Black Spot Gill Syndrome in the Northern Shrimp, *Pandalus borealis*, caused by the parasitic ciliate *Synophrya* sp.

Richard F. Lee[1*], Anna N. Walker[2], Stephen C. Landers[3], Tina L. Waters[1], Shirley A. Powell[2], Marc. E. Frischer[1]

[1] University of Georgia, Skidaway Institute of Oceanography, 10 Ocean Science Drive, Savannah, Georgia 31411, USA

[2] Mercer University School of Medicine, 1501 Mercer University Drive, Macon, Georgia 31207, USA

[3] Department of Biological and Environmental Sciences, 210A MSCX, Troy University, Troy, Alabama 36082, USA

*Corresponding Author

ABSTRACT

Black spot gill syndrome in the northern shrimp, *Pandalus borealis*, is caused by an apostome ciliate, *Synophrya* sp., found within the gill lamellae. Whole mount staining, thin section histology, electron microscopy, and molecular studies were carried out on infected gills. The *Synophrya* 18S rRNA from *Pandalus borealis* (Genbank accession no. KX906568) and from two portunid crab species, *Achelous spinimanus* (Genbank accession no. MH395150) and *Achelous gibbesii* (Genbank accession no. MH395151) was sequenced. Phylogenetic analyses confirmed the identity of these ciliates as apostomes. The 18S rRNA sequence recovered from *P. borealis* shared 95% nucleotide similarity with the sequences recovered from the portunid crab species suggesting that it is a different species of *Synophrya*. The invasive hypertrophont stages, with a distinctive macronuclear reticulum, ranged in size from 300 to 400 µm with as many as 5 large forms/ mm^2 of gill tissue. Histotrophic hypertrophont stages and hypertomont stages were observed in these studies. The presence of the parasite was linked to the formation of melanized nodules (up to 9 nodules/mm^2 of gill tissue) by the host and in some cases to extensive necrosis. Other studies have reported *Synophrya* sp. infections in *P. borealis* from Greenland, Labrador and Newfoundland, but further studies are necessary to determine the prevalence of this parasite in the dense schools of northern shrimp in the North Atlantic. Questions remain as to the possibility of epizootics of this pathogen and its impact on northern shrimp populations.

Keywords: Synophrya; Pandalus borealis; black gill; necrosis; gill; northern shrimp

1. Introduction

Crustaceans of commercial importance are infected by a variety of ciliated protozoan parasites. Apostome ciliates are responsible for some widespread epidemics. For example, severe outbreaks of darkened gill tissues, caused by a yet unidentified apostome ciliate and referred to as black gill, have resulted in ongoing epidemics in *Litopenaeus setiferus* (white shrimp) and *Farfantepenaeus aztecus* (brown shrimp) in the warm waters of the U.S. Southeast Atlantic (Frischer et al., 2017). Black gill is hypothesized to be a contributing factor in the observed decline of this fishery. Black spot gill syndrome in the commercially important pandalid shrimp, *Pandalus borealis*, from cold high latitude waters has been shown to result from infection by an unidentified ciliate (Apollinio and Dunstan, 1969; Rinaldo and Yevich, 1974). The goal of the present work was to describe and identify the protist causing black spot gill syndrome in the northern shrimp *Pandalus borealis* from coastal Maine.

Landings in Maine, USA of *P. borealis,* a shrimp species that often aggregates in dense schools (Bergström, 2000; Garcia, 2007; Shumway et al., 1985), are highly variable. Up to 18 million pounds were harvested in 1996, but this peak was followed by a dramatic decline in landings and moratoria have been instituted since 2014 (Atlantic States Marine Fisheries Commission, 2015). In addition to the New England area of USA, there are important fisheries for this shrimp in the Canadian Maritime Provinces, Greenland, Iceland, Norway, and Denmark. Northern shrimp surveys carried out in the North Sea have shown very low population levels of this shrimp since 2006 (Knutsen et al., 2015). Rinaldo and Yevich (1974) reported that 55% of *P. borealis* collected in the Gulf of Maine from 1967 to 1970 had damaged, heavily melanized gills, which they referred to as black spot gill syndrome. They suggested that this condition was reducing commercial catches of *P. borealis*. Maine shrimp with this syndrome were first noted in

1966 and tentatively identified as caused by the ciliate *Gymnodinioides* sp. (Apollinio and Dunstan, 1969). Rinaldo and Yevich (1974) could not confirm this identification but stated that an unidentified protozoan was the causative agent of the syndrome. In this study we investigated the identity of the black spot causing ciliate in *P. borealis* that remains present in epidemic proportions in the Gulf of Maine, USA.

2. Material and Methods

2.1 *Collections*

Northern shrimp (*Pandalus borealis)* were collected from the Gulf of Maine in April 2016 (specific locations not available) and in March 2018 (43°46.5”N, 69°30.66”W). From each collection, 10 shrimp representing a gradient of visible black spot syndrome symptoms (not visible to very dark) were collected and stored on ice. Replicate gill tissue samples were preserved within hours of collection in 70% non-denatured ethanol for PCR-based molecular analysis, and in Zinc Formalin Fixative (Sigma Aldrich # Z2902) for histological examination. For whole mount silver staining, thin section histology and electron microscopy, gill tissue was fixed in either 4% cold glutaraldehyde in 0.2 µm filtered seawater or in 2.5% cold glutaraldehyde buffered with 0.05M sodium cacodylate (pH 7.5). *P. borealis* collections were facilitated by the Maine Department of Marine Resources. Portunid crabs including *Achelous gibbesii, Achelous spinimamus* and *Achelous ordwayi* were collected (total of 48 crabs) during 3 cruises from the mid-continental shelf at ca. 40 m depth at 31°21.3” N, 80°17.9” W in the South Atlantic Bight. Collections were made on October 31, November 7 and December 5, 2017. Bottom trawls used a

12.2 m flat shrimp net with 4.8 cm stretch-mesh webbing throughout the body and bag. Tow duration was 15 min of bottom time at a speed of approximately 2-3 knots.

2.2 Whole mount analysis and staining

Whole gills were examined with a stereomicroscope for melanized lesions caused by *Synophrya*. Gill lamellae with the parasitic ciliates were separated, and stained with silver nitrate using a modification of the Chatton-Lwoff silver impregnation technique (Chatton and Lwoff, 1935, Landers, 2008) to reveal the surface pattern of the parasite's ciliary rows.

2.3 Routine Histology

Zinc formalin-fixed gill tissues from *P. borealis* were processed for routine light microscopy and embedded in paraffin. Sections (5 µm thick) were cut, mounted on glass slides, stained with hematoxylin and eosin, coverslipped and examined using a light microscope. The ciliates and nodules in 10 microscopic fields (400x) of each specimen were counted and reported as numbers/mm^2 of gill tissue.

