Endosymbiont Protistan Diversity in Zebra Mussels, Quagga Mussels and Other *Dreissena* spp. in North America and Eurasia

Daniel P. Molloy, PhD
Molloy & Associates, LLC

SCROLL DOWN TO SEE PRESENTATION SLIDES

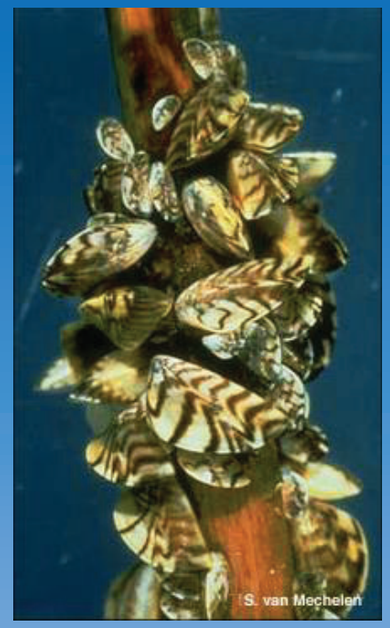
March 30, 2019

Midwest Protozoology Society Meeting
Peoria, Illinois

FUNDING ACKNOWLEDGEMENT

RECLAMATION
Managing Water in the West


ZEBRA MUSSEL
QUAGGA MUSSEL


S. van Mechelen

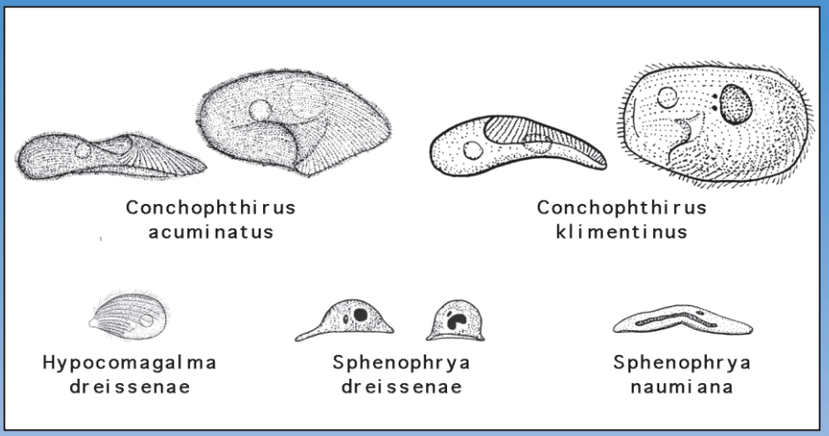
Journal of Shellfish Research, Vol. 18, No. 1, 251–257, 1999.

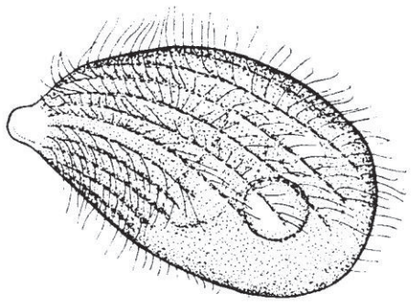
HISTOLOGICAL ANALYSIS OF MANTLE-CAVITY CILIATES IN *DREISSENA POLYMORPHA*: THEIR LOCATION, SYMBIOTIC RELATIONSHIP, AND DISTINGUISHING MORPHOLOGICAL CHARACTERISTICS

