

# **Do the Infectious Diseases of Dreissenids Represent a Threat to North American Unionid Populations?**

**Daniel P. Molloy, Ph.D.**

**State University of New York Great Lakes Center**

**Molloy & Associates, LLC**

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**Freshwater Mollusk Conservation Society**

**Freshwater Mollusk Health and Disease Assessment Workshop**

**La Crosse, Wisconsin**



Infectious diseases of dreissenids represent a threat to  
North American unionid populations?

## **Natural Enemies of Zebra Mussels: Predators, Parasites, and Ecological Competitors**

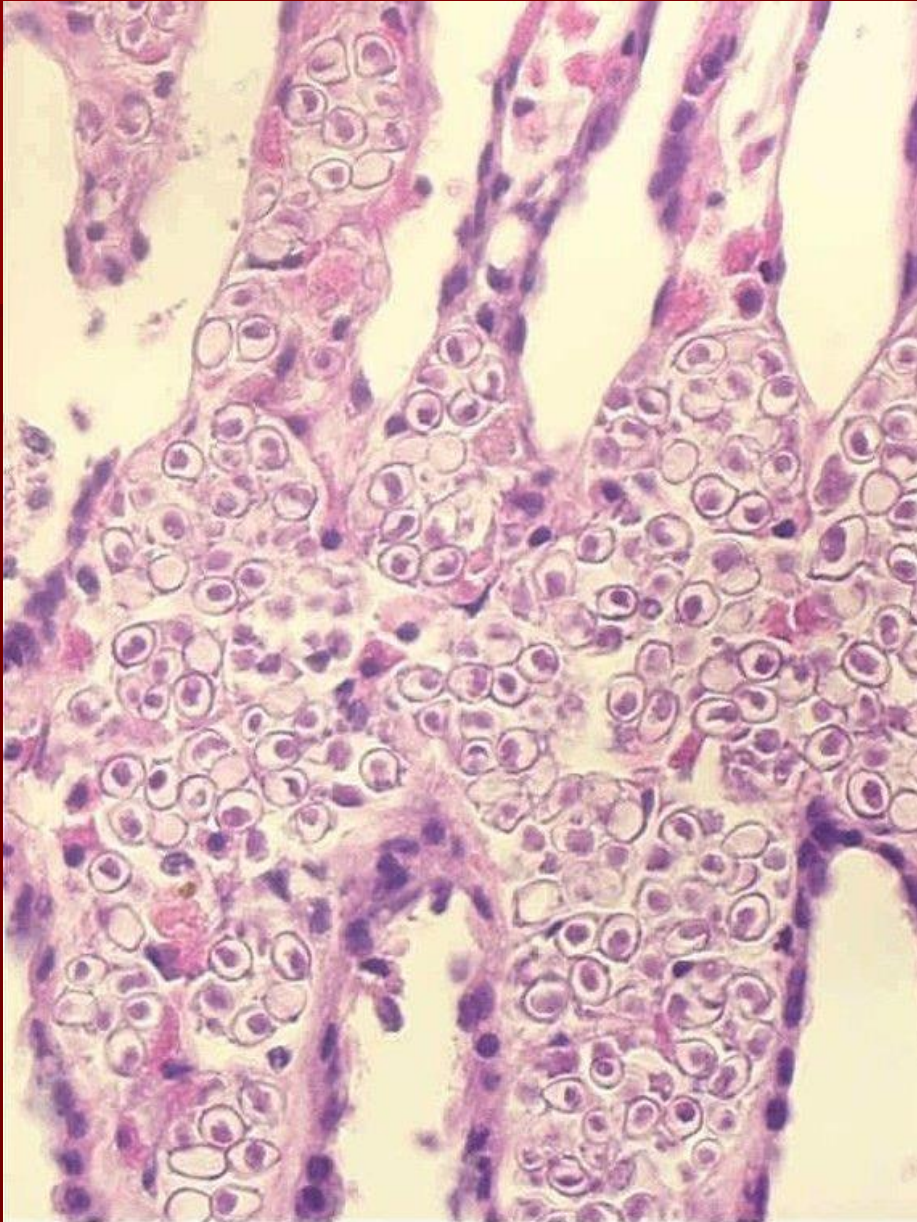
**Daniel P. Molloy,<sup>1</sup> Alexander Y. Karatayev,<sup>2</sup> Lyubov E. Burlakova,<sup>2</sup> Dina P. Kurandina,<sup>3</sup> and Franck Laruelle<sup>1</sup>**

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**ABSTRACT:** This paper reviews the international literature on the natural enemies of *Dreissena* spp. and discusses the biology and ecology of organisms known to be involved in their predation (176 species), parasitism (34 species), and competitive exclusion (10 species). Research on natural enemies, both in Europe and North America, has focused on predators, particularly birds (36 species) and fish (15 and 38 species eating veligers and

>30 species of parasites in Eurasian *Dreissena* spp.

Types of parasites in dreissenids.....



Haplosporidia in  
connective  
tissue

# *Haplosporidium raabei* n. sp. (Haplosporidia): a parasite of zebra mussels, *Dreissena polymorpha* (Pallas, 1771)

D. P. MOLLOY<sup>1\*</sup>, L. GIAMBÉRINI<sup>2</sup>, N. A. STOKES<sup>3</sup>, E. M. BURRESON<sup>3</sup>  
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(Received 15 March 2011; revised 28 July, 10 September and 13 October 2011; accepted 27 October 2011)

## SUMMARY

Extensive connective tissue lysis is a common outcome of haplosporidian infection. Although such infections in marine invertebrates are well documented, they are relatively rarely observed in freshwater invertebrates. Herein, we report a field study using a comprehensive series of methodologies (histology, dissection, electron microscopy, gene sequence analysis, and molecular phylogenetics) to investigate the morphology, taxonomy, systematics, geographical distribution, pathogenicity, and seasonal and annual prevalence of a haplosporidian observed in zebra mussels, *Dreissena polymorpha*. Based on its genetic sequence, morphology, and host, we describe *Haplosporidium raabei* n. sp. from *D. polymorpha* – the first haplosporidian species from a freshwater bivalve. *Haplosporidium raabei* is rare as we observed it in histological sections in only 0.7% of the zebra mussels collected from 43 water bodies across 11 European countries and in none that were collected from 10 water bodies in the United States. In contrast to its low prevalences, disease intensities were quite high with 79.5% of infections advanced to sporogenesis.

Key words: *Haplosporidium raabei* n. sp., Haplosporidia, *Dreissena polymorpha*, phylogeny, small subunit ribosomal DNA.

