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Short communication

Starch grain morphology of the seagrasses *Halodule wrightii*, *Ruppia maritima*, *Syringodium filiforme*, and *Thalassia testudinum*

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ABSTRACT

Starch grains are a ubiquitous component of plants that have been used in tandem with phytoliths, pollen, and macrofossils to reconstruct past floral diversity. This tool has yet to be fully explored for aquatic plants, specifically seagrasses, which lack phytoliths and are rarely preserved as macrofossils or pollen. If starch grains in seagrasses are morphologically distinct, this method has the potential to improve seagrass identification in the fossil record in such cases where its starch is preserved (e.g. scratches and occlusal surfaces of tooth enamel from seagrass consumers). The goals of this study were twofold: (1) to determine if starch is present in seagrass material and (2) to assess how starch grain morphology differs between different seagrasses.

This study focused on four abundant and ecologically distinct seagrasses from the Caribbean: *Halodule wrightii*, *Ruppia maritima*, *Syringodium filiforme*, and *Thalassia testudinum*. Starch grains were observed in all species except *S. filiforme*. Grains from *H. wrightii* are typically observed in side-on orientation, are sub-round to angular, and are fairly small (3-19 μm, end-on). Grains of *R. maritima* are small spherical grains (4–8 μm) that have a centric hilum and a straight extinction cross with a median angle between the arms of 90°. Grains from *T. testudinum* are large (9–31 μm, end-on), conical in side-on and round/sub-round in end-on orientation, have a slightly eccentric hilum with an obvious particle, and prominent lamellae.

Visual assessment and comparative statistics demonstrate that the morphology of starch grains from *T. testudinum*, *R. maritima*, and *H. wrightii* are significantly different. With more extensive research, there is potential for the positive identification of starch grains from an unknown seagrass. The ability to identify seagrass from starch grains could facilitate the identification of seagrasses in the fossil record and supply information on seagrass evolution and distribution, climate effects on seagrass distribution, and the diets of seagrass consumers.

1. Introduction

Fossil evidence of seagrasses is known since the late Cretaceous but fossil localities are limited to only a handful of sites (Brasier, 1975; Lumbert et al., 1984), as plant remains of seagrasses are rare and nearly impossible to identify without the preservation of reproductive parts (Brasier, 1975). Additionally, seagrasses do not have phytoliths and their pollen is not preserved because it lacks exine (Brasier, 1975; Domning, 1982). For these reasons, the identification of fossil seagrasses primarily relies on associated fauna (e.g. foraminifera, mollusks, echinoids, crustaceans, and sirenians) and distinctive sedimentary features of seagrass communities (Brasier, 1975). However these identification methods have limitations, some of which could be overcome if starch grains from different species of seagrasses are distinct and are preserved in the fossil record.

Starch is the energy source of a plant and, while present in all plant parts, it is most heavily concentrated in storage organs (i.e. roots, tubers, rhizomes, fruits, and seeds) (Gott et al., 2006). Starch grains occur in a variety of characteristic forms that can be used to identify plants to family and genus level, sometimes even species (Reichert, 1913). Starch is also highly resistant to alteration, which has enabled it to be preserved in a variety of climate regions ranging from arid to tropical and recovered from several substrates, including fecal material (Barton and Matthews, 2006); cracks, pits, and crevices in pottery, millstones, or other grinding tools and associated soils recovered from archaeological sites (Samuel, 1996; Piperno and Holst, 1998; Lentfer et al., 2002; Piperno et al., 2004; Barton and Matthews, 2006; Perry et al., 2007); and pyritized starch grains have even been found in rocks of Eocene age (Wilkinson, 1983). Unaltered starch grains could be preserved in the fossil record if they are protected from destructive elements such as microorganisms, soil moisture, soil pH, and oxygen following rapid

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burial (Barton and Matthews, 2006). One potential location for preservation could be in the cracks or occlusal surfaces of teeth from fossil seagrass consumers (i.e. manatees and dugongs) which could be similar to extracting starch from the grinding surfaces of tools.

Most of the studies that have characterized and described starch grain morphology have been descriptive studies working with a variety of terrestrial, aquatic, and estuarine vegetation (Reichert, 1913) or have focused on food crops used by early human cultures (Ugent et al., 1987; Piperno and Holst, 1998; Lentfer et al., 2002; Piperno et al., 2004; Perry et al., 2007). However, starch grains of seagrasses have not been described in detail except for the analysis of seeds and pollen from *Ruppia maritima*, seeds from *Zostera marina*, and rhizomes of *Z. noltii*, formerly *Z. nana* (Reichert, 1913). Leaves and rhizomes are the most common part of the plant to be eaten by marine consumers, so additional research on starch from these parts of the plants is necessary.

