Critical notes on the genus *Frenzelina* PENARD, 1902 (Protozoa, Testaceafilosia)*

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Abstract: This paper is a critical evaluation of the existing diagnosis of the genus *Frenzelina*, a testate amoeba with filopodia. All the authors who have reported on *Frenzelina* have assumed a rigid hemispherical shell, for example, like that described and figured by PENARD. However, we could not confirm the existence of this shell. The *Frenzelina* cell is enclosed by a flexible envelope and embedded in a gelatinous layer covered with xenosomes. Already PENARD concluded that there is a gelatinous layer invisible to the light microscope, whereas HOOGENRAAD designated it as ectoplasm. The gelatinous layer can be stained with neutral red. The *Frenzelina* individuals collected in Lake Stechlin and in the River Elbe closely resemble the *F. reniformis* PENARD, 1902 except that they not have a shell. It is currently impossible to answer the question whether PENARD made an observation error or whether there are indeed forms of *Frenzelina* with a rigid shell. Comparison between the *Frenzelina* specimens found in Lake Stechlin and those from the Elbe show that there is wide variability withwithin the species diagnosis. A *Frenzelina* without a shell may alter its systematic position (e. g. closer to the genus *Lecythium*). *Frenzelina* material from Lake Stechlin could be used for reconstruction of the division and the description of different stages of development of the species.

Key words: Cell division, Germany, ontogenesis, redescription, taxonomy, Testacea, testate amoeba.

Introduction

This description of *Frenzelina* is based partly on investigations made between 1965 and 1970 and partly on more recent work. In the early period samples were taken from Lake Stechlin (Brandenburg, Germany), a lake whose morphology, chemistry and physical characteristics, along with the settlement patterns of testate amoebae, have been described by SCHÖNBORN (1962). The more recent material derives from the River Elbe in the region of Magdeburg (Saxony-Anhalt, Germany).

The original plan was to produce a monograph of the genus, whose phylogenetic position is very uncertain (MEISTERFELD 2002). However, comparing the *Frenzelina* that we found with those described in the past faces insurmountable difficulties. The individuals from Lake Stechlin and those from the River Elbe precisely resemble those from Lake Geneva (PENARD 1902) except for one characteristic. This is that Lake Geneva individuals have a rigid hemispherical shell with a wide opening underneath. The question is therefore whether *Frenzelina* exists in two forms, with a shell and without. It is hard to believe that such a clear and unambiguous description could be the result of faulty observation by PENARD (1902). *Frenzelina* is mentioned relatively little in the literature. This may be because it attracts little attention or because it is simply overlooked. However, careful analysis of appropriate samples and the knowledge of the form reveal that *Frenzelina* is a frequently occurring and widespread genus that can even achieve high densities in sewage treatment plants. For this reason we offer some critical notes based on both the earlier and the recent collections.

Materials and methods

The earlier samples containing *Frenzelina* were collected from Lake Stechlin between 1965 and 1970. It was initially not clear whether the samples contained just one species or two. The most frequently occurring form was finally identified as *F. reniformis* PENARD, 1902. This species was not found during the early years of testacean research in Lake Stechlin, where it occurred only on stones covered with short algal growth. Once it had been found several times, and the investigators were looking out for it, it was regularly recorded. In fresh samples only a few individuals were present but the density of *Frenzelina* increased if the samples were left standing for a few days. There were then sufficient specimens for study. Dividers were also found, though infrequently. No controlled culture of the species was attempted at the time. To illustrate their structure, the

* The authors dedicate the paper to Univ.-Prof. Dr. Wilhelm FOISSNER on the occasion of his 60th birthday.

