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A Redescription of *Amphizonella violacea* (Amoebozoa: Arcellinida)

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Summary. We redescribe the lobose testate amoeba *Amphizonella violacea* Greeff, 1866. We have collected this conspicuous but often overlooked species in xerophilic mosses from six localities in Germany. The overall shape is much more variable than hitherto known. Non moving cells are usually roundish or oval but can emit pseudopods, moving amoebae adopt a more longish tongue or fan-like shape sometimes non moving amoebae take a stretched form with pseudopods at opposite ends. In contrast to previously published images the outer gelatine-like mucus layer is not always present. After excystation this layer is secreted *de novo*. Its thickness is variable and can reach up to 23 µm. Normally it contains numerous rod-shaped bacterial exobionts of unknown function. In SEM the outer surface is smooth. The untextured organic shell-wall is flexible and opens in a large not well defined aperture.

Key words: *Amphizonella violacea*, Arcellinida, ecology, Microcoryciidae morphology, SEM, testate amoebae, *Zonomyxa violacea*.

Abbreviations used: DIC - differential interference contrast, PBS - phosphate buffered saline, SEM - scanning electron microscope.

INTRODUCTION

The genus *Amphizonella* was erected by Greeff (1866) with *Amphizonella violacea* as type species. In 1888 the same author corrected and amended his original description. Penard (1902, 1906) provided a detailed redescription. Beside some secondary entries in keys and textbooks (Deflandre 1953, Harnisch 1958, Schönborn 1966, Meisterfeld 2002) only Thomas (1957) has published a short note dealing essentially with the nature of the conspicuous colour of the organism.

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This species has not been studied in further detail for about one hundred years until today. The aim of our study is to correct and supplement the previous descriptions and to document different life stages of this largely overlooked species with modern methods.

MATERIALS AND METHODS

A total of about fifty samples of xerophilic mosses (epiphytic or epilitic) from roofs and brick or concrete walls in different areas of Germany were studied. *Amphizonella violacea* could be discovered in six of these locations (Table l). Positive samples came exclusively from roofs. Usually only single or few cells were found, only two of these 50 samples had high abundances x = 14.8 (7-24) specimens per cover slip 18 × 18 mm.

Prior to extraction the samples were wetted for up to twenty four hours with none carbonated mineral water Volvic until saturated. Then the samples were squeezed out or washed out. To remove coarse particles the suspension was sieved through a small meshed screen (0.5-l mm). Mainly living specimens of *Amphizonella violacea* were studied.

If the suspension contains not too much detritus, the amoebae stay alive for days or up to two weeks. Pseudopodial activity can be observed best if the slides are left undisturbed in a moist chamber for several hours or with an inverted microscope. If the evaporated water is replaced daily such preparations can be studied for several weeks.

Cells for SEM preparation were first fixed in saturated sublimate solution ($HgCl_2$) for 10 min and after washing in tap water postfixed in glutaraldehyde (6.25% in PBS) for 20 min, washed in PBS and dehydrated in an alcohol series. From absolute alcohol the cells were transferred into HMDS (Hexamethyldisilazan, Merck) (Oshel 1997). The nuclei were stained in fixed material with bromophenolblue.

REDESCRIPTION

The outer shape of *Amphizonella violacea* is largely mutable (Figs 1-4). The envelope is flexible, organic, transparent, 1 to 5 µm thick, with undulations and a wrinkled surface (Fig. 13). The envelope is usually attached to the cell but sometimes small parts are not attached.

The colour depends on the stage and can vary from almost colourless over violet to brownish yellow (during cyst formation).

The pseudostome is large but its shape is not very well defined and changing continuously with the movement of the cytoplasm. Generally it looks like an indentured and ramified slit (Figs 4, 5, 11, 12) but it can sometimes be more or less circular.

The envelope is normally covered by a hyaline gelatine-like layer which can be up to 25 µm thick (Fig. 13). In many specimens this layer contains rod like bacteria (Figs 6-8, 14). The ovular nucleus is ovoid or spherical, the nuclear membrane is easily visible, the numerous (usually more than thirty) nucleoli are mainly concentrated below the nuclear membrane (Fig. 6)

Numerous (usually more than 30) contractile vacuoles are evenly distributed and discharge in the thin space between envelope and cell surface (Figs 5, 6). In most stages the cytoplasm contains countless small (< 1 µm) violet granu1es which give the whole cell a light to dark violet tint. We have also observed specimens lacking the violet pigment. The colour of these cells appeared yellowish to light brown. Prior to cyst formation the cytoplasm becomes dark brown.

