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RESEARCH ARTICLE

First record of donacid beetle larvae of *Macroplea appendiculata* (Panzer, 1794) (Coleoptera: Chrysomelidae: Donaciinae) within *Dreissena polymorpha* (Pallas, 1771) (Bivalvia: Dreissenidae) in the Kelkolovo quarry (Leningrad Oblast, Russia)

Oksana V. Travina[1], Yulia V. Bespalaya[1],
Alexander V. Kropotin[1], Alexander V. Kondakov[1]

1 *N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, 20 Nikolsky Ave., 163020, Arkhangelsk, Russia*

Corresponding author: Oksana V. Travina (travina.oksana136@yandex.ru)

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Abstract

Dreissena polymorpha is recognized as a powerful habitat engineer species in most aquatic ecosystems. An unusual association between the larvae of *Macroplea appendiculata* and *D. polymorpha* was discovered in the Kelkolovo quarry (Leningrad Oblast, Russia). Specifically, the larvae of *M. appendiculata* were found embedded within a small zebra mussel druse attached to the roots of aquatic plants. The possible nature of donacid beetle and zebra mussels association is discussed.

Keywords

Zebra mussel, aquatic leaf beetles, artificial reservoir, man-made water bodies, ecosystem engineers

Introduction

The zebra mussel *Dreissena polymorpha* (Pallas, 1771) is widely recognized as a potent ecosystem engineer that modifies aquatic environments (Karatayev et al. 2002; Zaiko et al. 2009; Burlakova et al. 2023). By forming dense, multi-tiered colonial aggregations (druses), this species creates novel habitats that provide shelter, promote the accumulation of organic detritus, and serve as foraging grounds for various macroinvertebrates (Karatayev et al. 1997, 2010; Shcherbina 2001; Pryanichnikova 2020). While the composition of macroinvertebrate communities associated with *D. polymorpha* is generally well documented, our recent studies in the Kelkolovo flooded quarry (Leningrad Oblast, Russia) revealed an entirely unrecorded, atypical association involving larvae of an aquatic leaf beetle.

Aquatic leaf beetles of the genus *Macroplea* Samouelle, 1819 (Coleoptera: Chrysomelidae) are widely distributed throughout the Palearctic region, spanning Europe, Northern Africa, and Asia (Pascal 2002; Kölsch et al. 2010; Nakahama et al. 2023; Geiser 2024). Within this genus, *Macroplea appendiculata* (Panzer, 1794) is of particular ecological interest. Its larvae develop entirely underwater on the roots and rhizomes of hydrophytes, from which they extract oxygen using specialized siphonal spurs (Bieńkowski 2004). The larvae of *M. appendiculata* are known to develop on a wide range of macrophytes, including the genera *Potamogeton*, *Myriophyllum*, and *Stuckenia* (Mohr 1985; Bieńkowski 2004; Kölsch and Krause 2011; Zaitsev and Medvedev 2018). Currently, however, the interaction between *M. appendiculata* larvae and other hydrobionts remains poorly understood.

The aim of this study is to describe the newly discovered association between *M. appendiculata* larvae and *D. polymorpha*, and to evaluate the potential ecological nature of this interaction.

Materials and methods

Field sampling and laboratory analysis

Fieldwork was conducted in July 2025 within the littoral zone of the Kelkolovo flooded quarry (59°47.88′N, 31°1.05′E; depth 0.5–1.5 m) located in the Kirovsky District of the Leningrad Oblast, Russia. This water body is a young anthropogenic reservoir characterized by high water clarity, sandy substrates, and a mosaic distribution of macrophytes. Currently, the maximum depth of the quarry does not exceed 10–15 m (Bolshiyanov et al. 2025).

Specimens of *D. polymorpha* were collected manually from the substrate. In the laboratory, the druses of *D. polymorpha* were examined under a Leica M165C stereomicroscope equipped with a FLEXACAM C1 digital camera (Leica Microsystems, Wetzlar, Germany). Larvae extracted from the druses were identified using an integrative taxonomic approach combining morphological keys and molecular data.