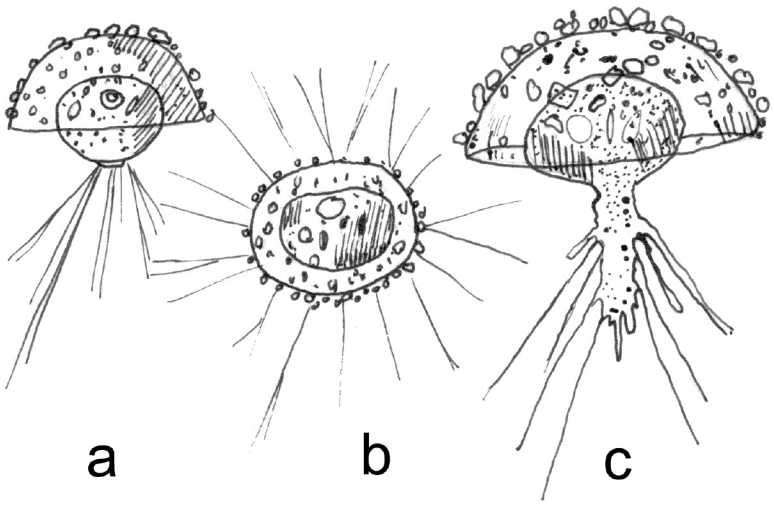

Fig. 1a–c: *Frenzelina reniformis* from Lake Geneva (after PENARD 1902). Note the rigid and hemispherical shell.

specimens were stained with neutral red. More recently, the species was frequently recorded in the River Elbe near the town of Magdeburg (BADEWITZ 2007).

Hitherto existing descriptions of *Frenzelina* species

Frenzelina was originally described by PENARD in 1902. He erected the genus on the basis of *F. reniformis* PENARD, 1902. According to his description, *Frenzelina* has a transparent, rigid, hemispherical shell with a very wide opening (Fig. 1a–c). The shell is covered with xenosomes, mainly tiny grains of quartz. The cell is enclosed in a flexible envelope, tapering to a tubal aperture (Fig. 1a), though the tube can also lengthen into a pseudopodial stalk (Fig. 1c). As there are no epipodia between cell-envelope and shell, PENARD assumed that both cell and surrounding envelope are embedded in a gelatinous layer. The cell body contains many inclusions, mostly food particles.

The species possesses numerous filopodia, both simple and forked. PENARD (1902) observed one nucleus and one contractile vacuole. The cell, including the gelatinous layer, is reniform, i.e. oval with one side indented. The shell-size varied between 26 and 30 µm. PENARD found the species fairly frequently in the littoral of Lake Geneva, but also at depths of 30–40 m.

It is already worth noting here that it is very difficult to understand how a shell with such a wide opening can support a gelatinous layer. PENARD's illustrations also show the hemispherical shell with a reniform indentation, a very improbable feature (Fig. 1b).

HOOGENRAAD (1910) described a second species, *Frenzelina minima* (Fig. 2). It is smaller (13–27 µm) than *F. reniformis* and the opening of the (presumed) shell is narrower. The cell is not reniform, the shell being more than hemispherical. There is no aperture tube. Only a few filopodia are observable, maximally six.

HOOGENRAAD (1910) believed that the cell is not enclosed in a flexible envelope but that the space be-

