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MORPHOLOGY, MOLECULAR DATA AND DEVELOPMENT OF *ZSCHOKKELLA MUGILIS* (MYXOSPOREA, BIVALVULIDA) IN A POLYCHAETE ALTERNATE HOST, *NEREIS DIVERSICOLOR*

Luís F. Rangel, Maria J. Santos*, Gábor Cech†, and Csaba Székely†

Universidade do Porto, Faculdade de Ciências, Departamento de Zoologia-Antropologia, Rua do Campo Alegre, s/n. Edifício FC4, 4169-007 Porto, Portugal, and CIMAR Laboratório Associado/CIIMAR, Centro Interdisciplinar de Investigação Marinha e Ambiental, Universidade do Porto, Rua dos Bragas, 289, 4050-123 Porto, Portugal. *e-mail: mjsantos@fc.up.pt*

ABSTRACT: The morphology of *Zschokkella mugilis* Sitjà-Bobadilla and Alvarez-Pellitero, 1993 (Myxosporea, Bivalvulida) in *Nereis diversicolor* O. F. Müller, 1776 is described for the first time. The molecular data show that the actinospore has 100% similarity with the myxospore of *Z. mugilis*. Fully mature actinospores are tri-radiate, the spore body has a small process, and the sporoplasm has 2 inner daughter cells. In the polychaete, the spores of the parasite develop in groups of 8 inside pansporocysts. The schizogony phase takes place in the intestinal epithelium, while gametogony and sporogony occur in the coelom of the polychaete. Observations indicate that mature spores are only released during the polychaete reproductive season. Infection was detected only in the winter and spring. In the Aveiro estuary (Portugal), the overall prevalence of infection of the polychaete was 0.5%.

Since the discovery of the life cycle of a myxozoan by Wolf and Markiw (1984), studies on this group of parasites have been aimed at freshwater species that use oligochaetes as invertebrate hosts (Lom and Dyková, 2006). However, it was suggested that, in the marine environment, polychaetes would be the best candidates to act as invertebrate hosts for marine species of Myxozoa (Køie, 2000, 2002). From those myxozoan life cycles already described, only 3 are marine species, i.e., *Ellipsomyxa gobii* Køie, 2003, that uses the polychaetes *Nereis diversicolor* O. F. Müller, 1776 and *N. succinea* Frey and Leuckart, 1847, as invertebrate hosts (Køie, 2000; Køie et al., 2004), *Gadimyxa atlantica* Køie, Karlsbakk and Nylund, 2007, which infects the polychaetes *Spirorbis* spp. (Køie et al., 2007), and *Ceratomyxa auerbachi* (Noble, 1950) that employs the polychaete *Chone infundibuliformis* Krøyer 1856, as invertebrate host (Køie et al., 2008).

In the marine environment, 2 other, still unidentified, myxozoan species are hosted by polychaetes (Køie, 2002, 2005). In freshwater, there are also 2 myxozoan species that occur in polychaetes, i.e., *Ceratomyxa shasta* Noble, 1950 (Bartholomew et al., 1997) and *Parvicapsula minibicornis*, Kent, Whitaker and Dawe, 1997 (Bartholomew et al., 2006), both reported in *Manayunkia speciosa* Leidy, 1858. Their life cycles are known.

The parasite of focus in the present study, *Zschokkella mugilis* Sitjà-Bobadilla and Alvarez-Pellitero, 1993, is a myxozoan originally described among fishes of the Mugilidae. In the Mediterranean Sea, *Z. mugilis* infects *Mugil cephalus* Linnaeus, 1758, *Liza ramado* (Risso, 1810) (=*M. capito* Cuvier, 1829), and *L. saliens* (Risso, 1810) (Sitjà-Bobadilla and Alvarez-Pellitero, 1993). These are euryhaline fishes found in estuaries (Cardona, 2000).

The main aim of the present work is to describe the morphology of the actinosporean phase of *Z. mugilis*, to present its molecular data, and to report on its development inside the invertebrate host, *N. diversicolor*. Moreover, data on the prevalence of infection and its seasonal variation in Aveiro estuary (Portugal) are also provided.

MATERIALS AND METHODS

Actinosporean survey

From January to October 2007, 1,048 specimens of *Nereis diversicolor* were collected by bait diggers from the Aveiro estuary (Portugal) (40º40'N, 8º45'W). The polychaetes were wrapped inside newspaper sheets with wet algae and kept refrigerated at 5-8 C. Under these conditions, they survive for at least 1 wk.

An examination for actinosporeans was performed using the coelomic fluid of *N. diversicolor*. Since the objective of this study was to follow the development of the spores within the polychaete, an innovative observation method, capable of preserving the host's life while still identifying the infected worms, had to be adopted. We believe that the new method for finding and isolating actinosporeans may be useful for other parasite surveys of polychaetes. In larger polychaetes, the coelomic fluid near a parapodium was obtained with the help of a hypodermic needle and syringe, while in smaller individuals 1 or 2 posterior segments were cut off and a drop of fluid placed on a slide. The coelomic fluid was then examined using a microscope, in a fresh mount with a drop of salt water (15‰ salinity).

The polychaetes were sexed by the examination of gametes. The maxillae of infected polychaete were collected and measured according to Olive and Garwood (1981).

Actinosporean development and morphology

In order to follow the actinosporean development, an infected polychaete was placed in quarantine. The polychaete was kept in a large Petri dish (15 cm in diameter), with 15‰ artificial salt water (Tropic Marin, sea salt**,** Wartenburg, Germany) and a small amount of sand. The sand helped the polychaete to remove the excess mucus from its surface and avoid body fragmentation. The water was exchanged every 3 or 4 days and the polychaete was fed with fish food flocks (Tetra, Anivite, S.A., Ribatejo, Portugal). The ambient air temperature was regularly measured and maintained at 21-24 C. Coelomic fluid of the quarantined polychaete was collected every 3 or 4 days. The actinosporean morphology was periodically examined and photographed using a microscope (Zeiss Axiophot, Grupo Taper, Sintra, Portugal) with differential contrast interference (DIC) and equipped with a Zeiss Axiocam Icc3 digital camera. The image analysis was performed with the software Axiovision 4.6 (Grupo Taper, Sintra, Portugal). Morphological descriptions and measurements were made according to Lom et al. (1997). Measurements of pansporocysts and actinospores were taken from fresh material as indicated in Figures 1 and 2. Mean values and their standard deviations are given in micrometers. For molecular analysis, coelomic spores released into the water by the quarantined polychaete were collected and preserved in 99.8% ethanol.

