Protistology 6 (4), 284–289 (2010/11)

Protistology

Morphological studies on two rare soil amoebae *Deuteramoeba algonquinensis* and *D. mycophaga* (Gymnamoebia, Amoebidae)

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Summary

The long-time unnoticed soil amoebae *Deuteramoeba algonquinensis* (Baldock, Rogerson et Berger, 1983) Page, 1987 and *D. mycophaga* (Pussard, Alabouvette, Lemaitre et Pons, 1980) Page, 1988 were recorded in the terrestrial habitats of Malé Karpaty Mts. (Slovakia) and its morphology investigated using live observations at the light microscopical level. The polypodial and monopodial locomotive form of *D. algonquinensis* is typical with a large hyaline cap on the anterior ends of the pseudopodia, spherical nucleus possessing many distributed nucleolar particles and refractile paired inclusions in the cytoplasm. *D. mycophaga* has a broad monopodial or polypodial locomotive form with a shallow hyaline cap on the anterior end, vesicular nucleus and many elongated bipyramidal crystals making its cytoplasm dense in appearance. Additional data and details of the morphology of both species were described and illustrated.

Key words: *Deuteramoeba algonquinensis, Deuteramoeba mycophaga*, lobose amoebae, soil, Slovakia

Introduction

The family Amoebidae Ehrenberg, 1838 includes about twenty valid species of large polypodial and monopodial amoebae from which fifteen have been relatively well investigated (Page, 1991; Smirnov and Goodkov, 1997, 1998; Goodkov et al., 1999). In general, they are considered as inhabitants of fresh water with the exception of a species of the genus *Deuteramoeba* Page, 1987, which have been assumed to be true soil species (Smirnov and Brown, 2004). Only two *Deuteramoeba* species were described: *D. algonquinensis* (Baldock, Rogerson et Berger, 1983) Page, 1987 and *D. mycophaga* (Pussard, Alabouvette, Lemaitre et Pons, 1980) Page, 1988. Though well investigated, after their initial descriptions (Pussard et al., 1980; Baldock et al., 1983), several taxonomical studies (Chakraborty and Old, 1986; Page, 1986, 1988, 1991), and two very brief recent records (Mrva, 2003, 2005), no data about their morphology, habitat preference and geographical distribution was published for about twenty years. During the research on naked amoebae in various terrestrial habitats in Slovakia,

both species were found. Their morphology was studied at the light microscopical level with special reference to the locomotive forms of both species.

Material and methods

Samples of leaf-litter, soil underneath the litter and moss were collected monthly in the period of 2000-2002 at five sites in oak-hornbeam forests of Malé Karpaty Mts. (Western Slovakia). In total 243 samples were examined. The material collected was analysed with a modification of the method used for ciliates (e.g. Foissner, 1987; Aescht and Foissner, 1995; Vd'ačný and Tirjaková, 2006). Briefly: dried sampled material was saturated with distilled water and incubated for 5 days at laboratory temperature under undirected light. Amoebae were examined directly in the suspension pipetted from the saturated sample. Observations were made using a Nikon Labophot microscope with phase contrast optics. Trophozoites were identified on the basis of morphological criteria according to works with detailed descriptions of the species (Pussard et al., 1980; Baldock et al., 1983; Chakraborty and Old, 1986; Page, 1988, 1991). Detailed examination of the locomotive and floating forms, the shape of the body and its dimensions, the type and dimensions of nuclei and the presence of crystals were used for identification. For ecological characteristics and situation of localities see Zlinská et al. (2005), for information on the biodiversity of the ecosystem with a checklist of species of protists and invertebrates, see Holecová et al. (2005).

