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PHYTOLITHS OF FERNS III : IN HIMALAYAN HORSETAIL AND SOME FERNS

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ABSTRACT

Phytolith morphology in root, stem, leaf & cone of *Equisetum diffusum*, leaf & spike of 4 species of eusporangiate ferns and 23 species of leptosporangiate ferns were studied. Commonly found forms were nearly triangular, cylindrical, square or rectangular and amorphous. Plates like phytoliths were found with distinct features in different species. Phytoliths resembling Spicular cells were found in 6 species of *Vittaria*. SEM studies of silicification patterns in *E. diffusum* revealed knob like and ridge like deposition over leaf and stem surfaces.

Key words : Opal phytolith, *Equisetum diffusum*, fern, leptosporangiate, eusporangiate

Introduction

Phytoliths are amorphous form of Silicon dioxide (known as 'opal') deposition found in many plants. However, cacti produce calcium oxalate phytoliths (Pearsall, 2000).

The discovery and exploration stage of phytolith research started from 1835, when a report of phytoliths in living plants was published by Struve (1835), a German botanist, to around 1895. Ehrenberg (1841, 1854) called them "Phytolitheria" from the Greek meaning "plant stone" and developed the first phytolith classification system (Piperno, 2006).

Phytoliths provide valuable information about vegetation in Archaeology and Paleoecology, crop processing (Harvey and Fuller, 2005), radiometric dating (Wilding, 1967) and monitoring vegetation and climate change (Kelly *et al.*, 1991). Particular plant taxon or plant part can be identified by Phytolith characters (Pearsall *et al.*, 1995; Zhao *et al.*, 1998).

The leaves of ferns, horsetails, and other plants that reproduce by spores can exhibit a considerable degree of silicification, and phytoliths display an impressive number of distinctive shapes (Piperno, 2006).

Phytoliths of pteridophytes have been studied in few taxa like *Trichomanes*, *Adiantum* & *Selaginella* (Piperno, 1988), *Equisetum* & *Metteuccia* (Bozarth, 1993), *Isoetes* and Polypodiaceae (cf. Piperno, 2006).

Recently, Mazumdar and Mukhopadhyay (2009a) reported the occurrence of opal phytoliths in three thelypteroid ferns viz., *Christella dentata* (Forssk.) Brownsey and Jermy, *Ampelopteris prolifera* (Retz.) Copel. and *Cyclosorus interruptus* (Willd.) H. Itô. Chauhan *et al.* (2009) reported biogenic silica deposition in different parts like leaf epidermis, hair base, stem cortex, spore wall, tracheid, parenchyma cell, etc of some ferns and fern allies and also discussed its ecological and evolutionary significance. Mazumdar and Mukhopadhyay (2009b) reported occurrence of opal phytoliths in fertile aerial branches of some *Selaginella* and *Lycopodium* species and sporophylls of *Isoetes coromondelina*. Characteristic plate like phytoliths were found in *Huperzia selago*, *Lycopodium clavatum*, *Palhinhaea cernua*, *Selaginella pentagona*, *S. bryopteris*, *S. inaequalifolia*, *S. tenera* and *Isöetes coromandelina*. Much work is required for proper characterization for phytoliths in Pteridophytes (Mazumdar and Mukhopadhyay, 2009a).

Pryer *et al.*, (2001) suggested horsetails and ferns belong to monophyletic group and is the closest living relatives to seed plants. *Equisetum* produces abundant biogenic silica. Giffford and Foster (1989) summarized the following importance of silica in *Equisetum*: maintaining erectness of the plant, normal growth (Chen and Lewin, 1969), viability of spores (Hoffman and Hillson, 1979), protection against pathogens & predators and prevention of excessive water loss (Kaufman *et al,* 1971).

Pattern of silica deposition in *Equisetum* has been studied in detail by some workers *viz*. Hauke (1978, 1979), Srinivasan *et al.* (1979), Perry and Fraser (1991), Sapei *et al.* (2007). Using Electron microscope analysis, Kaufman *et al.* (1971) found that silica was deposited primarily in discrete knobs and rosettes on the epidermal surface in *E. arvense* and essentially in a uniform pattern on and in the entire outer epidermal cell walls of *E. hyemale* var. *affine*. Using Scanning electron microscope (SEM) Page (1972) found differences in pilules (hemispherical or globose bead-like or knob-like objects), mamillae (rounded or conical projections) and stomatal cell area between Subgenus *Equisetum* and Subgenus *Hippochaete* and also suggested three principal taxonomic groups of species within subgenus *Equisetum*: Section I: Equisetum, Section 2: Subvernalia and Section 3: Palustria.

