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The *Centropyxis aerophila* Complex (Protozoa: Testacea)

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Summary. We show that the varieties *Centropyxis aerophila aerophila*, *C. aerophila sphagnicola*, and *C. aerophila sylvatica*, described by Deflandre in 1929 and later recorded by many workers worldwide, cannot be distinguished with the features provided in the original descriptions, and even not with refined morphometrical data. Very likely, this applies also to most varieties and forms described later. Some of these taxa are obviously extremes of variability clines, while others are distinct but morphometrically inseparable (sibling) species or subspecies, as indicated by concrete morphological traits and/or different ecologies. A representative example is the variety *sylvatica*, which is a distinct species because it has a unique inner pseudostome (ventral lip perforation) produced by material agglutinated on the dorsal and lateral shell wall. This peculiar feature, first recognised by Bonnet and Thomas (1955), is shown by clear light and scanning electron micrographs in the present paper. However, the inner pseudostome is recognisable only on refined investigation. Thus, we suggest to lump taxa, which are morphologically and/or morphometrically difficult to distinguish, into "complexes", as it has been done in sibling ciliate species. This would make testacean alpha-taxonomy more reliable and elegant, especially for field ecologists. Nomenclaturally, the varieties described by Deflandre (1929) are subspecies because he did not unambiguously indicate that the names were proposed for infrasubspecific entities.

Key words: *Centropyxis* spp., *Centropyxis sylvatica*, *Centropyxis aerophila sphagnicola*, morphology, morphometry, nomenclature, sibling species, varieties.

INTRODUCTION

Testate amoebae are common in terrestrial habitats and some authors consider them as the most important soil protozoa for their large biomass and production reaching that of earthworms (Lousier and Parkinson 1984; Foissner 1987, 1999; Schönborn 1992b). Accordingly, they are valuable bioindicators in a variety of terrestrial (Foissner 1987, 1999) and limnetic (Schönborn 1973) habitats. However, testacean ecology has been notoriously plagued by nomenclatural and taxonomical problems because many taxa are superficially described and taxonomy is almost entirely based on shell morphologies (Medioli and Scott 1985, Foissner and Korganova 1995). Recent studies show that shell features are valuable, if supported by sufficient morphometric data and used cautiously. Nonetheless, there is accumulating

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evidence that many of the subspecies, varieties, and forms described fall into the range of natural variability of a species (Wanner 1991, Schönborn 1992a, Wanner and Meisterfeld 1994, Bobrov *et al.* 1995, Foissner and Korganova 1995).

So far, several hundred testacean taxa have been recorded from terrestrial habitats worldwide, and some of these are very likely restricted to such biotopes and/or certain biogeographical regions (Bonnet 1975, Chardez and Lambert 1981, Foissner 1987). One of the most abundant and frequent species, contained in most species lists worldwide, is *Centropyxis aerophila* Deflandre, 1929 and its varieties, *C. aerophila sylvatica* Deflandre, 1929 and *C. aerophila sphagnicola* Deflandre, 1929. The variety *sylvatica* was later classified as a distinct species by Bonnet and Thomas (1955, 1960). They observed that it has an enlarged ventral pseudostome lip extending to the shell's dorsal wall, dividing it in an anterior and posterior compartment connected by a roundish opening in the lip through which the pseudopods can extend. The observations of Bonnet and Thomas (1955, 1960) were confirmed by Lüftenegger *et al.* (1988) and Rauenbusch (1987). Thus, if classical morphological traits are used, *C. sylvatica* must be considered as a distinct species, although the lip perforation is often difficult to recognise. In the fifties and sixties further varieties were described (see Discussion).

Problems in separating the varieties of *C. aerophila* were mentioned by many authors, for instance, Jung (1936), Schönborn (1966, 1975), and Chardez (1979). They became obvious also during our work, and several redescribers could not find better features for distinguishing these varieties, but usually emphasised their high similarity (Schönborn 1966, 1975; Rauenbusch 1987; Lüftenegger *et al.* 1988). On the other hand, the three taxa were mentioned in hundreds of ecological and faunistic studies, including our own (Foissner and Peer 1985, Korganova 1988, Todorov 1993, Aescht and Foissner 1994), and ecologists even provided different autecologies for *C. aerophila aerophila* and *C. aerophila sphagnicola* (Bonnet 1989). Finally, an examination of the original descriptions revealed that they contain very few, if any, features justifying the establishment of distinct taxa.

The purpose of our study was: (i) to investigate whether three taxa can be distinguished with the features provided by Deflandre (1929); (ii) to investigate how previous authors separated Deflandre's taxa; (iii) to confirm *C. aerophila* var. *sylvatica* as a distinct species with the features provided by Bonnet and Thomas (1955, 1960); and (iv) to suggest a practicable solution of the problem acceptable for both taxonomists and ecologists.

MATERIALS AND METHODS

Material

The specimens were isolated from the upper litter and soil layer of an about 110 years old secondary spruce forest (mixed with some *Corylus* and *Tilia*; ground covered mainly by *Carex* spp.) circa 50 km out of Moscow, that is, in the territory of the biogeocoenological experimental station "Malinki" of the Institute of Ecology and Evolution of the Russian Academy of Sciences. Soil was a "dern-podsol", that is, a podsol with a rather distinct, about 10 cm deep, greyish humus layer having 7% total organic matter and pH 5.2. It was covered by an up to 6 cm thick litter layer, depending on microhabitat and season. The sample was taken in August 1999, when soil water content was circa 40%, from the "F-horizon", that is, the upper 2 cm of the dern-podsol and the overlying 2cm of the rather strongly decomposed litter. The sample, which consisted of 5 subsamples taken from an area of about 25 m^2, was air-dried for four weeks and then stored in a paper bag.

Isolation of taxa and sampling protocol

In October 1999, the air-dried sample was rewetted with distilled water and gently shaken some minutes to separate shells from soil particles. *Centropyxis aerophila*-like shells were selected from the soil suspension with a micropipette under a dissecting microscope (x70), using "classical" features, such as shape, shell structure, size, and length:width ratio. Fortunately, the sample contained few other *Centropyxis* species, which could be easily distinguished by size (*C. orbicularis*) or size and shape (*C. constricta*). There was no selection for full (alive), empty (dead), and cystic specimens. The shells were put into glycerol drops to make them clearer and easier to handle while measuring. The following sample program was performed to meet the objectives mentioned in the introduction: (a) 127 specimens were taken at random for basic statistics and considerations; (b) Selected specimens, that is, shells identified with Deflandre's (1929) features either as *C. aerophila aerophila*, *C. aerophila sphagnicola* or *C. aerophila sylvatica*. We did not look for the perforation in the ventral pseudostome lip, which unequivocally separates *C. sylvatica* from *C. aerophila aerophila* and *C. aerophila sphagnicola* (Bonnet and Thomas 1955) in selecting these tests. 30 specimens from each "species" were collected and measured. All specimens (about 50%), which could not unequivocally be assigned to one of the three taxa, were discarded. Selected specimens were also used for scanning electron microscopy, using the technique described by Schönborn *et al.* (1983); (c) With respect to Deflandre's remark on *C. aerophila* var. *sylvatica* "Cette varieté differe de l'espèce type par ses dimensions généralement supérieurs" and because the inner pseudostome is difficult to recognise, we made the following experiment: 30 large (length ≥ 85 µm) *C. aerophila sylvatica*-

like looking shells were compared with 30 small (length 60-75 µm) shells more similar to either *C. aerophila aerophila* or *C. aerophila sphagnicola*.