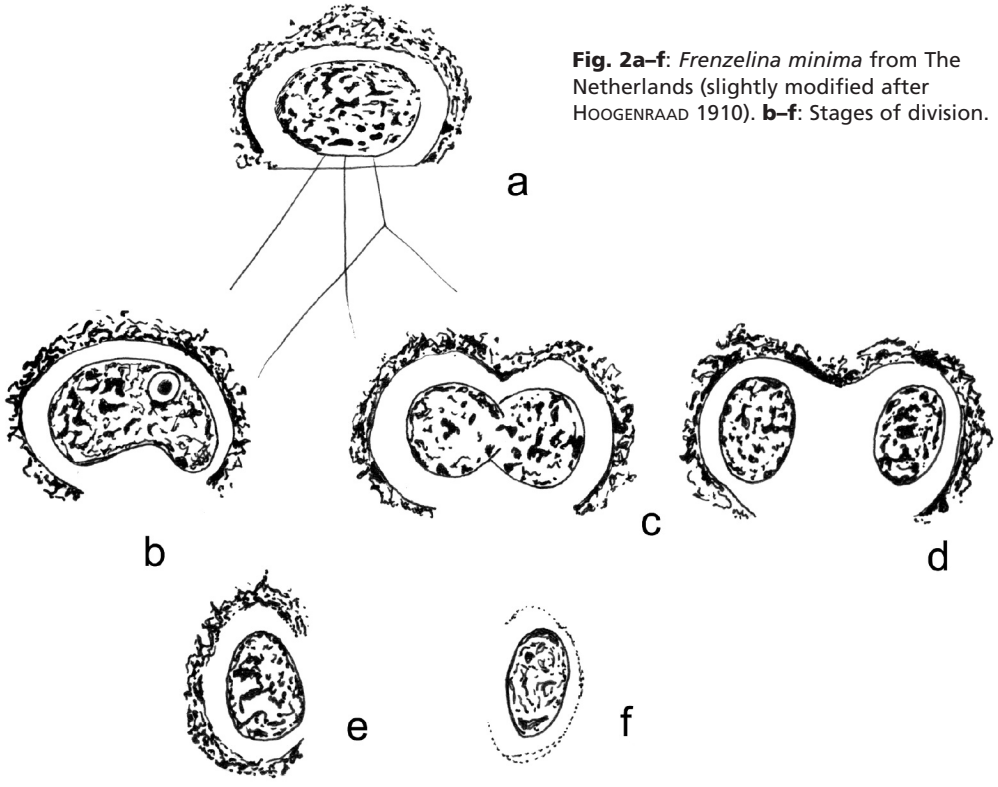

Fig. 2a–f: *Frenzelina minima* from The Netherlands (slightly modified after HOOGENRAAD 1910). **b–f**: Stages of division.

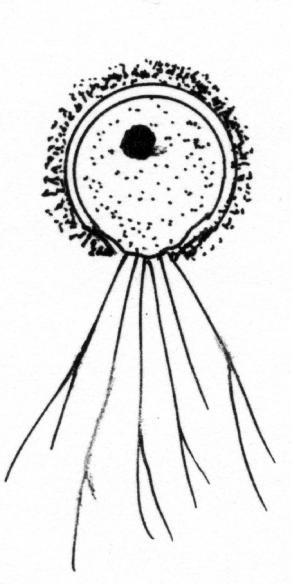

Fig. 3: *Frenzelina globosa* from Australia (slightly modified after PLAYFAIR 1917).

tween cell (= endoplasm) and shell is filled with ectoplasm. The surface of *F. minima* is also covered with xenosomes. The (completely transparent) shell that HOOGENRAAD presumed to exist does not appear in any of his figures.

HOOGENRAAD (1910) was able to reconstruct the division of *Frenzelina*. The ectoplasm (or gelatinous layer) subsequently also divides, which makes the existence of a rigid shell unlikely (Fig. 2b–f).

Frenzelina minima has so far only found in the Netherlands, in *Sphagnum* cushions, in sapropel of small pools with and without *Sphagnum*, and in other habitats such as lakes and ditches (HOOGENRAAD 1914, HOOGENRAAD & DE GROOT 1935, 1940).

A third species, *F. globosa* from Australia, is described by PLAYFAIR (1917). It is nearly spherical and the space between cell and (presumed) thin chitinous shell is very narrow (Fig. 3). The surface of the shell (or gelatinous layer) is also covered with xenosomes. The shell has a diameter of about 21–25 µm and almost entirely envelops the cell. The pseudostome protrudes slightly. The cell possesses one nucleus but there is no information about the number of filopodia. The species was found in a waterhole. HOOGENRAAD & DE GROOT (1940) synonymised *F. globosa* with *F. minima*, but this is not at all certain (see Discussion).

Results

Collections from Lake Stechlin

All of the individuals collected agree closely in shape and structure with *F. reniformis* except that they possess no shell (Fig. 4). The cell body is enclosed in a flexible envelope and the pseudostome (aperture) can be expanded into a small tube. The cytoplasm contains many inclusions, mostly algae and bacteria on which the cell feeds. These are concentrated in the central zone of the cell where digestion occurs. During digestion the algae change to a yellow-brown colour but do not become reddish. We observed one nucleus with a central nucleolus, and two contractile vacuoles. The time between vacuolar contractions was 30–40 s (measured on a slide under a coverslip at about 22°C).

