ORIGINAL ARTICLE

Mesnilia travisiae gen. nov., sp. nov. (Microsporidia: Metchnikovellida), a parasite of archigregarines *Selenidium* sp. from the polychaete *Travisia forbesii*: morphology, molecular phylogeny and phylogenomics

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Summary

Spore sacs and free spores of a metchnikovellid were found in archigregarines *Selenidium* sp. isolated from polychaetes *Travisia forbesii*. The studied worms were collected in the subtidal areas of the Kandalaksha Gulf of the White Sea and of Zelenetskaya Bay of the Barents Sea. Spore sacs of these hyperparasites had an elongated shape with a slight flexion. They had one polar plug and contained 12–14 rounded spores. Both spore sacs and free spores were in direct contact with the cytoplasm of the host cell. Contrary to a canonical idea about development of metchnikovellids, sac-bound sporogony was often observed in this parasite without traces of ongoing free sporogony. A combination of morphological features and host range distinguishes the studied isolates from any known genus and species of metchnikovellids. Phylogenetic analysis based on the SSU rRNA gene and BUSCO phylogenomics, showed that studied isolates form a new lineage of metchnikovellids. We proposed a new genus *Mesnilia* gen. nov. and described a new species, *Mesnilia travisiae* sp. nov. (Microsporidia: Metchnikovellida) to accommodate these organisms. Phylogenetic analysis showed that there is a mixed metchnikovellid infection in the population of polychaetes *T. forbesii* from Zelenetskaya Bay. We found molecular evidence for presence of the second metchnikovellid species in this host, which has yet to be characterised at the morphological level. In phylogenetic and phylogenomic trees, this 'cryptic' parasite grouped with another new metchnikovellid discovered in the populations of *Pygospio elegans* collected in Zelenetskaya Bay. New isolates described in this

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paper form two new lineages in the phylogenomic tree of metchnikovellids. This study confirmed widespread occurrence of mixed metchnikovellid infections in infrapopulations of gregarines from polychaetes.

Introduction

Metchnikovellida is a group of hyperparasitic microsporidia. They parasitise gregarines found in intestines of marine invertebrates, mainly polychaetes (Vivier, 1975; Sprague, 1977; Larsson, 2014). Recent in-depth investigations involving phylogenetic and phylogenomic analyses, have conclusively placed metchnikovellids as a basal branch within a broader clade that also encompasses canonical microsporidia (Mikhailov et al., 2017; Galindo et al., 2018; Nassonova et al., 2021; Bojko et al., 2022). Metchnikovellids differ from canonical microsporidia in many morphological and developmental traits including the structure of invasion apparatus (Larsson, 2014). Within the life cycle of metchnikovellids, two distinct types of sporogony can be identified: (1) 'free' sporogony resulting in formation of multiple spores located either directly in the host cytoplasm or within vacuoles, and (2) 'sac-bound' sporogony resulting in production of thick-walled spore sacs encapsulating several spores (Vivier and Schrével, 1973; Sokolova et al., 2014). The number of spores in the sac is a species-specific feature. In some it is strictly defined (Frolova et al., 2021), while in other species it can vary within a certain range (Paskerova et al., 2016). The shape of spore sacs, their size and presence of thicker polar parts (so-called 'polar thickenings' or 'polar plugs') are used as a key feature in the system of metchnikovellids developed over a century ago (Caullery and Mesnil, 1914; Caullery and Mesnil, 1919).

Since the first description (Caullery and Mesnil, 1897) about 30 species of metchnikovellids have been documented (Vivier, 1975; Larsson, 2014; Sokolova et al., 2014; Paskerova et al., 2016; Frolova et al., 2022). Caullery and Mesnil (1914, 1919) established three genera, *Metchnikovella, Amphiamblys* and *Amphiacantha*, originally grouped into the family Metchnikovellidae. These genera differ drastically in shape, size and the proportions between the length and width of the spore sacs.

Metchnikovella has oval, cylindrical or fusiform spore sacs with rounded ends. The sacs often have thicker polar plugs at one or at both ends. The length of spore sac exceeds the width less than ten times. *Amphiamblys* is characterised by long rod-shaped spore sacs with rounded ends. The length exceeds the width 10 times or more. *Amphiacantha* has fusiform spore sacs with sharp ends that usually extend with thread-like prolongations. Dogiel (1922) found an unusual metchnikovellid species in archigregarines *Selenidium* sp. from *Travisia forbesii* in the Barents Sea. This species has bottle-shaped spore sacs with one rounded end and one tapering end with a polar plug. He established a new genus and species for this organism, which he called *Caulleryetta mesnili* (Dogiel, 1922). This genus was not justified later in the revision of the family Metchnikovellidae by Vivier (1975) and in the further works of some authors (Sprague, 1977; Canning and Vávra, 2000; Schrével and Desportes, 2013). However, Sprague et al. (1992) included the genus *Caulleryetta* in the 'checklist of available generic names' of microsporidia and noted that it should be considered valid until proven otherwise. This genus was also listed by Issi and Voronin (2007), Becnel et al. (2014) and Cali et al. (2017).

The genus *Metchnikovella* Caullery et Mesnil, 1897 historically housed the majority of known metchnikovellids. Compared to the relatively uniform genera *Amphiacantha* and *Amphiamblys*, the genus *Metchnikovella*, as it was defined by Caullery et Mesnil (1914, 1919) and Vivier (1975), exhibits a remarkable diversity in the morphology of spore sacs. The shape of spore sacs varies in an exceptional way: from oval to cylindrical and fusiform. Depending on the species, they contain from 8 to 32 oval or rounded spores arranged in one − three rows. As it was mentioned above, the spore sacs of most *Metchnikovella* species possess one or two polar plugs. However, some species show no pronounced polar plugs. The type species, *M. spionis*, has remarkably elongated polar plugs at both ends of the spore sac. The spore sacs and free spores of

Metchnikovella spp. can be surrounded by vacuoles or located in direct contact with the host cytoplasm. Unfortunately, due to the low resolution of light microscopes in former times, older descriptions often lack information whether the hyperparasites develop within the vacuoles.

Therefore, despite having a limited set of distinguishing characters, species classified as *Metchnikovella* spp. displayed a greater variety of features compared to other metchnikovellid genera. Due to high polymorphism of the shape of spore sacs of the type species *M. spionis*, Caullery and Mesnil even suggested restricting the genus *Metchnikovella* to this only species, and noted that all other species within the genus were placed there provisionally (Caullery and Mesnil, 1919). Keeping in mind an exceptional morphological polymorphism of *Metchnikovella*, Larsson (2014) proposed to transfer the species that form oval spore sacs with a single polar plug and spherical spores to the genus *Caulleryetta* Dogiel, 1922.

Phylogenetic reconstructions further support the assumption that *Metchnikovella* is an artificial assemblage, as it does not form a monophyletic clade (Nassonova et al., 2021; Frolova et al., 2021; Frolova et al., 2022). However, lack of modern data for the type species *Metchnikovella spionis* Caullery et Mesnil, 1897, and *Caulleryetta mesnili* Dogiel, 1922, as well as the limited number of metchnikovellid taxa in phylogenetic trees, hinders the reconstruction of robust phylogeny of metchnikovellids.

Despite the years of intensive monitoring of the parasite fauna of *Travisia forbesii* in the Barents Sea, we have not succeeded in reisolating *Caulleryetta mesnili*. Instead, we isolated another metchnikovellid with a unique set of morphological features. This hyperparasite was also observed in archigregarines from *T. forbesii* collected in the White Sea. We present here its morphological description and provide molecular data for this organism, named *Mesnilia travisiae* gen. nov., sp. nov. Furthermore, our study provided molecular evidence for the existence of another yet hidden metchnikovellid from the same host species. This hyperparasite remains morphologically unidentified. In SSU rDNA trees and in phylogenomic reconstructions, this parasite groups with a new metchnikovellid found within the scope of our recent screenings of *Selenidium pygospionis* inhabiting populations of *Pygospio elegans* from Zelenetskaya Bay (Frolova et al., 2023). Here, we established the new genus and the new species of metchnikovellids from the *Travisia forbesii*, obtained sequences of four

new isolates of metchnikovellids from *T. forbesii* and *P. elegans* and demonstrated two new lineages in the phylogenetic tree of metchnikovellids.

Material and methods

Polychaetes *Travisia forbesii* Johnston, 1840 were collected from two locations: the subtidal zone near the White Sea Biological Station of M.V. Lomonosov Moscow State University (WSBS) in Velikaja Salma, the Kandalaksha Bay of the White Sea (66°33'12.0"N 33°06'17.0"E) in August 2017 and 2020, and near the Biological Station of Murmansk Marine Biological Institute of the Russian Academy of Sciences (MMBI) in Zelenetskaya Bay of the Barents Sea (69°06'43.3"N 36°05'56.1"E) in August 2020−2023. Polychaetes *Pygospio elegans* were collected in the littoral zone in the Zelenetskaya Bay in August 2021.