# *Haplosporidium raabei* n. sp. (Haplosporidia): a parasite of zebra mussels, *Dreissena polymorpha* (Pallas, 1771)

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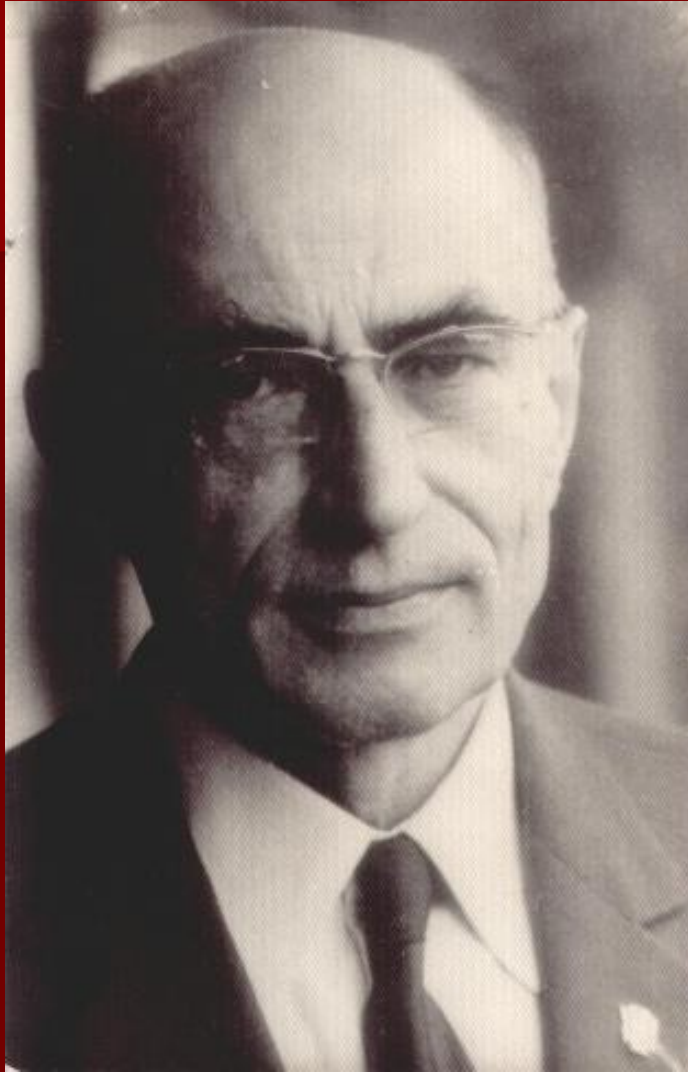
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## SUMMARY

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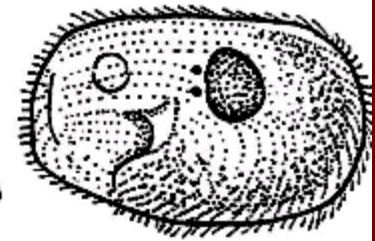
Dr. Z. Raabe



**Conchophthirus  
acuminatus**



**Conchophthirus  
klimentinus**



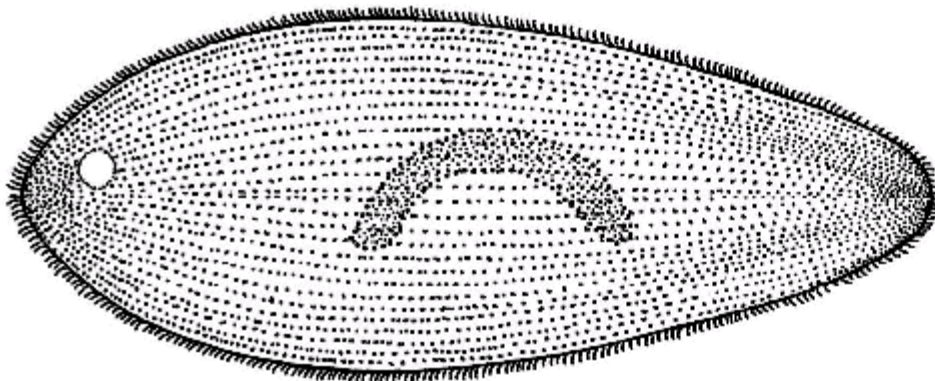
**Hypocomagalma  
dreissenae**



**Sphenophrya  
dreissenae**



**Sphenophrya  
naumiana**



**Large ophryoglene**



**Small  
ophryoglene**

**50  $\mu$ m**

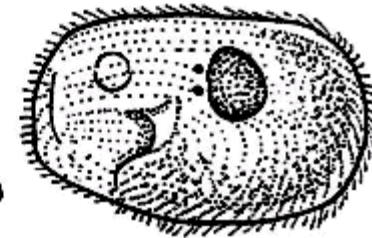
None of these species observed in unionids



**Conchophthirus  
acuminatus**



**Conchophthirus  
klimentinus**



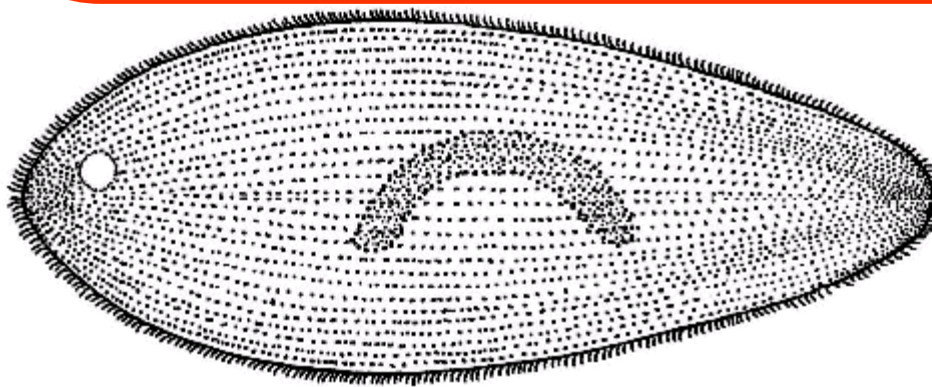
**Hypocomagalma  
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**Large ophryoglene**



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**50  $\mu$ m**

## HISTOLOGICAL ANALYSIS OF MANTLE-CAVITY CILIATES IN *DREISSENA POLYMORPHA*: THEIR LOCATION, SYMBIOTIC RELATIONSHIP, AND DISTINGUISHING MORPHOLOGICAL CHARACTERISTICS

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<sup>2</sup>Biological Survey, New York State Museum  
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Albany, New York 12230

<sup>3</sup>Biological Research Institute  
St. Petersburg State University  
St. Petersburg 198904, Russia

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Kiev-210 254655, Ukraine

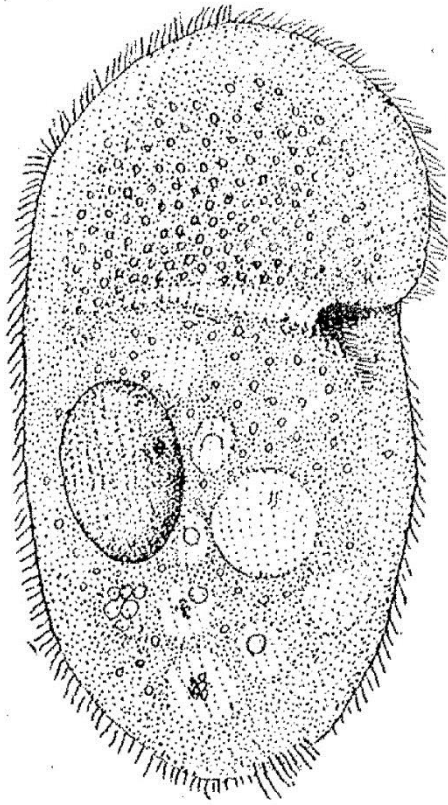
**ABSTRACT** Dissection has traditionally been the sole method used in investigations of the parasites and other endosymbionts of zebra mussels, *Dreissena polymorpha*. This study demonstrates the value of histological analysis as a complementary technique capable of precisely determining the location of ciliates within zebra mussels and characterizing their symbiotic relationships at the cellular level. The photomicrographs included herein represent the first published histological images of mantle-cavity ciliates of zebra mussels, and we have highlighted morphological characteristics useful in distinguishing individual ciliate species in histological sections. Although zebra mussels from both North America and Europe were sampled for this study, only European populations were found to harbor mantle-cavity ciliates, and five species were observed. The host-specific species *Conchophthirus acuminatus* (Scuticociliatida: Conchophthiridae) was frequently recorded from epithelium covering the outer gill surfaces and occasionally from visceral mass epithelium, but also found in four previously unreported regions: frequently within gill water tubes and occasionally on labial palps, mantle epithelium, and within suprabranchial cavities. Although we sometimes observed zebra mussel sperm in food vacuoles of *C. acuminatus*, epithelial tissues in contact with high densities of these ciliates showed no evidence of pathology, thus confirming this species' commensal nature. The host-specific species *Sphenophrya dreissenae* (Rhynchodida: Sphenophryidae) was frequently recorded attached to mantle cavity epithelium and outer gill surfaces, but also found in three previously unreported regions: frequently within the gill water tubes, occasionally on the visceral mass, and rarely within the suprabranchial cavities. High-intensity infections with this parasitic ciliate did induce hyperplasia, cell hypertrophy, and vacuolization of the epithelia. The host-specific species *Hypocomagalma dreissenae* (Rhynchodida: Ancistrocomidae) was most frequently observed attached to epithelial cells lining outer gill surfaces, but also in five previously unreported regions: occasionally on the visceral mass, the mantle cavity epithelium, and in gill water tubes, and rarely on labial palps and within the suprabranchial cavities. This parasitic ciliate feeds on the contents of epithelial cells using a suctorial tentacle. The intensity of *H. dreissenae* infection, however, was usually very low, and no adverse effects on parasitized cells or nearby tissues were evident. The ciliate *Ancistrumina limnica* (Scuticociliatida: Ancistridae), a nonhost-specific commensal of mollusks, was recorded frequently within gill water tubes, occasionally on outer gill epithelia, and rarely within suprabranchial cavities. This species was also observed to have ingested *D. polymorpha* sperm cells. Commensal Peritrichia ciliates were also occasionally observed within the mantle cavity, but were likely carried there passively by water currents from their typical location on shell surfaces. The presence of "mantle cavity" ciliate species in the gill water tubes and the suprabranchial cavities of zebra mussels suggests that these ciliates probably can exit into surrounding waters to infect other zebra mussels via the exhalant siphon.

