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## Freshwater and brackish bryozoan species of Croatia (Bryozoa: Gymnolaemata, Phylactolaemata) and their genetic identification

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**Abstract.** Freshwater and brackish species of bryozoans belong to the Phylactolaemata and Gymnolaemata class. Twelve species of bryozoans were recorded and morphologically determined at eight locations in the Black Sea and the Adriatic basin in Croatia. Twelve species of Bryozoa have been listed in the taxonomic index for Croatia (*Conopeum seurati*, *Lophopus crystallinus*, *Paludicella articulata*, *Cristatella mucedo*, *Fredericella sultana*, *Hyalinella punctata*, *Plumatella casmiana*, *Plumatella emarginata*, *Plumatella fruticosa*, *Plumatella fungosa*, *Plumatella geimermassardi* and *Plumatella repens*). For the purposes of gene identification of recorded species, molecular markers for nuclear 18S and 28S genes, ITS2 region and mitochondrial COI gene were amplified. Genetic identifications of morphologically determined bryozoan species were confirmed using highly similar sequences local alignment analysis. Proliferation of freshwater bryozoan species over long distances with the help of the vector animals was confirmed by defining haplotypes on the base of 18S, 28S and ITS2 sequences associated with the Black Sea-Mediterranean waterfowl flyway.

**Key words:** Adriatic basin, Black Sea basin, local alignment, haplotypes, long-distance transfer

### Introduction

Bryozoans are invertebrates that inhabit all types of aquatic ecosystems. Freshwater and brackish species of bryozoans are a poorly known group of animals even though they are widespread in most freshwater ecosystems. While the individual unit within colony has a size of only a few millimeters, by asexual budding it can form a large cooperative that can reach the size to tens of centimeters. With sponges and shellfish they belong to an important group of filter feeding animals (Wood *et al.* 2006). Phylum Bryozoa is divided into three classes: Phylactolaemata, Stenolaemata and Gymnolaemata (Woollacott & Zimmer 1977). Phylactolaemata contains about 80 freshwater and brackish species (Massard and Geimer 2008a; Wood 2002), Stenolaemata contains about 700 marine species, while Gymnolaemata contains about 5000 mostly marine species (Gordon 2003), whereas few species live in brackish waters. Although freshwater species are passively dispersed over large areas, their morphology shows little geographic variation (Frey 1995).

Their unique method of reproduction and small size allow them great potential for dispersal. Freshwater bryozoans create chitinous statoblasts, while brackish species generally produce hibernaculæ that are easily spread with water currents. Statoblasts and hibernaculæ are also transferred by waterfowl, fish, amphibians and reptiles so for that matter they serve as vectors for dispersal over long distances and therefore freshwater and brackish bryozoans can have cosmopolitan distribution. Recent studies have quantified transport in the field, confirming that a variety of long-distance migrants can carry invertebrates both internally and externally (Mayr 1963; Green & Figuerola 2005). Waterfowl feeding in swamp areas can ingest statoblasts, which can remain viable and germinate after passing through the digestive tract (Brown 1933; Charalambidou *et al.* 2003; Figuerola *et al.* 2005; Green *et al.* 2008). Those dispersal buds can attach to the feathers of waterfowl (Okamura & Hatton-Ellis 1995; Freeland 2001; Wood 2002) and can be dispersed over long distances. Distribution and gene flow are important factors for organisms such as bryozoans of fresh and brackish waters that pass through frequent local extinction because of drought or recolonization due to floods and dispersal vectors (Slatkin 1977; Whitlock 1992). Aforementioned statement is also important for bryozoans which inhabit discrete places such as ponds and lakes (Barrett *et al.* 1993; Berg & Garton 1994). In Croatia, freshwater and brackish bryozoan species have been reported in rivers, lakes and wetland areas of the Black Sea and the Adriatic basin.