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The role of charophytes (Charales) in past and present environments: an overview

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Abstract

Charophytes, i.e. extant and fossil members of the order Charales plus the members of the extinct orders Sycidiales and Moellerinales, are closely related to modern land plants. These algae have a complex morphology, and can tolerate salinities from freshwater up to hypersaline conditions, although they are not known to occur in fully marine habitats. Extant charophytes are found on all continents except Antarctica, in both lotic and lentic, natural and artificial habitats, ranging from ancient lakes to newly excavated gravel pits. The large size of the internode cells makes charophytes useful objects in plant cell biology. Charophytes can build up large biomasses in brackish and freshwater ecosystems, and contribute to a number of ecosystem services, including removal of nutrients from water, storage of carbon and nutrients in biomass and sediments, possible phytoremediation of organic chemicals and trace metal elements from water, as well as provision of habitat and food for a number of organisms. Charophytes are valuable indicators for lake and river ecological status assessment, and their oospores and gyrogonites are useful for paleolimnological

reconstructions of ecosystem properties such as trophic status or salinity. This paper introduces charophytes, and summarizes different aspects studied in these macroalgae.

Foreword

The International Research Group on Charophytes (IRGC; <http://irgc.uow.edu.au>) was established in 1989, and offers a forum for scientists dedicated to the study of fossil and extant charophytes. The organization has about 120 members from around the world, and holds international conferences every fourth year. The most recent conference, the 6th IRGC, was organized in Mendoza, Argentina in 2012, and was dedicated to honor Dr. Eduardo A. Musacchio, who began organizing the conference but died tragically in 2011. This Special Issue contains a selection of the papers presented at the 6th IRGC conference, and covers a wide variety of topics related to morphology, ecology, paleoecology, geochemistry, ecophysiology, and ultrastructure of charophytes. We here provide an overview of the articles published in this special issue, discussed within the broader context of charophyte research.

1. Introduction

The well-established term “charophytes” has been used for a long time to name the extant and fossil members of the order Charales plus the members of the extinct orders Sycidiales and Moellerinales (Feist et al., 2005). However, in the last few decades, the term “charophytes” or “charophyte-algae” has been used by some authors to name any of the living taxa within the Charales, Chlorokybales, Coleochaetales, Klebsormidiales, Mesostigmatales and Zygnematales (Karol et al. 2001). These six orders were grouped within a paraphyletic group that was called “Class Charophyceae” (Stewart and Mattox, 1984). According to www.algaebase.org, this former “class” is presently recognized as “phylum Charophyta”, with six classes corresponding to the six former orders mentioned above. However, phylogenetic analyses consistently show that these six classes do not form a monophyletic group (e.g. Timme et al., 2012). Since paraphyla should not be used in phylogenetic systematics (Wiley, 1981), and in order to avoid the confusing use of artificial groupings, we advocate restricting the term “charophytes” to its original meaning, i.e. the living and fossil species of the order Charales and their direct ancestors within the exclusively fossil orders Sycidiales and Moellerinales.

Charophytes are algae that have a complex morphology, consisting of a central axis or “stem” made of long unicellular internodal cells (which in some cases are covered by cortex cells), and short multicellular nodes, where whorls of branchlets (by other authors called “branches”) originate at more or less regular intervals (Wood and Imahori, 1965). According to Wood and Imahori (1965), the first records of charophytes date back to the 15th century, when they were called *Equisetum*. Indeed, many charophytes superficially resemble horsetails. In the 17th century, charophytes were described in greater detail, but still treated as a kind of horsetail. In fact, the Latin name used at that time translates to “smelly horsetail creeping under water” (*Equisetum foetidum sub aqua repens*). Only in the 18th century were they assigned to a new genus, *Chara*, by Vaillant (1721). This name was retained by Linnaeus some decades later (Linnaeus, 1753) and was kept since then. The fossil record of charophytes remained poorly understood until the late 19th century. For example, in one of the first reports of fossil charophyte remains, Lamarck (1804) wrongly stated that gyrogonites (the calcified remains of the oosporangium) from the Paris Basin belonged to foraminifers (Foraminiferida, = marine Rhizaria).