The objective the present study is to find occurrence and morphology of phytoliths in different plant parts and SEM study of *Equisetum diffusum* D. Don, an Indian species, commonly known as 'Himalayan horsetail' and some eusporangiate and leptosporangiate ferns.

Material and Methods

Preserved root, stem, leaf and cone of *Equisetum diffusum* D. Don (Equisetaceae) were obtained from Pteridology Laboratory of Botany Department, The University of Burdwan. In case of ferns, leaf segments were selected as Piperno (2006) mentioned that silicification of epidermis proper resulting in more useful forms appears to occur in leaves of the fern family Polypodiaceae and conifers. Fertile pinnules/leaves of 4 eusporangiate and 23 leptosporangiate species of ferns were studied. Leaf and spike of *Ophioglossum reticulatum* Linn. (Ophioglossaceae), *Botrychium virginianum* (L.) Sw. (Ophioglossaceae), *Helminthostachys zeylanica* (L.) Hook. (Ophioglossaceae) and fertile pinnules of *Angiopteris evecta* (Forst.) Hoffm. (Angiopteridaceae), *Gleichenia gigantea* Wall. ex Hook. (Gleicheniaceae), *Dicranopteris linearis* (Burm. f.) Und. (Dicranoptericaceae), *Arthromeris wallichiana* (Spreng.) Ching (Polypodiaceae), *Phymatopteris griffithiana* Pic. Ser. (Polypodiaceae), *Cheilanthes albomarginata* Clarke (Cheilanthaceae), *Antrophyum reticulatum* (Forst.) Kaulf. (Antrophyaceae), *Cyathea spinulosa* Wall. ex Hook. (Cyatheaceae), *Hypolepis punctata*

(Thunb.) Mett. ex Kuhn (Hypolepidaceae), *Lindsaea odorata* Roxb. (Lindsaeaceae), *Sphenomeris chinensis* (L.) Maxon (Lindsaeaceae), *Athyrium nigripes* (Bl.) Moore (Athyriaceae), *Diplazium esculentum* (Retz.) Sw. (Athyriaceae), *Polystichum squarrosum* (D. Don) Fee (Dryopteridaceae), *Oleandra wallichi* (Hook.) Pr. (Oleandraceae), *Leucostegia immersa* (Wall. ex Hook.) Presl (Davalliaceae) and *Blechnum orientale* Linn. (Blechnaceae), *Peranema cyatheoides* D.Don (Dryopterideceae) were also obtained from herbarium sheets/preserved materials in Pteridology Research Laboratory, Botany Department. Fertile leaves of *Vittaria carcina* Christ., *V. zosterifolia* Willd., *V. amboinensis* Fee, *V. sikkimensis* Kuhn, *V. elongata* Sw. and *V. himalayensis* Ching (Vittariaceae) were obtained from herbarium sheets, provided by Dr. J.B. Bhandari.

Phytolith extraction method by Piperno (2006) was followed with some modifications of steps (Mazumdar & Mukhopadhyay, 2009a). All materials were washed thoroughly several times with tap water and then with distilled water. About 0.2 gm of each of the plant materials were taken in test tubes and digested with concentrated Nitric Acid in water bath. Digested materials were washed with distilled water at 1700 rpm for 10 minutes and then boiled in 10 % Hydrochloric acid in water bath to remove calcium, then, washed with distilled water many times to remove the acid. The processed materials were then centrifuged with acetone at 1700 rpm for 10 minutes each time and dried with Acetone. Dried materials were mounted in 10 % glycerin on the glass slide. Photographs were taken with QWin (machine QG2-32, version V 3.2.0) in Leica microscope.

For SEM observations, leaf and stem of *Equisetum diffusum* were mounted on aluminium stub placed in IB_2 Ion Coater, Japan and evacuated prior to gold coating. SEM images were taken with a model of S 530 HITACHI, Japan in University Science Instrumentation Centre, The University of Burdwan.

