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Gregarines (Apicomplexa) and microsporidians (Microsporidia) of native and invasive gammarids (Amphipoda, Gammaroidea), occurring in Poland[1]

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ABSTRACT. The goal of our study was to recognize microparasites of alien gammarids inhabiting Polish inland and coastal waters versus those infecting local species. Twenty two localities including the Vistula, Oder and Bug Rivers, Vistula Lagoon, Gosławskie Lake, littoral of the Baltic Sea, as well as small rivers draining directly to the sea were investigated. In total, over 5000 individuals of 14 species of gammarids were collected and analyzed using light and electron microscopy. The studies have revealed five named and seven unnamed species of gregarines (Apicomplexa, Gregarinidae) as well as three named and seven unnamed species of microsporidians (Microsporidia, Nosematidae, Thelohaniidae) infecting six native and four invasive gammarid host species. All the above microparasites were new to Poland. Four species of gregarines (*Uradiophora ramosa*, *U. longissima*, *Cephaloidophora similis*, *C. mucronata*) and four microsporidians (*Nosema dikerogammari*, *N. pontogammari*, *Thelohania* sp. 2, *Thelohania* sp. 5) were associated with hosts of Ponto-Caspian origins. Evidently, these microparasites were transported to the area through the range expansion of their invasive hosts. Gregarines *Cephaloidophora* sp. 1 and *Uradiophora* sp. 1 were registered only in North American *Gammarus tigrinus. Uradiophoera ramosa* infects Ponto-Caspian (*P. robustoides*, *D. villosus*) and North-Americah hosts (*G. tigrinus*).

Key words: gregarines, microsporidians, native and invasive Gammaroidea, Poland

Introduction

Contamination of local ecosystems with alien species has become one of the most important problem in ecology. Only in the Baltic Sea, more than 120 species of alien hydrobionts have been recorded during the last twenty years [1]. Amphipods and particularly gammarids are among the fastest spreading invasive invertebrates in Europe. They are known to have deteriorative effects on the local fauna of colonized waterbodies – in big lowland rivers as Bug, Vistula and Oder they have completely replaced the native species [2–6]. So far, in Poland 8 species of alien gammarids were found – six of them are of Ponto-Caspian origin [3].

Transport of pathogens associated with invasive species is important yet still weakly studied field of parasitology. According to „pathogen release" hypothesis species invasion to new territory is enhanced for the migrating populations lose their parasites on subsequent stages of range expansion [7,8]. However, a possibility of recurrent uptake of pathogens by host migrating in subsequent waves through the same invasion route cannot be excluded.

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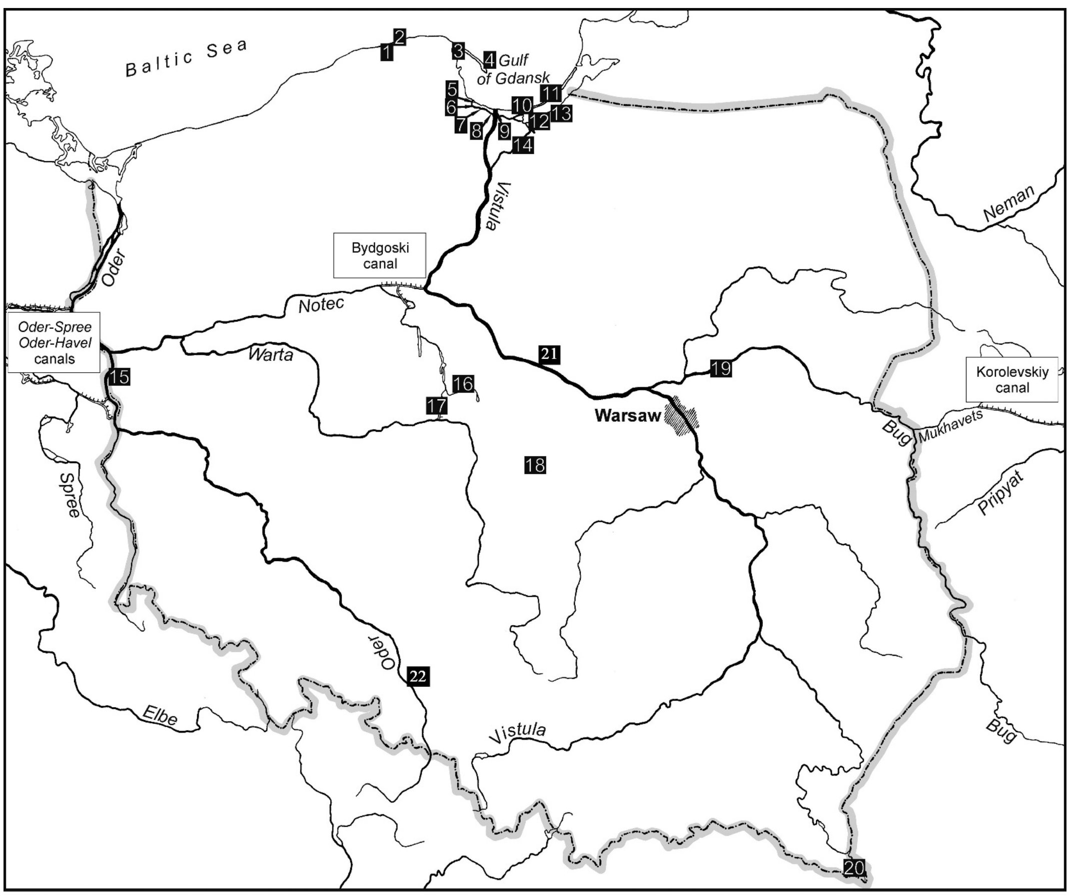
The knowledge on parasites infecting invasive gammarids is poor but even our preliminary studies revealed that alien species may transport their pathogens to new territories [9,10–14].