**KEY WORDS:** zebra mussels, ciliophora, *Conchophthirus*, *Sphenophrya*, *Hypocomagalma*, *Ancistrumina*, Peritrichia

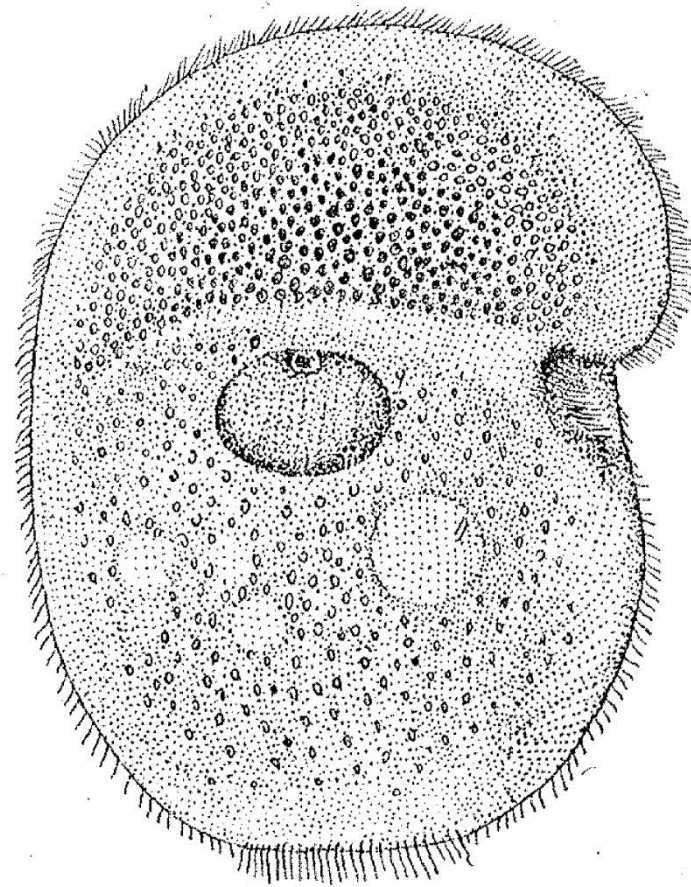
### INTRODUCTION

Zebra mussels, *Dreissena polymorpha*, were likely transported

raw-water conduits within infrastructures (O'Neill 1996, O'Neill 1997), they have also caused significant environmental impacts (MacIsaac 1996). Although considerable research has been carried



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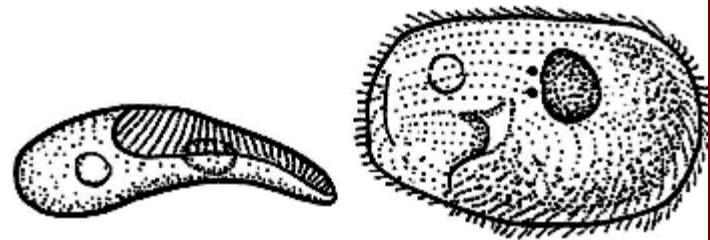
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Fig. 10. *C. unionis* sp. n. Nach dem Leben. Vergr.  $\pm 550$ .  
Fig. 11. *C. curtus* Englm. Nach dem Leben. Vergr.  $\pm 550$ .

.... and likewise *Conchophthirus* ciliates found in unionids are not found in dreissenids



**Conchophthirus  
acuminatus**



**Conchophthirus  
klimentinus**



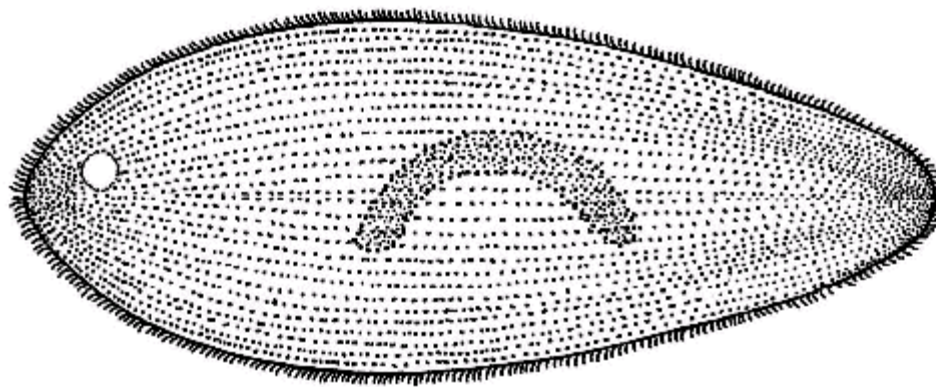
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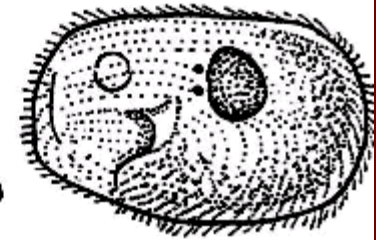
**50  $\mu$ m**



**Conchophthirus  
acuminatus**



**Conchophthirus  
klimentinus**



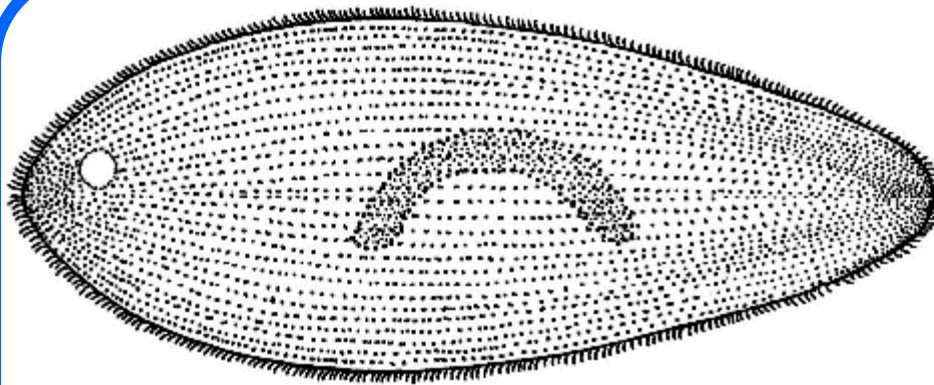
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**Large ophryoglene**



