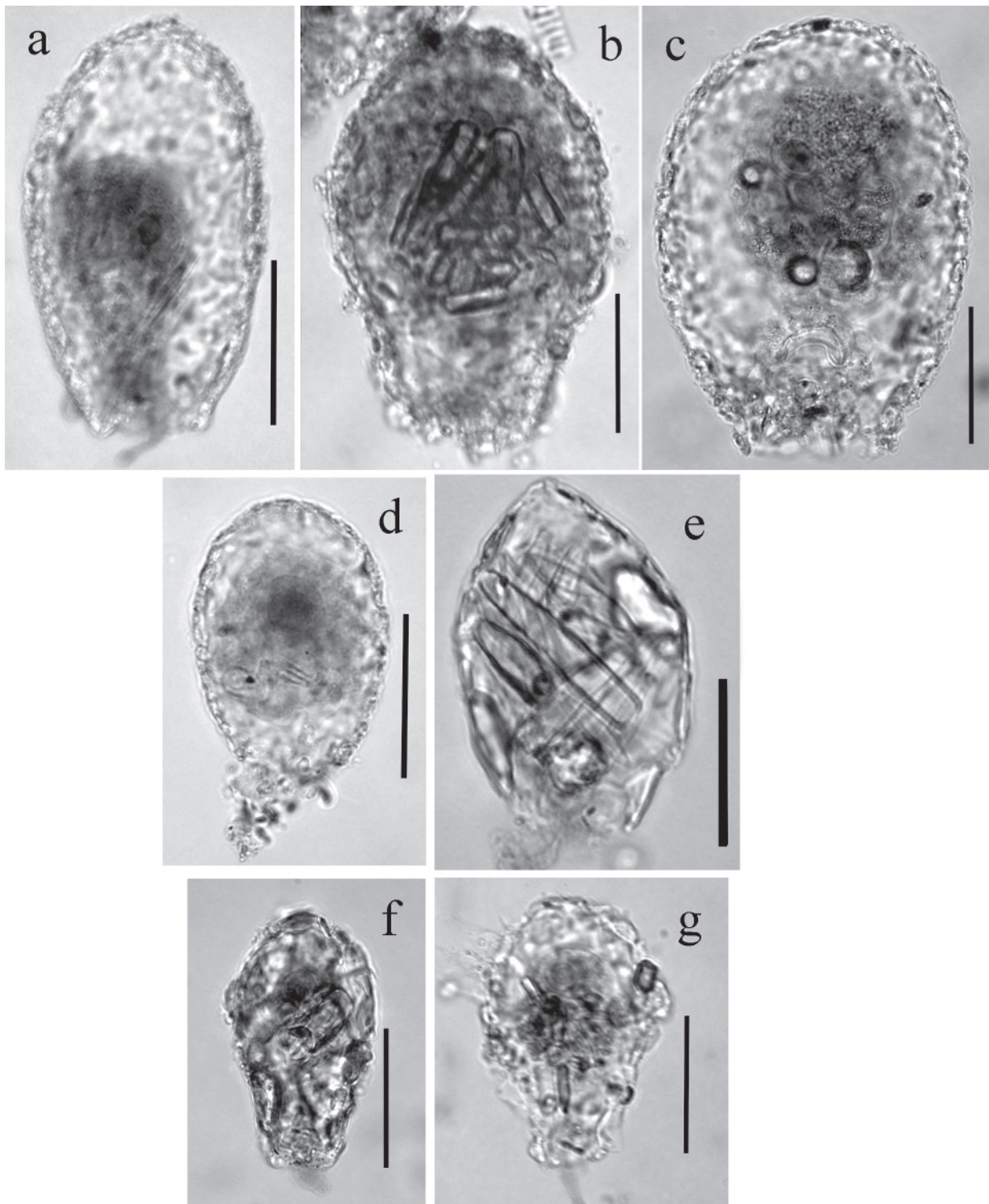
According to Ogden (1983) the shell of *D. ampullula* is hyaline, ovoid, and circular in cross-section (Fig. 36). Its wall is medium thickness and is composed mainly of small to medium-size pieces

of quartz, arranged to give a smooth surface. The aperture is circular, surrounded by a slightly raised collar of small particles; the edge of the collar is often irregular. Ogden measured 45 specimens of *D. ampullula*, 39 published (Ogden, 1983), six unpublished (Fig. 14). Based on these data, the shell dimensions are: length 54 to 95  $\mu\text{m}$ , width 35–72  $\mu\text{m}$ , aperture diameter 16–29  $\mu\text{m}$ .

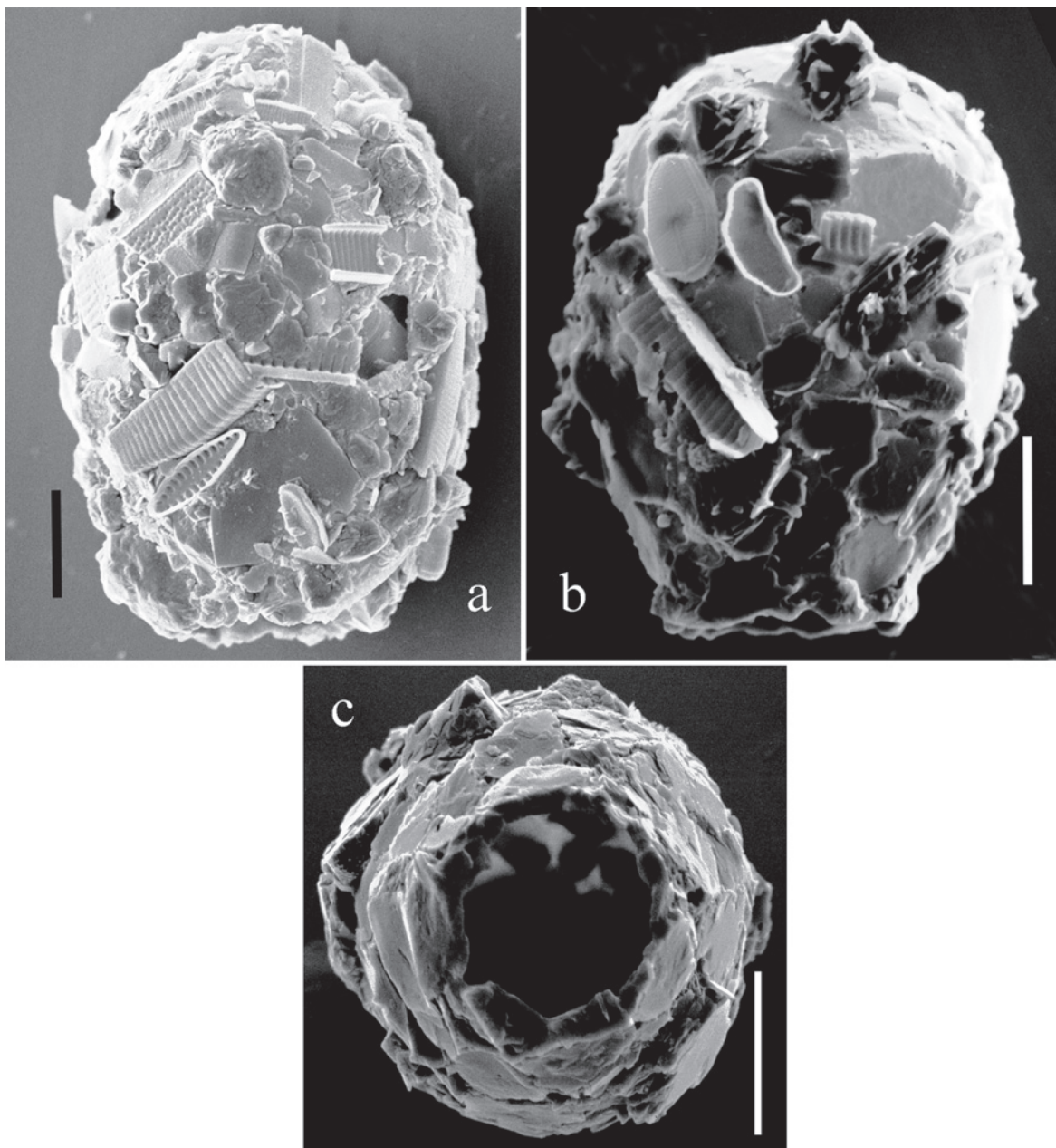
According to the original description (Playfair,



**Fig. 30.** Different specimens of *Diffugia pulex* from C.G. Ogden's SEM collection: a-c – lateral view (a – SEM CZ-10.242, b – SEM CZ-03.980, c – SEM CZ-09.337), d – apertural view (SEM CZ-09.352). Scale bars: a-d – 10  $\mu$ m.



**Fig. 31.** Different specimens of *Diffugia molesta* (a-c), *Diffugia pristis* (d), *Diffugia fallax* (e), and *Diffugia pulex* (f-g) from E. Penard's slides: a-g – lateral view (a – slide 04.5.9.121, d – slide 20.12.8.230, c – slide 20.12.8.231, d – slide 04.5.9.122, e – slide 04.5.9.100, f – slide 20.12.8.239, g – slide 20.12.8.240). Scale bars: 50  $\mu$ m.