For specimens collected near WSBS in 2017, dissections and gut symbiont examinations were conducted in the WSBS laboratory using an MBS 9 stereomicroscope (LOMO, Russia). Archigregarines displaying signs of infection, were placed on a cover glass and examined using a Leica DM2500 microscope equipped with differential interference contrast (DIC) and documented with a Leica DFC 420C digital camera. Polychaetes collected from the Barents Sea, were transported alive to the Department of Invertebrate Zoology, St. Petersburg University, and kept in containers at +6 °C with seawater. The seawater was refreshed every second day. Examination of potentially infected archigregarines was carried out using a Leica M205C dissection microscope equipped with Rottermann contrast. Infected archigregarines were examined using a Leica DM2500 microscope equipped with DIC, and documented with a Leica DFC295 digital camera. If the presence of metchnikovellids in archigregarines was confirmed with light microscopy, a large amount of Millipore-filtered (0.45 µm) seawater was added under the coverslip, which resulted in detaching the cells from the object slide. The archigregarines containing free spores and spore sacs were individually collected from the slide using a hair-thin tapered-tip Pasteur pipette, washed in a fresh portion of Millipore-filtered seawater, and placed in 200 µl PCR tubes with 1−2 µl of water. Each tube was checked for the presence of a gregarine using a Leica M205C dissection microscope.

DNA extraction from infected archigregarines was performed using Arcturus® PicoPure® DNA

Extraction Kit (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, DNA was amplified by Multiple Displacement Amplification (MDA) using a Repli-g Single Cell Amplification Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. For verification of MDA reactions, the SSU rRNA gene was amplified by PCR using a 1:10 diluted MDA product as a template with microsporidia-specific primers: 18F, 530R (Weiss and Vossbrinck, 1999) and 1353TnR (Nassonova et al., 2021). PCR program parameters were the following: initial denaturation (5 min at 95 °C) followed by 35 cycles of 30 s at 95 °C, 50 s at 50 °C and 90 s at 72 °C, followed by 7 min at 72 °C for final elongation. Amplicons were purified using a Cleanup Mini Purification Kit (Eurogen, Moscow, Russia) or with ExoSAP-IT™ PCR Product Cleanup Reagent (Thermo Fisher Scientific, Waltham, MA, USA). The Sanger sequencing reactions were carried out using the AppliedBiosystems™ BigDye™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Waltham, MA, USA) and sequenced using Applied Biosystems™ 3500×L Genetic Analyzer (Thermo Fisher Scientific, Waltham, MA, USA). The final length of the assembled contigs was around 1300−1400 bp.

Verified MDA products were used to prepare the libraries with TruSeq Nano DNA Library Preparation Kit (Illumina, San Diego, CA, USA) and sequenced using Illumina HiSeq 2500 system at the Core Facility Centre "BioBank" of the St. Petersburg University Research Park (https://researchpark.spbu.ru/en/biobank-eng) according to the manufacturer's protocols. Quality control check of raw sequence data was performed using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Reads were trimmed using Trimmomatic (http://www.usadellab.org/cms/?page=trimmomatic). The assembler SPAdes v.3.15 in a single-cell mode was used for *de novo* genome assembly (Nurk et al., 2013). To separate hyperparasite contigs from the host material and bacterial contamination in the obtained metagenome assemblies the binning was performed using MaxBin v.2.2.7. To determine the completeness of the identified target genomes, the BUSCO v.5.2.2 package (Manni et al., 2021) with the fungi_odb10 (with "parasitic check") and microsporidia_odb10 datasets was used.

Phylogenomic analysis was carried out using a set of single-copy orthologs in the Fungi BUSCO database (fungi_odb10) and BUSCO Phylogenomics utility script (McGowan, 2019), with de-fault parameters in the SUPERMATRIX mode. The analysis included a selection of genomes and transcriptomes of microsporidia, rozellids, aphelids, fungi available in the GenBank database. A set of genomes of Holozoa (choanoflagellates, ichthyosporeans, and filasterians) was used as an outgroup. For each identified ortholog, a multiple alignment was constructed using the MUSCLE algorithm (Edgar, 2004). The resulting alignments were filtered and trimmed with the trimAl tool (Capella-Gutiérrez et al., 2009). Alignments of individual orthologs were combined into a united concatenated alignment (53 taxa, 73 orthologs, 20783 amino acid positions) that was used for phylogenetic reconstruction using (a) maximum likelihood method (IQ-TREE v. 1.6.12, single partition, model LG+F+I+G4, ultrafast bootstrap) (Nguyen et al., 2015; Hoang et al., 2018) and (b) Bayesian analysis (MrBayes v. 3.2.7a, GTR model, gamma-distributed rate variation across sites and a proportion of invariable sites) (Ronquist et al., 2012).

For SSU rDNA phylogenetic analysis we constructed an alignment, containing all available sequences of metchnikovellids and a selection of 'core microsporidia'. A set of 'short-branch microsporidia' (*sensu* Bass et al., 2018) was used as an outgroup. Sequences were aligned using MAFFT v. 7.490 (Katoh and Standley, 2013) with the 'favour accuracy' mode as implemented in the CIPRES portal (Miller et al., 2010). A mask was created by the G-blocks algorithm (as implemented in SeaView v. 4.6.1 − Gouy et al., 2010) and was further manually expanded to include the maximal possible number of nucleotide positions.

The maximum likelihood (ML) phylogenetic analysis was conducted using IQ-TREE launched at the CIPRES portal, with all parameters estimated by the program. The best-fit model, TIM3+F+I+G4, was chosen according to the Bayesian information criterion. The tree was tested using non-parametric bootstrapping with 1000 pseudoreplicates. Bayesian analysis was performed with MrBayes v. 3.2.6 at the CIPRES portal, employing the GTR model with γ correction for intersite rate variation (eight categories) and the covarion model. Trees were run as two separate chains (using default heating parameters) for 5 million generations, at which point they had ceased converging (the final average standard deviation of the split frequencies was <0.01), and the first 25% of generations were discarded for burn-in.

The SSU rRNA gene sequences obtained in this study were deposited with the GenBank under

Table 1. Occurrence of archigregarines and metchnikovellid hyperparasites in polychaetes *Travisia forbesii* from the White Sea (WSBS) and the Barents Sea (MMBI) in 2017-2023.

Sampling site, year	Dissected worms	*Selenidium*-infected worms		Worms with metchnikovellid-infected *Selenidium* sp.		
	N	N	%*	N	%**	%***
WSBS, 2017	10	7	70	2	20	29
WSBS, 2020	4	3	75	0	0	0
MMBI, 2020	14	7	50	2	14	29
MMBI, 2021	35	13	37	3	9	23
MMBI, 2022	59	32	54	1	2	3
MMBI, 2023	102	56	55	11	11	20

Note: WSBS – the White Sea Biological Station of M.V. Lomonosov Moscow State University; MMBI – the Biological Station of Murmansk Marine Biological Institute of the Russian Academy of Sciences; N – the number of worms; * the ratio of *Selenidium*-infected worms to the total number of dissected worms; ** the ratio of worms with metchnikovellid-infected *Selenidium* sp. to the total number of dissected worms; *** the ratio of worms with metchnikovellid-infected *Selenidium* sp. to the total number of worms with archigregarines.

the accession numbers: PP057783-PP057786. The dataset used in the phylogenomic analyses were deposited in Figshare at https://figshare.com/s/912285b8 6769afe24e0f (doi: 10.6084/m9.figshare.24777507).

Results

OCCURRENCE, PREVALENCE AND MORPHOLOGY OF METCHNIKOVELLIDS FROM *TRAVISIA FORBESII*

The archigregarines *Selenidium* sp. were frequently found in the gut of *T. forbesii*. The rate of infection varied from 37 to 55% in the polychaetes collected in the Barents Sea and from 70 to 75% in the worms sampled in the White Sea (Table 1). Each worm hosted from one to about 30 archigregarines, which were either attached to the intestine epithelium or resided freely in the gut lumen. The frequency of occurrence of metchnikovellids in *T. forbesii* was relatively low. It varied from 2 to 14% in the samples from the Barents Sea and in a broader range (0–20%) in the specimens from the White Sea. The ratio of metchnikovellid-infected *Selenidium* sp. to uninfected ones varied significantly (from 0 to 29%) across different sites and years.