Morphometric measurements of both mussels and beetle larvae were performed using the same stereomicroscopic system. Taxonomic identifications followed the keys by Zaitsev and Medvedev (2009) and the monograph by Bieńkowski (2015).

The samples are stored in the collection of the Russian Museum of Biodiversity Hotspots (RMBH), N. Laverov Federal Center for Integrated Arctic Research of the Ural Branch of the Russian Academy of Sciences, Arkhangelsk, Russia.

DNA extraction, PCR, and sequencing

Total genomic DNA was extracted from ethanol-preserved larvae of the aquatic leaf beetle *M. appendiculata* according to standard phenol/chloroform procedures (Sambrook et al. 1989). The mitochondrial cytochrome c oxidase subunit I (COI) gene fragment was amplified by polymerase chain reaction (PCR) using the forward primer LCO1490 (Folmer et al. 1994) and reverse primer C1-N-2329 (Simon et al. 1994). Amplification was performed on a Veriti thermocycler (Thermo Fisher Scientific Inc., Waltham, MA, USA). Thermocycling was implemented with marker-specific PCR programs as follows: initial denaturation at 95°C (4 min), followed by 32 cycles at 94°C (50 sec), 52°C (50 sec), 72°C (50 sec) and a final extension at 72°C (5 min). Forward and reverse sequencing were performed on an ABI PRISM3730 (Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator v. 3.1 reagent kit.

The obtained COI sequence was edited using BioEdit v. 7.2.5 (Hall 1999) and aligned using the MUSCLE algorithm of MEGA12 (Kumar et al. 2024). To verify the taxonomic position and assess interspecific divergence, the obtained COI sequence was analyzed alongside sequences of the genus *Macroplea* retrieved from NCBI GenBank database (Suppl. material 1: Table S1). A Median-Joining (MJ) haplotype network was reconstructed using Network v. 5.0.0.1 (Bandelt et al. 1999). All sequences were trimmed to a consensus length of 583 bp, corresponding to the shortest sequence in the dataset.

Results

In the littoral zone of the quarry, *D. polymorpha* formed distinct colonies by overgrowing the root systems of aquatic macrophytes (Fig. 1A). In one of the discovered druses of *D. polymorpha* was found larvae of leaf beetle. The larvae were within the druse of zebra mussel (Fig. 1B).

In total, 12 specimens of *M. appendiculata* larvae were found. The body length of the collected larvae ranged from 5.1 to 5.5 mm (mean ± SD: 5.32 ± 0.13 mm, N = 12). Morphometric analysis indicates that all collected individuals belong to a single size group, presumably representing the senior larval instar of the same generation. The druse of *D. polymorpha* consisted of 31 individuals of *D. polymorpha*, with an average shell length of 3.5±0.85 cm.