F. LARUELLE,[1] D. P. MOLLOY,[2] S. I. FOKIN,[3] AND M. A. OVCHARENKO[4]

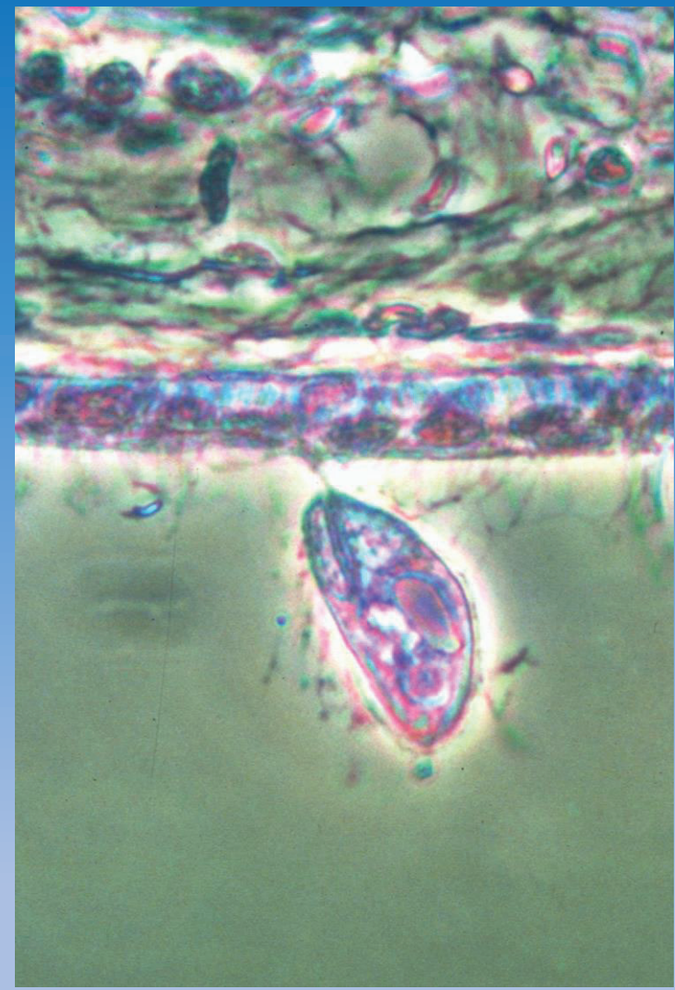
[1]*UMR CNRS 6539*
Institut Universitaire Européen de la Mer
UBO, Place Nicolas Copernic
Technopôle Brest-Iroise 29280 Plouzané, France
[2]*Biological Survey, New York State Museum*
The State Education Department
Cultural Education Center
Albany, New York 12230
[3]*Biological Research Institute*
St. Petersburg State University
St. Petersburg 198904, Russia
[4]*Institute of Hydrobiology*
Ukrainian Academy of Sciences
12 Prospect Geroyev Stalingrada
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.





Hypocomagalma dreissenae



Vol. 65: 237–243, 2005 | DISEASES OF AQUATIC ORGANISMS
Dis Aquat Org | Published July 18

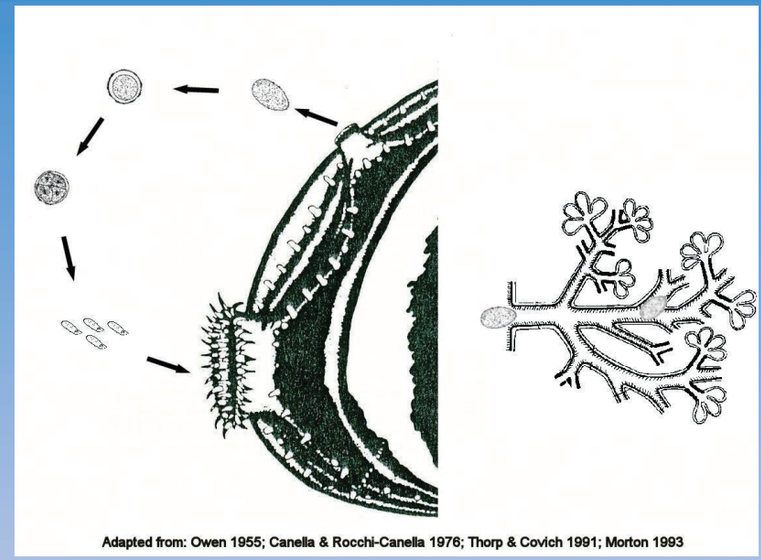
Ophryoglena hemophaga n. sp. (Ciliophora: Ophryoglenidae): a parasite of the digestive gland of zebra mussels *Dreissena polymorpha*

Daniel P. Molloy[1,*], Denis H. Lynn[2], Laure Giamberini[3]