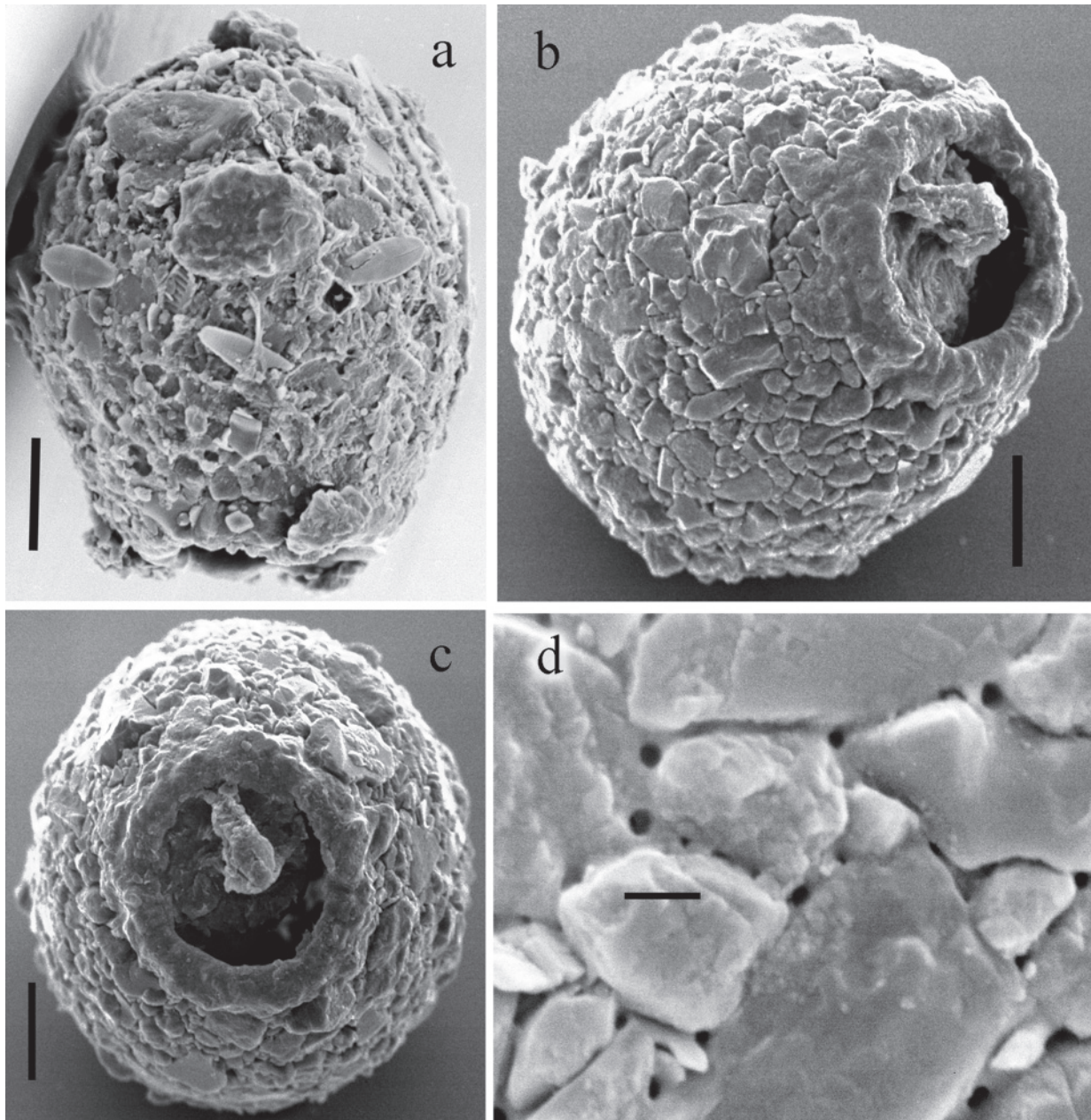


**Fig. 32.** Different specimens of *Diffugia pristis* from C.G. Ogden's SEM collection: a-b – lateral view (a – SEM CZ-09.402, b – SEM CZ-09.126), c – apertural view (SEM CZ-08.598). Scale bars: a-c – 10  $\mu$ m.

1918), the shell is broadly ovate, with a hemispherical dome, and sides gradually converging in almost straight lines to the broadly truncate base, where the test is suddenly constricted into a narrow, slightly everted collar. The dome is sometimes capped with a minute, pointed apex. The aperture is circular. The shell dimensions are: length 72–89  $\mu$ m, width 52–65  $\mu$ m. Playfair (1918) illustrated *D. ampullula* as being pointed aborally (Fig. 12i). However, he

also pointed out that this character is being absent sometimes. Moreover, Ogden (1983) noted that he never examined this feature among the 45 specimens that he investigated.

It is noteworthy that, among the individuals investigated by Ogden, it is possible to distinguish two distinct size classes with a shell length 54–61  $\mu$ m and 69–95  $\mu$ m (Fig. 37). In Volume 58 of his SEM collection deposited at NHM, Ogden



**Fig. 33.** Different specimens of *Diffflugia mica* from C.G. Ogden's SEM collection: a – lateral view (SEM CZ-05.484), b – ventro-lateral (SEM CZ-07.992), c – apertural view (SEM CZ-07.990), d – structure of organic cement (SEM CZ-07.994). Scale bars: a-c – 10  $\mu\text{m}$ , d – 1  $\mu\text{m}$ .

marked in yellow the smaller form and labeled it as *D. glans*. However, it was not reflected in the publication (Ogden, 1983). Furthermore, this does not correspond with the size-limits of *D. glans* according to the original description (Penard, 1902). Nevertheless, the presence of the short aperture collar in *D. ampullula* is sufficient to differentiate it from *D. glans*, thus we consider both species as valid.

***Diffflugia penardi* (Penard, 1890) Hopkinson, 1909**

According to Ogden and Hedley (1980) the shell of *D. penardi* is transparent or yellow, ovoid and circular in cross-section (Fig. 38). It is thin, usually with a regular outline, and is composed mainly of small diatom frustules arranged on an organic matrix. The aperture is small and circular. Ogden measured five specimens of *D. penardi* (Fig. 14),

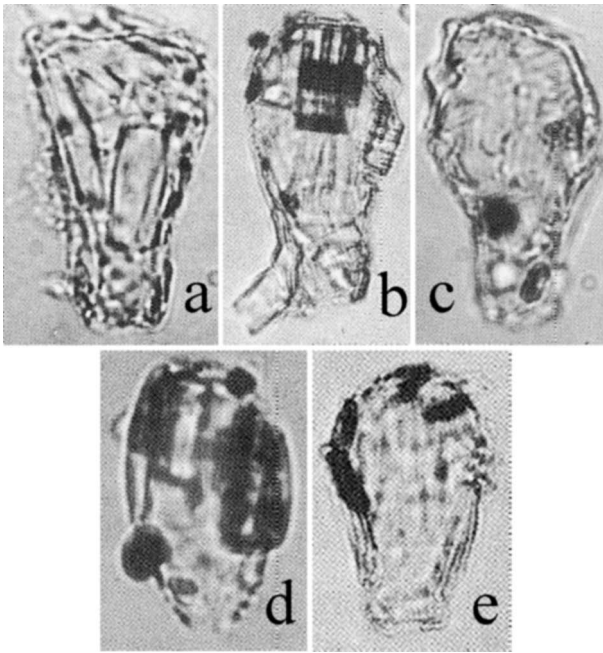


Fig. 34. Different specimens of *Diffflugia humilis*, after Chardez (1991, p. 46, figs 2–6).

one of which is unpublished and four are published (Ogden and Hedley, 1980). Based on these data, the shell dimensions are: length 75 to 94  $\mu\text{m}$ , width 47–54  $\mu\text{m}$ , aperture diameter 16–19  $\mu\text{m}$ .

According to the descriptions of Penard (1890, 1902) the shell of *D. fallax* is hyaline or yellowish, ovoid to elongate-ovoid, rounded or very rarely arched slightly aborally, uncompressed, except sometimes slightly at the oral side, terminating at a circular aperture, often bordered by overlapping scales (Figs 8, f-g; 31, e). The shell is chitinous and covered with amorphous flat scales, which sometimes seem to be particles either of mud or of mica plates. The size is highly variable and it is possible to recognize two different shell types within the species, the longer and narrow type and short and large type. Long individuals vary from 65 to 80  $\mu\text{m}$  in length, whereas short individuals are from 50 to 60  $\mu\text{m}$ , although according to the locality even smaller size shells can be found (Penard, 1890). Because of this heterogeneity in size, Penard (1902) established a new species for the small-size group, namely *D. pristis* (Fig. 31, d).

Hopkinson (in Cash and Hopkinson, 1909) renamed *D. fallax* as *D. penardi* (Cash and Hopkinson, 1909, p. 15, footnote) explaining that “this name [*D. fallax* Penard, 1890] having since the death of Mr. Cash been found to be pre-occupied”. Cash

and Hopkinson (1909) described this species as having short shell with a semi-circular dome tapering convexly downwards from the crown to the aperture, usually studded with diatom frustules, but frequently encrusted with minute sand grains; shell length 60–85  $\mu\text{m}$ , shell width about 30  $\mu\text{m}$ .

*Diffflugia penardi* can be separated from *D. glans* and *D. ampullula* by having a more elongated shell shape, thus we consider it to be a valid species. However, further investigations of transitional forms between these three species are awaited in order to make robust conclusions.

#### *Diffflugia manicata* Penard, 1902

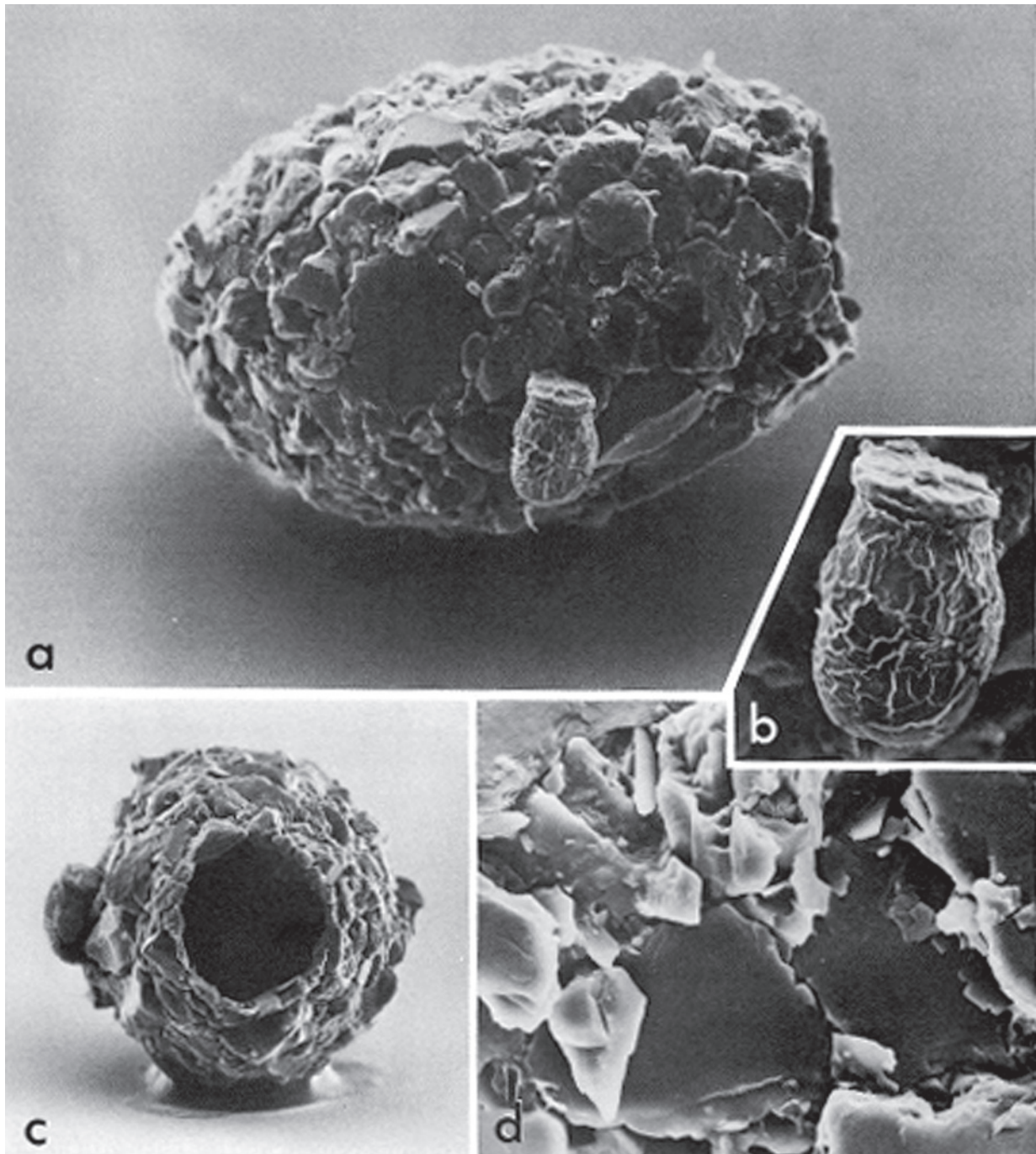
According to Ogden and Hedley (1980) and Ogden (1983) the shell of *D. manicata* is yellow or brown, elongate oval or pyriform, tapering evenly and gradually from a rounded aboral extremity towards the aperture (Fig. 39). The surface is rough and composed mainly of small to medium-size pieces of quartz, although the occasional specimen may also have larger particles. The aperture is circular and surrounded by a distinct pattern of small particles. Ogden measured 43 specimens of *D. manicata* (Fig. 14), all of which were published (Ogden and Hedley, 1980; Ogden, 1983, 1984; Ogden and Živković, 1983). Based on these data, the shell dimensions are: length 60 to 103  $\mu\text{m}$ , width 37–54  $\mu\text{m}$ , aperture diameter 12–20  $\mu\text{m}$ .