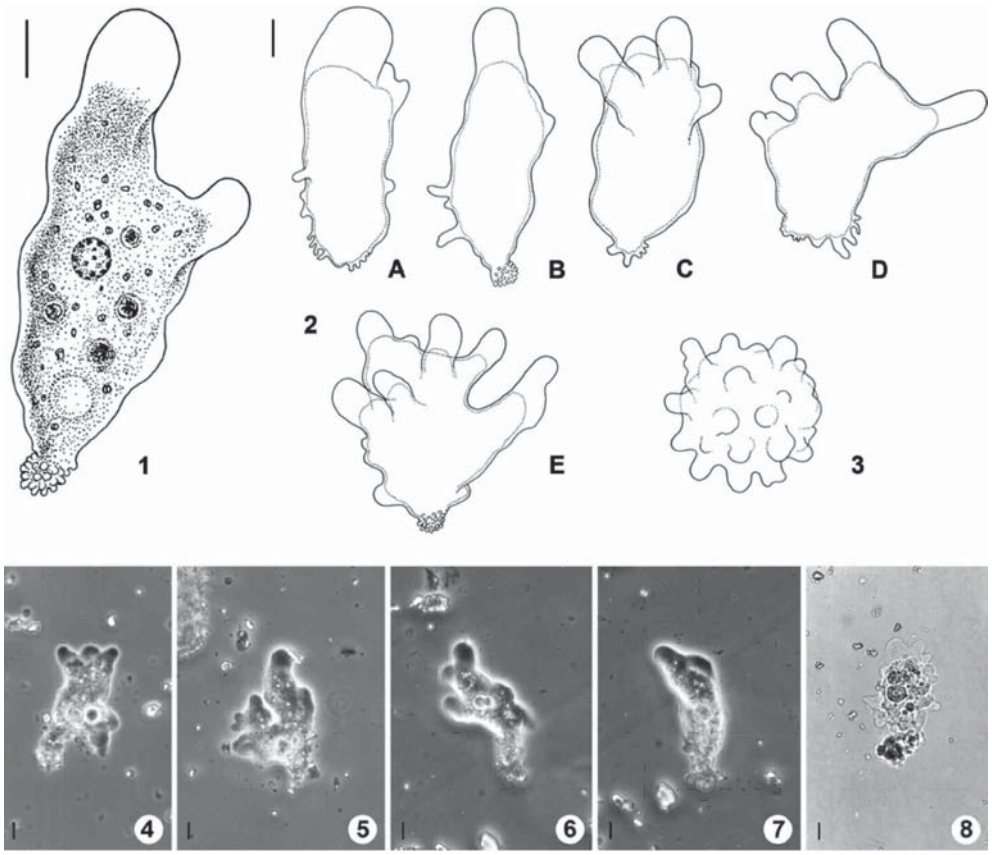
Results and discussion

Deuteramoeba algonquinensis (Baldock, Rogerson et Berger, 1983) Page, 1987 Polypodial amoeba with relatively short pseudopodia, during rapid locomotion broadly monopodial with body broadest at its anterior end (Figs 1, 2, 4-7). During the polypodial form, the leading pseudopodium always dominated. The hyaline zone on the anterior ends of the pseudopodia was large (7.5–15 μm) and crescent-shaped or hemispherical. The posterior end of the cell was narrowed and terminated with a morulate uroid or with a fasciculate uroid with short hyaline remnants of pseudopodia (Figs 1, 2). Sometimes 3–4 of them were presented near by the morulate uroid (Fig. 2, A, B). The uroidal part of the cell was occasionally covered with attached debris. The length of the locomotive form was 63–88 μm, breadth 15–25 μm, L/B 2.5–5.8. Amoebae moved by continual flow of cytoplasm. The adherence to glass surface was good. During changes of the movement direction a new pseudopodium, which later became the leading one, was formed in the anterolateral part of cell. The resting form was irregularly rounded, with many radiating short hyaline pseudopodia or protrusions (Figs 3, 8).

This species was found in the leaf-litter from Lošonec–lom quarry (17°23' E, 48°29' N) in April, 2000, in moss from Naháč–Kukovačník (17°31' E, 48°32' N) in June, 2000 and in moss from Lošonec–lom quarry in May, 2002. The species is known from freshwater in North America (Baldock et al., 1983; Page, 1991) and from leaf-litter in Slovakia (Mrva, 2005).

The observed specimens of *Deuteramoeba algonquinensis* matched well the type description in the shape of cell, shape of uroid, and typical crystalline inclusions (Baldock et al., 1983), and the differences were insignificant. The size range of the studied specimens was slightly wider than noted in the initial description (63–88 μm vs. 75–86 μm) however, the nuclear diameter fell within the known range of 7.5–11.5 μm (Page, 1991). During the locomotion two types of uroids were noted. From them, solely the morulate uroid (Figs 1; 2, B, E) was described by Baldock et al. (1983) as typical for this species. However, the present amoebae occasionally also possessed the fasciculate uroid consisting of short hyaline remnants of pseudopodia (Fig. 2, A, C-D). The observed resting form (Figs 3, 8) is morphologically similar to the floating form in the type description where it is characterized by short radiating pseudopodia. The major difference between them is in the appearance of the pseudopodia. In the resting form they were hyaline with a granular basis whereas the floating form is known as having the pseudopodia granular (Baldock et al., 1983).