The cell is slightly compressed and oval-reniform in dorsal and lateral view. The cell movement is without overall direction and amounts to about 20 µm min^{-1} (measured on a slide without a coverslip and at room temperature). The filopodia are very fine, numerous, and either simple or forked.

There is no shell like that described by PENARD (1902). The cell is embedded in a gelatinous layer that was made visible by neutral-red staining and may be completely enclosed by this layer. This layer is slightly flexible and able to adapt to the movements of the cell body. Its surface is covered with a more or less dense coat of xenosomes (detritus particles and sand grains) and is predominantly smooth although it may be very rough and irregular. The roughness would appear to be incompatible with the existence of an ectoplasm. The pseudostome tube can be retracted. The animals are completely transparent, as described by PENARD, and the aperture is thus also visible in dorsal view. The flexible envelope indicates that *Frenzelina* belongs to the testate amoebae.

Dividing specimens were also found in Lake Stechlin. During division the gelatinous layer remained intact and the cell, with its envelope, divided within it. After division, the daughter cell slips ventral out of the gelatinous layer. At this point the daughter cell has only a thin gelatinous layer (as can also be seen in HOOGENRAAD's figure). The emergence of the daughter cell unfortunately could not be directly observed, but could be reconstructed with a relatively high probability. Young stages occurred very frequently in the samples, especially in those that have stood for a few days. Under these conditions all stages of development were present. Growth occurred through an increase in the thickness of the gelatinous layer and in the density of xenosomes. The gelatinous layer of adult stages may reach a thickness equal to half of the dimensions of the cell body as described for *F. reniformis* by PENARD and for *F. minima* by HOOGENRAAD (Fig. 4).

In Lake Stechlin *Frenzelina* was found exclusively on submerged stones covered with a short algal aufwuchs. It was absent from sediment, and also from filamentous algae growing on higher plants that are colonised by numerous testacean species.

Lake Stechlin specimens were 13–40 µm long (usually 20 µm or more) and 10–30 µm, usually 15 µm wide (Fig. 5). The animals from Lake Stechlin seem to have a wider size spectrum than those from Lake Geneva.

Collections from the River Elbe

Frenzelina specimens from the Elbe were without shells like those from Lake Stechlin (Fig. 6). They clearly deviate, however, from these, although the deviations remain within the bounds of the species diagnosis. The gelatinous layer does not extend to the base of the cell body and the pseudopodia emerge from a long pseudopodial stalk. We assume this stalk to be an extension of the pseudostomal (or apertural) tube as also seen in PENARD's figures (Fig. 1c).

In both of these characters the forms from the Elbe resemble more those described by PENARD (1902) than those from Lake Stechlin. The Elbe forms are also similar in size (about 30 µm) to those from Lake Geneva.