For comparative purposes, histological studies were conducted using both infected and uninfected polychaetes. Tissue samples of *N. diversicolor* were fixed with Davidson's sea-water fixative for 24 hr, and preserved in 70% ethanol, after which they were processed for histology and stained with hematoxylin and eosin (H & E) and Giemsa.

Molecular analysis

For DNA extractions, samples preserved in ethanol were centrifuged at 5,000 *g* for 5 min to pellet the myxospores, and then the ethanol was removed. The DNA was extracted using a QIAGEN DNeasy^{TM} tissue kit (Qiagen, Hilden, Germany) and eluted in 50 µl of buffer AE.

The 18S rDNA was amplified using the primers 18e and 18g’ (Hillis and Dixon, 1991) in a 25 µl reaction mixture, which comprised 1 µl extracted genomic DNA, 5 µl 1 mM deoxyribonucleotide triphosphates (dNTPs, MBI Fermentas, Burlington, Ontario, Canada), 0.25 µl of each primer, 2.5 µl 10X Taq buffer (MBI Fermentas), 1.25 µl 25 mM $MgCl_2$, 1 µl Taq polymerase (2 U) (MBI Fermentas), and 12 µl DEPC water. The PCR cycle consisted of an initial denaturation step of 94 C for 4 min, followed by 35 cycles of 94 C for 50 sec, 56 C for 50 sec, 72 C for 80 sec and finished with terminal extension at 72 C for 7 min, then rested at 4 C.

This was followed by a second round of PCR with the ACT1F-MYX4R primer pair (Hallet and Diamant, 2001). The total volume of the nested PCR reactions was 50 µl, which contained 1 µl amplified DNA, 10 µl 1 mM deoxyribonucleotide triphosphates (dNTPs, MBI Fermentas), 0.5 µl of each primer, 5 µl 10X Taq buffer (MBI Fermentas), 2.5 µl 25 mM $MgCl_2$, 2 µl Taq polymerase (2 U)(MBI Fermentas), and 28.5 µl DEPC water. Amplification conditions in the second round were: 94 C for 50 sec, 52 C for 50 sec, 72 C for 60 sec for 35 cycles, and the cycle was terminated with an extension period at 72 C for 10 min, and then rested at 4 C. Both PCR cycles were performed in a PTC-200 thermocycler (MJ Research, Waltham, Massachusetts). The PCR products were electrophoresed in 1.0% agarose gels in Tris-acetate-EDTA (TAE) buffer gel stained with 1% ethidium bromide and then purified with the PCR-M^{TM} Clean Up System (Viogene, Taipei, Taiwan).

Purified PCR products were sequenced in both directions with primers mentioned above using the ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyser (Applied Byosystems, Foster City, California).

The forward and reverse sequence segments were aligned in BioEdit (Hall, 1999) and ambiguous bases clarified using corresponding ABI chromatograms. Nucleotide sequences were aligned with the software CLUSTAL W (Higgins et al., 1994). The alignment was corrected manually using the alignment editor of the software MEGA 4.0 (Tamura et al., 2007). DNA sequence similarities were calculated with the Sequence Identity Matrix of the software BioEdit (http://www.mbio.ncsu.edu/BioEdit/BioEdit.html).

RESULTS

Prevalence of infection in *N. diversicolor*

Actinospores and actinosporean developmental stages of *Z. mugilis* were found in infected *N. diversicolor*. The early stages (multinucleate and eventually uninucleate cells) were found in the intestinal epithelium (Fig. 3). All the other forms were found in the coelomic fluid (Fig. 4). Only 5 of 1,048 worms were infected (prevalence of 0.5%). Infected worms were all males or of undefined sex, with maxillae ranging from 1.7 to 2.5 mm in length. The seasonal prevalence was 3.2% for winter (January-March; n=126), 0.4% for spring (April-June; n=231) and 0% for summer (July-September; n=528) and autumn (October-December; n=163). In terms of pathology, no visible change of host morphology was detected, and only a continuous swelling of the body was noticed during the period of infection; however, this coincided with the sexual maturity.

Actinosporean development and morphology

One infected polychaete was examined regularly for 28 days. The worm was captured on 11 April 2007 in Aveiro estuary and the infection was initially detected on 16 April. The earliest stages observed in the coelomic fluid were round-shaped cells, 12 to 13 µm in diameter (Fig. 5A). Other cells were observed in division (Figs. 5B, 5C). These cells corresponded to the end of schizogony and the beginning of gametogony.

After 3 days, the coelomic fluid was filled with pansporocysts, all in the same state of development (Fig. 5D). The developmental stages previously observed on the first day were rarely seen. In this phase, the pansporocysts possessed 10 cells; 2 were large cells and 8 were smaller.

By the seventh day, all pansporocysts were in sporogony. Pansporocysts had 8 zygotes (Figs. 5E, 5F). Each zygote was undergoing development (Fig. 5E), with 3 peripheral cells present (those will form the capsulogenic and valvogenic cells) and 1 central cell (forming the sporoplasm-mother cell).

On the tenth day, the coelomic fluid was filled with pansporocysts possessing 8 actinospores in a more advanced stage of development (Fig. 5G). At that point, some pansporocysts were still in the same phase as described for the seventh day, showing some signs of asynchrony in pansporocyst development.

Most pansporocysts were in an advanced developmental stage by day 14 (Figs. 6A, 6B). Actinospores had reached their typical form by this time, and the 3 polar capsules were already visible (Fig. 6B).

On day 17, the coelomic fluid was filled with mature pansporocysts and some free actinospores (Fig. 6C). Some of the actinospores had already released their polar filaments spontaneously.

The coelomic fluid was filled with mature pansporocysts and a large number of free actinospores on day 21 (Fig. 6D). The round-shaped cells observed at the beginning of the quarantine period (Figs. 5A, 5C) reappeared again in the coelomic fluid.

On day 24, the coelomic fluid was filled with mature pansporocysts and a large number of free actinospores. However, other pansporocysts were still in their initial phase of development, i.e., gametogony (Fig. 6E).

On day 28, the infected polychaete died. Until that point, its body had been very swollen, but it suddenly became very slim, with an intense dark-green coloration. At that time, the water in the Petri dish was filled with mature spermatozoa, immature pansporocysts, and a large number of mature actinospores (Fig. 6F). No release of spores in the water was detected before that point. In total, the infected *N. diversicolor* was in quarantine for 28 days; it had completed 1 infection cycle (which took at least 21 days in the coelomic cavity) and started a second.