Dimensions

Greeff (1866) in his original description specifies a length of 150 µm. Later Penard (1906) in his redescription expanded the range to 125-250 µm. Our own measurements (rounded forms only, long axis exc1uding the galantine like layer) differ slight1y between different populations. The population from Magdeburg ranges from 120-239 µm with a mean length of 170 µm (n=15) while one population from Aachen is smaller: length of 95-170 µm, mean 125 µm (n=20).

Occasionally extremely large or small amoebae were observed. Stretched forms are often much longer than the average (Fig. 3). One stretched individual of 558 µm was observed as well as several small individuals (75-86 µm). The small individuals from Aachen lack the violet colour.

Motility and locomotion

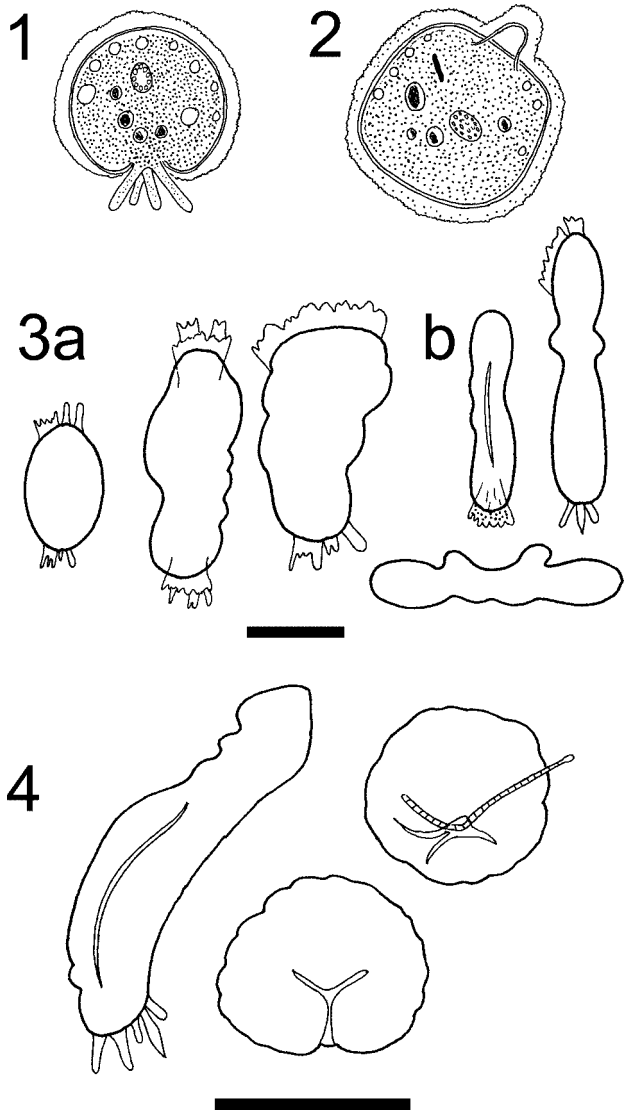
Amphizonella is able to change its shape to a large extent. Three distinct active forms are common: (i) Stationary non moving form (this is not a cyst), the cell is more or less spherical with flattened ventral sides; nevertheless this form can emit pseudopods (Figs 1, 2, 5). This is the most common form immediately after extraction from the moss. (ii) Moving form (with pseudopods). The cell is fiat, outstretched, ovoid, tongue or fanlike (Fig. 3a). (iii) Stretched form, irregularly longish, pseudopods often emitted simultaneously at both poles, without locomotion (Fig. 3b).

It takes several hours after rewetting of the moss until the moving and stretched forms can be observed.