The authors of the type description mentioned poor adherence of the amoeba to glass. However, the present amoebae adhered and moved on the glass without noticeable difficulties. In some cases the short hyaline remnants of the pseudopodia situated near by the uroid adhered to the glass this way making the attachment of the amoeba to the surface firmer. Similar short subpseudopodia were described recently for two hartmannellid amoebae. In the marine species *Hartmannella lobifera* they were no signs of adherence (Smirnov, 1997) however, in the freshwater species *Saccamoeba wellneri* which occasionally possessed subpseudopodia in the posterior part of the cell, the adhesive function was observed (Mrva, 2007).

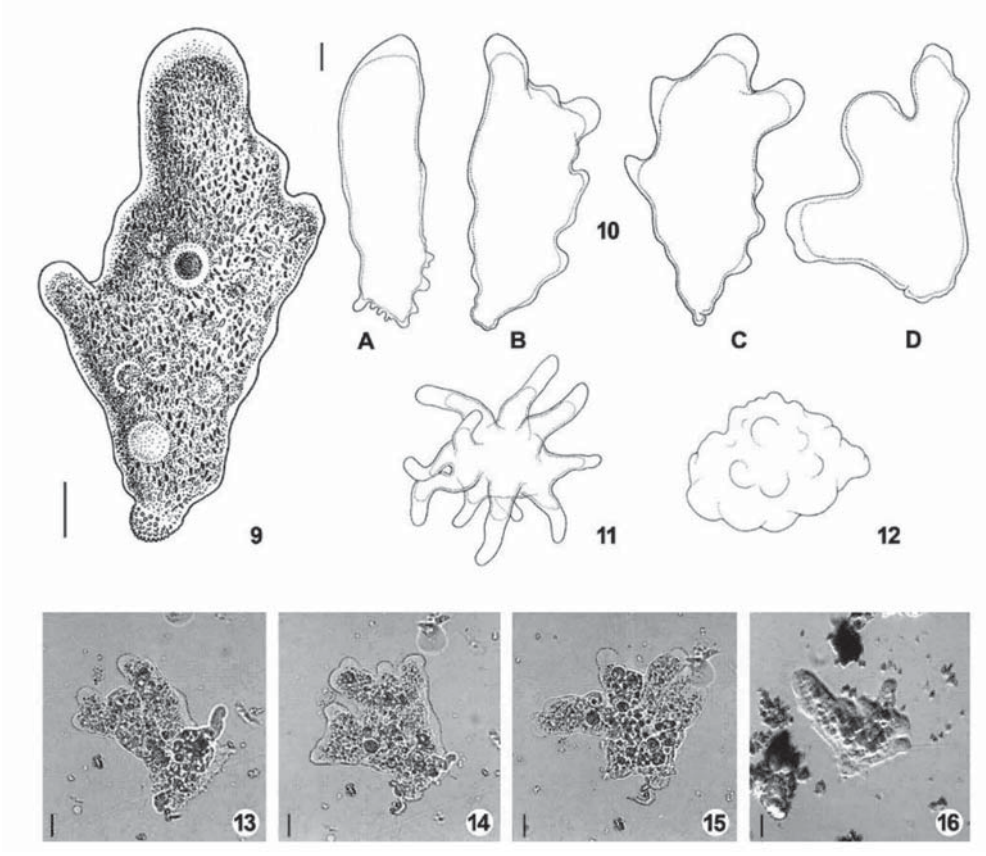


Figs 1-8. *Deuteramoeba algonquinensis* from life. 1 - Locomotive form; 2 - various locomotion stages and uroid types: A - monopodial with short remnants of pseudopodia in the posterior end, B - monopodial with morulate uroid and short remnants of pseudopodia, C, D -polypodial with fasciculate uroid, E - polypodial with morulate uroid; 3 - resting form; 4-6 - polypodial form, phase contrast; 7 - monopodial form, phase contrast; 8 - resting form, bright field. Scale bars: 10 µm.

In the cultures this amoeba was fed on fungal spores, trophozoites and cysts of *Hartmannella vermiformis* (Baldock et al., 1983) or *Chilomonas* (Page, 1986, 1991). In the cytoplasm of the presently studied specimens engulfed algae were observed. The consumption of bacteria was not observed. From this information it seems that, as in large amoebids (e.g. the genera *Amoeba* and *Chaos*) *D. algonquinensis* does not feed on bacteria and its feeding requirements include eukaryots such as small amoebae and flagellates, fungal spores and algae.

