

New data on the cyst structure of *Hartmannella vermiformis* Page, 1967 (Lobosea, Gymnamoebia)

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Summary

Isolates of widely distributed amoeba species - *Hartmannella vermiformis* differs in the details of cyst structure. This is unusual for gymnamoebae and is important in course of modern approaches to amoebae systematic. Cysts of the isolate of this species, described here, have regular separation of the cyst wall into endocyst and ectocyst, in contrast with the "original" isolates, described by F.C. Page. Fine structure of *H. vermiformis* cysts is studied and discussed.

Key words: amoebae, Lobosea, Gymnamoebia, *Hartmannella*, cyst, ultrastructure

Introduction

Hartmannella vermiformis was described by Page (1967), and re-investigated twice (Page, 1974, 1985). Trophozoites of *H. vermiformis* are very characteristic, and identification of this species does not seem to be a problem. However, cyst structure in this species varies from strain to strain. Cyst are double-walled, but ectocyst and endocyst may be so closely apposed in some strains, that the cyst seems to be single-walled at LM. Other known strains have cyst with outer wall somewhat separated in minority of cells (Page, 1988, 1991).

Cyst structure is considered to be important and stable systematic feature among gymnamoebae, and its considerable intraspecific variations are rare (op. cit.). Thus, more interesting is the case of varying cyst structure in *H. vermiformis*. Cysts of this species with closely apposed or partially separated walls were illustrated with LM and EM by Page (1985), but cysts with completely separated walls were never studied, and no detailed description of the cyst ultrastructure exists for *H. vermiformis*. This brief paper presents data on a new *H. vermiformis* strain with clearly separated cyst walls.

Material and methods

Hartmannella vermiformis was isolated in September, 1994 from the sediments in the water reservoir of a

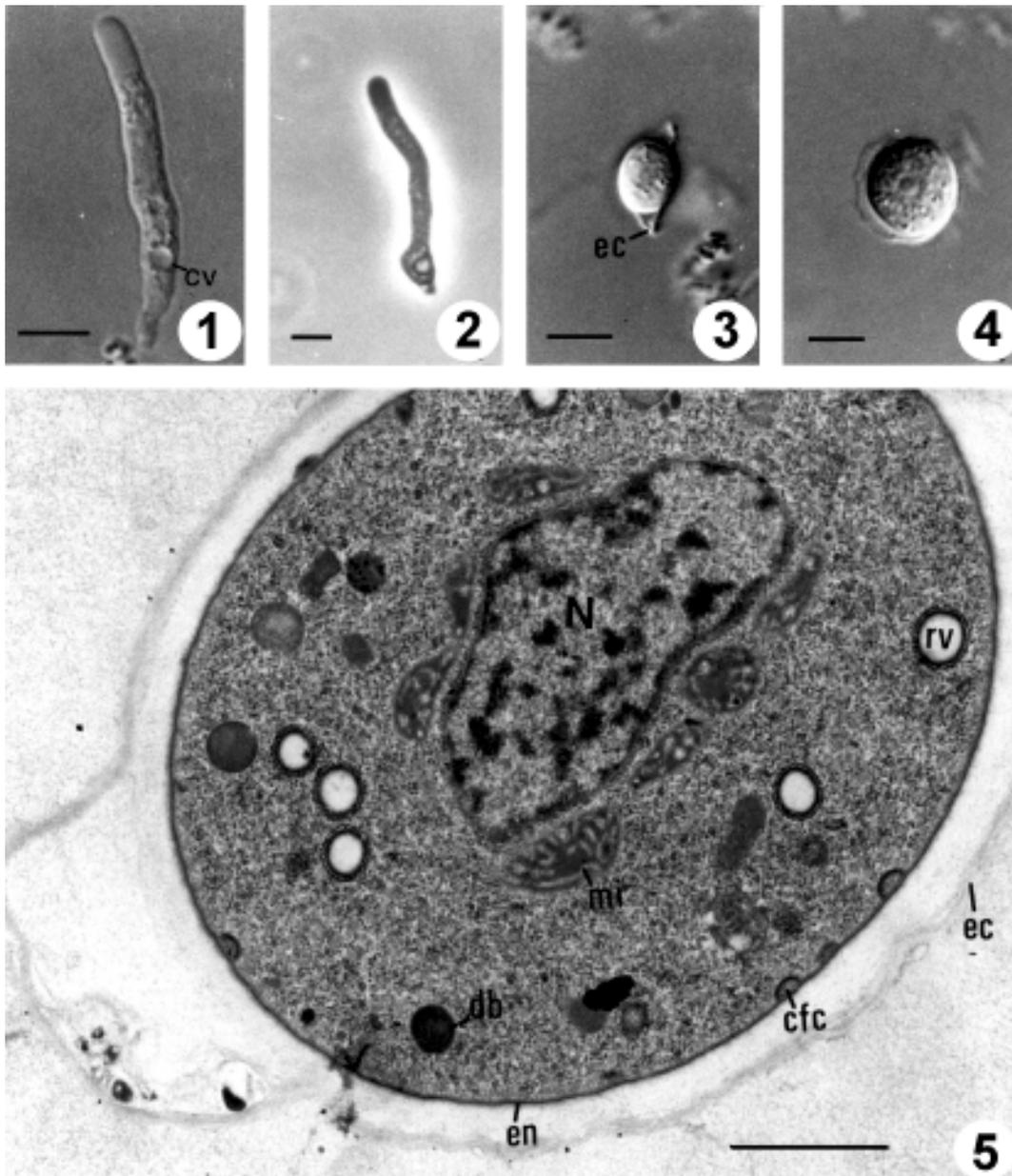
portable water-treatment plant near Bonn (Germany). Clonal cultures were maintained on NN-agar plates (Page, 1988) provided with *Enterobacter cloacae* as nutrient bacteria. All measurements and photographs were made from alive amoebae moving on glass surface.