Actinospore morphology

Spores were elongated, with a swelling in the capsule area, narrowing towards the opposite end, and possessed a slightly bent process (Fig. 6F; Table I). The mean size (spore body and process) was 17.3 ± 0.9 μm in length, and 8.8 ± 0.4 μm wide. The spore body measured 11.2 ± 0.6 μm in length and the process 6.1 ± 0.9 μm in length. The polar capsules were rounded, 3.4 ± 0.2 μm in diameter. In apical view, the polar capsules have a triangular arrangement (Fig. 6E). The polar filament had 5 or 6 coils; the fully distended polar filaments were 46-47 µm in length. The sporoplasm contained 2 inner daughter cells. They did not form nets. Suture lines were not observed. The development of spores occurs inside pansporocysts in groups of 8. The pansporocysts were elliptical in form, with a more or less median constriction (Figs. 6C, 6D; Table II). Mature pansporocysts measured 39.8 ± 1.1 µm in length and were 22.8 ± 0.6 µm wide. Some rare pansporocysts were round, without constriction, but still had 8 spores developing normally (Fig. 6A).

Molecular analysis

The 820 bp long fragment of the 18S rDNA sequence (EU867770) of our sample showed 99.5% similarity to *Ellipsomyxa gobii* (AY505126). There were 4 positions where substitutions were found.

In contrast, there was a 100% match with the gene sequence of *Z. mugilis* available in Genbank (AF411336). Strictly speaking, this similarity can only be revealed by thorough manual comparison of the sequence of *Z. mugilis.* Specifically, the *Z. mugilis* sequence available in GenBank contains 4 undetermined bases; therefore, only 99.5% similarity could be found without manual (visual) comparison of the 4 ambiguous positions (see Fig. 7). However, even when the 4 undetermined positions were included in the comparison, what we found fully corroborates the identification of *Z. mugilis.* Indeed, examining the alignment and the sequences of related species, it can be concluded that these positions are all in conservative regions. Moreover, the undetermined nucleotides could correspond to the nucleotides of the sequence of our material since, in every questionable position, 1 of the 2 possible nucleotides agrees with the aligned nucleotide base of the isolated actinospore.

DISCUSSION

Despite the 4 undetermined base pairs present in the available Genbank (AF411336), the alignment allowed us to definitively identify the actinospore isolated from *N. diversicolor* as the myxosporean *Z. mugilis*. Moreover, the morphological features of *Z. mugilis* actinospores and pansporocysts are very similar to those described for *E. gobii* in Denmark (Køie, 2000; Køie et al., 2004). This is consistent with the observation that *E. gobii* and *Z. mugilis* are also very closely related species, i.e., the difference between their 18S rDNA sequences (AF411336 and AY505126) is only 0.5%. In addition, phylogenetic analyses consistently indicate that the 2 species are grouped together (Fiala, 2006; Holzer et al., 2007).

In fact, these 2 species are so similar that they can be easily confused with one another. Fortunately, detailed observations in the present study showed that the actinospore morphology of *Z. mugilis* possesses a spore body process that is absent in *E. gobii*, a narrower body of 8.8 ± 0.4 µm in width and 10-12 µm in *E. gobii*, and a slightly longer polar filament of 46-47 µm and 32 µm in *E. gobii*. In addition, our genetic studies showed a 4 base pair difference, which allows differentiation of the 2 species.

However, an important conclusion stemming from the present observations is that both the genetic and morphological similarities between the actinosporean developmental stages of the 2 species (see figures in Køie, 2000 and Køie et al., 2004) are exceptionally high, strongly suggesting that a taxonomic revision is justified. The current placement of these 2 species in separate genera may prove incorrect following appropriate analysis.

Actinosporean developmental stages of *Z. mugilis* were found in the intestinal epithelium and in the coelomic spaces of infected *N. diversicolor*. The cells of the schizogony phase were found in the intestinal epithelium. All the other developmental stages were found in the coelomic space. In these polychaetes, and in marine oligochaetes except *C. shasta* (Bartholomew et al., 1997), actinosporean development has been described as occurring mainly in the host's coelomic space (Hallet, O'Donoghue, and Lester, 1998; Hallet, Erseus, and Lester, 1999; Køie, 2000, 2002; Køie et al., 2004; Bartholomew et al., 2006), something that is also now shown for *Z. mugilis* in *N. diversicolor*.

The first development phase in *N. diversicolor* is unknown, as is the period of time spent in the intestinal epithelium. The development of actinospores in the coelomic cavity took 21 days at temperatures ranging from 21 to 24 C (Fig. 8). Once started, cyclical development of this species in *N. diversicolor* continued until the polychaete's breeding season, at which time infected polychaetes succumbed. We did not observe any release of free actinospores throughout the quarantine period. In fact, mature actinospores were released only when the worm died, when it simultaneously released mature spermatozoa.

This coincidence of events suggests that the release of mature actinospores is connected with the breeding season of the host. *Nereis diversicolor* is a monotelic species, i.e., the polychaete dies immediately after spawning (Andries, 2001). In the breeding season, the ripe females deposit their eggs inside their galleries and the ripe males release their sperm into water near the openings of the female galleries (Bartels-Hardege and Zeeck, 1990). Sperm are released as the body of the male disintegrates (Scaps, 2002). It appears that, while gametes of the host mature, actinospores accumulate in the body of the host until it disintegrates during the breeding season, then releases spores and gametes.

Zschokkella mugilis in *N. diversicolor* exhibited a prevalence of 0.5% in 1,048 specimens examined during the study. Curiously though, only males and immature worms were infected. The infection was found either in small individuals (maxillae 1.7 mm in length) or in large ones (maxillae 2.5 mm in length) in a range of worms with maxilla sizes varying between 0.4 to 3.4 mm (Abrantes et al., 1999). The seasonal variation in prevalence showed that parasite recruitment occurred in winter (3.2%)

and spring (0.4%). *Nereis diversicolor* has 2 breeding periods in Aveiro estuary, one in March/April and another in September/October (Abrantes et al., 1999); it, therefore, appears that the development of this myxosporean species is somehow correlated at least with the first breeding period of *N. diversicolor*.

Zschokkella mugilis is also known to infect mugilid fishes (Sitjà-Bobadilla and Alvarez-Pellitero, 1993). The Aveiro estuary serves as a habitat for 6 mugilid species; these mugilids include *M. cephalus*, a resident species, and *L. ramado* and *L. saliens*, 2 migrant species (Pombo et al., 2007). Mugilids are euryhaline fishes and, in the case of *M. cephalus*, the juveniles of this species mature in brackish waters of the North Atlantic (Cardona, 2000). *Nereis diversicolor* is also a euryhaline species, and is more abundant in shallow waters with intermediate levels of salinity (Abrantes et al., 1999). There is, therefore, an overlapping habitat requirement by the myxozoan parasite and its mugilid host.

