ARTICLE IN PRESS

Journal of Archaeological Science xxx (2015) 1–8

Contents lists available at ScienceDirect

Journal of Archaeological Science

journal homepage: http://www.elsevier.com/locate/jas

Phytolith as a method of identification for three genera of woody bamboos (Bambusoideae) in tropical southwest China

Yansheng Gu [a, b, *], Hongye Liu [a], Hanlin Wang [c], Rencheng Li [d], Jianxin Yu [a]

[a] State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, China
[b] Hubei Key Laboratory of Wetland Evolution & Eco-Restoration (WEER), China University of Geosciences, Wuhan 430074, China
[c] School of Geography and Ocean Science, Nanjing University, Nanjing 210046, China
[d] College of Earth Science, Guilin University of Technology, Guilin 541004, China

ARTICLE INFO

Article history:
Received 26 April 2015
Received in revised form
19 July 2015
Accepted 3 August 2015
Available online xxx

Keywords:
Phytolith
Morphological parameters
Taxonomy
Woody bamboos
Southwest China

ABSTRACT

In order to explore the relation between phytolith morphology and plant taxonomy, we conducted a comparative research on the morphological features of phytoliths from 26 woody bamboo species within 3 genera of Bambusoideae in tropical southwest China. All morphological parameters were measured at 500× magnification using an Olympus BX51 light microscope. Three-dimensional scattered plots and hierarchical cluster analysis were performed using SPSS 13.0 software. The leaves of woody bamboos contain a great diversity of phytolith types including short cells, long cells, bulliform cells, hair cells, mesophyll and vascular tissues, of which cuneiform bulliform cells and oblong concave saddles were significant in clarifying the position of controversial *Dendrocalamopsis* group. Detailed research on phytolith morphology demonstrated that most of the overlap within this group occurs among long cells, bilobates, parallepipedal bulliform cells, hair cells, mesophyll and vascular tissues at the genus level. Oblong concave saddles exhibited taxonomical value both at the subfamily and genus levels. Comparative research on the morphological parameters of cuneiform bulliform cells and oblong concave saddles indicated *Dendrocalamopsis* has a separate partition from *Bambusa* and *Dendrocalamus*, which might provide strong evidence that *Dendrocalamopsis* should be an independent genus within Bambusoideae. Hierarchical cluster analysis on the cuneiform bulliform cells and oblong concave saddles indicated that *Dendrocalamopsis* is more closely related to *Bambusa* than *Dendrocalamus*. At the genus level, cuneiform bulliform cells and oblong concave saddles together exhibit taxonomical value.

1. Introduction

China is one of the world's bamboo largest distribution centers and is also one of the first countries to research the cultivation and utilization of bamboo (Fig. 1). The subfamily Bambusoideae in China comprises 37 genera and 500 species, of which twenty-nine genera and 250 species of bamboos grow in Yunnan (Hui et al., 1999; Yang et al., 2004). Bambusoideae is one of the largest and the only subfamily that contains woody members in the grass family of Poaceae (Soreng et al., 2015), and currently includes 115 genera with 1439 described species (Bamboo Phylogeny Group, 2006, 2012; Wang et al., 2013). The distinctive life history (infrequent flowering and predominance of asexual reproduction) of woody bamboos makes them an interesting but taxonomically difficult group to classify (Zhang et al., 2011). Taxonomists often use vegetative morphology (mostly bamboo shells) to conduct bamboo plant classification. The varied vegetative characteristics of bamboo, such as different habitats, growth stages, and geographic locations, often result in differences of opinion on the bamboo taxonomy. Due to the bamboo taxonomist' different views on the classification of the genus within the Bambusoideae, there is taxonomic confusion at the genus level, which causes classification position changing of some bamboo species (Zhao and Tang, 1993). For example, the classification of *Dendrocalamopsis* based on morphological identification remains a matter of debate (Yang et al., 2008). Keng (1982, 1983, 1987) and the Flora of China Editorial Committee (1996) proposed that *Dendrocalamopsis* should be an independent genus based on the morphological features of leaf, stem, inflorescence and fruit. However, Jia and Feng

* Corresponding author. State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences, Wuhan 430074, China.
E-mail address: ysgu@cug.edu.cn (Y. Gu).

