BALTIC COASTAL ZONE
Journal of Ecology and Protection of the Coastline

Vol. 20 pp. 55-61 2016

ISSN 1643-0115

Original research paper

Received: 19/05/2016
Accepted: 16/08/2016

AN INTRACELLULAR PROKARYOTIC MICROORGANISM ASSOCIATED WITH MICROSPORIDIOSIS OF INVASIVE AMPHIPODS *PONTOGAMMARUS ROBUSTOIDES*

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Abstract

The microsporidium *Nosema pontogammari* Ovcharenko and Kurandina, 1987 was detected in the muscles of *Pontogammarus robustoides* collected in the stream of South Baltic Coastal Zone (sample site N 54°16', E 19°25')
The parasite infects the cytoplasm of the muscle cells. The presence of remarkable intracytoplasmic vacuoles was noted inside of some parasite cells. Transmission electron microscopy showed that these intracytoplasmic vacuoles contained numerous microorganisms identified as rickettsia-like prokaryotic cells. Groups of pleiomorphic in shape bacilliform and rounded cells were enveloped in electron-dense cover 20-30 nm wide. The prokaryotic cells wall was constructed out of triple layered coat of different electron density. The cytoplasm of infected cells was degenerated with weakly developed endoplasmic reticulum and undeveloped organelles. The obtained data demonstrate pathogenic nature of host-parasite relationship between endosymbiotic rickettsia-like organism and microsporidia.

Key words: invasive amphipods, Microsporidia, prokaryotic endosymbionts

INTRODUCTION

The microsporidium *Nosema pontogammari* was firstly described in the Dnieper River based on light microscopic data (Ovcharenko and Kurandina 1987). A parasite was registered in the muscles of amphipods *Obesogammarus crassus* (Sars, 1894). Later *N. pontogammari* was detected in *Obesogammarus obesus* (Sars, 1894) and *Pontogammarus robustoides* (Sars, 1894) inhabiting the Dnieper Estuary and Ukrainian

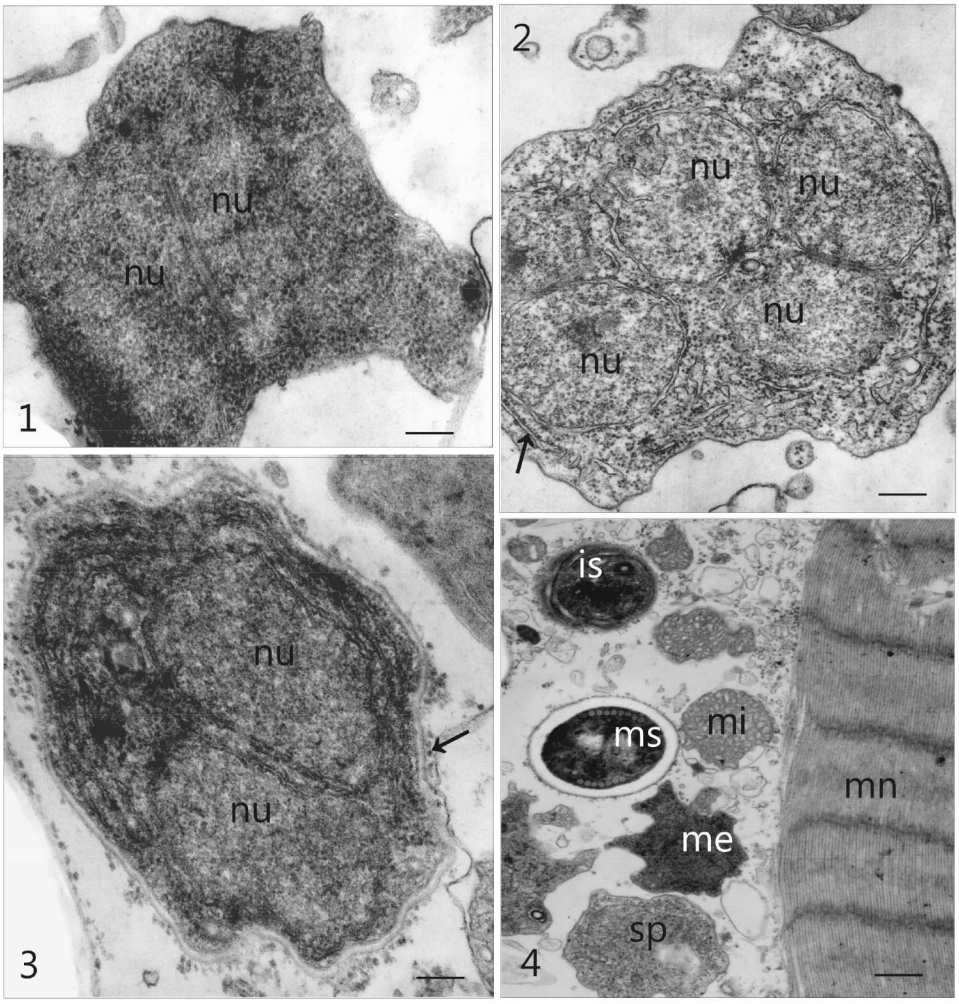
part of the Danube Delta (Ovcharenko 1994). In October 2005, infected specimen of *P. robustoides* was collected in the stream of South Baltic Coastal Zone (sample site N 54°16', E 19°25'), Poland and the electron microscopy preparations were done according to Ovcharenko et al. (2007, 2009). Ultrastructural study of infected specimen confirms the noticeable similarity between the analyzed species and Microsporidia belonging to the genus *Nosema* parasitizing Amphipoda. The shape and dimensions of the spores were similar to those of *N. pontogammari*as originally described (Ovcharenko and Kurandina 1987). In cytoplasm of vegetative developmental stages, some hyperparasitic prokaryotic organisms were noticed (Ovcharenko et al. 2007). The aim of the present paper is the morphological characterization of these intracellular pathogens. Particularly, the ultrastructure of the intracytoplasmic vacuoles, parasite cells and some host parasite relationships between endosymbiotic rickettsia-like organisms and microsporidians are explored.