Observations and Discussion

Crystalline biogenic silica deposition or Opal phytoliths were found in root, stem, leaf, branch and cone of *Equisetum diffusum* D. Don. Phytoliths, which were possible to extract during the present study, occur as hyaline, large plates resembling epidermal cast in stem (Fig.1A), leaf (Fig.1C) and root (Fig.1I). Some particles are three dimensional nearly triangular (Fig.1E,L), nearly cylindrical (Fig.1B,G), nearly square (Fig.1F) or rectangular (Fig.1K) and amorphous (Fig.1D,H,J). Piperno (2006) mentioned that phytoliths with granular or smooth surface and elevated projections along the edges are 'diagnostic' of genus *Equisetum*. Similar forms are found in abundance in stem and leaf (Fig.1A,C) with striations also (Fig.1A) in *E. diffusum*.

SEM studies revealed highly silicification in the form of pilules (hemispherical or globose bead-like or knob-like objects), mamillae (rounded or conical projections) and large irregular ridges in stem (Fig.2A) and leaf (Fig.1M). Areas filled with densely arranged pilules are surrounded by elevated branched and parallel ridges in stem (Fig.2A). In stoma of stem pilules are also surrounded by borders formed by adjacent pilules (Fig.1N).

In eusporangiate and leptosporangiate ferns, phytoliths, which were possible to extract during the present study, found commonly nearly cylindrical (Fig.3H,4I), nearly triangular (Fig.3M,4B), nearly rectangular (Fig.3E,L,4F) and amorphous (Fig.3F,G,4E) in most of the fern species.

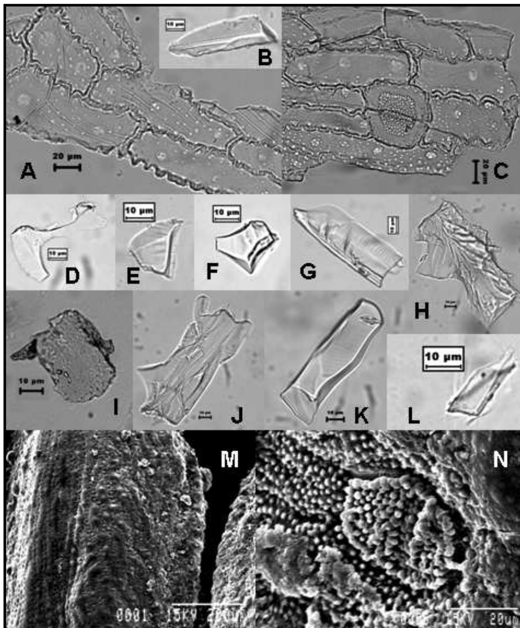

Fig.1: Phytoliths in stem (A, B, E, G, H), leaf (C, D, F), root (I, L), cone (J, K) and SEM images of leaf surface (M) & single stoma of stem (N) of *Equisetum diffusum*. (In A & C bar = 20 µm, in B & D-L bar = 10 µm, in M bar = 200 µm, in N bar = 20 µm).

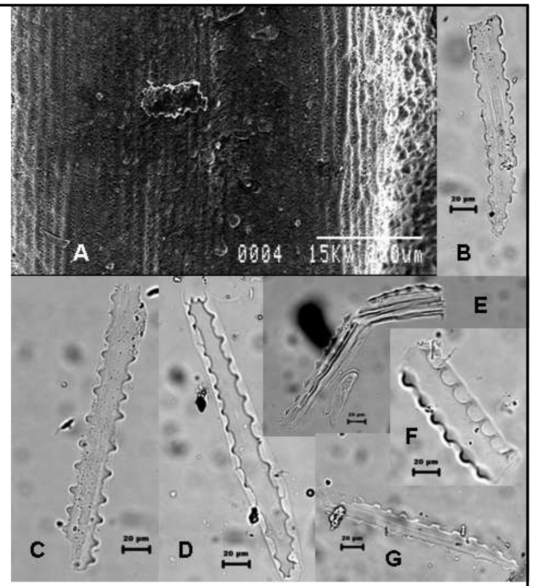

Fig.2: SEM image of stem surface of *Equisetum diffusum* (A). Phytoliths of *Vittaria carcina* (B), *V. elongata* (C), *V. sikkimensis* (D), *V. amboinensis* (E), *V. zosterifolia* (F) & *V. himalayensis* (G). (In A bar = 200 µm, in B-G bar = 20 µm).