Until now, only 3 marine myxozoan life cycles, all involving polychaetes, have been reported in the literature, i.e., for *E. gobii* (Køie et al., 2004), *Gadimyxa atlantica* (Køie et al., 2007), and *C. auerbachi* (Køie et al., 2008). The present work adds the description of a fourth marine myxozoan life cycle.

The results reported here also raise new problems for the survey of actinospores in invertebrate hosts. The method developed by Yokoyama et al. (1991), which is very useful in acquiring actinospores from oligochaete hosts, can not be applied to *N. diversicolor*. On the one hand, the maintenance of this species in the lab is more demanding because of its large size. On the other hand, if the release of actinospores is associated with the host's death, the use of the cell-well plate method for these polychaetes will be useless. Extracting coelomic fluid with the help of a hypodermic needle and syringe, or cutting off 1 or 2 posterior segments in younger individuals, will be an innovative, useful, and practicable method for monitoring actinospore development step by step, once we are able to keep the polychaete alive in the laboratory, with little disturbance, for several wk at a time.

Recently, efforts have been directed in fish farms to raise polychaetes, especially *N. diversicolor*, in conjunction with the fish. The idea is to use the aquatic systems already in use for the fishes and to add the polychaete cultures (Fidalgo-e-Costa, 1999; Fidalgo-e-Costa and Cancela-da-Fonseca, 2000). In many cases, the residues of food not ingested by the fishes and even their faeces can be directly used as food for polychaetes (Batista et al., 2003). If we consider that myxozoan parasites only need the simultaneous presence of fish and annelids to complete their life cycle, the polyculture of these 2 groups of animals could produce severe infections, which could surely perpetuate the parasite's life cycle under such conditions. Great vigilance and care must, therefore, be employed to prevent the establishment of the parasite.

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FIGURE 1. Measurements of pansporocysts. Abbreviations: Tl, pansporocyst total length; Pl1 and Pl2, partial length; Pw1 and Pw2, pansporocyst width; Cl: constriction length.

FIGURE 2. Measurements of actinospores. Abbreviations: Tl, actinospore total length; Sbl, spore body length; Sbw, spore body width, Pl, processes length; Pw, processes width.

FIGURE 3. Histological section of the intestinal epithelium of *Nereis diversicolor* infected with actinospores of *Zschokkella mugilis*. The arrows show earliest stages of *Z. mugilis*. Stained with Giemsa. Scale bar = 10 µm.

FIGURE 4. Histological section of *Nereis diversicolor* infected with actinospores of *Zschokkella mugilis*. The arrows show pansporocysts of *Z. mugilis* developing in the coelomic fluid between the muscle tissues and the intestine. Stained with H. & E. Scale bar = 20 µm.

FIGURE 5. Developmental stages of *Zschokkella mugilis* in the coelomic fluid of *Nereis diversicolor*. (**A-C**) The earliest stages observed on the first day of actinosporean detection. (**D**) Pansporocysts observed after three days. (**E**) Isolated zygotes on the seventh day. (**F**) Pansporocyst on the seventh day. (**G**) Pansporocysts observed on the tenth day. Scale bars = 10 µm.

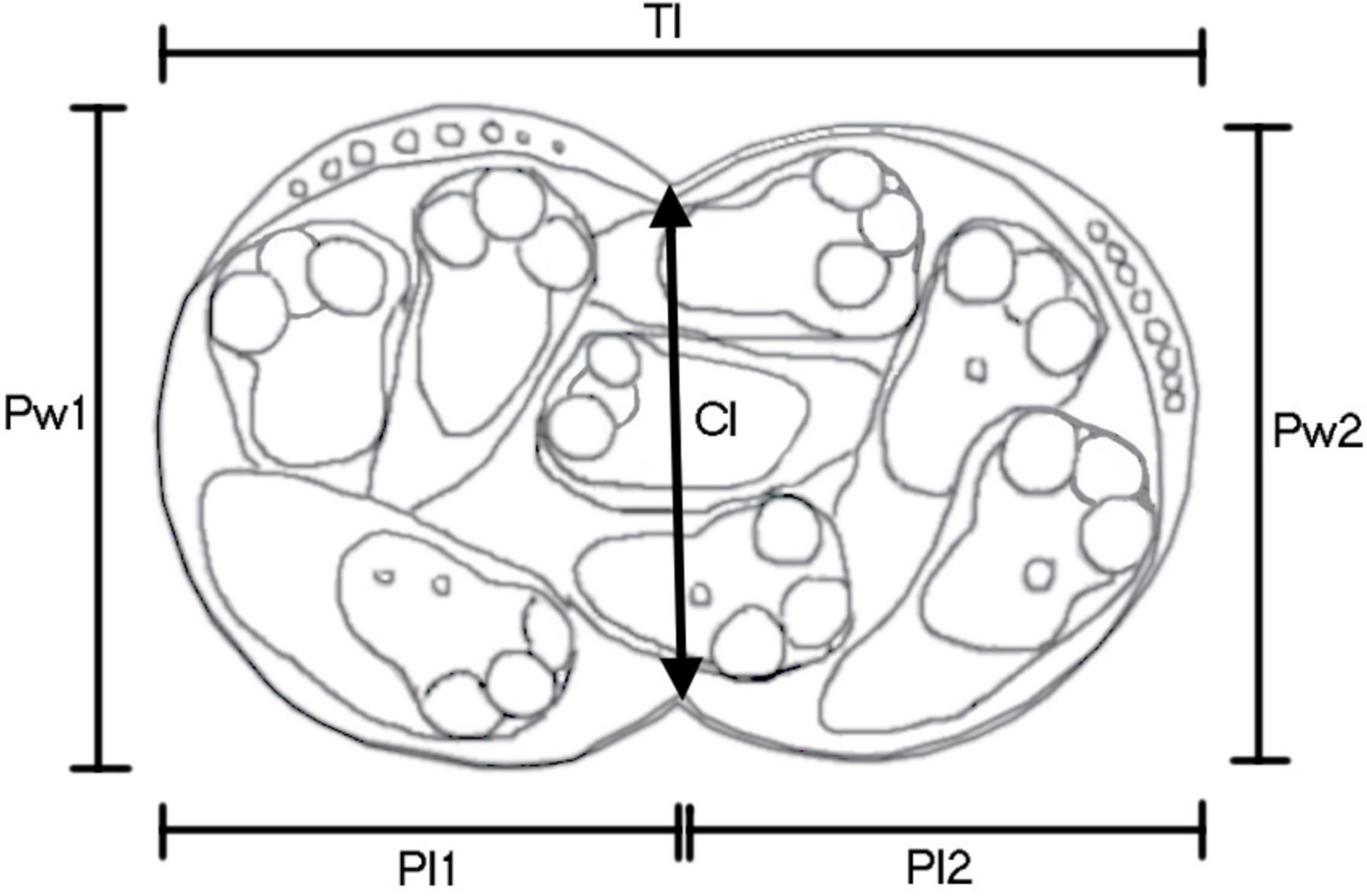
FIGURE 6. Developmental stages of *Zschokkella mugilis* in the coelomic fluid of *Nereis diversicolor*. (**A**) Pansporocyst observed on the fourteenth day. A rare pansporocyst lacking the typical median constriction. (**B**) Isolated immature actinospores on the fourteenth day. (**C**) Pansporocyst on the seventeenth day. (**D**) Pansporocyst observed on the twenty-first day. (**E**) Immature pansporocysts observed on the twenty-fourth day and mature actinospores. (**F**) Mature actinospores free in water, released after the death of *N. diversicolor* in quarantine. Scale bars = 10 µm.