Penard (1902) noted that this species closely resembles *D. pyriformis* var. *bryophila* in general shape although it is slightly more ovoid and stocky (Fig. 7, m). However, he failed to provide information about its size. Furthermore, although Penard (1902) discriminated between two similar forms, i.e. *D. manicata* and *D. fallax* (= *D. penardi*), he failed to compare the two directly with each other. Based on the descriptions supplied by Penard (1902 – compare Figs 7, m and 8, f) and Ogden (compare Figs 38 and 39), it is not possible to clearly separate these two species. We thus consider *D. manicata* Penard, 1902 to be a junior synonym of *D. penardi* (Penard, 1890) Hopkinson, 1909.

#### *Diffflugia tenuis* (Penard, 1890) Ogden, 1983

According to Ogden (1983) the shell of *D. tenuis* is usually transparent, cylindrical or slightly pyriform, composed of a mixture of mainly small to medium-size pieces of angular quartz, but occasionally with larger particles (Fig. 40). Sometimes additional particles give the aboral region a pointed outline instead of the usual rounded contours. The aperture is roughly circular, often having an irregular outline because of the mixture of particles surrounding



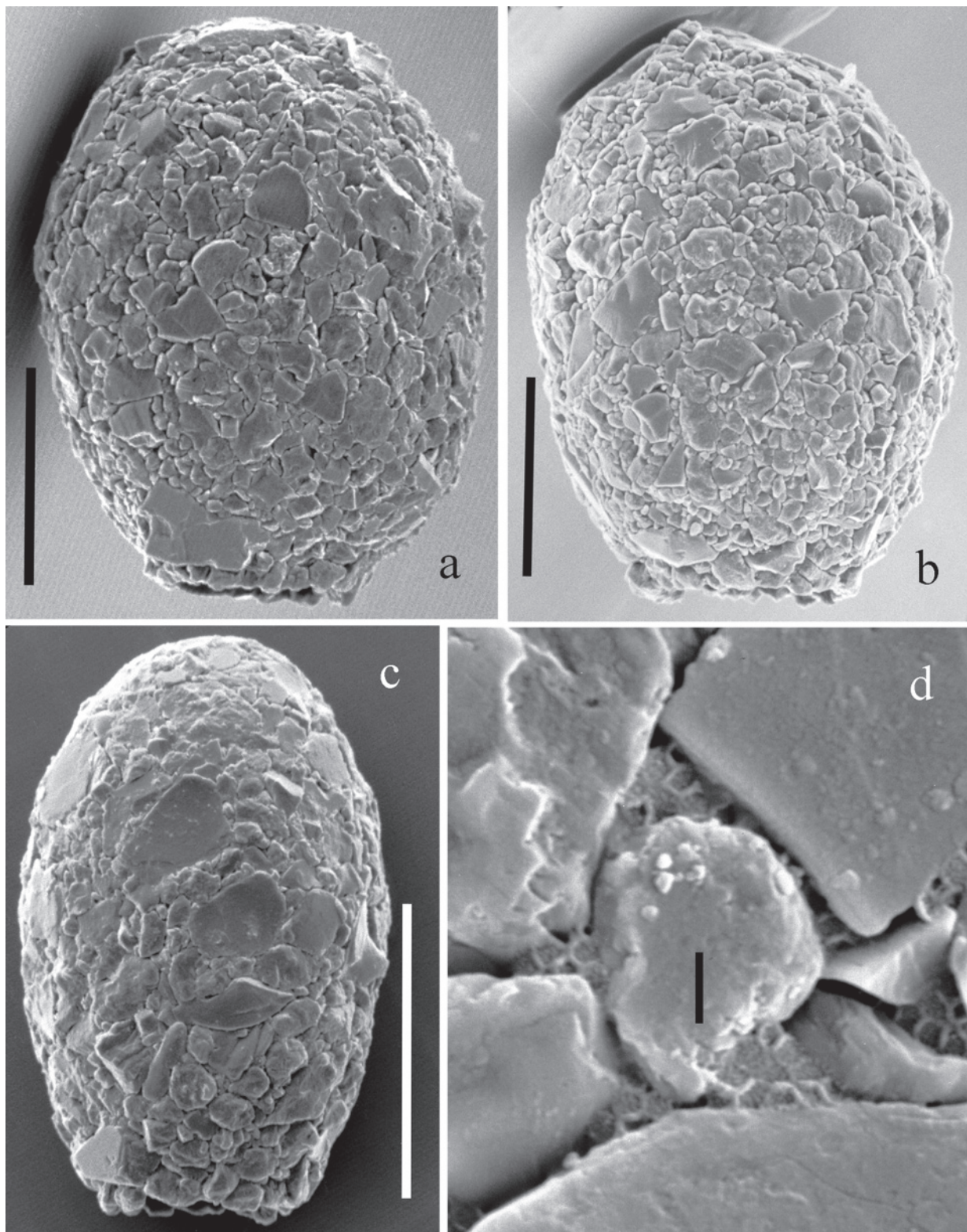


**Fig. 35.** *Diffugia glans* from Ogden (1983, p. 8, fig. 4): a – lateral view (x 1400), b – detail of small unidentified cyst (x 300), c – apertural view (x 1000), d – portion of shell surface showing the closely packed particles (x 3500).

it. This description is in good agreement with the original by Penard (1890). Ogden measured 30 specimens of *D. tenuis* (Fig. 14), 25 published (Ogden, 1983), five unpublished. Based on these data, the shell dimensions are: length 60 to 87  $\mu\text{m}$ ,

width 37–50  $\mu\text{m}$ , aperture diameter 17–27  $\mu\text{m}$ .

The slide deposited in NHM (Fig. 41) was labeled by Penard as *D. pyriformis* var. *tenuis*. However, this does not correspond with his original description (Penard, 1890 – see Figs 8 a-b) which



**Fig. 36.** Different specimens of *Diffugia ampullula* from C.G. Ogden's SEM collection: a-c – lateral view (a – SEM CZ-05.170, b – SEM CZ-04.507, c – SEM CZ-05.051), d – structure of organic cement (SEM CZ-04.949). Scale bars: a-c – 30 µm; d – 1 µm.

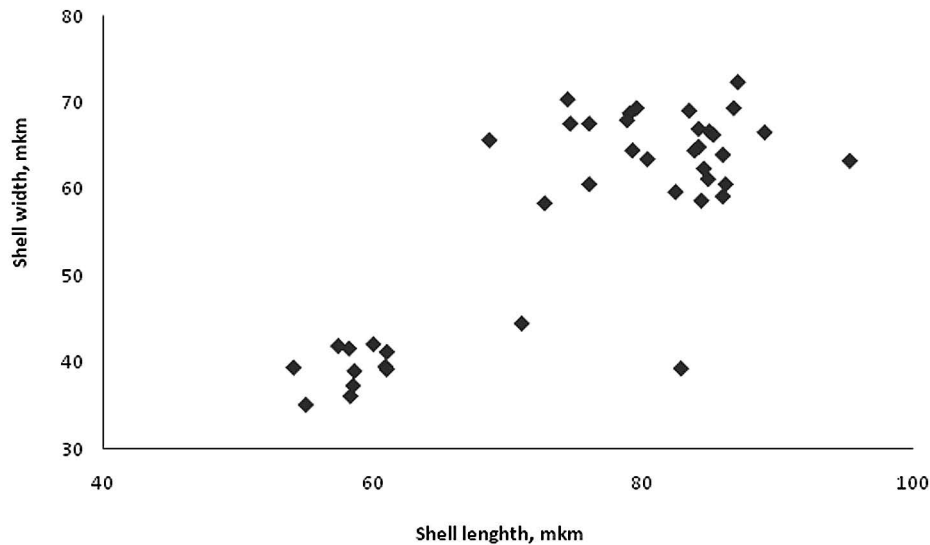


Fig. 37. Length-width bivariate scatter plot of *Diffflugia ampullula*, based on C.G. Ogden's measurements.

more closely resembles *D. bryophila* (see fig. 26 in Mazei and Warren, 2014). However, it is not evident that the differences between *D. tenuis* and *D. penardi* are sufficient for these taxa to be separated at species level (compare Figs 8, a-b vs 8, f-g and Figs 38 vs 40). According to all the images of specimens in the Penard and Ogden collections, both species are highly variable in terms of general shape and shell coverage. Perhaps, the only possibly significant difference is the larger aperture diameter in *D. tenuis*. However, size distributions of this character overlap considerably in both species. Thus we consider *D. tenuis* (Penard, 1890) Ogden, 1983 to be a junior synonym of *D. penardi* (Penard, 1890) Hopkinson, 1909.

#### *Diffflugia masaruzii* van Oye, 1958

According to Ogden (1983) the shell of *D. masaruzii* is transparent, ovoid and composed of a mixture of flattish siliceous particles including some diatom frustules, to give a fragile structure with an irregular surface and outline (Fig. 42). The aperture is circular, wide, and usually with an irregular margin. Ogden measured three specimens of *D. masaruzii* (Fig. 14), two published (Ogden, 1983), one unpublished: shell length 57–67  $\mu\text{m}$ , shell breadth 39–50  $\mu\text{m}$ , aperture diameter 24–25  $\mu\text{m}$ .

According to van Oye (1958) the shell of *D. masaruzii* is ovoid, transparent, chitinous, and bears a few quartz grains that are very large and conspicuous (Figs 11, f, g). The aperture is usually not surrounded by sand grains. Shell length 72–78