Infected archigregarines usually exhibited vacuolated cytoplasm containing numerous inclusions corresponding to various stages of metchnikovellid infection (Fig. 1, A). However, in some cases, the cytoplasm of infected cell appeared almost homogeneous (Fig. 1, B). Gentle pressure of an archigregarine cell with a coverslip revealed numerous spore sacs, positioned longitudinally in the host cytoplasm, as well as proliferative stages and free spores (Fig.1, C–D). The mature spore sacs had an elongated shape with a slight flexion and one prominent polar plug (Fig. 1, E). The sacs contained 12–14 rounded spores. Notably, differences in the size of spore sacs were observed between isolates collected in the White and Barents Seas, as detailed in Table 2. Furthermore, worms collected from the White Sea exhibited higher intensity of infection (Fig. 2), with archigregarines containing from 12 to 35 spore sacs per individual (n = 14). In contrast, infected archigregarines from the Barents Sea contained from 1 to 11 spore sacs per host cell (n = 19).

In immature spore sacs, no spores were visible inside, and the sac wall appeared thin (Fig. 1, A–B). The spores within mature sacs were rounded in shape and measured $1.3 - 2.5 \times 0.9 - 1.6$ µm (avg (average) = 1.95×1.2, n = 25). Free spores were observed alongside spore sacs of varying maturity, typically located in the terminal regions of the archigregarine cell (Fig. 1, D). Free spores were oval and measured $1.5 - 2.8 \times 0.8 - 1.7$ µm (avg = 2.1×1.4, n = 21).

NEW METCHNIKOVELLID ISOLATE INFECTING *SELENIDIUM PYGOSPIONIS* FROM *PYGOSPIO ELEGANS*

Four metchnikovellid species were described in the population of *P. elegans* in the White and Barents Seas (reviewed in Frolova et al. 2023). During our recent screenings of parasitic fauna in the populations of *P. elegans*, in addition to the four other species previously described, we unexpectedly found a new metchnikovellid, provisionally designated as MD2_b01_MMBI2021. It had oval (rarely
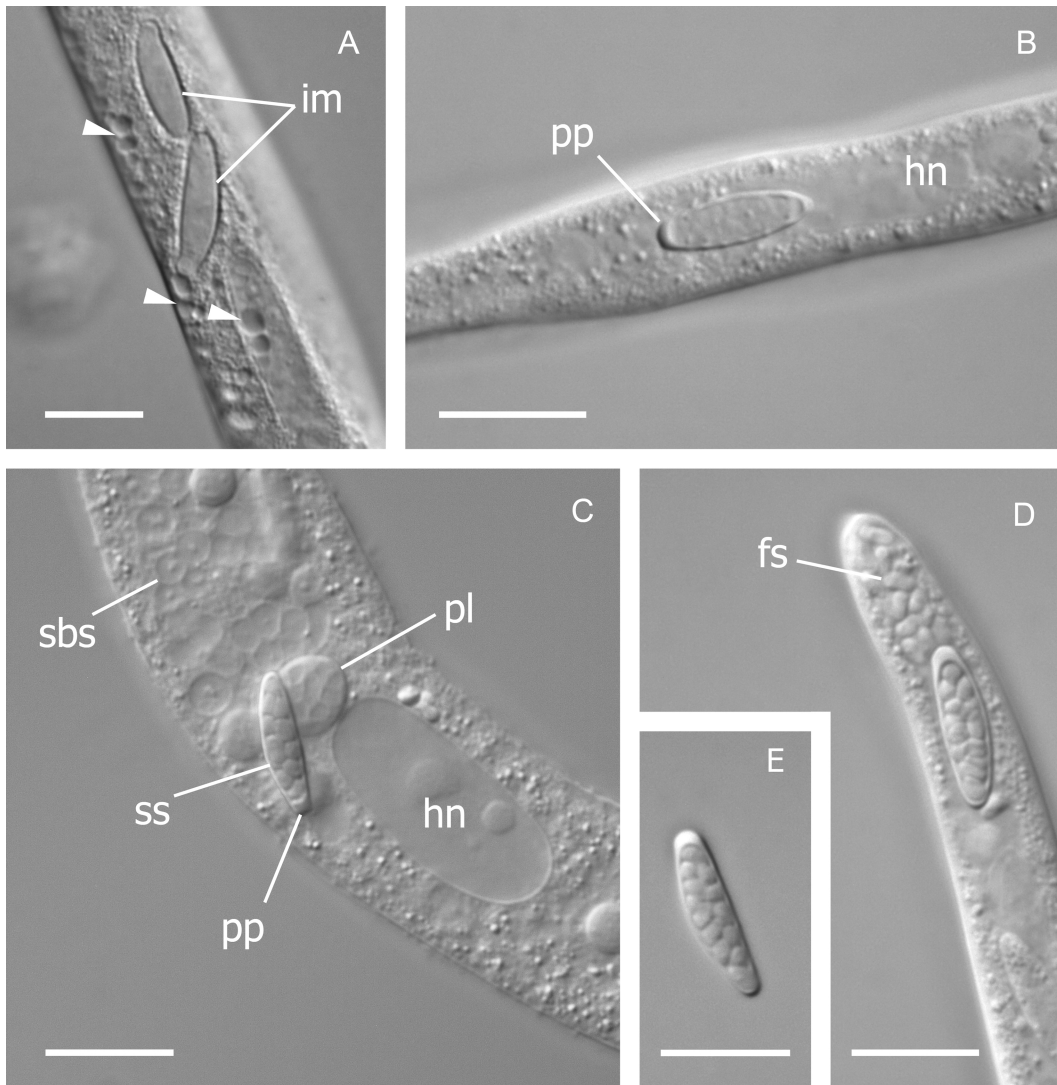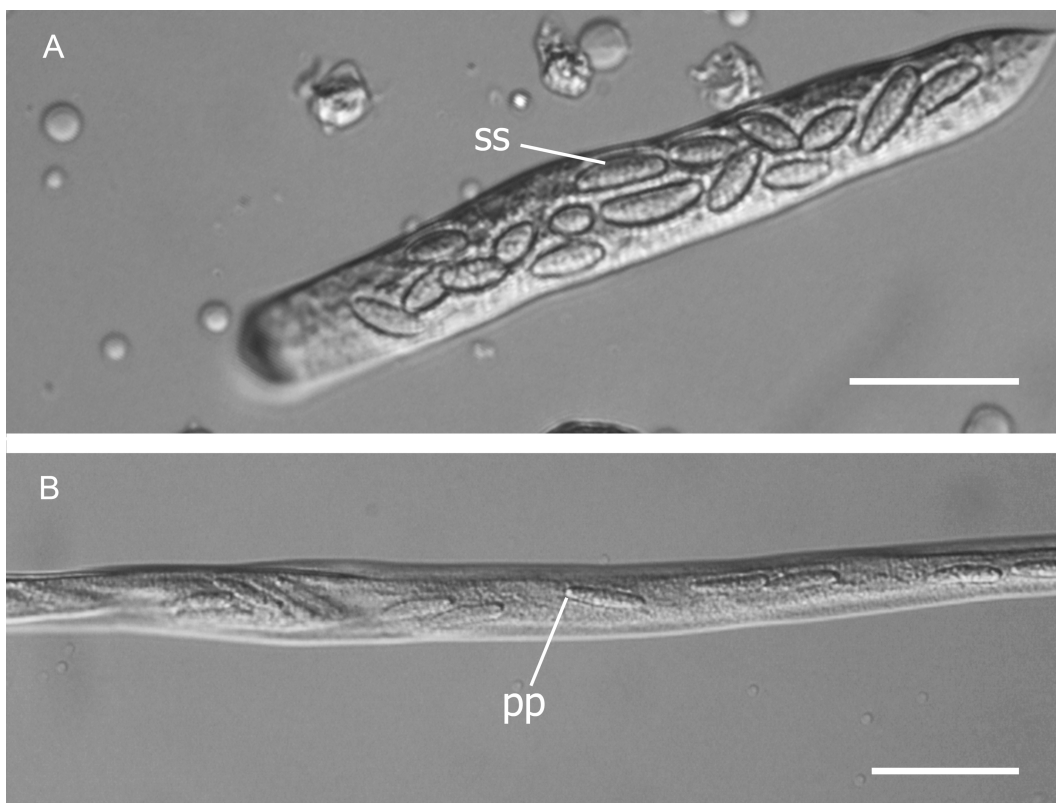
Fig. 1. Metchnikovellid *Mesnilia travisiae* TB2_b02_MMBI2020, a parasite of the archigregarine *Selenidium* sp. isolated from the polychaete *Travisia forbesii* collected in the Barents Sea, DIC. A, B – Early stages of infection and immature spore sacs; C – mature spore sacs and stages of free sporogony – plasmodium and sporoblasts; merged from two pictures taken at different focus depths; D – mature spore sac and free spores at the anterior end of the gregarine; E – an isolated mature spore sac of *M. travisiae*; note a polar plug at one pole of each spore sac. *Abbreviations*: im – immature spore sacs, pp – polar plug, hn – host nucleus, sbs – sporoblasts, pl – plasmodium, ss – spore sac, fs – free spores. Arrowheads point at early stages of sporogony. Scale bars: 10 μm.

irregularly oval, pear-shaped), sometimes slightly bent or curved spore sacs with one polar plug (Fig. 3). The spore sacs measured $8.9 - 11.1 \times 4.2 - 4.7$ μm in maximal dimension (avg = 9.9×4.4 μm, n = 8). They contained 8–12 spores per sac. Sac-bound spores were oval and measured $1.7 - 2.8 \times 1.3 - 1.5$ μm (avg = 2.2×1.4 μm, n = 14). Free spores were also oval and measured $2.3 - 3.2 \times 1.3 - 1.9$ μm (avg = 2.9×1.6 μm, n = 12). Both free spores and spore sacs seemed to be in direct contact with the host cytoplasm. Morphologically, it resembled

M. dogieli, but at the molecular level, these two metchnikovellids were very distant; the identity of SSU rRNA gene sequences was 65.5%.