FIGURE 7. 18S rDNA alignment containing the actinospore sample, *Zschokkella mugilis* and *Ellipsomyxa gobii*. The sections of alignment without substitutions were excised, the variable positions are highlighted with grey background. The scientific notation of the present undetermined nucleotides: M = A or C, R = A or G, Y = C or T.

FIGURE 8. Development of *Zschokkella mugilis* in *Nereis diversicolor* (schematic diagram). (**A**) Myxospores of *Z. mugilis*. (**B**) *Nereis diversicolor* infection and the first actinosporean development in the host's intestinal epithelium. (**C-H**) Actinosporean development in the host's coelomic cavity. (**C-D**) Gametogony phase. (**E-H**) Sporogonic phase. (**H**) Free actinospores.

*To whom correspondence should be addressed.

†Fish Pathological Research Team, Veterinary Medical Research Institute,
Hungarian Academy of Sciences 1143, Budapest, Hungária krt. 21, Hungary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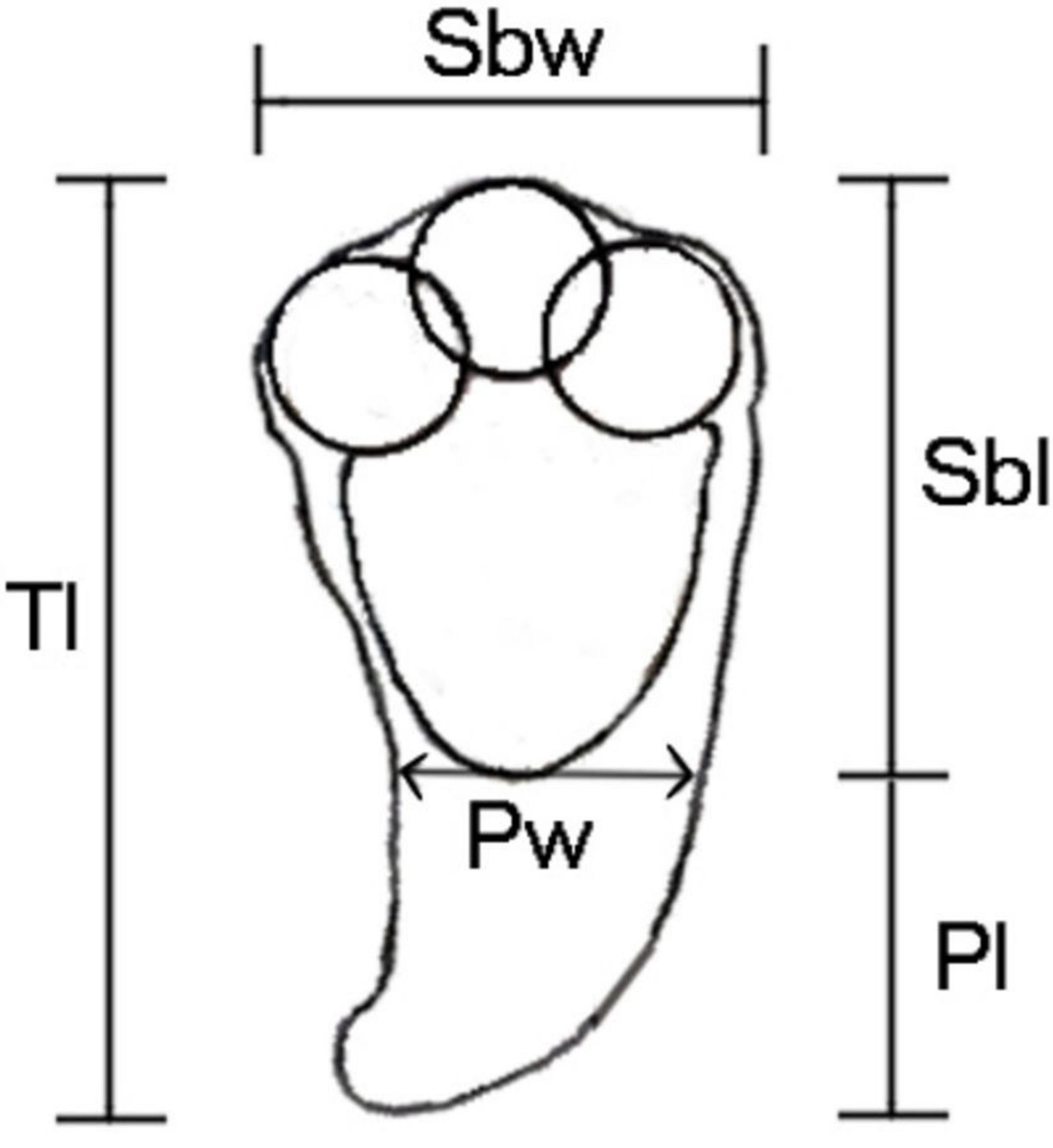
Pl2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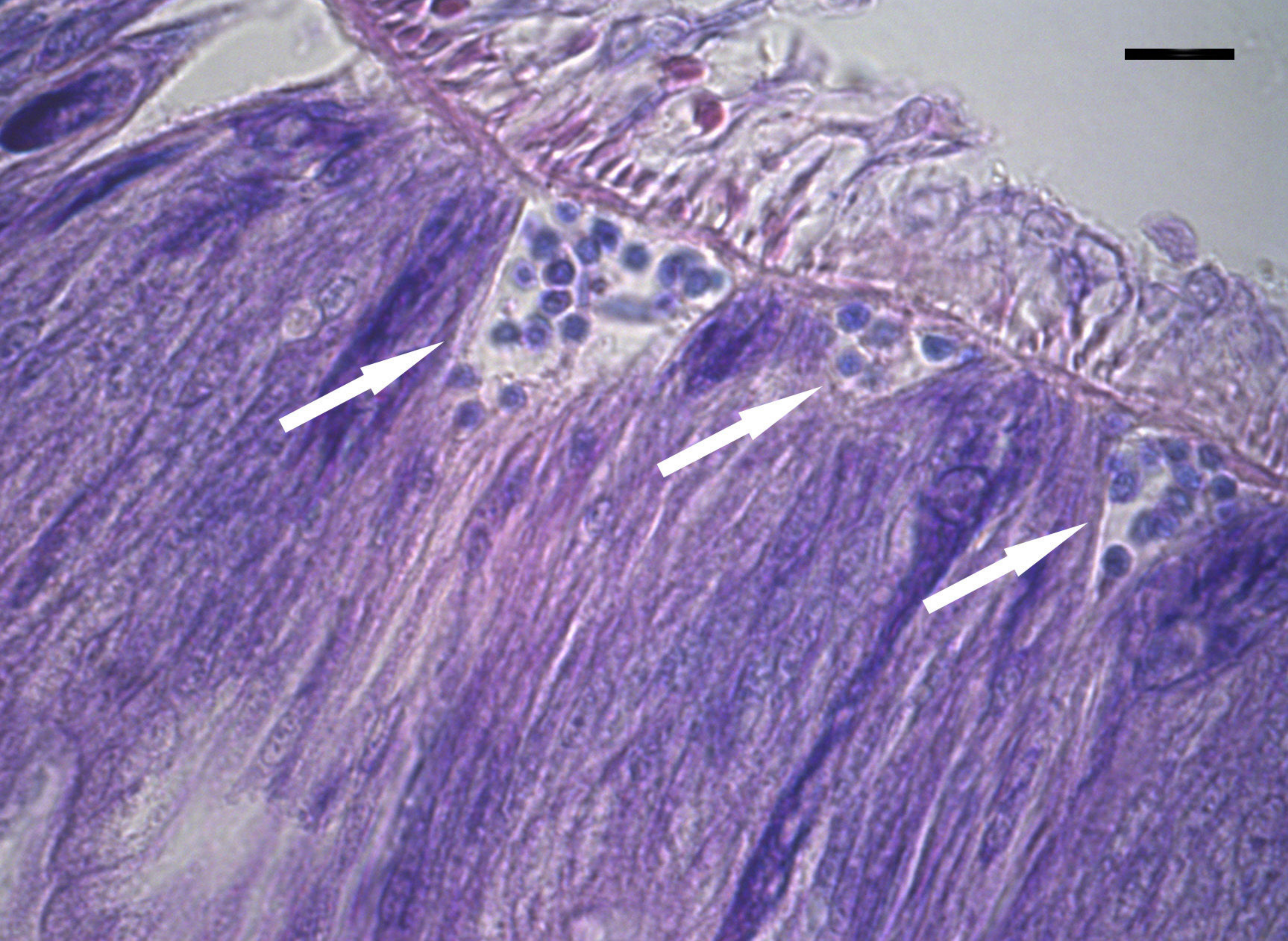

