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Research paper

The shape factors of phytoliths in selected plants from the Changbai Mountains and their implications

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ABSTRACT

Phytoliths as a climate proxy are one of the most important and effective tools for reconstruction of paleoenvironment. In this paper, twelve Gramineae species of the subfamilies Panicoideae, Pooideae and Arundinoideae from the Changbai Mountains were selected, and 3698 phytoliths were counted. We measured six parameters of dumbell, elongate, lanceolate, crenate, saddle and silicified stomata. The seven shape factors of different types of phytoliths were deduced by the six parameters measured above and analyzed by nonparametric testing and discriminant analysis.
The nonparametric testing indicated that the average value of the shape factor SF_4 among the three subfamilies was obviously different. Discriminant analysis for the three subfamilies, also revealed that the discriminant accuracy was high (89.81%). Total discriminant accuracy using shape factors of dumbell phytoliths of Panicoideae was 73.12%. Discriminant accuracies using shape factors of elongate, lanceolate and crenate phytoliths of Pooideae were respectively 60.87, 59.21 and 87.07%. The discriminant accuracies using shape factors of saddle phytoliths and silicified stomata of Arundinoideae were 59.06 and 71.77%. At the same time, the shape factors of phytoliths from *Setaria viridis* and *Leymus chinensis* were used to test whether the discriminant functions established at the subfamily level were effective or not. Results showed discriminant functions precisely classify them, thus verified discriminant functions were useful. In conclusion, using the seven shape factors of phytoliths not only may improve the accuracy of classifying Gramineae, but also provides basis reference data for better understanding the presence and function of Gramineae plants at archaeological sites.

1. Introduction

Phytoliths are microscopic silica bodies produced in the tissue of living plants, and can clearly reflect the environmental conditions of the time in which they were formed. At present, phytolith analysis mainly focuses on paleobiology, paleoenvironment, plant taxonomy (Brown 1984; Piperno 1988; Mulholland and Rapp 1992; Guiot et al. 1993; Fredlund and Tieszen 1994; Fredlund and Tieszen 1997; Horrocks et al. 2000; Huang et al. 2004; Xu et al. 2005; Iriarte & Paz, 2009), topsoil phytolith analysis (Lu et al. 1996; Huang et al. 2004; Lu et al. 2007), geology and archaeology (Zhang et al. 2003; Jin et al. 2007; Qin et al. 2008; Ge et al. 2010; Jie et al. 2010). However, research on phytolith morphology is fundamental prerequisite upon which the above disciplines are based. Consequently, many studies have focused on the analysis of phytolith morphology. For example, the phytoliths from Panicoideae and Festucoideae were classified by Prat (1936) on the basis of their morphologies; Twiss et al. (1969) further divided the phytoliths of Gramineae into four groups on the basis of the classification proposed by Prat. Brown (1984) categorized the phytoliths of Gramineae into eight broad groups and identified more than 130 phytolith types; Gramineae phytoliths were divided into 14 categories by Wang and Lu (1992). Piperno (1988) provided two different kinds of retrieval tables for phytolith analysis; and Mulholland and Rapp (1992) proposed a classification method for Gramineae phytoliths. In 2005, the ICPN (International Code for Phytolith Nomenclature) working group proposed an easy to follow, international-accepted protocol for describing and naming phytoliths (Madella et al. 2005). When taxonomic significance cannot be assigned to a single phytolith type, a group of phytolith types and their frequencies (phytolith assemblages) may have taxonomic significance and this should be considered (Madella et al. 2005).

Presently, the taxonomy of phytoliths has not yet been standardized. Different researchers use different terms and classification schemes that reflect differences in materials, classification criteria, and study areas (Madella et al. 2005). Additionally, it is difficult to classify Gramineae to genus or species level according to the morphology of phytoliths

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because one type of Gramineae plant can produce different types of phytoliths, and different species can produce the same type (Rovner 1971). Furthermore, the same type of phytolith from different kinds of plant can vary in size and shape, and it is difficult to further classify the plants according to phytolith geometric shape. So it may be important to describe phytoliths with a quantitative method to distinguish plants at the species level. As a result, many studies have tried to define the characteristic parameters of the different types of phytoliths, in an attempt to use small differences among the same type of phytoliths from different plants to easily classify plants. For instance, Wang and Lu (1992) measured the characteristic parameters of bulliform phytoliths from ten species of Oryzoideae, and tried to classify them using a quantitative method. However, the method they used is appropriate only for classifying plants that contain bulliform phytoliths, so the application of this method is limited. Furthermore, opinions on the characteristic parameters defining out of shape bulliform phytoliths vary among researchers and so this method is not widely used. Sato et al. (1990) studied 96 Asian rice cultivars, and identified *japonica* and *indica* rice by a discriminant function established by measuring the characteristic parameters of bulliform phytoliths. Wang et al. (1996) and Gu et al. (2013) also established a discriminant function according to characteristic parameters of bulliform phytoliths. As different studies in different areas have used the parameters of bulliform phytoliths of rice to establish discriminant functions, so the discriminant functions vary widely. Thus, there are many limitations when further classifying plants using this method. Fahmy (2008) established a classification system based on some important morphological parameters of bilobate phytoliths from 66 species belonging to tribe Paniceae in western tropical Africa. He found differences in the phytoliths shape and size, but was unable to distinguish the tribe Paniceae at the species level. To date there are many limitations when further classifying the plants, and studies on plant taxonomy based on the phytoliths at the species level are rare.