SSU rDNA PHYLOGENY

The obtained phylogenetic reconstructions of Metchnikovellida based on the SSU rRNA gene is congruent with the results of phylogenetic analyses published previously (Mikhailov et al., 2017; Galindo et al., 2018; Frolova et al., 2021; Nassonova

Fig. 2. Metchnikovellid *Mesnilia travisiae* MT_WSBS2017, a parasite of the archigregarine *Selenidium* sp. isolated from the polychaete *Travisia forbesii* collected in the White Sea, DIC. Mature (A) and immature (B) spore sacs of *M. travisiae* in the cytoplasm of the archigregarines. *Abbreviations*: ss — spore sac, pp — polar plug. Scale bars: 30 μm.

et al., 2021; Frolova et al., 2022; Mikhailov et al., 2022): metchnikovellids are a sister to 'core' microsporidia. In the SSU rDNA phylogenetic tree (Fig. 4), metchnikovellids from archigregarines and eugregarines form a robustly supported clade which, together with the sequence of morphologically unidentified parasite from the blastogregarine *Siedleckia* cf. *nematoides* (GHVV01457926, here and further the accession numbers of sequences in GenBank are provided) (Mikhailov et al., 2022), groups into a moderately supported superclade.

Three clades of metchnikovellids were always recovered. One was fully supported by all methods and comprised two sequences of *Amphiacantha* spp. (KX214676, KX214677), environmental clone p1_44 (KX214678) and the sequence of *Metchnikovella spiralis* (MW344837), as was previously shown by Frolova et al. (2021, 2022). In most reconstructions, the sequences of *M. dobrovolskiji* (OP225322) and *M. incurvata* (OXFS01000707) branched close to this clade, although with low support (Fig. 4). These species always branched sequentially in Bayesian analyses, but formed a clade in most ML analyses. The statistical support for both these topologies was negligible. The second clade of metchnikovellids united two sequences of

Table 2. Size variation in spore sacs of *Mesnilia travisiae* parasitising archigregarines *Selenidium* sp. from *Travisia forbesii* collected in the White Sea (isolate MT_WSBS2017) and in the Barents Sea (isolate TB2_b02_MMBI2020).

Isolates	Length (μm)	Width (μm)	Average size ± SE (μm); n
MT_WSBS2017	9.0 – 17.6	4.4 – 5.9	13.1 ± 0,28 x 5.2 ± 0,05; 52
TB2_b02_MMBI2020	7.7 – 16.3	2.4 – 4.6	12.4 ± 0,28 x 3.3 ± 0,11; 36

Note: n – a number of measurements; SE – standard error of the mean.
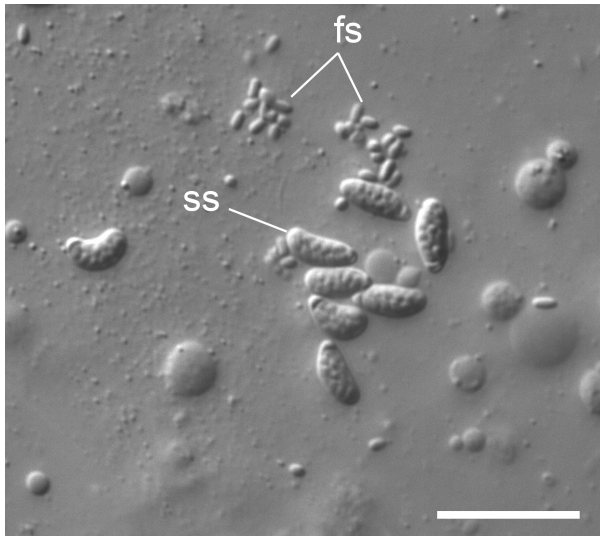
Fig. 3. Metchnikovellid isolate MD2_MMBI2021 ex *Selenidium pygospionis* from the polychaetes *Pygospio elegans* collected from the littoral zone of Zelenentskaya Bay of the Barents Sea, DIC. *Abbreviations*: ss — spore sac, fs — free spores. Scale bar: 20 μm.

*Amphiambly*s spp. (KX214672, KX214674) and the sequence of *Metchnikovella dogieli* (MT969020). This group had moderate support (BS = 73; PP = 0.95). The sequences of new isolates formed a third clade, which was moderately supported (BS = 76; PP = 1.0) and occurred to be a sister clade to the rest of metchnikovellids, except the basal lineage corresponding to an 'uncultured' parasite from *Siedleckia* cf. *nematoides*.

The SSU rRNA gene sequences of the metchnikovellid isolates from the White Sea (MT_WSBS2017) and Barents Sea (TB2_b02_MMBI2020) were almost identical (99.2% identity). Phylogenetic analysis showed that there was a mixed metchnikovellid infection in the infrapopulations of archigregarines *T. forbesii* polychaetes from Zelenetskaya Bay population. We found molecular evidence for the presence of a second metchnikovellid species (the sequence labelled TB2_b04_MMBI2020, Fig. 4), which remained uncharacterised yet at the morphological level. The sequence identity between this 'hidden' species and the isolates described above (MT_WSBS2017 and TB2_b02_MMBI2020) was 69.4%. This 'hidden' species branched together with a yet undescribed metchnikovellid parasitising *Selenidium pygospionis* from *Pygospio elegans* isolated in Zelenetskaya Bay

in 2021 (isolate MD2_b01_MMBI2021, Fig. 3). BUSCO PHYLOGENOMICS

To increase the resolution and robustness of phylogenetic reconstructions, we used the BUSCO-based phylogenomic analysis. The general topology of the phylogenomic tree corresponded to earlier published ones (Mikhailov et al., 2017; Galindo et al., 2018; Nassonova et al. 2021). Compared to the SSU rDNA tree, the clades of core microsporidia and metchnikovellids were fully supported (Fig. 5). Within the metchnikovellid clade, the grouping of *Amphiamblys* sp. and *Metchnikovella dogieli* was robustly supported. *M. incurvata* also branched together with them, like in earlier SSU rDNA trees with limited sampling of metchnikovellids (Frolova et al., 2021; Nassonova et al., 2021). The support for this branching was always high. New isolates studied in the present paper formed two clades. Both morphologically identified isolates MT_WSBS2017 and TB2_b02_MMBI2020 from *Travisia forbesii* grouped together. The yet hidden isolate TB2_b04_MMBI2020 from *T. forbesii* branched separately from other isolates from the same polychaete and grouped together with yet undescribed metchnikovellid MD2_b01_MMBI2021 parasitising *S. pygospionis* from *P. elegans*. Both these clades were fully supported.

Discussion

The metchnikovellid from *Travisia forbesii* de-picted here, has elongated spore sacs with one polar plug. Within the frames of the current taxonomy of Metchnikovellida, it clearly differs from the genera *Amphiamblys* and *Amphiacantha*. Based on the shape and size of spore sacs, it could be classified as a member of the morphologically heterogenous genus *Metchnikovella* Caullery et Mesnil,1914. Because of the presence of the polar plug only at one end of the spore sac, we can also consider it a member of the genus *Caulleryetta* sensu Larsson (2014). However, the latter genus is problematic and requires special consideration before classifying any new members in it.