[1]New York State Museum, The State Education Department, Cultural Education Center, Albany, New York 12230, USA
[2]Department of Zoology, University of Guelph, Guelph, Ontario N1G 2W1, Canada
[3]Laboratoire Ecotoxicité et Santé Environnementale, CNRS UMR 7146, Université Paul Verlaine-Metz, Campus Bridoux, rue du Général Délestraint, 57070 Metz Cedex, France

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



Adapted from: Owen 1955; Canella & Rocchi-Canella 1976; Thorp & Covich 1991; Morton 1993

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

D. P. MOLLOY[1]*, L. GIAMBÉRINI[2], N. A. STOKES[3], E. M. BURRESON[3] *and* M. A. OVCHARENKO[4,5]

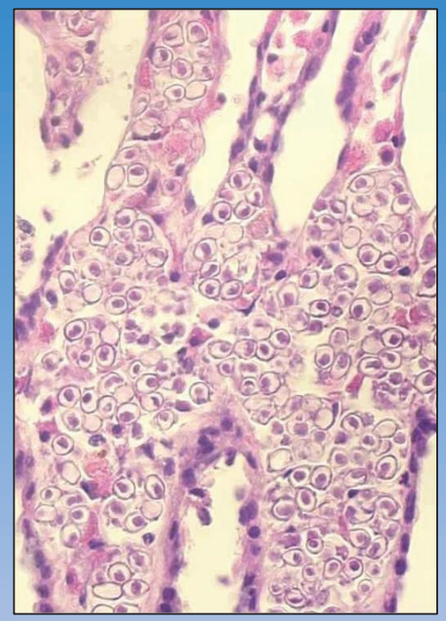
[1] *Division of Research and Collections, New York State Museum, Albany, NY 12230, USA*
[2] *Université Paul Verlaine – Metz, Laboratoire des Interactions, Ecotoxicologie, Biodiversité, Ecosystèmes (LIEBE), CNRS UMR 7146, Campus Bridoux, Rue du Général Delestraint, F-57070 Metz, France*
[3] *Virginia Institute of Marine Science, College of William & Mary, Gloucester Point, P.O. Box 1346, Virginia 23062, USA*
[4] *Institute of Parasitology, Polish Academy of Sciences, Twarda 51/55, 00-818 Warsaw, Poland*
[5] *Pomeranian Akademy, Arcishewski str. 22b, 76-200, Slupsk, Poland*

