Protist, Vol. 162, 222–236, April 2011
http://www.elsevier.de/protis

Protist

ORIGINAL PAPER

Dinoflagellates Associated with Freshwater Sponges from the Ancient Lake Baikal

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Submitted December 31, 2009; Accepted May 30, 2010
Monitoring Editor: Marina Montresor

Dinoflagellates are a diverse group of protists that are common in both marine and freshwater environments. While the biology of marine dinoflagellates has been the focus of several recent studies, their freshwater relatives remain little-investigated. In the present study we explore the diversity of dinoflagellates in Lake Baikal by identifying and analyzing dinoflagellate sequences for 18S rDNA and ITS-2 from total DNA extracted from three species of endemic Baikalian sponges (*Baikalospongia intermedia*, *Baikalospongia recta* and *Lubomirskia incrustans*). Phylogenetic analyses of these sequences revealed extensive dinoflagellate diversity in Lake Baikal. We found two groups of sequences clustering within the order Suessiales, known for its symbiotic relationships with various invertebrates. Thus they may be regarded as potential symbionts of Baikalian sponges. In addition, *Gyrodinium helveticum*, representatives from the genus *Gymnodinium*, dinoflagellates close to the family Pfiesteriaceae, and a few dinoflagellates without definite affiliation were detected. No pronounced difference in the distribution of dinoflagellates among the studied sponges was found, except for the absence of the *Piscinoodinium*-like dinoflagellates in *L. incrustans*. To the best of our knowledge, this is the first study of the diversity of dinoflagellates in freshwater sponges, the first systematic investigation of dinoflagellate molecular diversity in Lake Baikal and the first finding of members of the order Suessiales as symbionts of freshwater invertebrates.

Key words: Baikal; dinoflagellates; Lubomirskiidae; sponges; Suessiales; symbionts.

Introduction

Dinoflagellates are an important group of aquatic protists that appeared 245–208 million years ago and evolved a high diversity in forms and feeding strategies (Hackett et al. 2004). Dinoflagellates form a monophyletic lineage defined by several genetic, cytological and ultrastructural synapomorphies. They are the most closely related group to endoparasites from Apicomplexa, which have a great medical significance, and, along with the latter taxon and ciliates, are currently included in the group Alveolata (Saldarriaga et al. 2004).

Dinoflagellates often form symbiotic relationships with various marine invertebrates such as cnidarians (Karako-Lampert et al. 2004; Toller et al. 2001; Thornhill et al. 2009; van Oppen et al. 2005), sponges (Garson et al. 1998; Rosell 1993; Scalera-Liaci et al. 1999; Schönberg and Loh 2005), mollusks (Baillie et al. 2000; Farmer et al. 2001; Ishikura et al. 2004; Trench et al.

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doi:10.1016/j.protis.2010.07.002

1981), as well as some protists (Garcia-Cuetos et al. 2005; Gast et al. 2003; Pochon and Pawlowski 2006; Shaked and de Vargas 2006). Symbiotic dinoflagellates provide their hosts with various metabolites (Yellowlees et al. 2008) and appear to contribute significantly to their ecological success: *e.g.*, many corals die without them (Douglas 2003). There are also indications that many parasitic species of dinoflagellates may exist in nature (Taylor et al. 2008), including those associated with invertebrates (e.g., Mills and McLean 1991). The main diversity of dinoflagellates is found in marine environments (Taylor et al. 2008); freshwater dinoflagellates account for far fewer species and their ecological interactions as well the origin and phylogenetic relationships to marine taxa remain little-investigated.

Lake Baikal is the oldest (25-30 million years) and deepest (about 1637 m) lake on Earth (Stewart 1991). It is strongly oligotrophic, contains one-fifth of the world's total freshwater supply, and has oxygen levels >80% saturation in the whole water column, therefore even the abyssal area is inhabited (Peeters et al. 1997). It is often called a "natural laboratory to study species diversity and evolution" (Taliev 1955). There are more than 2500 animal species and subspecies and about 1000 plant species and subspecies in Lake Baikal (Timoshkin 1999). Dinoflagellates from this lake remain poorly studied, with only two systematic studies, both of which conducted by light microscopy (Antipova 1955; Kisselew 1950). To date six dinoflagellate species of different morphology (armored and non-armored) have been recorded in Baikalian planktonic open waters (Tanichev and Bondarenko 1995). The phylogenetic position of the dinoflagellates living in Baikal remains unstudied by modern methods.