**Small  
ophryoglene**

**50  $\mu$ m**

# *Ophryoglena hemophaga* n. sp. (Ciliophora: Ophryoglenidae): a parasite of the digestive gland of zebra mussels *Dreissena polymorpha*

Daniel P. Molloy<sup>1,\*</sup>, Denis H. Lynn<sup>2</sup>, Laure Giamberini<sup>3</sup>

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<sup>2</sup>Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada

<sup>3</sup>Laboratoire Ecotoxicité et Santé Environnementale, CNRS UMR 7146, Université Paul Verlaine-Metz, Campus Bridoux, rue du Général Délestraint, 57070 Metz Cedex, France

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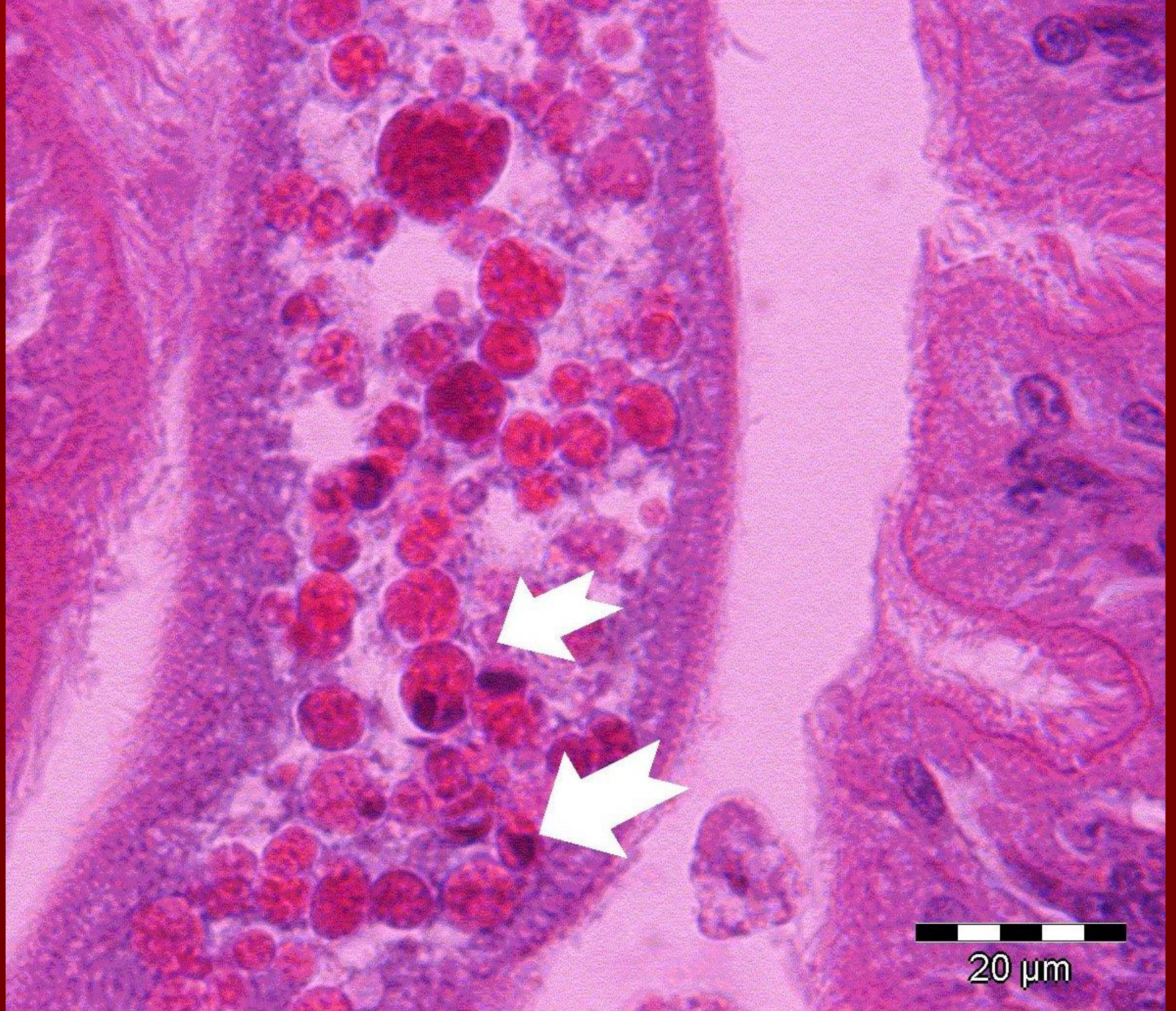
ABSTRACT: *Ophryoglena hemophaga* n. sp. is described from a freshwater *Dreissena polymorpha* population in the Rhine delta of the Netherlands. This is the first ophryoglenine species (order Hymenostomatida, suborder Ophryoglenina) recorded as a molluscan parasite. As is typical of ciliates in the suborder Ophryoglenina, *O. hemophaga* exhibits a polymorphic life history with cystment and reproduction by palintomy. Trophonts were observed within digestive gland lumina, and zebra mussel hemocytes were present in some of their digestive vacuoles. The presence of a single, longitudinal tract of multiple contractile vacuoles represents its most unique feature and distinguishes it from all other described *Ophryoglena* spp. The number of somatic kineties of *O. hemophaga* (range 50 to 62) is also a distinguishing feature, since it is the lowest described from any *Ophryoglena* sp. Other characteristics of this species include: ovoid to elongate trophonts 96 to 288 µm in length, with an elongate macronucleus 41 to 65 µm in length; tomonts 50 to 150 µm in diameter producing a clear mucous cyst envelope, whose thickness is approximately half of the tomont diameter; elongated theronts 96 to 131 µm in length which emerge after 1 to 3 cell divisions taking 36 to 48 h at 20 ± 3°C. Protomonts and theronts are, respectively, negatively and positively phototactic — characteristics that likely aid in maintenance of infection in zebra mussel populations.

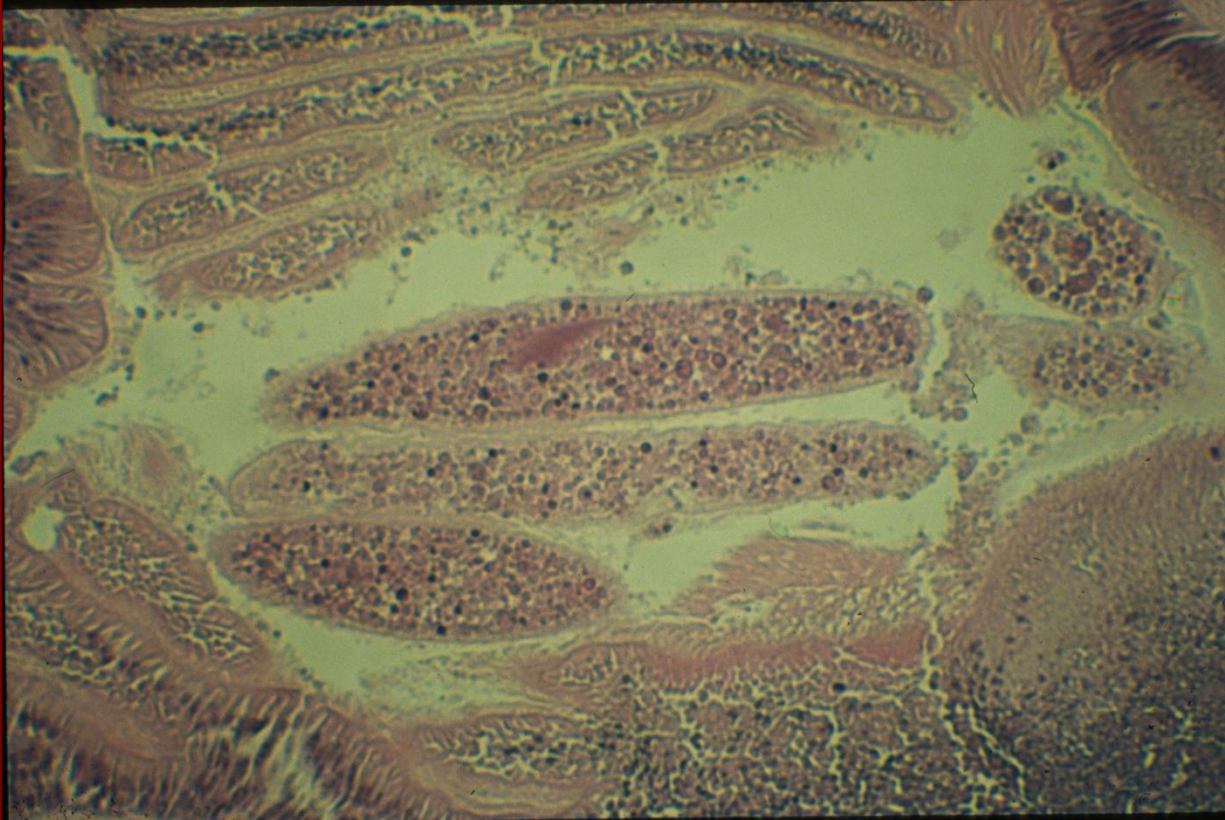
KEY WORDS: Contractile vacuoles · Trophont · Tomont · Theront · Zebra mussel · Palintomy · Phototaxis