Due to the pigment granules the movement and streaming of the cytoplasm can be easily observed. The amoebae move by lobose fan, finger or sword like pseudopods (Figs 1, 3, 4, 9) which are always emitted through a slit-like aperture. These pseudopods have a broad ectoplasmic seam. Short finger like pseudopodia can be completely ectoplasmic. The motion of *Amphizonella violacea* is extremely slow (0.65 µm s^{-1} at 20°C). Often the pseudopods of crawling amoebae are emitted under the cell and therefore difficult to observe.

Feeding

Active amoebae contain several food vacuoles. The content is often already digested but diatoms, testate amoebae e.g. *Arcella arenaria, Difflugia lucida* as well as members of the moss microflora like



Figs l-4. *Amphizonella violacea.* **1 -** lateral view (after Penard 1902); **2** - dorsal view (after Penard 1906); **3a** - stretched forms, often the pseudopodia that are emitted through the aperture are only visible at both poles of the cell; **3b** - aperture of the shell is sometimes only a long narrow slit; **4** - variability of apertural shapes. The individual on the right is ingesting a filamentous cyanobacterium. Scale bars: 200 µm.

cyanobacteria or green algae can be distinguished. The aperture of *Amphizonella violacea* is very large and flexible (Figs 4, 10-12) enabling the amoebae to ingest large food items like the lobose testate amoeba *Microcorycia flava* (diameter 125 µm). Faeces are excreted through the aperture by flat pseudopodia (Fig. 10).

Habitat

The preferred habitat of *Amphizonella violacea* are xerophilic mosses with extremely fluctuating water contents which can typically be found on roofs and with 1ower frequencies on walls, concrete surfaces or rocks. These biotopes are characterised beside the rapidly changing humidity by large temperature changes (daily and seasonally). In contrast to Bartoš (1940) and our results, Penard (1906) gives moist or even submersed mosses as preferred habitat. We do not believe that he had mistaken *Amphizonella violacea* with *Zonomyxa violacea* but it cannot be ruled out that he had taken xerophilic mosses that were only wet when the samples were taken.