In this paper, twelve Gramineae species comprising three subfamilies (four Panicoideae plants, five Pooideae plants, three Arundinoideae plants) from the Changbai Mountains are selected. We try to supply a theoretical basis and supporting evidence using phytoliths to accurately classify the Gramineae further.

2. Study site

The study region corresponds to the Changbai Mountain region, which is located from 42°13′20.5″ N to 46°25′45.2″ N and from longitude 126°29′39.8″ E to 103°51′47.7″ E. The area comprises the Dahei Mountain, the Jilinhada Ridge and the Zhangguangcai Ridge. The vegetation of the Changbai Mountains is mixed coniferous broad-leaved forest, with *Pinus koraiensis* the major tree species. The area has a warm temperate continental monsoon climate with high rainfall. Because of the humid climate, grassy marshland is widespread in the Changbai Mountains. Species of Gramineae are abundant and compose the grassy marshland.

3. Materials and methods

3.1. Materials

In this paper, leaf blades from twelve Gramineae species that grow well in the Changbai Mountains had been studied. There were four typical plants from Panicoideae, five typical plants from Pooideae and three kinds of plant from Arundinoideae (Table 1).

3.2. Methods

3.2.1. Phytolith extraction method

The wet-ashing method (Piperno 2006) was used to extract the phytoliths. Firstly, five individuals of each kind of plants were collected. We selected one piece of the consistent size leaf from the each individual plant and mixed them into one sample. The samples were cleaned in distilled water using an ultrasonic cleaning bath and dried. Then each sample was cut into small pieces. Secondly, 0.5 g of dry clean leaves for each sample was added to one test tube with 65% HNO_3 and heated by water bath until the organic matter was fully oxidized. Thirdly, distilled water was then added to the test tube to wash the acid and the mixture was centrifuged three times. Fourthly, absolute ethyl alcohol was added to each test tube and then centrifuged. Each time the mixture was centrifuged at 2000 revolutions/min for 15 min. Finally, after extraction, microscope slides were made and at least 300 phytoliths were counted at a magnification of 900× in each sample under a biological microscope (Motic, China). Only phytoliths with a diameter greater than 10 μm were counted. Photomicrographs of typical phytoliths from the twelve plants were shown in Plate I.

3.2.2. Phytolith measurement method

In this paper, a total of six parameters of phytoliths were measured using the measuring tools of MOTIC software installed on a computer: circumference (P), long axis length (L), area (A), the area of the minimum circumscribed (A_0) and the area of the maximum inscribed circle (A_i), as well as the circumference of the circle with the same area as the phytolith (P_0). Using the measuring tools, P and A were measured by the ‘irregular polygon’ button, L by the ‘line’ button, A_0 and A_i by the ‘circle (three points)’ button. Additionally, we calculated the value of P_0 based on the value A. Then the seven shape factors of the phytoliths were deduced according to the six parameters above (Table 2). Finally, we calculated the average values of the phytolith shape factors of the twelve

Table 1
Plant taxa used for phytolith analysis and location of wetlands from the Changbai Mountains.

Sites	Genera	Latin name	Phytoliths	Latitude	Longitude	Elevation (m)	Biotope
Chunguang	*Miscanthus*	*M. sacchariflorus*	Dumbell	43°53′15.9″	126°28′35.4″	296	Wetland
Xihe	*Echinochloa*	*E. phyllopogon*	Dumbell	44°16′5″	126°30′47.6″	175	Wetland
Taoshan	*Echinochloa*	*E. crusgalli* var. *caudata*	Dumbell	45°40′8.1″	125°15′13.3″	254	Wetland
Weihe	*Arthraxon*	*A. hispidus*	Dumbell	44°54′47.3″	128°42′55.6″	302	Wetland
Taoshan	*Calamagrostis*	*C. angustifolia*	Elongate lanceolate crenate	45°40′8.1″	125°15′13.3″	254	Wetland
Taoshan	*Calamagrostis*	*C. langsdorffii*	Elongate lanceolate crenate	45°40′8.1″	125°15′13.3″	254	Wetland
Taoshan	*Beckmannia*	*B. syzigachne*	Elongate lanceolate crenate	45°40′8.1″	125°15′13.3″	254	Wetland
Demoli Village 1	*Millium*	*M. effusum*	Elongate lanceolate crenate	45°52′8″	129°2′9.3″	234	Wetland
Demoli Village 3	*Calamagrostis*	*C. arundinacea*	Elongate lanceolate crenate	45°54′41.8″	129°8′18.3″	158	Wetland
Yuanchi	*Phragmites*	*P. communis*	Saddle -silicified stomata	40°01′53.8″	128°26′1.4″	1280	Wetland
Erdaobaihe	*Phragmites*	*P. hirsuta*	Saddle -silicified stomata	42°24′14.2″	128°05′54.4″	761	Wetland
Longwan	*Phragmites*	*P. jeholensis*	Saddle -silicified stomata	42°21′27″	126°22′03.5″	453	Wetland