*O. hemophaga* is in Mohawk River in *D. polymorpha*  
in New York State





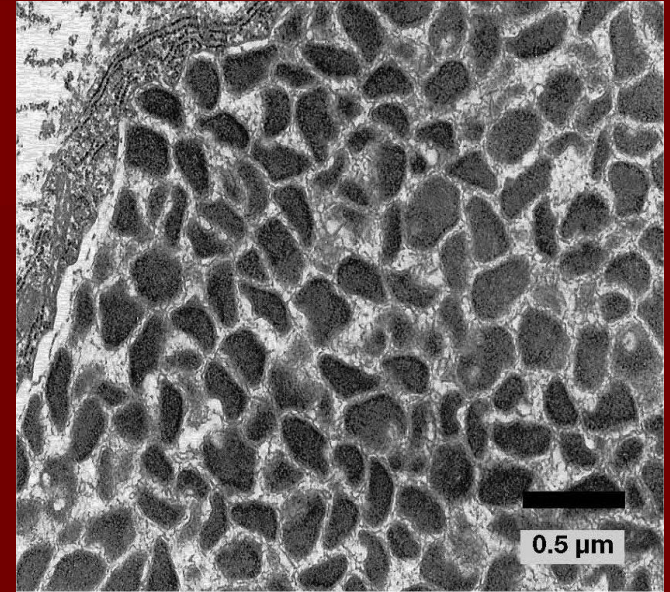
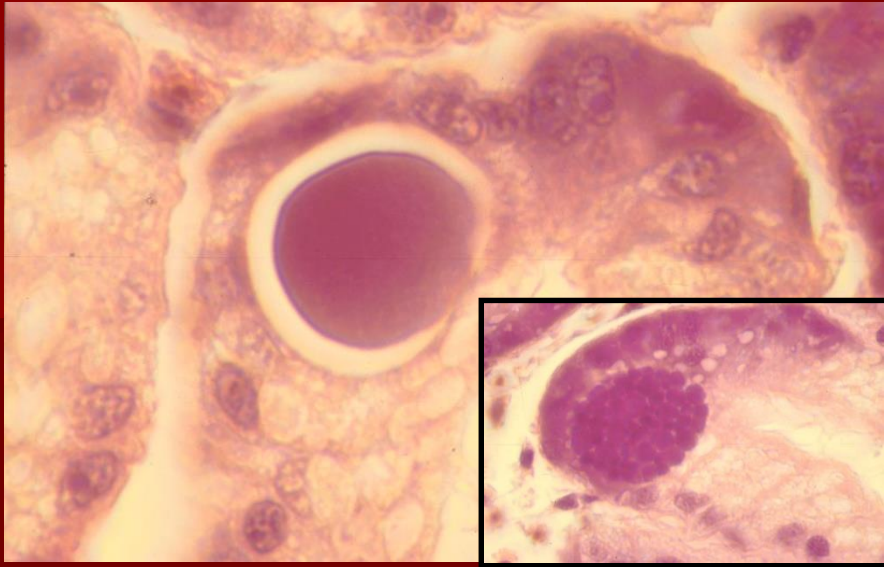


Yes, they are developing in the lumen of the digestive gland ducts, consuming food, blood cells, etc.....

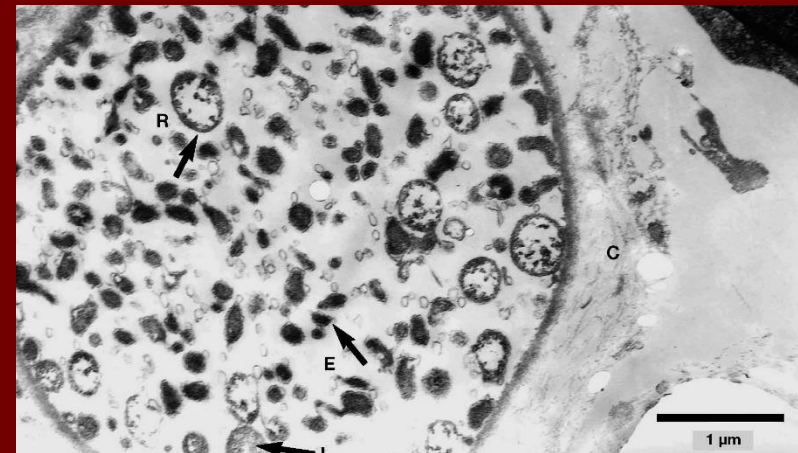
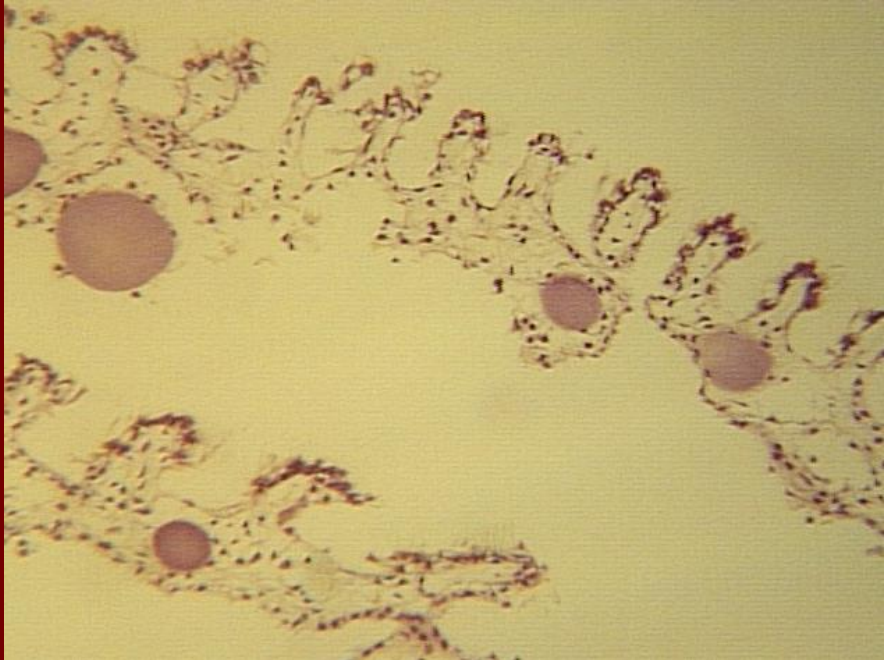
.... But no evidence otherwise of negative impact on host dreissenids....

