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Diversity of metchnikovellids (Metchnikovellidae, Rudimicrosporea), hyperparasites of bristle worms (Annelida, Polychaeta) from the White Sea

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Summary

In this paper we report the diversity of metchnikovellids discovered in two surveys at the silt littoral/sublittoral zone in vicinity of Marine Biological Station of St. Petersburg State University, Island Sredniy, Chupa Inlet, Kandalaksha Gulf of the White Sea performed in 1986-1987 and in 2009-2011. The list of metchnikovellid species that had been recorded for the locality includes: *Metchnikovella hovassei*, a parasite of the gregarine *Lecudina* sp. from the polychaete *Allita* (*Nereis*) *virens*; *M. selenidii*, a parasite of *Selenidium* sp. from *Ophelia limacina*; *M. spiralis* and *M. incurvata* from *Polyrhabdina* sp. from *Pygospio elegans*; and *Amphiamblys capitellae* from *Ancora sagittata* from *Capitella capitata*. Noteworthy, *M. incurvata* was never observed in the 1986-1987 survey. In 2009-2011 though, it became even more abundant than *M. spiralis*. Given the patchy distribution of metchnikovellids among gregarine and polychaete populations, *M.incurvata* might have been overlooked in 1986-1987 survey. Alternatively, the emergence of *M. incurvata* in the White Sea polychaetes can be the result of spreading of North Atlantic populations of *Pygospio elegans* species complex infected with *M. incurvata*, to the North and mixing them with local *M. incurvata*-free but *M. spiralis*-infected populations. Such tendency of expanding ranges for certain species presumably due to global warming and/or anthropogenic factors has been recently shown for several free living and parasitic organisms including microsporidia.

Key words: Metchnikovellidae, Microsporidia, gregarines, Apicomplexa, Polychaeta, White Sea, biodiversity, hyperparasitism

Introduction

All known representatives of the family Metchnikovellidae Caullery et Mesnil, 1914, order Metchnikovellida Vivier,1975, class Rudimicrosporea Sprague, 1977, phylum Microsporidia Balbiani, 1882, are hyperparasities of marine invertebrates. Most metchnikovellids parasitize gregarines from the alimentary tract of marine bristle worms (Annelida, Polychaeta). The life cycle of metchnikovellids includes pre-sporogonial stages represented by monokaryotic or dikaryotic uninuclear cells and multi-nucleate plasmodia, followed by two sporogonies. Free sporogony (FS) serves presumably for dissemination of the parasites within the same gregarine cell and results in production of free spores. Sac-bound sporogony yields in environmental "cysts" (by terminology of Caullery et Mesnil (1914), or "spore sacs" (by terminology of Larsson (2000))[1] (Larsson, 2000; Larsson, Køie, 2006; Sokolova et al., 2013, 2014). Family Metchnikovellidae constitutes a monotypic taxon, which has been routinely treated as an ancestral group within the phylum Microsporidia Balbiani 1982 basing mainly on presumably "primitive" ultrastructural features of metchnikovellidean spores, such as a short polar filament (manubrium) without the coiled region, absence of polaroplast and undeveloped spore wall (Larsson, 2000; Larsson and Køie, 2006; Sprague, 1977). Basal position of metchnikovellids in relation to other taxa of microsporidia was once proven by SSUrDNA-inferred phylogeny presented on XIII International Congress of Protistology (Simdianov et al., 2009). However, ultrastructurally metchnikovellid spores also resemble the ones of intranuclear parasites of amoeba, the representatives of the genus *Paramicrosporidium*, proved recently to belong to Rozellomycota (Corsaro et al., 2014), a basal or sister group to Microsporidia within the Aphelidea-Rozellomycota-Microsporidia (ARM) clade (Karpov et al., 2013; Letcher et al., 2013). Unfortunately, no sequences of any metchnikovellid genes are available through the public databases, and we can only guess whether Metchnikovellids belong to Microsporidia or Rozellomicota, or represent an independent lineage sharing a common ancestor with both groups. This intrigue adds interest to the research on these enigmatic and hardly accessible hyperparasites.