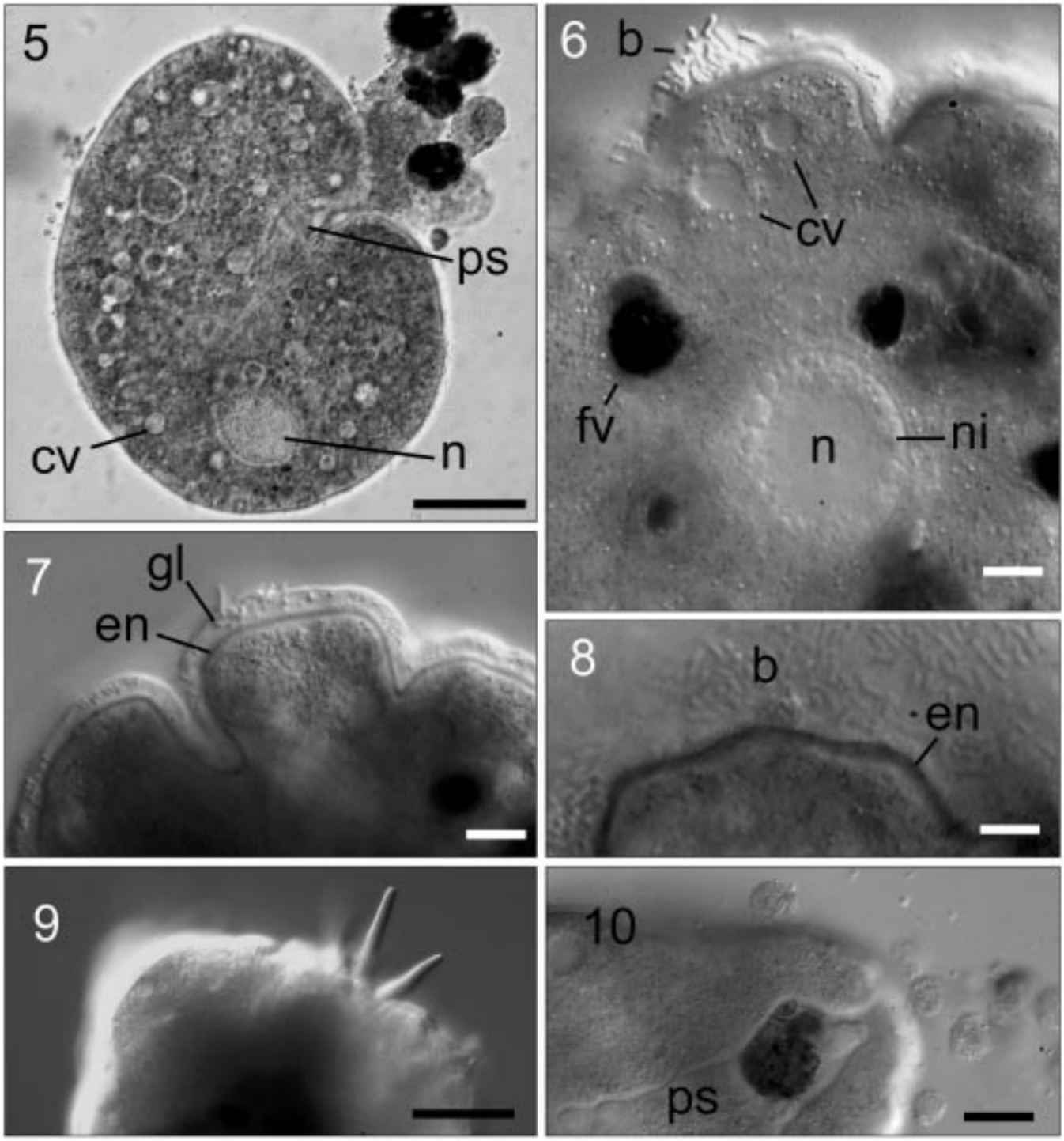
Cysts

Amphizonella violacea can survive under unfavourable environmental conditions as precysts or cysts. Precysts are formed in response to a rapid desiccation of the moss. In this case *Amphizonella* takes an irregular roundish shape, during dehydration the cell becomes dark brown and almost opaque.

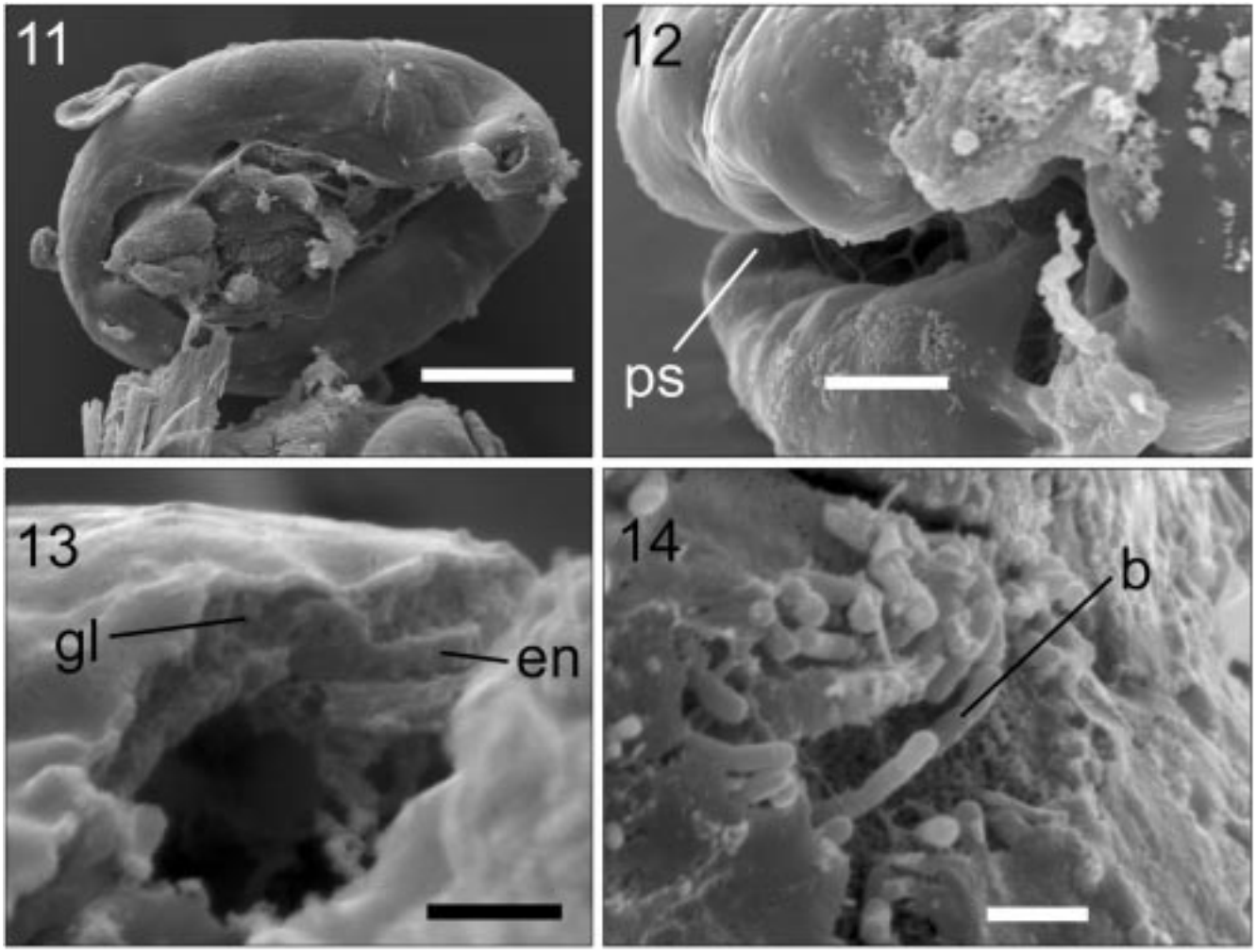
Prior to cyst formation the colour of the cytoplasm also changes from violet to brown. The cell becomes more or less spherical. In this early stage the surface of the envelope is still relatively smooth. The cytoplasm is now almost completely opaque. Details of the cell structure are hardly visible. Only peripheral vacuoles appear as pale spots. At the same time the outer hyaline layer, which has become thicker during encystment, spreads out over the surface of the substrate (slide). With further dehydration the interior of the cyst brightens partially and the spherical shape wrinkles more and more and becomes an irregular often relative flat cyst with a hyaline seam. In contrast to precysts the cysts are formed in moist or only slowly drying mosses.