Our studies led in years 2005–2007 aimed to answer the following questions: (1) does range expansion of alien gammarids lead to enrichment of microparasite assemblages in the newly colonized waterbodies; (2) is there any exchange of microparasites between native and invasive host species in such areas?

Materials and methods

Gammarids were collected from May 2005 to November 2007 in the following 22 sites: Włocławski Reservoir; Vistula River (in Świbno, Nowy Duninów, Przegalina, Trzcińsko, Górki Wschodnie); Vistula Lagoon (in Piaski, Krynica Morska, Połoniny); „Ptasi Raj" Lake; Bay of Puck in Kuźnica Helska; Baltic Sea near Dębki and Hel; Oder River in Pławidło, Zdzieszowice; Bug River (in Wyszków); Noteć River in Łysek (near Sępolno); Nogat River at the road Nowy Dwór Gdański – Elbląg, Stradanka stream in Tolkmicko, Piaśnica River at outflow from Żarnowieckie Lake; Gosławskie Lake, Struga Dobieszkowska stream near Łódź; San tributaries in Bieszczady Mts. (Map 1.). Altogether 5162 individuals of the following species: native freshwater *Gammarus pulex*, *G. fossarum*, *G. balcanicus*, *G. lacustris*, native brackishwater *G. zaddachi*, *G. locusta*, *G. duebeni*, alien Ponto-Caspian *Chaetogammarus ischnus*, *Dikerogammarus haemobaphes, D. villosus*, *Pontogammarus robustoides, Obesogammarus crassus*, alien Balkan *Gammarus roeselii* and alien North American *Gammarus tigrinus* were collected (Table 1). After identification, the gammarids were sectioned in order to make tissue samples for light and electron microscopy. Smears of infected organs were stained with Giemza stain. Gregarines were fixed with OsO_4 solution.

For ultrastructural analyses, infected tissues and gregarines were fixed in a 2.5% (v/v) glutaraldehyde and 2.0% (v/v) osmium tetroxide. Then the pieces were dehydrated and embedded in Epon–Araldite according to the standard methods [15,16]. The material was examined under JEOL-JEM-1200 electron microscope.



Map 1. Sampling sites (for number explanation see Table 1)

Table 1. Gammarid species found in the studied sites

Sampling site (site number)	Geographic coordinates (decimal degrees)	Gammarid species
Piaśnica River (1)	N 54,797865; E 18,048820	*Gammarus pulex*, *G. zaddachi*, *G. lacustris*
Baltic Sea at Dębki (2)	N 54,833523; E 18,064528	*G. zaddachi*, *G. tigrinus*, *Gammarus duebeni*
Puck Bay at Kuźnica Helska (3)	N 54,731708; E 18,586378	*G. tigrinus*, *G. zaddachi*
Baltic Sea at Hel (4)	N 54,638082; E 18,805161	*G. tigrinus*, *Gammarus locusta*, *G. zaddachi*
Ptasi Raj Lake (5)	N 54,359757; E 18,788338	*G. tigrinus*, *G. duebeni*, *G. zaddachi*
Vistula in Górki Wschodnie (6)	N 54,348002; E 18,801470	*Pontogammarus robustoides*, *Dikerogammarus haemobaphes*, *G. duebeni*, *G. tigrinus*
Vistula in Trzcińsko (7)	N 54,309463; E 18,869448	*G. tigrinus*
Vistula in Przegalina (8)	N 54,309313; E 18,918457	*P. robustoides*
Vistula in Świbno (9)	N 54,336144; E 18,936996	*P. robustoides*, *D. haemobaphes*, *Chaetogammarus ischnus*
Vistula in Nowy Duninów (21)	N 52,349940; E 19,286751	*P. robustoides*, *Dikerogammarus villosus*
Vistula Lagoon in Krynica Morska (10)	N 54,378583; E 19,445844	*G. tigrinus, Obesogammarus crassus, P. robustoides*
Vistula Lagoon in Piaski (11)	N 54,427369; E 19,599781	*P. robustoides*, *G. tigrinus*
Vistula Lagoon in Połoniny (12)	N 54,281011; E 19,425974	*G. tigrinus*, *O. crassus*, *P. robustoides*
Stradanka stream in Tolkmicko (13)	N 54,321079; E 19,528370	*G. pulex*
Nogat branch (14)	N 54,171177; E 19,251308	*D. haemobaphes*, *P. robustoides*
Oder in Pławidło (15)	N 52,440525; E 14,578686	*D. villosus*
Oder in Zdzieszowice (22)	N 50,244400; E 18,062335	*D. villosus*
Noteć in Łysek (16)	N 52,405404; E 18,504066	*Gammarus roeselii*
Gosławskie Lake (17)	N 52,302286; E 18,260479	*D. haemobaphes*
Struga Dobieszkowska stream (18)	N 51,838429; E 19,585190	*Gammarus fossarum*
Bug in Wyszków (19)	N 52,590600; E 21,460118	*D. villosus*, *D. haemobaphes*, *C. ischnus*
San tributaries in Bieszczady Mts. (20)	N 49,099948; E 22,845039	*Gammarus balcanicus*

Results

Parasite assemblages of native and alien gammarids in the studied sites composed of gregarines (Protozoa, Apicomplexa), microsporidians (Microsporidia) and acanthocephalan larvae (Metazoa, Acanthocephala) [11].