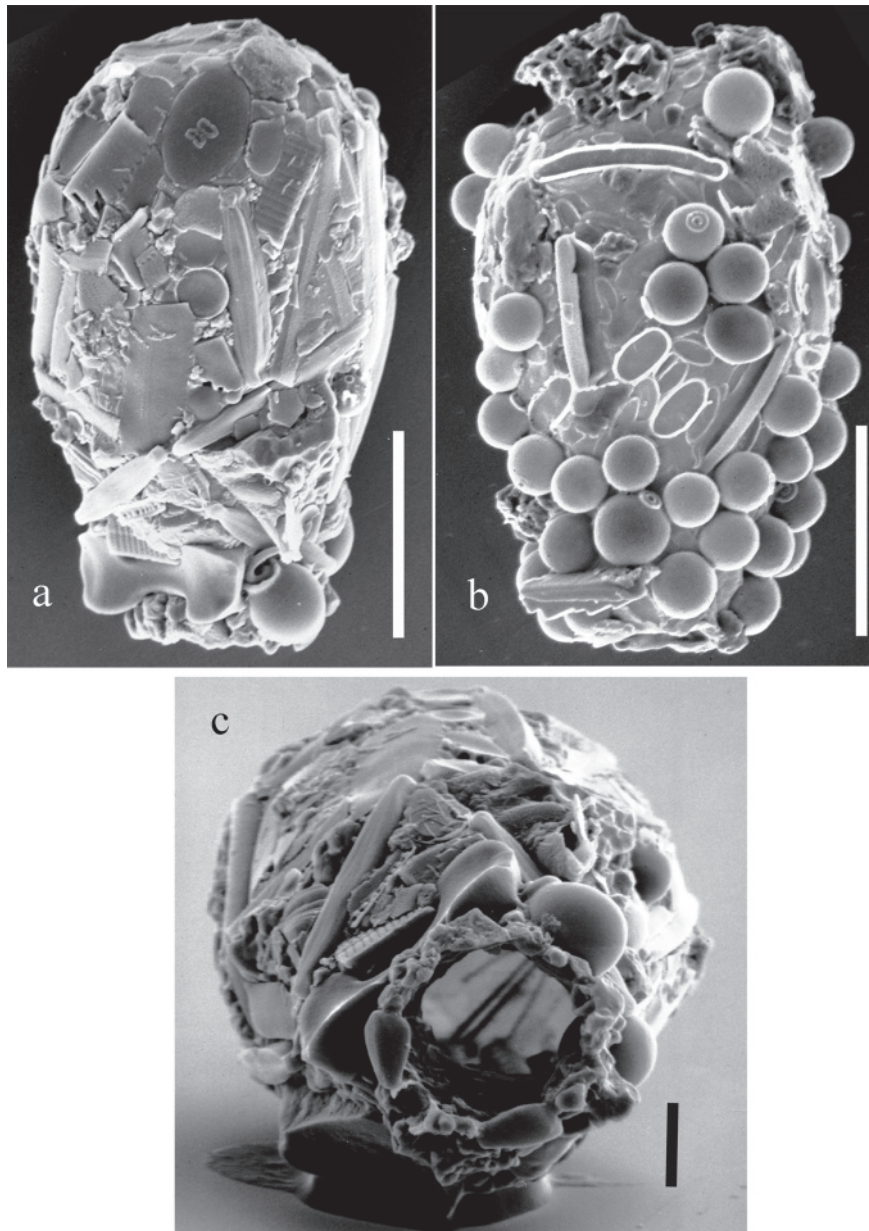
$\mu\text{m}$ , shell width 44–50  $\mu\text{m}$ , aperture diameter 28–30  $\mu\text{m}$ . In our opinion the validity of this species is highly questionable. Based on the description by van Oye (1958) it closely resembles *D. penardi* in terms of the size and general shape of the shell. By contrast, according to Ogden's data, it is more similar to *D. pristis*. A decision concerning the correct identity of this taxon must await a detailed redescription. In the meantime it should continue to be recognized as a nominal species of *Diffflugia*.

#### *Diffflugia molesta* Penard, 1902 species complex

The members of this group include *D. molesta*, *D. brevicolla*, *D. pyriformis* var. *atricolor*, *D. difficilis*, *D. microstoma*, *D. levanderi*, and *D. decloitrei* (Fig. 17, group '2b'; Figs 19, a–e).

#### *Diffflugia molesta* Penard, 1902

According to Ogden (1983) the shell of *D. molesta* is brown, ovoid or ovoid-elongate, sometimes with a small apertural collar and sometimes arched aborally. It is composed of a mixture of quartz particles and diatom frustules, the former usually being predominant (Fig. 43). The aperture is roughly circular, irregular in outline, and mainly surrounded by small particles. Ogden measured four specimens of *D. molesta* (Ogden, 1983) with the shell length ranging from 106 to 114  $\mu\text{m}$ , shell width 61–87  $\mu\text{m}$ , and aperture diameter 28–43  $\mu\text{m}$ . Ogden (1983) mentioned that the examination of four specimens is insufficient in order to make an



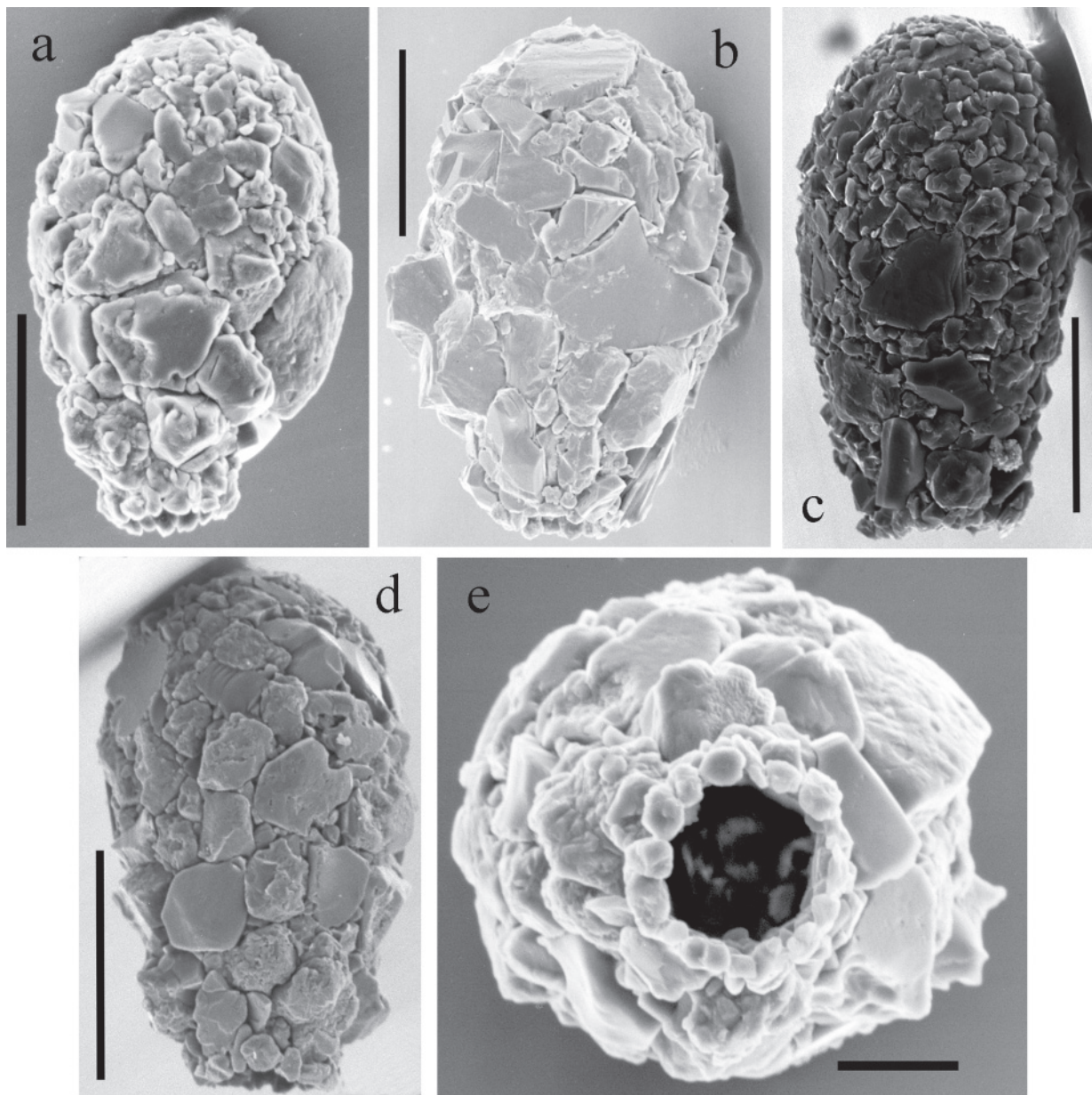
**Fig. 38.** Different specimens of *Diffugia penardi* from C.G. Ogden’s SEM collection: a-b – lateral view (a – SEM EM-11-624, b – SEM EM-11-713), c – apertural view (SEM EM-11-626). Scale bars: a-b – 30  $\mu\text{m}$ ; c – 10  $\mu\text{m}$ .

accurate identification noting that his identification was therefore tentative. In fact, his specimens do not match with the description and illustration of Penard (1902), a point which we discuss in the section on *D. levanderi* (see below).

In his original description (Fig. 7, a), Penard (1902) noted that the shell of *D. molesta* resembles *D. fallax* and *D. glans*, although narrower than the former and a little broader than the latter (Figs 31, a-c). He also notes that the shell is grayish or

brownish in color, and is much larger than other two species, the average length being 120–125  $\mu\text{m}$ . The aperture is large and circular and the scales that surround it are not conspicuously different from those of the rest of the shell.

Penard (1902, p. 248–249) acknowledged that “there are in fact four species on a cursory examination or take all ones for the others, *D. fallax* [= *D. penardi*], *D. glans*, *D. molesta*, and *D. pristis*, and it took me a long time and the multiplied



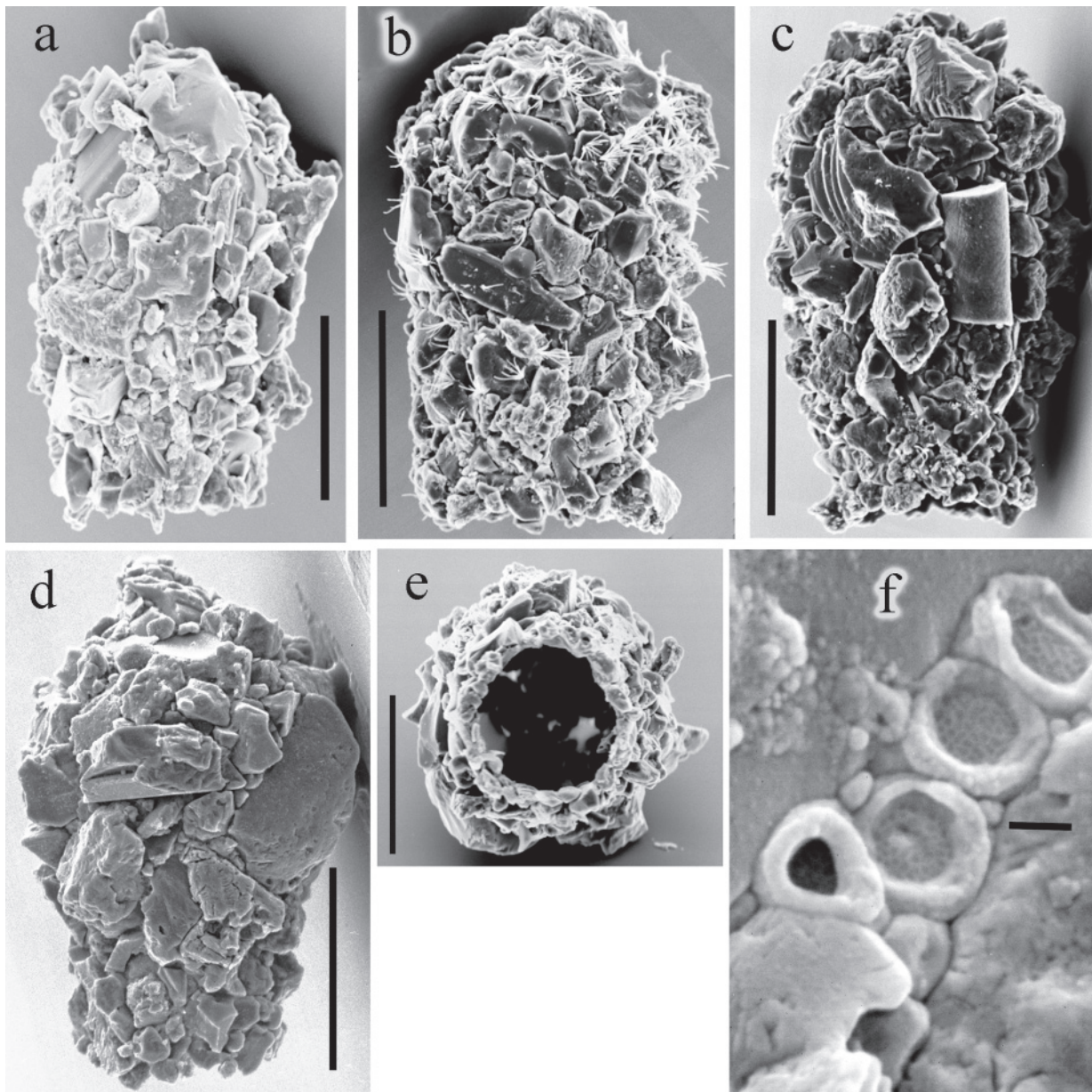
**Fig. 39.** Different specimens of *Diffugia manicata* from C.G. Ogden's SEM collection: a-d – lateral view (a – SEM CZ-01.123, b – SEM CZ-10.297, c – SEM CZ-07.026, d – SEM CZ-02.594), e – apertural view (SEM CZ-01.122). Scale bars: a-d – 30  $\mu\text{m}$ ;; e – 10  $\mu\text{m}$ .

comments to disentangle their special characters". We here support Penard's decision and separate these species by their size and shape, *D. molesta* being significantly larger than the other three.