Differential diagnosis

Amphizonella violacea can easily be confused with *Zonomyxa violacea* Nüsslin, 1884. *Zonomyxa violacea* can be distinguished from *Amphizonella violacea* by the following characters: A thinner test, no external mucus layer, and most significant1y numerous nuclei. According to our observations (Meisterfeld unpublished) *Zonomyxa* normally has 4 but Penard 1906 reports up to 32. These are smaller (~20 µm) than the single large nucleus of *Amphizonella violacea.* Nüsslin 1884 and Penard 1906 describe the locomotive form of *Zonomyxa violacea* as pointed at the front but broadly rounded at



Figs 5-10. *Amphizonella violacea.* **5 -** lateral view of a compressed individual during food uptake (algae). Numerous contractile vacuoles at the periphery of the cell and an ovular nucleus. The pseudostome is in this view a narrow cleft; **6** - ovular nucleus with numerous peripheral nucleoli, contractile vacuoles, food vacuoles, numerous dark pigment-granules and bright crystals; **7** - envelope of the cell with the transparent gelatine-like layer. Note the large embedded and attached bacteria; **8 -** detail of the shell surface with envelope and rod shaped bacteria in the gelatine layer. **9** - fingerlike pseudopodia (ectolobopodia); **10 -** apertural view (inverted microscope) of an excreting cell. A large pseudopodium is emitted from the apertural cleft. b - bacteria, cv - contractile vacuoles, en - envelope of the cell; fv - food vacuoles, gl - gelatine-like layer, n - nucleus, ni - peripheral nucleoli, ps - pseudostome. Brightfield (5), DIC (6-10). Scale bars: 40 µm (5); 10 µm (6, 7); 5 µm(8); 40 µm (9); 20 µm (10).



Figs 11-14. *Amphizonella violacea* SEM. **11 -** apertural view, the large pseudostome extends across the whole length of the specimen; **12 -** detail of the cleft-like shell aperture (ps). Smooth areas of the surface alternate with patches that show attached and embedded bacteria; **13 -** cut open shell surface showing the homogeneous envelope (en) and the gelatine-like layer (gl); **14 -** detail of the cell surface with embedded and attached bacteria (b). Scale bars: 50 µm (11); 10 µm (12); 2 µm (13, 14).