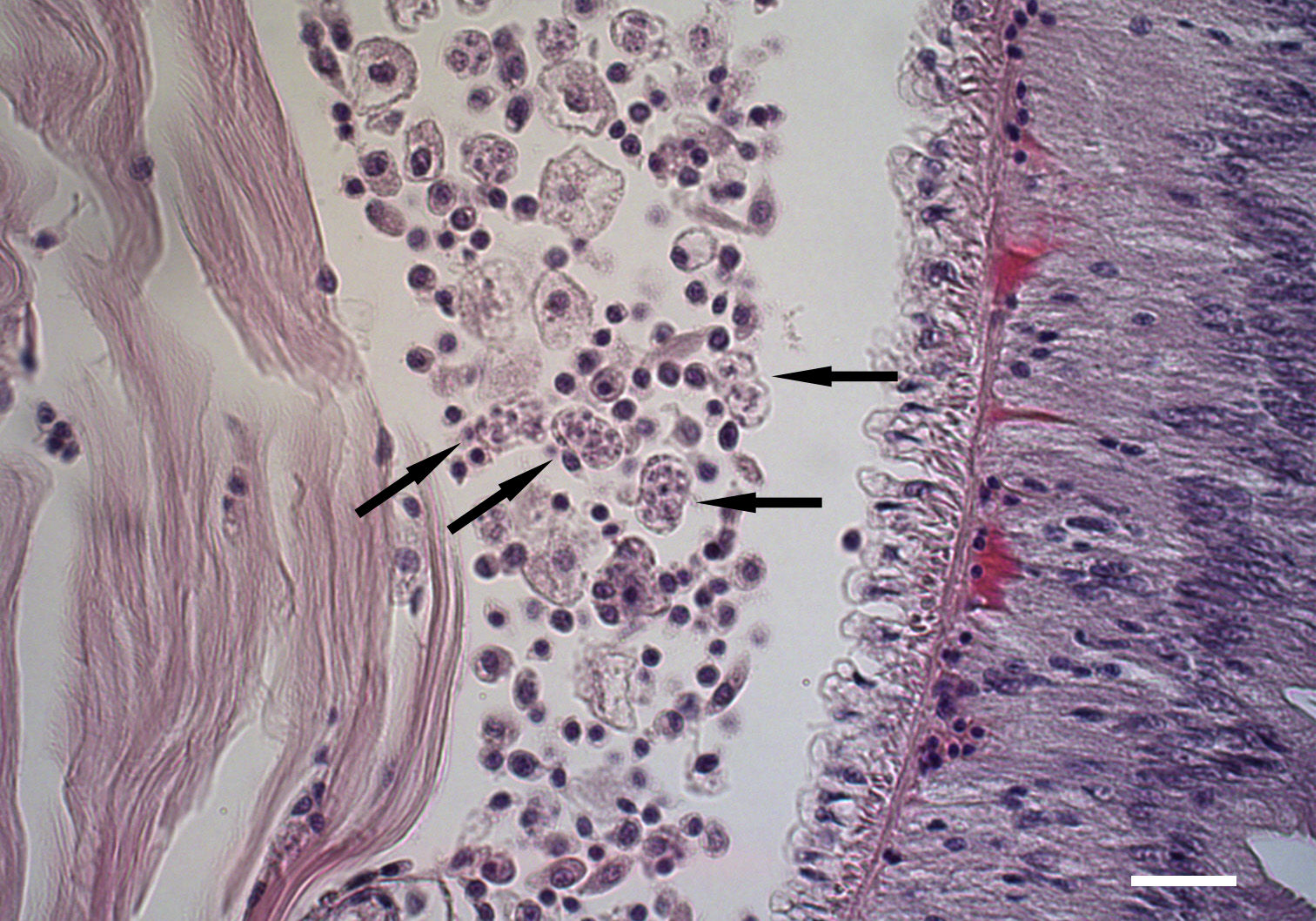

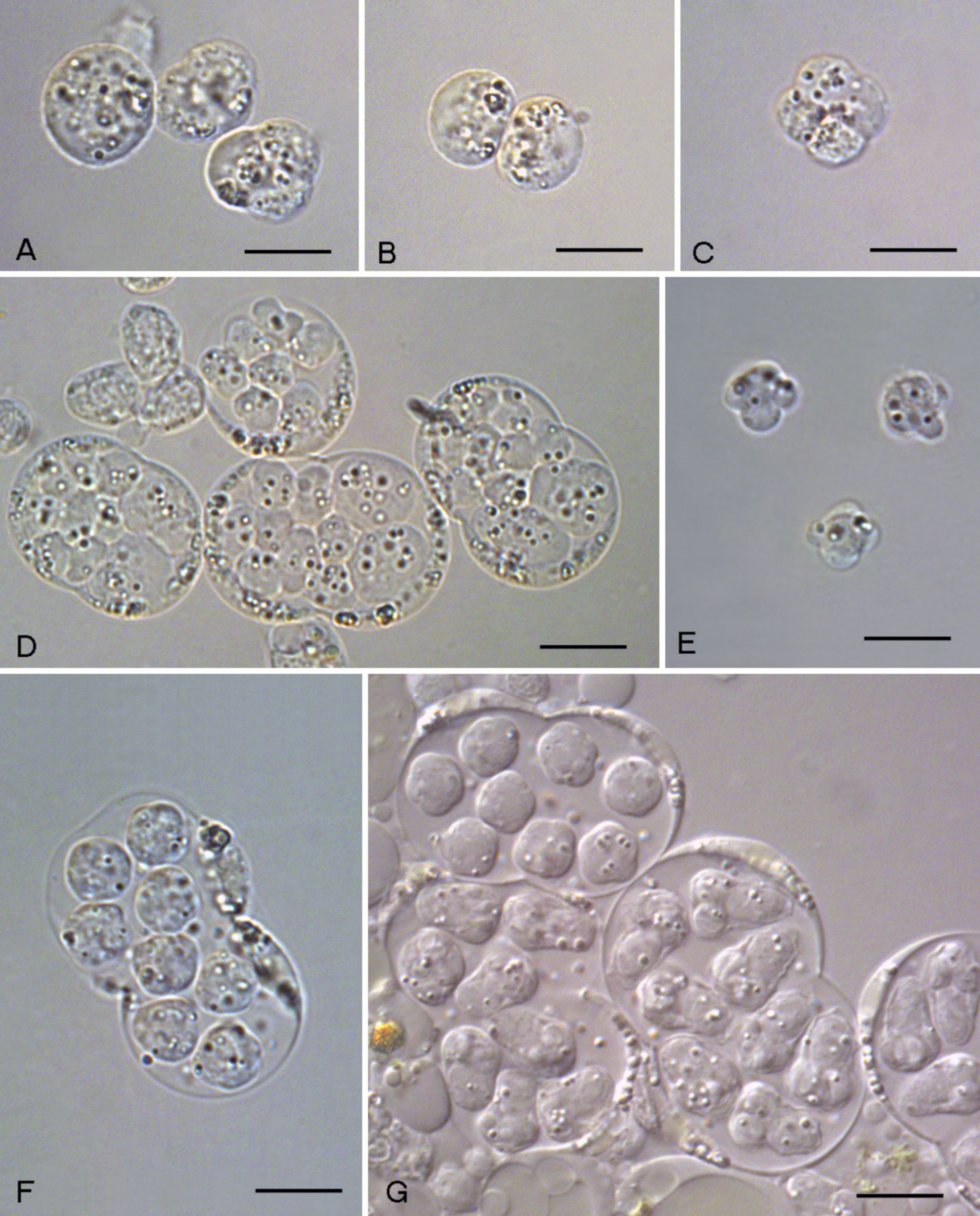
A
B
C
D
E