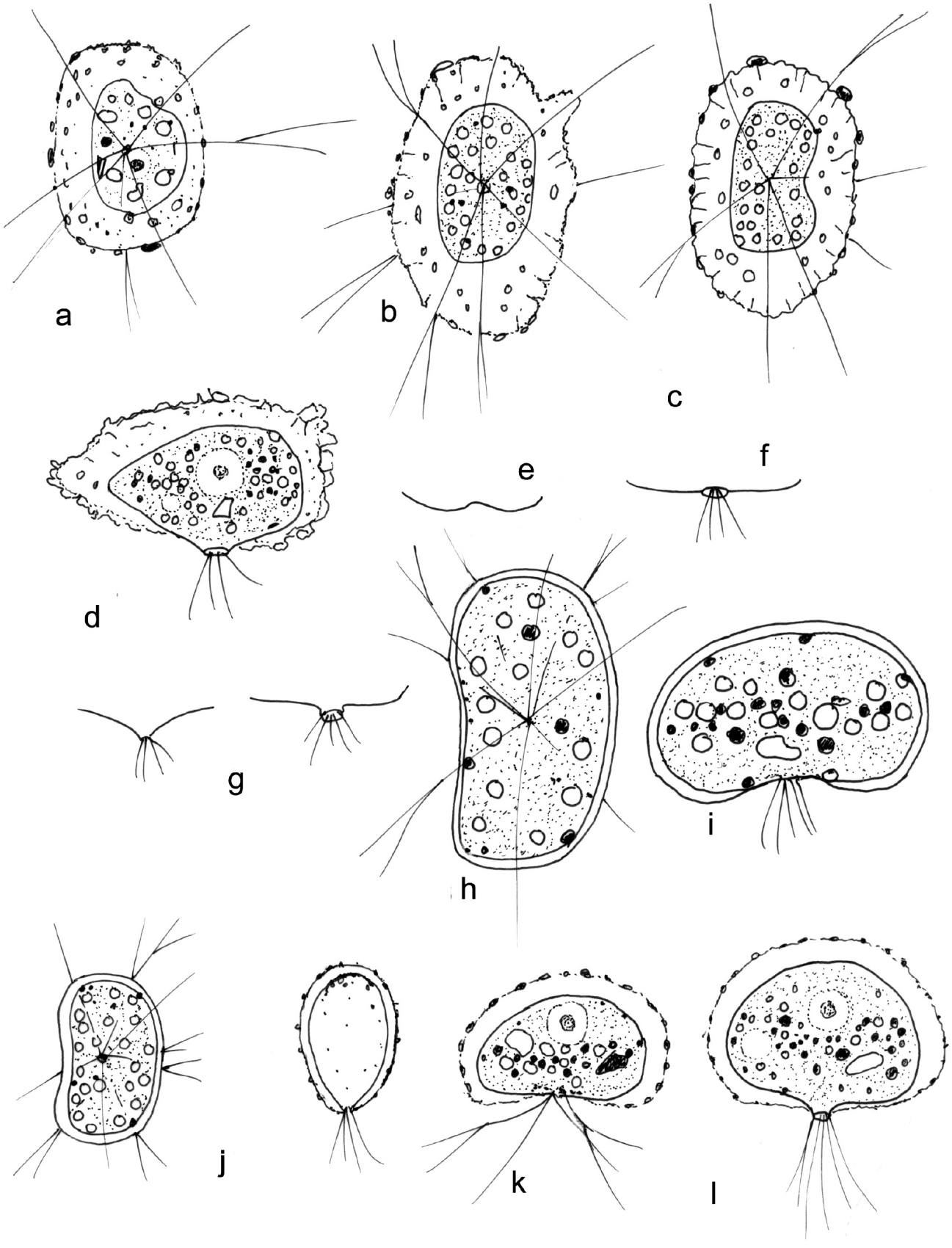

Fig. 4a–l: *Frenzelina* from Lake Stechlin. **a–c**: Adult individuals, dorsal view (note nucleus). **d**: Adult individual, lateral view (note nucleus and contractile vacuole). **e**: Aperture closed. **f**: Aperture opened. **g**: Aperture forming a tube. **h**: Young stage, dorsal view. **i**: Young stage, lateral view. **j**: Young stage, dorsal view and from behind: **k, l**: Middle life stages, lateral views (note nucleus and contractile vacuole).