MATERIAL AND METHODS

The study was carried out on original material, collected in the collected in the stream of South Baltic Coastal Zone, Vistula Basin, Poland (sample site N 54°16', E 19°25') in 2005. Amphipods were caught using a standard kick sampling procedure. Infected Ponto-Caspian gammarids *P. robustoides* were sampled in the littoral zone.
Fresh preparations of infected tissues and stained smears were studied. Smears were air dried, fixed in methanol and stained with Giemsa stain solution. Live and stained spores were observed and measured under an Olympus BX50F4 microscope. For measurements, the software „Analysis Pro 2.11" in combination with Olympus BX50F4 microscope was used.
For transmission electron microscopy (TEM), samples of were fixed with a 2.5% (v/v) glutaraldehyde in a 0.2 M sodium cacodylate buffer (pH 7.4) for 3-7 days. After washing and postfixation in 2.0% (w/v) osmium tetroxide in the same buffer for 1 h at 4°C, the pieces were dehydrated in a graded series of ethanol and acetone and embedded in Epon-Araldite solution using a standard procedure (Vávra and Maddox 1976). TEM blocks were sectioned with an LKB III ultra-microtome. Ultrathin sections were then mounted on copper grids. After contrasting with uranyl acetate (10 min.) and lead citrate (15 min.), the sections were examined in a JEOL 1010 electron microscope at 75-80 kV.

