Protistology 7 (3), 121–171 (2012)

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 1: Species with shells that are pointed aborally and/or have aboral protuberances

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Summary

We review the species of *Difflugia* with a shell that is pointed aborally and/or has aboral protuberances, 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 *Difflugia* taxa grouped into five species complexes, namely *D. acuminata* Ehrenberg, 1838, *D. bacillariarum* Perty, 1849, *D. elegans* Penard, 1890, *D. claviformis* (Penard, 1899) Ogden, 1979, and *D. scalpellum* Penard, 1899. Within the *D. acuminata*-complex we: (i) distinguish as separate taxa the typical form of *D. acuminata* Ehrenberg, 1838, as well as *D. acuminata* var. *magna* Deflandre, 1926, *D. giganteacuminata* (Chardez, 1958) Chardez et Gaspar, 1984, and *D. distenda* (Penard, 1899) Ogden, 1983; (ii) synonymise *D. aciminata* var. *umbilicata* Penard, 1902, *D. curvicaulis* Penard, 1899, and *D. venusta* (Penard, 1902) Ogden, 1983 with *D. acuminata*; *D. congolensis* Gauthier-Lièvre et Thomas, 1958 with *D. acuminata* var. *magna*; *D. bicruris* Gauthier-Lièvre et Thomas, 1958 with *D. distenda*, and; (iii) discuss the validity of *D. ventricosa* Deflandre, 1926 and *D. acutissima* Deflandre, 1931.
Within the *D. bacillariarum*-complex we: (i) distinguish as a separate taxon the typical form of *D. bacillariarum* Perty, 1849, and; (ii) synonymise *D. bicornis* Penard, 1890, *D. styla* Ogden et Živković, 1983 and *D. australis* var. *minor* Gautier-Lièvre et Thomas, 1958 with *D. bacillariarum*. Within the *D. elegans*-complex we: (i) distinguish as a separate taxon *D. elegans* Penard, 1890, and provide an improved diagnosis that includes the variability in the appearance and number of horns; (ii) synonymise *D. amphoralis* Hopkinson, 1909, *D. tricornis* (Jung, 1936) Ogden, 1983, *D. elegans* f. *tricornis* Jung, 1936, *D. elegans* f. *bicornis* Jung, 1936, *D. australis* (Playfair, 1918) Gautier-Lièvre et Thomas, 1958, *D. solowetskii* Mereschkowsky, 1877, *D. varians* Penard, 1902, *D. leidyi* Wailes, 1912, *D. borodini* Gassowsky, 1936, and *D. juzephiniensis* Dekhtyar, 1993 with *D. elegans*, and; (iii) discuss the validity of *D. elegans* var. *teres* Penard, 1899 and *D. elegans* var. *angustata* Deflandre, 1926.

Within the *D. claviformis*-complex we: (i) distinguish as a separate taxa *D. claviformis* (Penard, 1899) Ogden, 1979 and *D. microclaviformis* (Kourova, 1925) Ogden, 1983, and; (ii) synonymise *D. pyriformis* var. *venusta* Penard, 1902 with *D. microclaviformis* (Kourova, 1925) Ogden, 1983. Within the *D. scalpellum*-complex we: (i) distinguish as a separate taxon *D. scalpellum* Penard, 1899, and; (ii) discuss the validity of *D. præstans* Penard, 1905, *D. smilion* Thomas, 1953, and *D. sarissa* Li Sun Taï, 1931. 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: testate amoebae, *Difflugia*, taxonomic revision, morphospecies, species complex

Introduction

Difflugia Leclerc, 1815 is the oldest genus of testate amoebae (Leclerc, 1815). The type species, *D. proteiformis* Lamarck, 1816, has not been studied with modern methods and its nature is questionable (Ogden and Ellison, 1988). Members of the genus *Difflugia* have an agglutinate shell, with a terminal aperture that is round, oval, lobed or toothed (but never slit-like), sometimes with a collar or necklace (sensu Ogden and Meisterfeld, 1989) but never with an internal diaphragm. The shell is composed of mineral particles or diatom frustules, collectively called xenosomes, that are assembled on structured or sheet-like organic cement. All species of *Difflugia* acquire their xenosomes from their environment. Many select and arrange these xenosomes according to their size and shape in order to construct a shell with a morphology that is unique to that particular species. The nucleus is usually ovular, but in some species it is vesicular. Several freshwater species have green endosymbionts (Meisterfeld and Mitchell, 2008).

There is considerable uncertainty as to what characters may used to circumscribe species of *Difflugia*. The taxonomy of the genus is based mainly on shell size and shape. Comparison of cytoplasmic features is either difficult or impossible because most of it is encased by the shell which is usually opaque (Ogden, 1983). Only in a few cases have cellular structures, e.g. the shape and number of nuclei and the shape of the pseudopodia, been taken into consideration (Penard, 1902, 1905; Awerinzew, 1907; Štěpánek, 1952; Ogden and Meisterfeld, 1989; Chardez, 1991a).

Difflugia is the most speciose genus of the order Arcellinida with about 300 nominal species and varieties (Ogden, 1983; Meisterfeld and Mitchell, 2008). This abundance of species is, at least in part, due to a combination of inadequate descriptions and the lack of good diagnostic features. For example, in their detailed survey of the genus based on African specimens, Gauthier-Lièvre and Thomas (1958) had difficulty with several groups of individuals which shared common features and, as a result, about half of the 129 nominal species were designated as varieties or forms. Furthermore, Štěpánek (1952) noted that, because of their morphological variability, *Difflugia* populations often form a continuum from one species to another, even in one small pond. Nevertheless, it has often been the case that even slight deviations in shell shape have resulted in the establishment of new forms or species, regardless of the range of variability that individual *Difflugia* taxa may exhibit.

Since the genus was established by Leclerc (1815), there has been no taxonomic revision of *Difflugia*. This contrasts with other testate amoeba genera, many of which were revised during the 20th century including: *Arcella* (Deflandre, 1928; Decloître, 1976), *Centropyxis* (Deflandre, 1929; Decloître, 1978, 1979), *Cyclopyxis* (Deflandre, 1929; Decloître, 1977a), *Plagiopyxis* (Thomas, 1958), *Nebela* (Deflandre, 1936; Jung, 1942a; Gauthier-Lièvre, 1953, 1957; Decloître, 1977b), *Hyalosphenia* (Grospietsch, 1965), *Lesquereusia* (Thomas and Gauthier-Lièvre, 1959), *Cucurbitella* (Gauthier-Lièvre and Thomas, 1960), *Quadrulella* (Chardez, 1967a), *Paraquadrula* (Decloître, 1961), *Cryptodifflugia* (Grospietsch, 1964; Schönborn, 1965a), *Trinema* (Chardez, 1960), *Euglypha* (Decloître, 1962) and *Cyphoderia* (Chardez, 1991b).

Several approaches to the investigation of the taxonomy of the genus *Difflugia* can be recognized.

1. Descriptions of shell morphology using light-microscopical (LM) observations and, more recently, LM photomicrographs, as the main method of visualization. For many years light microscopy was the only method available for examining testate amoebae and many species are known from descriptions and illustrations based on such observations alone. In recent years, photomicrography has been routinely employed to record the morphology revealed by such methods. Consequently there is a large legacy of species descriptions based on such data (Ehrenberg, 1838; Dujardin, 1841; Perty, 1849; Carter, 1864; Wallich, 1864; Leidy, 1879; Penard, 1890, 1893, 1899, 1902; Rhumbler, 1891; Blanc, 1892; Cash and Hopkinson, 1909; Wailes, 1912; Playfair, 1914, 1918; Kourova, 1925; Deflandre, 1931; Oye van, 1932, 1958; Gassowsky, 1936; Rampi, 1950; Štěpánek, 1952, 1963b, 1967a, 1967b; Thomas, 1953, 1954; Chardez, 1957, 1958, 1985, 1987, 1991a; Ertl, 1965; Schönborn, 1965b, 1966; Godeanu, 1972; Chardez and Gaspar, 1984; Beyens and Chardez, 1994; Lopretto and Vucetich, 2001; Snegovaya and Alekperov, 2005, 2010a, 2010b).

2. Synthesis of existing data and proposing relationships within the genus based on morphological characteristics of shells revealed by LM. Two contrasting approaches have been made in the use of shell structure and shape for categorizing species of *Difflugia*. In the first of these, different types of shell morphology are identified and the species are grouped accordingly. In one example, Jung (1942b) split *Difflugia* into 8 nominal genera as follows: *Globonota* with spherical shells, *Difflugia sensu stricto* with ovoid, pyriform or cylindrical shells, *Acipyxis* with acuminate or horned shells, *Planodifflugia* with laterally flattened shells, *Picnochila* with small transparent fine-grained shells, *Loboforamia* with a lobed aperture, *Eustoma* with undescribed peculiarities, and *Cingodifflugia* with urceolate shells. However, he failed to provide detailed descriptions of the newly erected genera or to fix any type species, therefore these genera are invalid. In a second example, following a study of testate amoebae from Africa, Gauthier-Lièvre and Thomas (1958) divided the genus *Difflugia* into ten groups based on the morphology of the shell, i.e. lobed, collared, compressed, urceolate, globose, ovoid-globose, elongate, acute-angled, horned, and pyriform. Although no taxonomic significance was attached to these groupings, the study did serve to highlight the diversity of shell shape that exists within the genus. Similarly, Ogden (1983) recognized four basic shell shapes in *Difflugia* and grouped the species accordingly: (i) pointed or having aboral protuberances; (ii) pyriform and elongate; (iii) ovoid or spherical; (iv) compressed. Again, no taxonomic significance was attached to these groups.

In the second approach, the range of variablilty of shell morphology is recorded for a given habitat or species in order to determine clear distinctions between populations that are of presumed taxonomic importance. Štěpánek (1952) measured the range of morphological variations within *Difflugia oblonga* sensu lato inhabiting a pond near Prague. He found a series of intermediate forms between a number of different types that had previously been regarded as separate species. Forms lacking clear-cut distinctions were referred to as semi-species and these collectively formed a species complex or 'ultraspecies' (Štěpánek, 1952). Chardez (1961, 1967b, 1973) likewise described interspecies variability and distinguished a number of varieties and forms within three species: *D. oblonga, D. acuminata* and *D. ventricosa*. Subsequently, following a study of *Difflugia* diversity in a small pond, Chardez (1974) constructed hypothetical schemes of morphological correspondence between different shell shapes and defined a number of subspecies that collectively accounted for all the diveristy of *Difflugia* species with acuminate shells.

3. Comparative analysis of shell morphology using morphometric data and examination by scanning electron microscopy (SEM). The development of SEM enabled the testate shell to be examined in much greater detail, and for morphometric data to be recorded with greater accuracy, than was possible previously using LM. The widespread use of these techniques over the past 30 to 40 years has led to the redescription of many species and a better appreciation of intraspecific variability (Ogden, 1979, 1980a, 1980b, 1983, 1984, 1988, 1990, 1991, 1992; Ogden and Fairman, 1979; Ogden and Hedley, 1980; Ogden and Živković, 1983; Dekhtyar, 1993; Lüftenegger et al., 1988; Ogden and Meisterfeld, 1989, 1991; Lüftenegger and Foissner, 1991; Badewitz, 2000; Bobrov and Mazei, 2004; Yang et al., 2004, 2005a, 2005b; Yang and Shen, 2005; Lahr and Lopes, 2006; Mazei and Tsyganov, 2006b, 2006c; Todorov and Golemansky, 2007; Nicholls, 2007; Davidova et al., 2008; Liu et al., 2010). The use of SEM has also led to the recognition of additional characters of taxonomic importance for *Difflugia* such as the structure of the organic cement in the shell (Ogden and Ellison, 1988; Ogden, 1990; Wanner and Meisterfeld, 1994). However, following an investigation of shell ultrastructure in *Difflugia yorkui*, Nicholls (2007), casted doubt on the taxonomic value of

the organic cement noting that "at this stage of our understanding of shell ultrastructure, the morphology of the underlying organic layer of the shell may be of far lesser taxonomic significance than overall shell morphology, based on statistically defined size and shape variables as well as the nature of the agglutinated particles (e.g. thin and flat, large and angular, small and regular, etc.)".

4. Molecular studies of *Difflugia*. The first published molecular data concerning *Difflugia* was that of Gomaa et al. (2012). In this study, the small subunit rRNA (SSU rRNA) gene of nine species of testate amoebae, including five species of *Difflugia*, was amplified and sequenced in order to clarify the phylogenetic relationships among arcellinids at the generic and species levels and to evaluate the validity of the criteria used for their taxonomy. The nine species investigated were: *Difflugia bacillariarum, D. hiraethogii, D. acuminata, D. lanceolata, D. achlora, Bullinularia gracilis, Netzelia oviformis, Physochila griseola* and *Cryptodifflugia oviformis*. Phylogenetic analyses based on these data suggested that the genus *Difflugia* is not monophyletic. Moreover, the authors emphasized the importance of general shell shape in the taxonomy of arcellinid testate amoebae: four *Difflugia* species with a similar shell shape (*D. bacillariarum, D. hiraethogii, D. acuminata, D. lanceolata*) grouped together in the SSU rRNA gene tree despite differences in the structure of the organic cement that holds their xenosomes together. Furthermore, uniquely among arcellinids, each of these four species has a four nucleotide-sequence deletion in its SSU rRNA gene. This study thus provided independent evidence that general shell shape (e.g. pyriform vs. elongate) might be a reliable character in the taxonomy and phylogeny of *Difflugia*. On the other hand, the fifth species of *Difflugia, D. achlora*, which has a globular shell, was only distantly related to the other *Difflugia* species in the SSU rRNA gene tree and instead branched close to *Netzelia oviformis, Arcella hemisphaerica* and *A. vulgaris*. Sequence data from other species with shells of different shapes are needed in order to further investigate the significance of shell shape in the taxonomy and phylogeny of *Difflugia*.

As with many other taxa, there are two clear methodological tendencies among those working on the taxonomy of *Difflugia* which has led to their general categorization as either 'splitters' or 'lumpers' (Bobrov and Mazei, 2004). Ogden for example, may be said to belong to the former category having erected 17 new species of *Difflugia* previously considered as varieties or forms (e.g. Ogden, 1979, 1980b, 1983, 1984; Ogden and Živkovič, 1983). By contrast, careful analysis of some polymorphic species (e.g. *D. urceolata* – see Todorov and Golemansky, 2007) has demonstrated that there are many intermediate forms between nominal varieties (e.g. *D. urceolata olla* and *D. urceolata sphaerica*), and consequently it is not possible to distinguish them clearly. In this particular case it was concluded that these two varieties should be considered as synonyms (Todorov and Golemansky, 2007). In a similar approach, Foissner and Korganova (1995, 2000) concluded that the genera *Centropyxis* and *Cyclopyxis* contain many nominal species and varieties that are very likely invalid since they were established based on variations of shell morphology occurring as a result of shell aging and decomposition. As a result it was suggested that taxa as these, which are morphologically and/or morphometrically difficult to distinguish, should be lumped together into species "complexes". However, while this approach may be justified for well characterized forms, it is not reasonable to simply lump poorly described or polymorphic taxa into species complexes (*sensu* Foissner and Korganova, 2000) or ultraspecies (*sensu* Štěpánek, 1952) since such morphological variability is often closely associated with ecological conditions and may thus be of important indicator value for environmental change. For example, detailed investigations of testate amoebae from peatlands in western Russia revealed sub-species niche separation along a moisture gradient (Bobrov et al., 1999). Specifically, there was a clear decrease of shell size moving from wet to dry conditions in three different species groups, i.e. the *Trigonopyxis arcula* group (*T. arcula* var. *major* > *T. arcula* > *T. minuta*), the *Assulina-Valkanovia* group (*A. seminulum* > *A. muscorum* > *V. elegans*), and the *Trinema lineare* group (*T. lineare* var. *truncatum* / *T. lineare* > *T. lineare* var. *terricola*). In addition, spined forms within the genera *Euglypha* and *Placocista* consistently occurred in wetter habitats whereas spineless forms, or those with short spines, were more prevalent in drier habitats. It was therefore concluded that, in order to maximise the ecological indicator value of the assemblages recorded, identifications should be made at the lowest taxonomic rank possible within these groups (Bobrov et al., 1999).

In conclusion, aperture features appear to be the most reliable taxonomic characters within the family Difflugiidae (Ogden and Meisterfeld, 1989; Dekhtyar, 1995; Mazei and Tsyganov, 2006a), in particular the presence or absence of collars (either with or without lobes), necklaces and teeth. Species possessing such structures are referred to here as

'collared' and include those formerly referred to as lobed, collared and urceolate (sensu Gauthier-Lièvre and Thomas, 1958). In species lacking such structures, the aperture may be surrounded either by xenosomes (sand grains, diatom frustules etc.) or by organic lips. Difflugiids without collars or necklaces can further be categorized into one of four groups based on shell shape: (i) pointed aborally and/or having aboral protuberances; (ii) pyriform and elongate; (iii) ovoid and spherical; (iv) compressed or with irregular shape (Ogden, 1983).

This is the first 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 and shell measurements, and also on published literature. The aim of the present paper is to review those species of *Difflugia* with a shell that is pointed aborally and/or having aboral protuberances.

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

Leidy (1879) described *Difflugia acuminata* Ehrenberg, 1838 as varying considerably in size and shape and lumped together all previously erected taxa with shells that are acuminate and/or have aboral protuberances, namely: *D. acuminata* (Ehrenberg, 1838; Perty, 1852; Leidy, 1874a, 1874b, 1877), *D. acuminata* var. *acaulis* (Perty, 1849), *D. bacillariarum* (Perty, 1849), *D. proteiformis* var. *acuminata* (Wallich, 1863), *D. pyriformis* "acuminated variety" (Carter, 1864), *D. proteiformis*, subspecies *D. mitriformis*, var. *D. acuminata* (Wallich, 1864), and *D. Corticella acuminata* (Ehrenberg, 1871).

Penard (1890, 1899, 1902, 1905) accepted *D. acuminata* and described eleven new taxa: *D. elegans, D. bicornis, D. pyriformis* var. *claviformis*, *D. elegans* var. *teres*, *D. acuminata* var. *inflata*, *D. curvicaulis, D. scalpellum, D. pyriformis* var. *venusta*, *D. acuminata* var. *umbilicata*, *D. varians*, *D. bidens*, and *D. præstans*. Furthermore, Penard (1902) considered *D. bacillariarum* Perty, 1849, *D. bicuspidata* Rhumbler, 1891 and *D. Solowetskii* Mereschkovsky, 1878 as junior synonyms of *D. elegans* Penard, 1890.

Cash and Hopkinson (1909) accepted *D. pyriformis* var. *venusta* Penard, 1902, *D. pyriformis* var. *claviformis* Penard, 1902, *D. acuminata* Ehrenberg, 1838, *D. acuminata* var. *inflata* Penard, 1899, *D. bacillariarum* Perty, 1849, and *D. curvicaulis* Penard, 1899; they considered *D. elegans* Penard, 1890, *D. Solowetskii* Mereschkovsky, 1878 and *D. elegans* var. *teres* to be varieties of *D. bacillariarum*, i.e. *D. bacillariarum* var. *elegans*, *D. bacillariarum* var. *solowetskii*, and *D. bacillariarum* var. *teres*, respectively; and they erected the new taxon *D. acuminata* var. *curvata* (Cash and Hopkinson, 1909).

New taxa described during 20th century include: *Difflugia echinulata* (Penard, 1911), *D. leidyi* (Wailes, 1912), *D. acuminata* var. *levanderi* (Playfair, 1914), *D. oblonga* var. *microclaviformis* (Kourova, 1925), *D. ventricosa, D. acuminata* var. *magna*, *D. elegans* var. *angustata* (Deflandre, 1926), *D. acutissima* (Deflandre, 1931), *D. sarissa* (Li Sun Taï, 1931), *D. borodini, D. mamma* (Gassowsky, 1936), *D. elegans* f. *bicornis, D. elegans* f. *tricornis* (Jung, 1936), *D. acuminata* var. *inflata* f. *immanata* (Jung, 1942a), *D. curvicaulis* var. *inflata* (Decloître, 1951), *D. oblonga* var. *caudata*, *D. oblonga* var. *schizocaulis* (Štěpánek, 1952), *D. smilion* (Thomas, 1953), *D. acuminata* var. *inflata* f. *stenostoma* (Decloître, 1954), *D. acuminata* var. *brevicaulis* (Thomas and Mabille, 1956), *D. acuminata* var. *gigantea* (Chardez, 1958a), *D. oblonga* var. *heali* (Štěpánek, 1963a), *D. irregularis, D. spinosa* (Štěpánek, 1963b), *D. solowetskii* var. *stepaneki* (Schönborn, 1965a), *D. brychtai, D. elegans* var. *minor, D. elegans* var. *teres* f. *gali, D. elegans* var. *teres* f. *minor, D. immanata* var. *minor, D. acuminata* f. *minor, D. curvicaulis* f. *minor* (Štěpánek, 1967a), *D. mamella* (Laminger, 1971), *D. acutissimella* (Chardez, 1985), *D. longum* (Chardez, 1987), and *D. juzephiniensis* (Dekhtyar, 1993).

In their report on testate amoebae of Africa, Gauthier-Liиvre and Thomas (1958) grouped the various *Difflugia* spp. based on shell morphology. The following known species were included in the groups entitled "Acutangulaires", "Corniculees" and (in part) "Pyriformes", i.e. those with shells that are angular, horned or pyriform, respectively: *D. acutissima* Deflandre, 1931, *D. sarissa*, Li Sun Taï, 1931, *D. smilion* Thomas, 1953, *D. ventricosa* Deflandre, 1926, *D. acuminata* Ehrenberg, 1838, *D. acuminata* var. *magna* Deflandre, 1926, *D. acuminata* var. *inflata* Penard, 1899, *D. acuminata* var. *curvata* Cash, 1909, *D. acuminata* var. *umbilicata* Penard, 1902, *D. bacillariarum* Perty, 1849, *D. curvicaulis* Penard, 1899, *D. curvicaulis* var. *inflata* Decloître, 1951, *D. echinulata* Penard, 1911, *D. elegans* Penard, 1890, *D. elegans* var. *angustata* Deflandre, 1926, *D. elegans* f. *tricornis* Jung, 1936, *D. elegans* var. *teres* Penard, 1899, *D. leidyi* Wailes, 1912, *D. oblonga* var. *venusta* Penard, 1902, *D. oblonga* var. *microclaviformis* Kourova, 1925. Furthermore, they raised *D. bacillariarum* var. *australis* Playfair, 1917 to species rank as *D. australis* and described the following new taxa from northern Africa: *D.*

acutissima var. *gigas, D. smilion* var. *major*, *D. acuminata* var. *inflata* f. *bicornis, D. australis* var. *minor, D. bicruris*, and *D. congolensis* (Gauthier-Lièvre and Thomas, 1958).

Following a review of the diagnostic features of *Difflugia acuminata*, Chardez (1961) recognized 12 infraspecific forms (Fig. 1): *D. acuminata* Ehrenberg, 1838, *D. acuminata* var. *magna* Deflandre, 1926, *D. acuminata* var. *gigantea* Chardez, 1958, *D. acuminata* var. *curvata* Cash, 1909, *D. acuminata* var. *brevicaulis* Thomas et Mabille, 1956, *D. acuminata* var. *umbilicata* Penard, 1902, *D. acuminata* var. *acaulis* Perty, 1849, *D. acuminata* var. *levanderi* Palyfair, 1914, *D. acuminata* var. *inflata* Penard, 1899, *D. acuminata* var. *inflata* f. *stenostoma* Decloître, 1954, *D. acuminata* var. *inflata* f. *bicornis* Gauthier-Lièvre et Thomas, 1958, and *D. acuminata* var. *inflata* f. *immanata* Jung, 1942. In his review of *Difflugia* spp. with pyriform shells, Chardez (1967b) mentioned six taxa with aboral protuberances, all of which are varieties of *D. oblonga*, namely: *D. oblonga* var. *claviformis* Penard, 1899, *D. oblonga* var. *microclaviformis* Kourova, 1925, *D. oblonga* var. *caudata* Štěpánek, 1952, *D. oblonga* var. *schizocaulis* Štěpánek, 1952, *D. oblonga* var. *heali* Štěpánek, 1952, and *D. oblonga* var. *cornuta* Leidy, 1879 (Fig. 2).

In recent years, five species and varieties of *Difflugia* with pointed shells, or having shells with aboral protuberances, have been redescribed using SEM and biometric analysis: *D. acuminata* (Bobrov and Mazei, 2004; Mazei and Tsyganov, 2006b), *D. acuminata* var. *magna* (Yang et al., 2005b; Mazei and Tsyganov, 2006b), *D. claviformis* (Lahr and Lopes, 2006), *D. elegans* (Mazei and Tsyganov, 2006b), and *D. giganteacuminata* (Davidova et al., 2008).