***Diffugia brevicolla* Cash et Hopkinson, 1909**

According to Ogden (1980) the shell of *D. brevicolla* is transparent, yellow or light brown, and almost spherical with a short neck (Fig. 44). The neck, from the lateral view, varies from being

easily visible (Fig. 44, b) to being obscured by shell components (Fig. 44, d). The shell is composed mainly of a mixture of small diatom frustules, small pieces of quartz and siliceous cysts of chrysomonad flagellates. In addition, the empty shells of smaller testate amoebae, for example, *Trinema*, are often attached to the shell of *D. brevicolla* and aggregations of such material are sometimes attached to its aboral extremity. The aperture is circular, bordered by an arrangement of small particles and diatoms, and



**Fig. 40.** Different specimens of *Diffflugia tenuis* from C.G. Ogden's SEM collection: a–d – lateral view (a – SEM CZ-06.721, b – SEM CZ-06.728, c – SEM CZ-06.751, d – SEM CZ-07.364), e – apertural view (SEM CZ-06.713), f – structure of organic cement (SEM 060598). Scale bars: a–e – 30  $\mu\text{m}$ , f – 0.3  $\mu\text{m}$ .

usually has a regular outline. Ogden measured 84 specimens of *D. brevicolla* (Fig. 14), 79 published (Ogden, 1980, 1984), five unpublished: shell length 66–119  $\mu\text{m}$ , shell breadth 48–100  $\mu\text{m}$ , aperture diameter 22–48  $\mu\text{m}$ .

This species can be treated as an intermediate form between those with a pyriform shell (described in Mazei and Warren, 2014) and those with an ovoid or spherical shell. From all of the species with pyriform and ovoid shells it is easily distinguished by

high width/length ratio (0.81 on average which is close to those of species with spherical shells). The species that most closely resembles *D. brevicolla* in terms of general shape is *D. petricola*. The latter species is usually larger than the former, however, their size distributions only slightly overlap (see description of *D. petricola* in Mazei and Warren, 2014).

A variety of *D. brevicolla*, namely *Diffflugia brevicollavar. major*, with larger size (i.e. shell length

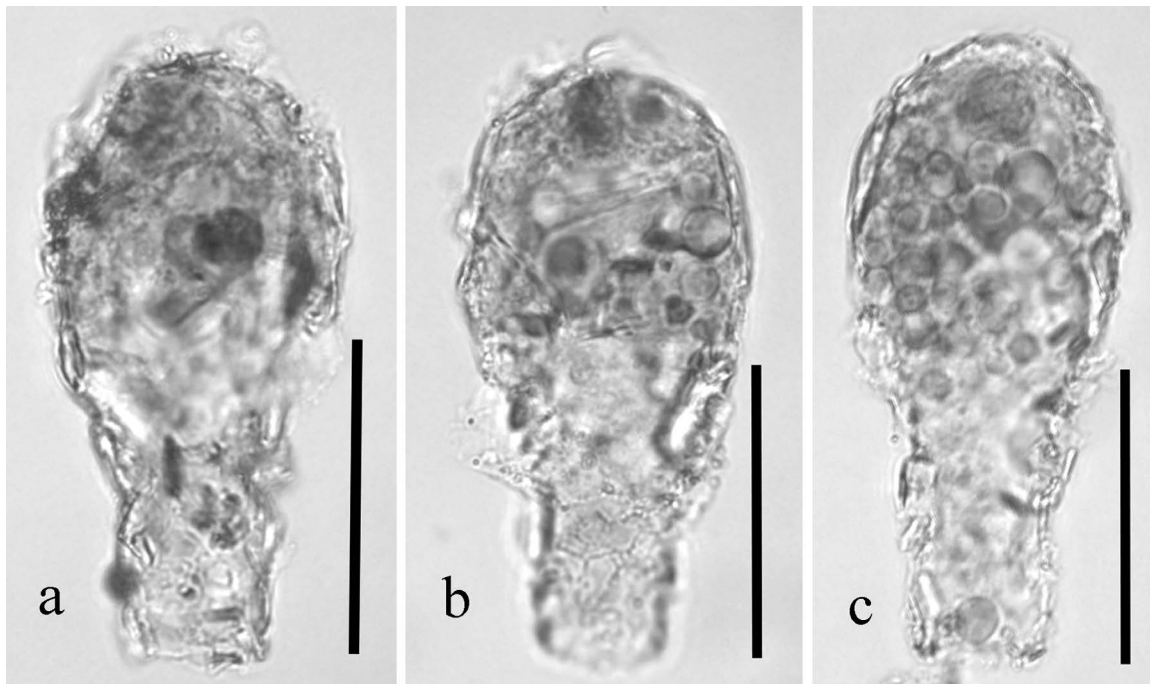


Fig. 41. Different specimens of *Diffflugia pyriformis* var. *tenuis* from E. Penard's slide 20.12.8.277. Scale bar: 50  $\mu\text{m}$ .

190  $\mu\text{m}$ , shell width 160  $\mu\text{m}$ , aperture diameter 60  $\mu\text{m}$ ) was described by Gauthier-Lièvre and Thomas (1958). Based on its size and short, prominent neck (see fig. 15c in Gauthier-Lièvre and Thomas, 1958) this taxon should be placed within the *D. pyriformis* species complex (see: Mazei and Warren, 2014). However, the position of *D. brevicolla* var. *major* remains unclear pending more data.

***Diffflugia pyriformis* var. *atricolor* Penard, 1902**

According to Penard (1902), *D. pyriformis* var. *atricolor* is characterized by its stocky shape, short, wide neck, and circular aperture; shell length is from 90 to 125  $\mu\text{m}$ . The individuals illustrated by Penard (1902) and deposited in the slide collection of NHM (Fig. 45) are almost identical with both description of *D. brevicolla* made by Cash and Hopkinson (1909) and Ogden (1980). We consider *D. pyriformis* var. *atricolor* a junior synonym of *D. brevicolla*.

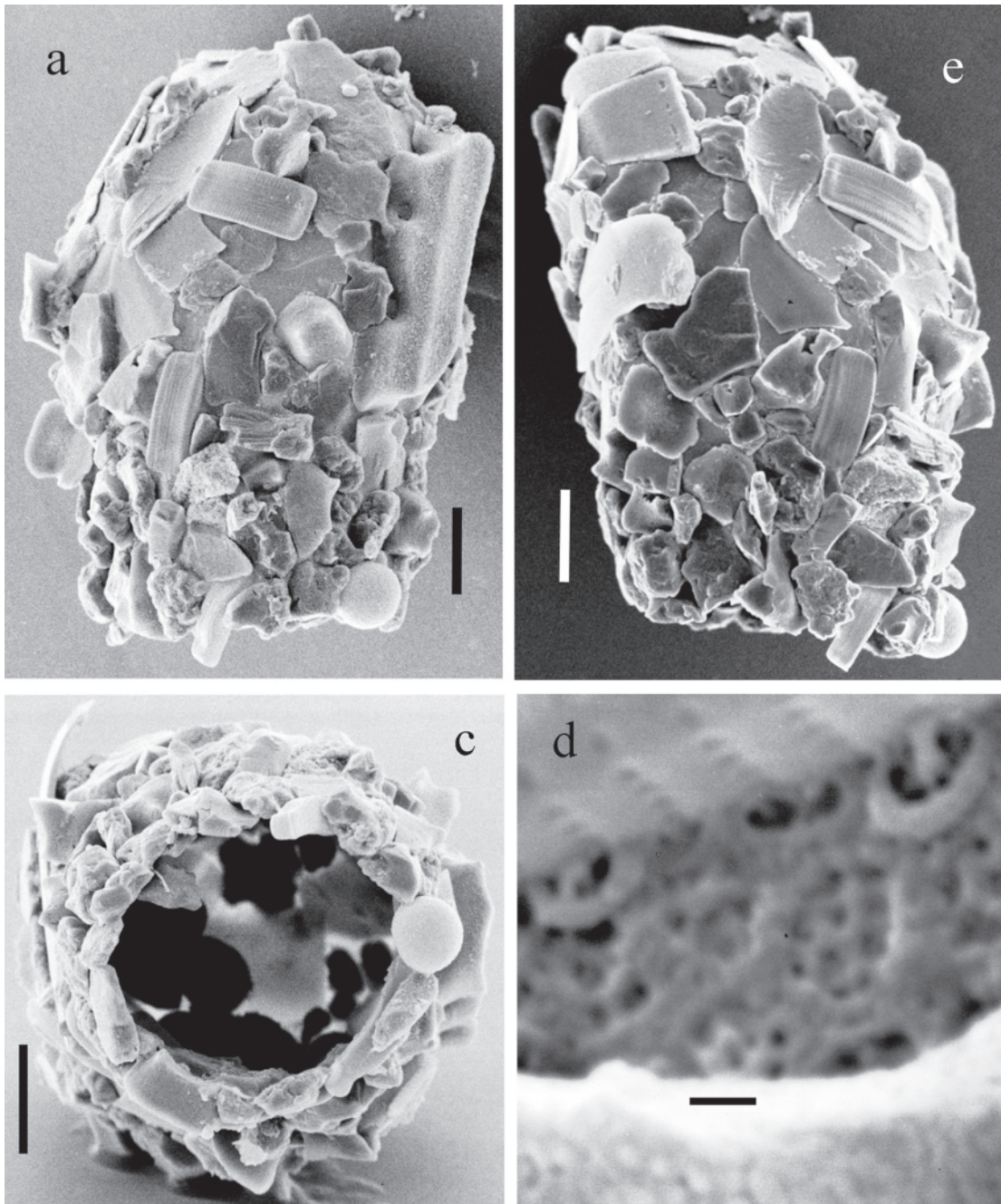
***Diffflugia difficilis* Thomas, 1954**

According to Ogden and Živković (1983) the shell of *D. difficilis* is transparent, ovoid, thin with a small apertural collar (Fig. 46). It is composed of small to medium pieces of angular quartz arranged to make a relatively smooth surface, with the occasional projection of some particles. A short collar made mainly of regularly arranged smallish

particles surrounds the circular aperture. Ogden measured three specimens of *D. difficilis* (Fig. 14), one published (Ogden and Živković, 1983), two unpublished. Based on these data, the shell length ranges from 81 to 114  $\mu\text{m}$ , shell width 56–69  $\mu\text{m}$ , and aperture diameter 18–23  $\mu\text{m}$ .

According to Thomas (1954) the shell of *D. difficilis* is ovoid, circular in cross-section (Figs 12, a, b). The base of the shell is rounded, very often with a few prominent protuberances. The aperture is circular with a diameter ranging between half and two thirds of the shell width and surrounded by a small collar. The shell is hyaline, transparent, and covered by regularly arranged sand grains. Shell length 83–93  $\mu\text{m}$ , shell width 20–27  $\mu\text{m}$ , aperture diameter 6–9  $\mu\text{m}$ .