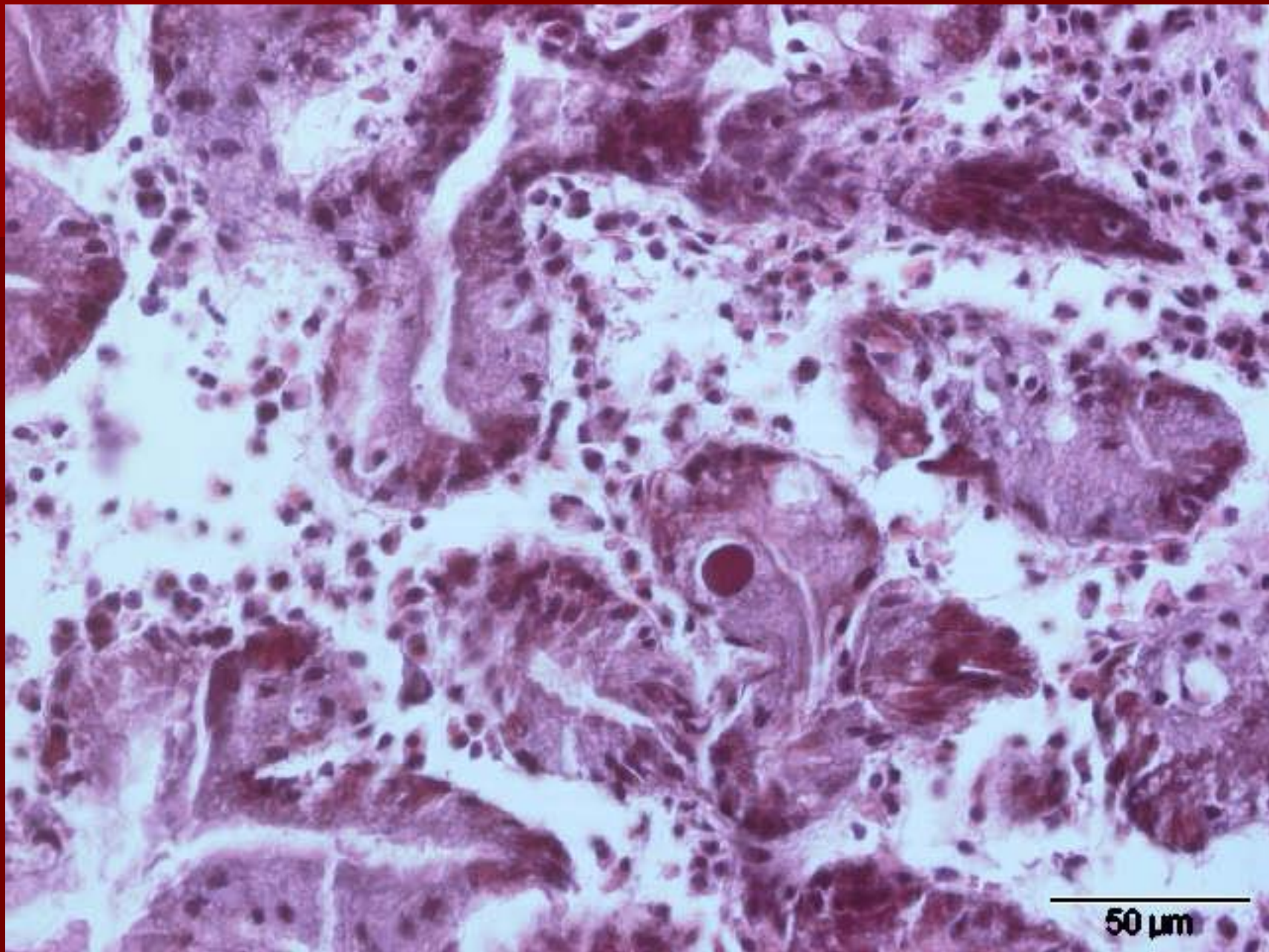
Ophryoglena has never been reported from digestive glands of Eurasian unionids.....

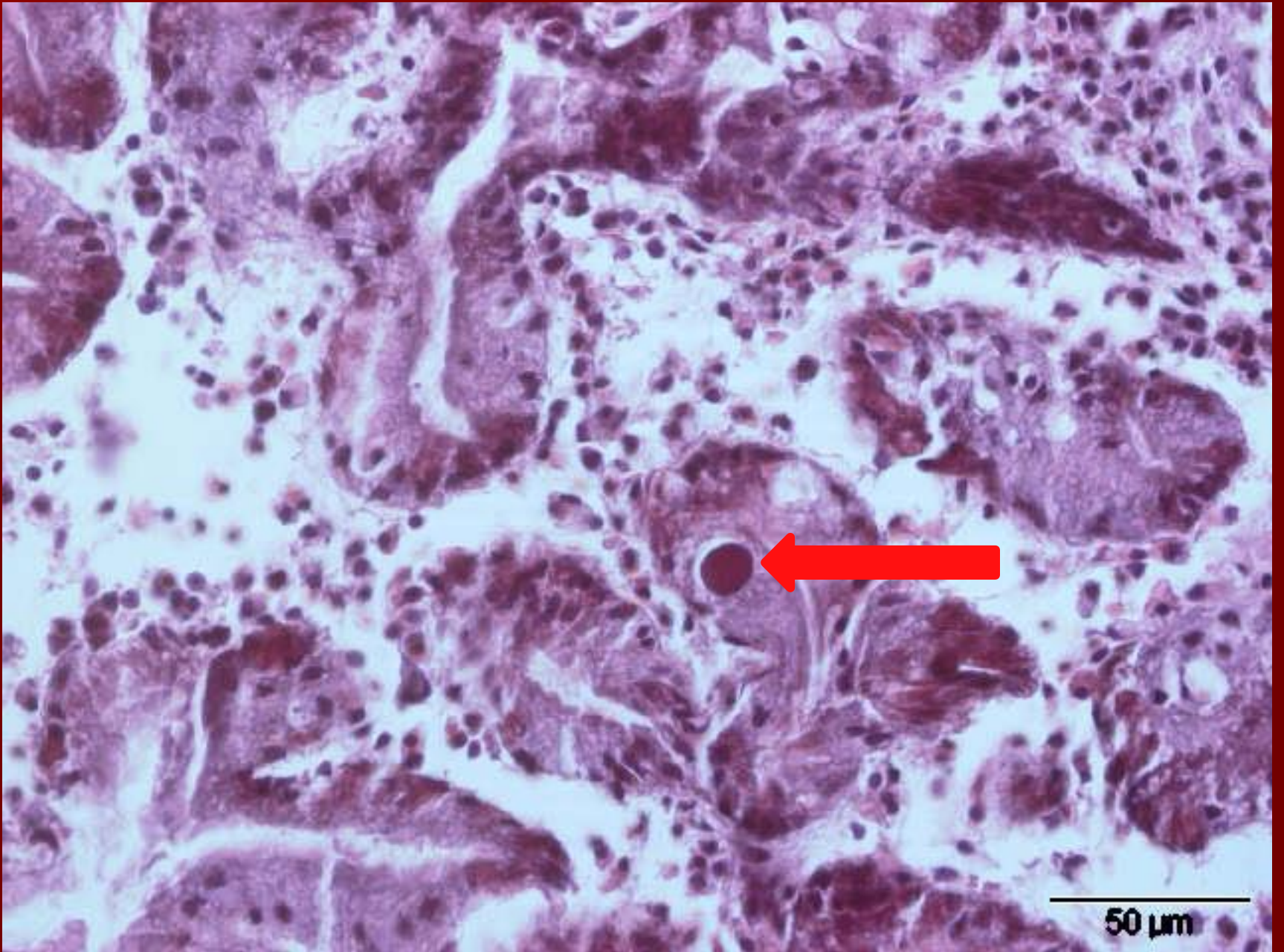
## Prokaryotes: Rickettsiales



## Prokaryotes : Chlamydiales







## Characterization of intracytoplasmic prokaryote infections in *Dreissena* sp. (Bivalvia: Dreissenidae)

Daniel P. Molloy<sup>1,\*</sup>, Laure Giamberini<sup>2</sup>, J. Frank Morado<sup>3</sup>, Sergei I. Fokin<sup>4</sup>,  
Franck Laruelle<sup>5</sup>

