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Zoochorous dispersal of freshwater peaclams (Bivalvia: Sphaeriidae): potential role of aquatic insects

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ABSTRACT. The present study reports the first observation of *Euglesa globularis* peaclams attached to dragonfly nymph *Aeshna juncea*, and *E. parvula* attached to the leg of the water boatmen nymph *Callicorixa* sp. from the Kolyma River basin in eastern Siberia (Asiatic Russia). It has been shown that the aquatic insects may play a potential role in the dispersal of bivalves both locally within one and between different water bodies.

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Зоохорное расселение пресноводных моллюсков (Bivalvia: Sphaeriidae): потенциальная роль водных насекомых

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РЕЗЮМЕ. В исследовании сообщается о первом наблюдении прикрепления двустворчатых моллюсков *Euglesa globularis* к нимфе стрекозы *Aeshna juncea* и *E. parvula* к ноге нимфы гребляка *Callicorixa* sp. из бассейна реки Колыма в Восточной Сибири (Азиатская Россия). Показано, что водные насекомые могут играть потенциальную роль в распространении двустворчатых моллюсков, как в локальном масштабе внутри одного, так и между различными водоемами.

Introduction

The small size of peaclams (family Sphaeriidae) and their capacity for passive dispersal with birds, reptiles, amphibians and insects facilitate their almost worldwide distribution (except of Antarctic) [Rees, 1965; Korniushin, Glaubrecht, 2002; Statzner *et al.*, 2008; Bespalaya *et al.*, 2020; Vinarski *et al.*, 2021]. The long-range dispersal of freshwater invertebrates helped Charles Darwin (in the "Origin of Species") to discuss some problems of evolutionary biogeography [Darwin, 1859; Gittenberger, 2012]. Most researchers agree that birds are the main agent of long-distance dispersal of freshwater mollusks through the air [Darwin, 1878, 1882; Rees, 1965; Boag, 1986; Wesselingh *et al.*, 1999; Coughlan *et al.*, 2017; Bespalaya *et al.*, 2020; Saito *et al.*, 2023]. At the same time, an analysis of the literature data shows that insects also play an important role in the dispersal of many freshwater mollusks, including the sphaeriid clams.

Indeed, the cases of the attachment of sphaeriid clams to the legs of aquatic insects were described for the first time more than 130 years ago, in the last half of the 19th century [Darwin, 1882; Standen, 1885; Kew, 1888, 1893]. Rees [1965], in his review on aerial dispersal of mollusks, provided a list of insect species for which the fact of attachment of small bivalves to their legs was observed. These

FIG. 1. A study area map in the Kolyma River basin (Magadan Oblast, Russia) (1) lakelet nearby Orotukan settlement; (2) Lebedinoye Lake nearby Omolon settlement. Photos by Aksenova O.V. (1) and Sokolova S.E. (2).

РИС. 1. Карта района исследований в бассейне реки Колыма (Магаданская область, Россия) (1) озеро рядом с поселком Оротукан; (2) Озеро Лебединое рядом с селом Омолон. Фотографии Аксёновой О.В. (1) и Соколовой С.Е. (2).

are swimming bugs of the genera *Nepa*, *Notonecta*, *Corixa*, and *Sigara* (Heteroptera), water diving beetles *Acilius* sp., and *Dytiscus marginalis* Linnaeus, 1758 (Coleoptera). Peaclams also known to be accidentally attached to the bodies of dragonfly larvae (Odonata) [Kew, 1893; Mackie, 1979; Soldan *et al*., 1989].

70 years ago, an experimental study was carried out on the possibility of attachment of peaclams to water bugs of the family Corixidae [Fernando, 1954]. A case of the attachment of sphaeriid clams to corixid bugs from Lake Titicaca in Bolivia was described by Zelaya and Marinone [2012]. Soldan *et al*. [1989] studied the phoretic association between *Sphaerium tasmanicum* and *Sigara truncatipala* (Corixidae). In this paper, we report a case of peaclam attachment to the legs of water boatmen nymph and dragonfly larvae observed in the Kolyma River basin in Eastern Siberia (Asiatic Russia).