This study focused on four abundant and ecologically distinct tropical seagrasses from the Caribbean: *Halodule wrightii*, *R. maritima*, *Syringodium filiforme*, and *Thalassia testudinum*. The objectives of this study were to: (1) determine if starch grains are present in seagrasses and (2) determine if these starch grains are morphologically distinct between species, potentially enabling the identification of seagrass species from starch grains of unclear origin. With more rigorous study, this method could be used to collect important dietary information for seagrass consumers and aid in identifying the distribution of seagrasses in the fossil record.

2. Methods

2.1. Sample selection

Seagrass samples were collected from the Indian River Lagoon (IRL) in Florida, USA in 2003 and 2004. The IRL is located on the east coast of Florida between approximately 28°24′N and 27°11′N. Samples of whole plants (i.e. leaves and rhizomes) were taken for study from seagrass beds of *H. wrightii*, *R. maritima*, *S. filiforme*, and *T. testudinum*. Samples were rinsed in deionized water (DIW) to remove epiphytes, sediments, and salts and then frozen and stored before preparation for starch grain analysis.

2.2. Sample preparation

Methods were adapted from Perry et al. (2007). For this study approximately 1 g of seagrass material (leaves and rhizomes together) was combined with 200 mL of DIW and then ground until fine using an immersion blender (approximately 5–10 min). For *H. wrightii*, *R. maritima*, and *S. filiforme* 5–10 individual plants were ground together; however given its larger size, only 2–3 plants of *T. testudinum* were used. Material was then passed through a stacked series of three sieves (250, 75, and 45 μm) to isolate starch grains from the bulk plant matter. Initial analyses demonstrated no starch grains larger than 45 μm were present and so this size was the smallest sieve used to remove other plant materials that would have obscured the view of the starch grains. This mixture was allowed to sit overnight and was then centrifuged for 5 min at 5000 rpm and the supernatant discarded. A few drops of 100% ethanol were added to the mixture to prevent molding. Starch grains were mounted in water; one drop of the <45 μm fraction/DIW mixture was placed on a slide for analysis with a polarizing light microscope and the edges of the coverslip sealed with clear nail polish. To prevent contamination between samples, sieves were cleaned by sonication with DIW heated to 68 °C for 30 min, which gelatinized and destroyed any starch grains adhering to the sieve.

2.3. Starch grain analysis

Slides were analyzed using a polarizing light microscope in both plane-polarized light (PPL) with one polarizing filter in place and cross-polarized light (XPL) with two polarizing filters set at 90° to one another. Photographs were taken in rapid succession to minimize the effect of starch grain movement and taken so that at least 30 starch grains in end-on orientation were available for measurement. Additional digital photomicrographs were obtained at the Paleo Research Institute in white light, partial-polarized light, and XPL. Starch grain identifications were based on a set of the most commonly used morphological characters (Piperno et al., 2004; Torrence et al., 2004; Holst et al., 2007; Field, 2008; Fullagar et al., 2008). Grain type, size, shape, and presence or absence of lamellae, hilum, particles, and fissures were determined under PPL whereas measurements of extinction cross size and shape, angle between arms, and strength of birefringence were made under XPL. While the shape, size, and relative position of the arms of the extinction cross can be important diagnostic features of modern starch grains (Reichert, 1913; Torrence et al., 2004), ancient starches often lose their birefringent properties and as such, extinction cross features will not always be preserved in ancient starch.

Statistical analyses were run using PAST (Hammer et al., 2001) with a significance level of $\alpha = 0.05$. Results from the Shapiro–Wilk test for normality indicated that only four of the 18 numerical variables resulted in normally distributed datasets. As such, the nonparametric Mann–Whitney–Wilcoxon rank-sum test was used to compare the difference in the sample medians.

3. Results

Starch grains were observed in three of the four species of seagrass analyzed. Table 1 lists the summary statistics (i.e. median and range) and comparative statistics of measurements of starch grains in end-on orientation. Statistical comparisons were limited to end-on orientation because starch grains of *R. maritima* did not occur in side-on orientation.

3.1. *H. wrightii*

Starch grains of *H. wrightii* (Fig. 1a and b) were relatively abundant and typically observed in side-on orientation. In end-on orientation starch grains were simple, ranged from 3 to 19 μm in diameter, were angular to sub-round, had an equal distribution of grains with a centric versus eccentric hilum, had the occasional particle and/or fissures, and occasionally had weak lamellae. The extinction cross was straight to rounded with median angle between the arms of 106° and strong birefringence. Grain characteristics did not change much in side-on orientation, although the grains typically had a greater length, which ranged from 8 to 24 μm in diameter.

3.2. *R. maritima*

Starch grains of *R. maritima* (Fig. 1a and b) were abundant; they were simple grains, roughly spherical and therefore only occurred in end-on orientation. The grains were small (4–8 μm diameter), round to sub-round, with a centric hilum that typically lacked a particle, and occasionally had weak lamellae. The extinction cross was straight with a median angle between the arms of 90° and strong birefringence.