For TEM amoebae were fixed for 1h in 3% glutaraldehyde with cacodylate buffer (pH 7.2), washed twice in the same buffer, postfixed for 1h in 1% osmium tetroxide, dehydrated in ethanol series, and embedded in Spurr resin. Sections were stained with uranyl acetate and Reynolds' lead citrate and examined with Zeiss EM 109 electron microscope.

Results and discussion

LM characteristics of the trophozoites of our isolate (Figs 1–2) and its ultrastructure were typical for *Hartmannella vermiformis* and correspond to Page's (1967, 1985) descriptions. Length of the locomotive amoebae of our isolate was 24 – 30 µm, breadth 2 – 4 µm.

Cysts of this isolate (Figs. 3–6) were 6–8 µm in diameter, double-walled, usually with completely separated cyst walls. EM (Figs. 5–6) indicated that the inner wall (endocyst) was amorphous, electron dense, about 50 nm in thickness. It was never seen to be separated from the plasma membrane, and was so closely apposed to it that looked to be integrated with the glycocalyx. In some sections dense, cup-like structures which we termed



Figs 1–5. *Hartmannella vermiformis*. **1.** Trophozoite (differential interference contrast). **2.** The same (phase contrast). **3–4.** Cysts, separation of the ectocyst is visible (differential interference contrast). **5.** TEM of portion of the cyst. EC – ectocyst, EN – endocyst, CFS – “cyst-forming caps”, N – nucleus, MI – mitochondria, DB – dense bodies. Scale bars: 1–4 – 10 μm , 5 – 1 μm .

“cyst-forming caps” (Fig. 5) were visible. Probably, they represent the last stage of the fusion of cyst wall-forming vesicles with the cell membrane. The outer wall (ectocyst) was separated with a distinct space from the inner wall. It was less electron dense, sometimes wrinkled, consisted of multilayered filamentous material and was 110–140 nm in thickness. Among other interesting details we should note RER-covered vesicles (Figs. 5–6) and dense bodies (Figs. 5–6) with finely granulated content, which were seen in all cysts. Both these inclusions were not found in trophozoites. The nucleus of encysted cells had dense, homogenous central nucleolus and numerous patches of

dense granular material, distributed in the caryoplasm, mostly below the nuclear envelope. An external nuclear membrane was covered with the ribosomes. The last was not seen in the trophozoites, and seems to be the specificity of the cyst.

All light-microscopical features of trophozoites, as well as its ultrastructure (not illustrated here, because is equal to that published by Page, 1985) corresponds to the description of *Hartmannella vermiformis* (Page, 1967, 1974, 1985, 1988, 1991). Cyst structure is also comparable in many characteristic details, particularly in the presence of RER-covered vesicles, which are visible (but

