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SHORT COMMUNICATION

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Natural infection with microsporidian organisms (KW19) in *Vannella* spp. (Gymnamoebia) isolated from a domestic tap-water supply

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Free-living amoebae (FLA) are distributed ubiquitously in aquatic and humid habitats. In addition to the role of a few species as causative agents of meningoencephalitis and keratitis, FLA are frequently considered to serve as natural hosts and vehicles of different bacteria – especially those that cause human illness. These intracellularly growing organisms are thus protected against chlorine – favoring their distribution within potable water systems and sanitary facilities. This important observation was first mentioned by Rowbotham (1986) for legionellae and was shown by in vitro investigations for various bacteria, finally sustained by recent findings of *Burgholderia pickettii* (Michel et al. 1997), *Chlamydia*-like organisms (Amann et al. 1997), *Pseudomonas aeruginosa* (Michel et al. 1995a), and *Ehrlichia*-like organisms (Michel et al. 1995b) multiplying as endocytobiotic organisms within amoebae isolated from natural habitats. Recently we isolated an obviously eukaryotic microorganism showing intracellular multiplication within *Vannella cirrifera* amoebae isolated from samples obtained from a domestic potable warm-water system.

For proof of the prevalence of FLA, 100-ml water samples were filtered through membrane filters (0.45 μm, nitrocellulose). The filters were placed upside down onto NN agar plates according to Page (1976) and then incubated at 30 °C. The best growth rates of the parasite and host were observed in cocultures with *V. miroides* on NN agar supplemented with 1% nonspecific ocean salt at 30 °C. Growth of the amoebae was investigated daily by light microscopy. For electron microscopy examinations, cultures of 5–8 days were prepared according to techniques described previously (Michel et al. 1995b).

An infection with intracellular microorganisms could be detected within 2 days of incubation in *V. cirrifera* trophozoites. The culture of host amoebae and their parasites appeared to be problematic because of a rapid growth of parasites (strain KW19) within their hosts, leading to decay of the amoebic culture. On evaluation of the host range among FLA, only one strain of *V. miroides* appeared sensitive for an infection with the parasite, showing the same characteristics as the wild-type strain, the exception being that the growth of the new host amoebae and their parasites stayed in a better balance, thus enabling continuous culture.

Phase-contrast microscopy revealed early stages of parasites within the caryoplasm of the host amoeba (**Fig**. 1). The fast-growing parasite expanded the nuclear membrane, finally occupying the whole cell after the nuclear membrane had torn. Within a period of 3–5 days the infection led to decay of the amoeba, resulting in the release of immobile coccoid stages of the parasite measuring 0.8–1.2 μm in diameter into the environment.

Electron microscopy examinations showed two different stages of the parasite in the amoebae: (1) the coccoid stage within the cytoplasm of the host cell and (2) the growing polymorphic stages within the caryoplasm (**Fig**. 2). The polymorphous stages in the nucleus grow up to a diameter of 6.5 μm, with intracellular vacuoles and compartments being surrounded by double membranes (**Fig**. 3). At the end of the developmental period the parasite differentiates into numerous coccoid forms, finally occupying the whole host cell. These coccoid stages are provided with a thick wall of low electron density.

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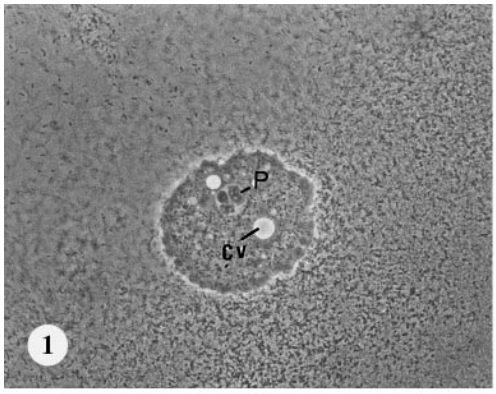