<sup>1</sup>New York State Museum, The State Education Department, Cultural Education Center, Albany, New York 12230, USA

<sup>2</sup>Equipe de Production des Ecosystèmes et Ecotoxicologie, Laboratoire EBSE, Université de Metz, Campus Bridoux, rue du Gal Délestraint, 57070 Metz Cedex, France

<sup>3</sup>National Oceanic & Atmospheric Administration, National Marine Fisheries Service, Alaska Fisheries Science Center, Resource Assessment & Conservation Engineering Division, 7600 Sand Point Way NE, Seattle, Washington 98115-0070, USA

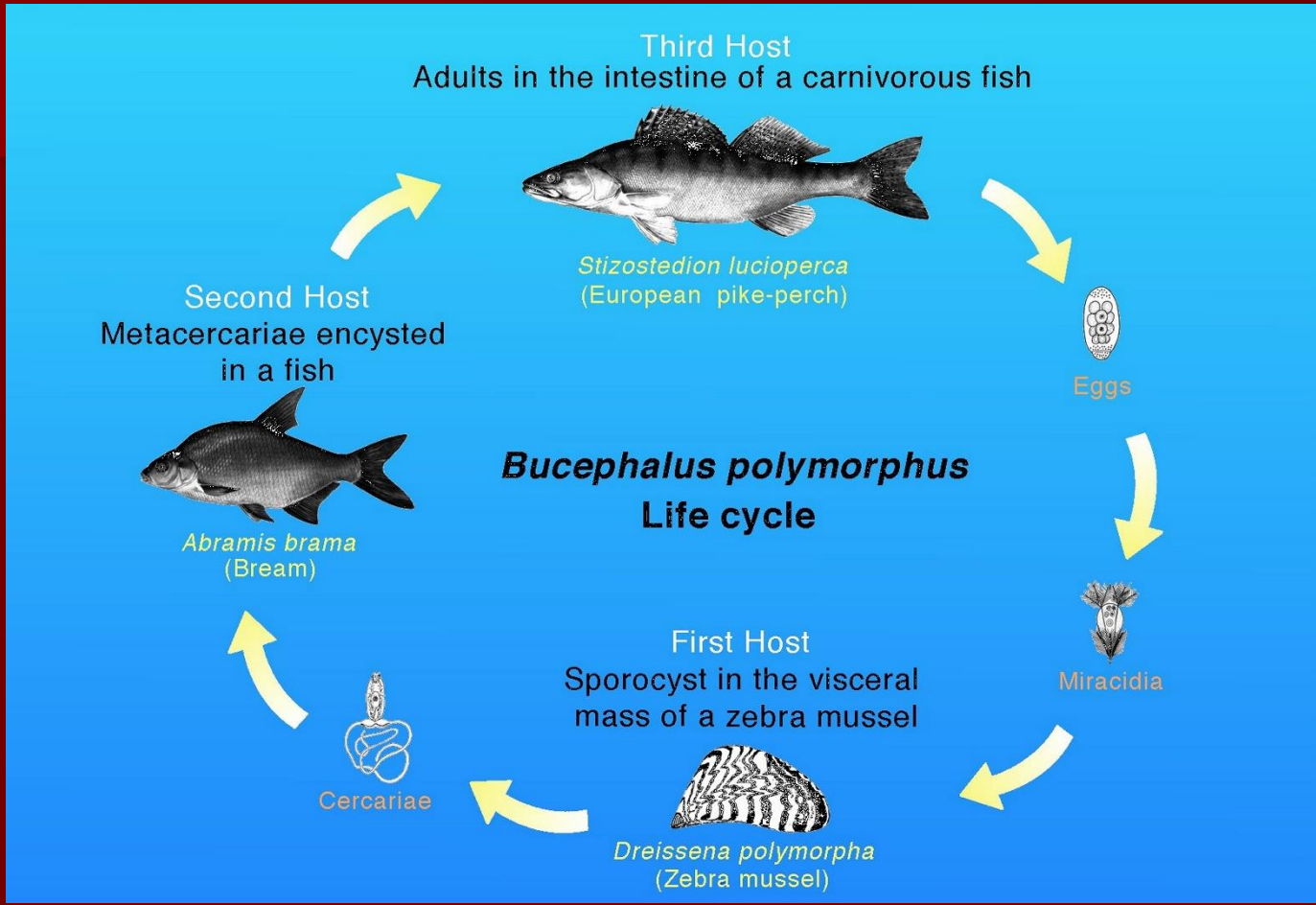
<sup>4</sup>Biological Research Institute, St. Petersburg State University, St. Petersburg 198904, Russia

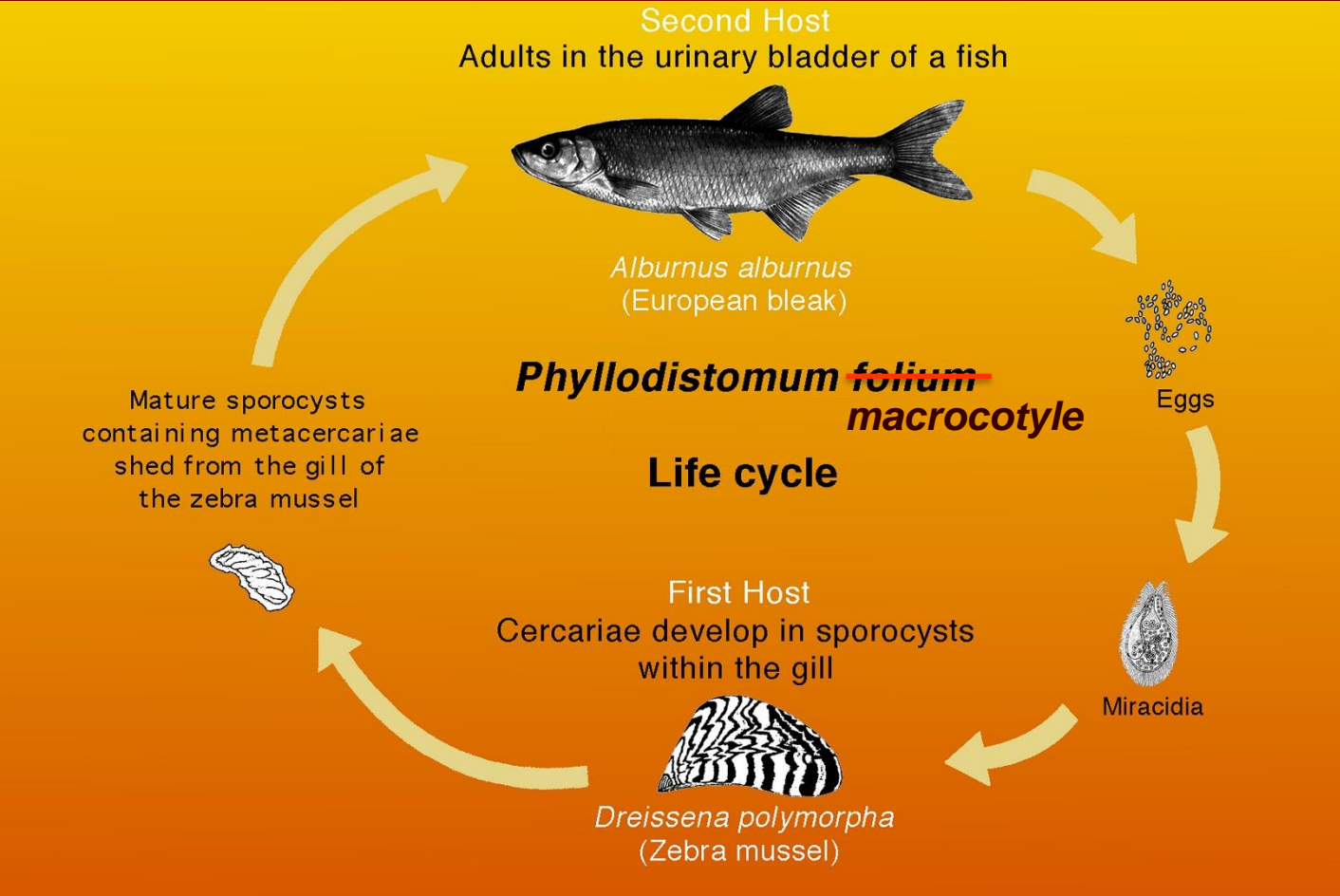
<sup>5</sup>UMR CNRS 6539, Institut Universitaire Européen de la Mer, UBO, Place Nicolas Copernic, Technopôle Brest-Iroise, 29280 Plouzané, France