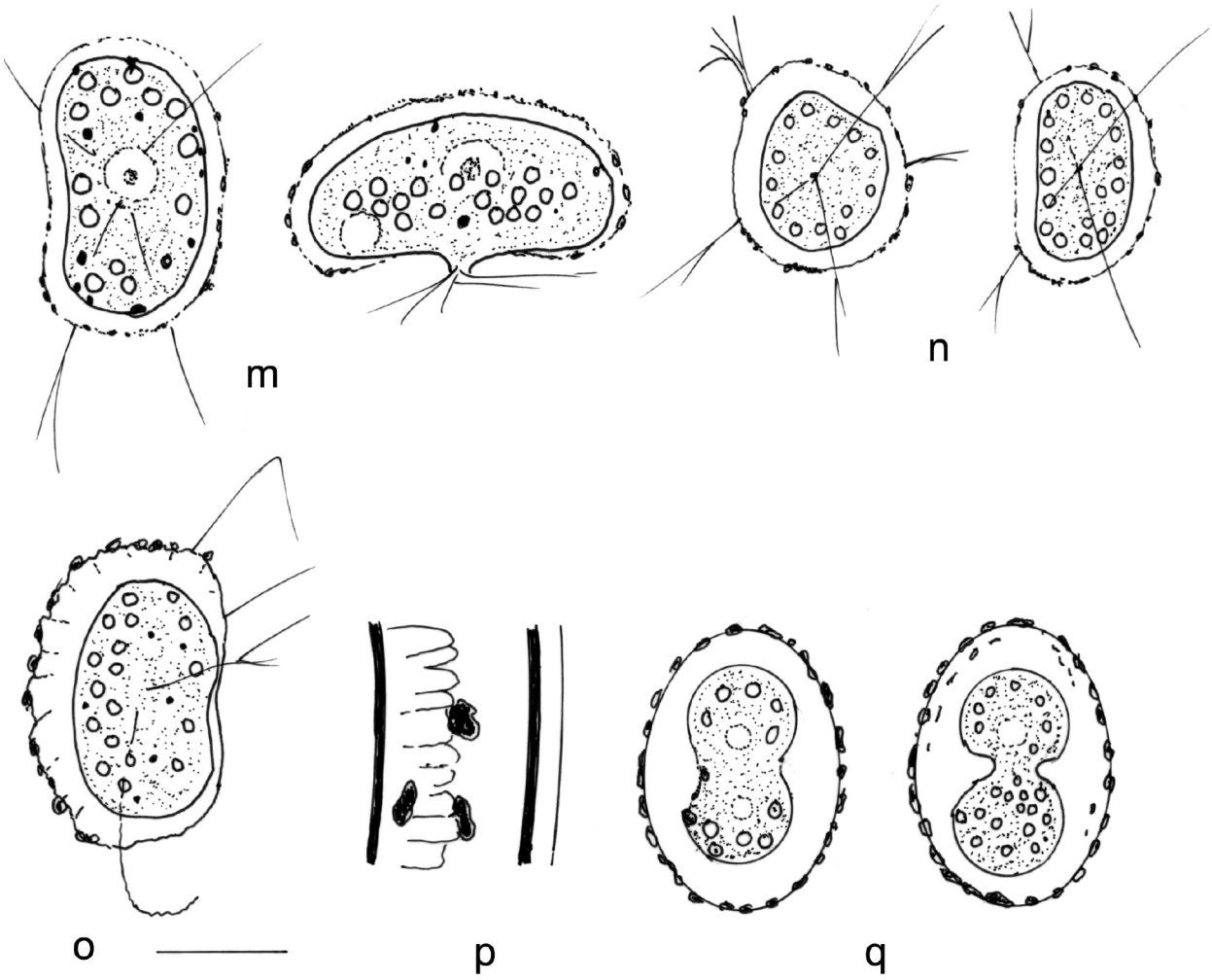

Fig. 4m–q: *Frenzelina* from Lake Stechlin. **m, n**: Middle life stages, dorsal and lateral views (note nucleus and contractile vacuole). **o**: Stage with different formation of the gelatinous layer. **p**: The gelatinous layer of an adult and young stage. **q**: Two stages of division. Scale 10 µm.

The cell envelope is flexible, the gelatinous layer is covered with xenosomes (detritus particles and sand grains). The long pseudopodial stalk of some individuals is understandable because the gelatinous layer is not narrowed basally and would thus impede extrusion of the pseudopodia (Fig. 6). In the Elbe, *Frenzelina* inhabits the thin sediment layer overlying a sandy substratum (BADEWITZ 2007).

Further observations on *Frenzelina*

Frenzelina reniformis is also found in the Ilm, a small river in Thuringia, Germany. It was absent from samples of algae and from sediment but was abundant in trap substrates of sheep wool and foamed plastic, a pore-rich material (SCHÖNBORN 1996). It also occurred on microscope slides exposed in the river.