It is amazing that all terrestrial plants, with their tremendous diversity have descended from a single streptophyte-alga that colonized the land somewhere between 430 and 470 million years ago (Timme et al., 2012). The complex morphology of charophytes, the fact that the egg-cell (oosphere) is surrounded by protective sterile cells and the existence of biflagellate spermatozoids were among the reasons why charophytes were long considered to be related to modern land plants (embryophytes). This relationship was later supported by molecular studies which proposed Charales as the sister group of land plants (McCourt et al., 2004). From a morphological standpoint, an increasingly complex development from single-celled algae via filaments to the complex morphology of charophytes and further to land-plants makes sense (Timme et al., 2012). Charophytes also have ultra-structural similarities (e.g. in the flagellar apparatus of spermatozoids) to terrestrial plants (Vouilloud et al., 2014; this issue). A peculiar morphological adaptation of some *Chara* species to high light intensities (Schneider et al., 2014; this issue) might be analogous to the acclimation to high light intensities found in land plants, and suggests that such a plasticity might have been important for the colonization of land. In spite of morphological and molecular support for Charales as sisters to land plants, however, other studies proposed alternative scenarios. Thus, recent phylogenetic analyses support the Zygnematales as direct ancestors of land plants (Timme et al., 2012).

In 1753, Linnaeus delimited four species within the genus *Chara*: *Chara flexilis*, *C. vulgaris*, *C. tomentosa* and *C. hispida* (Wood and Imahori, 1965). Since then, a vast increase in the number of described species and genera has occurred (e.g., *Chara flexilis* was renamed as *Nitella flexilis*). A search on www.algaebase.org shows that the family Characeae presently is assumed to comprise more than 690 species, sorted into 48 genera. Only six of these genera, however, encompass extant species: *Chara* Linnaeus, *Lamprothamnium* J. Groves, *Lychnothamnus* (Ruprecht) A. Braun, *Nitella* C. Agardh, *Nitellopsis* Hy and *Tolypella* (A. Braun) A. Braun (it is presently discussed whether an additional extant genus *Sphaerochara* should be separated from *Tolypella*; see Soulié-Märsche and García, 2014; this issue). The descriptions of the remaining 42 genera in this family are either based on fossil remains only, or they are synonyms of other currently accepted names. The genus *Chara* is assumed to be the most species-rich within the extant Characeae, closely followed by *Nitella* (www.algaebase.org). However, species discrimination of charophytes is more complicated than it might seem. This is due to the fact that many morphological traits used for species delineation overlap between “species”, both in living and fossil charophytes (Boegle et al., 2007; Soulié-Märsche, 1989, respectively). Such phenotypic plasticity may be induced by the environment, e.g., by light, water temperature, nutrient concentrations and salinity (Wood and Imahori, 1965), and this makes it difficult to know which morphotypes are environmentally induced and which are genetically controlled (Boegle et al., 2010).

Species delineation of extant charophytes is commonly based on morphological traits of the thallus, while fossil charophytes usually are discriminated based on the morphology of the oospore (= zygote plus its organic walls) or the gyrogonite (formed by calcification of the spiral cells surrounding the oospore) (Holzhausen et al., 2014; this issue; Soulié-Märsche and García, 2014; this issue). In order to link fossil charophyte remains to extant species, it is thus important to pay more attention to oospore and gyrogonite morphology of extant charophytes, and to investigate conditions under which the female gametangium calcifies to form a gyrogonite. The study of fossil gyrogonites and oospores found in Quaternary sediments could significantly improve our understanding of past biodiversity and permit the investigation of past natural versus human-induced ecological changes in aquatic ecosystems (Font and Chiesa, 2014; this issue; Yu et al., 2014; this issue). Previous studies of ancient charophytes from the Paleozoic, Mesozoic or Cenozoic eras have been commonly devoted to taxonomy (e.g. Feist et al., 2005) or biostratigraphy (e.g. Riveline et al., 1998), and charophytes have widely been used to establish the chronology of older sequences. However,

less-studied topics such as paleoecology or biogeography are equally relevant to understand ancient patterns of distribution and responses to environmental and climatic changes (Sanjuan and Martín-Closas, 2014; this issue).

Modern charophytes tolerate salinities from fresh to hypersaline waters, up to 58 g/L (García and Chivas, 2006), but they are not present, to our knowledge, in fully marine environments. This has probably been the case since their origin in the Early Paleozoic. Extant charophytes are present on all continents except Antarctica (Wood and Imahori, 1965), including Arctic regions like Spitzbergen (Langangen, 2000) and high mountain regions, e.g. the Chilean Andes (Schubert et al., 2014; this issue). They are found in both lotic and lentic, natural and artificial habitats, ranging from ancient lakes (like e.g. Lake Ohrid) to newly excavated gravel pits or newly created ponds (Rodrigo et al., 2014; this issue).