Štěpánek (1952) and Chardez (1974) proposed hypothetical schemes describing morphological relationships between pyriform and acuminate species of *Difflugia* (Figs 3–5). Unfortunately, Chardez (1974) did not provided any explanation for the relationships proposed in his figures (see Appendix). In his exhaustive study of *Difflugia* in a single pond, Štěpánek (1952) recorded numerous transitional forms within the *D. oblonga* 'ultraspecies'. For some of these he erected new names, e.g. *D. oblonga* var. caudata and *D. oblonga* var. *schizocaulis*. For others, he either accepted previously existing names, e.g. *D. acuminata* (as D. (*oblonga*) *acuminata*), *D. acuminata* var. *inflata, D. pyriformis* var. *claviformis*, and *D. acuminata* var. *umbilicata*, or simply provided brief descriptions of the shapes in the context of his scheme. However, with a few notable exceptions (e.g. *D. elegans* and *D. bacillariarum*), he did not include many acuminate taxa in the scheme because he did not find them in the pond. Thus, his scheme does not cover the diversity of *Difflugia* species with pointed shells or having shells with protuberances. It should also be noted that Štěpánek (1952) found a continuum of forms between those with shells covered by sand grains and those covered by diatom frustules and concluded that coverage by diatoms cannot be regarded as a good character for species (or even semi-species) separation. Consequently, he synonymised *D. bacillifera* with *D. oblonga*.

In a series of publications (Ogden, 1979, 1980b, 1983, 1984; Ogden and Hedley, 1980; Ogden and Živković, 1983) Colin Ogden redescribed, and in many cases changed the taxonomic status of several taxa of *Difflugia* with pointented shells or having protuberances on their shells, including: *D. acuminata, D. curvicaulis, D. elegans, D. claviformis, D. scalpellum, D. bacillariarum, D. acutissima, D. bicornis, D. microclaviformis, D. styla, D. elegans* var. *angustata, D. oranensis, D. amphoralis, D. tricornis, D. venusta, D. ventricosa, D. bicruruis*, and *D. distenda*. We have applied Ogden's morphometric data (both published and unpublished) to compare morphologically similar taxa (Fig. 6). On a logarithmic scale (Fig. 6a) we can distinguish three main size classes: (1) small, with a shell length of 55–105 μm, including *D. bacillariarum* and *D. bicornis*; (2) intermediate, with a shell length of 95–160 μm, including *D. elegans* and *D. amphoralis*; (3) large, with a shell length of 150–400 μm. The last group is divided into three subgroups on a linnear scale (Fig. 6b): (i) those with relatively short (150–250 μm) and narrow (60–100 μm) shells, including *D. acuminata* typical form, *D. curvicaulis, D. venusta*, and *D. ventricosa* (group 1a); (ii) those with relatively short (175–270 μm) and broad (90–135 μm) shells, including *D. distenda* and *D. acutissima* (group 1b); (iii) those with long (270–400 μm) shells, including *D. acuminata* var. *magna* and *D. giganteacuminata* (group 1c). Thus, according to size classes measured on a logarithmic scale, we can distinguish three species complexes (sensu Foissner and Korganova, 2000): *D. acuminata*-complex, *D. bacillariarum*-complex, and *D. elegans*-complex. Two other species complexes may also be recognised based on shell morphology: those having a pyriform shell with a prominent neck and thick mamilla-like aboral protuberance or pointed aboral region, which are included the *D. claviformis*-complex, and those having an elongated lanceolate shell that is sharply pointed aborally, which are included in the *D. scalpellum*-complex. Each species complex is here discussed in detail based on data from the

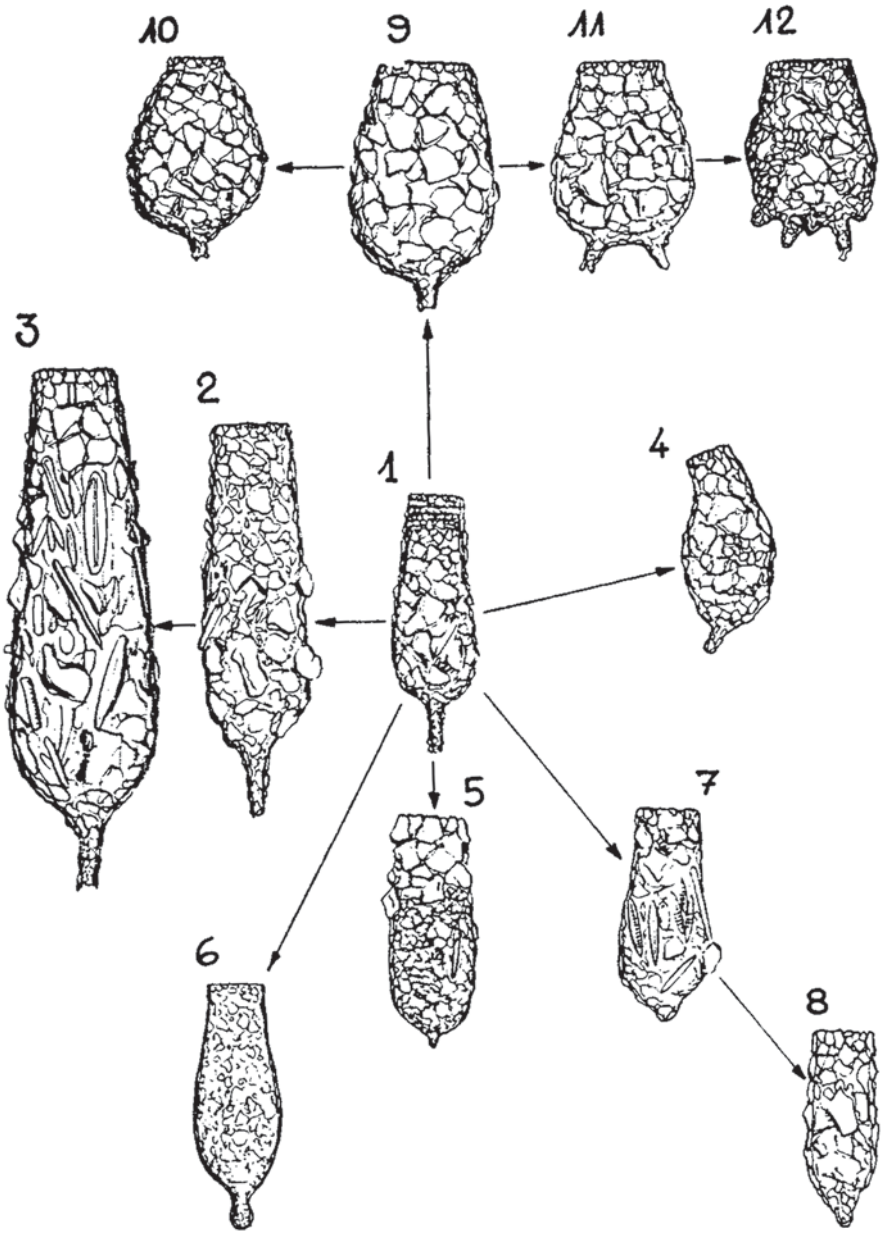

Fig. 1. Infraspecific taxa within *Difflugia acuminata* (after Chardez, 1961): 1 – *Difflugia acuminata* Ehrenberg, 1838, 2 – *D. acuminata* var. *magna* Deflandre, 1926, 3 – *D. acuminata* var. *gigantea* Chardez, 1958, 4 – *D. acuminata* var. *curvata* Cash, 1909, 5 – *D. acuminata* var. *brevicaulis* Thomas et Mabille, 1956, 6 – *D. acuminata* var. *umbilicata* Penard, 1902, 7 – *D. acuminata* var. *acaulis* Perty, 1849, 8 – *D. acuminata* var. *levanderi* Playfair, 1914, 9 – *D. acuminata* var. *inflata* Penard, 1899, 10 – *D. acuminata* var. *inflata* f. *stenostoma* Decloitre, 1954, 11 – *D. acuminata* var. *inflata* f. *bicornis* Gauthier-Lièvre et Thomas, 1958, 12 – *D. acuminata* var. *inflata* f. *immanata* Jung, 1942.

E. Penard and C. 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.

TAXONOMIC REVISION OF SELECTED *DIFFLUGIA* SPECIES.

All the species discussed below have shells that are pointed at the aboral end and/or have aboral protuberances. Illustrations comprise LM photomicrographs, scanning electron micrographs

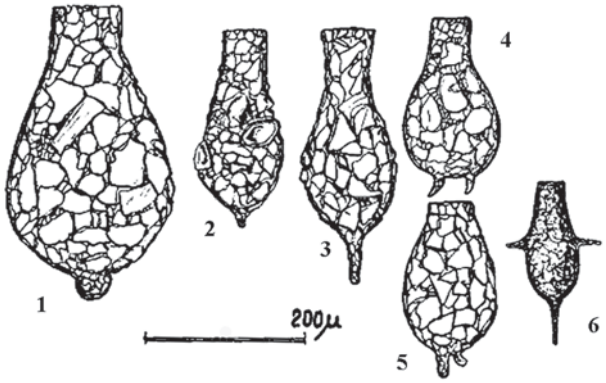

Fig. 2. Infraspecific taxa within *Difflugia oblonga* with protuberances (after Chardez, 1967b): 1 – *D. oblonga* var. *claviformis* Penard, 1899, 2 – *D. oblonga* var. *microclaviformis* Kourova, 1925, 3 – *D. oblonga* var. *caudata* Štěpánek, 1952, 4 – *D. oblonga* var. *cornuta* Leidy, 1879, 5 – *D. oblonga* var. *schizocaulis* Štěpánek, 1952, 6 – *D. oblonga* var. *heali* Štěpánek, 1952.

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 Štěpánek (1952) and Chardez (1961, 1967b, 1973, 1978).

Difflugia acuminata Ehrenberg, 1838 species complex.

A typical individual is shown in Fig. 7. The shell of *Difflugia acuminata* is typically cylindrical to elongate pyriform with a distinct aboral horn or pointed aboral region. The surface is rough to moderately smooth and covered with quartz particles of different size or with fragments of diatoms or chrysomonad cysts. The aperture is circular and usually surrounded by sand grains. Shell length 150–350 μm, shell breadth 70–120 μm, aperture diameter 30–50 μm.

In Ogden and Hedley (1980, p. 118–119) a rather unusual specimen, with a short horn and an aperture covered with a thin layer of organic cement, is presented. It is evident from Fig. 8 that shells of *D. acuminata* can vary in a number of respects including: shape, e.g. from lanceolate (Fig. 8a) to cylindrical (Fig. 8b) to pyriform (Figs 8e and 8f); the appearance of the aboral horn or protuberance, e.g. from pointed aboral region (Fig. 8d), to a thick, mammilla-like protuberance (Fig. 8f), to a long, narrow horn (Fig. 8e); and the building material used to construct the shell, e.g. from flat sand grains and chrysomonad cysts resulting in a smooth surface (Fig. 8a) to large, irregular sand grains resulting in a rough surface (Figs 8b, c). Ogden measured 36 specimens of *D. acuminata* (Fig. 6), of which 21 are unpublished and 15 are published (Ogden, 1979, 1984; Ogden and Hedley, 1980; Ogden and Živković, 1983). Based on these data, the shell length ranges from 193 to 382 μm, shell breadth 72–123 μm, and aperture diameter 32–48 μm.

The diversity of shell appearance in *D. acuminata* is also demonstrated in specimens on Penard's microscope slide collection (Figs 9, 10). These show the considerable variation in shell shape, surface patterns (including specimens covered by diatoms), and the size and shape of the horns and protuberances. In his review of *D. acuminata*, Chardez (1961) likewise illustrated its shell diversity (Fig. 11) and recognized several infraspecific taxa (Fig. 1). He subsequently accepted several other taxa that are closely related to *D. acuminata* (Figs 2, 3; Chardez, 1967b, 1974). Ogden subsequently redescribed some of these infraspecific taxa and, in many cases, raised them to a species rank (e.g. Ogden and Hedley, 1980; Ogden, 1983). However, Ogden and Živković (1983) noted that, due to the lack of data on natural variation of shell characters, many taxonomic decisions concerning infraspecific taxa within the *D. acuminata*-complex are questionable. No further attempts have been made to address this issue since that time. Here we present brief descriptions of subspecies taxa of *D. acuminata*, as well as those of closely related species, based on specimens in the Ogden and Penard collections at the NHM. In many cases the differences between certain taxa are not clear and thus the validity of such taxa is questionable.

***Difflugia acuminata* var. *magna* Deflandre, 1926 and *Difflugia giganteacuminata* (Chardez, 1958) Chardez et Gaspar, 1984**. Deflandre (1926) distinguished *Difflugia acuminata* var. *magna* for those specimens of *D. acuminata* with a shell length of 300–350 μm. Chardez (1958a) described *D. acuminata* var. *gigantea* Chardez, 1958, for those specimens with shell size of > 360 μm. Subsequently, Chardez and Gaspar (1984) considered that this variety was best treated as a distinct species, *D. giganteacuminata* (Chardez, 1958) Chardez et Gaspar, 1984, based on its unusually large size. Chardez and Gaspar (1984) noted that the size range of the new species is 360–550 μm. More recently, Davidova et al. (2008) showed that the size range for this species is 350–400 μm. Given that Ogden's measurements of *D. acuminata* cover the range from

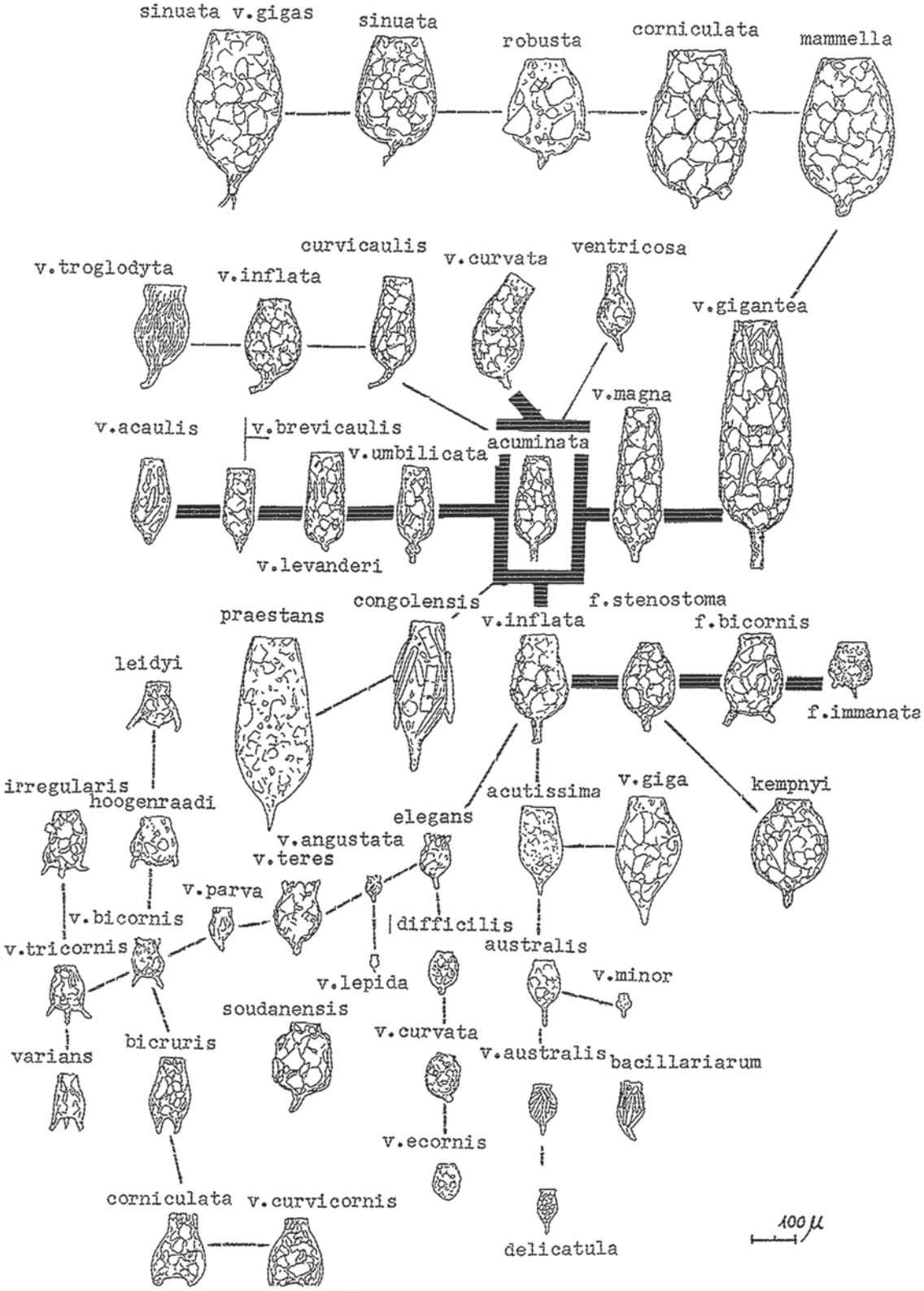

Fig. 3. Recognizable taxa of *Difflugia* with shells that are pointed aborally and/or have aboral protuberances (after Chardez, 1974).

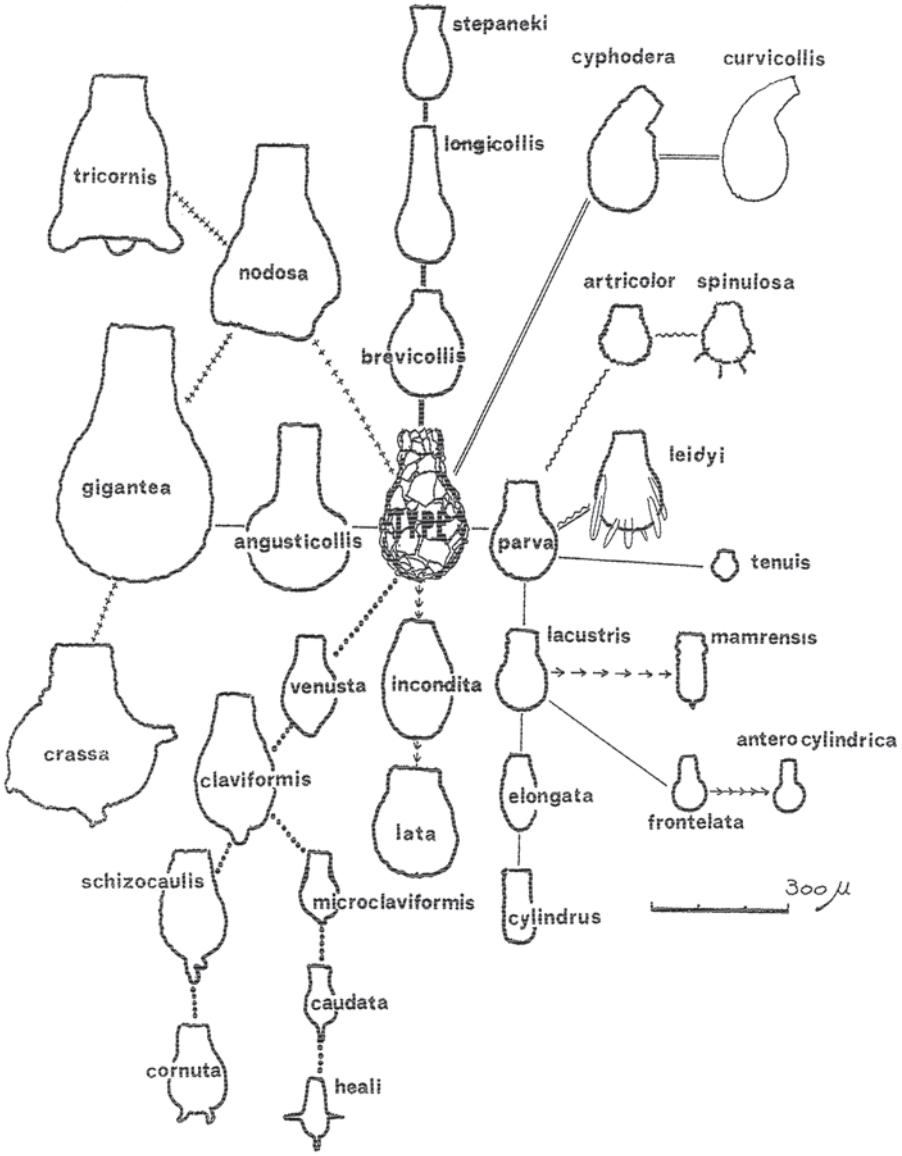

Fig. 4. Recognizable taxa of *Difflugia* with pyriform shells including those that have aboral protuberances (after Chardez, 1974).

190 to 380 μm, and that there is a clear difference in the size ranges between groups 1a and 1c (Fig. 6b) we agree that shell size is a useful character for distinguishing the typical form of *D. acuminata* (150–250 μm in length) from *D. giganteacuminata* (350–550 μm). Furthermore, we consider *D. acuminata* var. *magna* Deflandre, 1926, which has a shell length of 250–350 μm, to be an intermediate form which is probably more closely related to *D. giganteacuminata* since the structure of its organic cement is unlike that typically found in *D. acuminata* (Yang et al., 2005b).

***Difflugia congolensis* Gauthier-Lièvre et Thomas, 1958**. Gauthier-Lièvre and Thomas (1958) described *Difflugia congolensis* as having a large shell, i.e. length 300–350 μm, breadth 115–150 μm, aperture diameter 48–80 μm, that is elongated with a prominent horn, a circular aperture and is covered by a mixture of sand grains, diatom frustules and porifer spicules. These characters, however, are typical of *D. acuminata* var. *magna* so here we synonymise these two taxa, with *D. congolensis* the junior synonym of the former.

***Difflugia acuminata* var. *umbilicata* Penard, 1902**. Penard (1902) described a variety of *Difflugia acuminata* that differs from the typical form by having an aboral mammilla-like protuberance and shell dimensions of length 250 μm and breadth 100

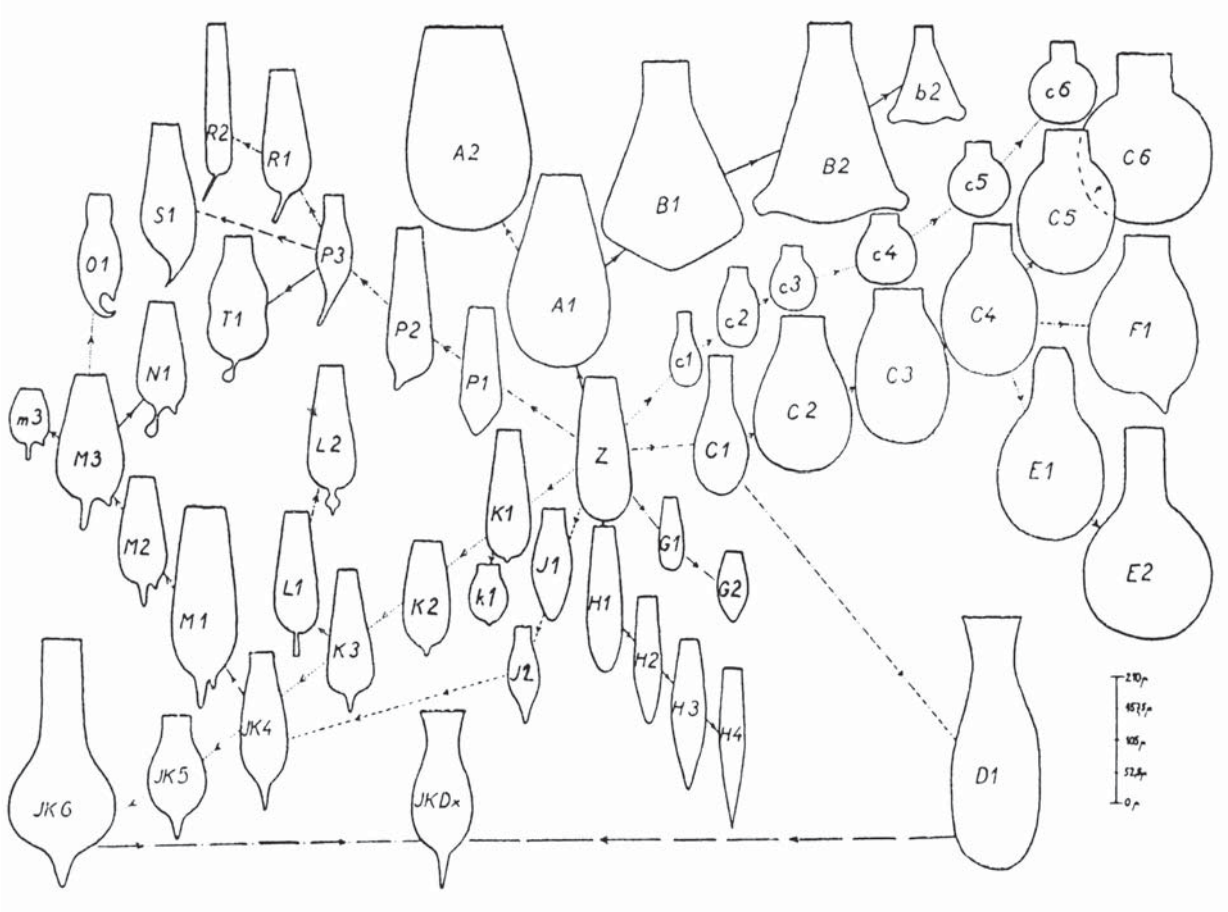

Fig. 5. Hypothetical phylogenetic scheme of *Difflugia oblonga* ultraspecies (after Štěpánek, 1952): abbreviations show different forms distinguished by Štěpánek (1952) in a single pond.