Chardez (1956) described *Diffflugia difficilis* var. *ecornis* which lacks visible protuberances but otherwise closely resembles the typical form in both size and shape (Fig. 12c). However, both Gauthier-Lièvre and Thomas (1958) and Ogden and Živković (1983) underlined high variability in shape including the presence of protuberances. We therefore consider *D. difficilis* var. *ecornis* to be a junior synonym of *D. difficilis* the main defining feature of which is the short collar surrounding the aperture.



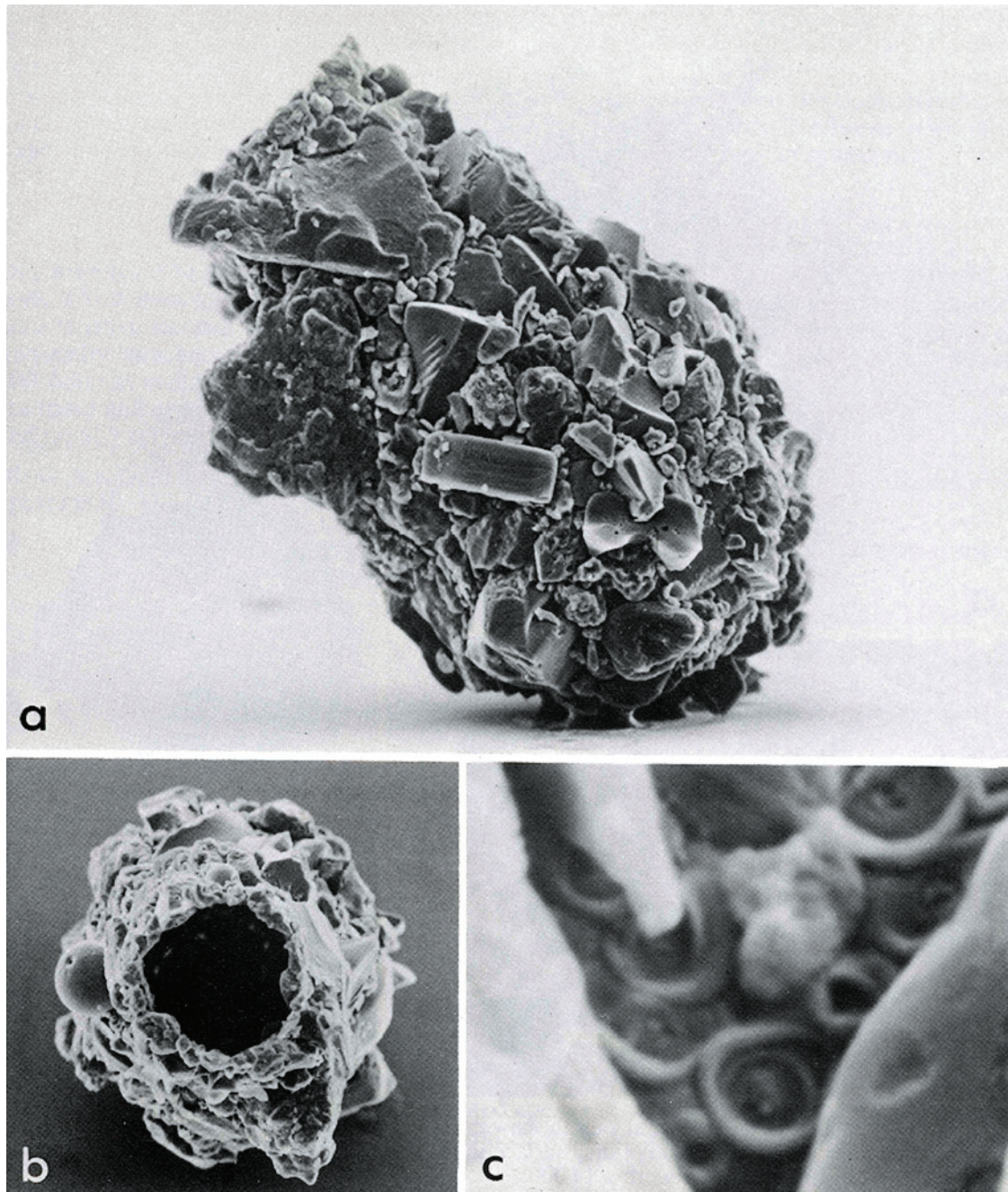
**Fig. 42.** Different specimens of *Diffflugia masaruzii* from C.G. Ogden's SEM collection: a-b – lateral view (a – SEM CZ-05.762, b – SEM CZ-08.752), c – apertural view (SEM CZ-05.767), d – structure of organic cement (SEM CZ-08.749). Scale bars: a-c – 10  $\mu\text{m}$ , d – 0.3  $\mu\text{m}$ .

***Diffflugia microstoma* (Thomas, 1954) Ogden, 1983**

According to Ogden (1983) the shell of *D. microstoma* is ovoid or subspherical, composed mainly of a mixture of diatom frustules and small to medium pieces of flattish quartz (Fig. 47). The particles are arranged to give a relatively smooth outline, with the

diatom frustules appearing to be additions to the main structure. The aperture is circular and usually surrounded by a border of small particles. Ogden measured 15 specimens of *D. microstoma* (Fig. 14), all published (Ogden, 1983): shell length 76–105  $\mu\text{m}$ , shell width 63–83  $\mu\text{m}$ , and aperture diameter 18–29  $\mu\text{m}$ .

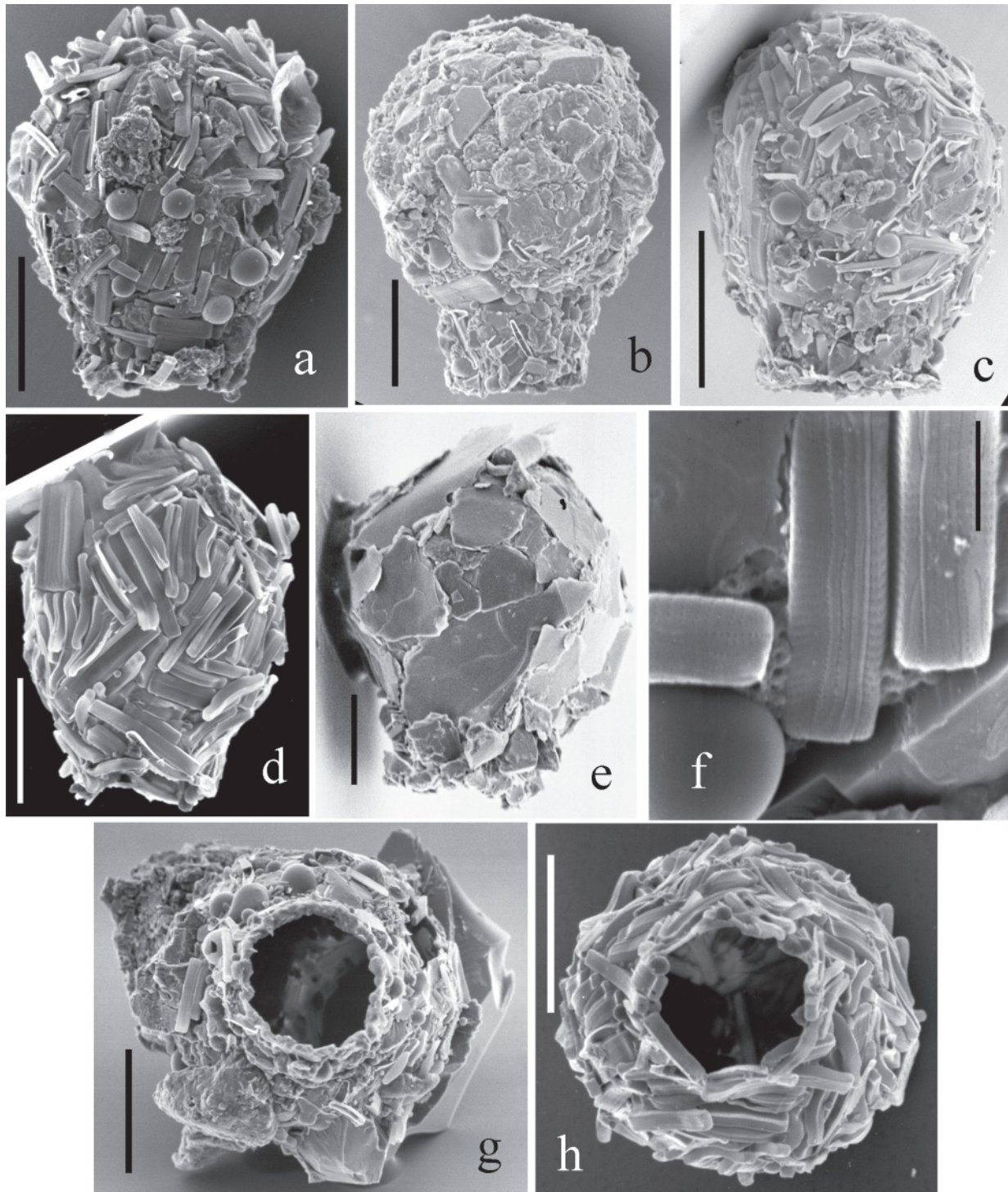




**Fig. 43.** *Diffflugia molesta* from Ogden (1983, p. 39, fig. 25): a – lateral view (x 910), b – apertural view (x 740), c – detail of organic cement (x 24000).

Thomas (1954, see his plate 2, fig. 9) described this taxon as *D. globularis* var. *microstoma* characterizing it as having an almost spherical shell (length 68–84  $\mu\text{m}$ ) and a small aperture (diameter 18–23  $\mu\text{m}$ ). Later Gauthier-Lièvre and Thomas (1958) proposed it as a synonym of *D. minuta* Rampi, 1950. However, *D. minuta* is much smaller than the

organism described by Thomas (1954). Consequently, Ogden (1983) elevated *D. globularis* var. *microstoma* to species rank as *D. microstoma*. However, if we compare this taxon with *D. difficilis* (Figs 46 vs 47 and Figs 19, a vs 19, b), it is not possible to find any significant differences between them in terms of shell size and shape. Thus, we consider *D.*



**Fig. 44.** Different specimens of *Diffflugia brevicolla* from C.G. Ogden's SEM collection: a-e – lateral view (a – SEM CZ-02.319, b – SEM CZ-02.683, c – SEM CZ-02.798, d – SEM CZ-02.723, e – SEM CZ-10.617), f – structure of organic cement (SEM CZ-02.320), g-h – apertural view (g – SEM CZ-02.329, h – SEM CZ-02.732). Scale bars: a-e, g-h – 30  $\mu$ m, f – 3  $\mu$ m.

*microstoma* (Thomas, 1954) Ogden, 1983 to be a junior synonym of *D. difficilis* Thomas, 1954.

#### *Diffflugia levanderi* Playfair, 1918

According to Ogden and Živković (1983) the shell of *D. levanderi* is ovoid, sometimes almost spherical, composed of flattish and angular pieces of quartz to give a regular outline (Fig. 48, a). The aperture is circular, well defined and surrounded by small particles. Ogden measured five specimens of *D. levanderi* (Fig. 14), all published (Ogden and Živković, 1983): shell length 95–104 µm, shell width 76–92 µm, and aperture diameter 32–40 µm.