For many decades, scientists from various disciplines have been studying charophytes, and this is due to several peculiarities of this group of algae. Firstly, the large size of the internode cells makes them convenient targets for studying processes in plant cells and across plant cell membranes. Secondly, some charophyte species can grow to more than one meter tall and build large biomasses in water-bodies. Thus, charophytes can form a significant part of the submerged vegetation in streams, ponds and lakes, thereby interacting with ecosystem properties such as carbon and nutrient balance, and water clarity. At the same time they are also sensitive to ecosystem changes, and this makes them good indicators of ecosystem status. Finally, some charophyte species can produce calcified parts, which in turn can easily fossilize. The fossil record of charophytes spans the last 425 Ma, and some of the fossil taxa are guides for non-marine sequences, making them valuable objects for biostratigraphic (e.g. exploration for hydrocarbon fields) and paleoecological studies.

2. Importance of charophytes

2.1 Charophyte cells are versatile plant cell models

More than 100 years ago, the first observations of cytoplasmic streaming, i.e. the movement of the cytoplasm inside a plant cell, were made on charophyte internode cells, and charophytes are still used when studying cytoplasmic streaming today (Woodhouse and Goldstein, 2013). Fully grown charophyte internode cells can have a diameter of about 1 mm, and a length of several centimeters (up to 40 cm long; García, 1990). Although these cells are

extraordinarily large, their structure and ultrastructure (Shimmen et al., 1994; Vouilloud et al., 2014; this issue) have similarities to that of many other plant cells, including those of higher plants. Thus, charophyte cells became convenient models for plant cells in general. The large size of charophyte internode cells makes them easy targets for various kinds of studies in plant cell biology, such as transport processes (Boot et al., 2012), wound healing (Foissner and Wasteneys, 2012), cell wall formation (Proseus and Boyer, 2006), cell elongation (Proseus and Boyer, 2012) or the uptake of persistent organic chemicals into cells (Schneider and Nizzetto, 2012). This is an incomplete list, and numerous studies have been published based on charophytes as models for plant cells in general.

2.2 Charophytes are providers of ecosystem services

In the last few decades, we have become increasingly aware that humankind benefits from a multitude of resources and processes that are supplied by ecosystems. These benefits are currently known as “ecosystem services” (MEA, 2005) and include products like clean water and processes such as carbon sequestration. Charophytes indeed contribute to a multitude of ecosystem services, and scientists have been aware of this for decades. Zaneveld (1940) summarized nine ways in which charophytes have been of more or less economic value. These include fish culture, water purification, food for aquatic animals as well as farm stock, fertilizers, polishes, mud baths, therapeutic applications, sugar purification, and insect control. For example, between the 18th and the 20th century, charophytes were harvested in Lake Constance, dried and used as fertilizer on vegetable fields (Schmieder, 2004). Although using charophytes for therapeutic applications may sound somewhat peculiar, *Chara intermedia* is used in homoeopathic medicine (Brand and Groeger, 2012). However, the most widely appreciated ecosystem services provided by charophytes probably are their role in providing habitat and food for various organisms, as well as their ability to enhance water clarity, and store carbon and nutrients.

Charophytes serve as food for a number of organisms, such as herbivorous fish (Lake et al., 2002), snails (Baker et al., 2010), waterfowl (Noordhuis et al., 2002; Schmieder et al., 2006) and crayfish (Cirujano et al., 2004). Since charophyte beds offer protection from predators and currents, dense charophyte patches are colonized by a multitude of different organisms. The most obvious are periphytic micro-organisms and invertebrates. Both seasonal variations in the biomass of aquatic invertebrates as well as overall species composition have been

shown to be related to *Chara* biomass (Van den Berg et al., 1997). Charophytes thus help maintaining biodiversity in fresh- and brackish water ecosystems.