μm. Chardez (1961, 1974) accepted this taxon. However, the NHM Penard collection contains specimens that are very similar to this variety (e.g. Fig. 9g) which were identified as *D. acuminata*. Thus we do not accept *D. aciminata* var. *umbilicata* as a valid taxon and here synonymyse it with *D. acuminata*.

***Difflugia curvicaulis* Penard, 1899**. Penard (1899) characterized this species as having a large, tubular, transparent shell formed by large flattened sand grains that are of increasing size nearer to the aperture. The aboral region ends as a curved horn, which is held at an angle to the longitudinal axis of the shell (Fig. 12). The length of the shell without the horn is 170–200 μm. Penard (1899) noted that two of these features distinguish *D. curvicaulis* from *D. acuminata*: its transparency and the curved aboral horn. Cash and Hopkinson (1909) questioned the validity of *D. curvicaulis* noting that the only significant difference between it and *D. acuminata* is the terminal horn. Although Chardez (1974) accepted this species (see Fig. 3), a previous paper that focused on the morphology of *D. acuminata* (Chardez, 1961) included illustrations of individuals that are very similar to *D. curvicaulis* (Fig. 11). Ogden (1979) and Ogden and Hedley (1980) described *D. curvicaulis* (Fig. 13) as having an elongate or ovoid shell that is circular in transverse section, and an aboral region terminating with a tubular horn that is often curved and perforated at its apex. They also described the shell as being composed of siliceous particles, often interspersed with a mesh of organic cement, arranged to give a smooth outline to the shell, and the aperure as being circular and surrounded by a rim of small particles. They concluded that *D. curvicaulis* differs from *D. acuminata* and *D. elegans* in the shape and smoothness of the shell and the appearance of the apertural region but failed to mention the curved horn (Ogden and Hedley, 1980). Furthermore, the shape of the specimens in the Ogden collection (Fig. 13) differs from the original (Fig. 12), thus the smoothness of the shell is the only distinguishing character that corresponds with the original description (Penard, 1899). Ogden measured 37 individuals of *D. curvicaulis* (Ogden,

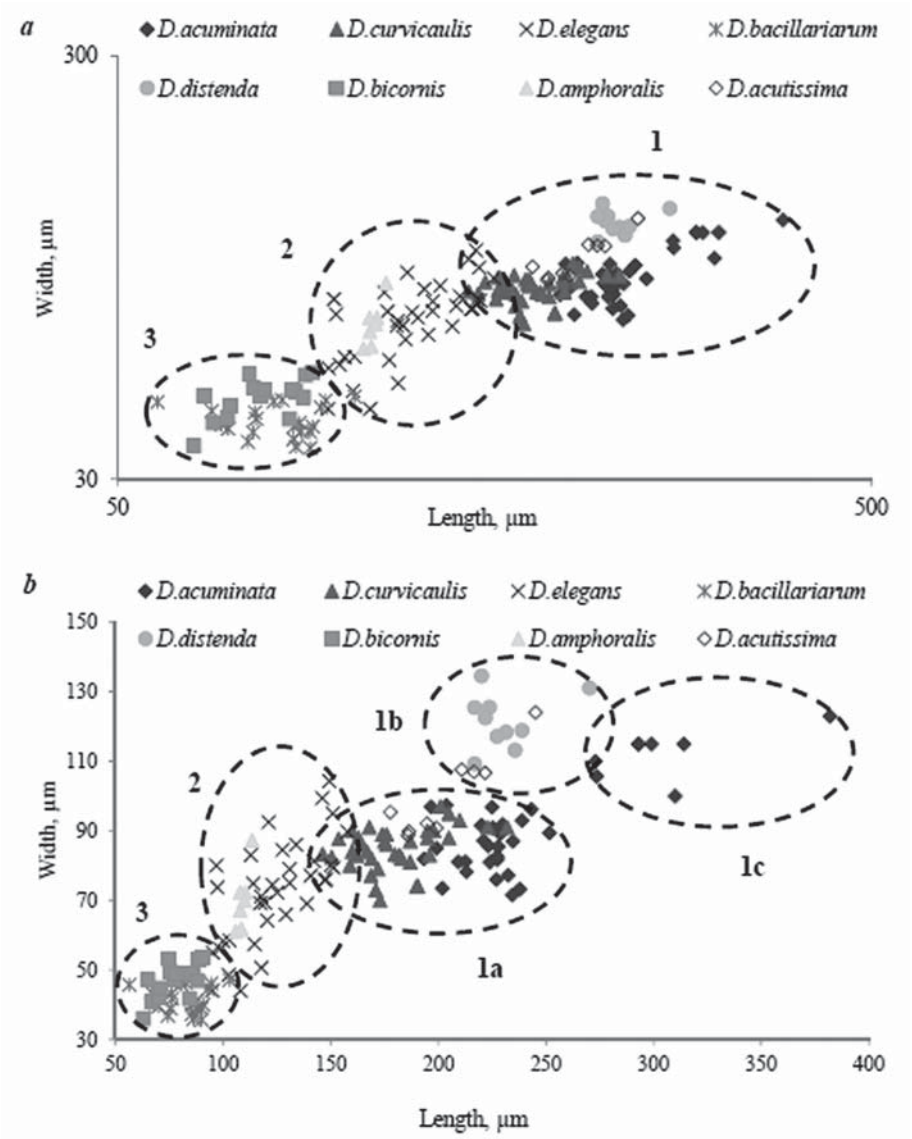

Fig. 6. Length-width bivariant scatter plots of *Difflugia* with shells that are pointed aborally and/or have aboral protuberances, based on C. Ogden's measurements: a – logarithmic scale, b – linear scale; 1a, 1b, 1c, 2, 3 – size groups.

1979; Ogden and Hedley, 1980) with the shell length ranging from 146 to 232 µm, shell breadth 70–97 µm, and aperture diameter 34–43 µm (Fig. 6). This overlaps considerably with the size distribution of the typical form of *D. acuminata*. Based on these data, we do not consider these two taxa sufficiently distinct to be recognised as separate species. We therefore consider *D. curvicaulis* to be a junior synonym of *D. acuminata*.

***Difflugia venusta* Ogden, 1983**. Ogden (1983) raised the variety *Difflugia pyriformis* var. *venusta* Penard, 1902 (*D. oblonga* var. *venusta* sensu Cash and Hopkinson, 1909) to the rank of species, as *D. venusta*. Ogden (1983) characterized *D. venusta* (Figs 14a–d) as having a basically cylindrical shell which gradually increases in diameter reaching a maximum at about two-thirds of the body length from the aperture and then tapering sharply in the last third to form a bluntly pointed apex. The shell is composed mainly of small to medium pieces of quartz and diatom frustules arranged to give a relatively regular and moderately smooth outline apart from the occasional addition of a larger angular piece of quartz or diatom frustules. The aperture is usually circular and surrounded by small particles that give it an irregular margin. Ogden (1983) measured three individuals of *D. venusta* with the shell length ranged from 174 to 188 µm, shell breadth 68–76 µm, and aperture diameter 30–32 µm. These

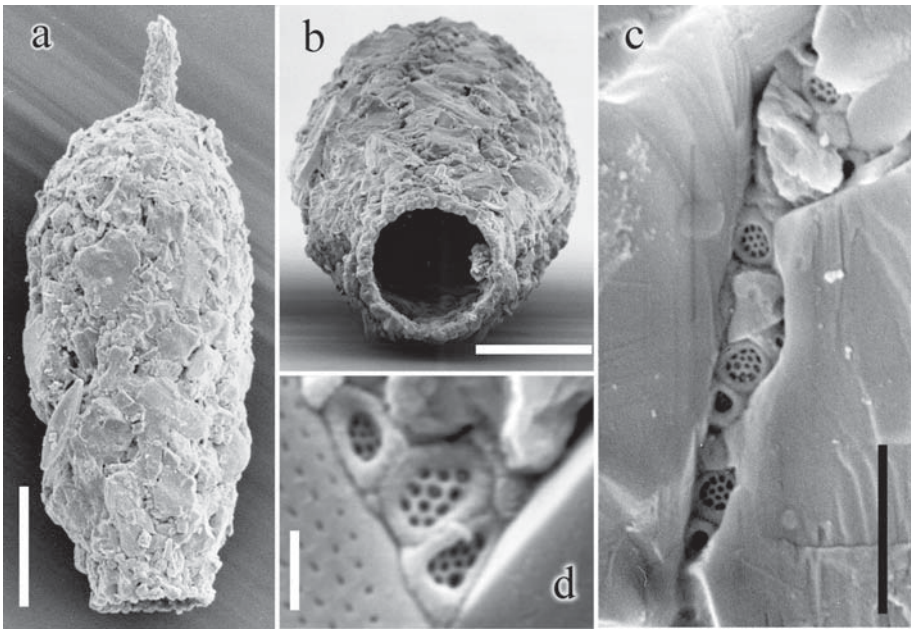

Fig. 7. *Difflugia acuminata* from C. Ogden's SEM collection: a – lateral view (SEM CZ-03.811), b – apertural view (SEM CZ-03.814), c, d – structure of organic cement (SEM CZ-03.852 and CZ-04.635). Scale bars: a – 50 μm, b – 30 μm, c – 3 μm, d – 0.5 μm.

three specimens closely resemble *D. acuminata* in having a pointed aboral region (Fig. 8d), albeit with a different pattern of organic cement (compare Figs 7d and 14c). However, the original descriptions of *D. pyriformis* var. *venusta* (Penard, 1902) and *D. oblonga* var. *venusta* (sensu Cash and Hopkinson, 1909), and the specimens in the NHM Penard collection (Fig. 33), all bear a close resemblance to *Difflugia microclaviformis* Ogden, 1983. We therefore here synonymise *D. pyriformis* var. *venusta* Penard, 1902 with *D. microclaviformis* (Kourova, 1925) Ogden, 1983 (see below) and treat *D. venusta* (Penard, 1902) Ogden, 1983 as a junior synonym of *D. acuminata* Ehrenberg, 1838.

***Difflugia ventricosa* Deflandre, 1926**. Deflandre (1926) described this species as having an elongate-pyriform shell that is rounded in the mid-third of the body, with a long, conical, usually chitinoid horn that may curve in different ways, and a circular aperture. Chardez (1973) reviewed this species (Fig. 15) and compared it with other species having pointed aboral extermities. He noted that the main differences between *D. ventricosa* and D. *acuminata* are the pyriform (vs. cylindrical) shape of the shell. Chardez (1973) summarized the shell measurements of *D. ventricosa* based on previously published data as follows: shell length 164–296 μm, shell breadth 50–89 μm, aperture diameter 23–36 μm. Ogden measured four individuals, two published (Ogden, 1983) and two unpublished, with the following size ranges: shell length 177–226 μm, shell breadth 64–77 μm, aperture diameter 29–40 μm. He described *D. ventricosa* (Figs 14e–j) as having an elongate shell with: a slight swelling in the aboral half of the body which then tapers to a sharp point; a wall comprising a mixture of quartz, diatom frustules and flagellate cysts to give a thin, irregular surface; an aperture that is circular and usually surrounded by small particles. He mentioned that this species is distinct in having a thin, elongate outline that is sharply pointed aborally (Ogden, 1983). However, this finding is inconsistent with those of Deflandre (1926) and Chardez (1973) described above. Ogden's *D. ventricosa* is likely a part of a broad spectrum of polymorphism of *D. acuminata*, e.g. compare the individuals identified by C. Ogden as *D. acuminata* (Fig. 8e) and *D. ventricosa* (Figs 14g, h). Thus, we believe that validity of *D. ventricosa* must await further investigation of the *D. acuminata*-complex.

***Difflugia distenda* (Penard, 1899) Ogden, 1983 and *Difflugia bicruris* Gauthier-Lièvre et Thomas, 1958**. Penard (1899) described the variety *Difflugia acuminata* var. *inflata* as having a shell that is large (length ranging from 230 to 250 μm) and ends in a terminal horn (Fig. 16). The maximal breadth is in the mid-third of the shell length, narrowing gradually towards the aperture. It can be distinguished from the typical form of *D. acuminata* by its broader shell and its thicker horn that is sometimes elongate-ovoid in shape. Penard (1899) mentioned finding

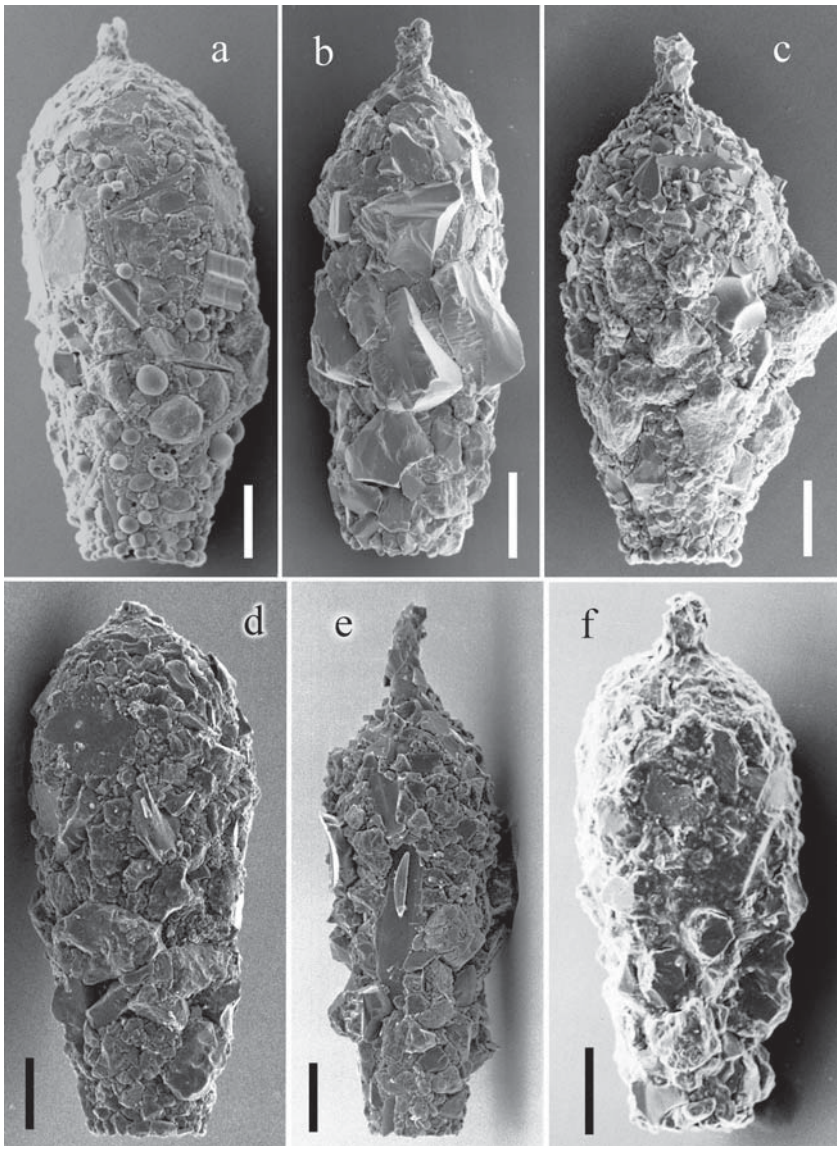

Fig. 8. Different specimens of *Difflugia acuminata* from C. Ogden's SEM collection: a-f – lateral view (a – SEM CZ-09.758, b – SEM CZ-02.870, c – SEM CZ-04.634, d – SEM CZ-06.504, e – SEM CZ-07.360, f – SEM CZ-08.167). Scale bars: 30 μm.

an individual with two horns. Ogden (1983, 1984) measured 13 specimens with shell dimensions as follows: length 199–270 μm, width 91–135 μm, aperture diameter 47–64 μm (Fig. 6). He elevated it to the rank of species as *Difflugia distenda* with the following definition (Fig. 17): shell pyriform with its aboral extremity acutely curved towards a small central horn; moderately smooth surface composed mainly of small to medium size pieces of quartz, occasionally also with diatom frustules; aperture circular and usually surrounded by small, evenly arranged particles. The description by Ogden (1983) is consistent with the original provided by Penard (1899). We agree with Ogden (1983) that *D. distenda* can be separated from *D. acuminata* based mainly by its significantly broader shell (see group 1b vs. 1a in Fig. 6b).

Ogden (1983) also redescribed *D. bicruris* Gauthier-Lièvre et Thomas, 1958, based on SEM observations of two specimens (Fig. 18). The shells of these two individuals were as follows: length (including horns) 202–207 μm, width 95–115 μm, aperture diameter 41–58 μm. Thus they were similar, but slighty larger, than the original specimens the dimensions of which were as follows: length (including horns) 188–193 μm, width 77–86 μm, aperture diameter 33–35 μm (Gauthier-Lièvre and Thomas, 1958). Therefore, given the similarity

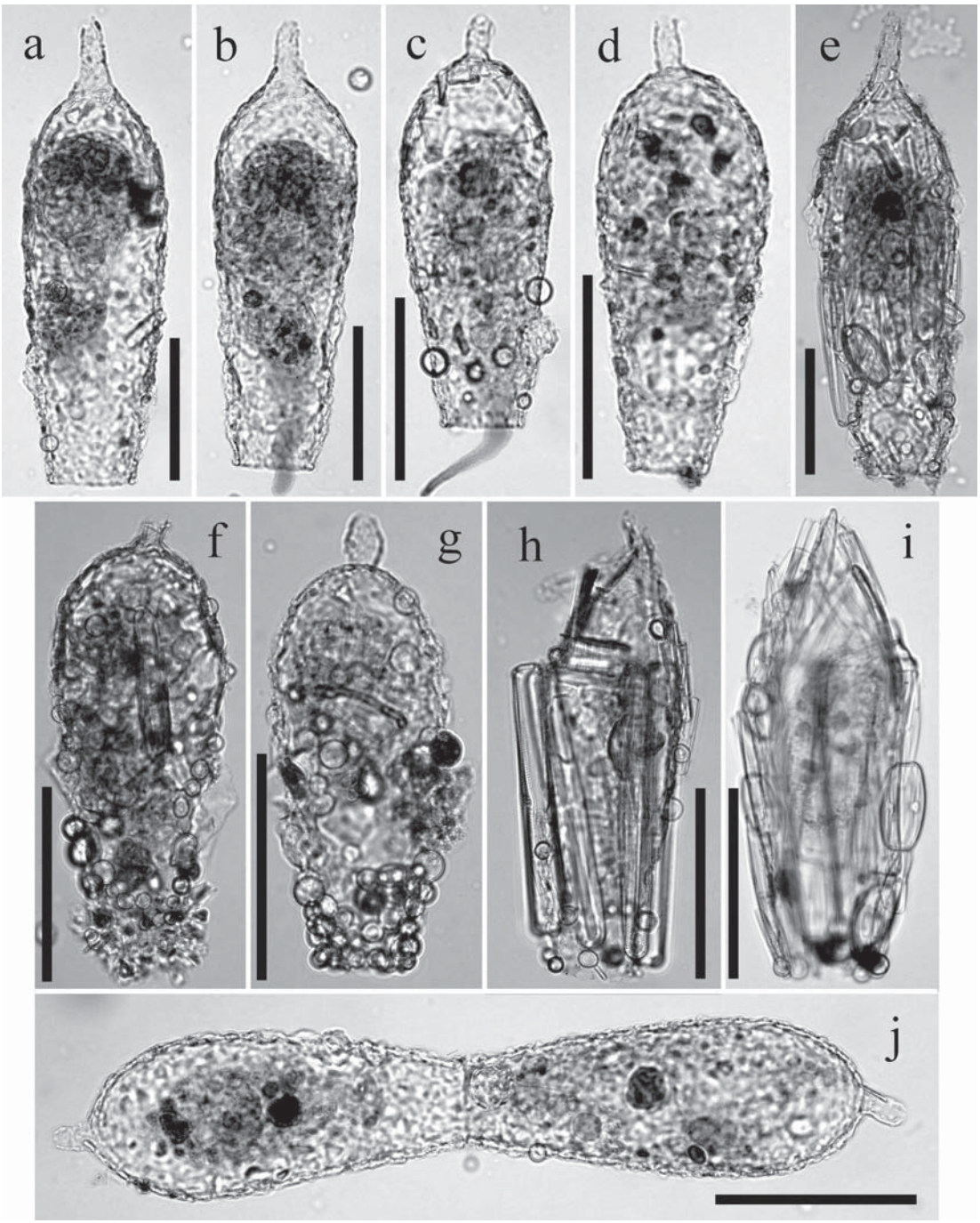

Fig. 9. Different specimens of *Difflugia acuminata* from E. Penard's slides: a-i – lateral view, j – dividing cells (a-d – slide 04.5.9.72, e – slide 04.5.9.75, f, g – slide 04.5.9.77, h – slide 04.5.9.73, i – slide 20.12.8.157, j – slide 04.5.9.78). Scale bars: 100 µm.

of shell size and shape of *D. bicruris* and *D. distenda* (Figs 17, 18), the similar structure of the organic cement (Ogden, 1983; Figs 17e, 18d), and that Penard (1899) reported an individual of *D. distenda* with two horns, we here synonymise *D. bicruris* with *D. distenda*.