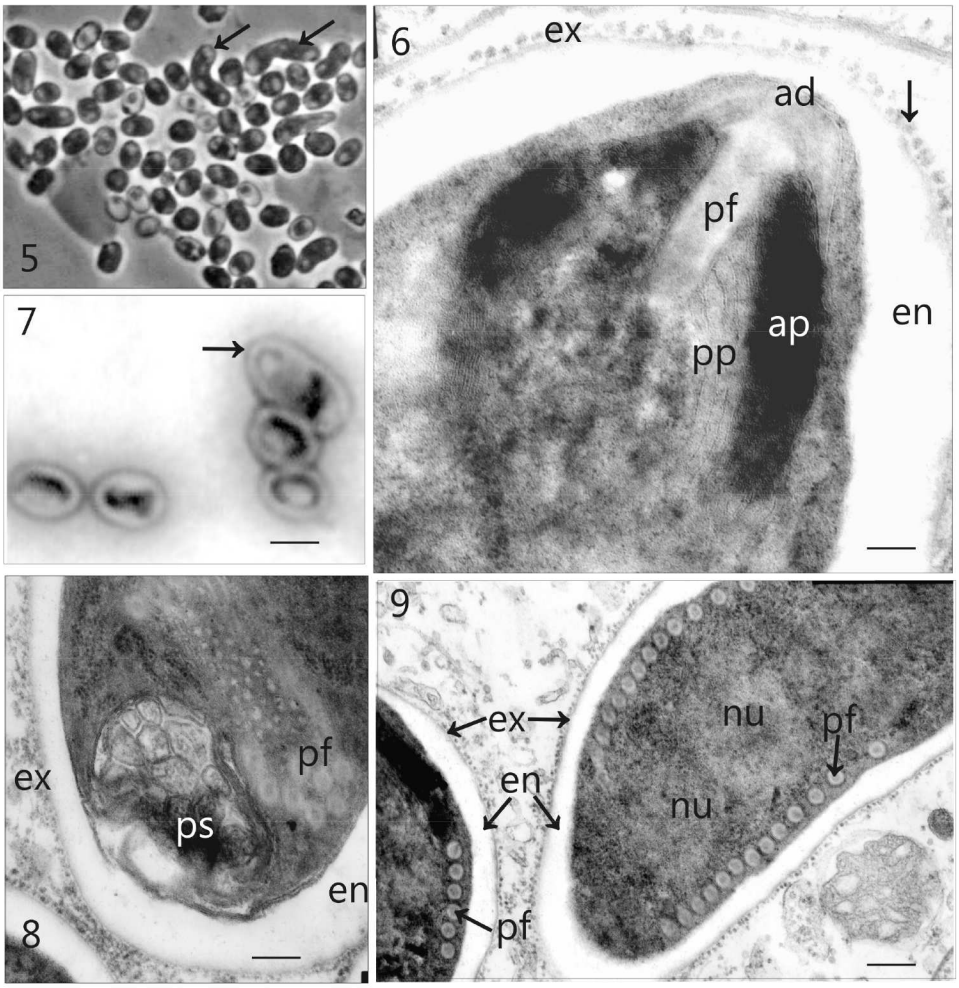
RESULTS AND DISCUSSION

Preliminary analysis allows to relate the obtained material with *Nosema pontogammari* Ovcharenko and Kurandina, 1987. The parasite infects the cytoplasm of the muscle cells (Fig. 4). The earliest observed stages were rounded merozoites approximately 2.0 × 3.2 μm in size (Fig. 1). They possessed two nuclei (nu) in diplokaryotic arrangement. The cytoplasm of the early merozoite was homogenously granular. Bi- and tetranucleate merogonial stages were distributed freely within the host cell cytoplasm (Figs 1, 2). Merozoites and early sporonts were multiplicated



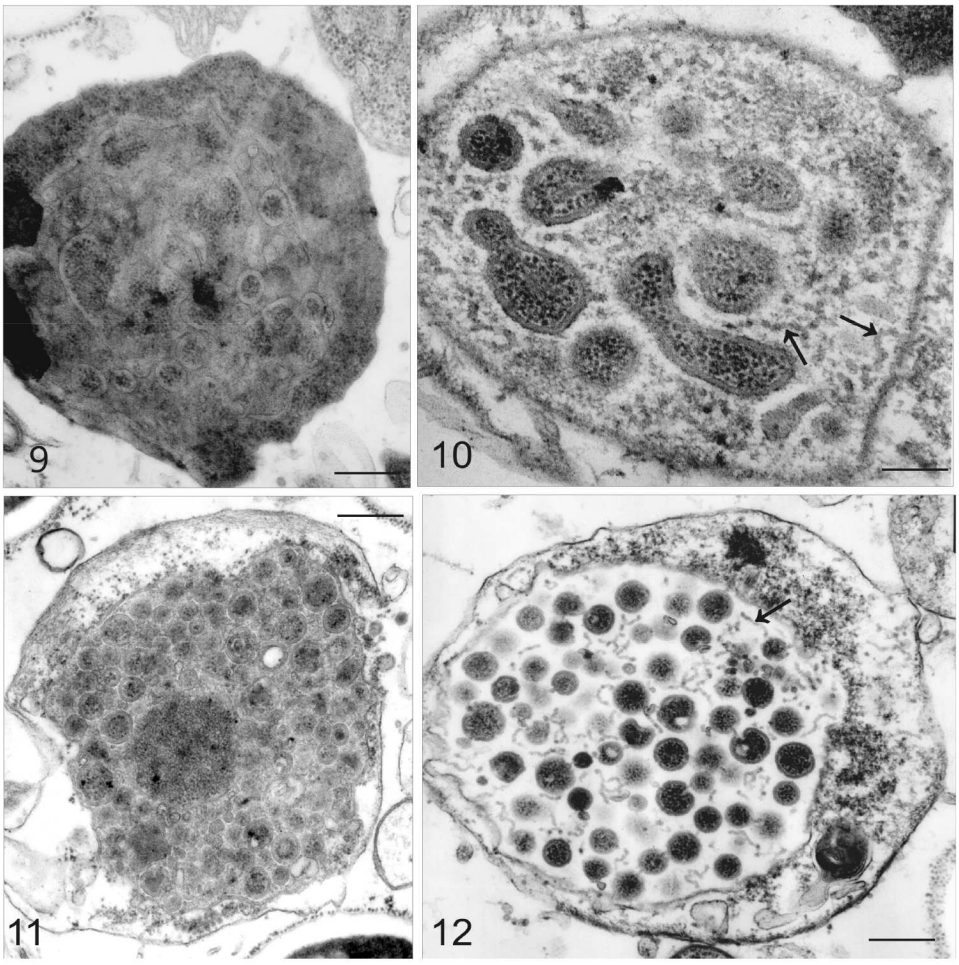
Figs 1-4. TEM micrographs of developmental stages of *Nosema pontogammari*. 1. Merozoite with dyplokaryotic nuclei (nu), and cytoplasm, filled with ribosomes. 2. Tetranucleate plasmodium. A few cisternae of rough cytoplasmic reticulum are displayed (arrows). 3. Sporont with two nuclei (nu) and numerous cisternae of endoplasmic reticulum surrounding the nuclei. Granular ornamentation surrounds developing exospores of future spore wall (arrowed). 4. Diplokaryotic merozoite (me), sporont (sp), immature and mature spores (is, ms) are located in sarcoplasm. Spores and developmental stages neighboring with host cell mitochondria (mi) and mionemes (mn) are visible. Bars: 100nm (1-3); 300 nm (4)