Parasitic Protozoa were represented by Gregarinomorpha (Eugregarina) belonging to genera *Cephaloidophora* Mavrodiadi, 1908 [*C. gammari* (Franzius, 1848), *C. mucronata* Codreanu-Balcescu, 1995, *C. similis* Codreanu-Balcescu, 1995 and four undetermined species], and *Uradiophora* Mercier, 1912 [*U. ramosa* Balcescu-Codreanu, 1974, *U. longissima* (Siebold, 1839) and three undetermined species] (Table 2, Fig. 1a–h).

Cephaloidophora gammari (Franzius, 1848), syn.: *Gregarina gammari* Franzius, 1848; *Cephaloidophora echinogammari* Poisson, 1921; *Rotundula gammari* Goodrich, 1949. The species has a large number of host and wide geographical distribution. It was initially described by Franzius from the gut of

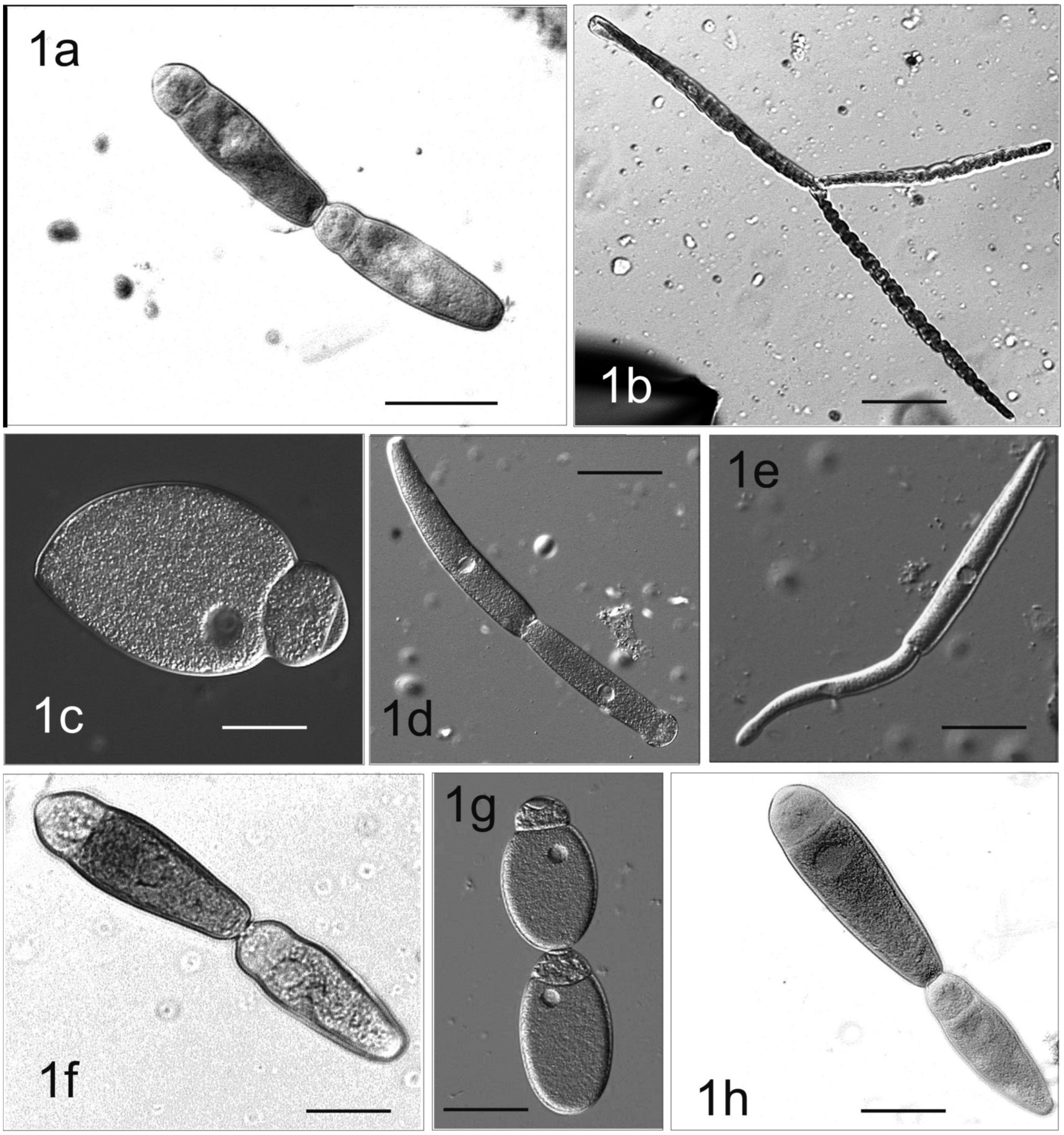


Fig. 1. Morphology of gregarines (adult trophozoites, syzygies) parasitizing digestive tracts of investigated gammarid species (1a, b, f, h – osmium fixation; 1c, d, e, g – living; interference contrast). 1a – *Cephaloidophora similis* from *Dikerogammarus villosus*; 1b – *Uradiophora ramosa* from *Pontogammarus robustoides*; 1c – *Cephaloidophora* sp. 1 from hepatopancreatic caeca of *Gammarus tigrinus*; 1d – *Uradiophora* sp. 1. from *G. tigrinus*; 1e – *Uradiophora* sp. 2 from *G. locusta*; 1f – *Cephaloidophora mucronata* from *D. villosus*; 1g – *Cephaloidophora* sp. 2 from *Gammarus locusta*; 1h – *Cephaloidophora* sp. 3 from *G. locusta*. Bars: 1a – 32 μm, 1b, e – 80 μm, 1c – 25 μm, 1d – 60 μm, 1f – 20 μm, 1g – 45 μm, 1h – 18 μm.

Gammarus pulex in France, and after it was identified in: *Echinogammarus berilloni* in France, *Gammarus olivii* (=*Echinogammarus olivii*, *Chaetogammarus olivii*) in France, *G. roeselii* in Germany, *G. balcanicus montanus* (=*G. balcanicus)* in Romania and *G. fasciatus* in North America [9,17]. In Poland we recorded *C. gammari* in *G. pulex* from Stradanka stream in Tolkmicko (October, 2005) [9,10, 14, 18, 19]. Solitary mature cephalins or sporadins associated in syzygy (35–75 μm by 24–33 μm) have ovoidal, mildly elongate in outline; button-like prominent epimerite; the length of calotte-shaped proto-

Table 2. Microparasites recorded from gammarids found in the studied sites

Microparasite species	Host species	Sampling site number
Gregarinomorpha		
Cephaloidophora gammari	*Gammarus pulex*	13
C. similis	*Dikerogammarus villosus*	15, 22
C. mucronata	*Pontogammarus robustoides*	11, 12
	Dikerogammarus villosus	15
Cephaloidophora sp. 1	*Gammarus tigrinus*	4
Cephaloidophora sp. 2	*Gammarus locusta*	4
Cephaloidophora sp. 3	*G. locusta*	4