***Difflugia acutissima* Deflandre, 1931.** This species was initially described by Deflandre (1931) who considered that it was distinct from *D. acuminata* and its varieties by the sharpness of the aboral extremity. Ogden and Živković (1983) provided the following definition of *D. acutissima*: shell pyriform with sides

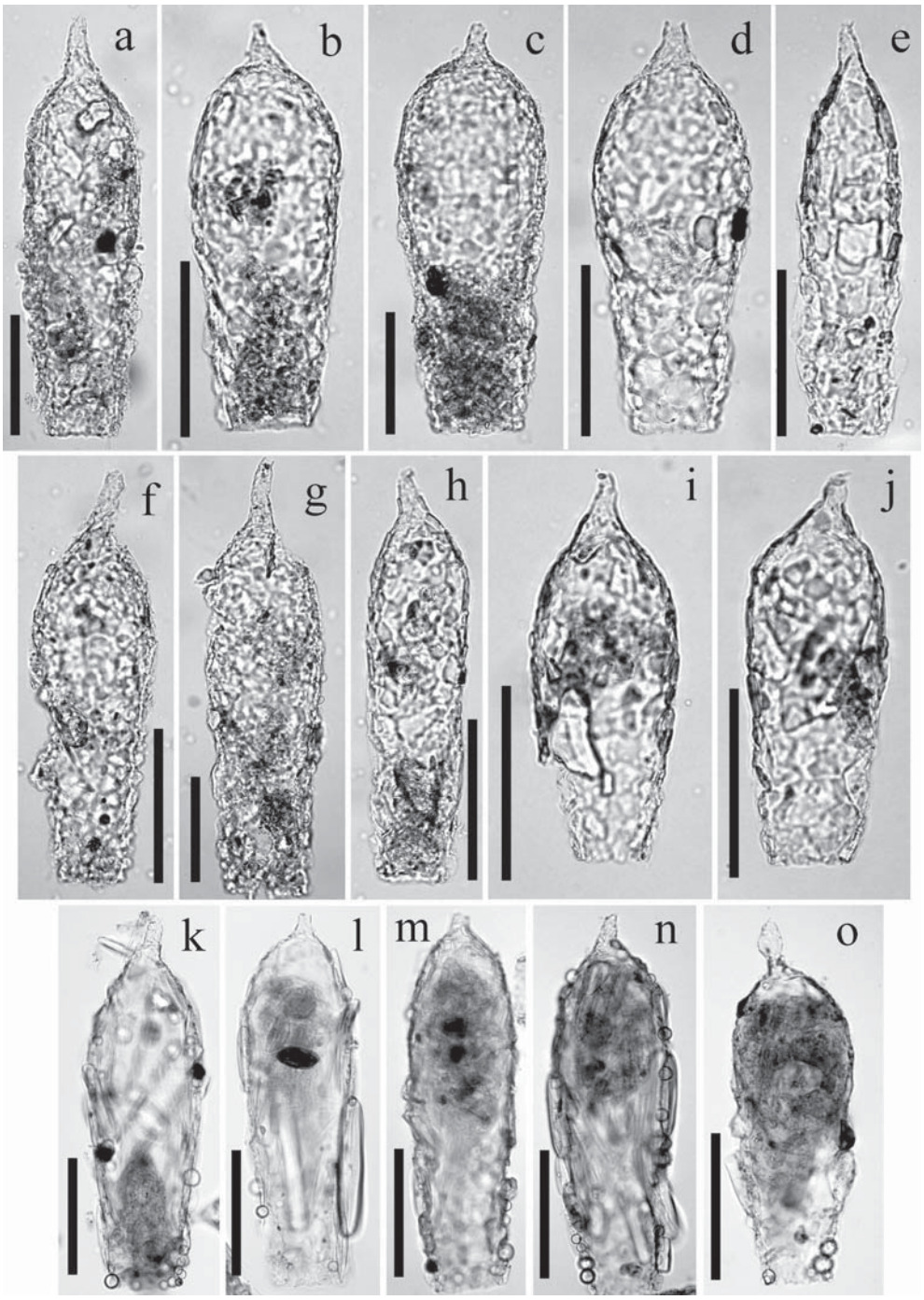

Fig. 10. Different specimens of *Difflugia acuminata* from E. Penard's slides. a–o – lateral view (a–h – slide 04.5.9.71, i, j – slide 4.5.9.103, k–n – slide 20.12.8.161, o – slide 20.12.8.160). Scale bars: 100 μm.

tapering evenly from about mid-body region and usually terminating in a sharp point (Fig. 19); shell composed mainly of flattish pieces of quarz to give a smooth surface; aperture circular and surrounded by small to medium size particles. Ogden measured 10 individuals, four of which were referred to in Ogden and Živković (1983), the other six being unpublished. The ranges of the shell dimensions were as follows: length 178–245 μm, width 80–124 μm, diameter of aperture 39–54 μm. The general dimensions are similar to those given for *D. distenda* (Fig. 6) which, in contrast with *D. acutissima*, is curved aborally forming a small tubular horn. The structure of the organic cement of *D. acutissima* (Fig. 19e and Fig. 1c in Ogden and Živković, 1983) is similar to that of *D. distenda* (Fig. 17e). Thus, we follow Ogden

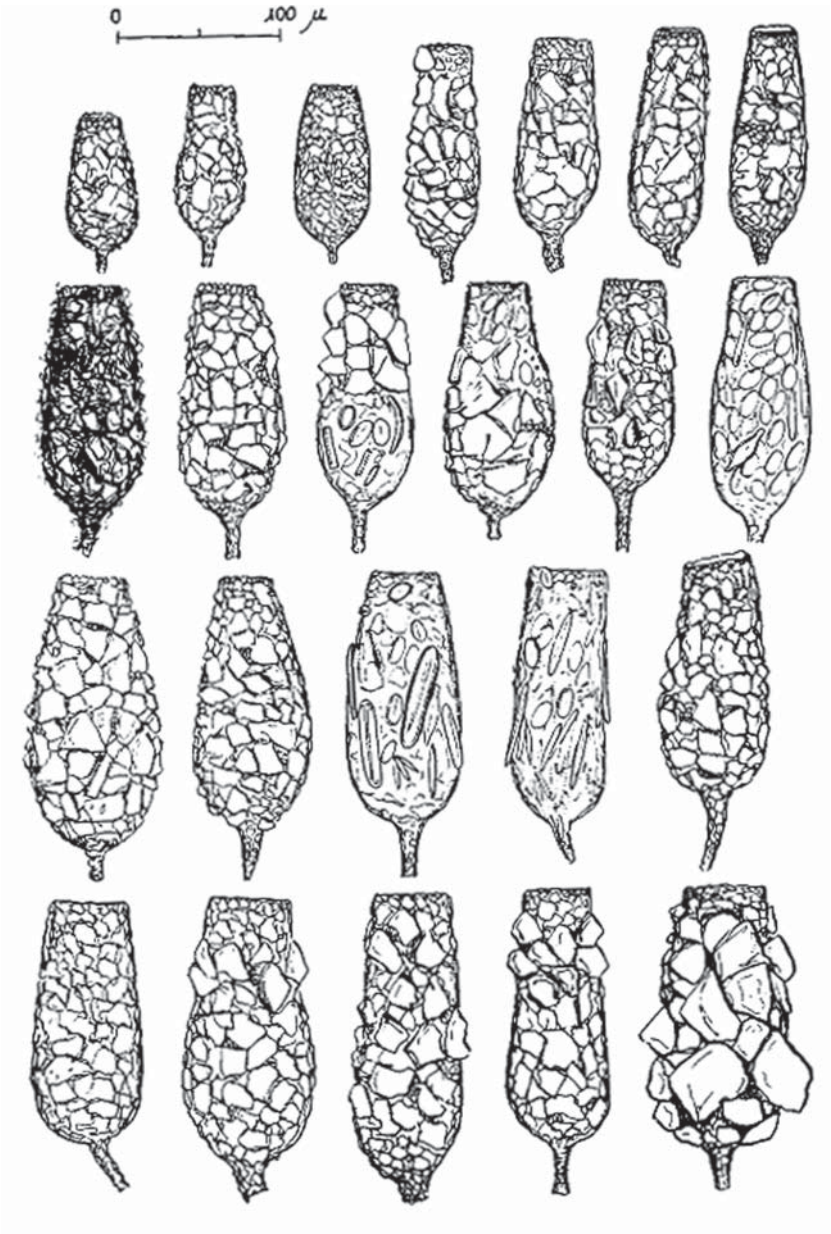

Fig. 11. Morphological variation of *Difflugia acuminata* (after Chardez, 1961).

and Živković (1983), who concluded that further data are required, particularly with respect to the natural variation of morphological features, in order to resolve the systematics of the *D. distenda* – *D. acutissima* group.

Difflugia bacillariarum Perty, 1849 species complex.

According to Ogden (1980b), *Difflugia bacillariarum* Perty, 1849 has an ovoid or pyriform shell that is circular in transverse section, usually with a distinct aboral protuberance or horn (Fig. 20a). Some specimens, however, (see Ogden and Hedley, 1980) do not have an aboral horn (Figs 20 b, c). The aboral horn is usually pointed, of varying length, and usually positioned centrally, but it may be inclined to the side if the tapering is uneven (Fig. 20e). Diatom frustules appear to comprise the bulk of the shell, and the compact nature of the shell material only leaves small areas in which either organic cement or small siliceous plates can be seen. Ogden (1980b) suggested that the basic shell is made of small plates and that the diatom frustules act as reinforcement. The shape of the aperture is dependent on the arrangement and size of the diatom frustules that surround it. Small diatom frustules are usually used and the aperture shape is circular (Fig. 20d), but in

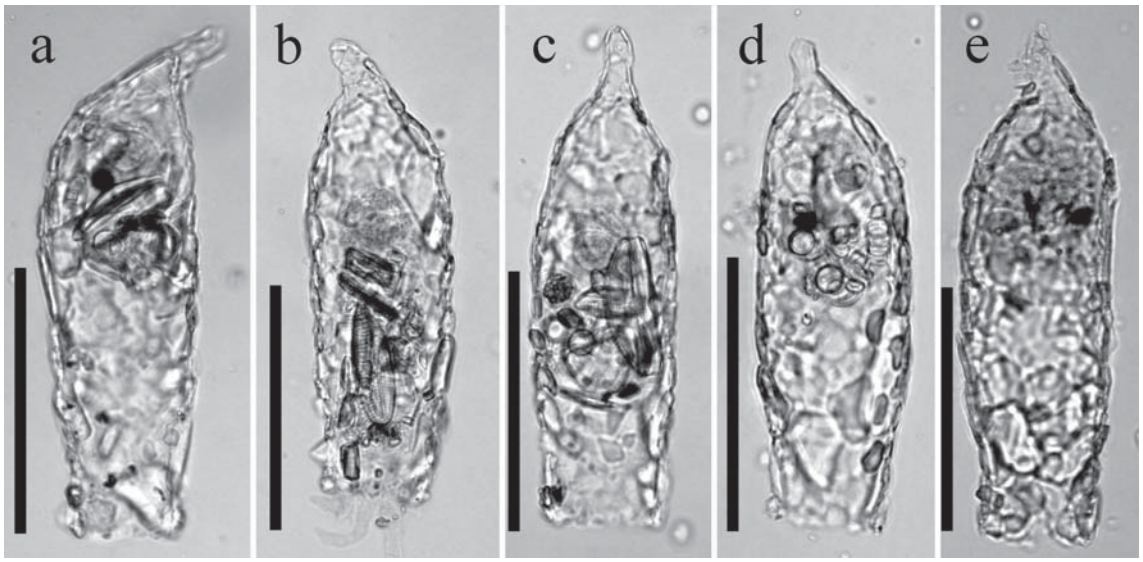

Fig. 12. Different specimens of *Difflugia curvicaulis* from E. Penard's slides. a–e – lateral view (a–d – slide 20.12.8.202, e – slide 04.5.9.93). Scale bars: 100 μm.

some specimens the aperture is triangular. Ogden measured 22 specimens (Fig. 6), 19 of which were published (Ogden, 1980b; Ogden and Hedley, 1980) and three unpublished. The ranges of the shell dimensions are as follows: length 57–103 μm, width 36–47 μm, aperture diameter 16–26 μm.

***Difflugia bicornis* Penard, 1890**. Penard (1890) initially described this as a distinct species that is characterized by its small, ovoid shell (length 50–60 μm (without horns), width 35–45 μm) with two large, aboral horns. The shell is composed of sand grains that are finer in the aperture region. However, Penard (1902) later considered this species to be a small variety of *Difflugia elegans*, and illustrated the variability of the shell of this form with several figures (Figs 3, 10–13 in Penard, 1902, p. 237), including specimens with either one or two aboral spines. In his slide specimens, deposited in the NHM and dated 1920, Penard again used the name *D. bicornis*. These specimens vary significantly in size, shape and general appearance (see Fig. 21), from ovoid (Fig. 21a) to roundish (Figs 21d–f) to pyriform (Figs 21b, c, h), having two large aboral spines (Figs 21a–c, g, i), one large aboral spine (Fig. 21h) or a pointed aboral region (Figs 21d–f), and different types of xenosomes from siliceous grains and plates (Figs 21a–c, h, i) to mixtures of diatom frustules and chrysomonad cysts (Figs 21d–g).

Based on SEM observations of three specimens, Ogden and Živković (1983) redescribed *D. bicornis* as having an ovoid or spherical shell with usually two, but occasionally only one, aboral spines; the shell wall as being thin with a rough surface composed of a mixture of small to medium size particles of quartz, with the occasional addition of portions of diatom frustules or siliceous flagellate cysts; aboral spine(s) roughly pointed and composed of small particles; the aperture usually circular and surrounded by a mixture of particles (Fig. 22a–d). Ogden measured 18 specimens (Ogden and Živković, 1983; Ogden, 1984) with shell dimensions as follows: length 63–91 μm, width 36–53 μm, aperture diameter 18–25 μm (Fig. 6).

We agree with Ogden and Živković (1983) that *D. bicornis* Penard, 1890 can be clearly distingushed from *D. elegans* Penard, 1890 by its size (Fig. 6) and by its fragile (vs. robust) shell. By contrast, we cannot find clear distinctions between *D. bacillariarum* Perty, 1849 and *D. bicornis* Penard, 1890. These two taxa overlap in their size distribution (Fig. 6) and closely resemble each other in shell shape and the composition of xenosomes, including the possession of diatom frustules as one of the possible types of xenosomes. Penard (1902) likewise did not accept *D. bacillariarum* as a valid species considering it to be a small form of *D. elegans*. We consider *D. bacillariarum* and *D. bicornis* to be synonymous, the former name taking priority by its seniority with *D. bicornis* Penard, 1890 a junior synonym. The diagnosis of *D. bacillariarum* Perty, 1849 is improved: shell ovoid, spherical or pyriform; shell xenosomes comprising diatom frustules, sand grains, or flagellate cysts; aboral region of shell usually with one or two prominent spines, occasionally pointed and lacking spines.

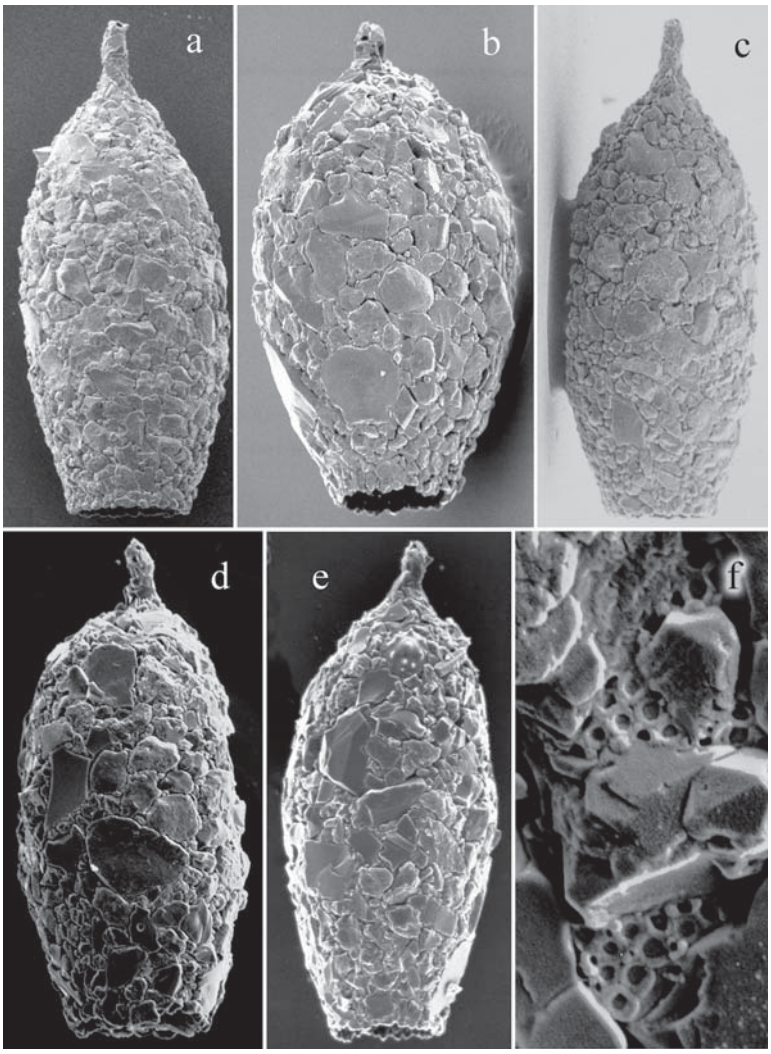

Fig. 13. Different specimens of *Difflugia curvicaulis* from C. Ogden's SEM collection. a–e – lateral view (a – SEM EM-10-485, b – SEM EM-10-980, c – SEM EM-10-481, d – SEM EM-12-342, e – SEM EM-12-799), f – structure of organic cement (SEM EM-10-969). Magnification: a – ×367, b – ×420, c – ×315, e – ×400, f – ×9700.

***Difflugia styla* Ogden et Živković, 1983**. Based on an SEM examination of a single specimen, Ogden and Živković (1983) described *Difflugia styla* as a new species with an ovoid shell composed of small to medium flattish pieces of quartz, a distinct aboral spine and circular aperture (Figs 22e, f). The general shape, appearance and dimensions of the shell of *D. styla* (length 97 μm, width 54 μm, aperture diameter 29 μm) correspond closely with those of *D. bacillariarum* (including *D. bicornis*). The only significant difference between these species is that *D. styla* uses flattish pieces of quartz (vs. diatom frustules, sand grains, or flagellate cysts) as xenosomes (Ogden and Živković, 1983). Thus, the validity of *D. styla* is highly questionable.

Difflugia elegans Penard, 1890 species complex

A typical individual is shown in Fig. 23. According to the original description, the shell of *Difflugia elegans* is urceolate-pyriform, 80–100 μm long and 30–40 μm wide, circular in transverse section and composed of sand grains (Figs 24a, b, d); the aboral region may be pointed or acuminate but usually has a long narrow horn that is sometimes slightly curved terminally; the aperture is circular (Penard, 1890). Ogden (1979) described this species as having a rough, pyriform shell covered by small to large pieces of angular quartz and often diatom frustules, usually with a constriction near the aperture to form a neck, and an aboral horn often curved and perforated at the

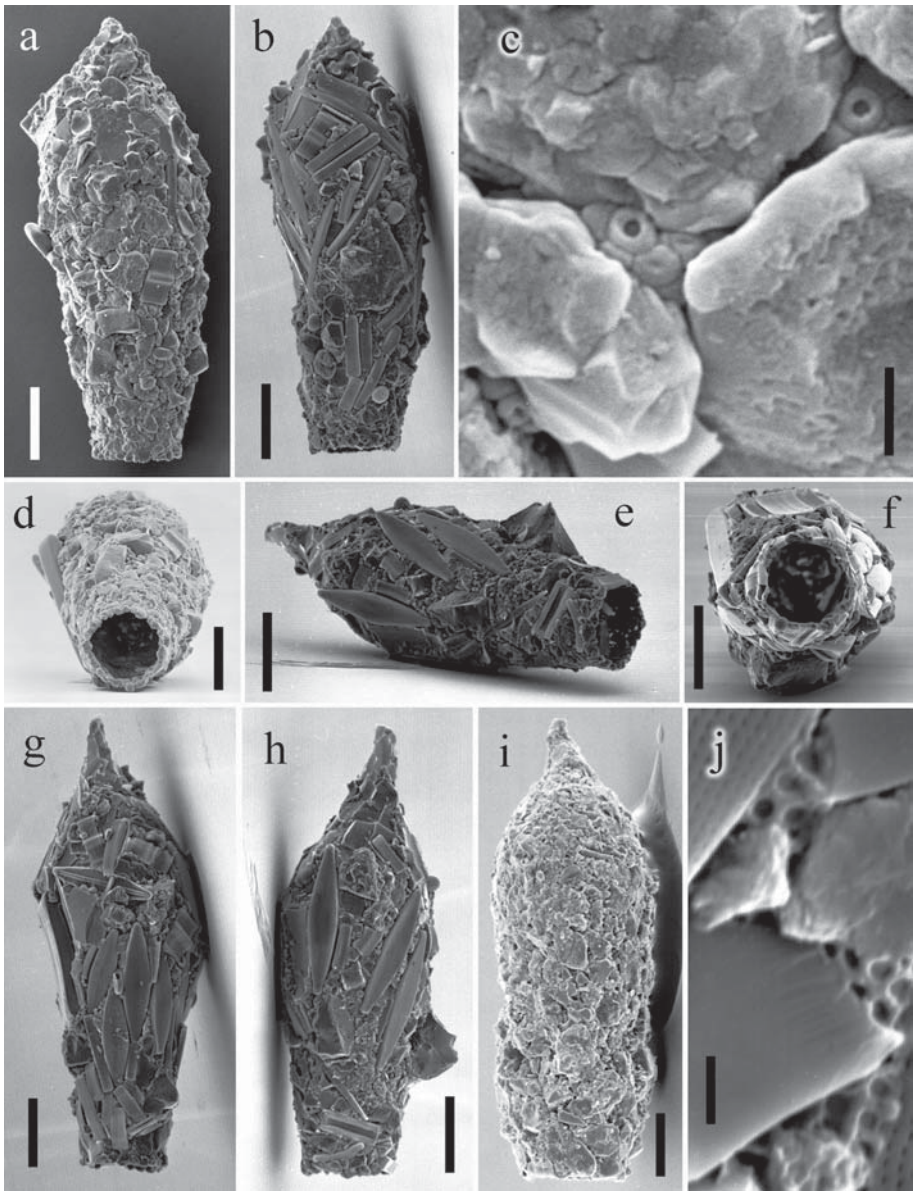

Fig. 14. Different specimens of *Difflugia venusta* (a-d) and *Difflugia ventricosa* (e-j) from C. Ogden's SEM collection. a, b, e, g-i – lateral view (a – SEM CZ-04-641, b – CZ-05-025, e – SEM-CZ-05-031, g – SEM-CZ-05-022, h – SEM CZ-05-028, i – SEM CZ-11-377), c, j – structure of organic cement (c – SEM CZ-04-642, j –SEM CZ-05-029), d, f – apertural view (SEM CZ-04-647, f –SEM CZ-05-034). Scale bars: a, b, d-i – 30 µm, c, j – 10 µm.

apex; the aperture is circular or oval, surrounded by an irregular mixture of particles. Ogden measured 33 specimens of *D. elegans*, 27 of which were published (Ogden, 1979, 1984; Ogden and Hedley, 1980) and six unpublished, with the ranges of shell dimensions as follows: length 95–158 µm, width 55–105 µm, aperture diameter 30–55 µm (Fig. 6).

***Difflugia elegans* var. *angustata* Deflandre, 1926 and *Difflugia elegans* var. *teres* Penard, 1899**. Ogden's collection of SEM micrographs contains examples showing the variability of *D. elegans* (Figs 25a–e) including individuals with a pointed aboral region (Fig. 25d), those with a horn that is either straight (Figs 25a–c) or curved (Fig. 25e) horn, and those with a shell covered by medium-sized particles (Fig. 25d) or a mixture of medium- and large-sized sand grains (Figs 25b, c, e). In some cases Ogden accepted the validity of infraspecific taxa. Based on four specimens, Ogden (1984) redescribed *D. elegans* var. *angustata* Deflandre, 1926 (which he erroneously referred to as *D. ele-*

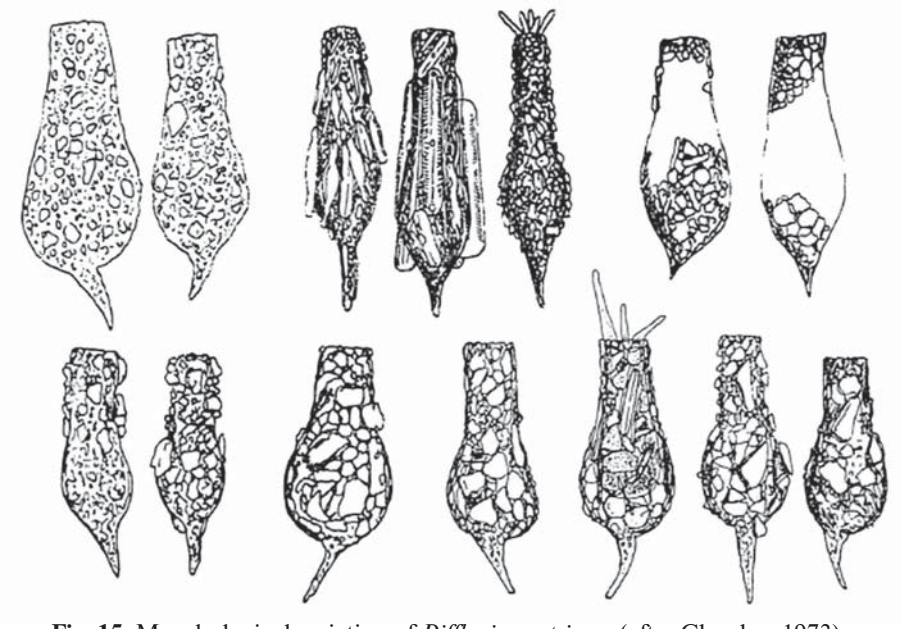

Fig. 15. Morphological variation of *Difflugia ventricosa* (after Chardez, 1973).

gans var. *angustata* Gautier-Lièvre et Thomas, 1958) which is characterized by its elongate shell (shell length 95–118 μm, shell breadth 44–51 μm, aperture diameter 24–27 μm) with an aboral horn and a surface composed of angular quartz and occasionally diatom frustules (Figs 25g, h). The only features that differentiate these specimens from *D. elegans* are the elongate shape of the shell and the absence of a constriction near the aperture. Ogden (1984) noted, however, that the number of specimens examined was insufficient on which to base an accurate assessment of the validity of this variety. Ogden (1984) also illustrated *D. elegans* var. *teres* Penard, 1899 (Figs 25f, i). According to the original description by Penard (1899), *Difflugia elegans* var. teres is characterized by its urceolate-ovoid shell that is acuminate or rarely roundish aborally and has a distinct constriction near the large, circular aperture (Fig. 24c). The shell is covered by large sand grains and has a length of 150–170 μm. This variety is distinguished from the typical *D. elegans* by its slightly larger size, a higher shell width/length ratio, the absence of a long horn and a surface covering of large, angular sand grains.