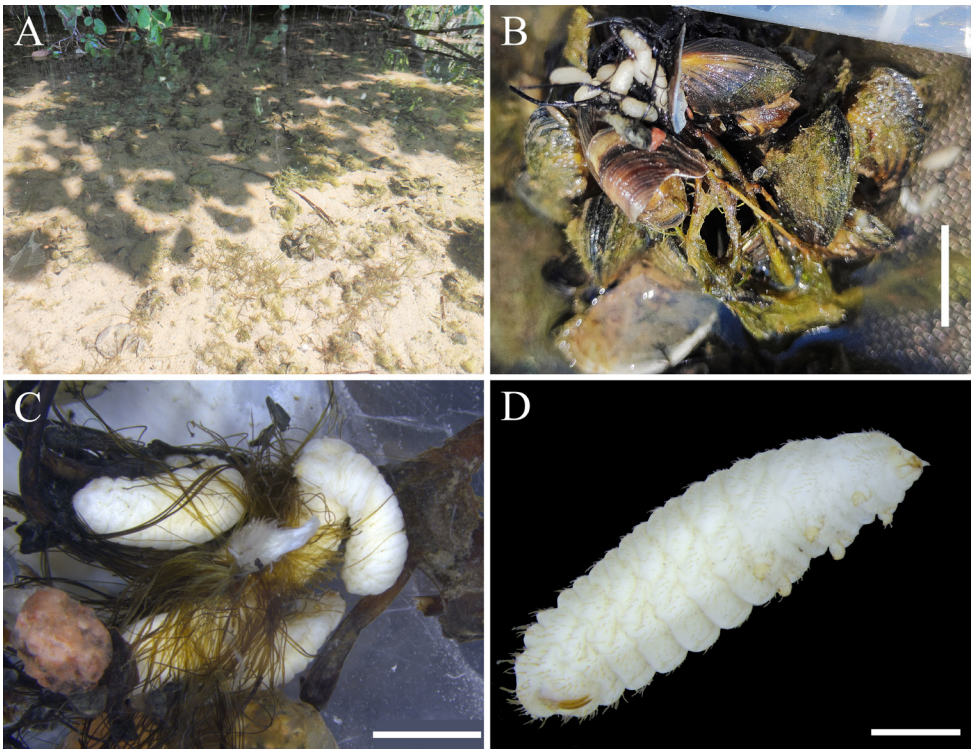

Figure 1. Association between *Macroplea appendiculata* larvae and *Dreissena polymorpha* in the Kelkolovo quarry: **A** – General view of a small zebra mussel druse encrusting the root system of an aquatic macrophytes (photo by Oksana V. Travina); **B** – Druse of *D. polymorpha* with larvae of *Macroplea appendiculata* (Scale – 1 cm); **C** – Larvae of *M. appendiculata* in byssal threads of *D. polymorpha* (Scale – 2.5 mm); **D** – Larva of *M. appendiculata* (Scale – 1 mm).

The median-joining network of COI sequences for the genus *Macroplea* revealed four distinct species-level clusters, corresponding to *M. appendiculata*, *M. mutica*, *M. pubipennis*, and *M. japana* (Fig. 2). Molecular analysis confirmed that discovered larvae belong to *M. appendiculata*. The newly obtained COI sequence (GenBank accession no. PZ463252) belongs to the most common central haplotype of *M. appendiculata*, which is widely distributed across Northern Europe (Fig. 2).

Discussion

The Kelkolovo quarry represents an anthropogenic reservoir characterized by high water clarity and a heterogeneous, mosaic distribution of macrophytes on sandy substrates. In such environments, *D. polymorpha* readily establishes high-density colonies on both artificial and natural hard substrates, including submerged veg-

etation (Lyakhnovich et al. 1994; Nalepa et al. 1995; Burlakova et al. 2006). Zebra mussels forming complex three-dimensional structures and accumulating organic detritus, zebra mussel druses function as specific habitats that support diverse macroinvertebrate assemblages (Karatayev et al. 1997; Shcherbina 2001; Pryanichnikova 2020). These aggregations offer substrate and refuge for multiple benthic taxa, including isopods, amphipods, trichopterans, chironomid larvae, and gastropods (Karatayev et al. 1997).

The widespread distribution of *D. polymorpha* in the Kelkolovo quarry has created conditions for the formation of complex consortia. *Dreissena* druses, which cover submerged macrophytes, probably accumulate detritus, creating favorable habitats for insect larvae (Ludyanskiy et al. 1993; Burlakova et al. 2005; Karatayev et al. 2010). The relationship between aquatic insects and filter-feeding bivalves is often commensalistic or involves using mollusks as additional substrates for attachment (Ricciardi et al. 1997).