Most findings of gregarines infected with metchnikovellids, were from polychaetes inhabiting littoral zone along the shores of water bodies of the Atlantic basin, including Northern Sea (Hebrides, Bretagne), English Channel (French shore), Øresund strait connecting Baltic and Northern Seas (Denmark and Sweden) and Mediterranean Sea (French coast) (Caullery and Mesnil, 1897, 1914, 1919; Larsson and Køie, 2006; Mackinnon and Ray, 1931; Reichenow, 1931; Vivier and Schrével, 1973; Vivier, 1975). Metchnikovellids have been also reported from the seas of the Arctic Ocean, namely from Murmansk shore, Kolski Gulf of the Barents Sea (Awerinzew, 1908; Dogiel, 1922), and Kandalaksha Gulf of the White Sea (Rotari, 1988; Sokolova et al., 2013; Sokolova et al., 2014). Such patchy distribution likely reflects the geography of sampling sites, rather than the factual range of metchnikovellids, which are probably globally distributed, similar to their polychaete hosts.

In this paper we report the diversity of metchnikovellids discovered in two surveys at the silt littoral/sublittoral zone in vicinity of Marine Biological Station of St. Petersburg State University, Island Sredniy, Chupa Inlet, Kandalaksha Gulf of the White Sea performed in 1986-1987 and in 2009-2011. The metchnikovellid diversity revealed in 1986-1987survey was reported in the diploma dissertation by the first author (Rotari, 1988) and has never been published. Basing on field studies performed in 2009-2011, two metchnikovellid species were described by light and by electron microscopy (Sokolova et al., 2013; Sokolova et al., 2014). In this paper we summarize the findings from both surveys and provide a list of metchnikovellid species that had been recorded for the locality, as well as the data on prevalence and general morphology for each of the recorded species.

Material and methods

Polychaetes of several species were collected in July-August-September in 1986-1987 at the silt littoral or sublittoral zones in Chupa Inlet, Kandalaksha Gulf of the White Sea at the Levin reach (sampling site 1, 66° 17.878' N, 33° 27.774' E) by hand sifting, and at the two other locations: Podpahta (sampling site 2, 66° 18.005' N, 33° 37.163' E), and Luda Cheremshiha (sample site 3, 66° 18.699' N, 33° 54.578' E) by dragging from boats. Additionally, polychaetes *Pygospio elegans* were collected at the silt littoral of the first sampling site

[1] Both terms "spore sacs" and "cysts" are used in this paper as synonyms. They reflect different aspects of these formations, which are, in fact, spore sacs by structure and origin, and environmental cysts by function.

in August 2009-2011. Polychaetes were dissected under a stereomicroscope using laboratory needles. Gregarines were found inside the gut, either attached to the gut epithelium, or freely in the gut lumen. Infected gregarines were identified under stereomicroscope by the presence of conspicuous cysts, and/or characteristic oval inclusions inside the cytoplasm. These gregarines were isolated on microscopic slides, observed and photographed. During the years 1986-1987 live gregarines, as well as the ones fixed in Bouin solution and stained with Boehmer hematoxylin, were studied using phase contrast Biolam microscope (Lomo, Russia), equipped with a film camera. In 2009-2011 observations were done using a field PZO Biolar microscope equipped with DIC optics and Cannon EOS 300 digital camera. Electron microscopy was performed as described before (Sokolova et al., 2013; Sokolova et al., 2014).

Results and Discussion

Metchnikovellids were noticed in the gregarines of genera *Lecudina* Mingazzini, 1891, *Selenidium* Giard, 1884, *Polyrhabdina* Mingazzini, 1891, and *Ancora* Labbé, 1899 from four species of polychaetes *Alitta* (*Nereis*) *virens* Sars, 1835, *Ophelia limacina* Rathke, 1843, *Pygospio elegans* Claparède, 1863, *Capitella capitata* Fabricius, 1780 (Table 1). In the survey 1986-1987, 100% of examined polychaetes contained intestinal gregarines. Their number was proportional to the worm size and varied from 1 to > 50. *P. elegans* and *C. capitata* were collected from littoral zone by hand sifting while *A. virens* and *O. limacina* - from sublittoral zone by dragging. Hand sifting allowed collecting greater numbers of worms. The largest number of metchnikovellids-infected gregarines were found in the two most abundant species, *Pygospio elegans* and *Capitella capitata*. Infection of gregarines was associated with high density of polychaete hosts: in the study 1986-1987 infected gregarines were observed only in the probes with >100 polychaetes in each. The survey of 2009-2011 was focused mainly on parasites of *Pygospio elegans* sampled from the same site as in 1986-1987. In this survey alimentary tracts of 43.5% (145 from 333) of the dissected polychaetes were parasitized by *Polyrhabdina* sp. Average number of gregarines per one polychaete was 3.5 ranging from 1 to 50. Gut lumens of 7.6% (11 of 145) of polychaetes contained at least one metchnikovellid-infected gregarine. The summarized data on parasites prevalence are presented in the Table 1. Beneath we describe each of the discovered metchnikovellid species and discuss comparative morphology of *Metchnikovella spiralis* and *M. incurvata*, both discovered in the gregarine *Polyrhabdina* sp. parasitizing *Pygospio elegans*.