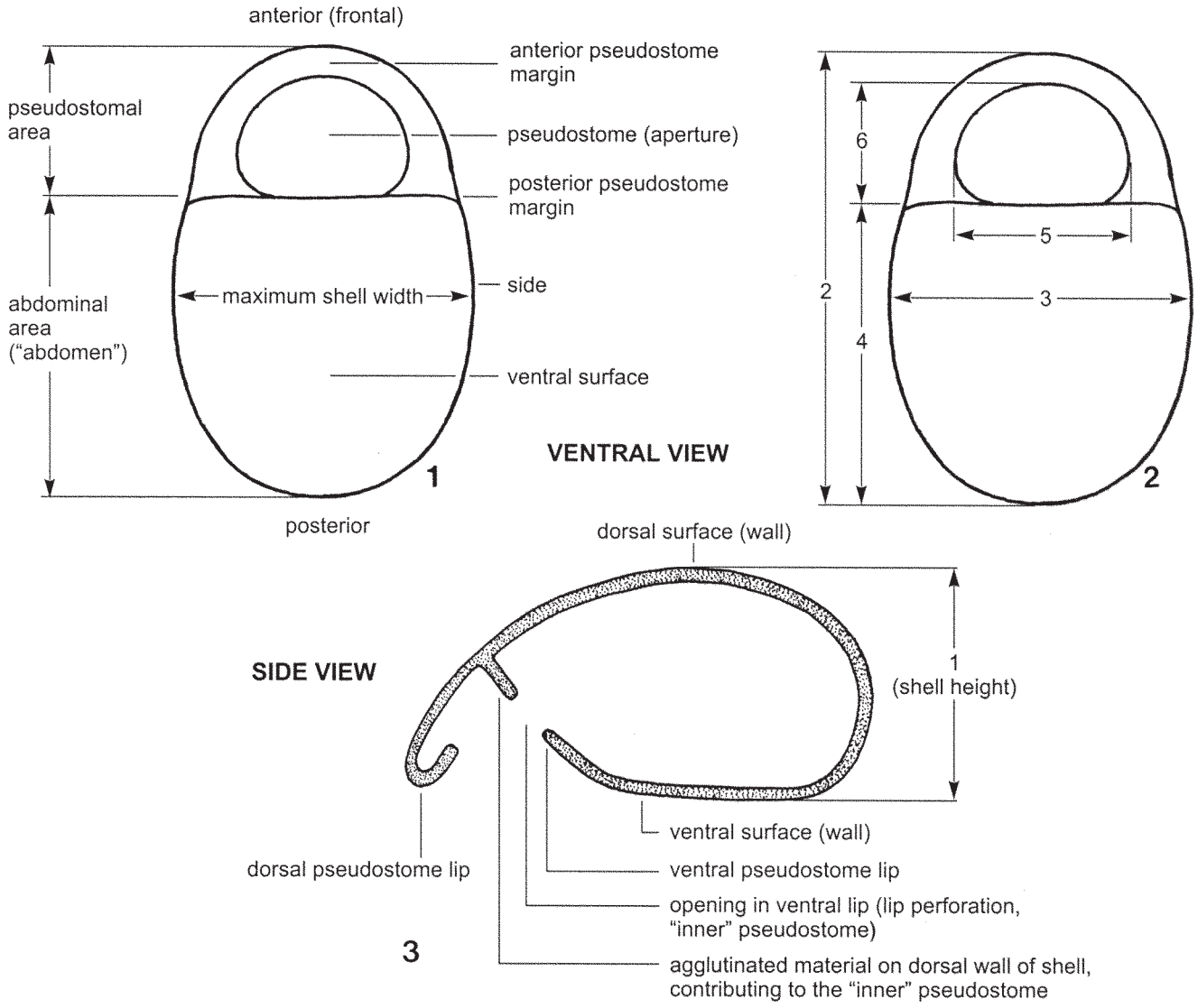
Measurements and statistics

Six classical features were measured in series (a) and (b), namely shell height (character 1 in Figure 3); shell length (character 2 in Figure 2); maximum shell width (3); "abdomen length" (4), that is, the distance between posterior margin of the pseudostome and posterior shell end; long axis of pseudostome (5), and short axis of pseudostome (6). Furthermore, ratios between characters 2 and 4 and 3 and 4 were calculated because they provide some overall measure for shell shape. All measurements were made in glycerinated specimens at middle magnification (x400), where a micrometer unit was 5 µm; accordingly, values were usually rounded up in 5 µm steps.

Statistics and diagrams were prepared according to textbooks using the computer program BIOMstat, version 3.3. As most variables were not normally distributed, the parameter-free analysis of variance (ANOVA) of Kruskal-Wallis was applied. To increase n (sample number) in some tests, we put together measurements from series (a) and (b).

Figures and terminology

Line drawings were taken from the literature because our investigations did not show significant new details. All micrographs were taken from the Moscow material under bright field illumination or in



Figs. 1-3. Terminology and morphometrics (numbers 1 - 6 in Figs. 2 and 3) used in this study.

the scanning electron microscope. Specimens usually must be strongly tilted and very carefully orientated to show the inner pseudostome of *C. sylvatica*.

Terminology of testate amoebae is rather confused and many terms, for instance, the so-called visor, vestibulum, and pseudostome are used in different ways by different or even the same authors (Bonnet 1964, 1975). Thus, we use "simple" terms, shown in Figures 1 and 3, for the scope of the present paper.

Permanent slides

The specimens used, and several others, were embedded in a drop of glycerol sealed by a rather thick ring of artificial resin (Histofluid, Merck Company, Germany) to prevent shells from desiccation and distortion by the cover glass pressure. All slides and the SEM-preparations have been stored in the Oberösterreichische Landesmuseum in Linz (LI).

RESULTS

Brief description of the taxa involved

To understand our argumentation it is crucial to know the original description and status of the taxa involved. Thus, we provide the original descriptions (translated from French) and supplement them with recent literature data and/or our observations from the Moscow material.

Centropyxis aerophila Deflandre, 1929 (Figs. 4-14, 28-33; Tables 1, 2)

"Test small, abdomen globular, dorsal wall strongly flattened towards pseudostome. Ventral outline oval with circular or slightly elliptical abdomen, sides not or only slightly converging towards pseudostome having semicircular outline. Sides only slightly curved, often almost straight. In ventral view, abdomen seemingly separated from pseudostomal area, looking like being attached to the abdomen. Pseudostomal region more transparent than abdominal region in resin preparations.

Pseudostome usually semicircular with straight, occasionally slightly concave (Figs. 7, 10) posterior margin. Abdomen distinctly inflated in lateral view, but steeply flattens towards pseudostome, whose anterior margin is more or less distinctly turned inside.

Test entirely chitinous, finely and irregularly punctuated or with rather distinct flakes, carries sometimes brown or dark organic debris and small quartz grains, colourless or yellowish, occasionally rather dark brownyellow. At certain sites, shell often appears entirely covered with foreign particles.

Pseudopods and nucleus not yet observed. Dimensions (number of specimens measured not given): overall size 53 - 85 x 42 - 66 µm, shell height about 2/3 of length; pseudostome 21 - 28 x 15 - 21 µm".

There are several notes and brief redescriptions available in the recent literature, adding significantly to the morphology of the shell and its inhabitant, but leaving untouched the basic features described by Deflandre (1929). Our selected material from Russia also matches Deflandre's description (Figs. 28-33; Table 2). No cement structures are recognisable in the scanning electron microscope.

Lobose pseudopods and their movements, as well as two contractile vacuoles, were described in detail by Bartoš (1954) and Bonnet (1961). Ogden and Hedley (1980) provided some helpful scanning electron micrographs of the shell, which is usually more chitinous in freshwater than terrestrial habitats, where it is often entirely covered by sand grains (Fig. 33). Measurements, but no detailed morphometrics, were given by various authors (Decloitre 1954, 1956; Laminger 1972; Ogden and Hedley 1980; Rauenbusch 1987), broadening, however, like our detailed data (Table 2), Deflandre's limits only slightly: 47 - 93 (length) x 32-77 (width) µm; pseudostome: 19-34 (long axis) x 10-22 (short axis) µm. Schönborn (1966) found very small specimens (about 30 x 30 µm) in the sediment of small Tundra lakes in Lapland.

Centropyxis aerophila var. *sphagnicola* Deflandre, 1929 (Figs. 15-20, 34-40, 58; Table 2)

"Test very similar to that of type species. In ventral view, it differs from *C. aerophila aerophila* and *C. aerophila sylvatica* in being more frequently circular. Pseudostome very eccentric, its outline forms two more or less convex arcs, ventral lip extends deeply into shell leaving free only a narrow slit between lip end and dorsal shell wall (Rauenbusch 1987). In lateral view less inflated than type and less flattened towards pseudostome. Wall chitinous, flaky, more frequently incrusted with foreign particles, small irregular platelets, or quartz grains mostly at anterior margin of pseudostome. Dimensions (number of specimens measured not given): diameter 49-66 µm, shell height 1/2 - 3/5 of diameter; pseudostome (long axis) 25-37 µm".