The genus *Caulleryetta* was established by Dogiel in 1922 for a metchnikovellid, isolated from the archigregarine *Selenidium* sp. inhabiting the polychaete *Travisia forbesii*. This species, which he named *C. mesnili*, had pyriform spore sacs with a short thin neck ending in a plug, typically containing 8–12 spores. Dogiel (1922, p. 574) compared them
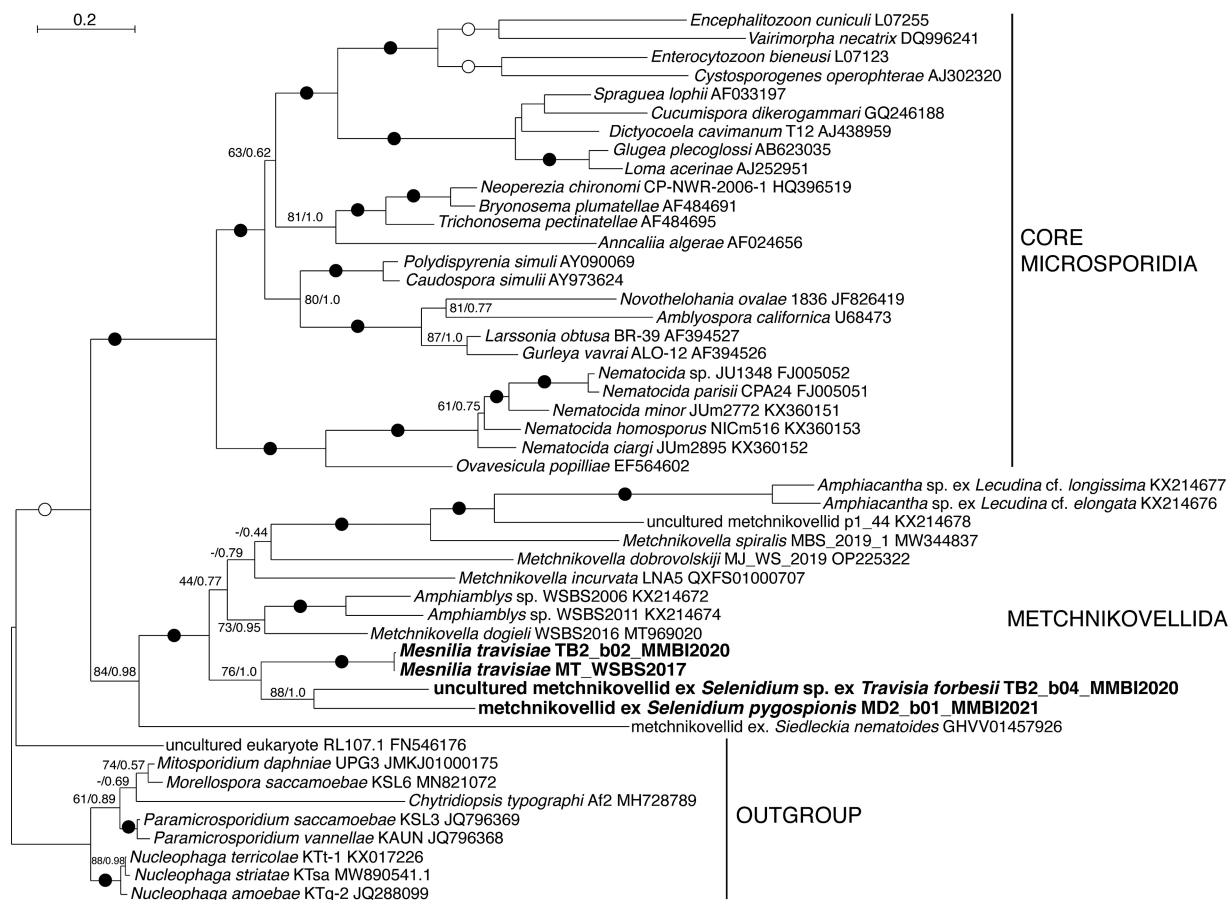
Fig. 4. SSU rDNA phylogeny of Microsporidia and related lineages including the sequences of *Mesnilia travisiae* and other isolates retrieved in this study (in bold). The tree was calculated using 1323 nucleotide positions. IQ-TREE (TIM3+F+I+G4 model; ultrafast bootstrap) / MrBayes (GTR model + gamma correction, 8 rate categories + covarion). Black dots indicate full support by all methods (bootstrap support [BS] ≥ 99%, posterior probability [PP] ≥ 0.99). Open circles correspond to BS ≥ 95% and PP ≥ 0.95.

with "an egg, elongated from the narrow end, or a small bottle". He provided description of young and mature spores sacs ('cysts') and several line drawings, but did not include a formal taxonomic diagnosis of the new genus and species. So, the genus *Caulleryetta* at that time was not formally established. Vivier (1975, p. 353) considered this genus as invalid and introduced a new combination "*Metchnikovella mesnili* (Dogiel, 1922)". Sprague (1977) also did not list *Caulleryetta* among metchnikovellid genera. However, in 1992 he provided English translation of selected sections of Dogiel's description (originally written in French) and composed a taxonomic summary for this genus, thus accepting its validity (Sprague, 1992, p. 310). Issi and Voronin (2007, p. 1017) provided a formal diagnosis of the genus *Caulleryetta* Dogiel, 1922 as "microsporidia with elongated oval sporophorous vesicles, one end of

which is rounded, and the other tapers like the neck of a bottle. Usually forms 5−10 sporophorous vesicles, located in a row before and after the nucleus of the host cell" (translated from Russian; the spore sacs characteristic of metchnikovellids are considered as one of the variants of sporophorous vesicles of microsporidia, therefore Issi and Voronin following Canning and Vavra (2000), called spore sacs as sporophorous vesicles).

No type material or slides of *Caulleryetta mesnili* was established by Dogiel. A careful search in the archives and collections at the Department of Invertebrate Zoology of Saint Petersburg University, where Dogiel worked, did not recover any additional data on this organism. So, the species *Caulleryetta mesnili* Dogiel, 1922 remains poorly studied and needs re-isolation, preferably from the type habitat (Strait Ekaterininskaya Gavan', Kola Bay, Barents
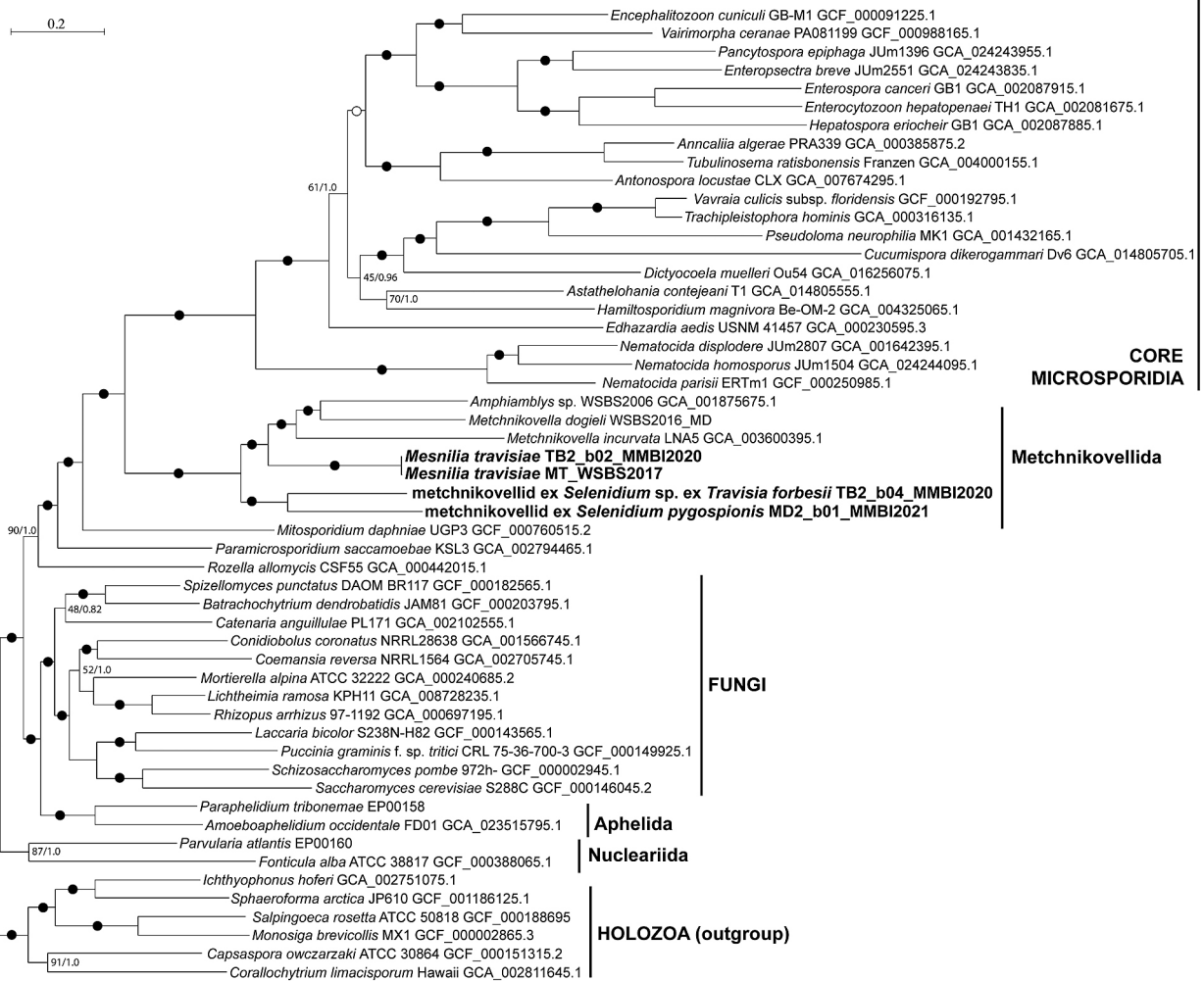
Fig. 5. Phylogenomic tree of Holomycota showing the position of a new species of metchnikovellids and other isolates retrieved in this study (in bold). The tree was reconstructed using a concatenated alignment ("supermatrix") prepared with a dataset of BUSCO single-copy protein domains (73 orthologs, 20783 amino acid positions) for 47 representatives of the Holomycota clade and 6 other Amorphea species used as an outgroup. The phylogeny was reconstructed using the maximum likelihood method (IQ-TREE v. 1.6.12, single partition, model LG+I+G4, ultrafast bootstrap) and Bayesian analysis (MrBayes 3.2.7a, GTR model, gamma-distributed rate variation across sites and a proportion of invariable sites). The support values are as follows: bootstrap values (BS, IQ-TREE), posterior probability (PP, MrBayes). Clades sharing full support gained with both methods (≥ 99% BS, ≥ 0.99 PP) are indicated by black dots. An open circle corresponds to BS ≥ 95% and PP ≥ 0.95.