Freshwater sponges are one of the most common animals in Baikal (Fig. 1) and form "underwater forests" on the bottom of the lake (Efremova et al. 2002). Most Baikalian sponges belong to the endemic family Lubomirskiidae, which branched off from the freshwater family Spongillidae and is clearly monophyletic (Itskovich et al. 2008). The family Lubomirskiidae is known for its greater morphological and species diversity than other freshwater sponge families (Efremova et al. 2002). It dominates the benthic communities in Lake Baikal at depths down to 40 meters in part because of the presence of photosynthetic symbionts (Kozhova and Izmest'eva 1998). Such presence is possible, in part, because of the high transparency of Baikal water (Stewart 1991), which is one of the major requirements for the prosperity of

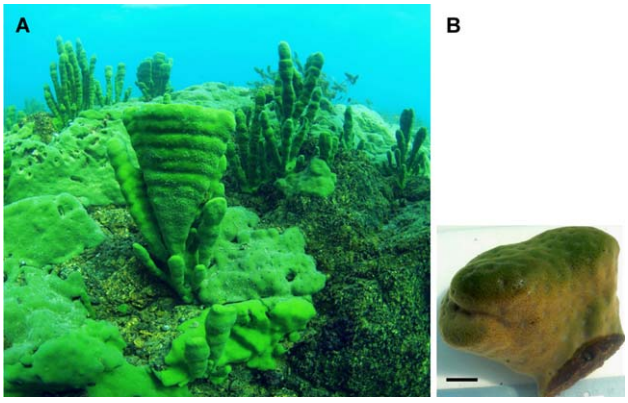

Figure 1. A. Underwater photo taken in Lake Baikal at about 9 m depth showing benthic community of sponges family Lubomirskiidae (with the permission of Dr. A. Kupchinskii). **B.** The sponge *Baikalospongia recta*. Bar = 2 cm.

photosynthetic organisms (Karlsson et al. 2009). Based on microscopy studies, it had been thought that freshwater sponges form symbiotic relationships exclusively with the algae from the order Chlorococcales, known as zoochlorellae (see for example Masuda 1990). However, Frost et al. (1997) uncovered the presence of symbiotic heterokonts from Eustigmatophyceae in a freshwater sponge. Furthermore, it appears likely that freshwater sponges can form symbiotic relationships with dinoflagellates because sponge-dinoflagellate associations are known in marine sponges. The first such association was observed in the marine bioeroding sponge *Cliona viridis* Schmidt (Sarà and Liaci 1964). Since then, two molecular studies investigated dinoflagellate biodiversity in marine sponges (Granados et al. 2008; Schönberg and

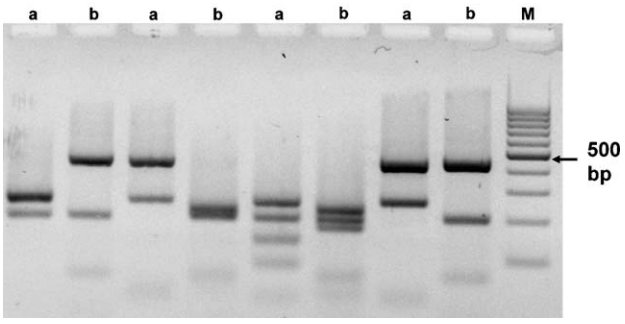

Figure 2. Restriction digest of cloned 18S rDNA sequences from *B. intermedia*. Agarose gel was stained with ethidium bromide. Each pair of tracks (a and b) corresponds to individual clones digested with a) Alu1 and b) Dde1; M = marker, 1 kilobase-pair ladder (Fermentas).