There are several notes and brief redescriptions available in the recent literature, adding significantly to the morphology of the shell and its inhabitant, but leaving

Table 1. Morphometric data on *Centropyxis aerophila*. Upper line: 127 randomly selected specimens (including varieties and *C. sylvatica*) identified with the features given by Deflandre (1929). Lower line: the 127 specimens mentioned above and the 90 specimens from Table 2 pooled

Characteristics[1]	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Shell, height (1)	42.3	43.0	7.8	0.7	18.5	15.0	65.0	127
	43.5	44.0	8.3	0.6	19.0	15.0	65.0	217
Shell, length (2)	70.8	70.0	10.2	0.9	14.4	51.0	110.0	127
	71.2	70.0	9.7	0.7	13.6	51.0	110.0	217
Shell, width (3)	66.1	65.0	9.9	0.9	14.9	47.0	95.0	127
	66.6	65.0	10.2	0.7	15.3	47.0	95.0	217
Abdomen, length (4)	45.0	45.0	6.6	0.6	14.7	30.0	65.0	127
	45.4	45.0	6.5	0.4	14.3	30.0	65.0	217
Pseudostome, long axis (5)	29.6	30.0	5.6	0.5	18.8	16.0	47.0	127
	29.6	30.0	5.6	0.4	19.0	16.0	47.0	217
Pseudostome, short axis (6)	16.3	15.0	3.8	0.3	23.5	10.0	27.0	127
	16.2	15.0	3.8	0.3	23.4	10.0	27.0	217
Shell length:abdomen length, ratio	1.6	1.6	0.1	0.0	7.1	1.3	1.9	127
	1.6	1.6	0.1	0.0	6.5	1.3	1.9	217
Shell width:abdomen length, ratio	1.5	1.5	0.2	0.0	11.5	1.0	2.0	127
	1.5	1.5	0.2	0.0	11.0	1.0	2.0	217

[1]Numbers in parenthesis designate features as shown in Figs. 2 and 3. Measurements in µm. CV- coefficient of variation in %; M - median; Max - maximum; Min - minimum; n - number of specimens investigated; SD - standard deviation; SE - standard error of mean; $\bar{x}$ - arithmetic mean

Table 2. Morphometric data on 30 " selected specimen " each (see Materials and Methods section) of *Centropyxis aerophila aerophila* (CAA), *C. aerophila sphagnicola* (CAS), and *C. aerophila sylvatica* (CS), identified with the features given by Deflandre (1929)

Characteristics[1]	Species	$\bar{x}$	M	SD	SE	CV	Min	Max	n
Shell, height (1)	CAA	36.6	36.0	5.7	1.0	15.6	25.0	45.0	30
	CAS	44.9	45.0	3.8	0.7	8.4	37.0	51.0	30
	CS	54.1	55.0	4.5	0.8	8.3	45.0	62.0	30
Shell, length (2)	CAA	66.6	65.0	4.5	0.8	6.8	55.0	80.0	30
	CAS	66.2	65.0	4.5	0.8	6.7	60.0	75.0	30
	CS	82.8	82.0	4.4	0.8	5.3	75.0	90.0	30
Shell, width (3)	CAA	56.4	55.0	4.5	0.8	8.1	50.0	70.0	30
	CAS	65.6	65.0	5.1	0.9	7.8	57.0	76.0	30
	CS	79.5	79.5	4.8	0.9	6.0	74.0	90.0	30
Abdomen, length (4)	CAA	42.2	42.0	3.9	0.7	9.3	35.0	50.0	30
	CAS	42.8	41.5	4.1	0.8	9.7	35.0	54.0	30
	CS	53.2	52.0	3.4	0.6	6.5	50.0	60.0	30
Pseudostome, long axis (5)	CAA	24.9	25.0	3.3	0.6	13.2	20.0	30.0	30
	CAS	28.9	26.5	4.6	0.8	15.7	25.0	37.0	30
	CS	35.2	35.0	3.6	0.7	10.3	27.0	42.0	30
Pseudostome, short axis (6)	CAA	14.3	15.0	2.6	0.5	18.3	10.0	20.0	30
	CAS	13.7	14.5	2.4	0.4	17.4	10.0	19.0	30
	CS	19.8	20.0	2.6	0.5	13.2	16.0	25.0	30
Shell length:abdomen length, ratio	CAA	1.6	1.6	0.1	0.0	6.5	1.4	1.9	30
	CAS	1.6	1.6	0.1	0.0	4.5	1.4	1.7	30
	CS	1.6	1.5	0.1	0.0	5.0	1.5	1.8	30
Shell width:abdomen length, ratio	CAA	1.3	1.3	0.1	0.0	11.0	1.1	1.6	30
	CAS	1.5	1.5	0.1	0.0	7.9	1.3	1.9	30
	CS	1.5	1.5	0.1	0.0	7.2	1.3	1.7	30

[1]Numbers in parenthesis designate features as shown in Figs. 2 and 3. Measurements in µm. CV - coefficient of variation in %; M - median; Max - maximum; Min - minimum; n - number of specimens investigated; SD - standard deviation; SE - standard error of mean; $\bar{x}$ - arithmetic mean

untouched the basic features described by Deflandre (1929). Our selected material from Russia also matches Deflandre's description (Figs. 34-40; Table 2). No cement structures are recognisable in the scanning electron microscope.

Lobose pseudopodia, which form rapidly and eruptively, were described by Bonnet (1963), who provided also detailed ecological data (Bonnet 1989). Detailed morphometrics and helpful scanning electron micrographs were published by Lüftenegger *et al.* (1988) and Rauenbusch (1987). Scattered measurements were provided by other authors (Laminger 1972, Chardez 1979), broadening, however, Deflandre's limits only slightly: shell diameter 47-75 µm, shell height 30-45 µm; pseudostome 18-44 x 9-23 µm. Decloitre (1956) and Rosa (1971) observed very small specimens with a length of 30-36 µm.

Centropyxis aerophila var. *sylvatica* Deflandre, 1929 (Figs. 21-27, 41-61; Table 2)

"This variety differs from the type species in that it is usually larger and more robust and the anterior pseudostome margin has a well-recognisable double contour.[1] The pseudostome is elliptical, more often it is semicircular (again, this is unclear and thus we provide the original text: "la bouche elle-même est elliptique plus souvent que semicirculaire"). In ventral view, the shell is usually exactly ("régulière") elliptical or subcircular. The sides are more strongly curved than in the type.

The shell is chitinous as in the type; usually, it carries a certain amount of small, angular quartz grains towards the posterior end. It appears more punctuated and flaky than the type and is reinforced by fine, transparent, irregular platelets and very small quartz grains. Dimensions (number of specimens measured not given): 68-102 x 63-85 µm; pseudostome 32-53 x 17-30 µm".

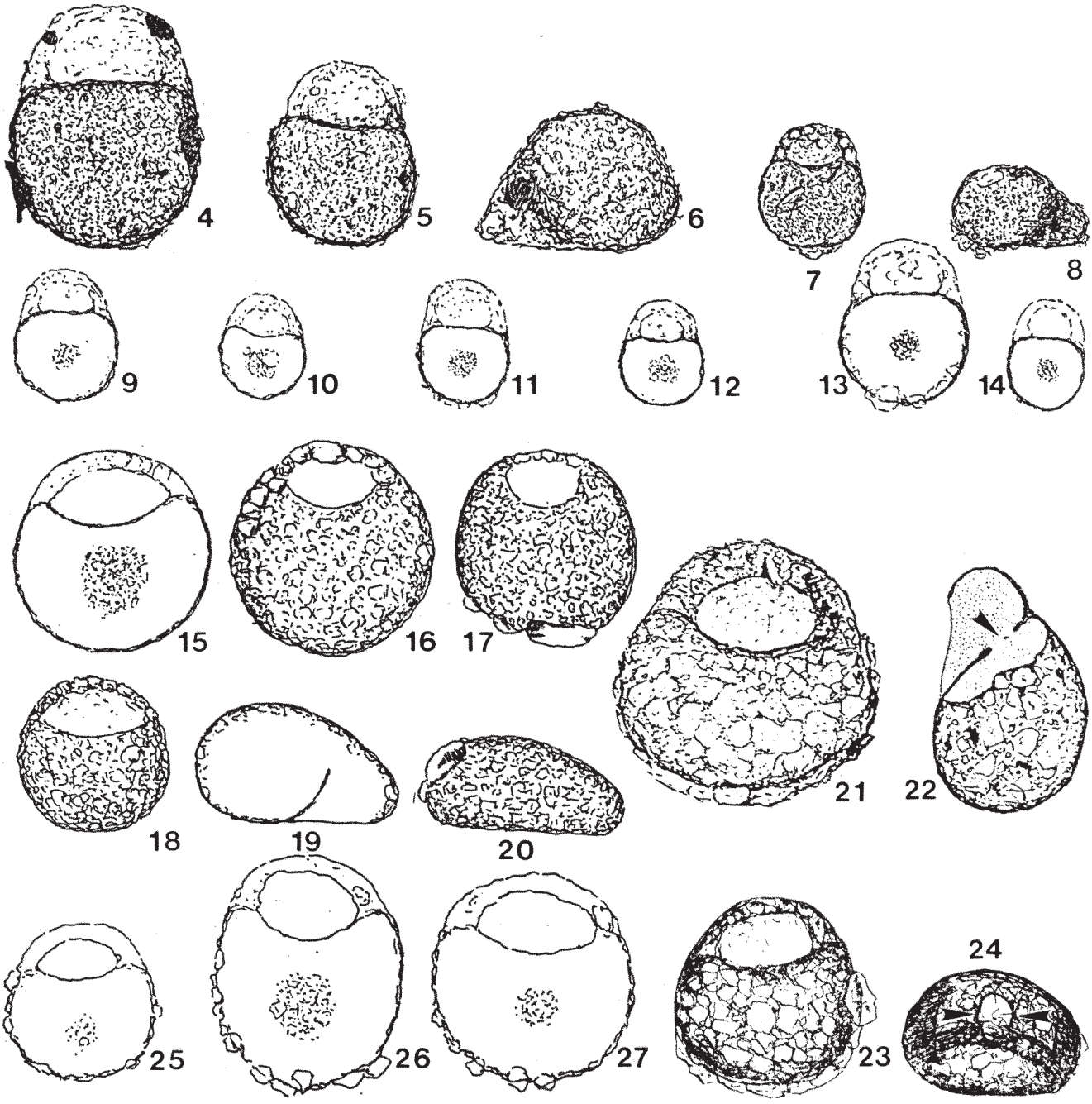
There are several notes and redescriptions available in the recent literature, significantly modifying Deflandre's original description. Most important is the observation by Bonnet and Thomas (1955) that the ventral pseudostome lip [called "membrane ventrale" by Bonnet and Thomas (1955) and "diaphragma" by Lüftenegger *et al.* (1988) and Rauenbusch (1987)] extends to the dorsal shell wall, dividing the test in a small "pseudostomal chamber" and a large "abdominal chamber" connected with each other by a more or less large opening (lip perforation) through which the pseudopods can extend. The observations of Bonnet and Thomas (1955) were confirmed by Lüftenegger *et al.* (1988) and Rauenbusch (1987). We put much effort in observing and documenting these details in our material and thus can, for the first time, clearly show the lip perforation which, in fact, is an inner pseudostome (Figs. 41-61).