Cephaloidophora sp. 4	*Gammarus zaddachi*	4
Uradiophora longissima	*D. villosus*	22
Uradiophora ramosa	*P. robustoides*,	21
	D. villosus,	22
	G. tigrinus	3
Uradiophora sp. 1	*G. tigrinus*	4
Uradiophora sp. 2	*G. locusta*	4
Uradiophora sp. 3	*G. zaddachi*	4
Microsporidia		
Nosema pontogammari	*P. robustoides*	9
Nosema dikerogammari	*D. villosus*	15, 19, 22
	Dikerogammarus haemobaphes	17, 19
Pleistophora muelleri	*G. pulex*	13
Thelohania sp. 1	*G. pulex*	18
Thelohania sp. 2 *(brevilovum)*	*D. haemobaphes*	17
Thelohania sp.3	*Gammarus duebeni*	6
Thelohania sp 4.	*G. zaddachi*	1
Thelohania sp. 5	*P. robustoides*	21
Thelohania sp. 6	*Gammarus balcanicus*	20
Thelohania sp. 7	*Gammarus fossarum*	18

merite is about a quarter from the total length (TL).

Cephaloidophora mucronata Codreanu-Balcescu, 1995 was originally found in the gut of Ponto-Caspian gammarids *Pontogammarus robustoides aestuarius* (=*P. aestuarius*), *Chaetogammarus tenellus behningi* (=*Ch. ischnus*) and *Dikerogammarus haemobaphes fluviatilis* (=*D. haemobaphes*) from the Danube Delta (Sulina branch) [17]. We recorded this gregarine species in Ponto-Caspian host *P. robustoides* living in Vistula Lagoon (October 2005) [2,8,11–13]. It was also found in *P. robustoides* from Włocławski Reservoir (June, 2006) as well as in *Dikerogammarus villosus* from Oder near Pławidło (June, 2006). Adult cephalins are fusiform, with a globular prominent hyaline epimerite; protomerite is flattened and the elongate-ovoidal, deutomerite is tapered to the posterior end (Fig. 1f). Biometrical values (sporonts in syzygies):

TL	LEpi	LP	LD	LP/TL	WP	WD	WP/WD	NØ
83 µm	7.5 µm	12 µm	64 µm	1:6.8	24 µm	31 µm	1:1.29	12.5 µm

Explanations: TL–total length; L Epi–epimerite length; LP–protomerite length; LD–deutomerite length; WP–protomerite width; WD–deutomerite width; NØ–nucleus diameter

Cephaloidophora similis Codreanu-Balcescu, 1995 was described in Ponto-Caspian gammarids *Chaetogammarus tenellus behningi* (=*Ch. ischnus*) and *Dikerogammarus haemobaphes fluviatilis* (=*D. haemobaphes*) (midgut) from the same stations as for *C. mucronata*; but never the two species were found simultaneously in the same host [17]. In Poland, we found *C. similis* in invasive Ponto-Caspian host *D. villosus* from Oder River, collected in June 2006. Adult cephalins of elongate rectangular form have a high cubic protomerite with lenticular flattened epimerite. The deutomerite has the same width as the protomerite, but it is slightly narrowed at its caudal fourth (Fig. 1a). Biometrical values (sporonts in syzygies):