No less interesting is the huge abundance of this species observed in a denitrification reactor near Dresden. The reactor bed contains cubes of foamed plastic,

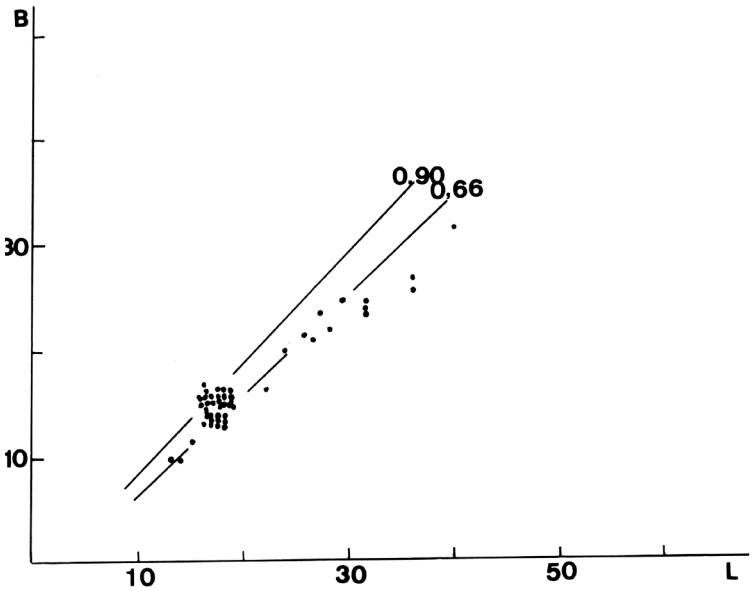

Fig. 5: Length and breadth spectrum of *Frenzelina* from Lake Stechlin. L – length, B – breadth (in µm).