The 30 large shells, which should have been *C. aerophila sylvatica* according to Deflandre's criteria (see "isolation of taxa and sampling protocol"), were very difficult to investigate because they were composed of rather large quartz grains making them opaque and refractive. Thus, nothing definite could be seen; only in five to six out of the 30 specimens there was a bright spot, possibly the lip perforation, at the right place. In contrast, we could see the lip perforation in 22 out of the 30 small, transparent shells, when they were optimally orientated, that is, viewed frontally; if debris adhered to the pseudostome, visibility of the lip perforation decreased. The transparent specimens showed that the site where the lip internally abuts to the dorsal shell wall is often marked by an inconspicuous groove (Figs. 52, 61), less distinct than those shown by Lüftenegger *et al.* (1988).

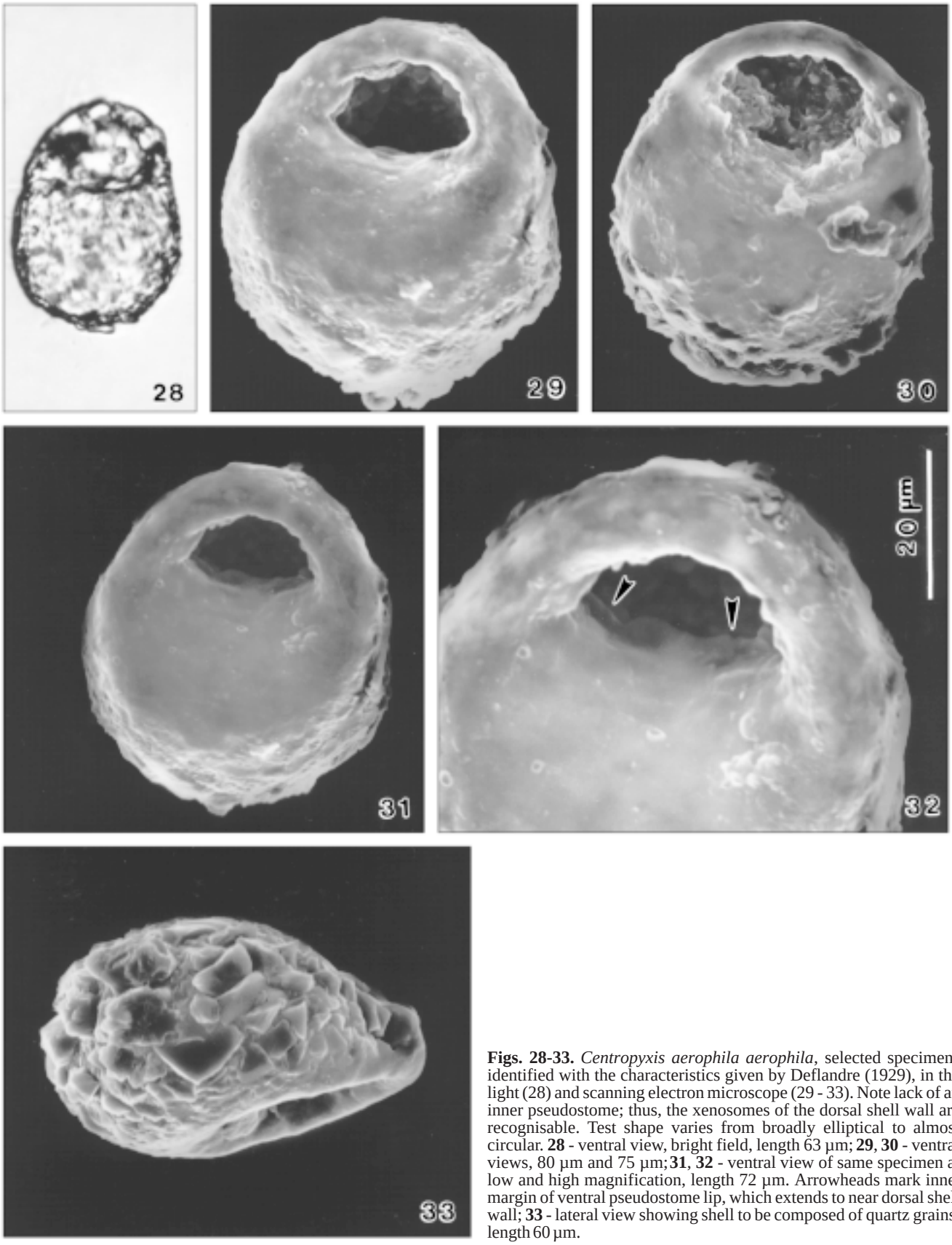
The lip perforation has a mean size of 17 x 11 µm (10-25 x 8-15 µm, n 22) in specimens with a pseudostome size of 28 x 15 µm (20-38 x 10-25 µm). Thus, it is elliptical and rather large; the margin is uneven. In the population investigated by Bonnet and Thomas (1955), the perforation is roundish with a diameter of only 5-8 µm. In the specimens studied by Lüftenegger *et al.* (1988), the lip perforation is very similar to our material having a mean size of 18 µm. In Rauenbusch's (1987) specimens the perforation is sickle-shaped (unfortunately, the scanning micrographs are too dark to be entirely convincing). Thus, there is considerable variability in the size and shape of the lip perforation; however, some of the variability might be caused by the observation problems described above.

In the scanning electron microscope, the inner pseudostome (lip perforation) was better recognisable in the large than in the small specimens (in contrast to the light microscope, see above). The scanning electron microscope investigations suggest that the lip perforation