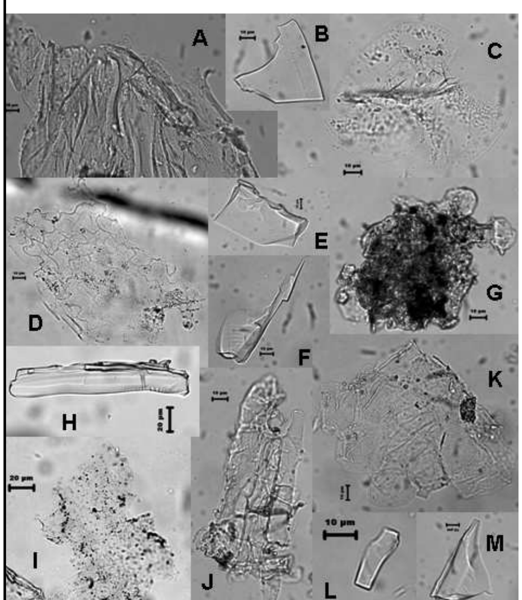

Fig.3: Phytoliths of spike (C) & leaf (D) of *Ophioglossum reticulatum*, spike (K, L) & leaf (F, I) of *Botrychium virginianum*, spike (B, E, G) & leaf (A, H) of *Helminthostachys zeylanica* and pinnule of *Angiopteris evecta* (J, M). (Bar = 10 µm, in H & I bar = 20 µm).

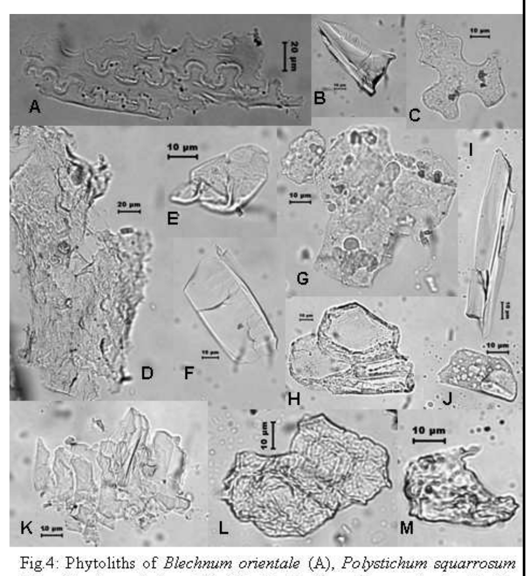

Fig.4: Phytoliths of *Blechnum orientale* (A), *Polystichum squarrosum* (B), *Athyrium nigripes* (C, D), *Cheilanthes albomarginata* (E, G), *Oleandra wallichi* (F), *Gleichenia gigantea* (H), *Cyathea spinulosa* (I), *Arthromeris wallichiana* (J), *Lindsaea odorata* (K), *Hypolepis punctata* (L) & *Diplazium esculentum* (M). (Bar = 10 µm, in A & D bar = 20 µm).

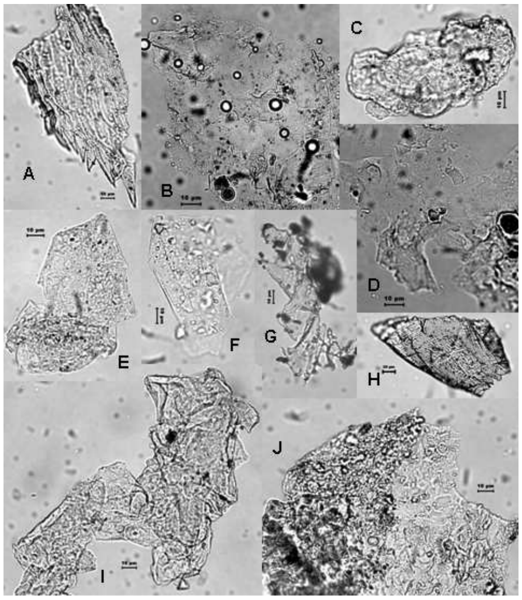

Fig.5: Phytoliths of *Sphenomeris chinensis* (A), *Polystichum squarrosum* (B), *Leucostegia immersa* (C), *Gleichenia gigantea* (D), *Antrophyum reticulatum* (E), *Phymatopteris griffithiana* (F), *Oleandra wallichi* (G), *Peranema cyatheoides* (H), *Cyathea spinulosa* (I) & *Dicranopteris linearis* (J). (Bar = 10 µm, in H bar = 20 µm).