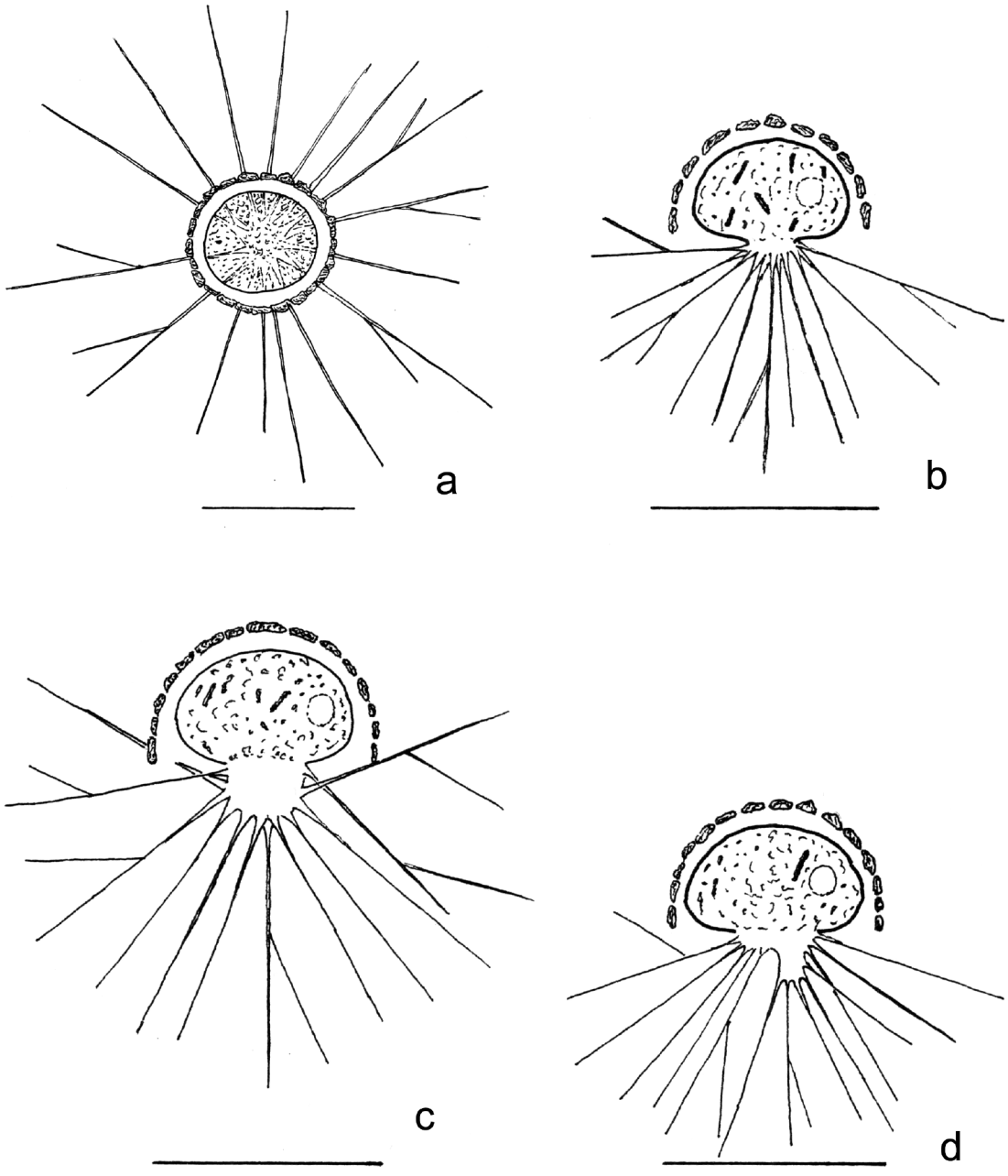

Fig. 6a–d: *Frenzelina* from the River Elbe. **a**: Dorsal view. **b–d**: Lateral views. Scale 10 µm.

the pores of which are colonised by many *F. reniformis* specimens which consume bacteria intensively. In this case the individuals examined possessed a thick gelatinous layer but no shell.

Discussion

It is difficult, as mentioned above, to consider the shell clearly drawn by PENARD (1902) a mere error of observation. However, the forms of *Frenzelina* that we collected in Lake Stechlin and the River Elbe agree completely with *F. reniformis* in their structure and in the kind of gelatinous layer. They must thus be considered as belonging to this species. This raises the supposition, therefore, that there are shelled and unshelled forms. The existence of two such forms is improbable but no evidence has been found to date that would solve the contradiction. A shell was not figured by either HOOGENRAAD (1910) or PLAYFAIR (1917) but they assumed it to be present, although very transparent.

PENARD (1902) has noted the great similarity of *F. reniformis* and the testate amoeba *Lecythium hyalinum*. This species resembles *F. reniformis* without its gelatinous layer and, in PENARD terms, without its rigid shell. The flexible envelope, aperture and the filopodia are very similar but, in contrast, the cell body is not reniform. This, however, is not a generic character in *Frenzelina* because neither of the other two species in the

genus is reniform. The resemblance of *Frenzelina* and *Lecythium* becomes closer still if, as we described here, *Frenzelina* has no shell. This suggests that the taxonomic position of *Frenzelina* might have to be changed. The relationship to *Lecythium* is still clearer when the comparison is extended to the young stages of *Frenzelina*.

There appears to be little differences in the habitat choice of the two genera. *Lecythium* occurs in cushions of filamentous algae and in sediment as well as on microscope slides and foamed plastic exposed in the water. *Frenzelina*, however, occurs particularly on planar surfaces like stones (with biofilm), and exposed slides or foamed plastic (the many pores of which provide a very large surface area). It occurs less frequently, however, in sediment or in growths of thin filamentous algae. It can occur in sediment (HOOGENRAAD & DE GROOT 1935; BADEWITZ 2007) and this may be most likely when the sediment has a coarse and flocky structure or the sediment layer is very thin. The occurrence of *Frenzelina* in sheep wool traps can be explained by the thickness of the wool filaments, which can be used as planes.

As already mentioned, the synonymisation of *F. minima* and *F. globosa* proposed by HOOGENRAAD & DE GROOT (1940) is morphologically doubtful. The space between the cell and the surface (whether shell or gelatinous layer) is very small in PLAYFAIR's specimens but amounts to half the size of the cell body in *F. minima*. PLAIFAIR's *Frenzelina* also has a slight aperture tube that is absent from *F. minima*.

It should be emphasised that young and adult stages of *Frenzelina* are morphologically different, something that is rare in Protozoa.

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