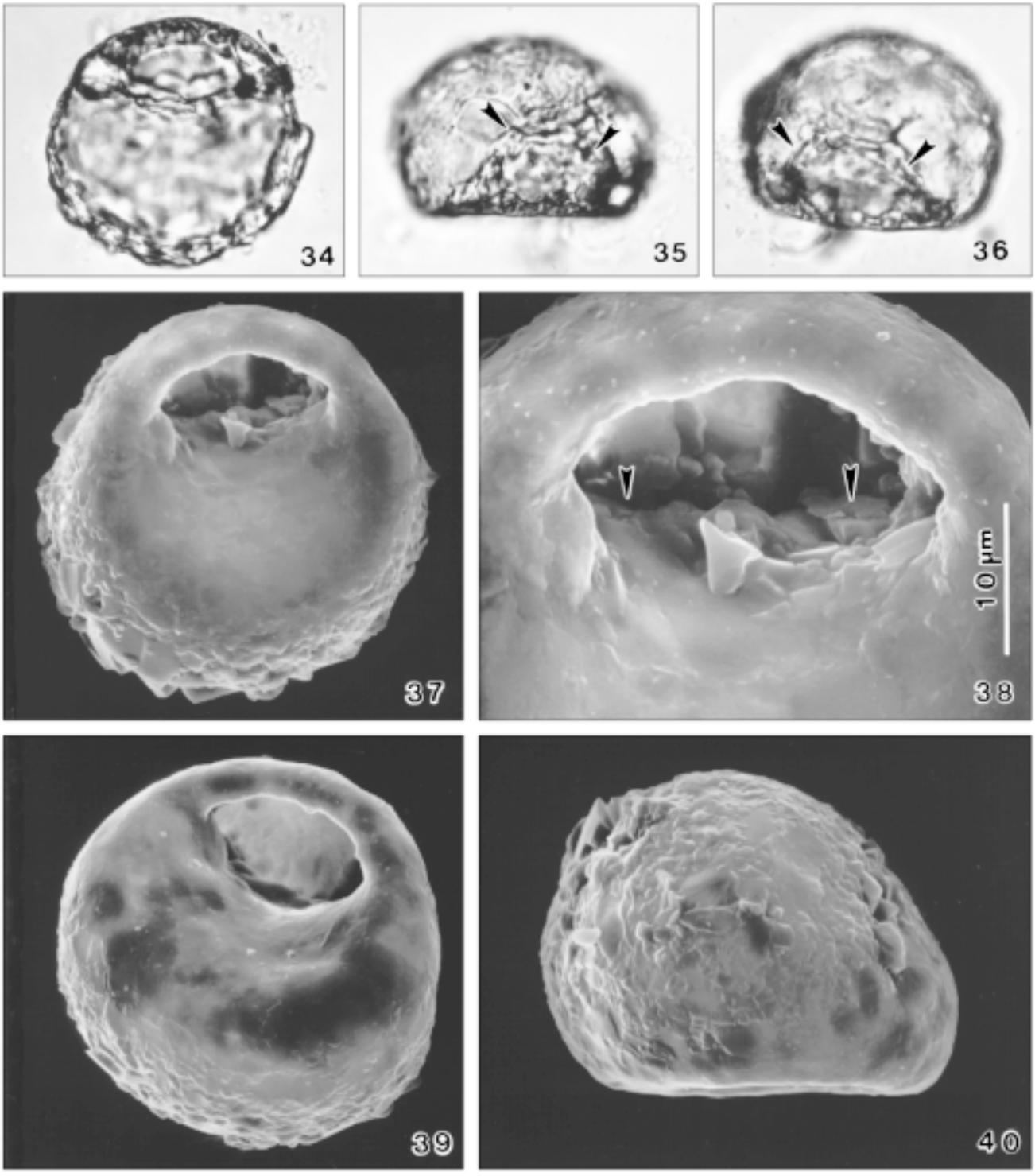
[1] "... vers la bouche dont le bord extérieur montre un double contour tres net". Very likely, Deflandre (1929) describes a dorsal pseudostome lip as shown in Figure 3. However, such a lip has not yet been convincingly shown and we could not find it in our material.



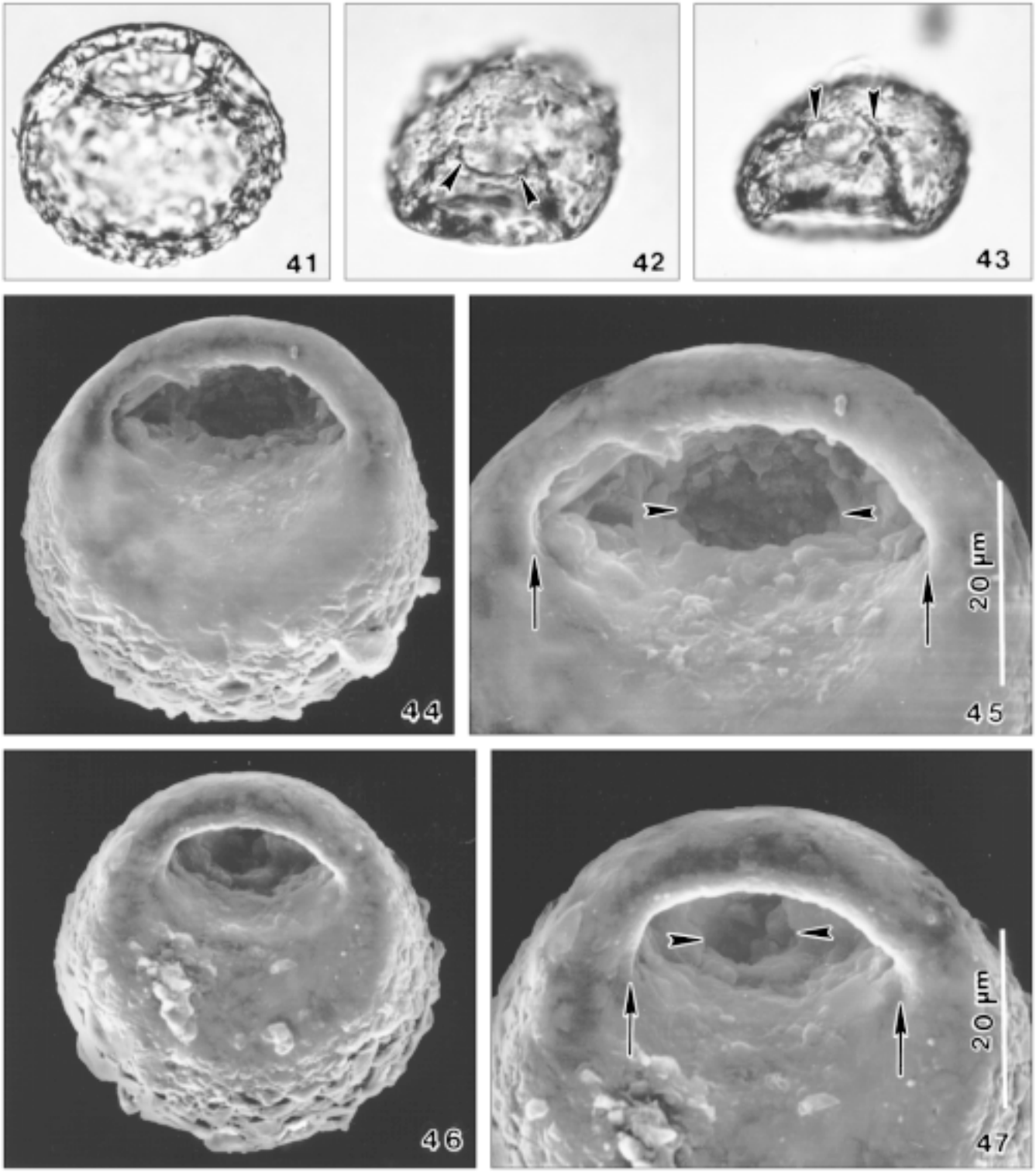
Figs. 4-27. Ventral (4, 5, 7, 9 - 18, 21, 23, 25 - 27), lateral (6, 8, 19, 20, 22), and frontal (24) views of shells of the *Centropyxis aerophila* complex in the light microscope (bright field illumination). All figures are reproductions (Xerox copies) from the original literature cited below. **4- 14** - *Centropyxis aerophila aerophila*, size 53 - 85 x 42 - 66 µm (from Deflandre 1929); **15-20** - *Centropyxis aerophila sphagnicola*, size 49 - 66 x 25 - 37 µm (from Deflandre 1929); **21-27** - *Centropyxis sylvatica*, size 65 - 105 x 60 - 87 µm (21, 22, from Bonnet and Thomas 1960; 23, 24, from Bonnet and Thomas 1955; 25 - 27, from Deflandre 1929). Arrowheads in Figs. 22 and 24 mark inner pseudostome, the main species character.