TL	LEpi	LP	LD	LP/TL	WP	WD	WP/WD	NØ
74 µm	4.5 µm	17 µm	53 µm	1:4.3	23 µm	24 µm	1:1.05	12.5 µm

***Cephaloidophora* sp. 1** was found in hepatopancreatic caeca of invasive North-American *G. tigrinus* in the Baltic Sea littoral (Hel Peninsula, September 2007). Isolate adult cephalins up to 95 µm long with thick discoidal epimerite (5 µm high and 16 µm width); protomerite globular slightly flattened (20 µm by 31 µm); large hearth-shaped deutomerite asymmetrical and tapered at the posterior end; LP/TL=1:4.65 and WP/WD=1:1.74 (Fig. 1c). In some hosts it was found mixed infection with an amoebozoan parasitizing in the hepatopancreatic caeca [20].

***Cephaloidophora* sp. 2** is a midgut parasite of native *Gammarus locusta* at Baltic Sea littoral (Hel Peninsula, September 2007). Sporadins (85–88 µm by 50–52 µm maximum width) associated in syzygies (TLsy=172 µm; ovoidal contour with simple lenticular flattened epimerite, calotte-shaped protomerite (LP/TL=1: 6.5) (Fig. 1g).

***Cephaloidophora* sp. 3.** infects the gut of *Gammarus locusta* at the Baltic Sea littoral (Hel Peninsula, September 2007). Syzygies (TLsy up to 90 µm) are formed by inequal sporadins elongate ellipsoidal (37 µm by 12 µm to 53 µm by 18 µm) narrowed to the posterior half of the deutomerite; approximately globular protomerite with flattened lenticular epimerite; LP/TL=1: 5.5 (Fig. 1h).

***Cephaloidophora* sp. 4** was registered in the gut of native *Gammarus zaddachi* (the same station and samples as above). Elongate rectangular cephalins (42–50 µm by 33–35 µm slightly tappered at the posterior end; protomerite rectangular with button-like epimerite; LP/TL=1: 4; TLsy=85–100 µm.

Uradiophora longissima (Siebold, 1839) Poisson, 1924, syn. *Gregarina longissima* Siebold, 1839; *Didymophies longissima* Franzius, 1848. This is widespread gregarine species parasitizing amphipod crustaceans; it was described in *Gammarus pulex* in France and found also in: *Orchestia littorea* and *Caprella aequilibra* in France; *G. pulex* and *G. roeselii* in Germany; and *G. balcanicus montanus* (=*G. balcanicus*) in Romania [21, 22]. In Poland, we found this species in the gut of *D. villosus* from Oder River. Elongate cephalins filiform (25–160 µm by 5–14 µm) with small cylindrical-conical (2–5 µm) decaying epimerite and globular protomerite (8–12 µm in diameter). Early end-wise syzygies with approximately equal partners (TLsy=200–320 µm).

Uradiophora ramosa Balcescu-Codreanu, 1974 was described from the midgut of Ponto-Caspian gammarids *Pontogammarus robustoides* in Danube Delta (Ghiolul Rosu, November, 1972); percentage of infection=14% [21]. This species was recorded from Poland in invasive *P. robustoides* in Vistula Deltaic system (October 2005) [9,10,14,18,19]. We found *U. ramosa* in Central Poland (June 2006) in *P. robustoides* (Włocławski Reservoir) and *Dikerogammarus villosus* (Oder near Zdzieszowice) as well as in North American invasive *Gammarus tigrinus* from Puck Bay (September, 2007); infection prevalence 90–95%. *U. ramosa* is characterized by frequent multiple syzygies with repeated dichotomously ramifications of satellites; morphological and cytologic differences between the primite and satellites. Primite sporadin (200–300 µm by 25–30 µm) were elongated, with rostrum-like anterior and rectangular posterior ends. Small conical protomerite; clear granulated cytoplasm of the deutomerite were observed. Satellites are filiform (400–450 µm by 18–23 µm) with dence cytiplasm and they tend to muffle oneself up (Fig. 1b).

***Uradiophora* sp. 1** was found in gut of *Gammarus tigrinus* inhabiting of the coastal zone of Baltic Sea (Hel Peninsula) in September 2007 (Table 1). Ribbon-like sporadins associated in elongate syzygies (TLsy about 280 µm by 22 µm maximum