2.4 Thin section histology and electron microscopy

Gill tissue fixed in cold glutaraldehyde was post-fixed in 2% osmium tetroxide. After post fixation the samples were dehydrated and embedded in plastic for light microscopy histology and transmission electron microscopy (TEM) (Landers, 2010) or were stored in 70 % ethanol for scanning electron microscopy (SEM). Plastic sections for light microscopy (Spurr epoxy, EMS Cat #14300) were cut at 1 micrometer thickness with a diamond knife on a Sorvall MT2B ultramicrotome and stained with sodium borate-buffered toluidine blue for 10 seconds on a hot plate. Tissue sections were observed using a light microscope. Tissues for SEM were dehydrated

to 100% ethanol, critical-point dried and sputter-coated with gold. The tissues were examined using a Zeiss EVO 50 SEM at the Auburn University Research Instrumentation Facility (AURIF) using both backscatter and secondary electron detectors. For TEM, -plastic-embedded gill tissues were cut at 80-90 nanometers thickness and stained with uranyl acetate and lead citrate. Sections were photographed using a Zeiss EM10 TEM at AURIF.

2.5 Routine PCR and microscopy screening for Synophrya

The presence of *Synophrya* in ethanol-preserved shrimp and crab gill tissue samples was initially assessed using a previously described diagnostic PCR assay and the primer set Hyalo-754F (5' GGA CAG TTG GGG GCA TTAGT) and Hyalo-952R (5' GAC CAA GTT ATA AAA TGG CCA) (Frischer et al., 2017). This primer set amplifies an approximately 207 bp fragment of the 18S rRNA gene from an apostome ciliate identified as *Synophrya* sp. detected in *P. borealis* (Frischer et al., 2018). The presence of *Synophrya* sp. was confirmed in representative crabs that screened positive for *Synophrya* sp. by microscopy.

2.6 Cloning, sequencing and phylogenetic classification

Nearly the complete small subunit rRNA (18S rRNA) gene of the ciliate associated with the portunid crab was sequenced as previously described (Frischer et al. 2017). Prior to this study the 18S rRNA gene of *Synophrya* from its originally described host (portunid crabs) had not been sequenced. Total genomic DNA was purified from 70% ethanol-preserved gill tissue collected from crabs that tested positive for *Synophrya* by PCR screening. DNA was purified using the DNeasy® blood and tissue kit following the manufacturer's instructions (QIAGEN inc., Valencia, CA, USA). The 18S rRNA gene was amplified utilizing the primers Hyalo-18SF-754 and reverse Hyalo-18SR-952 paired with universal eukaryotic 18S rRNA primers, Univ-

18SF-15 (CTG CCA GTA GTC ATA TGC) and Univ-18SR-1765S (ACC TTG TTA CGA CTT) (Gruebl et al. 2002). Cloning and sequencing was accomplished as previously described (Frischer et al., 2017). Sequence alignments were facilitated using ClustalW (version 1.4) as implemented in BioEdit software package using the default recommended weighting parameters (Hall, 1999). Phylogenetic analysis was facilitated using the MEGA7 evolutionary analysis software package (Kumar et al., 2016) after alignment of the recovered sequences with all Apostomatia ciliate 18S rRNA sequences available from the Silva ribosomal database (SSU r132) at the time of analysis. A total of 161 rRNA sequences from 8 Apostomatia divisions were included. The majority of these sequences were not from identified specimens (127) and were not represented by full-length sequences. To phylogenetically classify sequences from *P. borealis* and the portunid crabs, an alignment of near full-length sequences (60) was assembled. The 18S rRNA sequence from the hymenostome ciliate *Tetrahymena thermophila* (Genbank accession no. M10932) was also included as an outgroup. Utilizing the available maximum likely, neighbor joining, and maximum parsimony tree building algorithms implemented in the MEGA7 software package, evolutionary reconstructions were inferred using the Maximum Likelihood Tamura-Nei model (Tamura and Nei, 1993). Initial trees for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. All positions containing gaps and missing data were eliminated. There were a total of 1566 positions in the final dataset. All trees were constructed with the appropriate bootstrap (100) procedures as implemented in MEGA7 (Kumar et al 2016).

3. Results

3.1 Gross and light microscopic black spot gill syndrome pathology.

151 The visible symptoms of black spot gill syndrome in *P. borealis* typically present as
152 macroscopic black spots in gill tissues, often with gross tissue necrosis apparent. These
153 symptoms are distinct and easily distinguished from general gill fouling (Fig. 1, 2A). In contrast,
154 the condition known as shrimp black gill, which is currently impacting penaeid shrimp
155 populations in the Southwest Atlantic and Gulf of Mexico, is caused by a different apostome
156 ciliate and presents as diffuse darkening of gill tissue (Gambill et al., 2015). During this study
157 the prevalences of black spot in *P. borealis* during both the 2016 and 2018 sampling were
158 estimated to be >80% (M. Hunter, personal communication).

159 Chatton-Lwoff silver nitrate impregnation (staining) of *Synophrya* confirmed the genus
160 identification and identified many characteristic life cycle stages known for *Synophrya* (Figure
161 2B-D). The hypertrophont stage was varied in its structure, which ranged from a simple sphere
162 (Fig. 2B) to oblong or multilobed. The hypertrophont has nine kineties (ciliary rows), which run
163 from pole to pole and are termed *meridional*. Additionally, positioned between kineties 9 and 1
164 are three kineties, x, y, and z. After bloating to many times its original size after invasion, likely
165 in response to chemical cues from the host's blood, the hypertrophont divides in order to release
166 small swimming stages into the host's cast off exoskeleton at molting (the dividing
167 hypertrophont stage is named the hypertomont). This divisional process is called
168 hyperpalintomy, during which there are successive divisions without intervening growth (Fig.
169 2C). The division of the hypertomont results in smaller cells with the same distinctive ciliature
170 as the previous stage. Eventually, the hypertomont daughter cells form the ultimate product of
171 hyperpalintomy, the hypertomites (Fig. 2D), which are released into the host's exuvium where
172 they begin the next feeding stage in the life cycle as they transform into exuviotrophic trophonts.
173 The ciliature and life cycle stages of the parasite from *P. borealis* is completely consistent with

174 the apostome ciliate genus *Synophrya*, known to infect crabs and shrimp along the United States
175 Atlantic and Gulf coasts (Johnson and Bradbury, 1976; Haefner and Spacher, 1985; Landers,
176 2010).

177 Examinations of microscopic sections of infected *P. borealis* gills revealed invasive
178 *Synophrya* that presented as large cytoplasmic masses (hypertrophonts) within the gill lamellae
179 and contained darkened cords of nuclear material (Fig. 3A-C). These cords were disconnected in
180 some sections as the structures wound into and out of the plane of section, and formed a distinct
181 macronuclear reticulum known to occur in this genus. These hypertrophonts were not observed
182 within the raphe of the gill, but only within individual lamellae, and were associated with distinct
183 melanization from the host reaction (Fig. 3B, C). The hypertrophont stages ranged in size from
184 300 to 400 µm (Fig. 3B–C) and were numerous, with as many as five large forms/mm^2 (Fig. 3A,
185 Table 1). Additionally, later stages in the life cycle were observed, in which the hypertrophont
186 had divided (Fig. 3D). The small daughter cells, lacking the reticulate macronucleus, are the
187 hypertomites, which were also observed by whole mount silver staining (Figures 2D, 3D). In
188 some cases, small hypertomites (soon to develop into exuviotrophic trophonts) contained a
189 presumptive micronucleus visible within the cytoplasm next to the macronucleus (Figure 3D). In
190 addition to the feeding hypertrophont stages and the dividing stages, smaller ovoid cells attached
191 to the host's tissue were observed. These may have been settled phoront stages that had not
192 invaded the gill tissue to form hypertrophonts (Fig. 3C). These cells had a solid macronucleus
193 located within the central area of the cell.