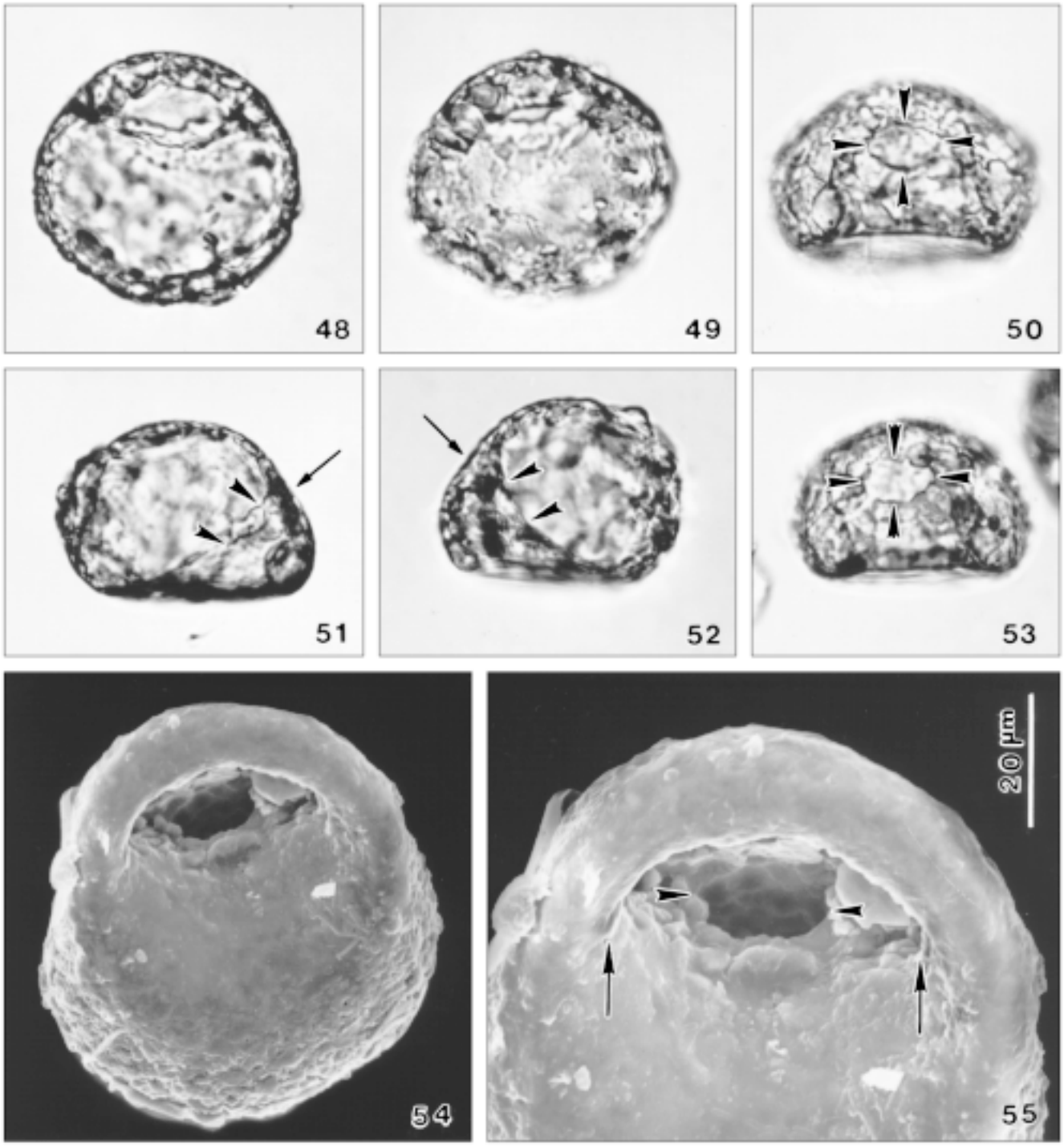
Figs. 28-33. *Centropyxis aerophila aerophila*, selected specimens identified with the characteristics given by Deflandre (1929), in the light (28) and scanning electron microscope (29 - 33). Note lack of an inner pseudostome; thus, the xenosomes of the dorsal shell wall are recognisable. Test shape varies from broadly elliptical to almost circular. **28** - ventral view, bright field, length 63 µm; **29**, **30** - ventral views, 80 µm and 75 µm; **31**, **32** - ventral view of same specimen at low and high magnification, length 72 µm. Arrowheads mark inner margin of ventral pseudostome lip, which extends to near dorsal shell wall; **33** - lateral view showing shell to be composed of quartz grains, length 60 µm.



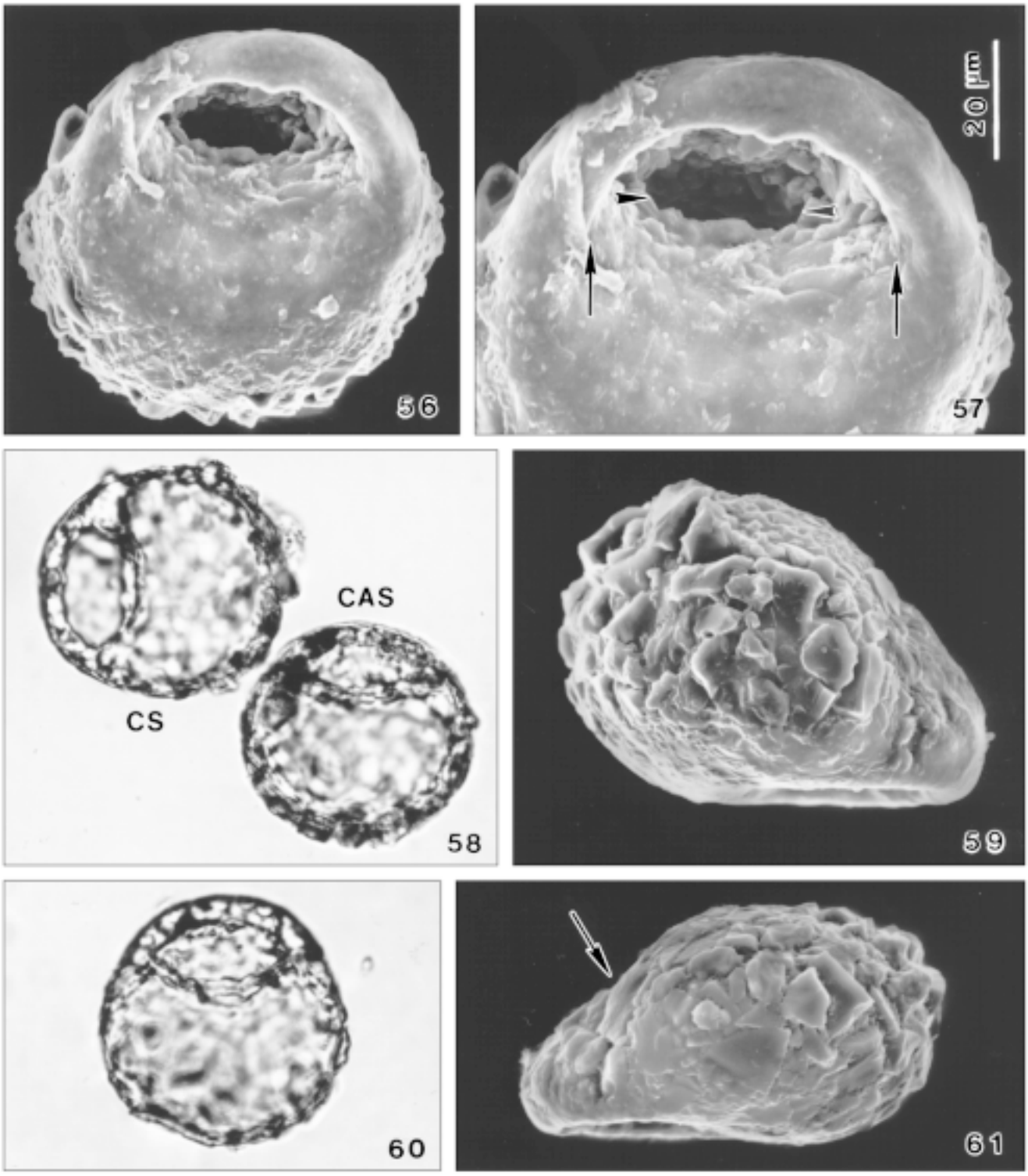
Figs. 34-40. *Centropyxis aerophila sphagnicola*, selected specimens identified with the characteristics given by Deflandre (1929), in the light (34 - 36) and scanning electron microscope (37 - 40). Note lack of an inner pseudostome; thus, the xenosomes of the dorsal shell wall are recognisable. The shells are circular or slightly broader than long and have agglutinated small quartz grains mainly in posterior portion. **34-36** - same specimen in ventral and frontal views, size 68 x 68 x 46 µm. Arrowheads mark ventral pseudostome lip; **37**, **38** - ventral view of same specimen at low and high magnification, length 61 µm. Arrowheads mark inner margin of ventral pseudostome lip, which extends to near dorsal shell wall; **39** - ventral view, length 63 µm; **40** - frontolateral view, length 58 µm.



Figs. 41-47. *Centropyxis sylvatica*, selected small ("transparent") specimens identified with the characteristics given by Deflandre (1929), in the light (41-43) and scanning electron microscope (44-47). Note that specimens are circular or slightly broader than long and have agglutinated small quartz grains mainly in posterior portion. **41-43** - same specimen in ventral view and two frontal focal plans showing lower and upper margin of inner pseudostome (arrowheads), size 68 x 74 x 53 µm; **44-47** - ventral views at low and high magnification showing the outer (arrow) and inner (arrowheads) pseudostome, length 61 µm and 66 µm. The inner pseudostome is formed by agglutinated material on the dorsal and lateral shell wall



Figs. 48-55. *Centropyxis sylvatica*, selected small ("transparent") specimens (48-53) and a large opaque specimen (54, 55) identified with the characteristics given by Deflandre (1929), in the light (48-53) and scanning electron microscope (54, 55). Arrow in Figs. 51 and 52 marks minute groove, where the inner pseudostome abuts to the dorsal shell wall. **48-51** - same specimen (size 68 x 74 x 46 µm) in ventral view, where the inner pseudostome is not recognisable; in oblique ventral view, where the pseudostome becomes minute; in frontal view, where the broadly elliptical inner pseudostome is well recognisable (arrowheads); and in lateral view, where the lip perforation (inner pseudostome, arrowheads) is difficult to recognise; **52**, **53** - lateral and frontal view of another specimen showing the inner pseudostome (arrowheads); **54**, **55** - ventral view of same specimen at low and high magnification showing impressively the outer (arrow) and inner (arrowheads) pseudostome, length 94 µm. The inner pseudostome is obviously made of small xenosomes attached to the dorsal wall of the shell and the lateral walls of the outer pseudostome