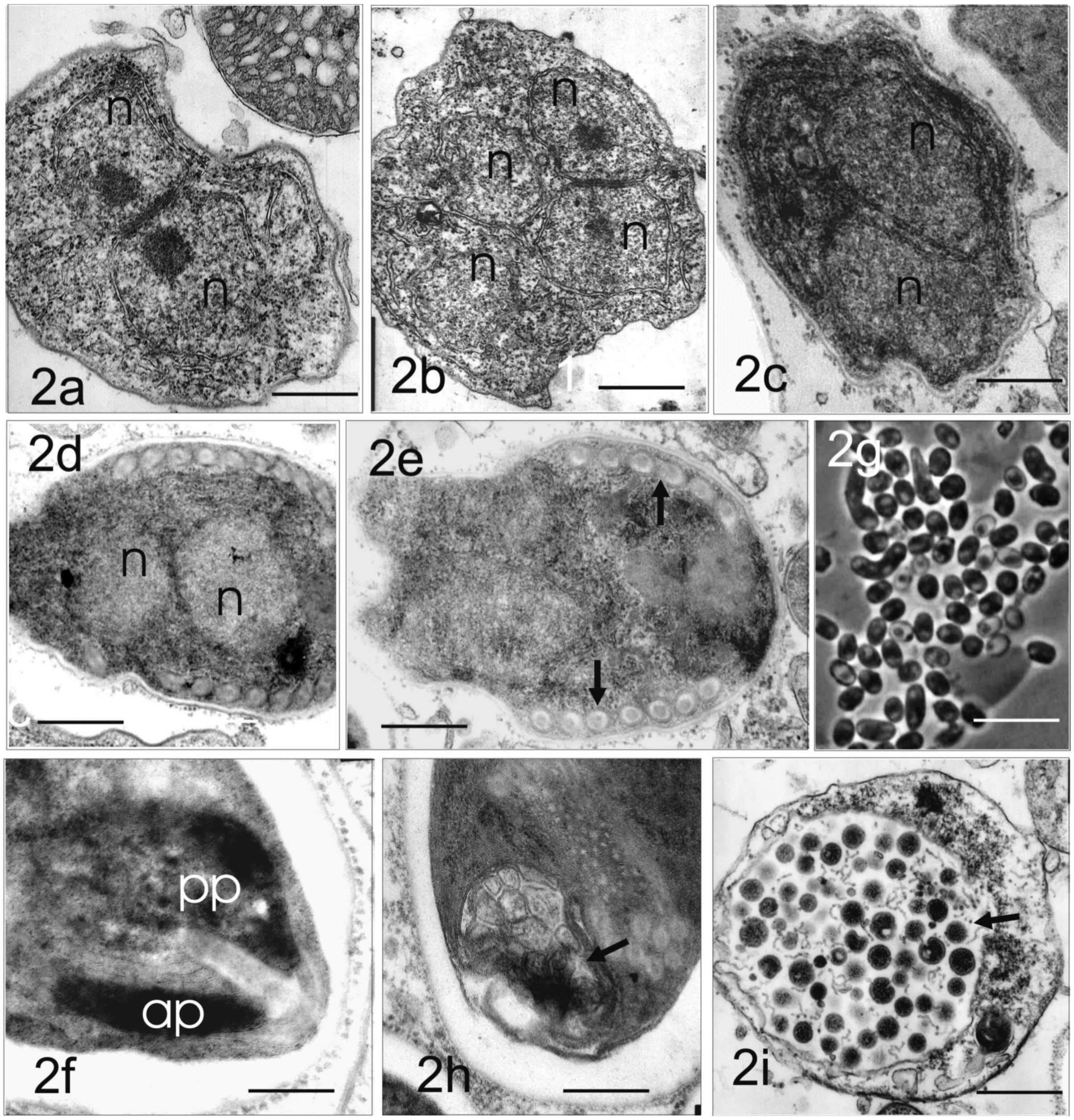


Fig. 2. Morphology and ultrastructure of microsporidium *Nosema pontogammari.* 2a – young sporont with diplokaryotic nuclei (n); 2b – tetranucleate sporogonal plasmodium; 2c – sporoblast; 2d – immature spore with diplokaryotic nuclei (n); 2e – sporoblast with developing polar filament (arrowed), 2g – live spores, phase contrast: 2f – anterior part of mature spores with bipartite lamellar polaroplast (ap, pp); 2h – posterior part of mature spores with posterior vacuole containing posterosome (arrowed); 2i – prokaryotic organismes (arrowed) in cytoplasm of the sporont of *N. pontogammari*. Bars: 2a – 2e, 2i – 1.0 µm; 2f, 2h – 0.5 µm; 2g – 6 µm.

width), the primite has uniform width and it is shorter than slightly curved and narrowed in its posterior half satellite (Fig. 1d).

***Uradiophora* sp. 2** was registered in the midgut of *Gammarus locusta* in the same station as *Uradiophora* sp. 1. Elongate syzygies (TLsy 320–390 µm) composed of sporadins different in sizes and morphology; smaller primite (TL 106–187 µm) filiform (about 5 µm width) and longer satellite (TL 204–216 µm) cylindrical (16–18 µm). tapered to the posterior fourth (Fig. 1e).

***Uradiophora* sp. 3** infects midgut of *Gammarus zaddachi* from the same station as above simultaneously with amebozoan infection in hepatopancreatic caeca [20]. Isolate cephalins (84 µm by 7–11 µm), small globular slightly flattened protomerite (4 µm ×6 µm) with a little knob-like epimerite, ribbon-like deutomerite mildly enlarged in its posterior half part.

Microsporidia are the most commonly found gammarid parasites. We found them in 5 native (*G. fossarum*, *G. pulex*, *G. balcanicus*, *G. zaddachi*, *G, duebeni*) and 3 alien (*D. villosus*, *D. haemobaphes*, *P. robustoides*) gammarid species. Based on light microscopy analysis we found spores of these parasites only in 3% of analyzed gammarid individuals. However, molecular analysis of *D. villosus* material from Bug and Vistula revealed infection of ca. 80% of studied individuals (Bącela, personal communication).

Nosema pontogammari Ovcharenko and Kurandina, 1987 was described as parasite of *Pontogammarus crassus* (=*Obesogammarus crassus*) from the Dnieper river in Ukraine [23]. Later this parasite was found in *Obesogammarus obesus* and *Pontogammarus robustoides* from the Dnieper River and Danube Delta [24–26]. In Poland it was found in *P. robustoides* from the Vistula Delta [10].