1. *Metchnikovella hovassei* Vivier, 1965 from *Lecudina* sp. from the polychaete *Allita* (*Nereis*) *virens*

One of 14 bristle worms *Allita virens* collected in one of the dragging net probes at the sampling site 2 (Podpahta) carried gregarines *Lecudina* sp. One of those contained an ovoid metchnikovellid cyst of about 10 μm long and 4 μm wide with a thickening at one pole. Inside the cyst there were 8 roundish spores assembled in two rows (Fig. 1a). All this characters as well as general morphology of the cyst resembled the one of *Metchnikovella hovassei* (see Fig. 16 in Vivier, 1975). Vivier and Schrével (1973) described this metchnikovellid from the gregarine *Lecudina* sp., a parasite of the polychaete *Perinereis cultrifera*. Similarity in spore sac morphology, the

Table 1. White Sea metchnikovellids and their hosts.

Polychaete species	Sample site	Year	Number of worms		Gregarine species	Metchnikovellid species	Infected gregarines per worm
			Examined	With metchnikovellids			
Allita (Nereis) virens	2,sl	1986-1987	14	1(7%)	*Lecudina sp.*	*Metchnikovella hovassei*	1
Ophelia limacina	3,sl	1986-1987	31	2 (6%)	*Selenidium sp.*	*M. selenidii*	1
Pygospio elegans	1, l	1986-1987 2009-2011	106 145	6 (6%) 3 (2%)	*Polyrhabdina sp.*	*M. spiralis*	1-3
Pygospio elegans	1, l	2009-2011	145	11 (8%)	*Polyrhabdina sp.*	*M. incurvata*	1-4
Capitella capitata	1, l	1986-1987	244	32 (13%)	*Ancora sagittata*	*Amphiamblys capitellae*	1-6

Notes: 1-3 – Sample sites in Chupa Inlet, Kandalaksha Gulf of the White Sea (1 – Levin reach, 2 – Podpahta, 3 – Luda Cheremshiha); sl – sublittoral, l – littoral.

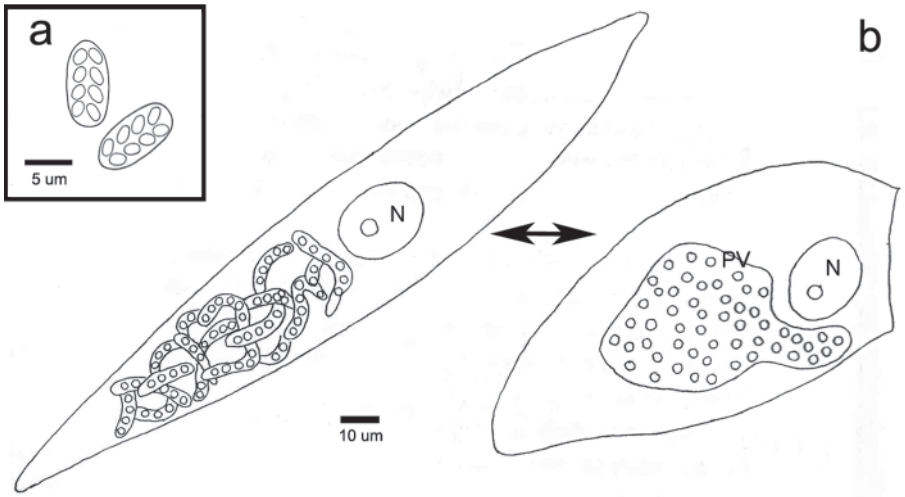

Fig. 1. *S*chematic drawings: a – *Metchnikovella hovassei* spore sacs; b – *M. selenidii spore* sacs (left image) and presporogonic stages of free sporogony enclosed in the parasitophorous vacuole within the cytoplasm of *Selenidium* sp. isolated from a polychaete *Ophelia limacina* (right image). *Abbreviations*: N – gregarine nucleus, PV – parasitophorous vacuole.

same number of spores within a spore sac, and related hosts allowed identifying the species as *Metchnikovella hovassei* Vivier, 1965.