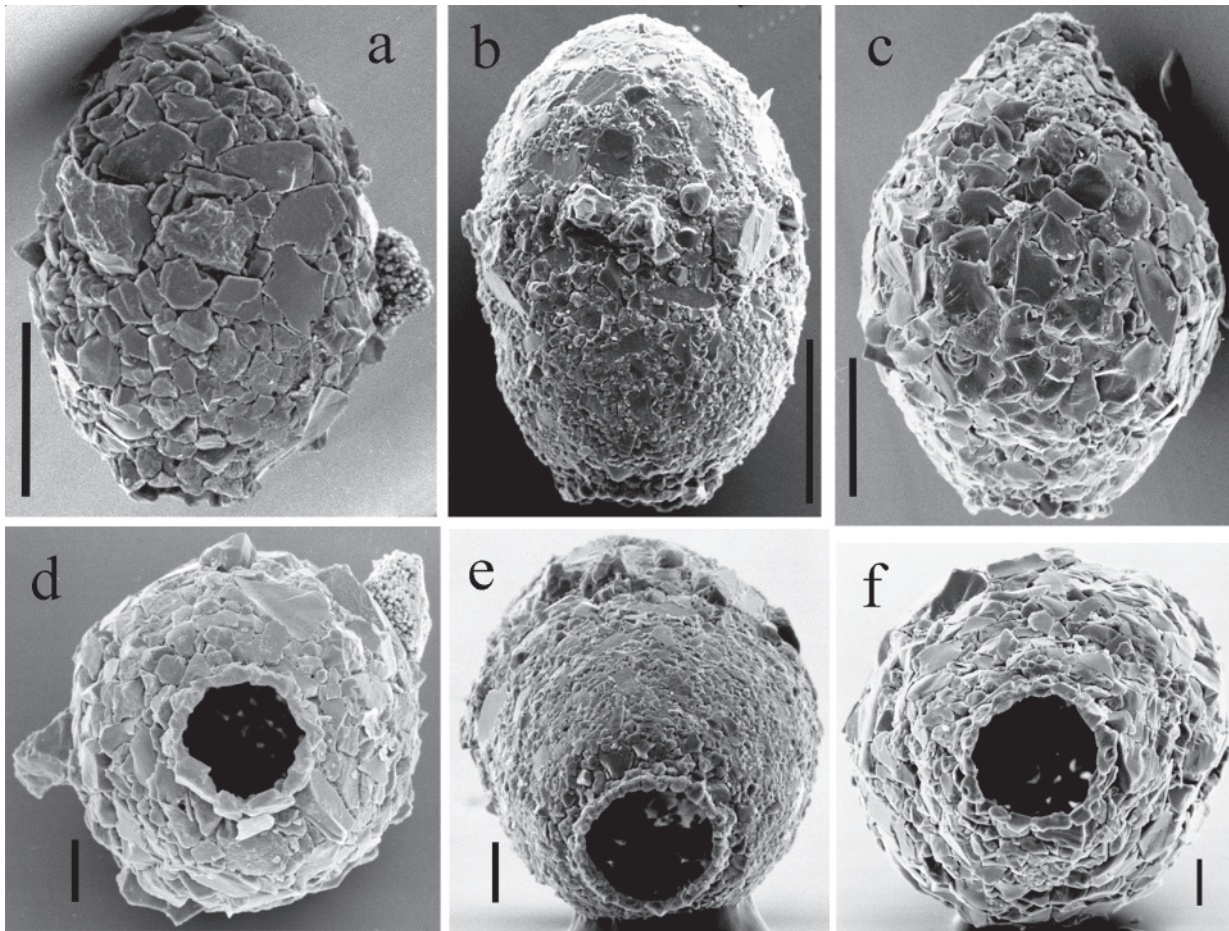
According to Playfair (1918) the shell of *D. levanderi* is lanceolate or mitriform, short and broad, with a pointed, dome-shaped posterior region, and arched sides that converge towards the aperture which is wide, circular and without either a rim or collar. Shell length 78–116 µm, shell width 51–78 µm, aperture diameter 32–42 µm. Note that these dimensions represent those of two shell types: large with coarse particles (Fig. 12 e), as well as small and chitinous, with small, scattered particles (Fig. 12, f). Ogden (1983) left the group of larger specimens within *D. levanderi* and transferred the group of smaller individuals to *D. decloitrei*. Although we agree with the latter conclusion, we question the validity of *D. levanderi* which bears a strong resemblance to another species of similar size, namely *D. molesta*. Comparing the illustrations from the original descriptions (Fig. 7, a vs Fig. 12, f) it can be seen that both forms have an ovoid shell with a simple aperture (i.e., without a collar), and an overlapping size distribution. It is noteworthy that the specimens of *D. molesta* and *D. levanderi* described by Ogden (1983) and Ogden and Živković (1983) respectively are different in shape (Fig. 48, a vs Fig. 43, a). However, Ogden (1983) noted that he identified *D. molesta* only tentatively, and his specimens do not much resemble the original illustrations of *D. molesta* made by Penard (1902). Therefore it is likely that the specimens shown in Fig. 43 belong to another species. Furthermore, only four specimens of *D. molesta* were investigated by Ogden (1983), which is insufficient to make reliable decisions concerning its taxonomy. Given that original illustrations of these two species are almost identical (compare Figs 7, a and 12, f) we consider them to be conspecific with *D. levanderi* Playfair, 1918 – a junior synonym of *D. molesta* Penard, 1902.



Fig. 45. *Diffflugia pyriformis* var. *atricolor* from E. Penard's slide 04.5.9.128. Scale bar: 50 µm.

#### *Diffflugia decloitrei* Godeanu, 1972

According to Ogden (1983) the shell of *D. decloitrei* is ovoid, tapering evenly from the mid-body towards both the aperture and the aboral extremity (Figs 48, b-d). It has a well defined outline, and the arrangement of flattish pieces of quartz gives it a smooth surface. The aperture is circular often with a rugged outline due to the placement of the flattish particles. Ogden measured 15 specimens of *D. decloitrei* (Fig. 14), 10 published (Ogden, 1983), five unpublished: shell length 68–95 µm, shell width 35–55 µm, and aperture diameter 15–27 µm. According to Godeanu (1972) the shell of *D. decloitrei* is broad-oval, similar to that of *D. levanderi*, and covered with sand grains of different size (Fig. 13, b). The aperture is large and circular. The shell dimensions are: length 62–86 µm, width 40–56 µm, aperture diameter 20–23 µm. *Diffflugia decloitrei* differs from *D. penardi* in several respects, most notably its ovoid (vs. pyriform) shape (compare Figs 19, d and 19, f). It is uncertain whether such differences are sufficient for species separation. Thus we retain this taxon as a separate but questionable species and transfer it to the *D. molesta* species complex.



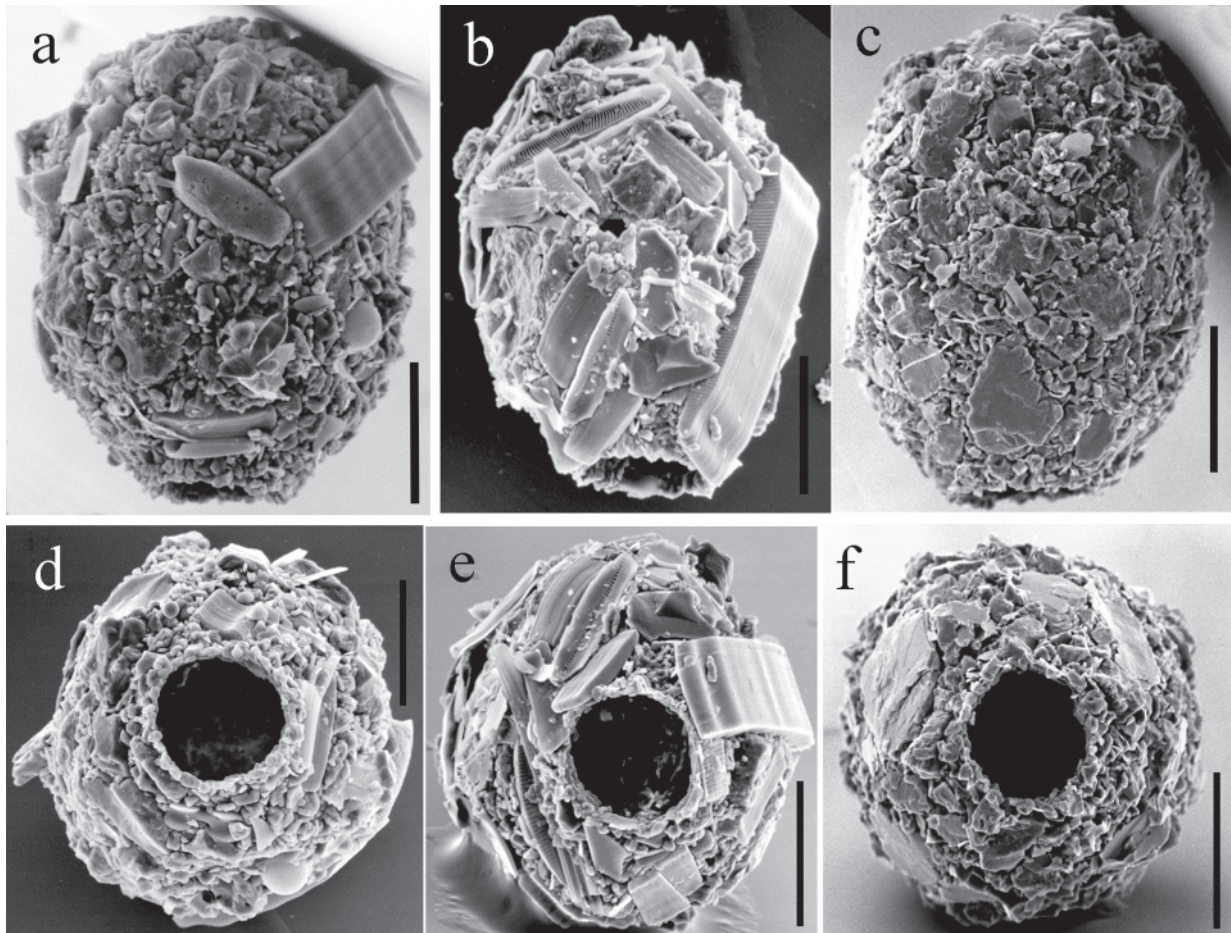
**Fig. 46.** Different specimens of *Diffflugia difficilis* from C.G. Ogden's SEM collection: a-c – lateral view (a – SEM CZ-03.087, b – SEM CZ-11.310, c – SEM CZ-11.287), d-f – apertural view (d – SEM CZ-03.098, e – SEM CZ-11.308, f – SEM CZ-11.284). Scale bars: a-c – 30  $\mu$ m, d-f – 10  $\mu$ m.

## Acknowledgements

This work was financially supported by the Russian Scientific Foundation (14-14-00891) and the award (to YM) from the Royal Society International Travel Grant Scheme (2009R1).