194 The distribution of both large (hypertrophonts, Fig. 3A) and smaller forms (phoronts, Fig.
195 3C) was highly variable from specimen to specimen and also within the tissues of a given
196 specimen, ranging from non-detectable to 12 phoronts/mm^2 (Table 1). The hypertrophonts

observed in the gill lamellae were associated with melanized nodules (Fig 3B, C, 4A, B), with up to 9 nodules/mm^2 (Table 1). On occasion, necrosis of host tissues was observed and the parasite was separated from adjacent host tissues by an accumulation of necrotic debris and melanin (Fig 3C). The hypertrophonts and divided stages were encapsulated by a cyst wall (Fig 3B, 4B).

3.2 *Electron microscopy.*

Scanning electron microscopy of infected gills revealed the bloated nature of individual gill lamellae that contained *Synophrya* (Fig. 5A). No damage to the exoskeleton of the host was visible, however. Transmission electron microscopy confirmed the presence of the *Synophrya* cyst wall, which was approximately 2 µm thick, homogeneous, and electron dense (Fig. 5B). The cyst wall is known to be semi-permeable because nutrients from the host's tissue must cross this structure to be absorbed by the mouthless parasite. The cyst wall from *Synophrya* in the Gulf of Maine was indistinguishable from that of the *Synophrya* species in the Gulf of Mexico (Landers 2010).

3.3 Phylogenetic Identification

Nearly the complete 18S rRNA gene from the *Synophrya* infecting *P, borealis,* and from the portunid crabs *A. spinimanus* and *A. gibbesii* collected from the South Atlantic Bight continental shelf were sequenced. All sequences were deposited in Genbank (accession nos. KX906568, MH395150, MH395151). The *Synophrya* sp. 18S rRNA sequences obtained in this study clustered most closely with *Gymnodinioides* sp. JCC-2008 (Genbank accession no. EU503535) and *Vampyrophrya pelagica* (Genbank accession no. EU503539) in a clade distinguished from a clade consisting of representatives of the genera *Hyalophysa* and the ciliate that causes black gill in penaeid shrimp (sBG cliliate, Genbank accession no. KX906567; bootstrap = 79%). The

similarity between the two *Synophrya* sp. strains associated with the portunid swimming crabs *A. spinimanus* and *A. gibbesii* was high. Only a single nucleotide difference over the full gene was observed indicating that the infecting ciliates in both crab species are almost certainly the same species. Slightly greater differences were observed between the sequences recovered from the portunid crabs and the shrimp *P. borealis*. Sequences were 95% similar with a total of 80 nucleotide differences over the entire gene (Figure 6). This level of difference is consistent with at least a species level difference although it may indicate a higher order (genus) difference (Clamp et. 2008).

4. Discussion

The cause of the black spot gill syndrome in the northern shrimp, *P. borealis*, is parasitization by the apostome ciliate, *Synophrya* sp. The earliest studies on *Synophrya hypertrophica* were carried out by Chatton and Lwoff (1926a, b; 1927; 1935) who described the structure and complex life cycle of this this apostome ciliate in a number of crab species from the Brittany coast and the Mediterranean coast of France. Later studies reported *Synophrya* sp. in portunid crabs from the offshore waters of Spain and Tunisia (Sprague and Couch, 1971), in decapods collected off the US South Atlantic (Johnson and Bradbury, 1976; Haefner and Spacher, 1985), and in the swimming crab, *Portunus* (now *Achelous*) *spinicarpus*, from the Gulf of Mexico (Landers, 2010). The ciliate seems to be restricted to high salinity offshore waters and is absent in decapods from estuaries (Johnson and Bradbury, 1976). While most studies have reported on *Synophrya* sp. in portunid crabs, the genus has also been reported parasitizing the brown rock shrimp (*Sicyonia brevirostris*) and one specimen of the white shrimp (*Litopenaeus setiferus*) (Johnson and Bradbury, 1976; Frischer, unpublished data). Annual surveys (2001 to 2009) of *P.*

borealis in waters off Labrador and Newfoundland reported the presence of black spot gill syndrome in each year of the survey with parasite prevalence ranging from 1 to 13% (Orr et al., 2011). Black spot gill syndrome in *P. borealis* from the Maine coast is highest (55%) in the winter months (Rinaldo and Yevich, 1974) and thus the relatively low prevalence reported from Labrador and Newfoundland may have differed due to collection in the fall (Orr et al., 2011). Rinaldo and Yevich (1974) noted that *P. borealis* collected off Greenland were parasitized by ciliates similar to those observed in northern shrimp from Maine waters Based on these observations, we suggest that *Synophrya* is present in *P. borealis* from most North Atlantic waters.

It remains unclear why black spot gill syndrome occurs at epidemic levels in the Gulf of Maine. One factor may be related to relatively warmer temperatures in the Gulf of Maine compared to more northern regions where northern shrimp currently support a major commercial fishery. In the Gulf of Maine, *P. borealis* is at the southern extreme of its range and therefore experiences subsurface water temperatures that are often above the 2 to 4 ^{o}C preferred by this species (Shumway et al., 1985; Kavanaugh et al., 2017). Furthermore, in association with climate change processes, water temperatures have been increasing in the Gulf of Maine at a rapid rate, having increased at a rate of 0.1 to 0.4 ^{o}C per decade over the past 4 decades, faster than most other oceanic regions (Kavanaugh et al., 2017; Poppick, 2018). This warming trend may be associated with an increase in black spot gill syndrome and possibly other diseases. Warming water temperatures have been associated with increased prevalence of disease in other coastal invertebrate species. For example, on the east coast of the USA warming waters have been linked to the spread of several oyster diseases caused by protist parasites (Harvell et al.,

1999; Ford, 1996). Although strictly speculation at this time, we suggest that if high latitude waters continue to warm, black spot gill syndrome and could spread in *P. borealis* populations.