2. *Metchnikovella selenidii* Awerinzew, 1908 from *Selenidium* sp. inhabiting the polychaete *Ophelia limacina*

In one of the dragging net probes taken at the sample site 3 (Luda Cheremshiha), among 31 polychaetes *Ophelia limacina* one gregarine contained 24 elongated and slightly bent spore sacs measured 16 × 5-8 μm. Each sac contained 14-18 oval spores. The second gregarine isolated from another worm of the same species from the same probe, contained an irregular shaped net-like structure with numerous spherical bodies of 2.5-3 μm in diameter inside (Fig. 1b). When the gregarine pellicle disrupted, the plasmodium-like structure kept its integrity. We presume that we observed a free sporogony within parasitophorous vacuole that resulted in producing roundish or slightly oval spores. This species is likely *Metchnikovella selenidii*, described by Awerinzew (1908) from the same host species. In addition to slightly bent cysts containing elongated spores, he also described plasmodia with round bodies inside (Awerinzew, 1908).

3. *Amphiamblys capitellae* (Caullery et Mesnil, 1914) from *Ancora sagittata* inhabiting the polychaete *Capitella capitata*

In two probes sampled at the silt littoral at Levin Reach (sample site 1) in the 1986-1987 survey bristle worms *Capitella capitata* carried numerous gregarines *Ancora sagittata* in their intestines. Many of those were infected with elongated cysts characteristic for the genus *Amphiamblys* (Caullery and Mesnil, 1914, 1919; Desportes and Théodoridès, 1979; Larsson and Køie, 2006; Ormières et al., 1981; Vivier, 1975). In the first probe 17 of 122 (13.9%) polychaetes contained infected gregarines; in the second - 15 of 132 (11.4%) (data from both probes are pooled together in the Table 1). Number of spore sacs per a gregarine varied from 1 to 60. Bigger and more mature gregarines contained larger numbers of cysts. Parasites within the spore sacs located in one host gregarine were at the same stage of sporogenesis. Spore sacs of this metchnikovellid were shaped as elongated, slightly bent cylinders, rounded at the ends. They measured 30-70 μm in length and about 4-5 μm in width. A spore sac enclosed at least 20 spindle-shaped centrally curved spores arranged in pairs (Fig. 2, a-c). Spores were about 9 μm long and 2 μm wide. Some spore sacs contained 4, 8 or 16 roundish sporoblasts (Fig. 2, d-f). Vegetative stages were represented by roundish or shapeless plasmodia (Fig. 2, g-h) with 4-6 paired nuclei. By the shape and measurements of the cysts, number of spores within a cyst, and related host species this metchnikovellid can be identified as *Amphiamblys capitellae* (Caullery et Mesnil, 1914, 1919). As many as 6 species of the genus *Amphiamblys* have been recorded, 3 of them,