Sea) and type host (polychaete *Travisia forbesii*).

Larsson (2014, p. 622-623) re-defined the borders of the genus *Caulleryetta*. He believed the number of plugs to be the primary character, and proposed the following diagnosis for the genus *Caulleryetta*: "Spore sacs oval, one end with a polar plug. Both sporogonies produce spores of the same shape. Spores almost spherical, slightly pointed over the polar sac" (Larsson, 2014, p. 622). He transferred to this genus all metchnikovellids with the single polar plug. As a result, six more species, formerly classified as members of the genus *Metchnikovella*, became members of the genus *Caulleryetta* (see Larsson, 2014, p. 623). According to his classification, only species forming spore sacs with two polar plugs remained within the genus *Metchnikovella*. He re-defined the latter genus as follows: "Spore sacs cylindrical or fusiform, more or less curved, with rounded ends containing polar plugs. Length not exceeding 10 times the width. Spores are oval. Both sporogonies produce spores of approximately the same shape" (Larsson, 2014, p. 621).
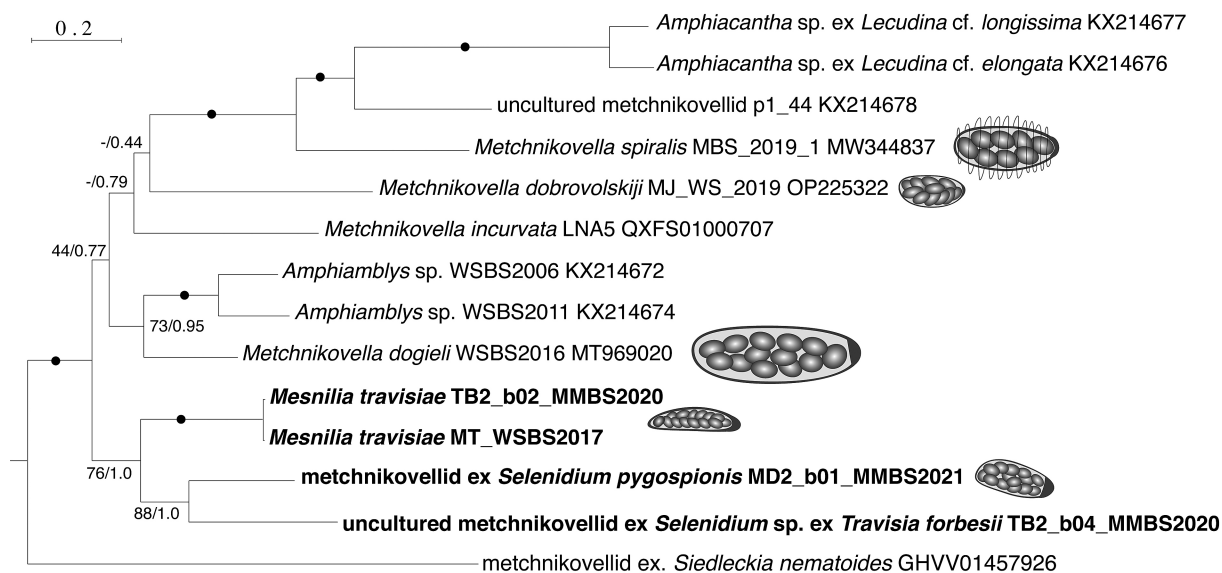
Fig. 6. Cut-off from the tree shown in Fig. 4, demonstrating the wide distribution and broad variation in shape and size of hyperparasites with *Caulleryetta*-like morphology (sensu Larsson, 2014) among the metchnikovellid lineages. Drawings of the spore sacs are to scale.

Molecular phylogeny did not support the revision proposed by Larsson (2014). It was shown that species with oval sacs having one polar plug are scattered throughout the phylogenetic tree of metchnikovellids and do not form a monophyletic clade (Frolova et al., 2021; Frolova et al., 2022) (Fig. 6). The species that, according to Larsson's definition, should be included in this genus, belong to different phylogenetic lineages of metchnikovellids. Thus, the available data suggest that the genus *Caulleryetta* sensu Larsson (2014) is an artificial group. It appears to be a paraphyletic assemblage. Moreover, the type species of this genus – *C. mesnili* Dogiel, 1922 differs in morphology of spore sacs and spores from all other metchnikovellids forming spore sacs with one polar plug (six species transferred by Larsson (2014) to the genus *Caulleryetta*: *C. berliozi, C. brasili, C. hovassei, C. nereidis, C. oviformis, C. wohlfarthi*, three species sequenced and described recently by us under the generic name *Metchnikovella*: *M. dogieli* (Paskerova et al., 2016), *M. spiralis* (Frolova et al., 2021) and *M. dobrovolskiji* (Frolova et al., 2022) and three isolates characterized in the present paper). In this situation, the most parsimonious solution seems to return to the initial definition of *Caulleryetta* as a monotypic genus (Dogiel, 1922; Sprague, 1992; Issi and Voronin, 2007) and return other species transferred by Larsson back to the genus *Metchnikovella*. In fact, it returns us to the classifications by Sprague (1992) and Issi and Voronin (2007).

The isolates that we described in the present paper are from the same host and super-host as *C. mesnili* Dogiel, 1922. However, in contrast to this species, they have elongated and slightly bent spore sacs with 12−14 spores, which appear to be slightly oval, not rounded. These spores and spore sacs resemble to some extent those of *M. dogieli* (Paskerova et al., 2016) and to a lesser extent those of *M. incurvata* (Sokolova et al., 2013), but certainly not those of *C. mesnili*.

All lifecycle stages of studied metchnikovellids from *T. forbesii* as well as *M. incurvata* and *M. dogieli* develop in direct contact with the host cytoplasm. However, unlike the metchnikovellids from *T. forbesii*, *M. incurvata* has fusiform, slightly incurved (boomerang-shaped) spore sacs, they are larger (22-27 μm versus 7.7-17.6 μm), possess two polar plugs and more spores per sac (up to 16 versus 12-14). Compared to *M. dogieli*, which also has slightly bent spore sacs (however, oval rather than elongated) and only one polar plug, the shape and size of the spore sacs of the studied isolates were more uniform. Regardless of their size or degree of maturity, the sacs of the studied isolates remain elongated with a slight flexion. This is probably caused by different structure of the sac wall and its rigidity between the compared hyperparasites. The spore sacs of metchnikovellid from *T. forbesii* are generally smaller and the size polymorphism is significantly less than in the case of *M. dogieli*. Among

sequenced metchnikovellids, *M. dobrovolskiji* is also characterized by spore sacs with 12 spores and one polar plug, however these sacs are irregularly oval, sometimes pear-shaped, both free spores and spore sacs are enclosed in vacuoles (Frolova et al., 2022); that sharply contradict with the morphological features of isolates described from *T. forbesii*.