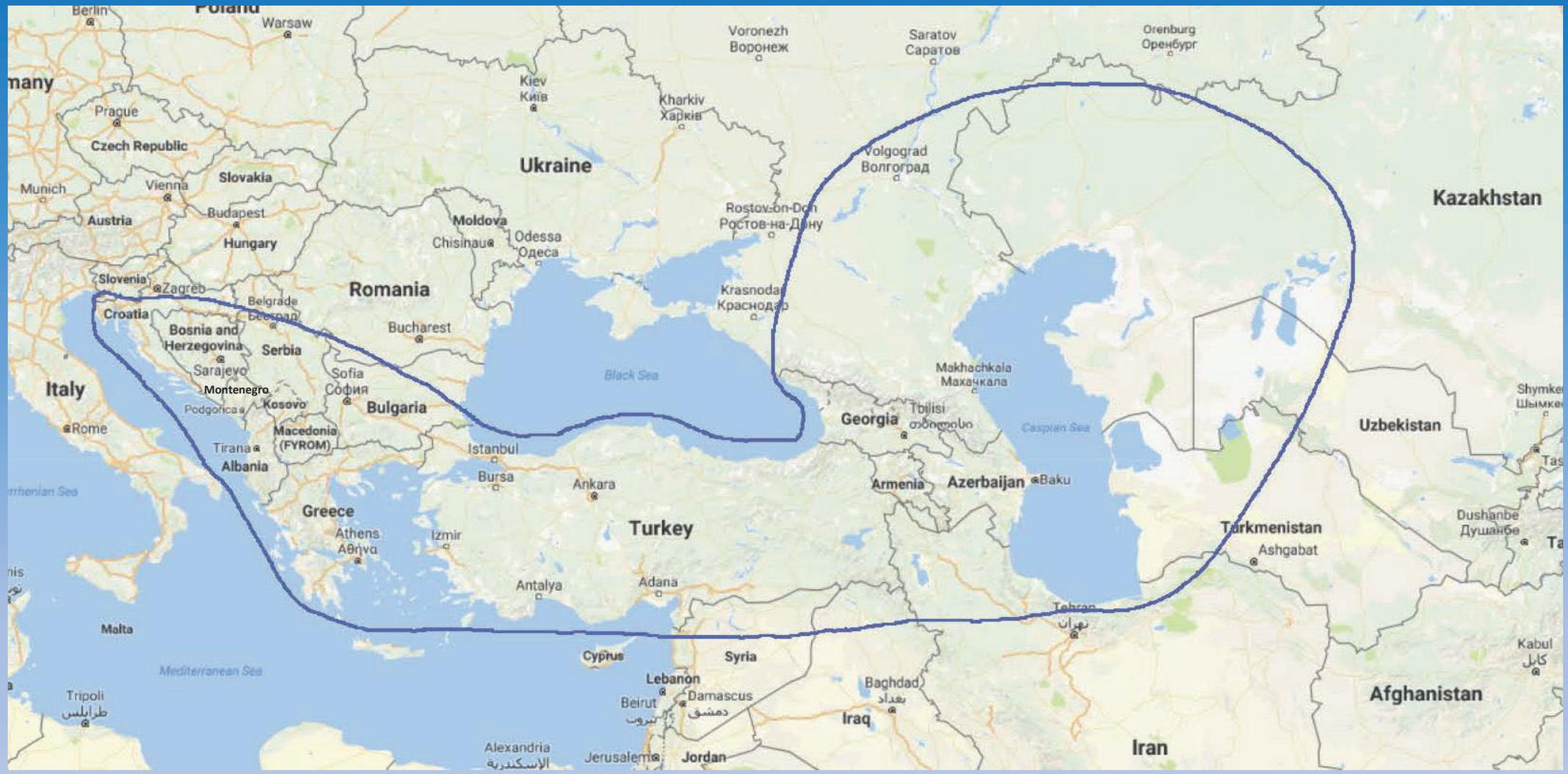
(*Received 15 March 2011; revised 28 July, 10 September and 13 October 2011; accepted 27 October 2011*)

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.

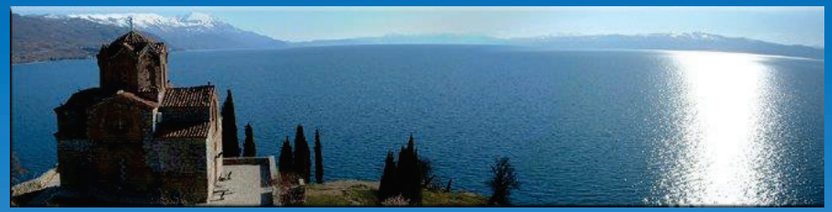



Berlin
Poland
Warsaw
Voronezh
Воронеж
Saratov
Саратов
Orenburg
Оренбург
Kiev
Київ
Kharkiv
Харків
Prague
Czech Republic
Ukraine
Volgograd
Волгоград
Slovakia
Munich
Vienna
Austria
Budapest
Hungary
Moldova
Chisinau
Odessa
Одеса
Rostov-on-Don
Ростов-на-Дону
Kazakhstan
Slovenia
Zagreb
Romania
Krasnodar
Краснодар
Croatia
Belgrade
Београд
Bucharest
Bosnia and
Herzegovina
Serbia
Sarajevo
Sofia
София
Black Sea
Makhachkala
Махачкала
Italy
Montenegro
Podgorica
Kosovo
Bulgaria
Tbilisi
თბილისი
Georgia
Caspian Sea
Uzbekistan
Rome
Macedonia
(FYROM)
Tirana
Albania
Istanbul
Bursa
Ankara
Armenia
Azerbaijan
Baku
Greece
Athens
Αθήνα
Izmir
Turkey
Dushanbe
Душанбе
Turkmenistan
Ashgabat
Antalya
Adana
Tehran
تهران
Malta
Cyprus
Syria
Kabul
کابل
Mediterranean Sea
Lebanon
Baghdad
بغداد
Tripoli
طرابلس
Beirut
بيروت
Damascus
دمشق
Afghanistan
Iraq
Alexandria
الإسكندرية
Jerusalem
Jordan
Iran

“cousin” Dreissena spp…….