***Difflugia tricornis* (Jung, 1936) Ogden, 1983 and *Difflugia elegans* f. *bicornis* Jung, 1983**. Ogden examined by SEM two individuals that closely resemble *Difflugia elegans* but which have either three (Figs 26a–c) or two (Figs 26d–g) horns. The former was identified as *D. elegans* forma *tricornis* Jung, 1936 which he redescribed based on a single specimen (with body length 116 μm, breadth 82 μm, aperture diameter 40 μm) and elevated it to species rank as *D. tricornis* (Ogden, 1983). This organism is characterized by its pyriform shell which is slightly broadened near the aperture, has three, evenly spaced, aboral spines, a surface that is rough and mainly composed of a mixture of medium- and large-sized pieces of angular quartz, and a circular aperture that is surrounded by irregular particles (Figs 26a–c; Ogden, 1983). The second individual has a shell shape that is very similar to that of *D. elegans* in terms of shape and size (shell length 135 μm, shell breadth 92 μm, aperture diameter 73 μm), but has two aboral horns and is slightly compressed laterally (unpublished; Figs 26d–g). Jung (1936) described two forms of *D. elegans*, namely *tricornis* and *bicornis* (not *D. bicornis* sensu Penard, 1890) but failed to supply measurements or illustrations of either. Gautier-Lièvre and Thomas (1958) illustrated *D. elegans* f. *tricornis* with three irregularly spaced horns and gave its shell dimensions as 110–130 μm in length and 65–70 μm in width. However, the number of horns on the shell of *Difflugia* is a highly variable character, as seen in *D. bacillifera* (see above), *D. tuberspinifera* (Yang et al., 2004) and *D. corona* (Jennings, 1916), and therefore has little or no taxonomic value. Thus we do not accept the validity of *D. tricornis, D. elegans* f. *tricornis* or *D. elegans* f. *bicornis*, all of which are junior synonyms of *D. elegans.*

***Difflugia amphoralis* Hopkinson, 1909**. This species was characterized by Hopkinson (in Cash and Hopkinson, 1909) as having a shell that is composed

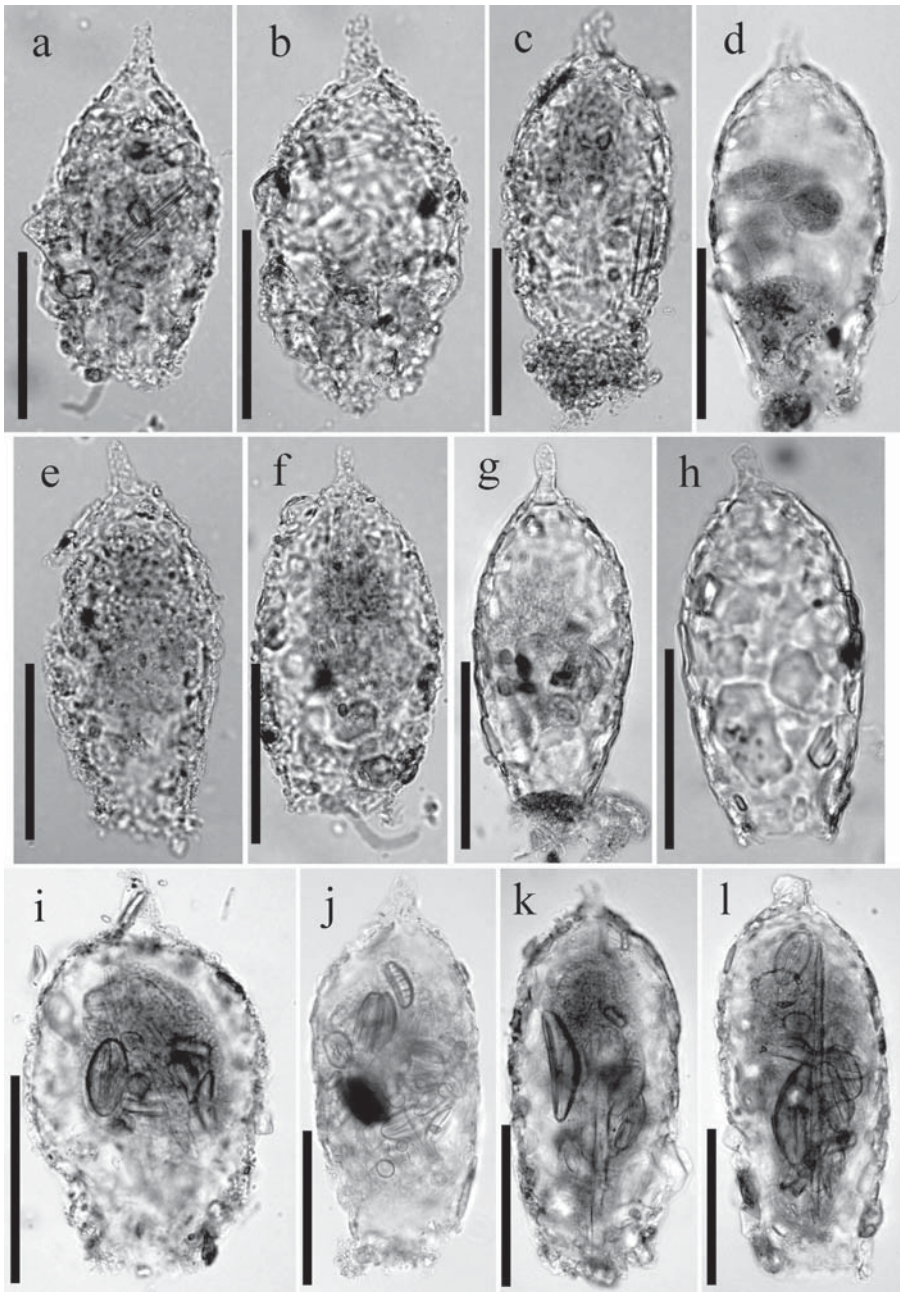

Fig. 16. Different specimens of *Difflugia acuminata* var. *inflata* from E. Penard's slides. a-l – lateral view (a-c, e, f – slide 04.5.9.70, d, j – slide 20.12.8.163, g, h – slide 20.12.8.164, i – slide 20.12.8.159, k, l – slide 20.12.8.162). Scale bars: 100 μm.

of chitinous material, covered by amorphous scales and few sand grains, 115 μm long and 70 μm wide, neither convex nor spined but pointed like a Gothic arch, and with an aperture that has an expanded but not upturned rim below the constricted neck. Ogden (1983) redescribed this species based on SEM observations (Fig. 27) as having a squat, pyriform shell with the aboral extremity tapering evenly to a point. The shell is composed mainly of medium pieces of quartz along with some smaller pieces arranged in such a way to produce a moderately smooth surface. The circular aperture is surrounded by a small rim of mainly small particles giving a poorly defined border. Ogden measured a total of seven specimens, including one described in Ogden (1983), two in Ogden (1984) and four unpublished, with the range of shell dimensions as follows: length 106–114 μm, width 61–87 μm, aperture diameter 28–43 μm (Fig. 6). According to Ogden (1983) the shell is composed mainly of quartz particles (see Fig.

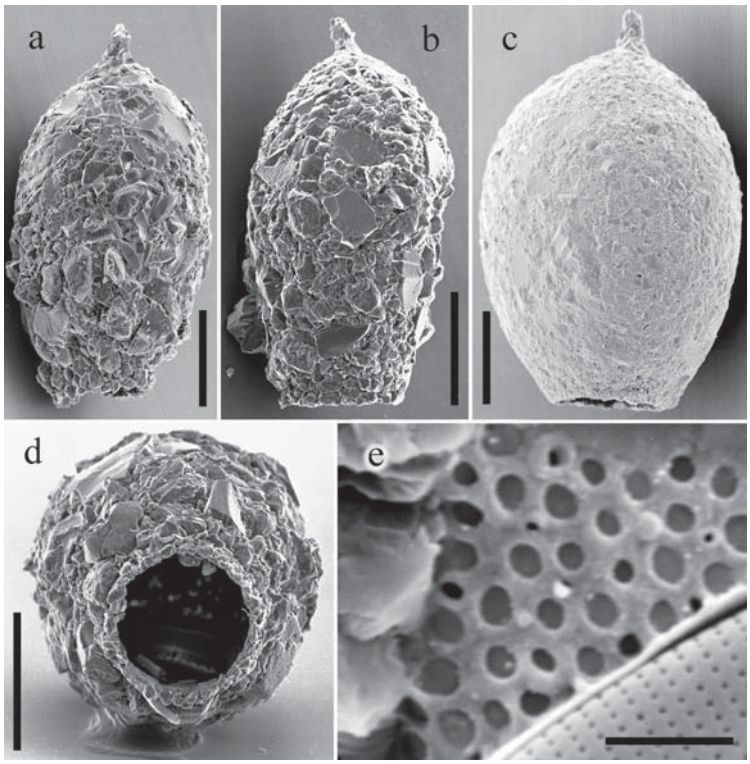

Fig. 17. Different specimens of *Difflugia distenda* from C. Ogden's SEM collection. a-c – lateral view (a – SEM CZ-06-378, b – SEM CZ-07-406, c – SEM 096691), d – apertural view (SEM CZ-05-361), e – structure of organic cement (SEM CZ-05-372). Scale bars: a-d – 60 µm, e – 20 µm.

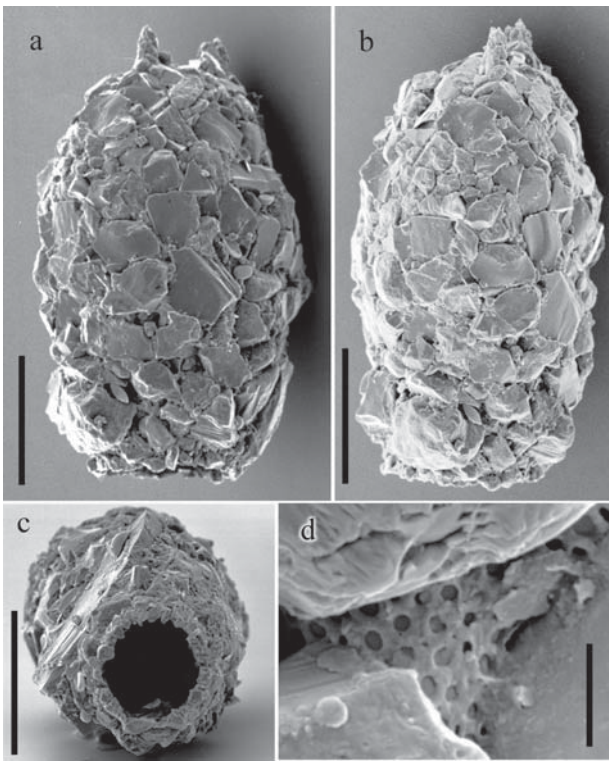

Fig. 18. Different specimens of *Difflugia bicruris* from C. Ogden's SEM collection. a-b – lateral view (a – SEM CZ-05-373, b – SEM CZ-05-369), c – apertural view (SEM CZ-07-416), d – structure of organic cement (SEM CZ-06-380). Scale bars: a-c – 60 µm, d – 20 µm.

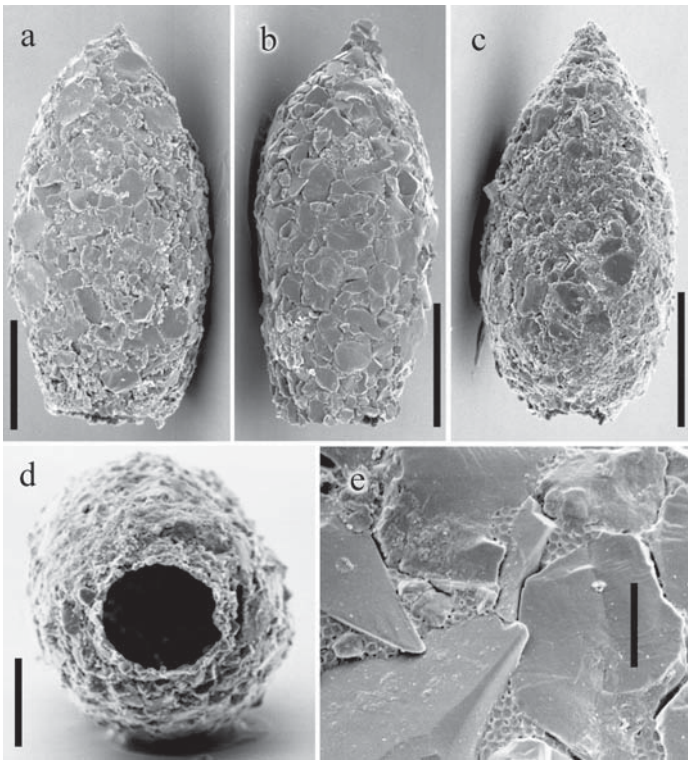

Fig. 19. Different specimens of *Difflugia acutissima* from C. Ogden's SEM collection. a-c – lateral view (a – SEM CZ-03-210, b – SEM CZ-03-213, c – SEM CZ-11-295), d – apertural view (SEM CZ-11-298), e – structure of organic cement (SEM CZ-03-616). Scale bars: a-c – 60 µm, d – 30 µm, e – 20 µm.

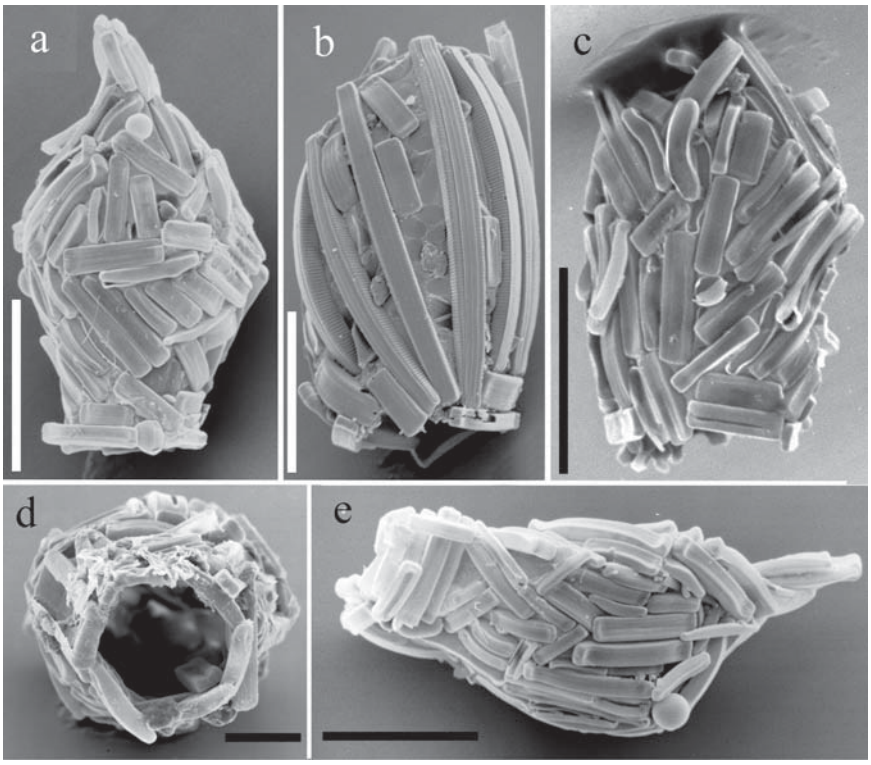

Fig. 20. Different specimens of *Difflugia bacillariarum* from C. Ogden's SEM collection. a–c, e – lateral view (a – SEM CZ-01-601, b – SEM EM-12-126, c – SEM CZ-02-740, e – SEM CZ-01-712), d – apertural view (SEM CZ-02-971). Scale bars: a–c, d – 30 µm, e – 10 µm.

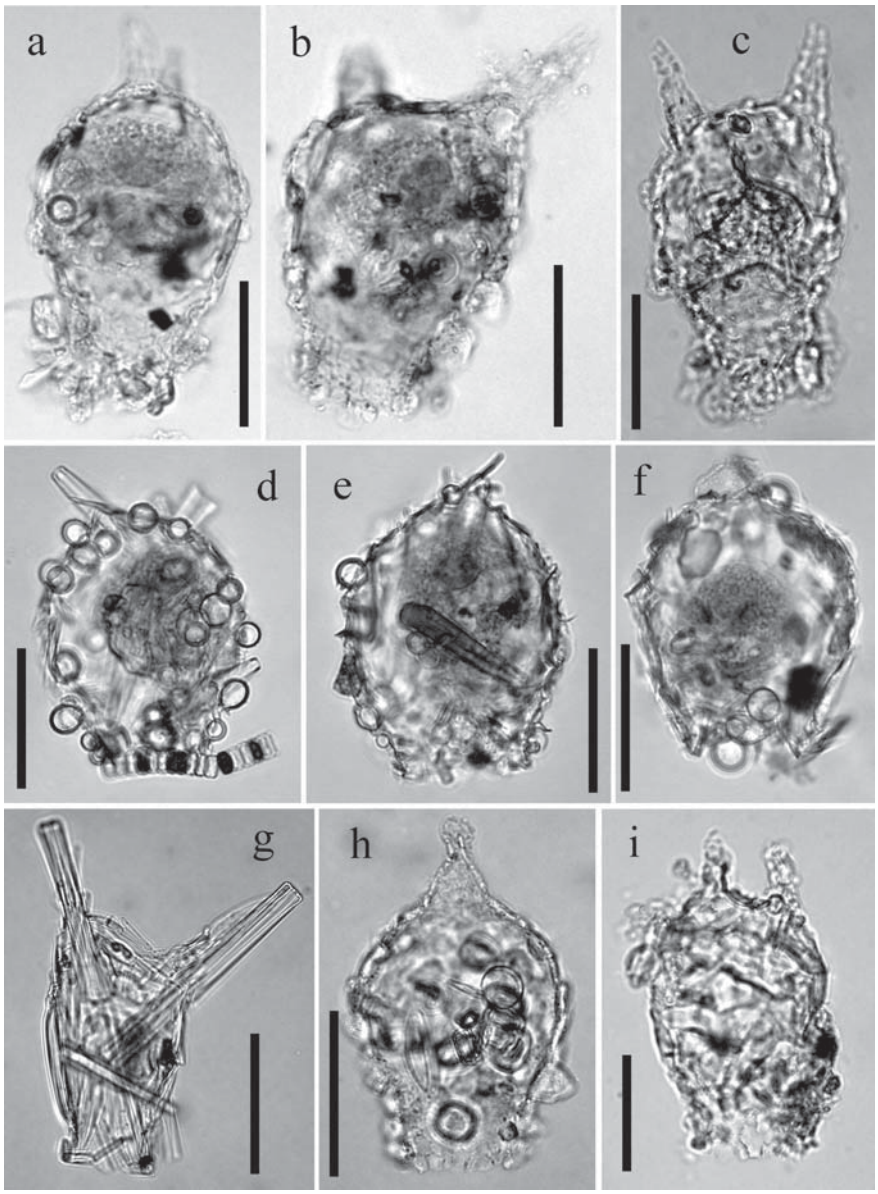

Fig. 21. Different specimens of *Difflugia bicornis* from E. Penard's slides. a–i – lateral view (a–c – slide 20.12.8.176, d–f – slide 04.5.9.99, g – slide 20.12.8.178, h – slide 20.12.8.177, I – slide 04.5.9.94). Scale bars: 50 μm.

27), rather than "amorphous (siliceous?) scales" as stated by Cash and Hopkinson (1909). Ogden (1983) also noted that the specimens described by Leidy (1879), which Cash and Hopkinson (1909) considered to be synonymous with *D. amphoralis*, are also composed mainly of quartz sand.

Cash and Hopkinson (1909) compared *D. amphoralis* with *D. urceolata* and distinguished these taxa by the absence of an upturned rim (collar) in the former species. However, neither Cash and Hopkinson (1909) nor Ogden (1983) compared *D. amphoralis* with *D. elegans*, despite the fact that the shells of these two species are very similar in size and shape (see Figs 6, 25d, 27d and also Fig. 13, plate XXI and Fig. 3, plate XX in Cash and Hopkinson, 1909). In our opinion these two taxa are indistinguishable so we consider *D. amphoralis* Hopkinson, 1909 a junior synonym of *D. elegans* Penard, 1890.

***Difflugia australis* (Playfair, 1918) Gautier-Lièvre et Thomas, 1958**. Playfair (1918) described *Difflugia bacillariarum* var. *australis* with a shell that is broadly ovate and dome-like with convex sides that converge to within a very short distance of the aperture, at which point they diverge sharply

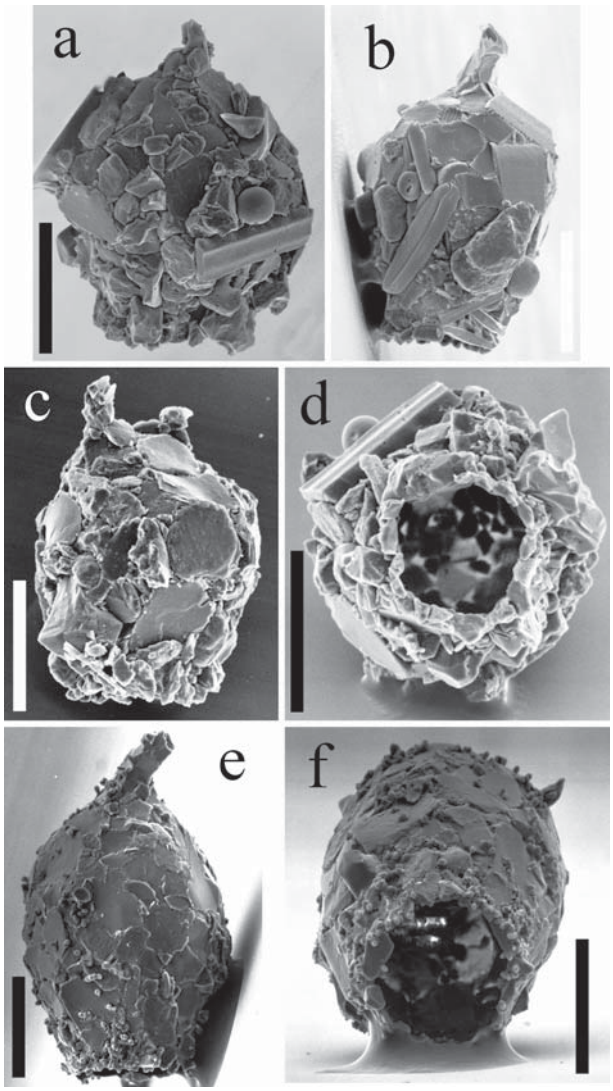

Fig. 22. Different specimens of *Difflugia bicornis* (a-e) and Difflugia styla (f, g) from C. Ogden's SEM collection. a-c – lateral view (a – SEM CZ-09-527, b – SEM CZ-10-386, c – SEM CZ-3-716), d – apertural view (SEM CZ-3-724), e – lateral view (SEM CZ-03-161A), f – apertural view (SEM CZ-03-168). Scale bars: 30 μm.

forming a rim. The shell is usually asymmetrical, one side being more convex than the other. There is often a slanting spine at the aboral end of the shell. The shell is smooth, composed of flat siliceous plates of irregular shape and size, mixed with fine grains. The aperture is circular. The dimensions of the shell are: length 100–120 μm, width 57–72 μm, aperture diameter 23–36 μm. Gautier-Lièvre and Thomas (1958) elevated this taxon to species level as *D. australis* stating that it is clearly separated from both *D. bacillariarum* and *D. elegans*, although they did not supply any evidence to support this decision.

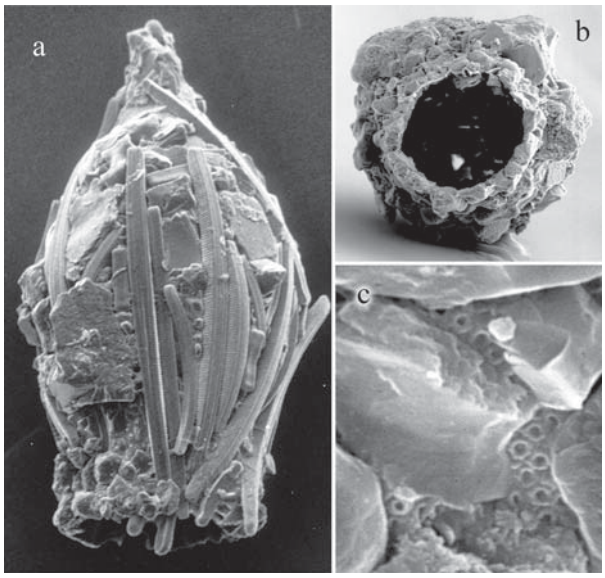

Fig. 23. Different specimens of *Difflugia elegans* from C. Ogden's SEM collection. a – lateral view (SEM EM-10-063), b – apertural view (SEM EM-11-851), c – structure of organic cement (SEM Z-15-841). Magnification: a – ×477, b – ×791, c – ×11200.

Chardez (1978) illustrated high variability of *D. australis* (Fig. 28) although he did not compare it with either *D. elegans* or *D. amphoralis*, despite the fact that his description of *D. australis* is almost identical to those of both these species. Thus, based on their shell size and shape (Figs 25, 27, 28), we synonymise *Difflugia australis* (Playfair, 1918) Gautier-Lièvre et Thomas, 1958 with *Difflugia elegans* Penard, 1890 and *Difflugia australis* var. minor Gautier-Lièvre et Thomas, 1958 with *Difflugia bacillariarum* Perty, 1849.

***Difflugia varians* Penard, 1902**. In his original description of *Difflugia varians*, Penard (1902) noted the high variability of the shell structure, in particular the spines (which may or may not be present) and the number (two, three or four) and distribution of the horns (Figs 1–8, page 241 in Penard, 1902). In the NHM Penard collection we found one individual with two horns and another with three horns (Fig. 29). Ogden likewise observed individuals with either two or three horns (Fig. 26). In terms of its general morphology, including shell size and shape, *D. varians* closely resembles *D. elegans*, the only significant difference being the structure of the nucleus, a character which does not normally have applications in the systematics of difflugiids. Thus we consider *D. varians* Penard, 1902 to be a junior synonym of D. *elegans* Penard, 1890.