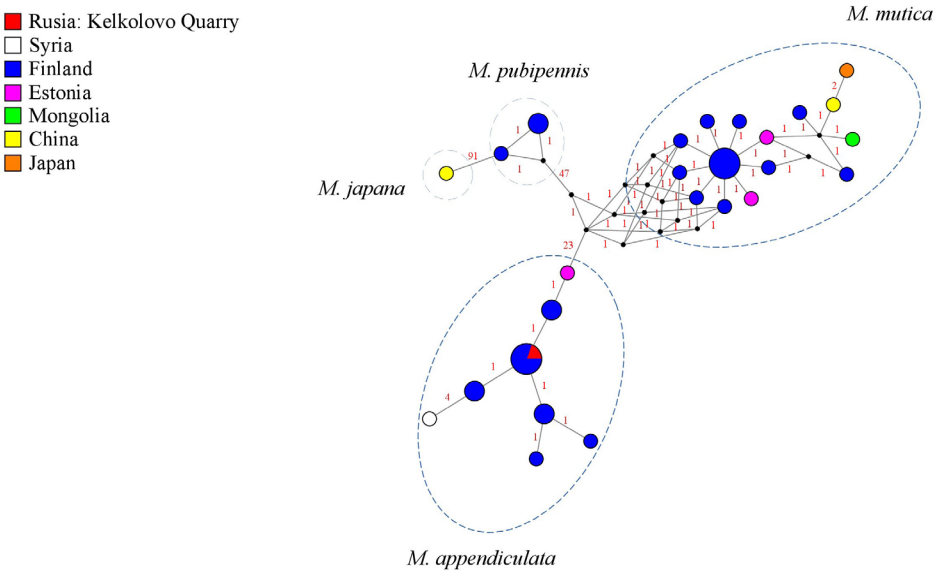

Figure 2. Median-joining haplotype network of the genus *Macroplea* based on a 583-bp fragment of the mitochondrial COI gene. The red numbers near branches are number of nucleotide substitutions between haplotypes. The size of circles corresponds to the number of COI sequences that were used in the analysis (the smallest circle = one sequence).

The detection of *M. appendiculata* larvae within *D. polymorpha* druses in the flooded Kelkolovo quarry represents a non-trivial ecological case. Perhaps that the discovered association is a random event. It is well established that *Macroplea* larvae successfully survive and develop within benthic sediments on host plant roots (Bienkowski 2004). Traditionally, the life cycle of *Macroplea* is understood to be asso-

ciated exclusively with macrophytes, where larvae lead a sessile lifestyle, extracting oxygen directly from the plant aerenchyma (Mohr 1985; Bieńkowski 2004; Türkgülü et al. 2011). The larvae of this genus possess hollow chitinous siphonal spurs, which they insert into the host plant tissue to consume the oxygen released during photosynthesis (Bieńkowski 2004). Female *M. appendiculata* are capable of ovipositing underwater within leaf axils or directly on submerged substrates (Goecke 1935). Consequently, the larvae might have actively migrated into the pre-existing zebra mussel druse, or female of *M. appendiculata* can to lay eggs into the druse.

Possible other explanations of this unusual finding could include: first, *D. polymorpha* druses act as shelters that capture fragments of food plants and create a protected environment. Second, druses provide protection for the larvae from predation. Obviously, these hypotheses need to be tested and further investigated.

This work is not aimed on confirmation of associative relationship between the zebra mussel and the *M. appendiculata*. Here we present discovery of *M. appendiculata* larvae in *D. polymorpha* druses.

The discovered relationship highlights the ecological plasticity of donacid beetle *M. appendiculata* and the role of zebra mussels as an "engineering" species that expands the habitat for other hydrobionts in artificial reservoirs. Future research should be aim to study the nature of the relationships between zebra mussels and other invertebrates.

Acknowledgments

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Supplementary material 1

Table S1. Specimen data for the COI dataset

Authors: Oksana V. Travina, Yulia V. Bespalaya, Alexander V. Kropotin, Alexander V. Kondakov

Data type: table

Link: https://journal.asu.ru/biol/article/view/@@@@@@@