The role of charophytes for water clarity, carbon and nutrient storage has been reviewed by Kufel and Kufel (2002). They point out that especially dense charophyte beds can control nutrient cycles and lake biogeochemistry in several ways: directly by taking up nutrients into plant biomass, indirectly by co-precipitation of phosphorus with calcium carbonate, and by enhancing sedimentation and counteracting resuspension of sediment particles (Vermaat et al., 2000). Because many charophytes are ever-green and their biomass generally decomposes rather slowly, carbon and nutrients may be effectively stored over a long time in the sediment of charophyte meadows. Charophytes can precipitate large amounts of CaCO_3 and thus markedly enhance water clarity (Rodrigo et al., 2014; this issue), and decrease the concentration of Ca^{2+} (Pełechaty et al., 2014; this issue). Moreover, a significant part of the total phosphorus in charophytes has been shown to be associated with CaCO_3 , a fraction that is insensitive to redox changes and may be stored in sediments for a long period of time (Kufel et al., 2013). Charophytes may also effectively remove organic chemicals such as hexachlorobenzene (Schneider and Nizzetto, 2012) as well as metals such as uranium (Kalin et al., 2005), cadmium and lead (Sooksawat et al., 2013) from the water, and they may mitigate cyanobacterial blooms in surface waters (Pakdel et al., 2013).

Although charophytes usually are considered “advantageous” in an ecosystem, it is worthwhile mentioning that they sometimes also may be considered a “problem” or “nuisance”. Already in 1940, Zaneveld mentioned that charophytes can clog channels and reservoirs, and even today, it happens that extensive *Chara hispida* stands are removed from channels leading to fish farms, because they clog the inlet to the farm ponds (own observations in the Rotbach, south of Munich, Germany).

2.3 Charophytes are indicators of past and present ecosystem properties

Dense charophyte beds enhance water clarity and direct nutrients and carbon from the water to the sediment beneath charophyte meadows. They thus help maintain oligotrophic conditions in ecosystems. On the other hand, charophytes are also sensitive to environmental changes such as eutrophication (Blindow, 1992). This makes them vulnerable to continuing

ecosystem changes, such that many charophytes have become rare or even endangered in recent decades (see e.g. references in Auderset and Rey-Boissezon, 2014; this issue).

Marked differences occur among charophyte species both with respect to the water chemistry and size of habitat they prefer (Rey-Boissezon and Auderset, 2014; this issue). Consequently, many charophyte species are used as indicators for ecosystem status assessment, both with respect to eutrophication (Melzer, 1999) and ecological status according to the European Water Framework Directive (Stelzer et al., 2005). There are also species-specific differences in light acclimation capabilities among charophyte taxa (Rubio et al., 2014; this issue), and this may well be part of the explanation why different charophyte species occur in different habitats, such as small ponds versus large lakes (Rey-Boissezon and Auderset, 2014; this issue). Knowledge about the ecological conditions under which charophytes occur may be used, in turn, for predictive modeling of charophyte distribution over larger areas (Torn et al., 2014; this issue), as well as for predicting future charophyte distribution under given climate scenarios (Auderset and Rey-Boissezon, 2014; this issue).

What is valid for the present and the future, may well be applied to the past. Thus, charophytes are also a useful tool for paleoecological reconstructions. Species-specific paleoenvironmental constraints are being defined for fossil charophytes on the basis of sedimentological, taphonomical and geochemical analyses (e.g. Villalba-Breva and Martín-Closas, 2011). Their most common use in paleoecology is as indicators of paleosalinities in non-marine water bodies (e.g. García and Chivas, 2006; Rodrigo et al., 2010). Charophytes have also successfully been used as indicators of lake water level (e.g. García, 1999; Soulié-Märsche et al., 2010) and to document changes in the trophic status of fossil lakes (Martín-Closas et al., 2006). Current developments involve experimental cultures to be able to use geochemistry (trace-elements and oxygen isotopes) to reconstruct paleo-salinities and paleo-temperatures (Dux et al., 2014; this issue).

There is a multitude of additional examples, where charophytes have successfully been used for gaining relevant information about both the present and the past. Establishing closer links between the disciplines of paleoecology and ecology is a challenge, but charophytes indeed can provide such a link. For example, the historical biogeography of fossil charophytes helps to understand how the distribution of both fossil and extant species occurred (Bhatia, 2006; Sanjuan and Martín-Closas, 2014; this issue).

The manuscripts collected in this special issue reflect that charophytes are of interdisciplinary interest, and are useful tools to answer questions related to the past, the present and the future. We hope that this special issue can stimulate further work on an admittedly somewhat peculiar group of organisms. We wish to thank the authors for contributing their manuscripts, Elisabeth Gross and Jan Vermaat for giving us the opportunity to prepare this special issue and for their support, and many reviewers for their help in improving the quality of the manuscripts.

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