Material and methods

Data collection and DNA analyses

The samples of freshwater invertebrates were collected by authors in 2022 during fieldwork carried out in the Kolyma River basin (Magadan Oblast, Russia), from the Lebedinoye Lake (near Omolon settlement, the Omolon River basin) (65.50741°N; 159.81429°E), and in a small unnamed lakelet (near Orotukan settlement) (62.25955°N; 151.71263°E) (Fig. 1). Although our research mainly concerned the species diversity of freshwater bivalves, we were able to collect representative material on phoresy of mollusks by aquatic insects. The invertebrates were sampled using a hand net and a sieve, and then fixed in 96% ethanol. A total of 247 specimens of mollusks of various species and three water boatmen nymphs were collected from the small unnamed lakelet; 48 individuals of mollusks and nine dragonfly nymphs from the Lebedinoye Lake.

The studied material is stored in the collection of the Russian Museum of Biodiversity Hotspots (RMBH) of the N. Laverov Federal Center for Integrated Arctic Research of Russian Academy of Sciences, Arkhangelsk, Russia (Table 1).

The species identification of insects was based on GenBank data for both species and morphological data using Kharitonov's key [1997] for the dragonfly larvae. The identification of freshwater bivalves was based on the integrative taxonomic approach, when the morphological, anatomical [Korniushin, 1996; Glöer, Meier-Brook, 2003] and molecular data are analyzed together.

Total DNA was extracted from 96% ethanol-preserved specimens. The performed molecular genetic analysis included amplification and sequencing of 16S rRNA marker for mollusks and mtDNA COI marker for insects. The 16S rRNA and mtDNA COI genes were chosen because data for only these

Table 1. Accession numbers of the studied invertebrate specimens.

Табл. 1. Регистрационные номера для изученных образцов беспозвоночных.

RMBH Voucher No.	Species	NCBI accession number
Mollusks		
MSph-0868	*Euglesa globularis* (Clessin in Westerlund, 1873)	PP425941
MSph-0869	*E. parvula* (Clessin in Westerlund, 1873)	PP425942
Insects		
N80	*Aeshna juncea* (Linnaeus, 1758)	PP425930
N81	*Callicorixa* sp.	PP425931

genes are available in fairly large numbers in the GenBank database for most of the nominal species of the groups studied. The methods of DNA isolation, primer combinations, PCR conditions, sequencing, primary sequence processing are described in detail in our previous articles [Bolotov *et al.*, 2019; Bespalaya *et al*., 2022, 2023].

Images of studied animals were taken using a Leica M165C stereomicroscope (Leica Microsystems, Germany) and Canon EOS 7D digital camera with Canon EF 100mm f/2.8L Macro IS USM lens.

Results

During our field study in the Lebedinoye Lake (Omolon River basin) we collected a specimen of *Euglesa globularis* attached to the claw of fore leg of a dragonfly nymph *Aeshna juncea* (Fig. 2 A-B). In the lakelet near Orotukan settlement an individual of *E. parvula* clipped to the leg of the water boatmen nymph *Callicorixa* sp. was collected (Fig. 2 C-D). The invertebrates were living in aquatic vegetation at a depth of 0.2-0.3 m.

All sequenced peaclam specimens from the sample collected in the lakelet near Orotukan settlement shared the same haplotype 16S rRNA or differed from *E. parvula* specimens from Yakutia by 1–2 mutational substitutions [Bespalaya *et al.*, 2023]. Based on our molecular data, individuals of bivalves collected in the Lebedinoye Lake have one mutational substitution compared to *E. globularis* sampled in the outskirts of Magadan city [Bespalaya *et al.*, 2020].

Discussion

Phoretic relationships between one organism and another, more mobile animal, can facilitate the dispersal of various species [Tomlin, 1910; Coughlan *et al.*, 2017; Bartlow, Agosta, 2021]. Dispersal is one of the key processes in determining the genetic structure and demography of populations [Walther *et al*., 2008; Zelaya, Marinone, 2012; Kappes, Haase, 2012; Bespalaya *et al.*, 2020].