Figs. 56-61. *Centropyxis sylvatica*, selected small ("transparent") specimens (58-60) and large opaque specimens (56, 57, 61) identified with the characteristics given by Deflandre (1929), in the light (58, 60) and scanning electron microscope (56, 57, 59, 61). **56**, **57** - ventral view of same specimen (length 87 µm) at low and high magnification showing the outer (arrow) and inner (arrowheads) pseudostome; **58**, **60** - ventral views of *C. sylvatica* (CS) and *C. aerophila sphagnicola* (CAS), size of specimen shown in Fig. **60**, 61 x 65 x 43 µm; **59**, **61** - lateral views showing shells to be composed mainly of quartz grains, length 73 µm and 88 µm. Arrow marks minute depression, where the inner pseudostome attaches to the dorsal shell wall (cp. Figs. 51, 52)

is mainly brought about by accumulation of agglutinated material on the dorsal and lateral shell wall. Thus, the ventral lip is possibly not "perforated" in the strict sense of the word; this is also indicated by its general appearance, which is as in the other varieties (Figs. 32, 38). In the scanning electron microscope, a lip perforation was seen in several small (transparent; Figs. 41-47, 48-53) and large ("typical") *C. sylvatica* specimens (Figs. 54, 55, 56, 57), showing that the species cannot be recognised by size.

Lobose pseudopods and their movements, as well as two contractile vacuoles, were described by Bonnet (1961) in *C. sylvatica* var. *minor*. Rauenbusch (1987) and Lüftenegger *et al.* (1988) provided some helpful scanning electron micrographs showing that shell wall structure highly depends on the substrate the organisms live. Lüftenegger *et al.* (1988) provided also detailed morphometrics showing that pseudostome features are more variable than the length and width of the shell. Scattered measurements were given by other authors (Bonnet and Thomas 1955, Rosa 1971), broadening, however, Deflandre's limits only slightly: 56-113 (length) x 47-100 (width) x 45-68 (height) µm; pseudostome 23-55 (long axis) x 20-32 (short axis) µm.

Morphometry

Basic statistics show that most variables have usual coefficients of variation and the number of specimens investigated is sufficient because mean and median hardly change if 127 or 217 specimens are analysed (Table 1). Of course, variation is distinctly lower in the selected specimens (Table 2). Only a few of the variables measured are normally distributed, viz. shell width, ratio shell length: abdomen length, and ratio shell width: abdomen length. All other features are slightly skewed to the left.

Analysis of variance: If the 30 selected specimens of each are compared (all intermediate shells removed, see Method section!), all variables tested (length, width…) are significantly different ($p \leq 0.001$), except the ratio shell length: abdomen length, that is, the three taxa can be clearly distinguished. If the 127 randomly chosen specimens are compared with the 30 selected specimens of either *C. aerophila aerophila* or *C. aerophila sylvatica*, highly significant differences ($p \leq 0.001$ to $p \leq 0.05$) still occur in most variables, except for the ratios; in contrast, only shell height, shell length, short pseudostome axis, and the length: width ratio are different ($p \leq 0.05$) in *C. aerophila sphagnicola*, indicating that this variety is intermediate between the two others. Finally, when the 30 selected specimens of each are pooled (= 90 specimens) and compared with the 127 randomly chosen specimens, all variables become indistinguishable ($p \geq 0.05$).

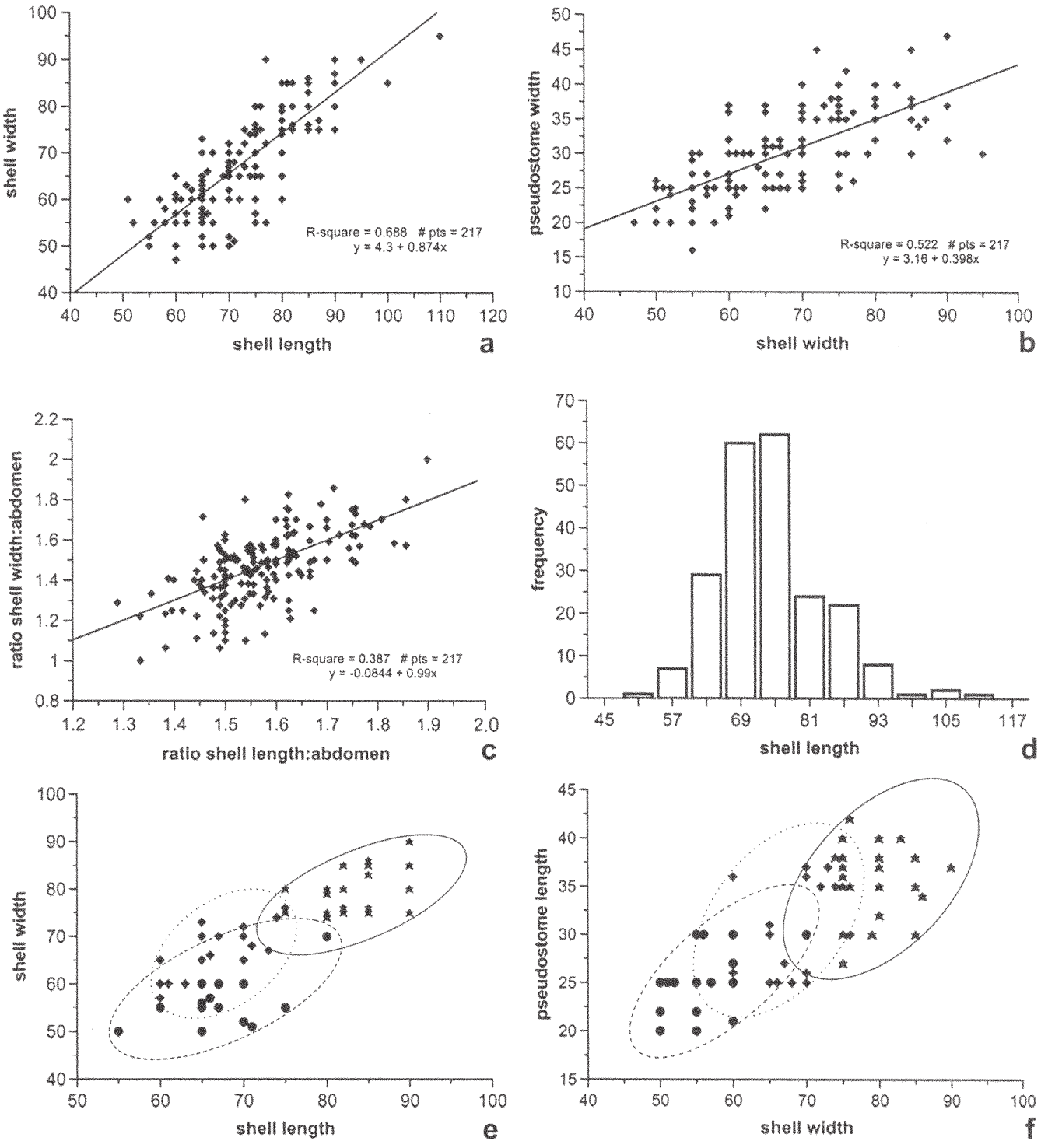
Frequency distributions and relationships between variables (only some representative examples each are shown, Figs. 62a-f): Frequency distributions show curves with a single peak (Fig. 62d). Likewise, rather homogenous clusters are formed in the randomly chosen specimens, when variables are plotted against each other (Figs. 62a-c). In contrast, two distinct clusters are usually formed, if the selected specimens are plotted (Figs. 62e, f): one contains *C. aerophila aerophila* and *C. aerophila sphagnicola*, the other *C. aerophila sylvatica*.

DISCUSSION

Morphometry

Although species cannot be proven mathematically, some basic statistics are often useful to distinguish them more properly. As concerns the present material, neither the coefficients of variation (Table 1) nor frequency distributions (Fig. 62d) and relationships between the variables tested (Figs. 62a - c) give any indication that the randomly chosen specimens consist of more than one species. Variation coefficients (Tables 1, 2) are of the same order of magnitude as in multicellular organisms (Mayr 1975) and other protozoans, e.g. ciliates (Foissner 1984, 1993), and as in testate amoebae in general (Lüftenegger *et al.* 1988, Wanner 1991, Foissner and Korganova 1995). Only the pseudostome variables have coefficients higher than 20%; however, this seems to be a general feature of testacean shells (Lüftenegger *et al.* 1988, Wanner 1991).