Deuteramoeba mycophaga (Pussard, Alabouvette, Lemaitre et Pons, 1980) Page, 1988 The shape of the amoeba was most frequently broadly monopodial and rarely polypodial with short pseudopodia (Figs 9-10, 13-16). The narrow crescent-shaped hyaloplasm on the anterior ends of the pseudopodia was faintly visible due to its cloudy appearance and was sometimes obliterated during locomotion. Between the hyaloplasm and endoplasm a zone of very fine granules was observed (Fig. 9). The endoplasm was divided into the anterior finer part (about one third) and the posterior roughly granular part (two thirds). The posterior end of the cell was terminated with short remnants of pseudopodia or bulbous uroid, occasionally covered with minute papillae (Figs 9; 10, A, C). The uroid was frequently not present (Fig. 10, B, D). The length of the locomotive form was 60–118 µm, breadth 30–50 µm, L/B 1.2–4. The floating form was rounded with massive radiating pseudopodia with blunt ends. The distal third of half of the pseudopodia consisted of hyaloplasm (Fig. 11). The resting form was irregular with many bulges (Fig. 12).

The nucleus was spherical, vesicular, about 10–12 µm in diameter, with a large central 7.5 µm nucleolus. A single contractile vacuole was situated in the posterior part of the cell. In the cytoplasm, many small elongated bipyramidal crystals were present. Their high quantity created a dark, densely



Figs 9-16. *Deuteramoeba mycophaga* from life. 9 - Locomotive form; 10 - various locomotion stages and uroid types: A - monopodial with short remnants of pseudopodia in the posterior end, B - polypodial without uroid, C - polypodial with small bulbous uroid, D - polypodial without uroid; 11 - floating form; 12 - resting form; 13-15 - locomotion stages of the same specimen with bright field;16 - locomotive form, side illumination. Scale bars: 10 μm.

granulated appearance of the endoplasm (Fig. 9).

This species was found in the leaf-litter of Naháč–Kukovačník (17°32' E, 48°33' N) in August, 2002 and Lindava (17°22' E, 48°22' N) in July, 2002, and in the soil underneath the litter from Lošonec–lom quarry (17°23' E, 48°29' N) in August, 2002. The species is known from the soil of France and Australia (Page, 1991) and from leaf-litter and moss in Slovakia (Mrva, 2005).

The observed characteristics (the shape, size, shallow hyaline cap) of the Slovakian populations were congruent with the original descriptions of the species. The size of the studied amoebae falls within the known range of 45–180 μm (Pussard et al., 1980; Chakraborty and Old, 1986), and also meets the average of 110 μm mentioned by Page (1988, 1991). The nuclear diameter was slightly smaller than noticed in the type description (10–12 μm vs. 12–20 μm) by Pussard et al. (1980). However, similarly small or even smaller nuclei of only 4–10 μm have been detected by Chakraborty and Old (1986).

The hyaloplasm and the endoplasm of the observed specimens were remarkably separated by a zone of very fine granules (Fig. 1). This zone had not yet been noticed. Although it was markedly similar to the zone of fine granules typical in *Dermamoeba granifera* (Page 1977) it was not permanently present and the fine granules most likely originated from the frontal endoplasmic border. The observed separation of the endoplasm in two zones is in accordance with Pussard et al. (1980). They described it as containing smaller organelles as mitochondria in the anterior third while the posterior major part contained other more voluminous organelles. Furthermore, the unusually dark, densely granulated endoplasm with high number of elongated bipyramidal crystals (Chakraborty and Old, 1986; Page, 1988) makes the appearance of the species distinctive and connected with its characteristic shape, size and nucleus type it considerably facilitates the identification of the species.

The finding of studied amoebae in the leaf-litter, soil and mosses stresses the opinion by Smirnov and Brown (2004) that species of the genus *Deuteramoeba* are true soil inhabitants. Their relatively small average size of up to, or around 100 µm enables them locomotion in tiny microhabitats of soil and leaf-litter pores. Their diet includes various eukaryotic organisms such as flagellates, small amoebae, algae, and in particular fungi (Pussard et al., 1980; Page, 1991) which are massively abundant in the terrestrial habitats rich in disintegrating or-ganic matter, also corresponds with their preference for soil habitats. Although *D. mycophaga* was isolated from soil in the type description and subsequent studies, *D. algonquinensis* was firstly isolated from fresh water and only recently also from terrestrial habitats (Mrva, 2005). The present knowledge including the literary data, prevent us from a statement on the geographical distribution of both species. Particularly it is caused by a deficit of publications on the diversity of Gymnamoebae, and also probably by difficulties in the identification of the species, which is based on the morphological criteria. However, both these species are relatively easy to distinguish. Their structures of nuclei, crystalline inclusions and considerable difference in the size of the hyaline cap are good features for identification. Their rare occurrence in the diversity studies supposes that they are perhaps not as prevalent as other soil amoebae.

Acknowledgements

The study was supported with grants VEGA 1/0124/09 and APVV 0566-07. Special thanks are due to Mária Garajová for her close support.

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