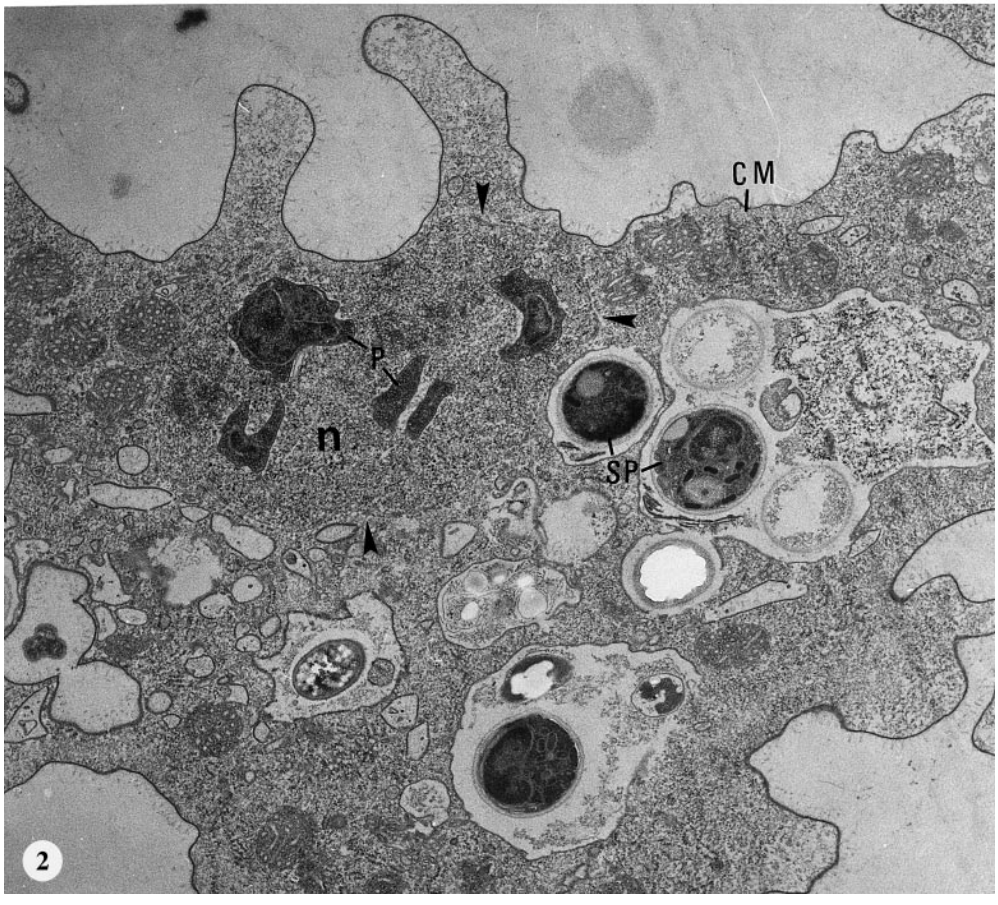

Fig. 1 Trophozoite of *Vannella* sp. Early stage of infection. Two intranuclear parasites (*P*) can be distinguished within the caryoplasm adjacent to the endosome. ×1760 **Fig. 2** *Vannella* sp. with different stages of parasite KW19: within the nucleus (*n*) – located beneath a food cup – several sections of one or more sporoblasts can be observed. Within some of them the nucleus has differentiated. Spores (*S*) are visible within vacuoles with loose-fitting membranes. A real nucleus and a vacuole are consistant features of these infectious agents. The cytoplasm of the host amoeba is *limited* by a cellular membrane (*CM*) bearing the glycostyli characteristic of the genus *Vannella*. ×14 400

They possess a nucleus, a vacuole, and a tubular structure resembling polar filaments (**Fig**. 4).

Because of the morphological similarity of these coccoid stages to spores of microsporidian organisms, we suppose this amoebal parasite to be related to *Microsporidia*. In the case of confirmation of the microsporidian nature of this unique amoebal parasite by further investigations, we wish to designate the scientific name *Amoebosporidium minutum* for this organism.