Another unusual trait of the metchnikovellids from *T. forbesii* is that the stages of sac-bound sporogony may occur in the absence of free sporogony. It contradicts the current ideas about the life cycle of metchnikovellids. Typically, free sporogony precedes sac-bound one, resulting in the occurrence of gregarines with free spores or with both free spores and spore sacs (Frolova et al., 2023). In the studied metchnikovellids, the archigregarines, only with spore sacs were often seen. Early formation of spore sacs could be considered as an adaptation enabling fast production of thick-walled spore sacs, resistant to environmental conditions. It may be a situational response to unfavourable environmental conditions.

The unique morphological features of the isolates from *T. forbesii* are complemented by their independent position in the phylogenetic and phylogenomic trees.

There are the following variants for taxonomic placement of described here metchnikovellid from *T. forbesii*:

(1) to place it in the genus *Caulleryetta* using expanded definition by Larsson (2014). This choice appears to be weak, as we just discussed above the need to limit *Caulleryetta* back to Dogiel's description and diagnosis of Issi and Voronin. The studied metchnikovellid evidently does not fit this stringent definition;

(2) to describe it as one more *Metchnikovella*, thus increasing the heterogeneity of this genus and postponing the taxonomic problems for the future, until (maybe) more data on similar organisms will become available;

(3) to create a new genus for it, basing on its isolated phylogenetic position and clear differences from *Caulleryetta* sensu Dogiel. No one of species currently placed in the genus *Metchnikovella* is phylogenetically close to the isolates from *T. forbesii*. In this case we avoid adding more paraphyly to the assemblage called *Metchnikovella*. This solution seems to be preferable. Therefore, based on the morphological features and phylogenetic position of the studied isolates, we suggest the establishment of a new genus — *Mesnilia*, in honour of Félix Étienne Pierre Mesnil (1868−1938), a French zoologist, biologist, botanist, mycologist and algologist, one of the founders of research on the metchnikovellids. The type species for the new genus is named *Mesnilia travisiae* gen. nov., sp. nov. after the super-host, *Travisia forbesii*.

Our study highlights the existence of a 'hidden' metchnikovellid species even in well-studied hosts, as evidenced by molecular detection of uncultured metchnikovellid TB2_b04_MMBI2020 from the super-host *T. forbesii* and by discovery of isolate MD2_b01_MMBI2021 from *Pygospio elegans*. These findings are further evidence of widespread co-occurring infections of metchnikovellid in infrapopulations of gregarines from polychaetes (Sokolova et al., 2014; Frolova et al., 2023). The concept of the host specificity (also known as common assumption 'one host − one parasite') obviously does not work for metchnikovellids. As of now, no less than five species of metchnikovellids are known from the super-host *P. elegans*, of them, no fewer than three species (including MD2_b01_MMBI2021) parasitise the archigregarine *Selenidium pygospionis* and two species infecting the eugregarine *Polyrabdina pygospionis* (Frolova et al. 2023). Our study once again stressed this complication in isolating, studying and identifying hyperparasitic microsporidia.

We were specially searching for *Caulleryetta mesnili* in the locations at the Barents Sea during 2020−2023, but never isolated it. Theoretically, we cannot exclude that yet hidden and morphologically undescribed isolate TB2_b04_MMBI2020 from *T. forbesii* corresponds to *C. mesnili*. The isolate MD2_b01_MMBI2021, infecting *Selenidium pygospionis* in the polychaete *Pygospio elegans*, groups together with the above mentioned isolate TB2_b04_MMBI2020 in the phylogenomic tree (Fig. 5). The former isolate is not well-characterised yet at the morphological level, but from the available field images (Fig. 3) we cannot conclude that it is similar in morphology to Dogiel's *Caulleryetta*. It has oval, sometimes slightly bent or curved spore sacs with one polar plug, and only rarely irregularly oval, pear-shaped spore sacs were seen. This reduces, but does not completely exclude the chances that the still morphologically unstudied isolate TB2_b04_MMBI2020 from *T. forbesii* is *C. mesnili*.

The isolates described in this study (two isolates of new species *Mesnilia travisiae*, one yet hidden species from *T. forbesii* and a new isolate infecting *Selenidium pygospionis* from *Pygospio elegans*), form two new lineages in the tree of metchnikovellids. Despite the increment in the number of obtained sequences, the phylogenetic tree of

metchnikovellids based on the SSU rRNA gene sequences remains unstable. The phylogenomic analysis shows better resolution and results in highly supported tree; however, it still includes limited set of metchnikovellid taxa.

It is becoming evident that Metchnikovellida is a widely distributed and species-rich group of microsporidia. This stresses the need for further study of metchnikovellid diversity in order to improve phylogenetic analyses by adding more species and to study complex multilevel parasitic systems involving hyperparasites.

Taxonomic summary

Phylum Microsporidia Balbiani, 1882
Class Rudimicrosporea Sprague, 1977
Order Metchnikovellida Vivier, 1975
Genus *Mesnilia* gen. nov.

Diagnosis. Spore sacs are elongated, with one polar plug. Both sporogonies are in direct contact with host cytoplasm. Spore sacs non-accompanied by free spores may be observed in host cytoplasm.

Mesnilia travisiae sp. nov.

Diagnosis. Free spores are oval (1.5−2.8 × 0.8 −1.7 μm). Spore sacs are elongated with a slight flexion (7.7−17.6 × 2.4−5.9 μm), with rounded ends and a prominent polar plug at one end. Sac-bound spores counted 12−14 per sac. Sac-bound spores are rounded (1.3−2.5 × 0.9−1.6 μm).

Differences from closely related species. The species differs from other metchnikovellids by the combination of characters: the size and shape of the spore sacs, the number of spores per sac, the number of polar plugs, the super-host and host range. It exhibits significant differences in the SSU rRNA gene sequence and in protein-coding gene sequences.

Type locality. Zelenetskaya Bay of the Barents Sea (69°06'43.3"N 36°05'56.1"E). Subtidal zone.

Type habitat. Marine.

Type host and super-host. Archigregarine *Selenidium* sp. (Apicomplexa: Selenidiidae) from the polychaete *Travisia forbesii* (Annelida: Travisiidae).

Location in the host. Gregarine cytoplasm.

Type material. Images of the live archigregarines are stored in the image collection of the Department of Invertebrate Zoology, St Petersburg University. Frozen purified genomic DNA of the infected archigregarines as well as the individual infected

gregarine cells fixed in 96% ethanol are stored at the same department.

Etymology. This genus was named in honour of Félix Étienne Pierre Mesnil (1868−1938), one of the founders of the studies on metchnikovellids, French zoologist, biologist, botanist, mycologist and algologist. The species was named after the super-host, *Travisia forbesii*.

Gene sequences. SSU rRNA gene sequences of *M. travisiae* have been deposited in the GenBank under the accession numbers OR887354-OR887355

References

Bass D., Czech L., Williams B.A.P., Berney C. et al. 2018. Clarifying the relationships between Microsporidia and Cryptomycota. J. Eukaryot. Microbiol. 65: 773−782. https://doi.org/10.1111/jeu.12519

Becnel J.J., Takvorian P.M. and Cali A. 2014. Checklist of available generic names for Microsporidia with type species and type hosts. In: Microsporidia: pathogens of opportunity (Eds: Weiss L.M. and Becnel J.J.). John Wiley and Sons, Inc., Ames, Iowa, pp 671−686. https://doi.org/10.1002/9781118395264.app1

Bojko J., Reinke A.W., Stentiford G.D., Williams B. et al. 2022. Microsporidia: a new taxonomic, evolutionary, and ecological synthesis. Trend. Para-

sitol. 38: 642−659. https://doi.org/10.1016/j.pt.
2022.05.007

Cali A., Becnel J.J. and Takvorian P.M. 2017.
Microsporidia. In: Handbook of the protists. 2nd
ed. (Eds: Archibald J.M. et al.). Springer, Cham,
Switzerland, pp. 1559−1618. https://doi.org/10.
1007/978-3-319-28149-0_27

Canning E. and Vávra J. 2000. Phylum Micro-
sporida Balbiani, 1882. In: An illustrated guide to
the protozoa: organisms traditionally referred to as
protozoa, or newly discovered groups. Vol. 1, 2nd
ed. (Eds: Lee J.J. et al.). Allen Press, Lawrence,
Kan, pp. 39−126.

Capella-Gutiérrez S., Silla-Martínez J.M. and
Gabaldyn T. 2009. trimAl: a tool for automated
alignment trimming in large-scale phylogenetic
analyses. Bioinformatics. 25 (15): 1972−1973.
https://doi.org/10.1093/bioinformatics/btp348

Caullery M. and Mesnil F. 1897. Sur un type
nouveau (*Metchnikovella* n.g.) d'organismes para-
sites des Grégarines. C. R. Séances Soc. Biol. 4 (49):
960−962.