To the best of our knowledge, this study is the first report of phoretic relationships between sphaeriid species and aquatic insects in the Kolyma River basin and in Russia in general. The analysis of the literature and our data shows that aquatic insects (both in the adult and larval stages) can serve as potential agents of the peaclam dispersal [Rees, 1965; Zelaya, Marinone, 2012]. The water boatmen have high migration rates varying between 10 and 90 km/day [Fernando, 1954; Zelaya, Marinone, 2012; Srayko *et al.*, 2022]. The mobile insects facilitate spread of bivalves not only between different water bodies, but also help their movement to deeper areas within the same reservoir [Weinrauch, Borcherding, 2002; Zelaya, Marinone, 2012]. It is well known that corixids are fast swimmers regularly moving from the bottom to the water surface to replenish the atmospheric air supply [Zelaya, Marinone, 2012; Srayko *et al.*, 2022]. The *Aeshna juncea* nymphs lead an ambush predator lifestyle, rarely crawling or swimming while avoiding danger or chasing prey. However, if a nymph is disturbed, the water, forcefully expelled through its anal pyramid, creates a water jet that allows it to move extremely fast [Galliani *et al.*, 2017]. According to Darwin [1882], some sphaeriid species can remain attached to the legs of the aquatic beetles for up to six days. The sphaeriids are firmly tight attached to the limbs of animals [Voskresensky, 1966; Gutleb et al., 2000; Zelaya, Marinoni, 2012]. It was established that the attachment of bivalves may have a negative impact on animals and usually damages their limbs [Gutleb *et al*., 2000; Wood *et al*., 2008; Laza-Martínez *et al*., 2012; Zelaya, Marinoni, 2012; Baker *et al*., 2019]. In addition, damage to the edge of the shell was observed after attachment to the insect's claw [Zelaya, Marinoni, 2012]. Consequently, the excellent mobility of aquatic insects, as well as long and strong attachment of bivalves to their extremities, make them suitable means of peaclam dispersal