## References

- Beyens L. and Chardez D. 1994. On the habitat specificity of the testate amoebae assemblages from Devon Island (NWT, Canadian Arctic), with the description of a new species: *Diffflugia ovalisina*. Arch. Protistenkd. 144, 137–142.
- Blanc H. 1892. Les difflogiés de la faune profonde du Lac Léman. Recueil inaugural de l'Université de Lausanne. Lausanne, pp. 377–387.
- Carter H.J. 1864. On freshwater Rhizopoda of England and India. Ann. Mag. Nat. Hist. Ser. 3. 13, 18–39.
- Cash J. and Hopkinson J. 1909. The British freshwater Rhizopoda and Heliozoa. Vol. II. Rhizopoda. Part II. The Ray Society (London), publ. no 89.
- Chardez D. 1956. Contribution a la faune rhizopodique de Belgique. Rev. verviet. Hist. Natur. 5–6, 42–49.
- Chardez D. 1957. Contribution a la faune rhizopodique de Belgique. II. Thécamoebiens de la Fagne (I.). Rev. Verviet. Hist. Natur. 14, 62–79.
- Chardez D. 1962. Sur *Diffflugia globularis* var. *sphaerica* Chardez. Rev. Verviet. Hist. Natur. 19, 82–83.
- Chardez D. 1991. Note sur *Diffflugia humilis* sp. n. (Protozoa: Rhizopoda: Testacea). Acta Protozool. 30, 45–47.
- Dujardin F. 1837. Sur une nouvelle espèce de



**Fig. 47.** Different specimens of *Diffflugia microstoma* from C.G. Ogden's SEM collection: a-c – lateral view (a – SEM CZ-01.223, b – SEM CZ-01.017, c – SEM CZ-07.897), d-f – apertural view (d – SEM CZ-01.225, e – SEM CZ-01.018, f – SEM CZ-07.901). Scale bars: a-f – 30  $\mu$ m.

*Gromia* et sur les difflogiés. Annales des Sciences Naturelles. Paris. Zoologie. 8, 310–314.

Dujardin F. 1841. Histoire naturelle des Zoo-phytes. Infusoires, comprenant la physiologie et la classification de ces animaux, et la manière de les étudier à l'aide du microscope. Librairie Encyclopédique de Roret, Paris.

Ehrenberg G.C. 1838a. Die Infusionstierchen als Vollkommene Organismen. Verlag. von Leopold Voss, Leipzig.

Ehrenberg G.C. 1838b. Atlas von Vier und Sechzig Kupfertafeln zu Christian Gottfried Ehrenberg über Infusionstierchen. Verlag. von Leopold Voss, Leipzig.

Foissner W. and Korganova G.A. 2000. The *Centropyxis aerophila* complex (Protozoa: Testacea). Acta Protozool. 39, 257–273.

Gauthier-Lièvre L. and Thomas R. 1958. Les genres *Diffflugia*, *Pentagonia*, *Maghrebina* et

*Hoogenraadia* (Rhizopodes testaces) en Afrique. Arch. Protistenkd. 103, 241–370.

Godeanu S. 1972. Especies nuevas de thécamoebiens (Protozoa, Rhizopoda, Arcellinida). Rev. Roum. Biol. (sér. Zool.). 17, 227–236.

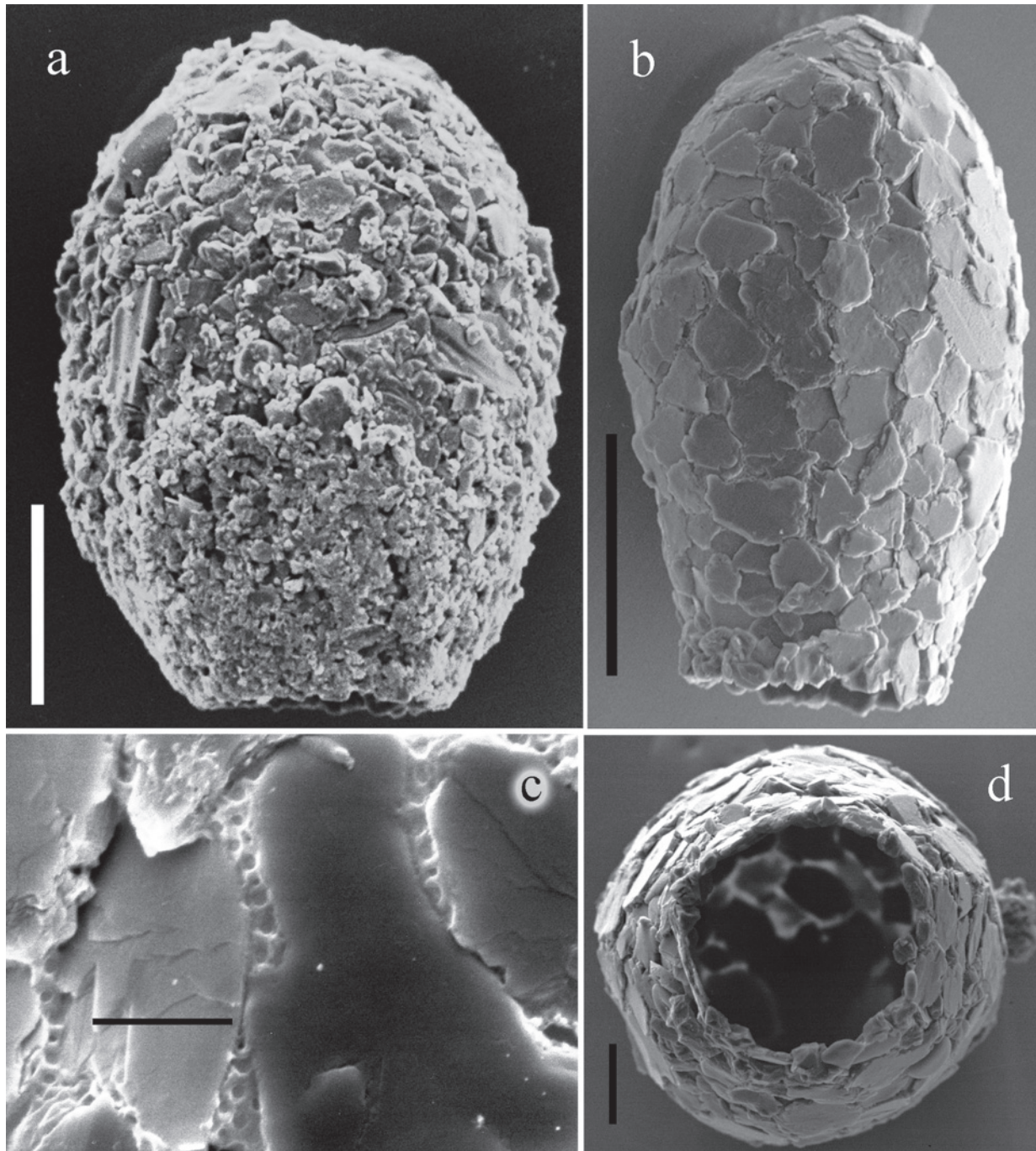
Laminger H. 1971. Sedimentbewohnende Schalenamöben (Rhizopoda Testacea) der Finstertaler Seen (Tirol). Arch. Hydrobiol. 69, 106–140.

Leclerc M. 1815. Note sur la Difflogie, nouveau genre de Polype amorph. Mémoires du Muséum d'Histoire Naturelle (Paris). 2 (12), 474–478.

Leidy J. 1877. Remarks upon American species of *Diffflugia*. Proceedings of the Academy of Natural Sciences of Philadelphia, 3rd series, 29, 306–308.

Leidy J. 1879. Fresh-water Rhizopods of North America. Rep. US Geol. Surv. Terr. 12, 1–324.

Mazei Yu. and Warren A. 2012. A survey of the testate amoeba genus *Diffflugia* Leclerc, 1815 based on specimens in the E. Penard and C.G.



**Fig. 48.** Different specimens of *Diffflugia levanderi* (a) and *D. decloitrei* (b-d) from C.G. Ogden's SEM collection: a-b – lateral view (a – SEM CZ-03.691, b – SEM CZ-06.144), c – structure of organic cement (SEM CZ-07.858), d – apertural view (SEM CZ-06.143). Scale bars: a-b – 30  $\mu\text{m}$ , c – 3  $\mu\text{m}$ , d – 10  $\mu\text{m}$ .

Ogden collections of the Natural History Museum, London. Part 1: Species with shells that are pointed aborally and/or have aboral protuberances. *Protistology*. 7(3), 121–171.

Mazei Yu. and Warren A. 2014. A survey of the testate amoeba genus *Diffflugia* 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. *Protistology*. 8(4), 133–171.

Ogden C.G. 1980. Shell structure in some pyriform species of *Diffflugia* (Rhizopoda). *Arch.*

Protistenkd. 123, 455–470.

Ogden C.G. 1983. Observations on the systematics of the genus *Diffflugia* in Britain (Rhizopoda, Protozoa). Bull. Brit. Mus. nat. Hist. (Zool.). 44, 1–73.

Ogden C.G. 1984. Notes on testate amoebae (Protozoa: Rhizopoda) from Lake Vlasina, Yugoslavia. Bull. Brit. Mus. nat. Hist. (Zool.). 47, 241–263.

Ogden C.G. 1988. Morphology of the organic shell matrix of *Diffflugia* (Rhizopoda) in culture, including modification by the addition of agglutinate particles. Arch. Protistenkd. 136, 365–376.

Ogden C.G. 1991. The biology and ultrastructure of an agglutinate testate amoeba *Diffflugia goesphaira* sp. nov. Arch. Protistenkd. 140, 141–150.

Ogden C.G. and Hedley R.H. 1980. An atlas of freshwater testate amoebae. Oxford Univ. Press, London.

Ogden C.G. and Živković A. 1983. Morphological studies on some Diffflugidae from Yugoslavia (Rhizopoda, Protozoa). Bull. Brit. Mus. nat. Hist. (Zool.). 44, 341–375.

Penard E. 1890. Études sur les Rhizopodes d'eau douce. Mémoires de la Société de Physique et d'Histoire Naturelle de Genève. 31, 2, part 1, 1–230.

Penard E. 1902. Faune rhizopodique du Bassin du Léman. Henry Kundig, Genève.

Penard E. 1905. Les Sarcodinés des grands lacs. Librairie de l'Institute, Genève, pp. 1–134.

Penard E. 1908. Recherches sur les Sarcodinés de quelques lacs de la Suisse et de la Savoie. Rev. Suisse Zool. 16, 441–471.

Playfair G.I. 1914. Contributions to the knowledge of the biology of the Richmond River. Proc. Linn. Soc. New South Wales. 39, 93–151.

Playfair G.I. 1918. Rhizopods of Sydney and Lismore. Proc. Linn. Soc. New South Wales. 42, 633–675.

Rampi L. 1950. Su alcuni laghetti alpini del Massiccio dell'Abisso (Alpi Marittime). Bollettino di Pesca, Piscicoltura e Idrobiologia, new series. 5, 207–224.

Schönborn W. 1962. Neue Testaceen aus dem Grossen Stechlinsee und dessen Umgebung. Limnologica. 1, 83–91.

Schönborn W. 1965. Die Sedimentbewohnenden Testaceen einiger Masurischer Seen. Acta Protozool. 3, 297–309.

Thomas R. 1954. Thécamoebiens de la region bordelaise. Bull. Soc. Hist. Natur. Toulouse. 89, 245–264.

Oye P., van. 1958. Étude sur les rhizopodes des marais du Sud-ouest d'Uvira (Congo-belge). Hydrobiologia. 10, 85–127.

Wallich G.C. 1864. On the extent, and some of the principal causes, of structural variation among the Diffflugian Rhizopods. Annals and Magazine of Natural History, Ser. 3. 13, 215–245.

**Address for correspondence:** Yuri Mazei, Department of Zoology and Ecology, Penza State University, Krasnaya str. 40, 440026 Penza, Russia; e-mail: [yurimazei@mail.ru](mailto:yurimazei@mail.ru)