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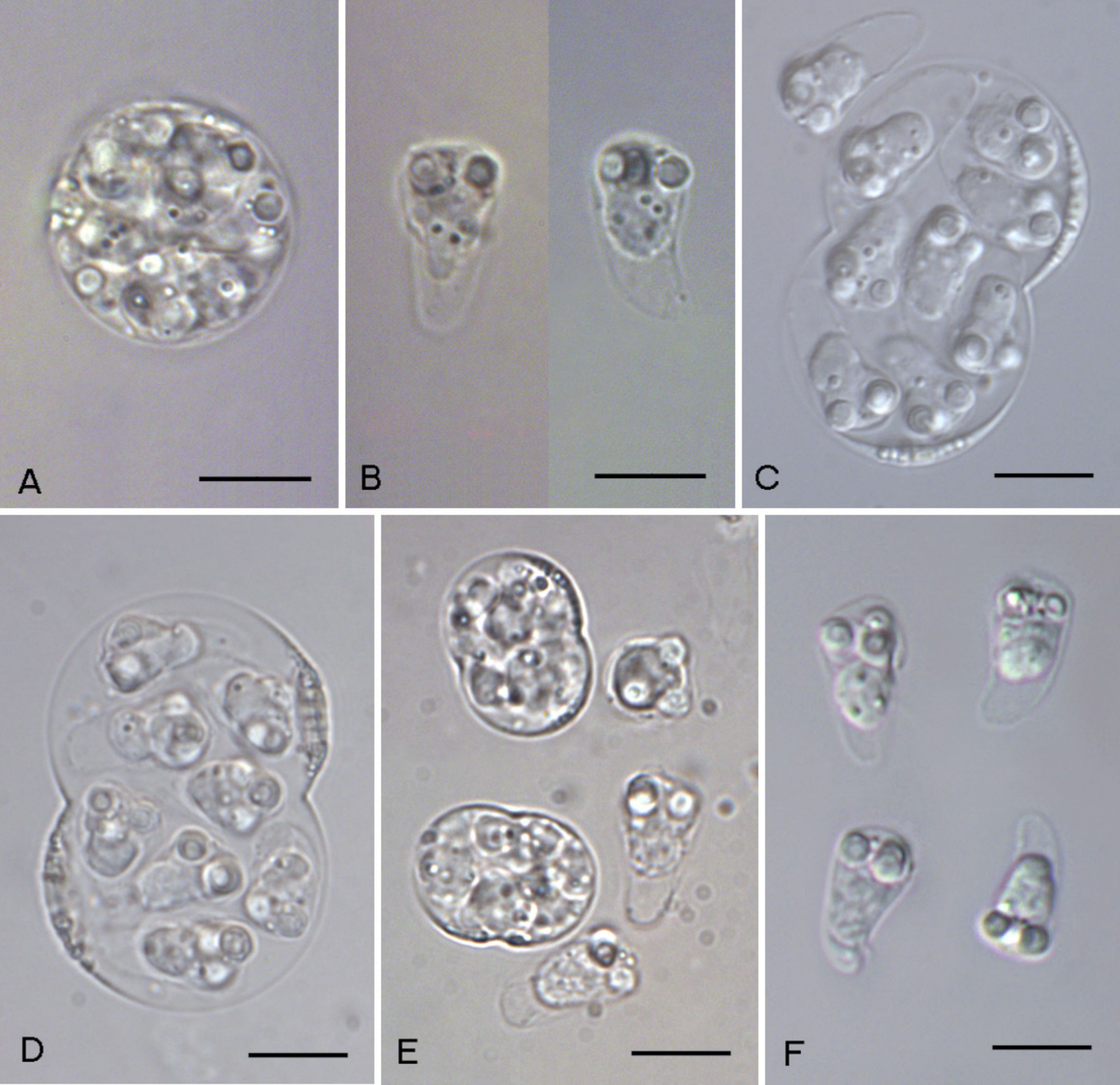
G