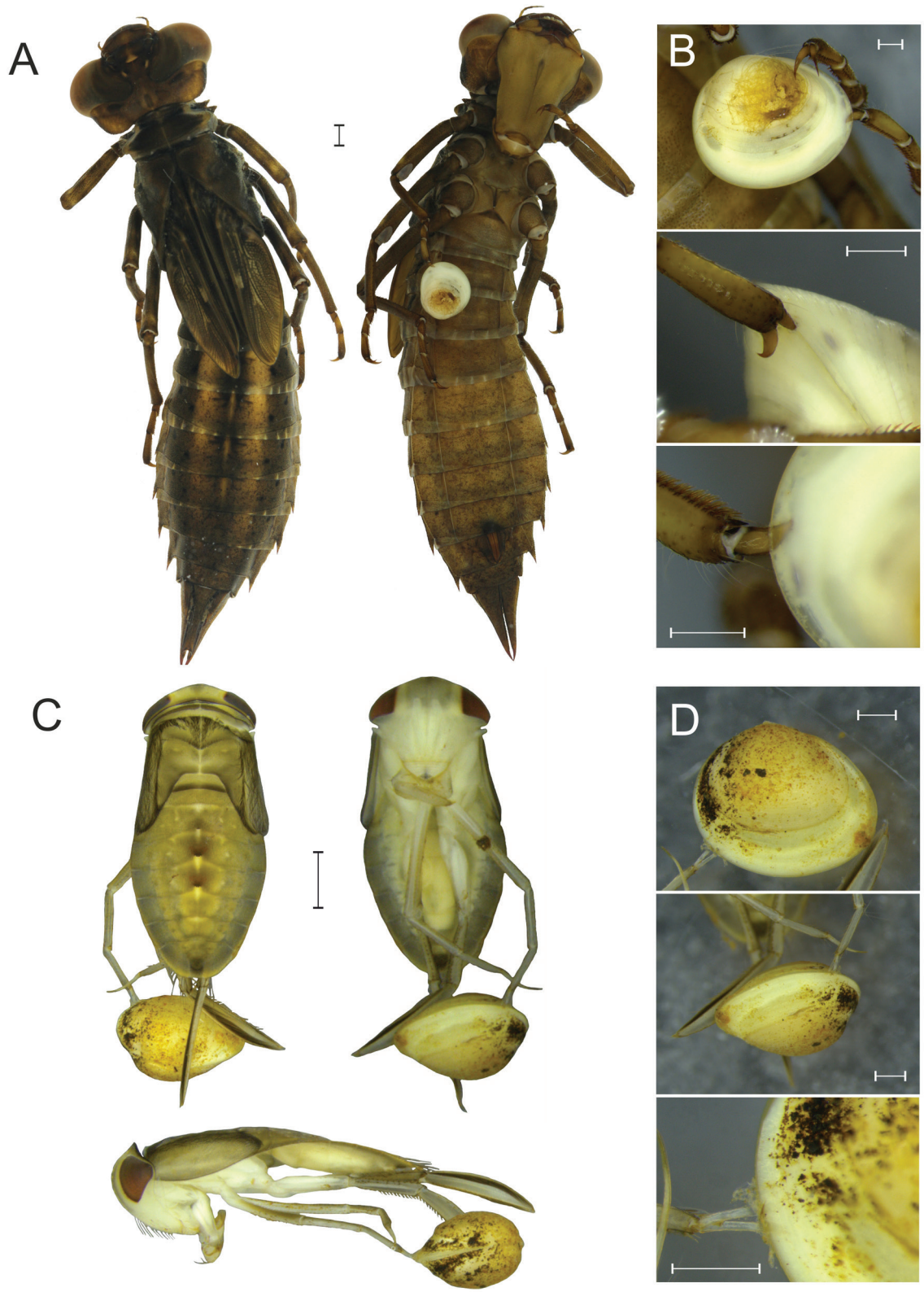

FIG. 2. **A.** Dorsal and ventral views of the *Aeshna juncea* (Linnaeus, 1758) dragonfly nymph (Omolon River basin, Magadan Oblast, Russia). **B**. *Euglesa globularis* attached to the leg of the Common Hawker dragonfly nymph. **C.** Dorsal, ventral, and lateral views of the *Callicorixa* sp. nymph (a small unnamed lakelet near Orotukan settlement, Magadan Oblast, Russia). **D**. *Euglesa parvula* attached to the leg of the water boatmen nymph.
Scale bars: (A, C) – 1 mm; (B, D) – 0.5 mm. Photos by Aksenova O.V.

РИС. 2. **A.** Вид нимфы стрекозы *Aeshna juncea* (Linnaeus, 1758) со спинной и брюшной стороны (бассейн реки Омолон, Магаданская область, Россия). **B**. *Euglesa globularis*, прикрепленный к ноге нимфы стрекозы коромысла. **C.** Виды нимфы *Callicorixa* sp. со спинной, брюшной и латеральной сторон (безымянное озеро в окрестностях поселка Оротукан, Магаданская область, Россия). **D**. *Euglesa parvula*, прикрепленный к ноге нимфы водяного клопа гребляка.
Масштабная линейка: (A, C) – 1 мм; (B, D) – 0,5 мм. Фотографии Аксёновой О.В.

[Darwin, 1882; Fernando, 1954; Ress, 1965; Zelaya, Marinone, 2012].

In this study, we observed the attachment of peaclams only to insect nymphs, i.e. the preimaginal stages, whose dispersal abilities are limited. At the same time, a number of studies have reported the attachment of freshwater bivalves to adult specimens (imago) of various Corixidae bugs [Fernando, 1954; Zelaya, Marinone, 2012]. Thus, we assume that the dragonfly and water boatmen may potentially play some role in the dispersal of bivalves, both locally within a lake and between different water bodies.

In addition, it should be borne in mind that aquatic insects are a significant component of the diet of many fish and bird species [Srayko *et al.*, 2022]. It has been proven that freshwater molluscs can survive by passing through the intestines of fish and birds. [Mackie, 1979; Alonso, Castro-Díez, 2008; Wada *et al.*, 2012; Coughlan *et al.*, 2017]. It can therefore be assumed that water insects with a clam attached to their leg can be eaten by a bird or fish. Accordingly, the molluscivorous fish or waterfowl can potentially contribute to their dispersal [Coughlan *et al.*, 2017]. However, this hypothesis remains speculative until convincing evidence is provided.

In conclusion, the results of the present study bring new instances of phoretic relationships between aquatic insects and peaclams. The ability of water insects to disperse freshwater bivalves as well as the true importance of this dispersal mechanism require further investigation. In particular, the knowledge on the role of environmental conditions, retention time, and the frequency of cases of phoresy and successful colonization should be explored.

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Conflict of interest

The authors declare no conflict of interest.

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