http://dx.doi.org/10.1016/j.jas.2015.08.003

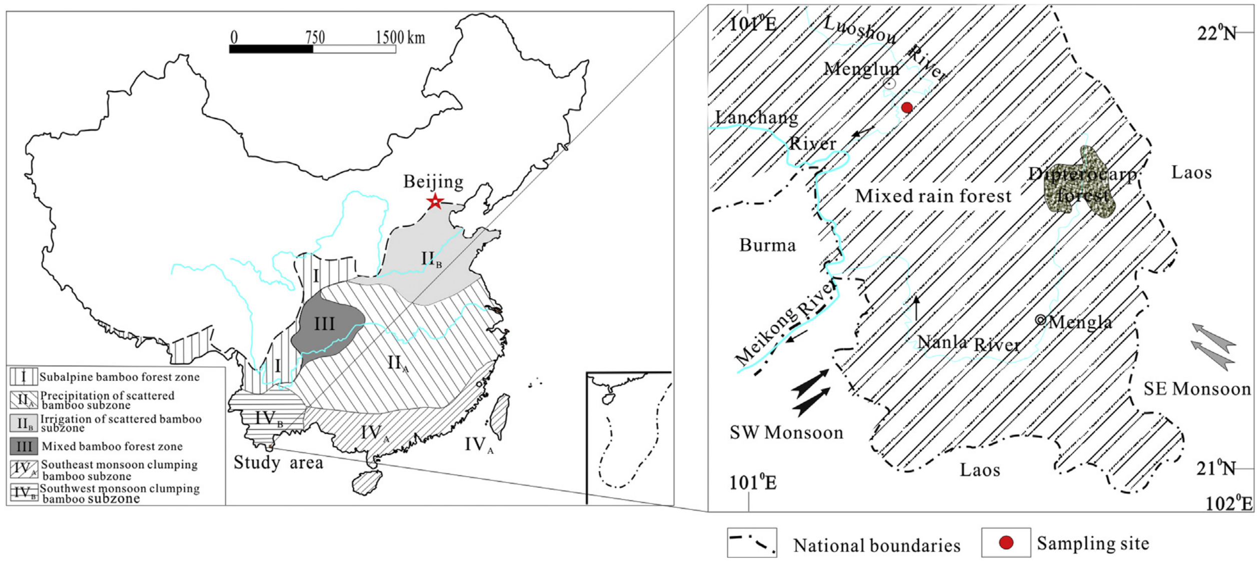

Fig. 1. Map showing the bamboo forests in China and location of the Xishuangbanna Tropical Botanical Garden where the samples of bamboo leaves were collected (Bamboo forest map modified from Liang (1990) and Gu et al. (2008)).

(1980), Wang et al. (2002) and the Flora of China Editorial Committee (2014) proposed that *Dendrocalamopsis* should be a subgenus within *Bambusa* based on the numbers of papillae in subsidiary cells of the stomata. In addition, the chloroplast (cp) genome of Bambusoideae evolved slowly and the limited variation of DNA sequences among bamboos makes their molecular classification difficult, particularly at the genus and subgenus levels (Zhang et al., 2011).

Bamboos have a high phytolith content and are widely distributed in subtropical and temperate zones, forming a significant organic silicon pool which plays an important role in the global silicon cycle (Li et al., 2006; Ding et al., 2008). Phytoliths are microscopic silica bodies that precipitate in or between cells of living plant tissues. Bambusoideae produce a large number of phytoliths specific at the tribal, subtribal, and genus levels (Piperno and Pearsall, 1998). Phytoliths diagnostic to bamboos include oblong concave saddles (collapsed saddles or tall saddles), saddles with ridged platforms, and five types of cross-shaped phytoliths with unique three-dimensional structures (Li et al., 2005; Piperno, 2006).

To date, little phytolith data are available to evaluate their taxonomic value for woody bamboos at genus level. Comparative research was first conducted on the leaves of phytoliths from 26 species within three genera of *Dendrocalamopsis*, *Bambusa*, and *Dendrocalamus*, respectively. Our research provides important evidence for the relation between phytolith morphology and plant taxonomy.

2. Material and methods

A total of 26 woody bamboo species of three genera, supplied by the Xushuangbanna Tropical Botanical Garden of the Chinese Academy of Sciences, were studied (Table 1). Thousands of tropical and sub-tropical plants, including 100 species of woody bamboos, grow in the Xishuangbanna Tropical Botanical Garden. The regional climate is dominated by the southwest monsoon (Indian Ocean monsoon) and marked by an annual alternation of dry and wet periods. At Menglun Station of the Tropical Botanical Garden, the annual mean precipitation is 1463.3 mm, 85% of which falls during the summer wet season. The monthly mean temperature (21.7 °C) varies little, although it rises slightly in the wet seasons to 25.7 °C (Liu and Li, 1996). Tropical rain forest dominates the lower hills, mountain valleys and basins in this region (Fig. 1) (Gu et al., 2008).

Phytoliths were extracted through chemical oxidation and slide-mounted in Canada balsam following procedures outlined in Pearsall (1989, 2000). Identification and counting for 26 phytolith slides was carried out at the State Key Laboratory of Biogeology and Environmental Geology, China University of Geosciences (Table 1). Identification and counting for 250–300 phytolith grains per slide was carried out at the lab. The cuneiform bulliform cells and oblong concave saddles are abundant and thus 60 bodies on each slide were carefully measured for 26 bamboo species, respectively. For each sample, all morphological parameters were measured at 500 × magnification under an Olympus BX51 microscope.

Following Li et al. (2005), two morphological measurements were carried out for oblong concave saddles under an eyepiece micrometer: horizontal length (W, width of base) and vertical length (L, height of base). Three morphological measurements of cuneiform bulliform cells were taken following Fujiwara (1993): horizontal length (HL, length of base), lateral length (LL, width of side), and vertical length (VL). All data are processed by SPSS 13.0 software. Three-dimensioned plots and cluster analysis are performed by the function of the Graphics and Hierarchical Cluster Dendrogram.

3. Results & discussion

3.1. Phytolith morphotypes and classification system

To date, there are very little data about classification systems of phytoliths in bamboos despite extensive research on phytoliths in Poaceae and Poaceae subfamilies (Pearsall, 1995; Lu et al., 1997; Piperno and Pearsall, 1998; Li et al., 2005). The classification scheme used here follows the classification system of the Poaceae phytoliths of Wang and Lu (1993), Madella et al. (2005) and Gu et al. (2008, 2013). Phytoliths for anatomical terms inside 26 bamboo species are divided into six groups: short cells, long cells, bulliform cells, hair cells, mesophyll, tracheid and vascular tissues. The first four phytolith types are originating from epidermis, whilst the last two are from mesophyll and vascular tissues, respectively. Short