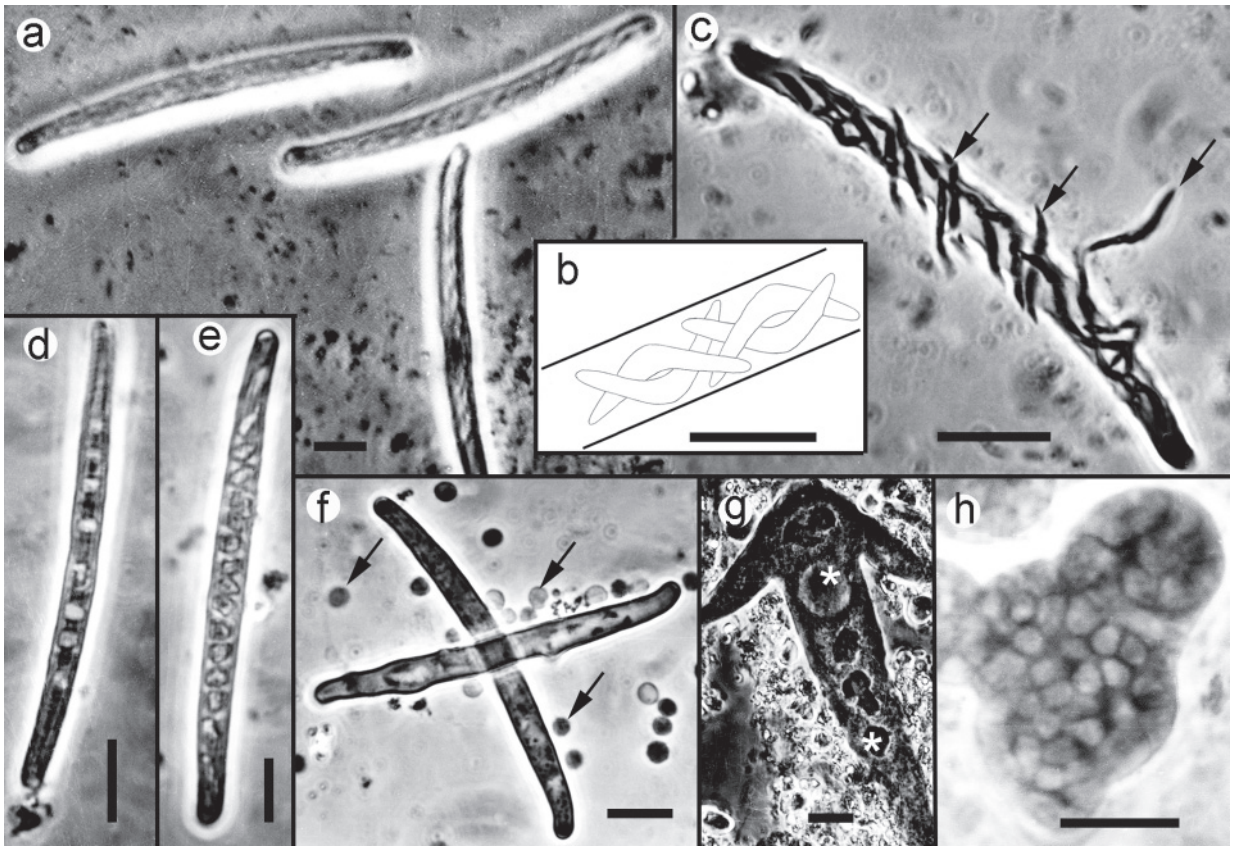

Fig. 2. Light microscopy of *Amphiamblys capitellae* discovered in *Ancora sagittata*, a parasite of *Capitella capitata*. a – Elongated spore sacs with spindle-shaped and slightly curved spores arranged in pairs; b – schematic drawing of spores arrangement in the sac; c – matures spores liberating from the spore sac (*arrows*): at least 20 spores are in the view; d – spore sac with 8 sporoblasts; e – spore sac with 16 sporoblasts; f – roundish sporoblasts (*arrows*) released from spore sacs; g – roundish plasmodia (*asterisk*) in the cytoplasm of *Ancora sagittata*; h – vacuoles liberated from the gregarine cytoplasm, contained sporonts of the free sequence resulted from plasmodia division. Scale bars: a-f – 10 μm, h – 5 μm.

A. capitellides (the type species), *A. laubieri*, and *A. bhatiellae*, have been studied ultrastructurally (Desportes and Théodoridès, 1979; Larsson and Køie, 2006; Ormières et al., 1981). Coupled nuclei in plasmodia were also reported for *A. capitellides* inhabiting a polychaete *Capitella giardi* collected in Øresund strait (Larsson and Køie, 2006). In a view of the presence of synaptonemal complexes observed in two species, *A. bhatiella* (Ormières et al., 1981) and *A. capitellides* (Larsson and Køie, 2006), it was presumed that the life cycle of *Amphiamblys* spp. included meiosis at the onset of sac-bound sporogony (Larsson and Køie, 2006). Coupled nuclei in presporogenic plasmodia, and formation of 4, 8, or 16 uninucleate sporoblasts (likely the products of post-meiotic divisions) in *A. capitellae* from the White Sea, is in a good accord with this presumption.

4. *Metchnikovella spiralis* Sokolova et al., 2014 from *Polyrhabdina* sp. parasitizing the polychaete *Pygospio elegans*

During the survey in 1986-1987, 6 of 106 *P. elegans* collected in one probe at Levin Reach contained gregarines infected with spiral-shaped spore sacs. Because of the peculiar organization of these spore sacs the new found metchnikovellid was presumed to belong to a new yet undescribed genus and was provisionally named *Vivierus spiralis* (Rotari, 1988), later amended to *Vivieria spiralis* (Rotari and Paskerova, 2007). In 2009-2011 a metchnikovellid with identical structure of spore sacs was re-isolated from the same hosts and locality. The life cycle of the parasite was re-examined by DIC light microscopy and by electron microscopy (Sokolova et al., 2014). It was found out that free sporogony (FS) in the life