Some plate or sheet like phytoliths were found and they are with variable characters in each species. In *Ophioglossum reticulatum* plates are hyaline, slightly granular (Fig.3C) or hyaline, with sinuous margins resembling epidermal cells (Fig.3D). In *Botrychium virginianum* plates are hyaline with fine granules and folded surface (Fig.3K) or hyaline with rough surface (Fig.3I). In *Helminthostachys zeylanica* plates are hyaline with highly folded surface (Fig.3A). In *Angiopteris evecta* plates are hyaline with sinuous margins resembling epidermal cells (Fig.3J).

In *Gleichenia gigantea* plates are hyaline with rough & folded surface (Fig.5D) or smooth resembling epidermal cells (Fig.4H). In *Dicranopteris linearis* plates are hyaline with dense granules & ridges (Fig.5J). In *Arthromeris wallichiana* plates are opaque with pitted and rough surface (Fig.4J). In *Phymatopteris griffithiana* plates are hyaline with rough surface and entire margin (Fig.5F). In *Cheilanthes albomarginata* plates are hyaline with rough surface and blunt elevated projections (Fig.4G). In *Antrophyum reticulatum* plates are hyaline with pitted surface (Fig.5E). In *Peranema cyatheoides* plates are opaque with granular and folded surface (Fig.5H). In *Cyathea spinulosa* plates are hyaline with rough and highly folded surface (Fig.5I). In *Hypolepis punctata* plates are hyaline with rough surface and ridges (Fig.4L). In *Lindsaea odorata* plates are hyaline with smooth surface and furrows (Fig.4K). In *Sphenomeris chinensis* plates are hyaline with granules and furrows (Fig.5A). In *Athyrium nigripes* plates are hyaline with rough surface and sinuous margins resembling epidermal cell (Fig.4C) or irregular margins & folded surface (Fig.4D). In *Diplazium esculentum* plates are hyaline with less granular surface (Fig.4M). In *Polystichum squarrosum* plates are hyaline with less granular and highly folded surface (Fig.4B). In *Oleandra wallichi* plates are hyaline with smooth surface and sinuous margins resembling epidermal cell (Fig.5G). In *Leucostegia immersa* plates are opaque with granular surface (Fig.5C). In *Blechnum orientale* plates are hyaline with smooth surface and sinuous margins resembling epidermal cells (Fig.4A).

Elongated plate like phytoliths with dentate margins resembling 'spicular cells' (Bhandari and Mukhopadhyay, 2009) were found in all six studied species of *Vittaria* viz, *V. carcina* (Fig.2B), *V. elongata* (Fig.2C), *V. sikkimensis* (Fig.2D), *V. amboinensis* (Fig.2E), *V. zosterifolia* (Fig.2F) and *V. himalyensis* (Fig.2G) and possibly it might be a distinguishing character of genus *Vittaria*. However, further investigation is required with other species to confirm it.

Studies of phytoliths extracted from pteridophytes during present study suggest that phytoliths morphology could be useful for taxonomy of Horsetails & ferns and they could be used for distinguishing different species as some phytoliths show different characters.

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REFERENCES

Bhandari, J.B. and Mukhopadhyay, R. (2009). The Vittarioid Ferns of India, Bishen Singh Mahendra Pal Singh, Dehra Dun.

Bozarth, S. (1993). Biosilicate assemblages of boreal forests and aspen parklands. In *Phytolith database of University of Missouri Paleoethnobotany Laboratory* (http://web.missouri.edu/~umcasphyto/prodrefs.htm).

Chauhan, D.K., Tripathi, D.K., Sinha, P. and Tiwari, S.P. (2009). Biogenic silica in some Pteridophytes. *Bionature* **29(1):** 1-9.

Chen, C. and Lewin, J. (1969). Silicon as a nutrient element for *Equisetum arvense*. *Can. Jour. Bot.* **47:** 125-131.

Ehrenberg, C.G. (1841). Über Verbreitung und Einfluss des Mikroskopischen Lebens in Süd- und Nordamerika. *Monatsberichte der Königlich Preussischen Akademie der Wissenschaften zu Berlin.* pp. 139-144.