by binary division of diplokaryotic cells. Pairs of diplokaryotic nuclei (n) have separated prior to division of the cytoplasm (Fig. 2). During the development of the sporonts numerous rows of rough endoplasmic reticulum surrounding diplokaryon were formed (Fig. 3). When the sporoblasts matured, differentiation of the spore organelles could be observed. The shape of the future spore became more regular, and the cytoplasm grew denser (Fig. 4). Mature spores were binucleate (Figs 4, 9).



Figs 5-9. Morphology and ultrastructure of the spores. 5. Live spores. Teratospore (arrows) and ovoid spores are displayed. 6. Oblique section of anterior part of mature spore. Manubroid part of polar filament (pf), anchoring disk (ad), anterior and posterior polaroplast regions (ap, pp), endospore and exospore with granular ornamentation (arrows) are showed. 7. Giemza-stained spores. Macrospore is arrowed. 8. Posterior fragment of immature spore. The spore wall consists of plasmalemma, structureless endospore (en) and not stratified exospore (ex). Golgi derived posterosome (ps) and developing polar filament coils (pf) are presented. 9. Fragments of posterior parts of two mature spores. Paired nuclei (nu) polar filament (pf) with six and thirteen coils and components of the spore wall (ex, en) are visible. Bars: 10 μm (5); 15 nm (6); 2.5 μm (7); 30 nm (8); 100 nm (9)

Thickness of the spore increases during spore maturation (Fig. 4). The live spores were ovoid, slightly irregular with equally rounded ends 3.54 ± 0.78 (2.71 – 6.34) × 2.26 ± 0.18 (1.99 – 2.64) μm in size (N = 50) (Fig. 5). Macrospores and teratospores were often recorded on the slides (Fig. 5). Giemsa stained spores were ovoid, different in size (Fig. 7). The spore wall exhibited the three classic layers: an internal



Figs. 9-12. Prokaryotic endosymbionts in cytoplasm of presporal stages of *N. pontogammari*. 9. Microcolonies of bacteria-like cells in cytoplasm of merogonal stage. 10. Intracytoplasmic vacuoles (ICV) enveloped in structureless electron-dense cover. The cell wall supporting the plasma membrane of rickettsia-like organisms (RLO) is composed of two electron-dense and electron-lucent layers. Septate filamentous structures (arrows) fill ICV. 11. Microcolonies of RLO inside cytoplasm of early sporont. Filamentous structures inside ICV are not produced. Electron-dense ribosome zone is weakly developed in cytoplasm of RLO. 12. Microcolonies of RLO inside cytoplasm of late sporont. ICV contains filamentous structures (arrows). RLO exhibit clearly recognizable ultrastructural characteristics of prokaryotic bacteria-like cells, including trilaminar membranes, and electron-dense periplasmic ribosome zone. The cytoplasm of infected sporonts is degenerated. Bars: 120 nm (9); 60 nm (10); 100 nm (11, 12)