The life cycle of *Synophrya hypertrophica*, described by Chatton and Lwoff (1927, 1935) and recently reexamined based on slides archived by Chatton (d'Avila et al. 2016), has two phases, an invasive histotrophic phase and an exuviotrophic phase. The histotrophic phase is the hypertrophont, which grows within the host's gill and is eventually stimulated to divide prior to the molt of the host. The dividing stage is the hypertomont, that likely develops based on a chemical cue from the pre-molt host. The related apostome *Hyalophysa chattoni*, while attached to the shrimp's exoskeleton, is also known to metamorphose in response to the molting stage of the host (Landers 1986). As the hypertomont divides, it produces hypertomite stages which are released into the exoskeleton cast off by the host, where they mature further and feed on exuvial fluid as trophonts. These exuviotrophic trophonts eventually encyst and divide to form swimming stages that will attach to a host to begin again the invasive phase of the life cycle. Thus, *Synophrya* sp. is unique, feeding both on tissue and on exuvial fluid during two different phases of the life cycle. *Synophrya* sp. is also evidently related closely to the common exuviotrophs, such as *Hyalophysa* and *Gymnodinioides* (Fig 6), and is morphologically indistinguishable from *Gymnodinioides* in the exuviotrophic feeding stage (this feeding stage is the basis for species identification in the apostomatida). *Synophrya* sp. can be easily diagnosed by its large hypertrophont stages with the reticulated macronucleus, located within the gill tissue of decapods. The reticulated macronucleus is a characteristic shared with other apostomes that live in quite different hosts; for example, *Foettingeria* in the coelenteron of anemones, *Chromidina* in kidneys of cephalopods, and *Pericaryon* in the lumen of ctenophores (Chatton and Lwoff 1935). Further confirmation of the *Synophrya* genus is established by silver staining,

which reveals the characteristic ciliary rows (kineties) 1–9 and xyz in the hypertrophont as well as the divisional stages. However, identification of *Synophrya* to the species level is difficult. It is tempting to assign the ciliate reported herein to the only known species, *S. hypertrophica*, as our results are consistent with that identification. However, comparison of the 18S rRNA gene sequences between the ciliates collected from portunid crabs and *P. borealis* suggest that the *Synophrya* species found in *P. borealis* is a different species. Because different species may exist that either have few morphological differences, or have differences in the life cycle that we have not observed, the parasite from *P. borealis* is therefore identified at this time as *Synophrya* sp.

In the present study hypertrophonts and divisional stages were observed within gill tissues of *P. borealis* and presumably were responsible for the pathologic changes. The observed dense ring of melanin and focal necrosis are believed to be the result of the host immune effector cells that attempt to surround, encapsulate and isolate the pathogen (Cerenius et al., 2010; Landers et al., 2010). Additionally, it is interesting that the expanding hypertrophont stage absorbs its nutrition from the host's blood across a cyst wall, which was observed by light microscopy and transmission electron microscopy (TEM) in the current study. The TEM cyst wall data from the current *Pandalus* study is consistent with the TEM images of *Synophrya* in the gills of the portunid crab, *Achelous spinicarpus (*Landers, 2010).

The small attached ciliates observed in this study are consistent with apostome phoronts in cell shape and nuclear shape. Given the *Synophrya* sp. hypertrophont infection, it is likely that many of these cells are *Synophrya* phoronts that have not invaded the host's gill tissue. However, as *Synophrya* is known to be sympatric with *Gymnodinioides* (Chatton and Lwoff, 1935) not all of the attached phoronts can be assumed to be invasive.

5. Conclusions

Macroscopic black speckled spots in *P. borealis*, referred to as black spot gill syndrome, is caused by the apostome ciliate, *Synophrya* sp. The invasive histotrophic forms, referred to as hypertrophonts, are associated with heavy melanization and tissue necrosis, and are likely responsible for observed pathologic changes. A different apostome ciliate is responsible for the more diffuse black gill found in penaeid shrimp in the SE United States. We suggest that the dense schools of *P. borealis* in the north Atlantic are susceptible to infection by the pathogenic *Synophrya* sp. and because of the commercial importance of this shrimp stock, it is a priority to monitor the spread and impact of black gill spot syndrome in northern shrimp populations. The recent dramatic decreases in the northern shrimp catches in North Atlantic fisheries may be, in part, due to the spread of black spot syndrome.

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Figure Legends

Figure 1. *Pandalus borealis* with black spot gill syndrome.

Figure 2. Light microscopy of unsectioned *Synophrya*. A. Low magnification image of *Pandalus borealis* gills, with multiple *Synophrya* sp. parasites surrounded by the shrimp's melanin reaction. B-D. Chatton-Lwoff silver nitrate staining. B. The hypertrophont stage. Kineties 1–9 and x, y, and z are visible as dark lines that run from pole to pole (meridional) on the enlarged cell (arrows). C. The dividing hypertomont, with daughter cells revealing the characteristic meridional kineties. D. Daughter cells from a hypertomont dissected from the gill, revealing larger divisional stages as well as small hypertomite stages (arrow), characteristic of *Synophrya* sp.

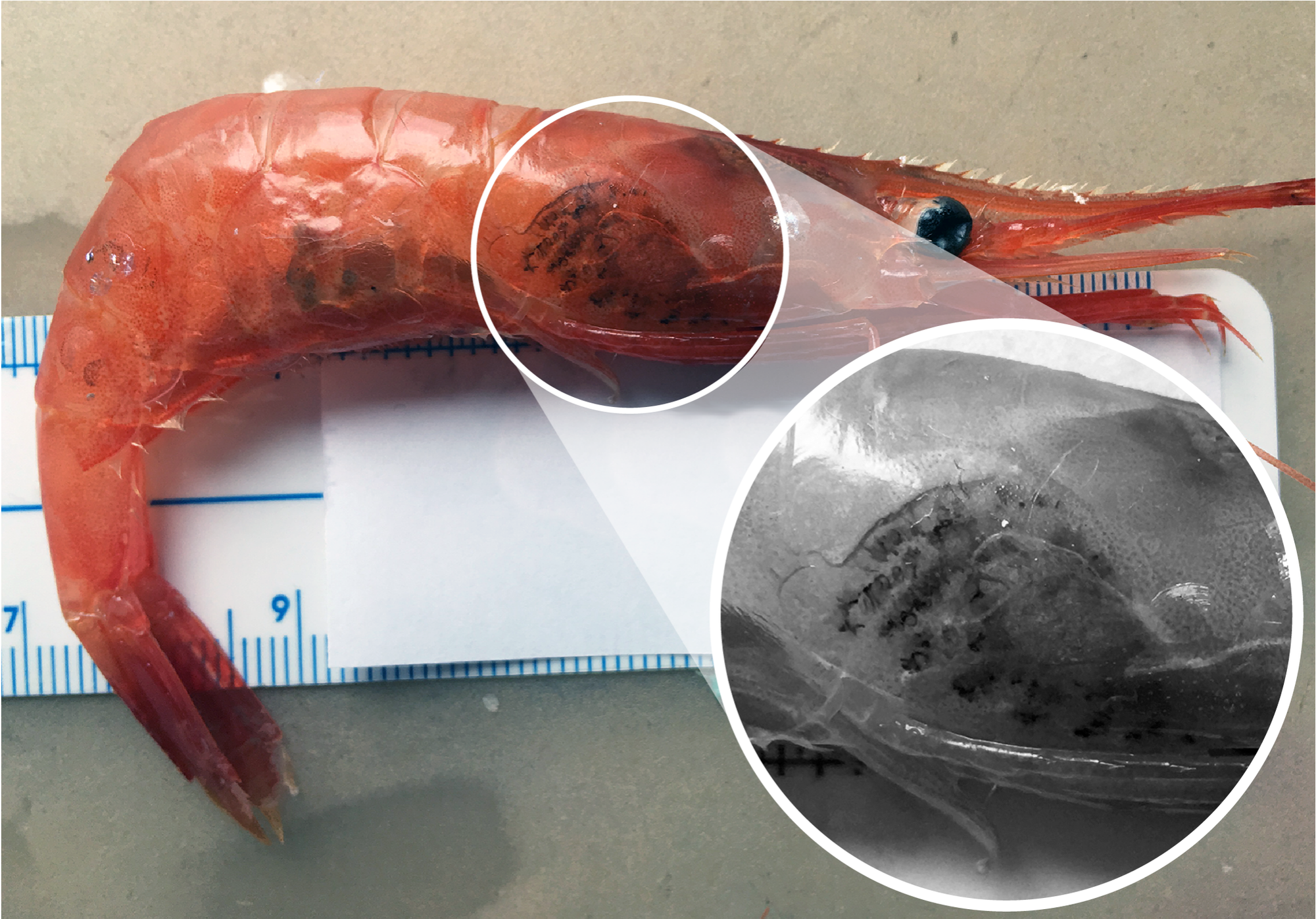
Figure 3. Paraffin sections of *Synophrya* sp. from *Pandalus borealis*. A. Overview of a gill region with four parasites (arrows). B. A *Synophrya* sp. hypertrophont within a gill lamellum, revealing the distinctive winding macronucleus within the cytoplasm. The host's melanization reaction, used to isolate the parasite, is indicated (arrow) along with the cyst wall of the parasite (arrowhead). C. A *Synophrya* sp. hypertrophont (star) revealing the complex macronucleus and multiple lobes of this single cell. The host's melanization reaction is indicated (arrows). Non-invasive apostome phoronts are indicated by arrowheads (Scale bar = 300 µm). D. A *Synophrya* sp. cyst wall filled with daughter cells following division. In two of the cells, a micronucleus is visible (arrowheads).