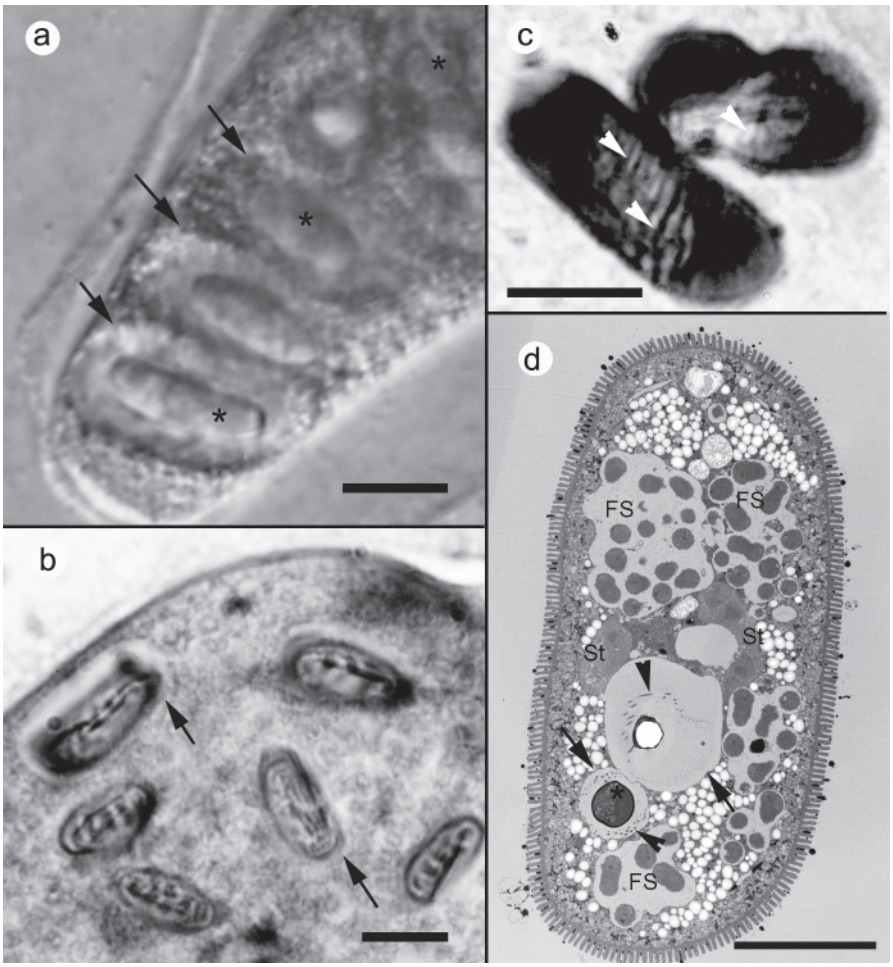

Fig. 3. *Metchnikovella spiralis,* a parasite of *Polyrhabdina* sp. from P*ygospio elegans* (a-c – light microscopy; d – electron microscopy). a – A portion of a live gregarine filled with spore sacs (asterisks) enclosed in the parasitophorous vacuole (arrows) under DIC optics; b, c – an infected gregarine fixed in Bouin solution and stained with Boehmer hematoxylin (b – spore sacs are indicated by arrows, c – isolated spore sacs display striation corresponding to spiral carcasses around spore sacs, *arrowheads*); d – an ultrathin section through the infected gregarine: *arrows* point to vacuoles containing spore sacs (*asterisk*), *arrowheads* – to spiral structures surrounding the sac. *Abbreviations*: FS – free spores within parasitophorous vacuoles; St – presporogonic stages. Scale bars: a-b, d –10 μm, c – 5 μm.

cycle of this metchnikovellid occured concurrently with sac-bound sporogony (SBS). Free spores measured on sections 2.5 ± 0.03 μm (n=18, range 2.2-2.8) × 1.5 ± 0.07 μm (n=8, range 1.2 -1.7).The life cycle included pre-sporogonial stages represented by dikaryotic cells and 4-nucleate cells with coupled nuclei. A multinucleate sporogonial plasmodium of FS split in numerous (>10) sporoblasts. In SBS, segregation of sporoblasts occurred within thick-walled cysts by internal budding. Live spore sacs were oval and measured 11.6 ± 0.24 μm (n = 19, range 10.0 - 13.5) × 4.7 ± 0.10 (n=22, range 3.5 - 5.3); fixed - 9.8 ± 0.27 μm (n= 8, range 9.0 -11.3) × 3.3 ± 0.1 (n=8, range 2.7 -3.6). Spore sacs contained 8 barrel-shaped spores measured 3.9 ± 0.10 μm (n=10, range 3.3 - 4.4) × 2.4 ± 0.06 μm (n=7, range 2.3 - 2.8). Spore sacs were enclosed in external spiral carcass composed of a dense cord embracing the sac, and their number per a gregarine varied from one to >20. (Fig. 3). Comparative ultrastructural analysis of both spore types and intracellular development of "*Vivierus spiralis*" and *Metchnikovella* spp. suggested that the former species was in fact *Metchnikovella*. We described this metchnikovellid as a new species, *M. spiralis* (Sokolova et al., 2014).