plasma membrane, a structureless endospore (en), and thin not layered exospore (ex) (Figs 6, 8, 9). Exospore was covered by microgranules. Granular ornamentation firstly appeared on the surface of late sporonts (Fig. 3).The cytoplasm of a microsporidian spore consists of a nucleus in a diplokaryon arrangement (nu), an anterior anchoring disk (ad), a bipartite membranous lamellar polaroplast (ap, pp), endo-

plasmic reticulum, ribosomes, and a posterior vacuole containing posterosome (ps) (Figs 6, 8, 9). The isofilar polar filament (pf) emanates from the anchoring disk and coils 6-13 times within the posterior region of the spore. The polaroplast has two regions with regularly arranged lamellae, anteriorly narrow (ap), posteriorly wider (pp) (Fig. 6). Multimembranous posterosome was observed inside posterior vacuole (Fig. 8).

Numerous prokaryotic endosymbionts were registered inside the cytoplasm of some Microsporidia belonging host cells (Figs. 9-12). Microcolonies of round and bacilliform bacteria-like cells were enveloped in structureless or layered electron-dense cover 20-30 nm wide. The intracytoplasmic vacuoles, which were spherical, measured up to 900 nm in diameter (Figs 9-12). TEM showed that these intracytoplasmic vacuoles contained numerous microorganisms identified as rickettsia-like organisms (RLO). Many published reports have documented that rickettsia-like organisms are involved in the complete membrane-limited vacuoles and form colonies which consist of purely or structurally complete organisms (Elston and Peacock 1984, Fryer and Lannan 1994, Azevedo et al. 2005). The studied life form exhibit clearly recognizable ultrastructural characteristics of prokaryotic bacteria-like cells, including trilaminar membranes, an increasing electron-dense periplasmic ribosome zone (Figs 9-12). These organisms are pleiomorphic in shapeand in size, as measured in cross sections of transmission electron micrographs. Microcolonies of RLO were detected inside merogonal (Figs 9, 11) and sporogonal stages (Figs 10-12). Delicate nest of septate filamentous structures filled intracytoplasmic vacuole of the sporonts (Figs 10-12).The cytoplasm of infected microsporidian cells was degenerated, with weakly developed endoplasmic reticulum and undeveloped the spore organelles. Rickettsia-like infections have been reported in 3 amphipod species (Frederici et al. 1974, Larsson 1982, Graf 1984). RLO infection characteristics in *Crangonyx floridanus* (Frederici et al. 1974), and *Rivulogammarus pulex* (Larsson 1982) were similar to infections in microsporidian cells observed in this study.

CONCLUSION

The obtained data demonstrate the pathogenic nature of the host-parasite relationship between endosymbiotic rickettsia-like organisms and microsporidians. The ultrastructural morphology of the microorganisms presented here is the first description of intracellular prokaryotic Rickettsiae (rickettsia – like organisms or RLO), from the aquatic fauna of Poland.

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O WEWNĄTRZKOMÓRKOWYCH PROKARIOTYCZNYCH MIKROORGANIZMACH, POWIĄZANYCH Z MIKROSPORYDIOZĄ INWAZYJNYCH OBUNOGÓW *PONTOGAMMARUS ROBUSTOIDES*

Streszczenie

W komórkach mięśni kiełży *Pontogammarus robustoides* zebranych w małej rzece przybrzeża Bałtyku Południowego (N 54°16', E 19°25') stwierdzono Microsporidia *Nosema pontogammari* Ovcharenko i Kurandina 1987. Z zarażonych tkanek wykonano preparaty do transmisyjnej mikroskopii elektronowej. *N. pontogammari* infekuje cytoplazmę komórek mięśniowych, wewnątrz niektórych komórek pasożyta odnotowano występowanie nietypowych wakuoli wewnątrzcytoplazmatycznych. Transmisyjna mikroskopia elektronowa wykazała, że wymienione wakuole zawierają skupiska komórek zidentyfikowanych jako Rickettsia-podobne komórki prokariotyczne. Skupiska pleomorficznych okrągłych i pałeczkowatych komórek były otoczone przez pokrywę o dużej gęstości elektronowej i szerokości 20-30 nm. Ściana komórek prokariotycznych składa się z wewnętrznej błony komórkowej i dwuwarstwowej otoczki o różnej gęstości elektronowej. Zdegradowana cytoplazma zainfekowanych komórek zawierała zarówno słabo rozwinięte retikulum endoplazmatyczne, jak i wakuole. Uzyskane dane sugerują, że relacje pomiędzy endosymbiotycznymi Rickettsia-podobnymi organizmami a ich żywicielami mają charakter patogeniczny.