However, the situation changes drastically in the selected material, that is, when all intermediate specimens, which could not unequivocally assigned to one of Deflandre's varieties, are excluded (Table 2). Then, three taxa can be distinguished by analysis of variance and at least two in scatter diagrams (Figs. 62e, f), just as different species were compared (Lüftenegger *et al.* 1988). *Centropyxis aerophila sylvatica* is separated by its larger dimensions, while *C. aerophila aerophila* and *C. aerophila sphagnicola* are distinguished mainly by the shell proportions (Table 2): the former is broadly elliptical (66.6 x 56.4 µm), the latter almost circular (66.2 x 65.6 µm); furthermore, the long pseudostome axis is distinctly larger in *C. aerophila sphagnicola*



Figs. 62a - f. Representative examples from the measurements (for details, see chapter on morphometry). **62a - c** - when all (217) specimens of *Centropyxis aerophila aerophila*, *C. aerophila sphagnicola* and *C. sylvatica* are pooled and the main characteristics plotted, rather homogenous clouds of dots are formed, indicating that the taxa are morphometrically inseparable; **62d** - likewise, frequency distributions of the 217 specimens do not provide any indication that several taxa are mixed; **62e, f** - when the 30 selected (all intermediates discarded, see Materials and Methods) specimens each are plotted, at least two distinct clouds are formed, one contains *C. sylvatica* (*), the other *C. aerophila aerophila* (•) and *C. aerophila sphagnicola* (♦). We hypothesise that most authors distinguished Deflandres varieties by a similar (mental) selection process

than in *C. aerophila aerophila* (28.9 vs. 24.9 µm). However, such result is expected and can be obtained with most living things, even with man, when intermediate specimens are removed. In fact, the experiment was performed specifically with the goal of obtaining information as to how previous authors probably distinguished Deflandre's varieties (see next but one chapter).

Centropyxis sylvatica is a distinct morphospecies

We agree with Bonnet and Thomas (1955) that the lip perforation of *C. sylvatica* is difficult to observe and usually recognisable only in optimally orientated and transparent specimens. Indeed, when looking at an unorientated shell assemblage one gets doubts whether the lip perforation exists at all! And this doubt is strengthened by the fact that, contrary to Deflandre's (1929) claim, *C. sylvatica* is obviously not larger than *C. aerophila aerophila* and *C. aerophila sphagnicola* (Figs. 62a - c), this being also evident from the size (69-72 x 67-73 µm) of the specimens studied by Bonnet and Thomas (1955). Nonetheless, the lip perforation exists and distinguishes *C. sylvatica* from the other varieties (Figs. 41 - 61). This conclusion is supported by the investigations of Lüftenegger *et al.* (1988) and Rauenbusch (1987). Furthermore, the lip perforation has been clearly shown, i.e. by scanning electron microscopy, in a larger, related species, *Centropyxis matthesi* Rauenbusch, 1987; and a similar, probably homologous opening occurs in *Paracentropyxis mimetica* Bonnet, 1960. Thus, we agree with Bonnet and Thomas (1955, 1960) that *C. aerophila* var. *sylvatica* should obtain species rank. It is well defined by its extraordinary lip perforation, forming a second, inner pseudostome.

Much more difficult is the question whether *C. sylvatica* is a member of the genus *Centropyxis* at all! There are at least two other, well-defined species with a distinct lip perforation, viz., *Centropyxis matthesi* and *C. deflandriana*. We cannot exclude that these three taxa represent a specific (homologous) evolutionary line different from that of *Centropyxis*, that is, it could happen that the similarities in shape and size of *C. aerophila* and *C. sylvatica* are an analogy. The solution of this question will require genetic and molecular methods.

How did previous authors distinguish Deflandre's *Centropyxis aerophila* varieties?

As mentioned in the introduction, Deflandre's varieties of *C. aerophila* have been reported by many testacean researchers from terrestrial and freshwater habitats worldwide, usually even from the same sample. Obviously, all used Deflandre's traits to distinguish the varieties, at least none mentioned to have applied other and/or additional features. Furthermore, none mentioned having looked for the lip perforation, even after the pioneering paper of Bonnet and Thomas (1955), although it is the sole feature unequivocally separating *C. aerophila sylvatica* from *C. aerophila aerophila* and *C. aerophila sphagnicola*.

We showed that it is impossible to distinguish three varieties in *C. aerophila* with the morphological and morphometrical features given by Deflandre (1929); and our data give no indication that other, as yet undescribed reliable characteristics exist (Figs. 62a-d; Table 1). How then, could so many authors distinguish Deflandre's varieties, although some mentioned problems (Jung 1936; Schönborn 1966, 1975; Chardez 1979)? In our opinion it is because they considered mainly the extremes of a variability cline, which fit to Deflandre's descriptions, obviously assigning intermediate specimens more or less arbitrarily to one of the varieties. We could also distinguish three taxa when we sorted out all intermediates, that is, about half of the shells (Figs. 62e, f; Table 2).

A practicable solution for the problem: the "*Centropyxis aerophila* complex"

The species problem in general and of testate amoebae in particular has been extensively investigated and discussed recently (Schönborn and Peschke 1988, Medioli *et al.* 1990, Schönborn 1992a, Foissner and Korganova 1995, Bobrov *et al.* 1995, Wanner 1999). These studies showed the lack of a simple answer and emphasised the need for thorough species descriptions and avoidance of infrasubspecific taxa, unless they can be proven by reliable morphological and/or morphometrical features. They also showed that testacean shells are not extraordinarily variable, in contrast to the widespread believe, because the variation coefficients are hardly greater than in other organisms. This is emphasised by the present results (Tables 1, 2).

We showed that it is impossible to distinguish varieties in *C. aerophila* with the features used by Deflandre (1929). The situation became even worse when the varieties described later were taken into account (for reviews, see Decloitre 1978, 1979).

Basically, there are two ways to solve the problem. First, one might recognise only a single species, namely, *Centropyxis aerophila*, and classify all varieties and forms as falling into the species natural range of variability. Actually, this has been done by the describers of the

varieties and forms. This, however, resulted in an extraordinary variability of a single species casting doubts on the decision because such a broad range indicates the inclusion of several species or, at least, several cryptic (sibling) species (Mayr 1975), which is emphasised by the varying proportions the varieties were found in field samples (Chardez 1979, Bonnet 1989, Aescht and Foissner 1994). On the other hand, a reliable separation and identification of the varieties is often impossible and will be difficult also with modern molecular techniques (Wanner *et al.*1997), if they exist at all. Accordingly, we prefer the second way at the present state of knowledge, that is, to unite all taxa in a "*Centropyxis aerophila* complex", as has been done in several "difficult" (cryptic) ciliate species [*Paramecium aurelia*, *Tetrahymena pyriformis*, *Sterkiella histriomuscorum*; see Foissner and Berger (1999) for a brief review]. This will at least relieve ecologists and practitioners of work which can hardly be done during field investigation, e.g., counting work. The theoretical background of this suggestion is the assumption that the phenospectrum *aerophila-sphagnicola* is an adaptive polymorphism to meet wetter (*aerophila*) and drier (*sphagnicola*) environmental conditions by selective shifting of certain genes.

We suggest that *C. sylvatica* is also included in the *C. aerophila* complex, although it is a distinct species, because its overall appearance is so similar and the species character, the lip perforation, is too difficult to recognise in routine counting work. Certainly, taxonomists and biogeographers (faunists) must look for this feature to get a reliable species list. Very likely, further taxa need to be put into the complex, for instance, *C. cassis* and *C. constricta*. However, these and other candidates, especially the subspecies and varieties of *C. aerophila aerophila* and *C. aerophila sphagnicola* (for reviews, see Decloitre 1978, 1979), are still poorly known and thus a final decision must await further investigations.

Nomenclature

There is great uncertainty whether Deflandre's "varieties" should be considered as infrasubspecific taxa or as subspecies. Indeed, many authors cited the varieties like subspecies or species, but none formally raised *C. aerophila sphagnicola* to subspecies or species rank. Fortunately, the matter can be unambiguously decided by the International Code of Zoological Nomenclature (1999), article 45.6. Deflandre (1929) described *C. aerophila sphagnicola* and *C. aerophila sylvatica* as "var. n."; however, his work does not **unambiguously** reveal that the names were proposed for infrasubspecific entities, which are not regulated by the code, and accordingly *sphagnicola* and *sylvatica* have subspecific rank from their original publication. Thus, Deflandre (1929) is author of the subspecies *C. aerophila sylvatica*, raised to species rank by Bonnet and Thomas (1955). Accordingly, the species formally must be cited as "*Centropyxis sylvatica* Deflandre, 1929". The same applies to *C. aerophila* var. *sphagnicola*, if it is not considered as synonym of *C. aerophila*, and to most other varieties and forms described before 1961 (see previous chapter). All varieties and forms described after 1961 have infrasubspecific rank, unless they were adopted as valid name of a species or subspecies before 1985 (e.g. *C. aerophila* var. *globulosa* Bonnet and Thomas, 1955).

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