After ultrastructural analysis we could support that *N. pontogammari* was properly placed in genus *Nosema* based on diplokaryotic life cycle, during which oval spores (Fig. 2g) with homogenous exospore (Fig. 2d, f, h) and layered (lamellar) bipartite polaroplast were produced (Fig. 2f).

Vegetative stages of *N. pontogammari* (Fig. 2a, b) reproduce by division of tetrakaryotic sporogonal palsmodium (Fig. 2b). After division, diplokaryotic sporonts initate emergence of sporoblasts (Fig. 2e) that eventually transform into spores (Fig. 2d, g, h). The spore posterior vacuole embeds crystal-like structured posterosome (Fig. 2h). In cytoplasm of vegetative developmental stages, some hyperparasitic ricketsia-like prokaryotic organisms were observed (Fig. 2i).

Nosema dikerogammari Ovcharenko and Kurandina, 1987 occurs in Dnieper basin and Danube Delta inhabiting gammarids of Ponto-Caspian complex, mostly *Dikerogammarus villosus, D. haemobaphes* and rarely *Chaetogammarus ischnus*, *Obesogammarus crassus* (one report from Dnieprovsko--Bugski liman in Ukraine) [12,13,23,26]. In Poland, we registered *N. dikerogammari* in *D. vilosus* and *D. haemobaphes* (Table 2). Preliminary data on the ultrastructure of this parasite supported its place in genus *Nosema*, however some of the observed features (short polar filament twisted in a spiral with turns inclined at various angles to longitudinal axis of a spore) differentiate it from other congeneric species [12]. Based upon molecular studies published in 2007, microsporidians parasitising *D. villosus* were defined as a separate species named provisionally *Microsporidium* sp. D [16]. Our detailed ultrastructure (Fig. 3a–g) analysis of microsporidia registered in *D. villosus,* and *D. haemobaphes* in Poland accompanied by some molecular studies (Bącela, Ironside, personal communication) support the above results. The earliest known developmental stages of this parasite are diplokaryotic meronts found in sarcoplasm of muscle cells (Fig. 3a). After series of mitotic divisions the parasite produce oval sporonts (Fig. 3b), which after double division transform into elongated sporoblasts (Fig. 3c, d). After cytoplasm rearrangement and forming extrusive organelles (Fig. 3d), the sporoblasts produce elongate-oval thin-wall spores (Fig. 3e, g). Polaroplasts fills them to ca. L of their length (Fig. 3e, g). In Poland, we found *N. pontogammari* and *N. dikerogammari* to occur only in alien Ponto-Caspian gammarids (*P. robustoides*, *D. haemobaphes*, *D. villosus*).

The rest of microsporidians found in the analysed gammarids belonged to ***Thelohania*** group – parasites similar to above, having octosporous sporogony. They are commonly found in both, European and Ponto-Caspian gammarids. We found *Thelohania like* microsporidia in various species of native and invasive gammarids inhabiting Vistula and Oder basins, Stradanka and Struga Dobieszkowska streams (Table 2). So far, ca. 20 species inhabiting gammarids and having octosporous sporogony were described, among which only *Pleispothora muelleri* was studied thoroughly based on ultrastructure and molecular data [27]. Recently a new genus *Dictyocoela* Terry *et al.*, 2004 [28] was proposed, with 10 species described exclusively on a base of molecular data. The only published picture of *Dictyocoela mulleri* Terry *et al*., 2004 found in *Gammarus duebeni celticus* presents a fragment of sporophorous vacuole including several spores of ultrastructure typical for *Thelohania*-like microsporidians (multilayered spore wall, bipartite polaroplast, isofilar polar filament, presence of microtubular structures inside of episporontal space). Ultrastructural data shows high host-specificity of *Thelohania*-like microsporidians. Previous molecular identification supports identity of *Thelohania* sp. 2. and *Thelohania* sp. 3 with *Dictyocoela brevilovum* and *D. muelleri* (Ironshide, personal communication). So far, we identified seven *Thelohania-Dictyocoela*-like species inhabiting the analyzed gammarids, but precise definition of their taxonomic position is not possible without particular molecular analysis. *Thelohania--Dictyocoela*-like microsporidians were found in native *G. pulex*, *G. fossarum*, *G. balcanicus*, *G. zaddachi* as well as in alien *Pontogammarus robustoides and Dikerogammarus haemobaphes*.

Discussion

In the studied sites in Poland, unicellular parasites were found in native gammarids as well as in Ponto-Caspian and North-American invaders.

In the gregarine genus *Cephaloidophora* above 60 species are known, parasitizing in aquatic crustaceans. More than 30 species are parasites of Amphipoda. Ponto-Caspian gammarids *Pontogammarus robustoides*, *Chaetogammarus* spp., and *Dikerogammarus haemobaphes* are the hosts of *Cephaloidophora mucronata*; *C. similis* was found in *Dikerogammarus villosus.* Both species were previously reported from Danube Delta [2]. In the studied Polish waterbodies we found these gregarine species parasitizing the invasive gammarids *Pontogammarus robustoides* and *D. villosus*. In North-American *G. tigrinus* from Baltic Sea was found one undeterminated species of the genus *Cephaloidophora*.