Ehrenberg, C.G. (1854). *Mikrogeologie*. Leopold Voss, Leipzing.

Gifford, E.M. and Foster, A.S. (1989). Morphology and evolution of vascular plants, 3 rd ed., W.H. Freeman and Company, New York.

Harvey, E.L. and Fuller, D.Q. (2005). Investigating crop processing using phytolith analysis: the example of rice and millets. *Journal of Archaeological Science* **32:** 739-752.

Hauke R. L. (1978). Microreplicas as a Technique for Rapid Evaluation of Surface Silica Micromorphology in *Equisetum*. *American Fern Journal* **68(2):**37-40

Hauke R. L. (1979). *Equisetum ramosissimum* in North America. *American Fern Journal* **69(1):** 1-5.

Hoffman, F.M. and Hillson, C.J. (1979). Effects of silicon on the life cycle of *Equisetum hyemale* L. *Bot. Gaz.* **140:** 127-132.

Kaufman, P.B., Bigelow, W.C., Schmid, R. and Ghosheh, N.S. (1971). Electron microprobe analysis of slilica in epidermal cells of *Equisetum*. *Amer. J. Bot.* **58:** 309-316.

Kelly, E.F., Amundson, R.G., Marino, B.D., Deniro, M.J., (1991). Stable isotope ratios of carbon in phytoliths as a quantitative method of monitoring vegetation and climate change. *Quaternary Research* **35:** 222–233.

Mazumdar, J. & Mukhopadhyay, R. (2009a). Opal Phytoliths in Three Indian Thelypteroid Ferns, *Bionature* **29(1):** 11-15.

Mazumdar, J. & Mukhopadhyay, R. (2009b). Phytoliths in Lycopods. *Indian Fern J.* **26** (In Press).

Page C. N. (1972), An Assessment of Inter-Specific Relationships in *Equisetum* Subgenus *Equisetum*, *New Phytologist* **71(2):** 355-369.

Pearsall, D.M. (2000). Paleoethnobotany: A Handbook of Procedures, 2 ed., Academic Press, San Diego.

Pearsall, D.M., Piperno, D.R., Dinan, E.H., Umlauf, M., Zhao, Z., Benfer, Jr. R.A. (1995). Distinguishing rice (*Oryza sativa* Poaceae) from wild *Oryza* species through phytolith analysis: results of preliminary research. *Economic Botany* **49(2):** 183-196.

Perry, C. C. and Fraser, M. A. (1991), Silica deposition and ultrastructure in the cell wall of *Equisetum arvense*: the importance of cell wall structures and flow control in biosilicification? *Philosophical Transactions: Biological Sciences*, **334(1269):** 149-157.

Piperno, D.R. (1988). Phytolith Analysis: An Archaeological and Geological Perspective, Academic Press, San Diego.

Piperno, D.R. (2006). Phytoliths: A Comprehensive Guide for Archaeologists and Paleoecologists, AltaMira Press, New York.

Pryer, K. M., Schneider, H., Smith, A. R., Cranfill, R., Wolf, P. G., Hunt, J. S. & Sipes, S. D. (2001). Horsetails and ferns are a monophyletic group and the closest living relatives to seed plants. *Nature* **409:** 618-622.

Sapei L., Gierlinger, N., Hartmann, J., Nöske, R., Strauch, P. & Paris, O. (2007), Structural and analytical studies of silica accumulations in *Equisetum hyemale*, *Anal. Bioanal. Chem.* **389:** 1249-1257.

Srinivasan, J., Dayanandan, P. and Kaufman, P.B. (1979). Silica Distribution in *Equisetum hyemale* Var. *Affine* L. (Engelm) in Relation to the Negative Geotropic Response, *New Phytologist*, **83(3):** 623-626.

Struve, G.A. (1835), *De silica in Plantis Nonnullis*. Dissertation, Berlin.

Wilding, L.P. (1967). Radiocarbon dating of biogenetic opal. *Science* **156:** 66-67.

Zhao, Z., Pearsall, D.M., Benfer Jr, R.A. and Piperno, D.R. (1998). Distinguishing rice (*Oryza sativa* Poaceae) from wild Oryza species through phytolith analysis II: Finalised method, *Economic Botany* **52(2):** 134-145.