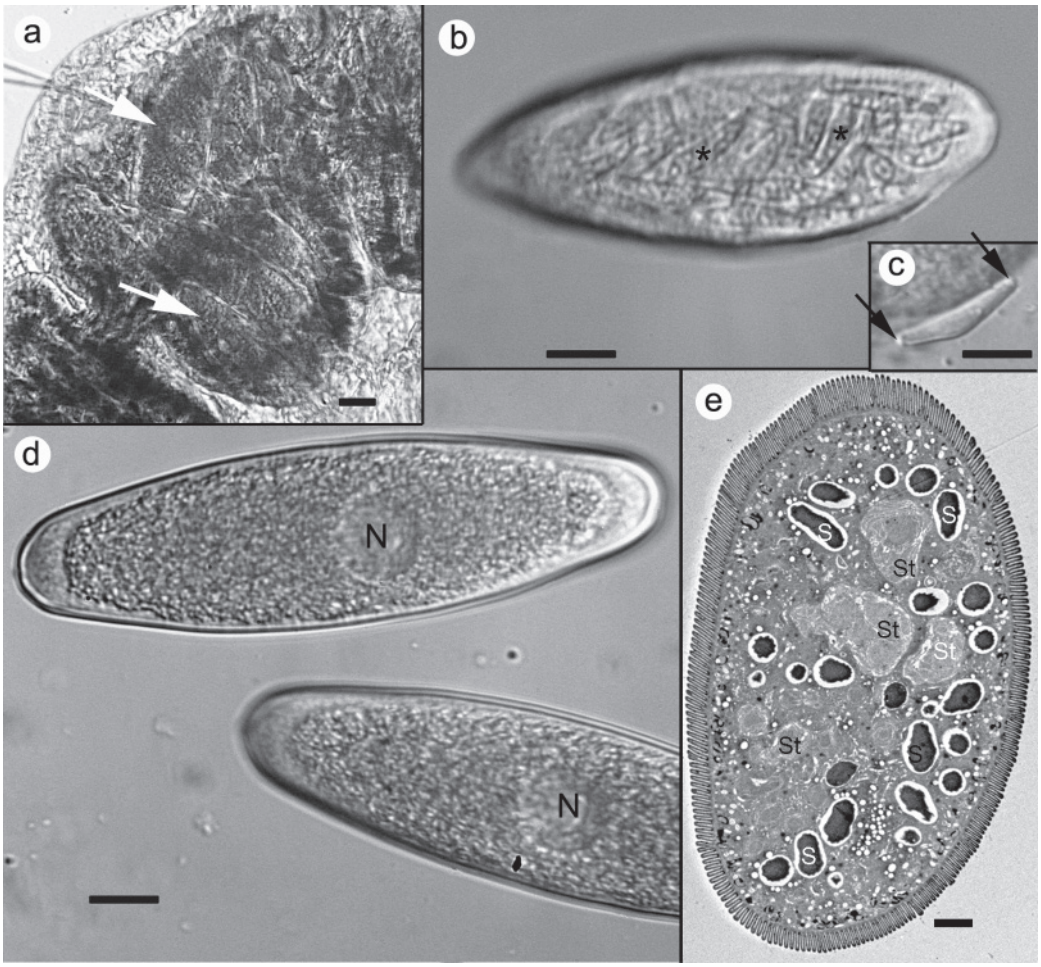

Fig. 4. *Metchnikovella incurvata*, a parasite of *Polyrhabdina* sp. from a polychaete *Pygospio elegans* (a-d – light microscopy, DIC; e – electron microscopy). a – Gregarines inside the gut are visible through transparent teguments of the polychaete host (*white arrows*); b – gregarines with multiple spore sacs (*asterisks*) arranged chaotically; c – an elongated boomerang-shaped spore sacs released from the gregarine display characteristic "plugging" structures at both ends (*black arrows*); d – uninfected gregarines, uniform in shape, with homogenous cytoplasm, roundish nucleus (N) and nucleolus located centrally; e – an ultrathin section through the infected gregarine. *Abbreviations*: N – nucleus, S – free spores, St – presporogenic stages. Scale bars: a – 20 μm, b-d – 10 μm, e – 2 μm.