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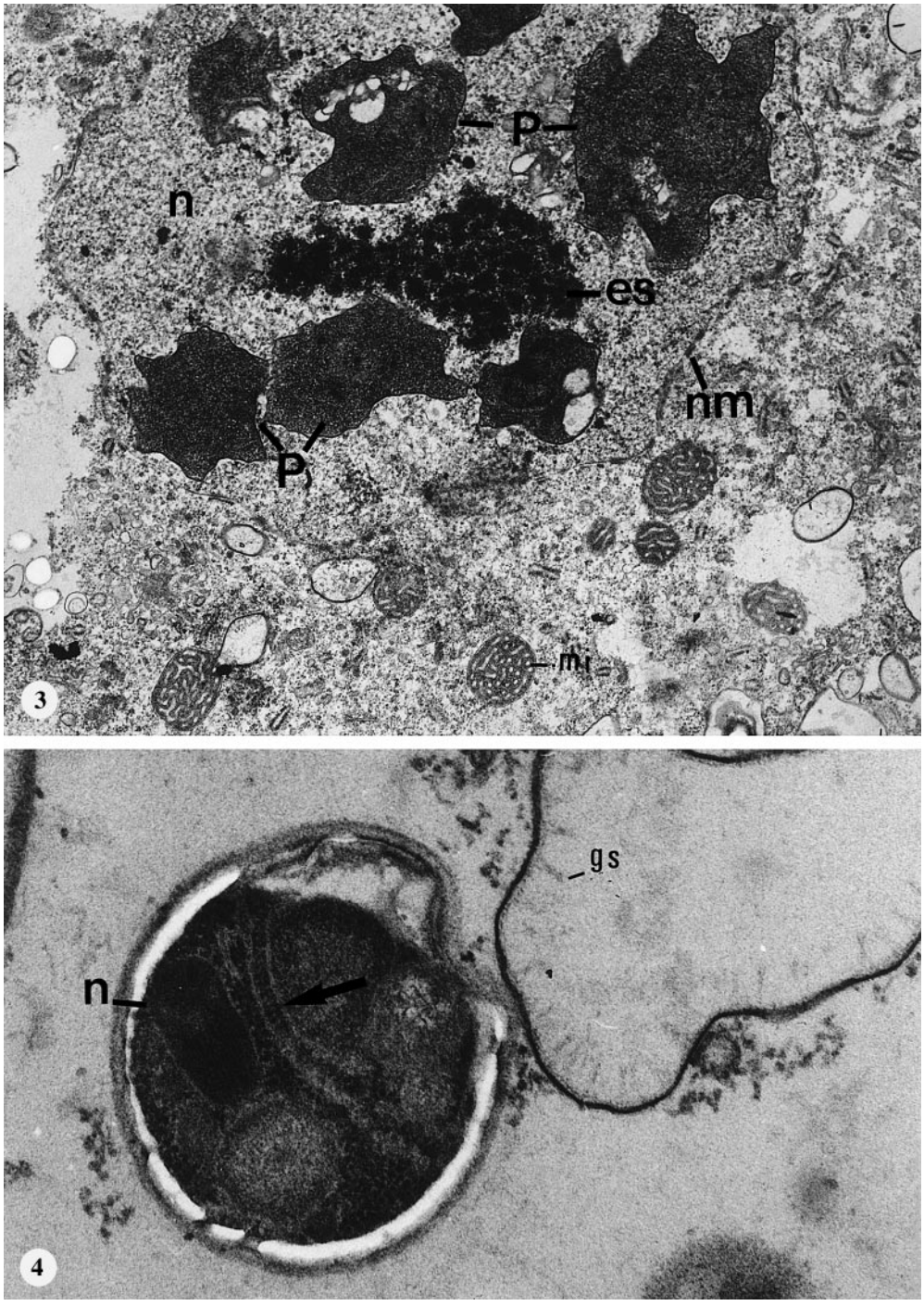

Fig. 3 *Vannella* sp.: nucleus (*n*) with six parasitic stages (*P*), all but one not leaving differentiated to nucleus-bearing stages. The endosome (*es*) shows signs of disintegration (*nm* Nuclear membrane, *mi* mitochondria) ×12 400 **Fig. 4** A single spore within the host amoeba *Vannella* sp. surrounded by a thick wall of low electron density. The nucleus (*n*) and a tubular structure resembling polar filaments of microsporidia can clearly be distinguished. On the *right side* of the photograph an invagination of the host cell membrane can be seen decorated by the glycostyli (*gs*) characteristic of *Vannella* sp. ×44 200

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