-- the Balkans (e.g., *D. blanci*, *D. carinata*)

-- Turkey (e.g., *D. caputlacus*, *D. anatolica)*



Lake Ohrid

Skadar Lake

There’s only one dreissenid species in these lakes: *D. carinata*

Collecting mussels in the field


TANGO

Dissecting mussels in the lab

International Team of Collaborating Scientists

UNITED STATES
Dan Molloy

ITALY
Sergei Fokin

ITALY
Wanying Liao

ITALY
Mahesh Nitla

FINLAND
Jouni Taskinen

MACEDONIA
Sasho Trajanovski

ALBANIA
Spase Shumka

RUSSIA
Yulia Bespalaja

TURKEY
Zeki Yildrim

FRANCE
Laure Giamberini

MONTENEGRO
Mihailo Jovićević

MONTENEGRO
Vladimir Pešić

UNITED STATES
Jacque Keele

UNITED STATES
Yale Passamaneck

UNITED STATES
Sherri Pucherelli

Morphological Evidence of an *Ophryoglena* n. sp. Parasitic in *Dreissena carinata*

Sergei I. Fokin[a], Wanying Liao[b], Mahesh M. Nitla[c], Sasho Trajanovski[d], Mihailo Jovićević[e], and Daniel Molloy[f]

[a] University of Pisa, Italy, sifokin@mail.ru
[b] University of Pisa, Italy, wanyingl1992@gmail.com
[c] University of Pisa, Italy, venkatamahesh.nitla@gmail.com
[d] Hydrobiological Institute of Ohrid, Macedonia, trajsa@hio.edu.mk
[e] Environment Program, Montenegro, mihajov@gmail.com
[f] Molloy & Associates, United States, dan@danielpmolloy.com

Dreissena mussels are regarded as disruptive invasive species, yet little effort has been made to investigate the biodiversity and virulence of their parasites. This imbalance is currently being addressed in a new international project, of which the present investigation is part of. Few *Ophryoglena* spp. (Ciliophora) have been described as parasites of invertebrates, but one of these, *O. hemophaga,* is a parasite in the digestive gland lumina of *D. polymorpha* (Molloy et al. 2005), and it remains the only *Ophryoglena* sp. described from a mollusc. Herein we report morphological evidence supporting the discovery of a new species of *Ophryoglena* from *D. carinata* in Lake Ohrid (Macedonia/Albania). As is similar to *O. hemophaga*, this presumptive new species is a parasite of the digestive gland of *D. carinata.* Due to this similarity, our research focused on conducting a comprehensive morphological comparison between *O. hemophaga* and the *Ophryoglena* from *D. carinata. Ophryoglena* spp. exhibit a polymorphic life history, and thus various life stages will differ by size and shape. We collected trophonts (the feeding stage) from *D. carinata* by dissection and investigated the morphology both of live cells as well as cells fixed and stained by Feulgen and protargol techniques. Trophonts were typically elongated. Young trophonts selected for staining (150-220 μm long and 50-150 μm in maximum width) had 90-140 somatic kineties, an elongate macronucleus (Ma) (71,66±8,74x15,83±2,73 μm), and one subspherical micronucleus (Mi) (3,5x3,2 μm) located in close proximity to the middle of the Ma. The Feulgen-positive organelle of Lieberkühn (OL) was 7,0x5,4 μm. In contrast to the relative transparency of younger trophonts, mature trophonts (200-400 μm long) were opaque. These mature trophonts appeared to have a higher number of somatic kineties (>140), but due to technical difficulties the kineties on such big cells could not be accurately counted. Trophonts had 6-10 CV typically scattered in a field – a distribution in sharp contrast to the linear arrangement of CV in *O. hemophaga*. In summary, key morphological features – such as CV distribution, OL size, nuclei size, and cell dimensions – support description of this *Ophryoglena* from *D. carinata* as a new species, but molecular analysis will also be required (and is now planned) in order to fully support establishment of this *Ophryoglena* as a new species.