Caullery M. and Mesnil F. 1914. Sur les Met-
chnikovellidae et autres protistes parasites des Gré-
garines d'Annélides. C. R. Séances Soc. Biol. 2 (77):
527−532.

Caullery M. and Mesnil F. 1919. Metchniko-
vellidae et autres protistes parasites des Grégarines
d'Annélides. Ann. Inst. Pasteur. 33 (4): 209−240.

Dogiel V.A. 1922. Sur un nouveau genre de Met-
chnikovellidae. Ann. Inst. Pasteur. 36: 574−577.

Edgar R.C. 2004. MUSCLE: multiple sequence
alignment with high accuracy and high throughput.
Nucleic Acids Res. 32: 1792−1797. https://doi.org/
1093/nar/gkh340

Frolova E.V., Paskerova G.G., Smirnov A.V.
and Nassonova E.S. 2021. Molecular phylogeny and
new light microscopic data of *Metchnikovella spiralis*
(Microsporidia: Metchnikovellidae), a hyperparasite
of eugregarine *Polyrhabdina* sp. from the polychaete
Pygospio elegans. Parasitology. 148 (7): 779−786.
https://doi.org/10.1017/S0031182021000603

Frolova E.V., Paskerova G.G., Smirnov A.V.,
Nassonova E.S. 2022. *Metchnikovella dobrovolskiji*
sp. nov. (Microsporidia: Metchnikovellida), a pa-
rasite of archigregarines *Selenidium pygospionis* from
the polychaete *Pygospio elegans*. Protistology. 16 (3):
226−235. https://doi.org/10.21685/1680-0826-
2022-16-3-7

Frolova E.V., Paskerova G.G., Smirnov A.V.
and Nassonova E.S. 2023. Diversity, distribution,
and development of hyperparasitic microsporidia in
gregarines within one super-host. Microorganisms.
11 (1): 152. https://doi.org/10.3390/microorga-
nisms11010152

Galindo L. J, Torruella G., Moreira D., Tim-
pano H. et al. 2018. Evolutionary genomics of *Me-
tchnikovella incurvata* (Metchnikovellidae): an early
branching microsporidium. Genome Biol. Evol. 10:
2736−2748. https://doi.org/10.1093/gbe/evy205

Gouy M., Guindon S. and Gascuel O. 2010.
SeaView version 4: a multiplatform graphical user
interface for sequence alignment and phylogenetic
tree building. Mol. Biol. Evol. 27: 221−224. https://
doi.org/10.1093/molbev/msp259

Hoang D.T., Chernomor O., von Haeseler A.,
Minh B.Q. and Vinh L.S. 2018. UFBoot2: Impro-
ving the ultrafast bootstrap approximation. Mol.
Biol. Evol. 35: 518−522. https://doi.org/10.1093/
molbev/msx281

Issi I.V. and Voronin V.N. 2007. Phylum Mic-
rosporidia Balbiani 1882. In: Protista: handbook on
zoology. Pt 2. (Eds: Frolov A.O. and Krylov M.V.).
Nauka, St. Petersburg, pp. 994−1045 (in Russian
with English summary).

Katoh K., Standley D.M. 2013. MAFFT mul-
tiple sequence alignment software version 7: Im-
provements in performance and usability. Mol.
Biol. Evol. 30: 772−780. https://doi.org/10.1093/
molbev/mst010

Larsson J.I.R. 2014. The primitive Microspori-
dia. In: Microsporidia: Pathogens of opportunity. 1st
ed. (Eds: Weiss L.M. and Becnel J.J.). John Wiley
and Sons, Inc., Ames, Iowa, pp. 605−634. https://
doi.org/10.1002/9781118395264.ch24

Manni M., Berkeley M. R., Seppey M., Sim-
ão F. A. et al. 2021. BUSCO update: novel and
streamlined workflows along with broader and
deeper phylogenetic coverage for scoring of euka-
ryotic, prokaryotic, and viral genomes. Mol. Biol.
Evol. 38 (10): 4647−4654. https://doi.org/ 10.1093/
molbev/msab199

McGowan J. 2019. BUSCO phylogenomics
utility script. https://github.com/jamiemcg/BUS-
CO_phylogenomics

Mikhailov K.V., Simdyanov T.G. and Aleo-
shin V.V. 2017. Genomic survey of a hyperpara-
sitic microsporidian *Amphiamblys* sp. (Metchni-
kovellidae). Genome Biol. Evol. 9 (3): 454−467.
https://doi.org/10.1093/gbe/evw235

Mikhailov K.V., Nassonova E.S., Shishkin
Y.A., Paskerova G.G. et al. 2022. Ribosomal
RNA of the metchnikovellids in gregarine trans-
criptomes and rDNA of the microsporidia *sensu*

lato in environmental metagenomes. Biol. Bull. Rev. 12 (3): 213−239. https://doi.org/10.1134/S2079086422030069

Miller M. A., Pfeiffer W. and Schwartz T. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. In: 2010 Gateway Computing Environments Workshop (GCE). Presented at the 2010 Gateway Computing Environments Workshop (GCE), IEEE, New Orleans, LA, USA, pp. 1−8. https://doi.org/10.1109/GCE.2010.5676129

Nassonova E.S., Bondarenko N.I., Paskerova G.G., Kováčiková M. et al. 2021. Evolutionary relationships of *Metchnikovella dogieli* Paskerova et al., 2016 (Microsporidia: Metchnikovellidae) revealed by multigene phylogenetic analysis. Parasitol. Res. 120 (2): 525−534. https://doi.org/10.1007/s00436-020-06976-x

Nguyen L.-T., Schmidt H.A., von Haeseler A. and Minh B.Q. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum likelihood phylogenies. Mol. Biol. Evol. 32: 268−274. https://doi.org/10.1093/molbev/msu300

Nurk S., Bankevich A., Antipov D., Gurevich A.A. et al. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J. Comput. Biol. 20 (10): 714−737. https://doi.org/10.1089/cmb.2013.0084

Paskerova G.G., Frolova E.V., Kováčiková M., Panfilkina T.S. et al. 2016. *Metchnikovella dogieli* sp. n. (Microsporidia: Metchnikovellida), a parasite of archigregarines *Selenidium* sp. from polychaetes *Pygospio elegans*. Protistology. 10 (4): 148−157. https://doi.org/10.21685/1680-0826-2016-10-4-4

Ronquist F., Teslenko M., van der Mark P., Ayres D. L. et al. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61: 539−542. https://doi.org/10.1093/sysbio/sys029

Schrével J. and Desportes I. 2013. Biology of gregarines and their host-parasite interactions. Ch. 2. In: Treatise on zoology−anatomy, taxonomy, biology. The gregarines (Eds: Desportes I. and Schrével J.). Brill, Leiden, Boston, pp 25−195. https://doi.org/10.1163/9789004256057_004

Sokolova Y.Y., Paskerova G.G., Rotari Y.M., Nassonova E.S. and Smirnov A.V. 2013. Fine structure of *Metchnikovella incurvata* Caullery and Mesnil, 1914 (Microsporidia), a hyperparasite of gregarines *Polyrhabdina* sp. from the polychaete *Pygospio elegans*. Parasitology. 140: 855−867. https://doi.org/10.1017/S0031182013000036

Sokolova Y.Y., Paskerova G.G., Rotari Y.M., Nassonova E. S. and Smirnov A. V. 2014. Description of *Metchnikovella spiralis* sp. n. (Microsporidia: Metchnikovellidae), with notes on the ultrastructure of metchnikovellids. Parasitology. 141: 1108−1122. https://doi.org/10.1017/S0031182014000420

Sprague V. 1977. Classification and phylogeny. In: Comparative Pathobiology, Vol. 2. Systematics of the Microsporidia (Eds.: Bulla L. A. and Cheng T. C.), Plenum Press, New York, USA, pp. 1−30.

Sprague V., Becnel J.J. and Hazard E.I. 1992. Taxonomy of phylum Microspora. Crit. Rev. Microbiol. 18 (5−6): 285−395. https://doi.org/10.3109/10408419209113519

Vivier E. 1975. The microsporidia of the protozoa. Protistologica. 9 (3): 345−361.

Vivier E. and Schrével J. 1973. Étude en microscopie photonique et électronique de différents stades du cycle de *Metchnikovella hovassei* et observations sur la position systématique des Metchnikovellidae. Protistologica. 9: 95−118.

Weiss L.M. and Vossbrinck C.R. 1999 Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the Microsporidia. In: The Microsporidia and microsporidiosis (Eds: Wittner M. and Weiss L.). Amer. Soc. Microbiol., Washington D.C., pp 129−171. https://doi.org/10.1128/9781555818227.ch4