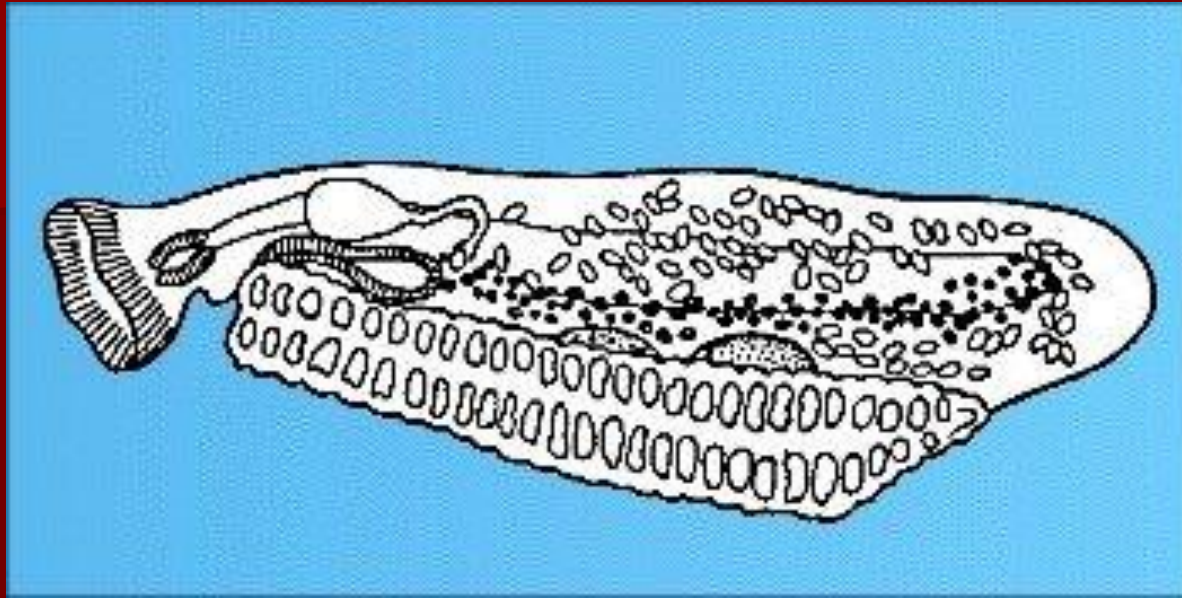
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**ABSTRACT:** This study characterizes intracytoplasmic infections with prokaryote microorganisms in *Dreissena* sp. (near *Dreissena polymorpha*) from northeastern Greece and represents the first report of such infections in freshwater bivalves. Light microscope observations of stained tissues revealed basophilic, cytoplasmic inclusion bodies in 87.5% (28/32) of the mussels sectioned. Inclusions in









Aspidogastrea trematodes can cause internal host damage, but clear documentation is lacking in the literature that they kill their unionid hosts.

*Aspidogaster conchicola* has been reported from both unionids and dreissenids, and other freshwater bivalves.

## HISTOLOGICAL ANALYSIS OF TREMATODES IN *DREISSENA POLYMORPHA*: THEIR LOCATION, PATHOGENICITY, AND DISTINGUISHING MORPHOLOGICAL CHARACTERISTICS

Franck Laruelle, Daniel P. Molloy\*, and Vitali A. Roitman†

CEDRE, Rue Alain Colas, BP 20413-29604 Brest Cedex, France. e-mail: [dmolloy@mail.nysed.gov](mailto:dmolloy@mail.nysed.gov)

**ABSTRACT:** Four families of trematodes were observed in histological sections during a 1992–1997 investigation of the parasites of zebra mussels *Dreissena polymorpha*. These included Aspidogastridae, i.e., *Aspidogaster*, Echinostomatidae, Bucephalidae, i.e., *Bucephalus polymorphus*, and Gorgoderidae, i.e., *Phyllodistomum folium*. This article describes the precise location of these trematodes in the tissues of *D. polymorpha*, provides graphic evidence of their effect on the organs they inhabit, and highlights the distinguishing morphological characteristics. Evidence of defense reaction of host to trematode infection, i.e., encapsulation of *Aspidogaster* and nacreization of *B. polymorphus*, is also presented and is the first such report for zebra mussels. The histological photomicrographs included represent the first comprehensive series published on trematode infection of zebra mussels. These images, in conjunction with the morphological descriptions presented, should assist researchers in identifying the 4 major trematode taxa that they are likely to encounter in the tissue sections of zebra mussels.

Zebra mussels, *Dreissena polymorpha*, were likely transported from Europe to North America in the ballast water of transoceanic vessels (Carlton, 1993). Within a few years after their discovery in Lake St. Clair in 1988 (Hebert et al., 1989), these freshwater, macrofouling bivalves were found in high densities throughout the Great Lakes Basin. Populations have thus far been reported as far south as Louisiana and as far west as Oklahoma (New York Sea Grant, 2001). In addition to the economic impact of the fouling of raw-water conduits within infrastructures (O'Neill, 1996, 1997), zebra mussels have also caused significant environmental impacts (Strayer, 1999) similar to those reported from their native European range (Karatayev et al., 1997).

Although extensive research efforts have been made since the 19th century to understand the ecological interrelationships of these bivalves with other aquatic organisms in their habitats (MacIsaac, 1996; Karatayev et al., 1997; Strayer, 1999), relatively little effort had been made to investigate the diversity, distribution, and significance of endosymbiotic organisms of

of zebra mussels. In addition, these photos illustrate the precise location of trematodes in the organs of zebra mussels and their effects on the surrounding tissues. A report detailing the infection intensity and prevalence of all parasitic infections recorded during this 5-yr research project is being prepared separately for publication.

### MATERIALS AND METHODS

During 1992–1997, several hundred *D. polymorpha* were collected from the eastern United States and several thousand from across Europe as part of an investigation of the endosymbionts of this species. Mussels (ca. 5–30 mm in length) were fixed in 10% neutral formalin (buffered with sodium phosphate), dehydrated in a graded series of alcohols and toluene, and embedded in paraffin. Approximately 4 serial, oblique-longitudinal sections (5 µm thick) were cut per mussel, stained with hematoxylin and eosin, and examined using light microscopy (×450–×1,000). Unless mentioned in the text, the photomicrographs included in this article are representative of the typical appearance of infection observed during the study.

### RESULTS

# Take Home Message

Q: Infectious diseases of dreissenids represent a threat to North American unionid populations?

# Take Home Message

Q: Infectious diseases of dreissenids represent a threat to North American unionid populations?

A: No evidence to warrant any concern yet