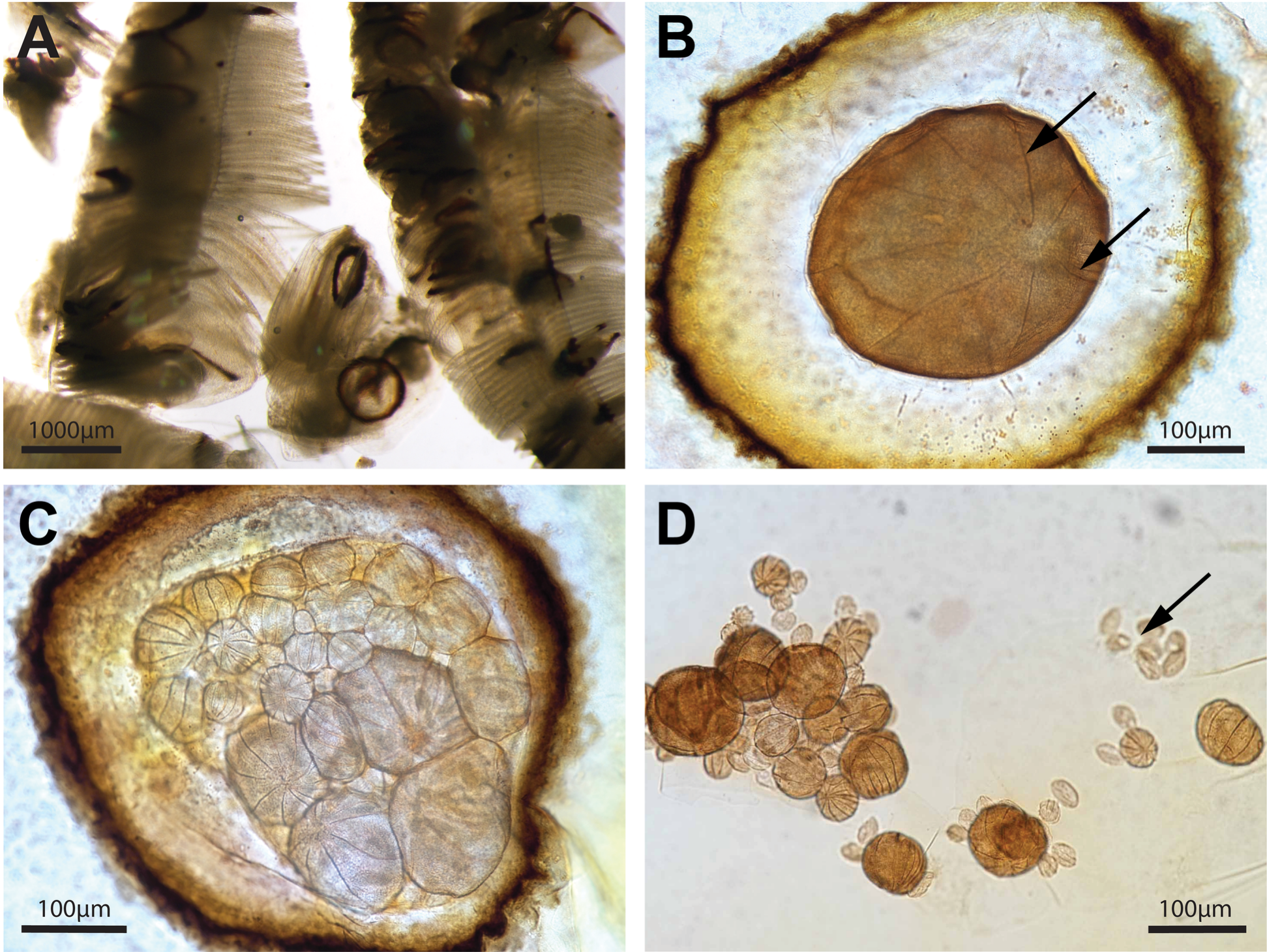
Figure 4. One-µm thin sections of *Synophrya* sp. from *Pandalus borealis*. A. Two *Synophrya* sp. parasites within the gill lamellae. The organism on the left is greatly enlarged and has caused the lamellum to rupture. This cell may be in an early divisional stage, and thus, a hypertomont. The host's melanization reaction typically encircles the parasite and is evident on either side of

the smaller hypertrophont (arrows). B. Higher magnification reveals the semipermeable cyst wall of the parasite (arrow) as well as the host exoskeleton (arrowhead). The melanization reaction is visible on the right.

Figure 5. Electron microscopy of *Synophrya* sp. from *Pandalus borealis*. A. Scanning electron microscopy of two enlarged lamellae (arrows) with *Synophrya* sp. parasites reveals little damage to the outer exoskeleton. B. Transmission electron microscopy reveals the host's exoskeleton (EX), the parasite cyst wall (CW), and the parasite cytoplasm (C) filled with lipid droplets (L).