5. *Metchnikovella incurvata* Caullery and Mesnil, 1914 from *Polyrhabdina* sp. parasitizing the polychaete *Pygospio elegans*

This metchnikovellid was not recorded in 1986-1987 survey and was discovered in 2009-2011 from the probes collected at the Levin Reach silt littoral. Average number of infected gregarines *Polyrhabdina* spp. per one polychaete was 3.8 ranging from 1 to 4. Gut lumens of 7.6% (11 of 145) of polychaetes contained at least one metchnikovellid-infected gregarine. Either cysts with spores or rounded to oval inclusions inside the cytoplasm were observed in 3.9% (20 from 509) of examined gregarines. Spore sacs were elongated and boomerang-shaped, 22-27 μm long and 4-5 μm wide with characteristic plugging structures ("thickenings") at both ends. Sac-bound spores were oval and measured 3.6 ± 0.3 × 1.8 ± 0.1 μm (n=8) (Fig.4). Fresh and epoxy resin-embedded gregarines showed up to 16 spores in each spore sac. Together with the sacs, cytoplasm of infected gregarines contained numerous free spores (3.7 ± 0.4 x 1.8 ± 0.2 μm, n=12) with the same morphology as sac bound spores. Spore sacs were arranged chaotically inside the host cytoplasm. As many as 30 sacs were visible in one focal plane (Fig. 4). Morphology of spores and spore sacs was undistinguishable from the one described for *M.*

incurvata. The gregarine and polychaete hosts were identical or closely related to the isolate collected at the French shore of the British Channel (Caullery and Mesnil, 1914) and the one described from the White Sea. Fine structure of *M. incurvata* have been described previously (Sokolova et al., 2013).

The phenomenon of two congeners, *Metchnikovella spiralis* and *M. incurvata*, parasitizing the same gregarine (*Polyrhabdina* sp.) and polychaete host (*Pygospio elegans*), needs special attention. Like all so far described *Metchnikovella* spp. these two species produce spores very similar in size and internal structure. However both species can be readily distinguished from each other by the following features. (i) Organization of spore sacs strikingly differs in two species by the shape, size, and especially by the presence of a conspicuous spiral carcass surrounding *M. spiralis* cysts. (ii) All stages of FS and SBS in *M. spiralis*, except for early dikaryotic stage, are enclosed in interfacial envelopes, while in *M. incurvata* free spores and spore sacs reside free in the cytoplasm. (iii) In *M. incurvata* SBS and FS occur consequently: SBS always follows FS upon depletion of cell resources, while in *M. spiralis* both types of sporogony develop concurrently. The mentioned differences strongly suggest that these two parasites rather belong to two different species, than represent two morphs of one polymorphic species. *M.incurvata* and *M.spiralis* were never found to co-occur simultaneously within one gregarine, so it is unlikely that congeners have been diversified by specializing to a particular intracellular microniche. Noteworthy, *M. incurvata* was never observed in the 1986-1987 survey. In 2009-2011 though, it became even more abundant than *M. spiralis* (8% versus 3%) (Table 1). Given the patchy distribution of metchnikovellids among gregarine and polychaete populations, *M.incurvata* might have been just overlooked in 1986-1987 survey. Alternatively, the emergence of *M. incurvata* in the White Sea polychaetes, can be the result of spreading of North Atlantic populations of *Pygospio elegans* species complex infected with *M.incurvata* to the North and mixing them with local *M. incurvata*-free but *M. spiralis*-infected populations. In the White Sea area the elevations of temperatures by 5°C per decade have been recorded basing on 30-year observations (Chapmann and Walsh, 1993; Johannessen et al., 2004). This factor together with synergistically acting other disturbances (i.e. shelf drilling, introduction of aquacultures and acclimatization of new species) resulted in essential rearrangements of marine biotas recorded worldwide (Lejeusne et al., 2010), and, specifically, of the White Sea bentic littoral ecosystems (Naumov et al., 2009). One of the outcomes of global warming is shifting of faunas, with the main routes of species expansions going northbound (Lejeusne et al., 2010). *M. incurvata* might have spread among the White See populations of *Pygospio elegans* similar to the situation with *Nosema ceranae*, a microsporidium of Asian origin, that have spread in European populations of honey bees due to transport of infected honey bees by beekeepers (Klee et al., 2007). Another example of emerging new microsporidia and gregarine parasites was recorded in invasive gammarids in Europe and has been also explained by the expansion of hosts' ranges (Ovcharenko et al., 2009, 2010). Long term observations on *Metchnikovella* spp. prevalence in *P. elegans* populations would be desirable to prove this hypothesis and to exemplify an effect of large scale perturbations like a climate change on every biological level including hyperparasites of marine polychaetes.

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