A
B
C
D
E
F

```
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Z. mugilis   TCCAGACATT GGGAGGTAGT GACGAGAAAT ACCAAAGTGT GCCTTTYGGT
E. gobii     TCCAGACATT GGGAGGTAGT GACGAGAAAT ACCAAAGTGT GCCTTTTGGT

                     60         70         80         90        100
actinospore  TCACTATTGG AATGAACGTA ACATAGTACC TTCGATGAGT ACCTACTGGA
Z. mugilis   TCACTATTGG AATGAACGTA ACATAGTACC TTCGATGAGT ACCTACTGGA
E. gobii     TCACTATTGG AATGAACGTA ACATAGTACC TTCGCTGAGT ACCTACTGGA

                    210        220        230        240        250
actinospore  CGTTGATCAA GCACTAGTTT GGTTGGCGTC GGGCTTTTTT ATCGCAAGAA
Z. mugilis   CGTTGATCAA GCACTAGTTT GGTTGGCGTC GGGCTTTTTT ATCGCAAGAA
E. gobii     CGTTGATCAA GCACTAGCTT GGTTAGCGTC GGGCTTTTTT ATCGCAAGAA

                    260        270        280        290        300
actinospore  TCACTCGTGC ATTTAACCGT GTGCGGGTGA CCACTTGCGG AGCGTGCCTT
Z. mugilis   TCACTCGTGC ATTTAACCGT GTGCGGGTGA CCRCTTGCGG AGCGTGCCTT
E. gobii     TCACTCGTGC ATTTAACCGT GTGCGGGTGA CCACTTGCGG AGCGTGCCTT

                    310        320        330        340        350
actinospore  GAATAAAGCA CAGTGCTCAA AGCAAGCGTA ACGCTCGAAT GTTTAAGCAT
Z. mugilis   GAATAAAGCA CAGTGCTCAA AGCAMGCGTA ACGCTCGAAT GTTTAAGCAT
E. gobii     GAATAAAGCA CAGTGCTCAA AGCAAGCGTA ACGCTCGAAT GTTTAAGCAT

                    360        370        380        390        400
actinospore  GGAACGAATA ACTATCTGAC ACATGGTTCA GTTGTTGGTT ATCTGAGCCG
Z. mugilis   GGAACGAATA ACTATCTGAC ACATGGTTCA GTTGTTGGTT ATCTGAGCCG
E. gobii     GGAACGAATA ACTATCTGAC ACACGGTTCA GTTGTTGGTT ATCTGAGCCG

                    710        720        730        740        750
actinospore  GGCACCACCA GGAGTGGAGC CTGCGGCTTA ATTTGACTCA ACACGGGGCA
Z. mugilis   GGCACCACCA GGAGTGGAGC CTGCGGCTTA ATTTGACTCA ACACRGGGCA
E. gobii     GGCACCACCA GGAGTGGAGC CTGCGGCTTA ATTTGACTCA ACACGGGGCA
```


E
F
D
day 7
day 3
day 10
G
C
day 21
day 1
day 21-28
Unknown
H
B
A

Table I - Free actinospores measurements of *Zschokkella mugilis*. Details of the measurements can be seen in figure 2. All measurements in micrometers.

Measurements	n	Mean ± SD	Range
Polar capsules diameter	32	3.4 ± 0.2	2.9 - 3.8
Total length (Tl)	10	17.3 ± 0.9	16.1 - 18.6
Spore body length (Sbl)	10	11.2 ± 0.6	10.5 - 12.6
Spore body width (Sbw)	10	8.8 ± 0.4	8.0 - 9.5
Caudal process length (Pl)	10	6.1 ± 0.9	5.3 - 8.0
Caudal process width (Pw)	10	5.9 ± 0.4	5.0 - 6.4

Table II. Mature pansporocysts measurements of *Zschokkella mugilis* in the coelomic fluid of *Nereis diversicolor*. Details of the measurements can be seen in figure 1. All measurements in micrometers.

Measurements	n	Mean ± SD	Range
Total length (Tl)	7	39.1 ± 1.0	37.9 - 40.8
Partial length (Pl1)	7	20.1 ± 0.5	19.2 - 20.8
Width (Pw1)	7	22.3 ± 0.8	20.8 - 23.4
Partial length (Pl2)	7	19.0 ± 0.5	18.2 - 19.8
Width (Pw2)	7	21.3 ± 0.5	20.8 - 22.2
Constriction length (Cl)	7	13.4 ± 1.0	11.9 - 14.5