Figure 6. Inferred phylogenetic identity of *Synophrya* spp. Phylogenetic analysis was based on the nearly complete 18S gene sequences from the *Synophrya* spp. associated with the Northern shrimp *Pandalus borealis* (Genbank accession no. KX906568) and from the swimming crabs *Achelous spinimanus* (Genbank accession no. MH395150) and *Achelous gibbesii* (Genbank accession no. MH395151). Phylogenetic analyses were conducted in MEGA7 (Kumar et al 2016). The percentage of trees in which the associated taxa clustered together is shown next to the branches (values < 75 are omitted). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The tree shown was constructed based on an alignment of all available (60) near full length apostome ciliate rRNA sequences obtained from the Silva SSU RNA database (SSU r132) although the portion of the tree shows only the *Synophrya* and *Hyalophysa* clades. The hymenostome ciliate *Tetrahymena thermophila* (Genbank accession no. M10932) was also included as an outgroup.

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B
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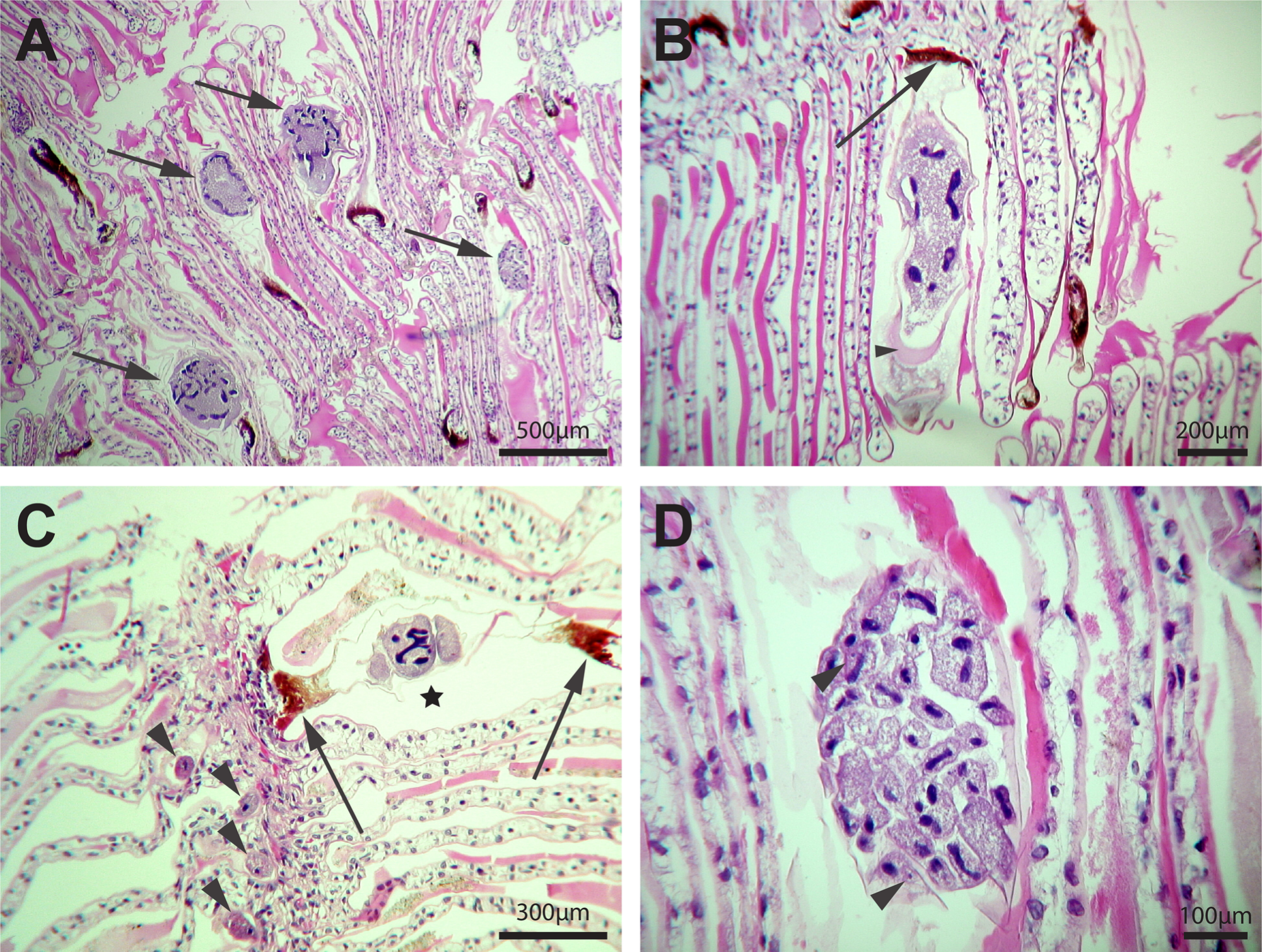
C
100µm
D
100µm