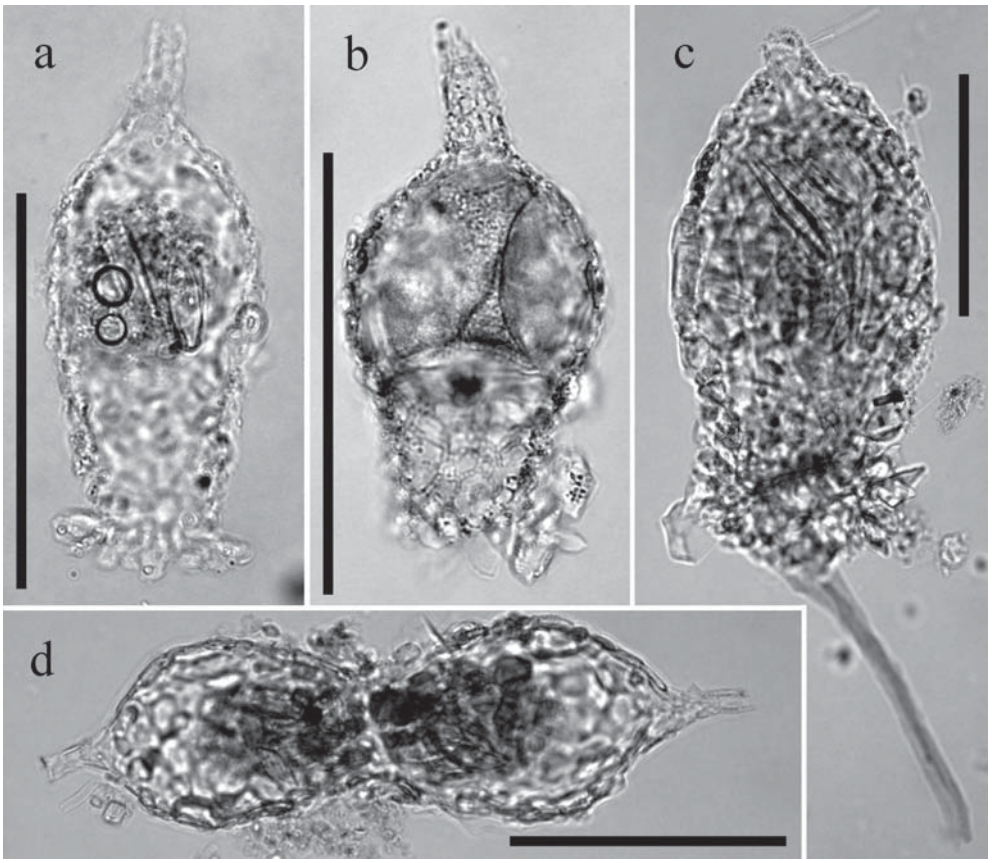

Fig. 24. Different specimens of *Difflugia elegans* (a, b, d) and *Difflugia elegans* var. *teres* (c) from E. Penard's slides. a–c – lateral view (a – slide 20.12.8.204, b – slide 04.5.9.97, c – slide 4.5.9.96) d – dividing cells (slide 04.5.9.98). Scale bars: 100 μm.

***Difflugia Solowetskii* Mereschkowsky, 1877 and *Difflugia borodini* Gassowsky, 1936**. *Difflugia Solowetskii* was initially described in paper published in Russian (Mereschkowsky, 1877). However, Penard (1902) and Cash and Hopkinson (1909) discussed this taxon using information from another manuscript (Mereschkowsky, 1879). *D. Solowetskii* is very similar in shape and size to *D. elegans*. Penard (1902) considered Mereschkowsky's (1879) descriptions of this species as insufficient and synonymised *D. Solowetskii* with *D. elegans*. Cash and Hopkinson (1909) considered *D. Solowetskii* to be a variety of *D. bacillariarum*, i.e. *D. bacillariarum* var. *solowetskii*. However, subsequent workers treated *D. Solowetskii* as a valid species (Gassowsky, 1936; Schönborn, 1965b) with following shell dimensions: length 72–136 μm, width 36–71 μm, aperture diameter 21–45 μm (Gassowsky, 1936) and length 80–100 μm, width 30–35 μm, aperture diameter 15–18 μm (Schönborn, 1965b). Furthermore, Gassowsky (1936) considered *D. elegans* Penard, 1890 as a junior synonym of *D. Solowetskii* Mereschkowsky, 1877. Schönborn (1965b) described small variety with a shell length 30 μm, shell width 15–18 μm, and aperture diameter 7–8 μm, as *D. solowetskii* var. *stepaneki*. Gassowsky (1936) described *D. borodini* (length 76–131 μm, width 45–69 μm, aperture diameter 24–43 μm) separating it from *D. Solowetskii* by its broader shell and rough surface caused by a mixture of large- and medium-size sand grains. These characters are very similar to those of *D. elegans* var. *teres*. Furthermore, data on the natural variability of both *D. borodini* and *D. Solowetskii* as well as comparisons with closely related taxa, are scant. Thus the validity of *D. Solowetskii* Mereschkowsky, 1877 and *D. borodini* Gassowsky, 1936 is highly questionable and we here synonymise both taxa with *D. elegans* Penard, 1890.

***Difflugia leidyi* Wailes, 1912**. This species was described by Wailes (1912) and is characterized by its medium-size shell (length including horns 130–150 μm, width 78–80 μm) that is circular or semicircular in transverse section and composed of siliceous particles and sometimes diatoms frustules; the shell has two (rarely three) horns arranged symmetrically in the aboral region; the aperture is circular. This

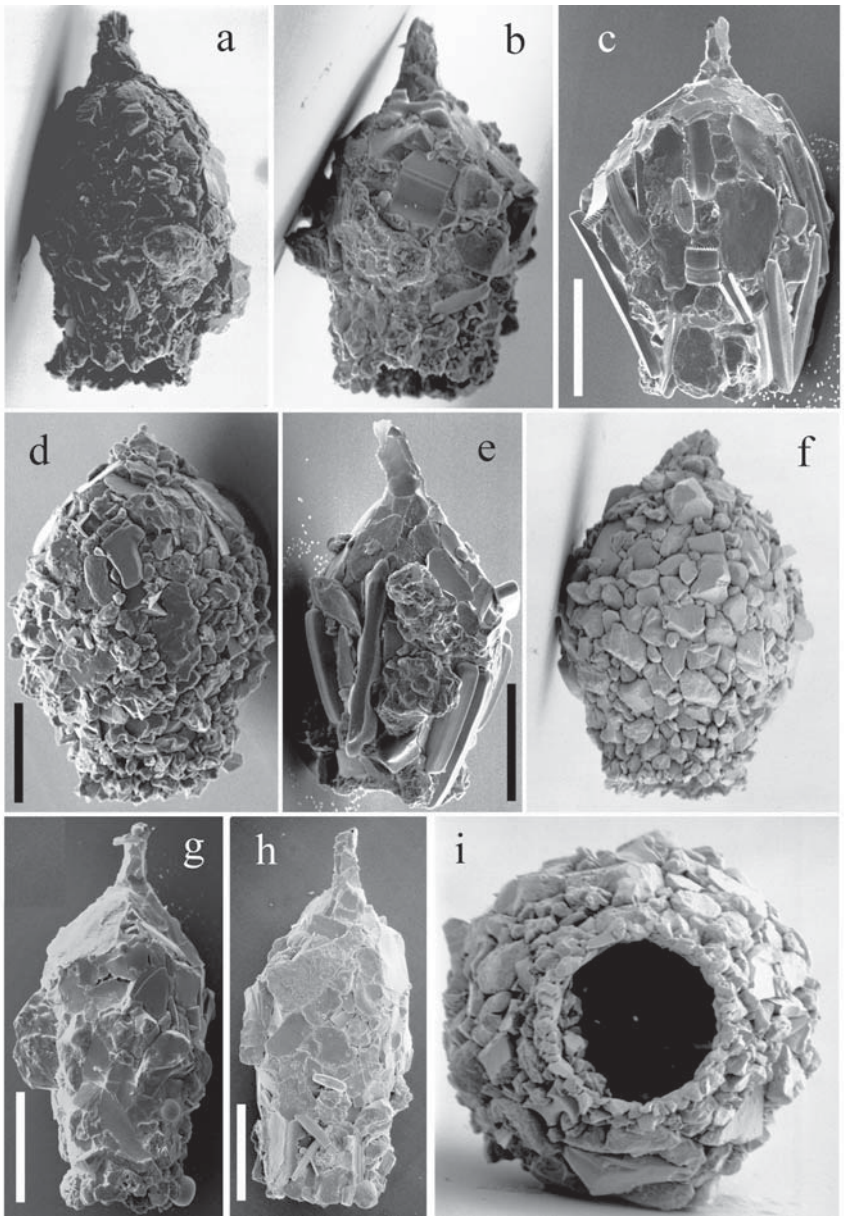

Fig. 25. Different specimens of *Difflugia elegans* (a-e), *Difflugia elegans* var. *angustata* (g, h), *D. elegans* var. *teres* (f, i) from C. Ogden's SEM collection. a-h – lateral view (a – SEM EM-11-563, b – SEM EM-11-963, c – SEM CZ-10-454, d – SEM CZ-06-150, e – SEM CZ-10-447, f – SEM EM-11-817, g – SEM CZ-10-423, h – SEM 007271), i – apertural view (SEM EM-11-818). Scale bars: 30 µm.

species was redescribed by Ertl (1956) and Štěpánek (1957) who concluded that careful investigation of the closely related taxa, including *D. leidyi, D. bacillifera*, and *D. elegans*, is necessary in order to determine the validity of *D. leidyi*. *Difflugia leidyi* is almost impossible to separate from *D. elegans* f. *tricornis* or *D. elegans* f. *bicornis*. Thus we treat *D. leidyi* Wailes, 1912 as a junior synonym of *D. elegans* Penard, 1890.

***Difflugia juzephiniensis* Dekhtyar, 1993.** This species was described by Dekhtyar (1993) from sedge-*Sphagnum* bogs in Ukraine and is characterized by its wedge-shaped shell with two horns symmetrically placed in the aboral region, a rough shell surface composed of mixture of sand grains, diatom frustules, flagellate cysts, and shell length 89–104 µm, width 59–86 µm, aperture diameter 26–48 µm. Dekhtyar (1993) noted that this species differs from *D. elegans* and *D. elegans* f. *bicornis* by its lateral compression. The specimen illustrated by Dekhtyar (1993; p. 6, Fig. 1a) is almost identical with those of *D. elegans* investigated by Ogden (Figs 26e–g). We therefore consider *Difflugia juzephiniensis* Dekhtyar,

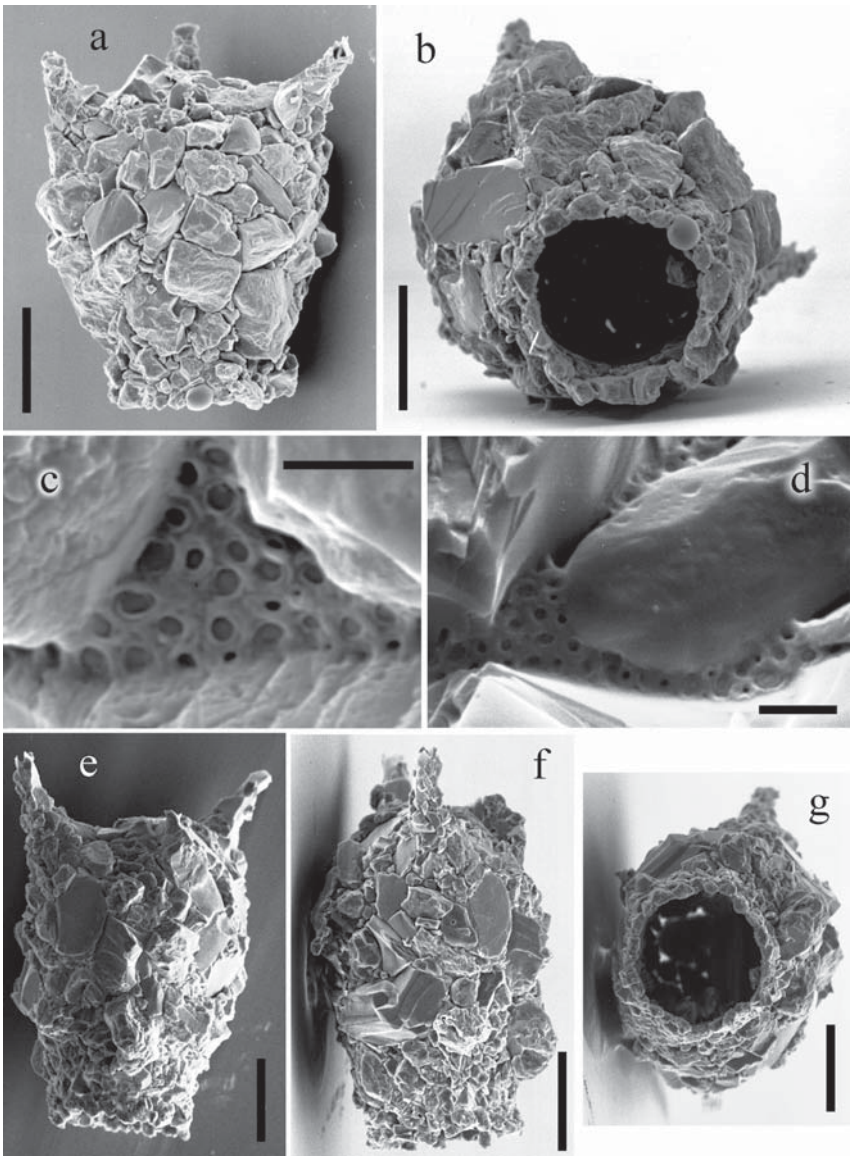

Fig. 26. Different specimens of *Difflugia elegans* with three (a-c) and two (d-g) horns from C. Ogden's SEM collection. a, e, f – lateral view (a – SEM CZ-05-332, e – SEM CZ-10-566, f – SEM CZ-10-570), b, g – apertural view (SEM CZ-05-342 and SEM CZ-10-572), c, d – structure of organic cement (SEM CZ-05-333 and SEM CZ-10-568). Scale bars: a, b, e–g – 30 µm, c, d – 2 µm.

1993 to be a junior synonym of *D. elegans* Penard, 1890.

Considering the highly variable shell morphology of members of the *D. elegans*-complex, we believe the following statement by Foissner and Dragesco (1996, p. 62) made in reference to the polymorphic ciliate species *Tracheloraphis phoenicopterus* also applies to the *D. elegans*-complex: "Whether the ... complex consists of a single, highly variable species, of several distinct, still insufficiently characterized morphospecies or, as we believe, of a set of sibling species, needs further investigation. At the present state of knowledge, the populations ... are hardly distinguishable. Further studies should thus try to characterize such populations in more detail", i.e. morphometric data linked with environmental characteristics, molecular data and SEM observations are needed in order to resolve the systematics of the *D. elegans*-complex.

Difflugia claviformis (Penard, 1899) Ogden, 1979 species complex

This taxon was initially described by Penard (1899) as *Difflugia pyriformis* var. *claviformis* (Fig. 30). Ogden (1979) redescribed it based on SEM

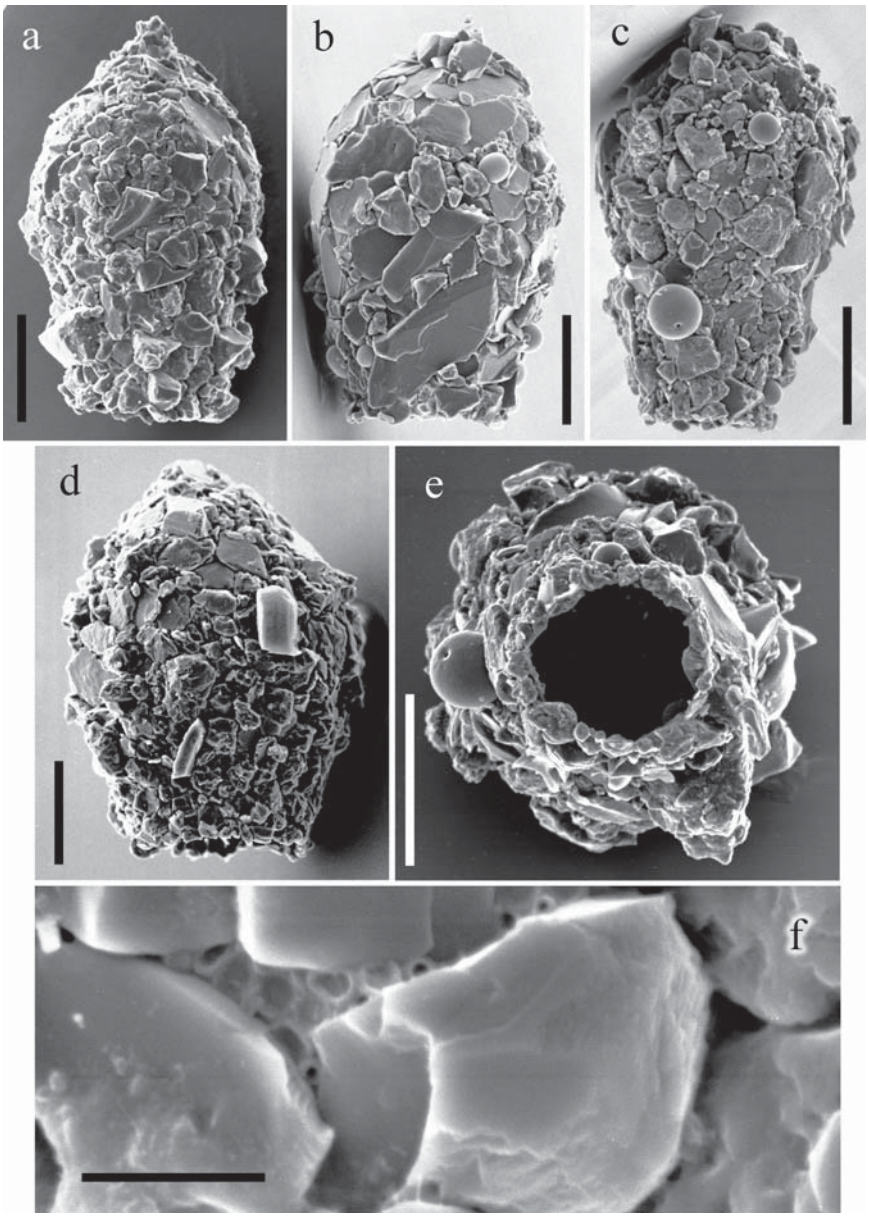

Fig. 27. Different specimens of *Difflugia amphoralis* from C. Ogden's SEM collection. a-d – lateral view (a – SEM CZ-07-818, b – SEM CZ-10-997, c – SEM CZ-05-050, d – SEM CZ-09-328), e – apertural view (SEM CZ-05-047), f – structure of organic cement (SEM CZ-07-291). Scale bars: a-e – 30 μm, f – 3 μm.

observations (Fig. 31) and raised it to species rank as *D. claviformis*. Further details were given by Ogden and Hedley (1980). The shell of *D. claviformis* is pyriform with the aboral region having a terminal conical protuberance that is often curved and perforated at the apex (Fig. 30c), although sometimes the protuberance is absent and the aboral region only slightly narrowed (Figs 30b, d–f). The sides of the shell curve out evenly from the aperture to reach the widest diameter in the mid-region, and then taper evenly towards the conical protuberance. The shell surface ranges from smooth (Fig. 31) to rough (Fig. 30) and is composed mainly of sand grains. The aperture is circular and surrounded by small, evenly arranged particles. Ogden measured 20 specimens, including two in Ogden (1979), 12 in Ogden and Hedley (1980) and eight unpublished, with shell size ranges as follows: length 247–414 μm, width 97–196 μm, aperture diameter 33–62 μm. The shell size ranges given by Penard (1899) were: length 390–435 μm, width 130–200 μm.

***Difflugia microclaviformis* (Penard, 1902) Ogden 1983**. This taxon was initially described as a *Difflugia*

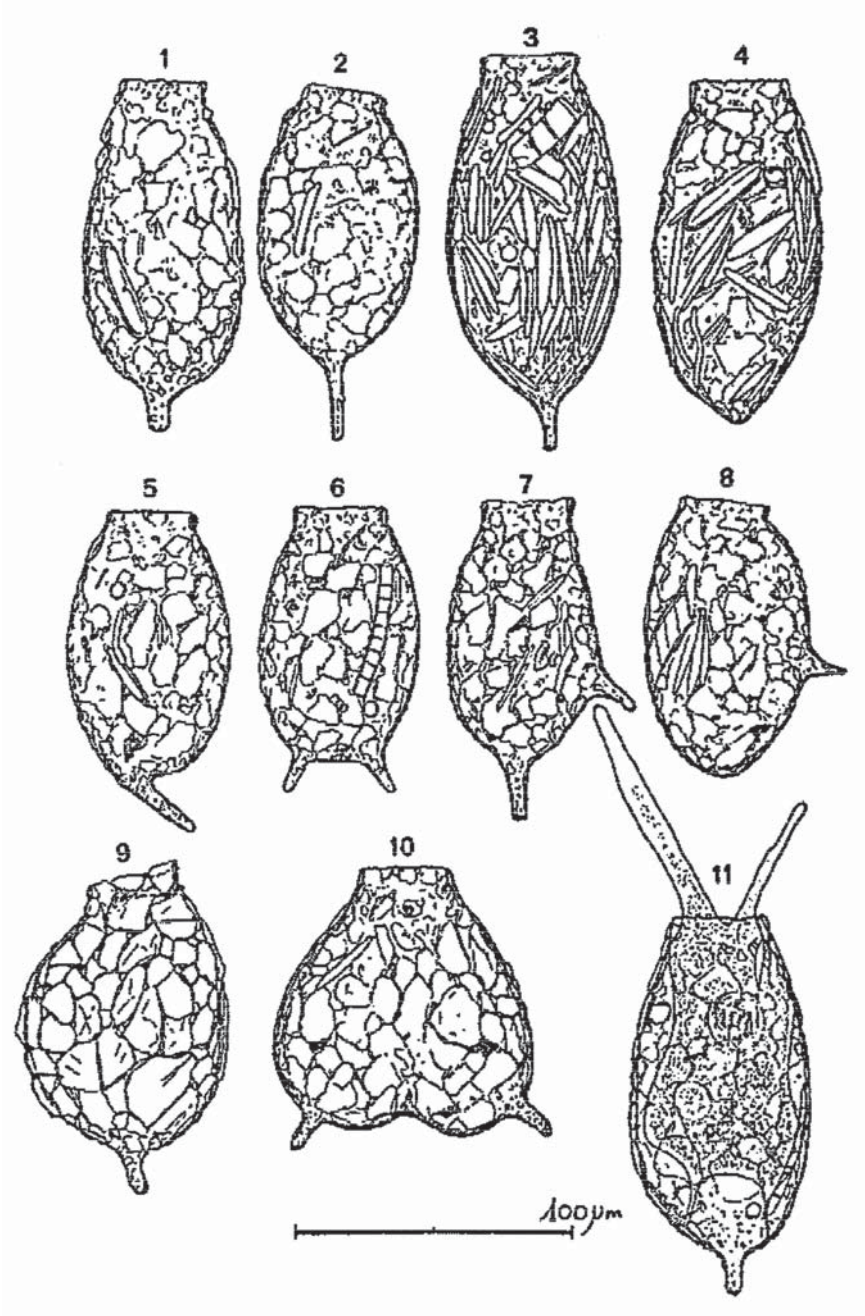

Fig. 28. Morphological variation of *Difflugia australis* (after Chardez, 1978). 1–3, 11 – specimens with one straight long horn, 4 – specimen without horn (pointed aborally), 5 – specimen with curved horn, 6 – specimen with two horns equally spaced aborally, 7 – specimen with one aboral and one lateral horn, 8 – specimen with one lateral horn, 9 – specimen with asymmetrical shell, 10 – teratic individuals (Siamese twins).

pyriformis var. *venusta* by Penard (1902). Cash and Hopkinson (1909) renamed it *D. oblonga* var. *venusta*. Later Kourova (1925) described *D. oblonga* var. *microclaviformis*, the shell of which is identical in both size and shape with the original description of *D. pyriformis* var. *venusta* Penard, 1902 (Fig. 32). Unfortunately, Ogden (1983) mistakenly occupied the name *venusta* for the specimens discussed above (Figs 14a–d) when treating the polymorphic species *D. acuminata*. Moreover, he accepted the name *microclaviformis* for specimens (Fig. 33) identical with those from Penard's slides (Fig. 32). Thus, here we consider *D. pyriformis* var. *venusta* Penard, 1902 to be a junior synonym of *D. microclaviformis* (Kourova, 1925) Ogden, 1983 which Ogden (1983) redescribed as follows: shell pyriform with distinct

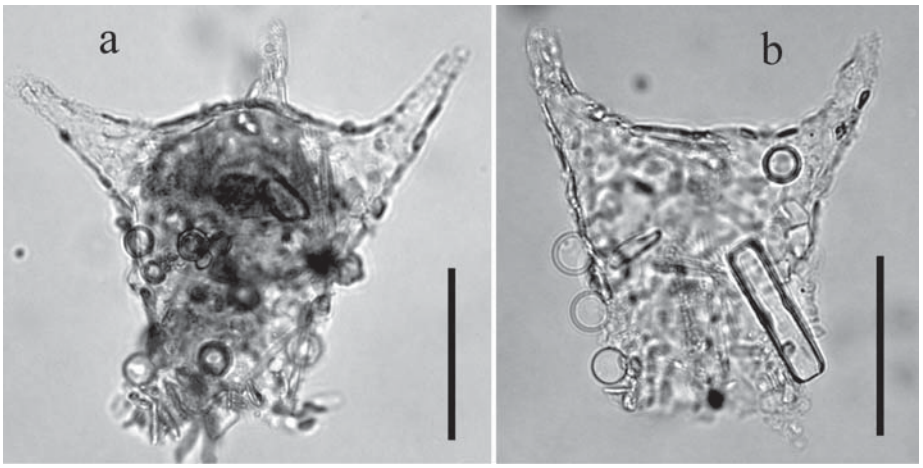

Fig. 29. Different specimens of *Difflugia varians* from E. Penard's slides. a-b – lateral view (slide 04.5.9.152). Scale bar: 50 μm.

aboral protuberance; shell wall composed of small to medium pieces of quartz, some flattish diatom frustules within a network of organic cement; shell surface usually smooth with a well defined outline, although sometimes the outline is masked by larger sand grains; aperture circular, surrounded by small particles. Ogden measured four specimens, two in Ogden (1983) and two in Ogden and Živković (1983), with shell size ranges as follows: length 141–206 μm, width 66–89 μm, aperture diameter 19–28 μm.