In native *Gammarus pulex* we found the gregarine species *C. gammari*, widespread in European and North-American hosts. Native gammarids *G. zaddachi* and *G. locusta* were parasitized by three undeterminated species of *Cephaloidophora* different from those found in *G. tigrinus.* For instance we can note that Ponto-Caspian gammarids keep own gregarine parasites that are not transferred to the native gammarid hosts. Also no cases of *Cephaloidophora* gregarine transfer from native species to Ponto-Caspian aliens was observed to date. We are not yet able to decide on origin of *Cephaloidophora* sp. found in North-American invader – *G. tigrinus.*

The genus *Uradiophora* groups six species, which parasitize crustaceans. Four species [*U. gammari* Poisson, 1924; *U. longissima* (Siebold, 1839); *U. mercieri* Poisson, 1921; *U. ramosd*] were found in gammarids [21]. Five taxons of gregarines belonging to the genus *Uradiophora* (*U. ramosa, U. longissima* and *Uradiophora* sp. 1–3) were registered by us during 2005–2007 in Polish waterbodies. Two of them (*U. ramosa, U. longissima)* were found in Ponto-Caspian host (*P. robustoides* and *D. villosus*). *Uradiophora ramosa* was reported also from North-American invasive amphipod *G. tigrinus*. It is the only gammarid species in Poland, which was parasitized by a gregarine infecting Ponto-Caspian hosts with very high prevalence of infection (about 95% in Hel Peninsula, September 2007). It is also interesting to point out that *D. villosus* was infected by three species of gregarins (*C. mucronata, C. similis, U. longissima*). Two of them (*C. mucronata, C. similis*) infect only Ponto-Caspian gammarids, but *U. longissima* is the widespread parasite of fresh and brackishwater hosts, excluding species of Ponto-Caspian origin. Presence of *U. longissima* in a pontocaspian host (*D. villosus*) in Poland can be explained partially by the changes of ecological status of the hosts in colonized area. *D. villosus* is voracious predator, destroying native invertebrate fauna to absolute domination in colonized biotopes [3].

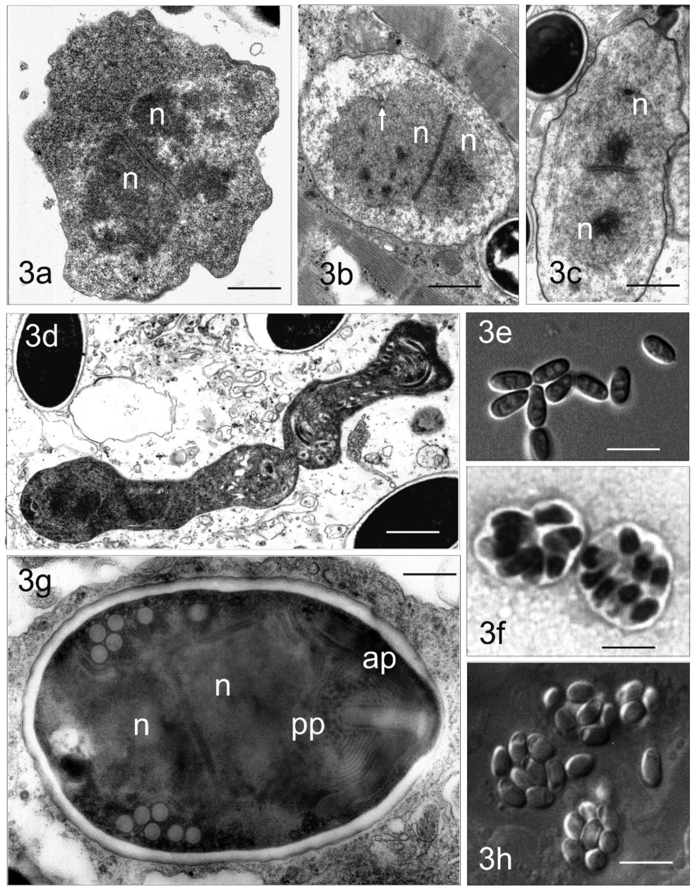


Fig. 3. *Nosema dikerogammari* from sarcoplasm of *Dikerogammarus haemobaphes* (3a – 3g) and some *Thelohania*-like species. 3a – diplokaryotic meront; 3b – young sporont during initialization of mitotic division (arrowed); 3c – sporont; 3d – dividing sporoblast; 3e – live spores; 3g – ultrastructure of the spore with bipartite (ap, pp) and diplokaryotic nuclei (n); 3f – Giemza stained spores of *Thelohania* sp. from the muscles of *Gammarus balcanicus*; 3h – *Dictyocoela mulleri* from the muscles of *Dikerogommarus haemobaphes* (live spores, Nomarski contrast). Bars: 3a – 0.9 μm; 3b – 1.5 μm; 3c – 0.6 μm; 3d – 0.7 μm; 3e – 10 μm; 3g – 0.3 μm

Summarizing, the transport of alien pathogens with gammarids invading Polish waters can be estimated as significant. Each species of invasive Ponto-Caspian gammarid is accompanied by its parasites. This lack of „pathogen release" can be explained by a continuous migration of host through the artificial canals joining the two see basins. On the other side, lack of alien parasites in North-American *G. tigrinus* may possibly be related to just few intentional introductions of this species into European waters. The very first record of still unidentified *Cephaloidophora* sp. in this gammarid may be a result of its infection with some local gregarine species.

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