A
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B
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C
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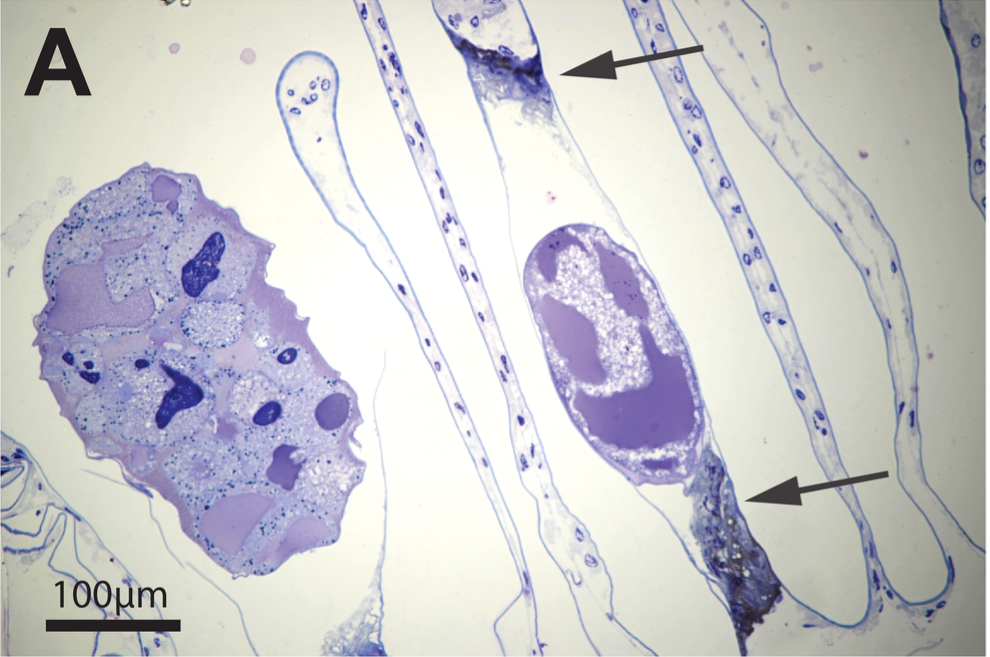
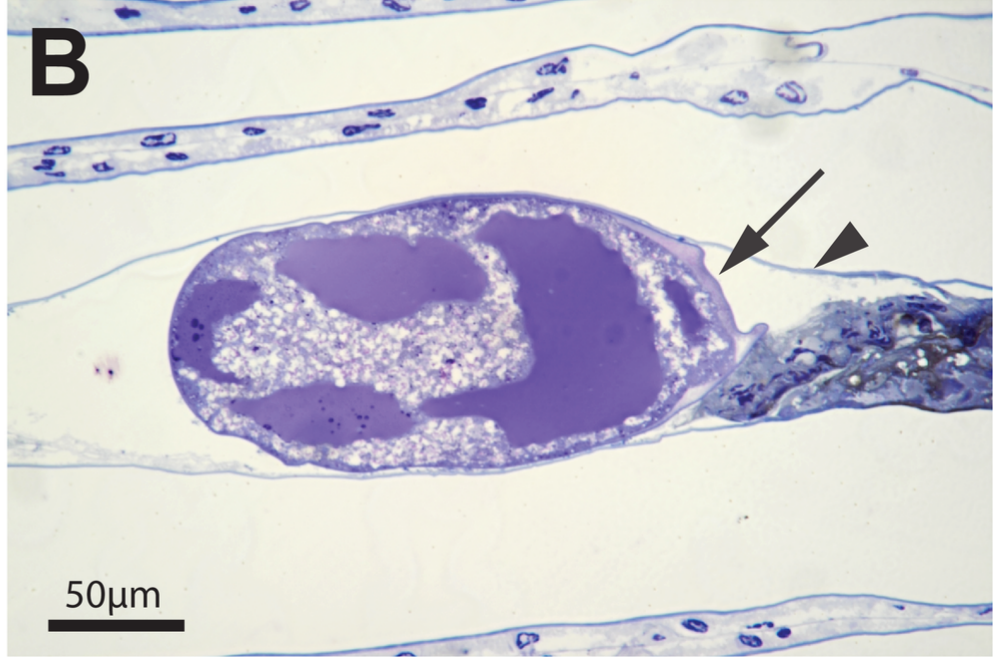
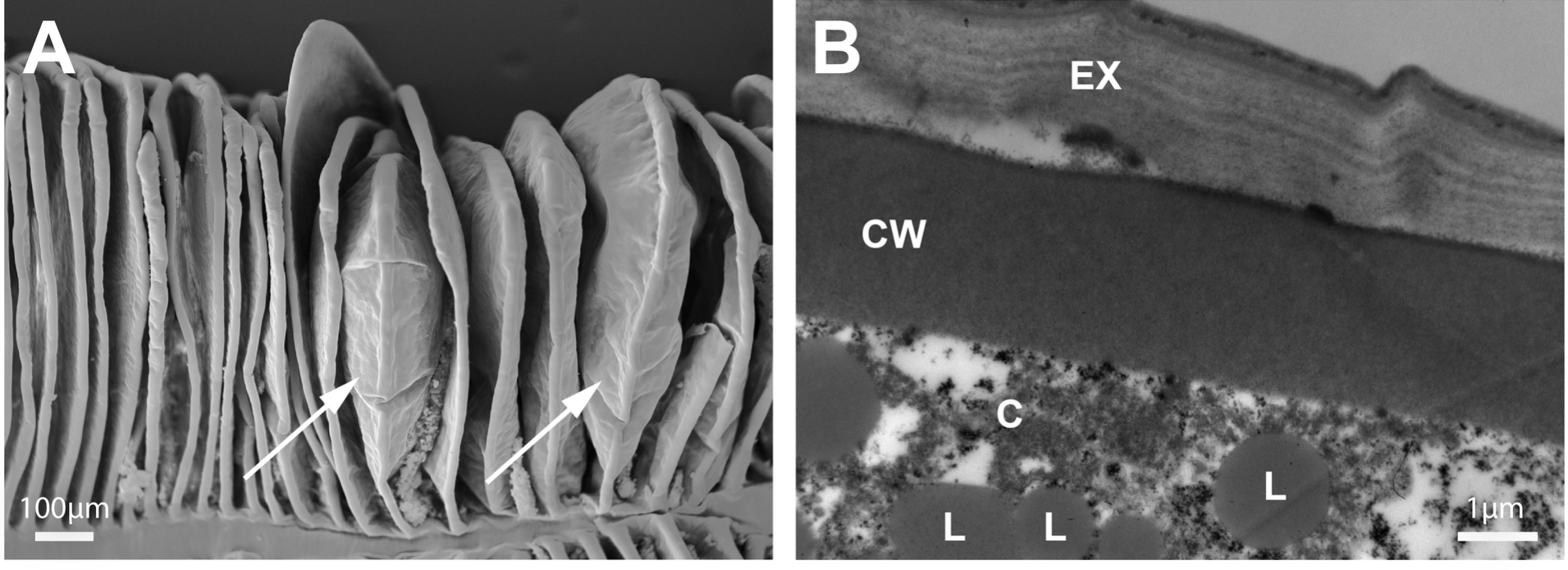
D
100µm