Table 1. New locations in Germany where *Amphizonella violacea* has been found during this study.

Location	District	State
Klaber	Güstrow	Mecklenburg- Vorpommem
Warnkenhagen OT Tellow	Güstrow	Mecklenburg-Vorpommem
Gardelegen	Altmark-Kreis Salzwedel	Sachsen-Anhalt
Loburg	Anhalt-Zerbst	Sachsen-Anhalt
Magdeburg		Sachsen-Anhalt
Aachen OT Seffent		Nordrhein-Westfalen

the posterior end. But this character is of little value because active cells can emit several finger-like pseudopodia giving the cells an outline similar to *Amphizonella violacea* (Meisterfeld unpublished). The average dimension is 150 µm but large individuals can reach up to 200 µm. Stretched, moving cells can be up

to 250 µm long (Nüsslin 1884). The habitat requirements for *Zonomyxa* are completely different: so far it has only been reported from Sphagnum or aquatic vegetation (e.g. Nüsslin 1884, Penard 1906).

DISCUSSION

Greeff (1866) describes the shape of *Amphizonella violacea* as more or less spherical. Even Penard (1902/1906) depicts *Amphizonella violacea* in his drawings as a rather spheroid rhizopod. These figures later served other authors as templates for their own illustrations (Deflandre 1953, Harnisch 1958, Schönbron 1966, Meisterfeld 2002). For this reason the published picture of *Amphizonella violacea* dos not reflect the actual range of existing life stages. Based on our observations of a large number of individuals, *Amphizonella violacea* is only spherical in the non locomotive form. Moving amoebae display a considerable variability of shapes. A so called peripheral gelatine or mucus layer was reported in several descriptions of the species although it was not mentioned in the original description (Greeff 1866). Not until his description of 1888 did Greeff explicitly describe the existence of the double envelope, a peripheral hyaline usually broad layer and underneath the actual shell. According to Greeff (1888) the outer layer is only formed by larger seemingly full-grown individuals but he gives no further information about the nature of this layer. In his redescription Penard (1902/1906) interprets it as mucus layer and illustrates *Amphizonella violacea* constantly with this layer. This led to the impression that *Amphizonella violacea* is always covered by a peripheral mucus layer which is definitely not the case. After excystation the amoeba initially do not have it, only during the course of the active phase a mucus layer is eventually formed. This layer is usually colonised by large rod like bacteria. Occasionally we have found single individuals with a thick mucus layer of 13 to 23 µm (sampling site 4, Table 1). Such a thick mucus layer is obviously only rarely formed. The conditions that induce this process are unclear.

Little is known about the nature of the violet pigment. Already Penard (1906) had shown that the pigment granules bleach and eventually become rose if the cells are kept in an acid medium. Thomas (1957) hypothesized that the colour is a result of the feeding of *Amphizonella* on *Gloeocapsa compacta* and related Cyanobacteria which were common in the samples he had studied and were frequently found in the food vacuoles. *Gloeocapsa compacta* contains gloeocapsin, a pigment that together with scytonemin is believed to be a powerful protective agent against UV-radiation (Garcia-Pichel and Castenholz 1993). These pigments are widely distributed among cyanobacteria of extreme habitats like rock surfaces. Whether the pigment of *Amphizonella* is Gloeocapsin and *Gloeocapsa* is really the source of the pigment has not been proven. In our samples these or related species were not present but filamentous cyanobacteria could be observed.

Classification

According to recent classifications (Meisterfeld 2002, Cavalier-Smith *et al*. 2004, Adl *et al*. 2005) the position of *Amphizonella* in the system is as follows:

Phylum: Amoebozoa Lühe, 1913
Class: Lobosea Carpenter, 1861
Order: Arcellinida Kent, 1880
Suborder: Arcellinina Haeckel, 1884
Family: Microcoryciidae

Below Arcellinida this classification is based on morphological characters alone. The relation to typical lobose testate amoebae like *Arcella, Difflugia* or *Nebela* remains uncertain until molecular data are available.

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