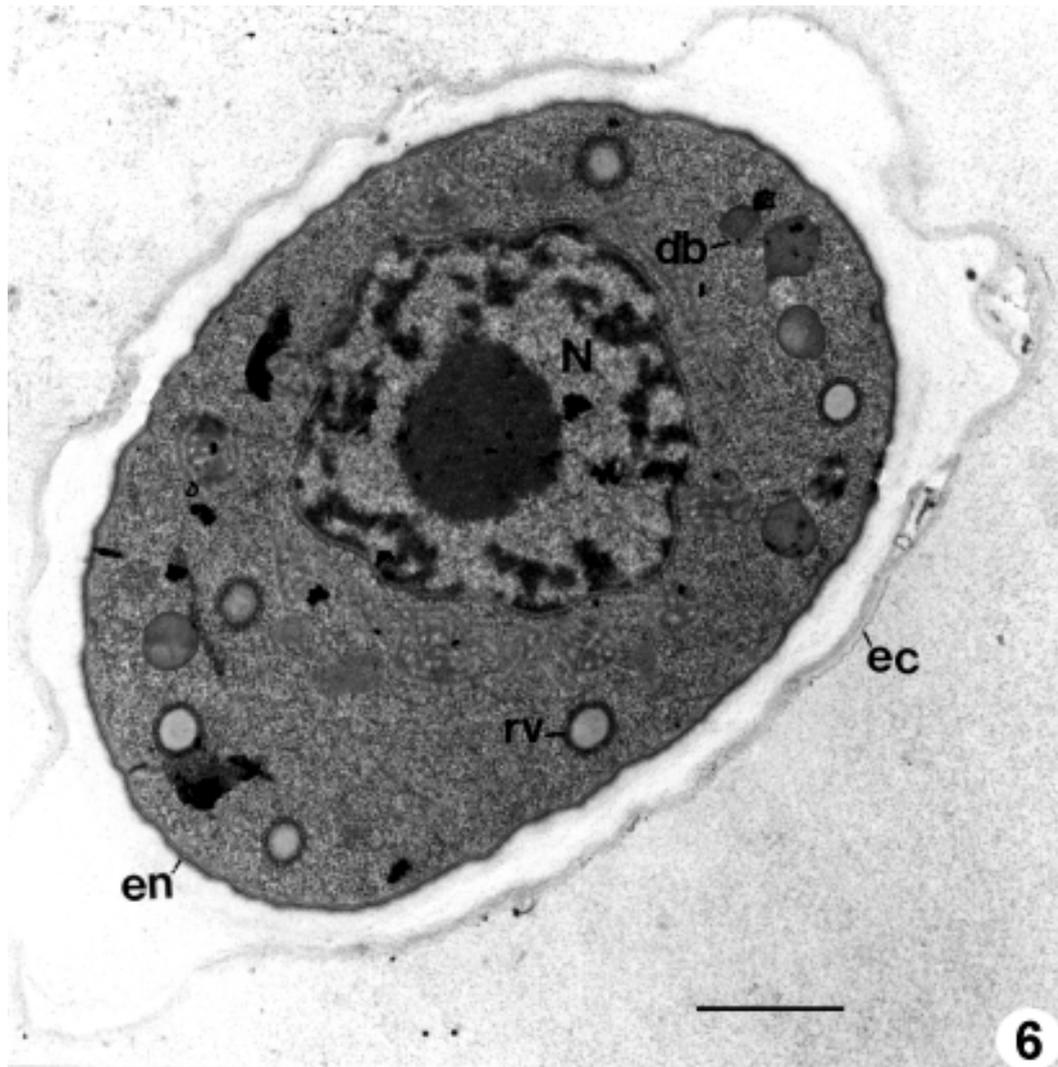


Fig.6. Total section of the cyst of *Hartmannella vermiformis*. RV – RER-covered vesicles, other abbreviation as at figs 1–5. Scale bar 1 μ m.

not noted) in Page (1985, fig. 46). The only difference of our strain from *H. vermiformis* Page 1967 is a regular, distinct space between cyst walls. But despite the last mentioned difference we concluded that the present strain belongs to the species *H. vermiformis* Page, 1967.

Taking into account the data by Page (1967, 1974, 1985) and our results we can conclude, that *H. vermiformis* may have different cyst appearance, depending on the strain. It vary from cysts with closely apposed walls (op. cit.), which on LM level may seem to be single-walled to the cysts with distinct separation of the cyst walls, as described here. These cysts are clearly double-walled when observed with LM. Intermediate variants, with partial separation of the cyst walls, is also known (Page, 1985). In all cases, both cyst wall, different in structure as described here, are present, and truly single-walled cyst are not known for this species. It seems, that the separation of cyst walls does not depend on the age of cysts, and is a strain-spe-

cific property. In our cultures double-walled cysts were seen from the very beginning of the encystment.

In terms of the modern microsystematics of gymnamoebae, cyst structure considered to be very important and stable characteristic. Most species of *Gymnamoebia* seems not to have considerable strain-specific variations of cysts, with the exception for *Acanthamoeba* (see Pussard & Pons, 1977). Thus, sufficient polymorphism shown by species like *H. vermiformis* may result in misidentification of strains. This indicates once more the importance of ultrastructure in amoebae identification. In this relation, researches perhaps should pay special attention to the deviation of single morphological characters not only in newly described, but also in well-known species.

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References

- Page F. C. 1967. Taxonomic criteria for limax amoebae, with descriptions of 3 new species of *Hartmannella* and 3 of *Vahlkampfia*. *J. Protozool.* 14, 499–521.
- Page F. C. 1974. A further study of taxonomic criteria for limax amoebae, with description of new species and a key to genera. *Arch. Protistenk.* 116, 149–184.
- Page F. C. 1986. The limax amoebae: comparative fine structure of the Hartmannellidae (Lobosea) and further comparisons with the Vahlkampfiidae (Heterolobosea). *Protistologica.* 21, 361–383.
- Page F. C. 1988. A new key to freshwater and soil Gymnamoebae. *Freshwater Biological Assoc., Ambleside.*
- Page F. C. 1991. Nackte Rhizopoda. *Protozoenfauna*, band 2. Gustav Fisher Verlag, Stuttgart-New, York., 1–170.
- Pussard M. and Pons R. 1977. Morphologie de la paroi kystique et taxonomie du genre *Acanthamoeba* (Protozoa, Amoebida). *Protistologica.* 13, 557–598.

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