A
100µm


B
50µm


A
100µm
B
EX
CW
C
L
L
L
1µm

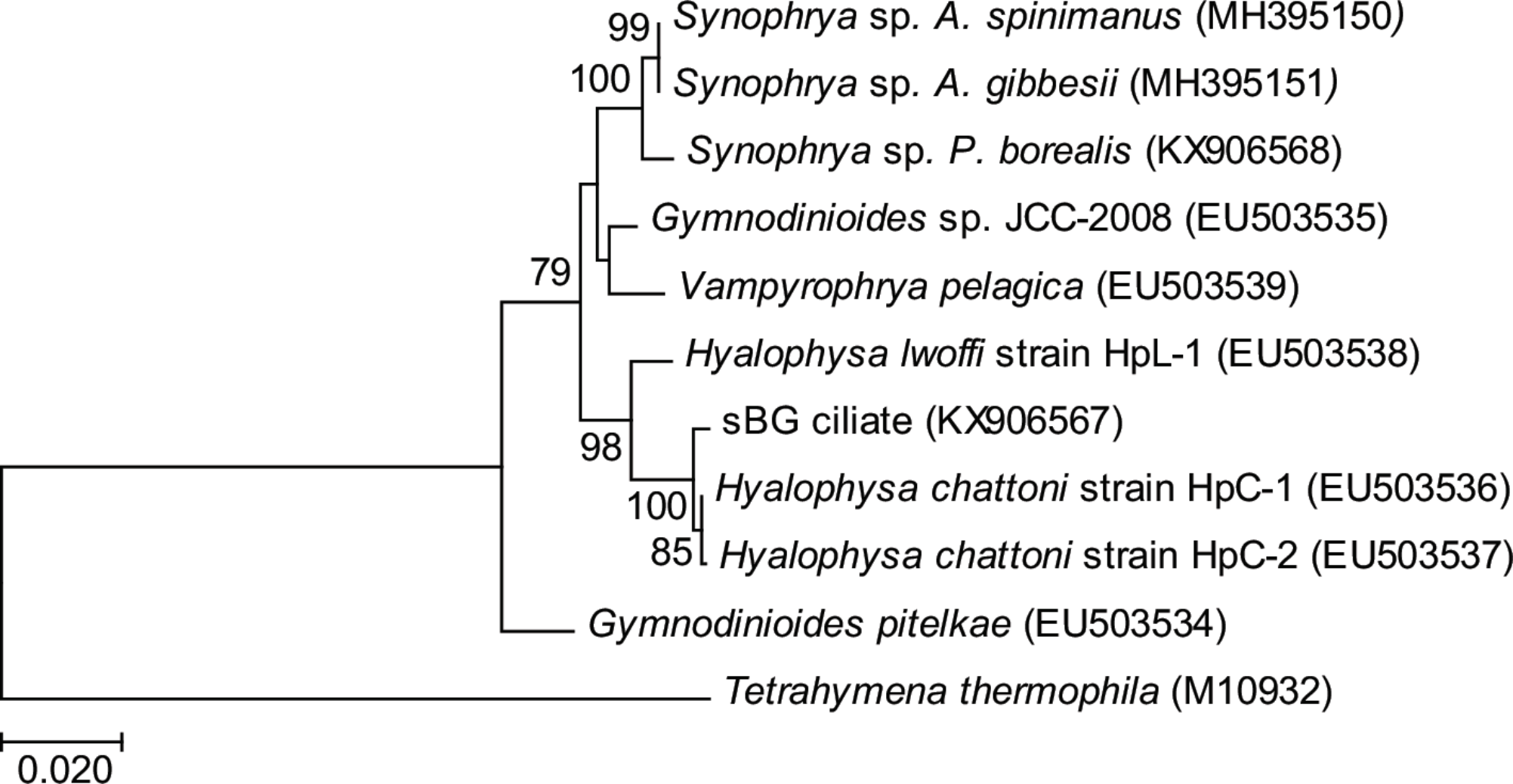
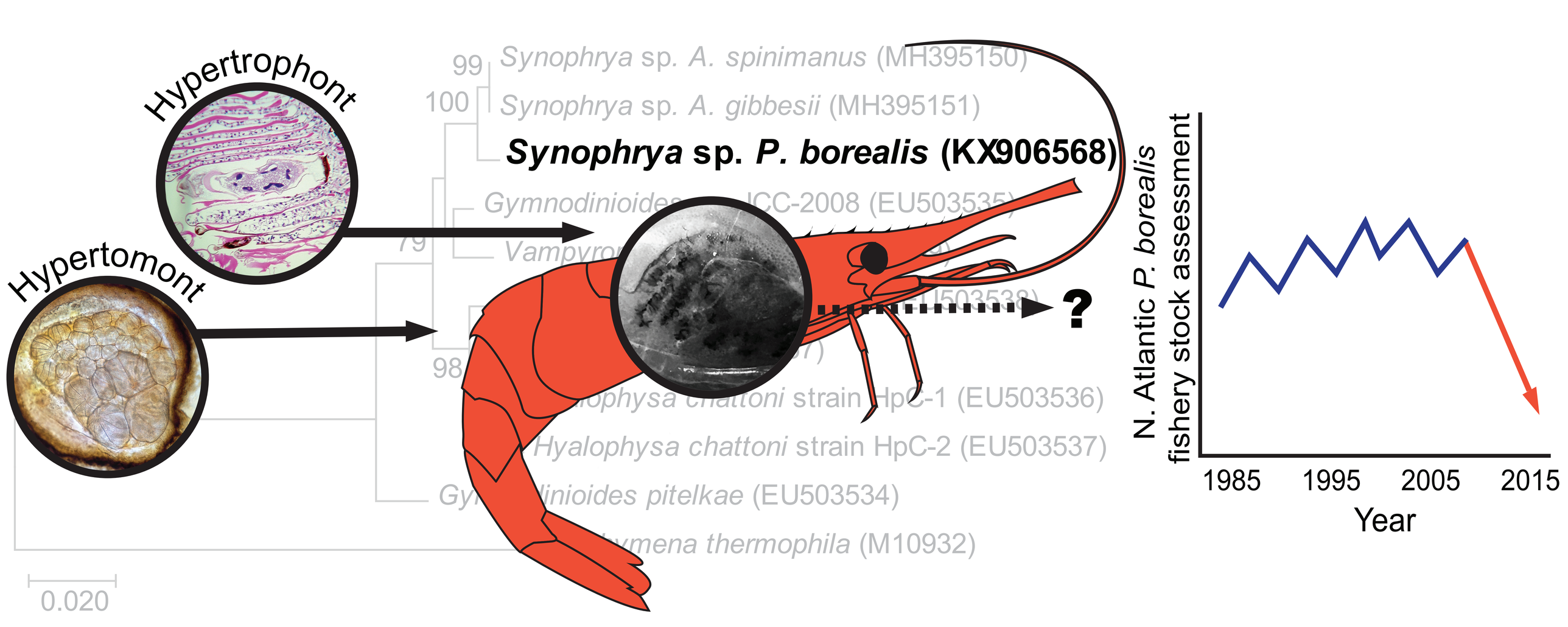

99
100
79
98
100
85
Synophrya sp. A. spinimanus (MH395150)
Synophrya sp. A. gibbesii (MH395151)
Synophrya sp. P. borealis (KX906568)
Gymnodinioides sp. JCC-2008 (EU503535)
Vampyrophrya pelagica (EU503539)
Hyalophysa lwoffi strain HpL-1 (EU503538)
sBG ciliate (KX906567)
Hyalophysa chattoni strain HpC-1 (EU503536)
Hyalophysa chattoni strain HpC-2 (EU503537)
Gymnodinioides pitelkae (EU503534)
Tetrahymena thermophila (M10932)
0.020

Table 1. Concentrations of black gill ciliates, hypertrophonts and hypertomonts, and nodules in *Pandalus borealis* gill tissues.

Sample Number	Ciliates/1mm^2	Hyper- forms/mm^2	Nodules/mm^2
1 (PB1)	7	0	1
2 (PB2)	5	0	0
3 (PB3)	12	0	0
4 (PB4)	3	1	1
5 (PB5)	6	0	1
6 (PB6)	1	2	9
7 (PB7)	3	5	6
8 (PB8)	1	0	6
9 (PB9)	4	0	1
10 (PB10)	0	4	4


Hypertrophont
Hypertomont
99
100
79
98
Synophrya sp. A. spinimanus (MH395150)
Synophrya sp. A. gibbesii (MH395151)
Synophrya sp. P. borealis (KX906568)
Gymnodinioides
ICC-2008 (EU503535)
Vampyro
(EU503538)
?
lophysa chattoni strain HpC-1 (EU503536)
Hyalophysa chattoni strain HpC-2 (EU503537)
Gy linioides pitelkae (EU503534)
ymena thermophila (M10932)
0.020
N. Atlantic P. borealis
fishery stock assessment
1985
1995
2005
2015
Year