Difflugia scalpellum Penard, 1899 species complex

Difflugia scalpellum was originally described by Penard (1899) from Lake Geneva (Fig. 34). Ogden (1979) redescribed it based on SEM observations (Fig. 35). The shell is colourless and usually almost transparent, especially in the aboral region. It is elongate, tubular, swelling gradually from the aperture to the mid-body region and then tapering rapidly to a fine point in the aboral region. Small pieces of quartz and thin, flat portions of diatom frustules are arranged to make a mostly smooth surface. The aperture is circular and surrounded by a fairly regular arrangement of small quartz particles. The aboral region may be curved to resemble the cutting edge of a knife, hence the specific name. Ogden (1979) stressed that the most striking feature of this species is the transparent shell, which is unusual for such a large agglutinate form. Ogden (1979) measured two specimens with shell dimensions as follows: length 252–264 μm, width 70–79 μm, aperture diameter 32–33 μm. Penard (1899) reported the shell length as ranging from 260 to 300 μm.

***Difflugia præstans* Penard, 1905**. Penard (1905) described this species, the shell of which is very similar in appearance to that of *D. scalpellum* but is significantly larger, i.e. shell length 350–420 μm (Fig. 36).

***Difflugia sarissa* Li Sun Taï, 1931**. In terms of the general appearance of the shell, *D. sarissa* closely resembles *D. scalpellum* and *D. præstans* but is significantly smaller (Li Sun Taï, 1931). Chardez (1966) described it as elongate-lanceolate, circular in transverse section, acuminate posteriorly with the sides tapering symmetrically in the posterior two thirds forming a slender cone (Fig. 37). The shell is covered by sand grains. The aperture is circular. The dimensions of the shell are: length 120–160 μm, width 60–70 μm, aperture diameter 28–30 μm.

***Difflugia smilion* Thomas, 1953**. Thomas (1953) described this species (Fig. 38) which is similar in general shell shape with *D. scalpellum* but slightly smaller in size, i.e. length 210–220 μm, width 40–60 μm, aperture diameter 30–50 μm. Furthermore, Thomas (1953) noted ecological differences between these two species. Ogden and Živković (1983) illustrated one broken specimen (shell length 226 μm) characterized by an elongate shell with a distinct conical protuberance, circular aperture and surface composed of medium to large, angular or flat, pieces of quartz resulting a rough surface. However, Ogden and Živković (1983) did not compare *D. smilion* with *D. scalpellum*.

Difflugia sarissa, D. smilion and *D. præstans* are relatively poorly known and there is a lack of data on their natural variability. Therefore any decision concerning their relationship with similar species

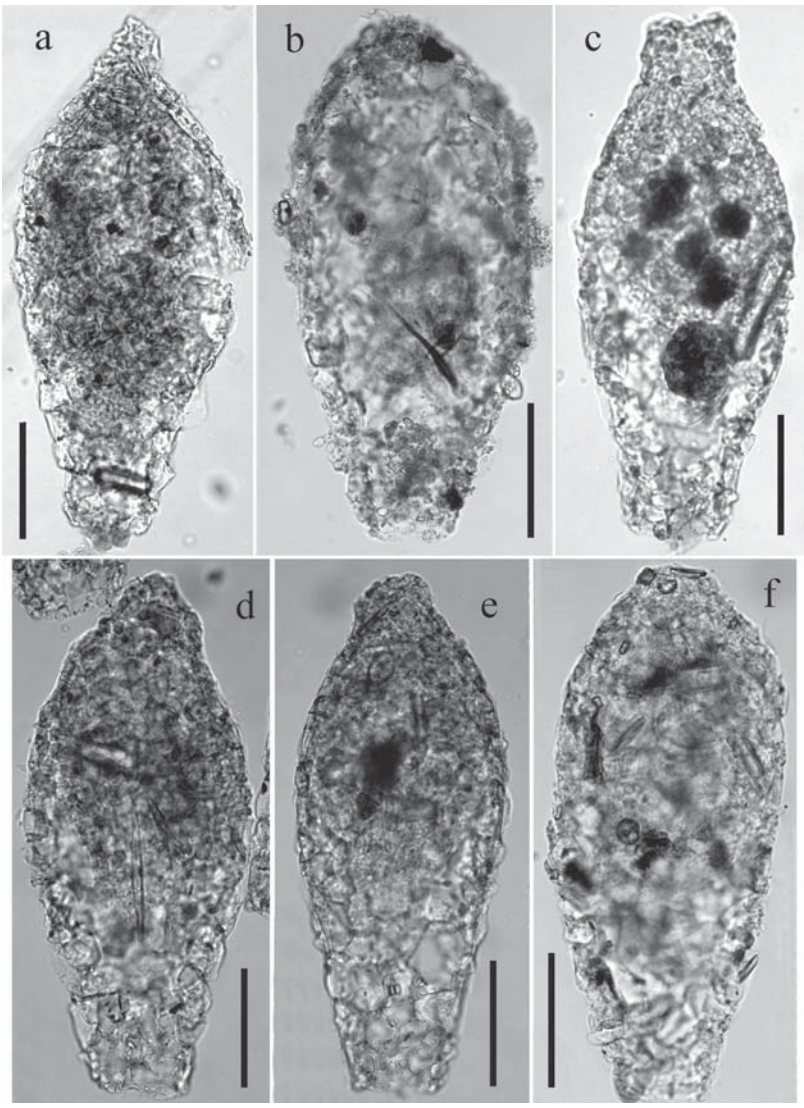

Fig. 30. Different specimens of *D. pyriformis* var. *claviformis* from E. Penard's slides. a-f – lateral view (a – slide 04.5.9.129, b, f – slide 20.12.8.267, c–e – slide 20.12.8.271). Scale bars: 100 µm.

such as *D. scalpellum* or *D. acuminata* must await further study.

Acknowledgements

This work was financially supported by the award (to YM) from the Royal Society International Travel Grant Scheme (2009R1).

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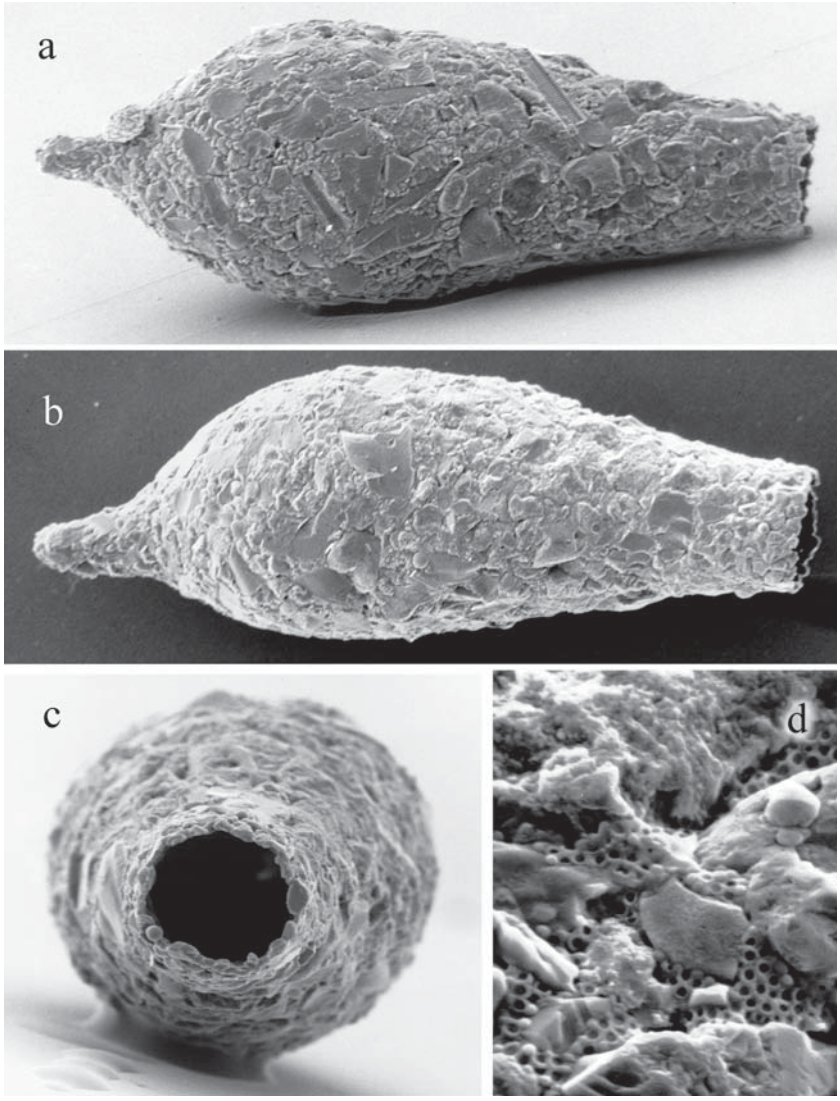

Fig. 31. Different specimens of *Difflugia claviformis* from C. Ogden's SEM collection. a, b – lateral view (SEM EM-12-219 and EM-12-215), c – apertural view (SEM EM-12-217), d – structure of organic cement (SEM EM-12-340). Magnification: a – x305, b – x307, c – x631, d – x5400.

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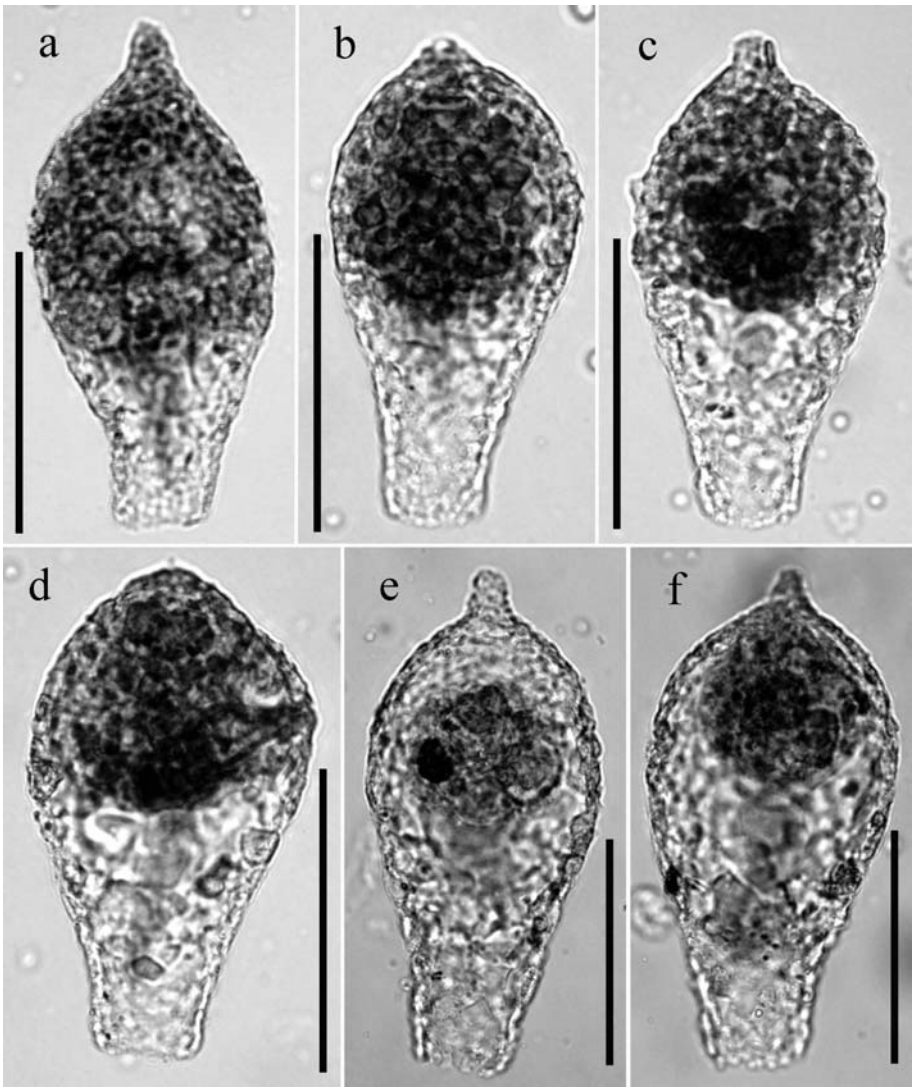

Fig. 32. Different specimens of *Difflugia pyriformis* var. *venusta* from E. Penard's slides. a–f – lateral view (a-d – slide 4.5.9.126, e, f – slide 20.12.8.278). Scale bars: 100 μm.

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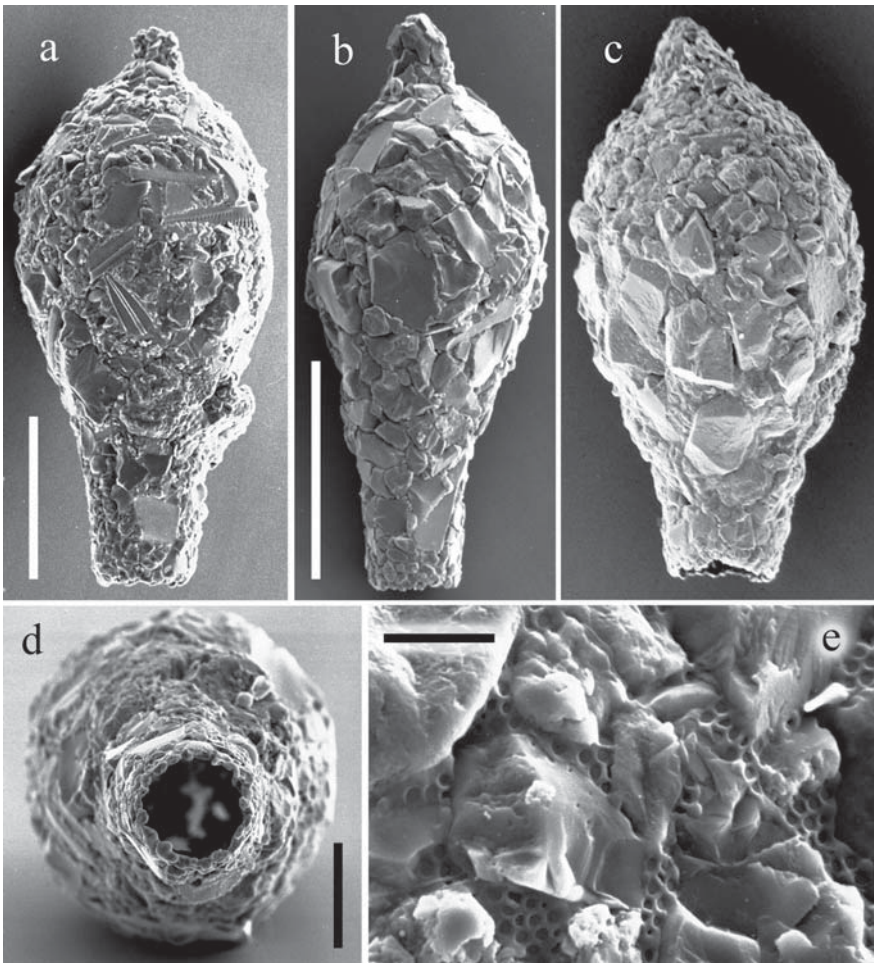

Fig. 33. Different specimens of *Difflugia microclaviformis* from C. Ogden's SEM collection. a-c – lateral view (a – SEM CZ-07-476, b – SEM CZ-03-172, c – SEM EM-12-623), d – apertural view (SEM CZ-07-463), e – structure of organic cement (SEM CZ-07-471). Scale bars: a–c – 60 μm, d – 30 μm, e – 3 μm.

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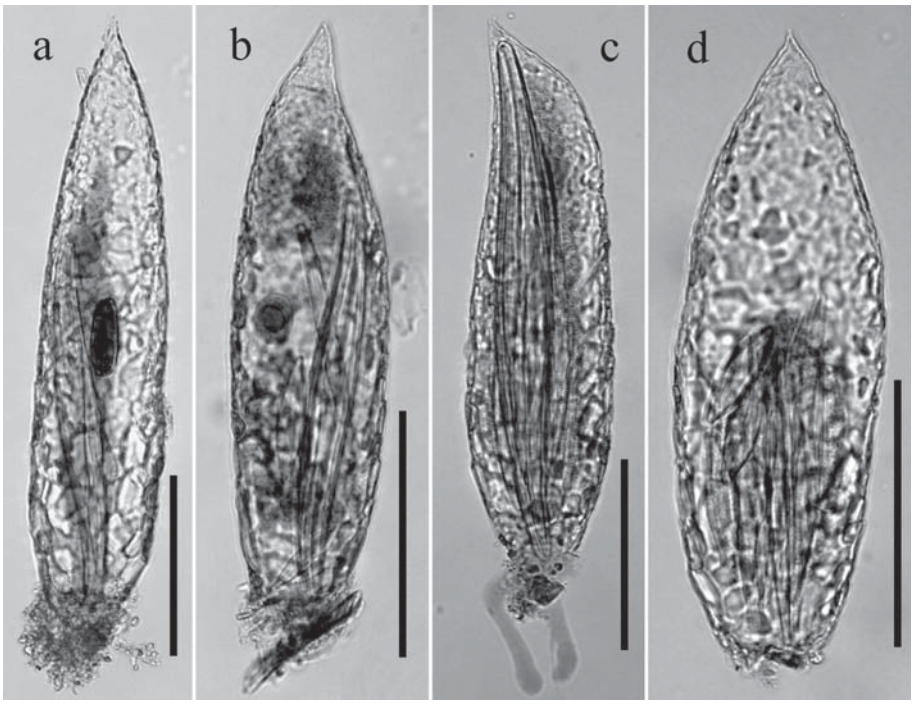

Fig. 34. Different specimens of *Difflugia scalpellum* from E. Penard's slides. a–d – lateral view (a, b – 04-5-9-140, c, d – 20.12.8.281). Scale bars: 100 μm.

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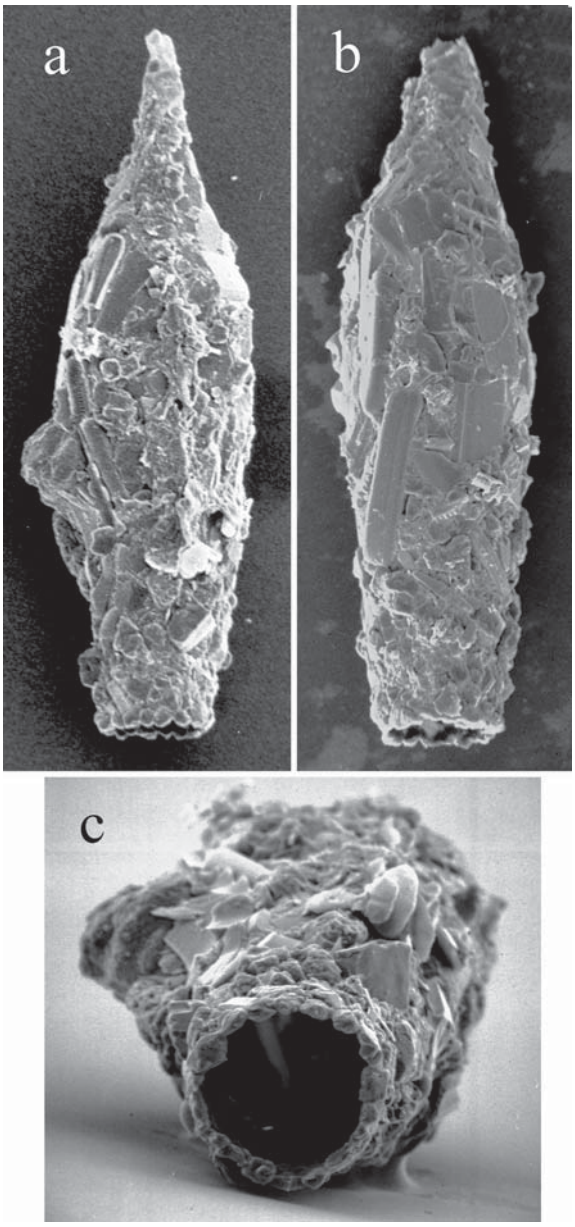
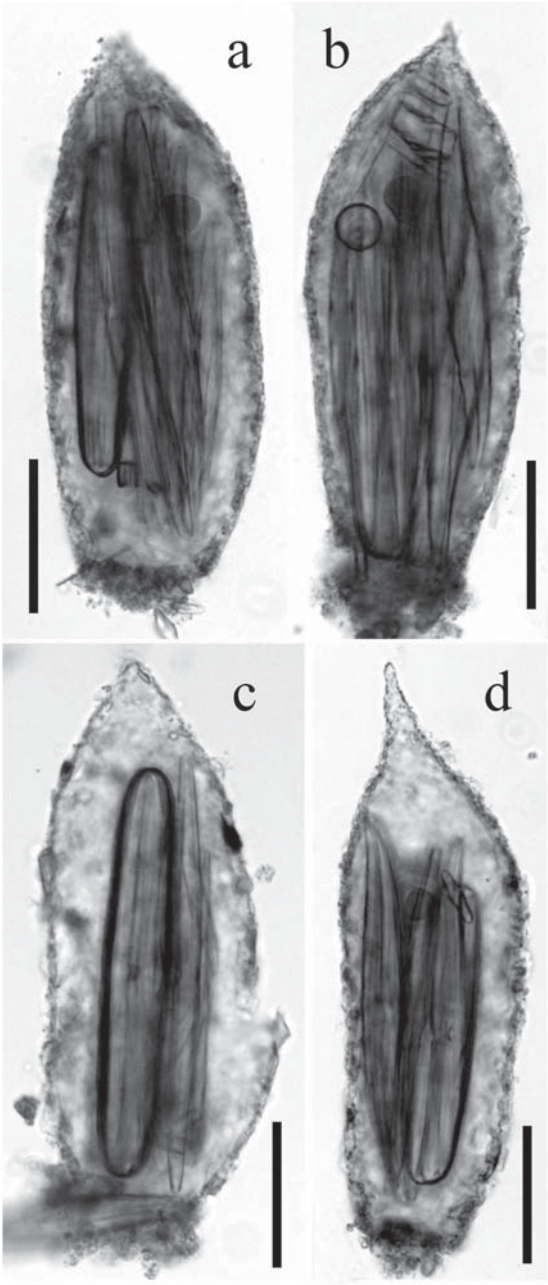


Fig. 35. Different specimens of *Difflugia scalpellum* from C. Ogden's SEM collection. a-b – lateral view (SEM Z-15-803 and EM-13-064), c – apertural view (SEM EM-12-777). Magnification: a, b – ×317, c –×430.



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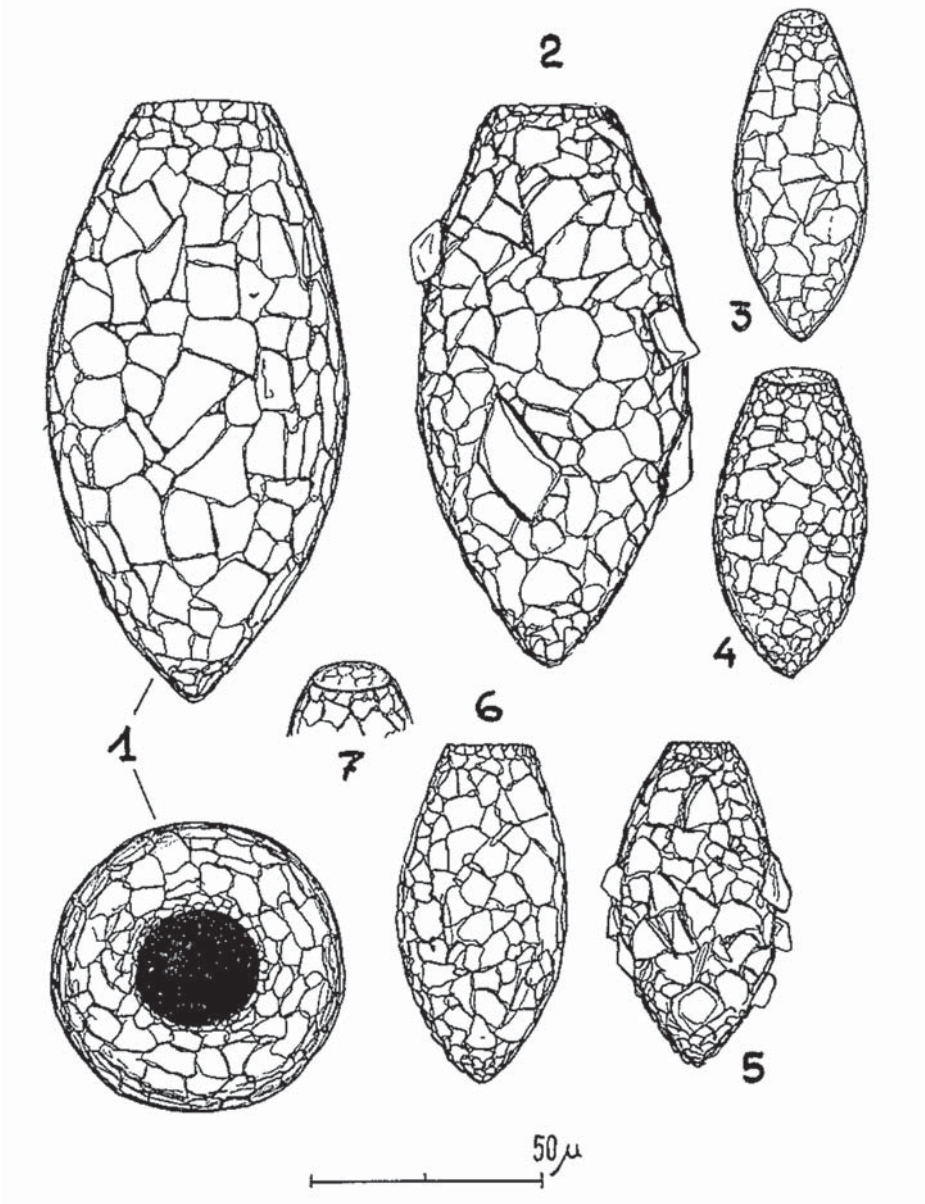

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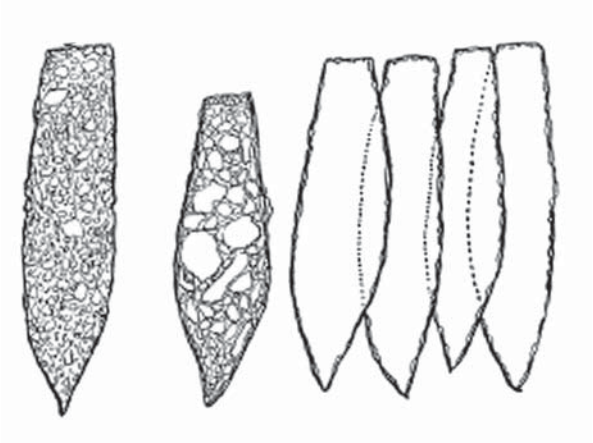

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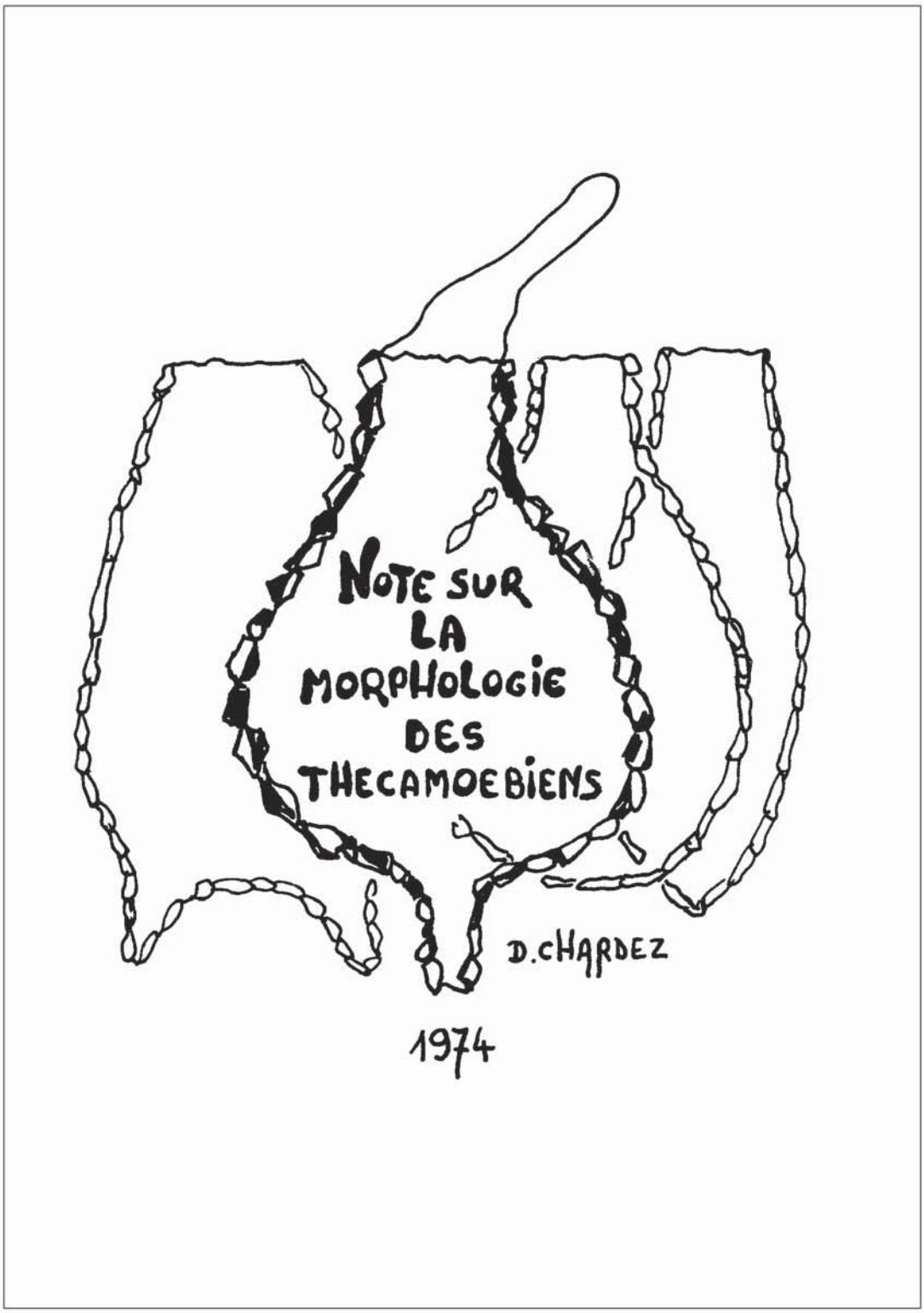
Appendix



1

Dans son étude morphologique du Genre Centropyxis, Deflandre écrivait en 1929 : "... Ces espèces et variétés proviennent sans nul doute les unes des autres par mutation, mais nous pouvons les considérer comme fixées actuellement. Il est nécessaire de nommer ces réunions de lignées qui se présentent à nous comme ayant une valeur systématique positive par suite des caractères qui les différencient les unes des autres. "

Comme cet auteur nous dirons que les idées acquises pour les Genres Arcella et Centropyxis sont applicables à d'autres genres de Thécamoebiens.

En 1966, nous avons publié une étude sur les variations morphologiques de Centropyxis aculeata soulignant que les espèces les plus cosmopolites et les plus ubiquistes sont également les plus riches en variétés et formes considérées comme stables et qu'il faut y voir essentiellement une conséquence des adaptations stationnelles. Toutes nos observations sur le sujet tendent à démontrer l'influence des conditions du milieu sur la génèse des variations morphologiques.

Dans l'état actuel des connaissances, la systématiques des Thécamoebiens reste une chose fort compliquée même pour les espèces les plus communes et les plus fréquentes, ceci en raison de ces grandes variations intraspécifiques.

Nombreuses sont les espèces qui ont été subdivisées en variétés et formes sur des critères morphologiques de la thèque en réalité peu apparents et souvent sujet à interprétation.

Dans ce travail, nous avons étudié l'ensemble de la population du Genre Difflugia d'un même petit biotope pendant plusieurs années et comparé synoptiquement la morphologie des espèces à symétrie axiale et à pseudostome non lobé.

On remarque en analysant un grand nombre de formes, qu'il est facile de passer de l'une à l'autre, que les formes de transition sont nombreuses.

On remarque également que lorsque les matériaux qui constituent les thèques sont de même origine les déterminations deviennent très difficiles.

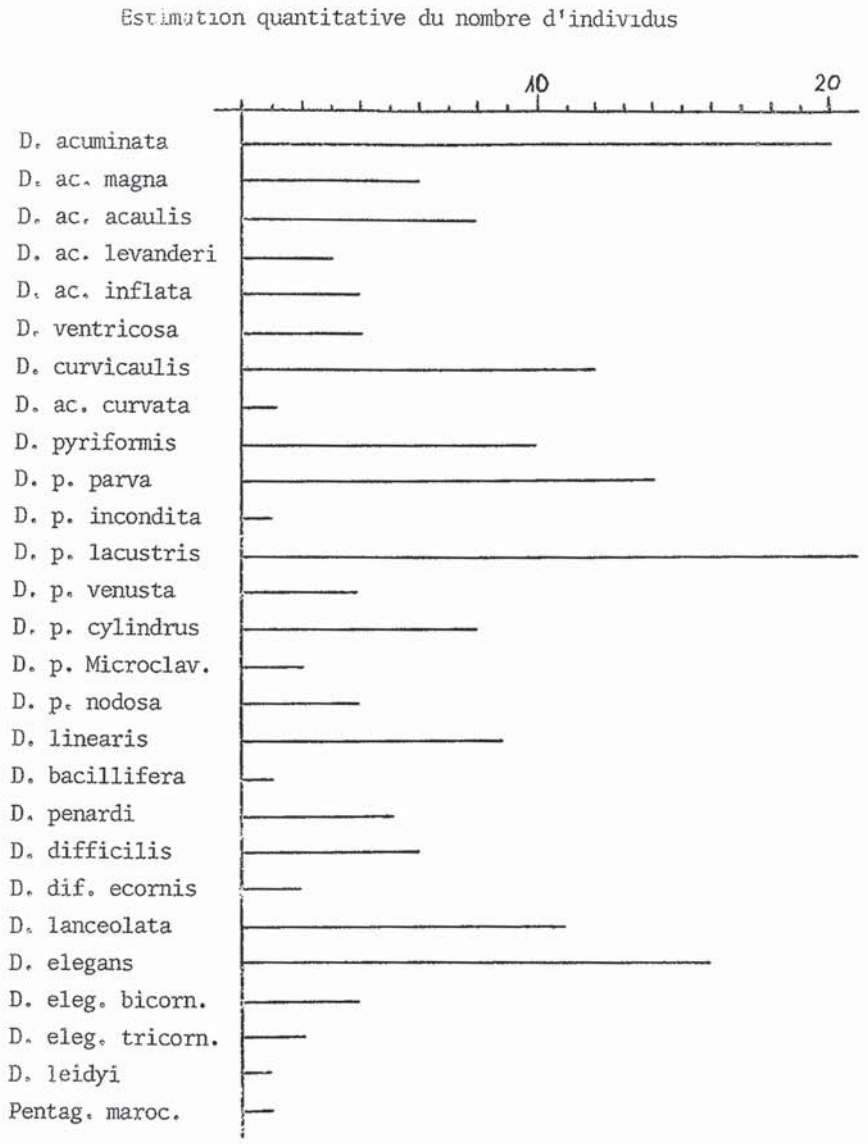
Le BIOTOPE

Petite mare d'eau stagnante (Heusy-Verviers) de 12 m^2 de superficie, profondeur environ 1 m. Couverte de Lemna minor, entourée de grands arbres (Hêtres, Chênes) de ce fait très peu éclairée.

Le fond est garni d'une épaisse couche de feuilles mortes en décomposition, formant un sapropèle noir, pH variant entre 8 et 9.

LISTE DES ESPECE

Fig. 1, 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1I, 1J : Difflugia acuminata Ehrbg
1K : D. acuminata v. levanderi Playfair
1L : D. acuminata v. inflata Penard
1M : D. acuminata v. magna Deflandre
1M, 1N : D. acuminata v. brevicaulis Mabille et Thomas
1B' : D. acuminata (forme intermédiaire entre 1 et 2)
1D' : D. acuminata (forme intermédiaire entre 1 et 4)
2 : D. ventricosa Deflandre
3, 3A, 3B, 3C : Difflugia curvicaulis Penard
3D : D. acuminata v. curvata
4, 4A, 4B, 4C, 4D, 4E, 4F, 4G : Difflugia smilion Thomas
5, 5A, 5B, 5C, 5D : Difflugia pyriformis Perty
5E, 5F, 5G, 5H, 5I, 5J : D. pyriformis v. parva (Th et Mab.) Ch. et Decl.
5K : D. pyriformis v. incondita (Gl et Th) Chardez et Declaitre
5L, 5M, 5N : D. pyriformis v. lacustris (Pen) Ch. et Decl.
5P : D. pyriformis v. venusta Penard
5Q, 5R, 5S : D. pyriformis v. cylindrus (Thomas) Ch. et Decl.
5U : D. pyriformis v. nodosa Leidy
5V : D. pyriformis v. microclaviformis (Kaurov) Ch. et Decl.
6 : D. bacillifera Penard
7 : D. linearis (Penard) Gauthier-Lièvre et Thomas
8 : D. penardi (Penard) Hopkinson
8A : D. difficilis v. ecornis Chardez
8B, 8C : D. difficilis Thomas
9A, 9B : D. lanceolata Penard
10, 10A, 10B, 10C, 10D, 10E : D. elegans Penard
10H, 10I : D. elegans f. bicornis Jung
10J : D. elegans f. tricornis Jung
11 : Pentagonia marocana Gauthier-Lièvre et Thomas
12 : Difflugia leidyi Wailes.


Estimation quantitative du nombre d'individus
10
20
D. acuminata
D. ac. magna
D. ac. acaulis
D. ac. levanderi
D. ac. inflata
D. ventricosa
D. curvicaulis
D. ac. curvata
D. pyriformis
D. p. parva
D. p. incondita
D. p. lacustris
D. p. venusta
D. p. cylindrus
D. p. Microclav.
D. p. nodosa
D. linearis
D. bacillifera
D. penardi
D. difficilis
D. dif. ecornis
D. lanceolata
D. elegans
D. eleg. bicorn.
D. eleg. tricorn.
D. leidyi
Pentag. maroc.


1A
1B
1C
1D
1E
1F
1G
1H
1I
1J
1B′
1D′
1K
1M
1N
1L
3A
3B
3C
3D
4A
4B
4C
4D
4E
4F
4G
5E
5F
5G
5H
5D
5C
5B
5A
5P
5V
5I
5J
5K
5L
5M
5N
5T
5U
5S
5R
5Q
1P
10A
10H
10B
10C
10I
10D
10E
10F
10G
10J
9B
9A
8B
8C
8A
100 μ
I

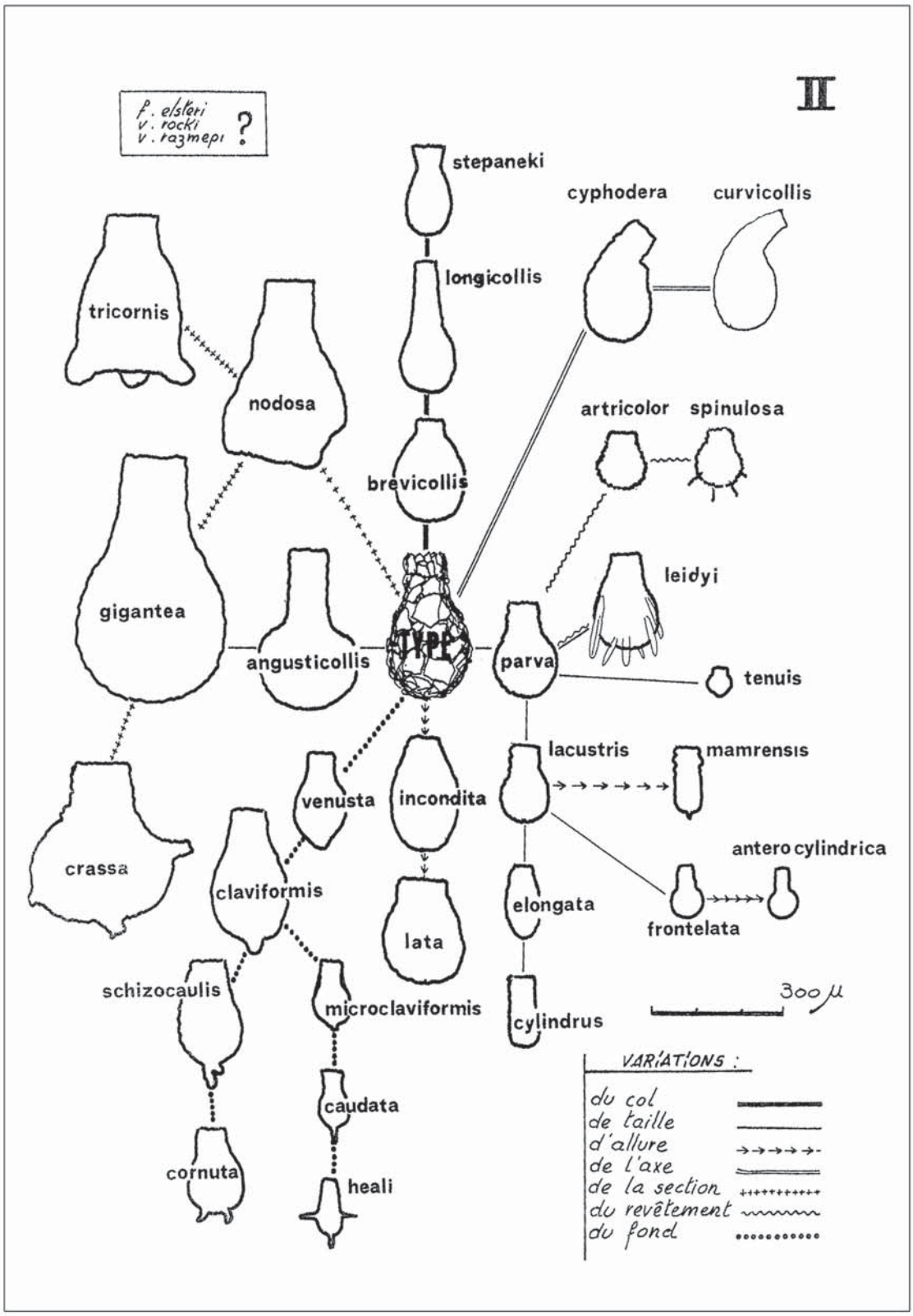
6

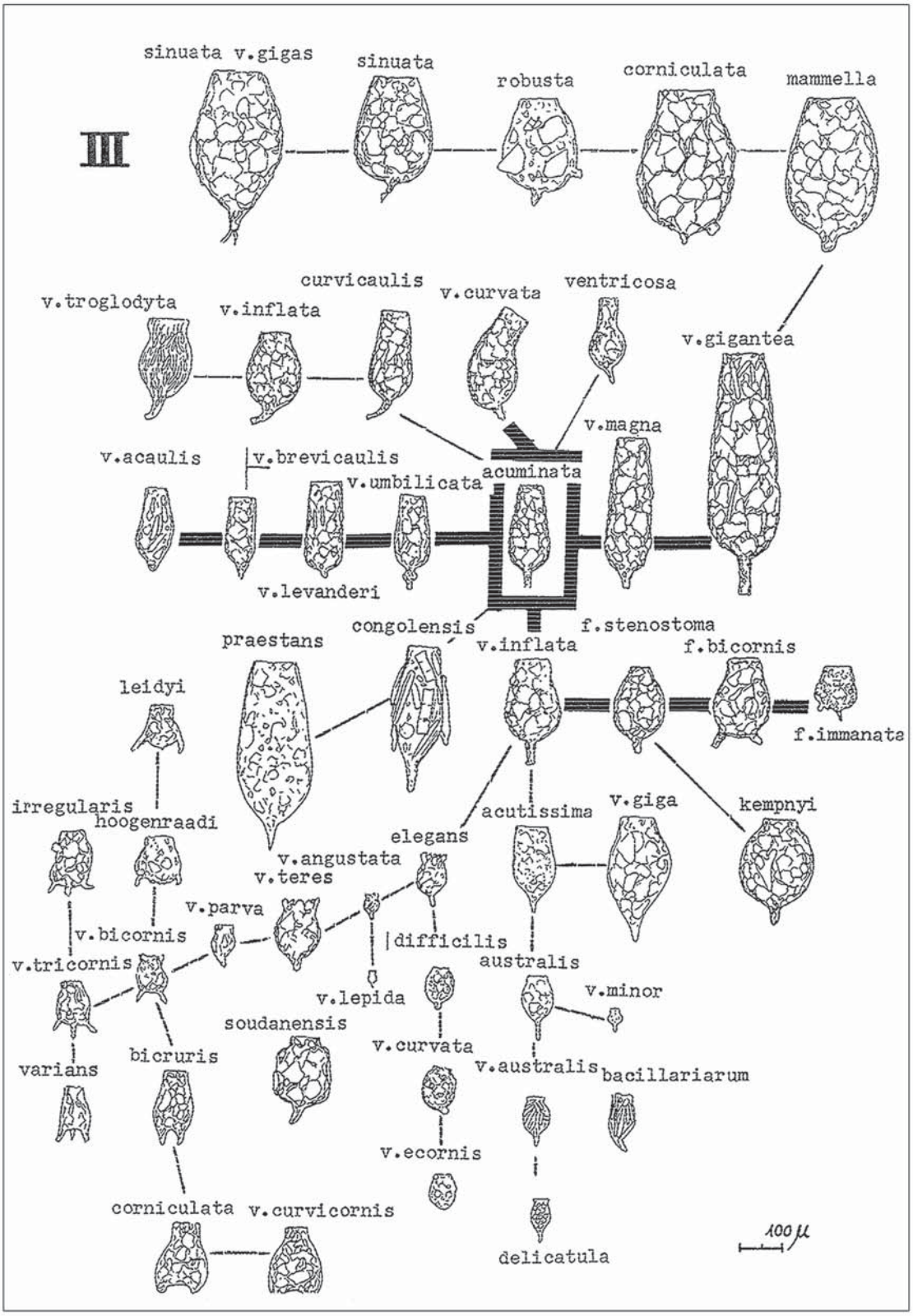
Note

Outre les espèces figurées sur la planche I et reprises dans la liste précédente la population de ce biotope se compose de :

Difflugia lobostoma Leidy
Difflugia glans Penard
Centropyxis aculeata (Ehrenbery) Stein
Cucurbitella mespiliformis Penard
Arcella hemisphaerica Perty
Arcella hemisphaerica v. depressa Playfair
Arcella rotunda Wailes
Euglypha tuberculata Dujardin
Trinema lineare Penard

Les planches II et III, sont des schémas hypothétiques des correspondances morphologiques pour l'ensemble des pyriformes et des corniculées, mettant en évidence les variations conduisant aux espèces, variétés et formes actuelles. Seules des études très poussées des différents cas particuliers mettront en évidence les phylum réels.


II
f. elsteri
v. rocki
v. razmepi ?
stepaneki
cyphodera
curvicollis
longicollis
tricornis
nodosa
artricolor
spinulosa
brevicollis
leidyi
gigantea
angusticollis
TYPE
parva
tenuis
lacustris
mamrensis
venusta
incondita
antero cylindrica
crassa
claviformis
elongata
frontelata
lata
schizocaulis
microclaviformis
cylindrus
300 μ
VARIATIONS :
du col
de taille
d'allure
de l'axe
de la section
du revêtement
du fond
caudata
cornuta
heali


III
sinuata v.gigas
sinuata
robusta
corniculata
mammella
v.troglodyta
v.inflata
curvicaulis
v.curvata
ventricosa
v.gigantea
v.magna
v.acaulis
v.brevicaulis
v.umbilicata
acuminata
v.levanderi
congolensis
f.stenostoma
praestans
v.inflata
f.bicornis
leidyi
f.immanata
irregularis
hoogenraadi
acutissima
v.giga
kempnyi
elegans
v.angustata
v.teres
v.parva
v.bicornis
difficilis
v.tricornis
australis
v.lepida
v.minor
soudanensis
v.curvata
varians
bicruris
v.australis
bacillariarum
v.ecornis
corniculata
v.curvicornis
delicatula
100 μ