A liliaceous inflorescence

Recently, a liliaceous inflorescence[1] was described from the Deccan Intertrappean beds of Mandla District, Madhya Pradesh. After going through this publication carefully we observed that there are several lapses on the part of the authors in interpreting the fossil as a liliaceous inflorescence. We failed to understand how the authors have described the fossil specimens as an inflorescence since the photographs (Figures 1 *a*, *b*) in ref 1 do not show any flower-like structure nor have they given its description. The spirally arranged small structures measuring 0.8×0.8 cm in size are in fact small cavities with or sometimes without seeds which may be called as infructescence. Similar specimens showing better preservation are already known as infructescence, *Callistemonites indicus*[2], from near the same fossil locality of Mandla District. Recently[3], this infructescence was reinvestigated and found very similar to *Musa cardiosperma* Jain[4], a fruit described earlier from Mohgaonkalan cherts.

The authors have further claimed that the pollen recovered from the chert pieces belong to the so-called inflorescence. When nothing is known about the floral parts, especially the anthers, how one can consider them *in situ* pollen. However, they could be the dispersed pollen of different taxa as evidenced from their exine structures which vary from monosulcate to trichotomosulcate and inaperturate. Therefore, they cannot be referred to a single taxon as suggested by the authors.

Among known pollen taxa, the authors have assigned them to *Matanomadhiasulcites*[5] despite being different in many features. The reticulation of the pollen was described as coarse at the periphery and faint towards the centre, whereas in *Matanomadhiasulcites*[6] it is more or less constant throughout the sexine. Therefore, they cannot be referred to this taxon. Further, the authors themselves are not certain to which liliaceous genera the pollen belong.

In view of the above, the described megafossil should not be considered as an inflorescence unless and until more structural details are known. The pollen too need critical re-examination from their type slides in order to ascertain their affinities.

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S. D. Bonde and K. P. N. Kumaran reply:

1. The authors were fully aware of the publication of *Callistemonites indicus*[1] and its reinvestigation (personal discussion with Dr Awasthi). However, we have not taken cognizance of the latter as the results are yet to be published. A mere occurrence near the same fossil locality and a superficial resemblance with an infructescence having 'cavities' do not justify to assign our specimens either to *Callistemonites* or to *Musa*. Had the authors (N.A. & R C.M.) studied the material and pollen grains their comments would have been addressed in a better perspective. Besides, they have not processed the cherts and recovered any dispersed pollen grains from the rock matrices to support their comments. If the authors feel that the described megafossil is an 'infructescence', there is no rationale in their comment on the taxonomy and affinity of the pollen grains and as such it is unwarranted.
2. The described specimens are parts of spikelets of an inflorescence in which the flowers were compressed and preserved in such a way that the structural details are not easily distinguished.
3. In order to ascertain the organic connection the chert pieces of the inflorescence were carefully macerated separately in triplicate along with a control of chert sample and it has been proved that the pollen grains are derived from the samples having the inflorescence parts. Had it been otherwise the control sample should have also yielded similar results. The rock matrices after the extraction of pollen grains have been cleaned and preserved for further reference. The pollen grains are of the same type and occur in abundance as they belong to the inflorescence and such is not the case in dispersed condition. Apart from some fungal spores, no other dispersed species of pollen grains were recovered although minor variation in size and exine ornamentation has been observed. We wonder how the authors have come to the conclusion that the pollen grains illustrated in our paper belong to different taxa. Minor variations are common even in the extant taxa. Therefore, we have referred the pollen grains to a known taxon.
4. We have sought comments on the pollen grains from experts and in fact a liliaceous affinity has been

suggested. Affinity to a dispersed pollen taxon *Matanomadhiasulcites* is based on gross morphological characters as one of us (KPN) had observed similar pollen grains in the West African sediments. Further investigation of materials and critical comparison with extant forms could suggest its affinity with a liliaceous taxon.

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Characterization of structural proteins of A/Equine-2/Ludhiana/87

A/Equine/Ludhiana/87 (H3N8) equine influenza virus isolated from horses during the 1987 epizootic in India[1], resembled recent origin A/Equine-2 viruses by monoclonal antibody typing and sequencing of the HA-1 gene[2]. Since characterization of the structural polypeptides of this virus is not reported earlier, efforts were made to study these by polyacrylamide gel electrophoresis (PAGE).

The virus under study was grown in allantoic cavity of 9–10-day-old chick embryonated eggs. It was concentrated and purified by gradient centrifugation[3]. A/Equine-2/Kentucky/81 (H3N8) virus used as standard strain for comparison was similarly grown, concentrated and purified.

For polyacrylamide gel electrophoresis (PAGE), the technique of Oxford *et al*[4] was used with slight modification. It comprised a separating gel of 20% polyacrylamide, above which was a 5% polyacrylamide stacking gel. The upper and lower surfaces of the gels were in contact with a buffer containing glycine, Tris HCl and sodium dodecyl sulphate (SDS) which gave low conductivity and low ionic strength. Virus samples were boiled with SDS and mercaptoethanol to split the virus into constituent polypeptides and to provide a net negative charge The treated virus samples were then applied to the wells in the stacking gel and an electric current of 24 milliamps was applied across the gel Virus polypeptides migrated rapidly through the stacking gel and were then separated in the 20% gel according to their molecular weights. After 18 h of run, the separating gel was removed and the polypeptides were stained with Coomassie Blue dye (0 1%). The relative amounts of the virus polypeptides were analysed by scanning the gels with a densitometer (Biorad 620 Video Densitometer)

Polypeptide bands as revealed by densitometric analysis of PAGE of Ludhiana/87 and Kentucky/81 viruses are shown in Figure 1. The structural polypeptides seen were nucleoprotein (NP), matrix protein (M) and haemagglutinin (HA) which was dissociated into HA1 and HA2 under the reducing conditions of the gel system. Ludhiana virus contained 17% NP, 52% M and 31% HA (HA1 plus HA2) which were almost similar to 17% NP, 47% M and 34% HA of Kentucky virus. Also present was a high molecular weight aggregate which was composed of NP, M and HA (detected by immunoblotting in separate experiment). Both Ludhiana and Kentucky virus polypeptides were found to have similar molecular weights. The PAGE results are in agreement with antigenic[5] and genetic analysis[2] performed elsewhere which indicate the similarity of the Ludhiana/87 virus with other equine influenza viruses circulating in America and Europe during the 1980s. The results will form the basis of attempts to produce and standardize an equine influenza vaccine in India.

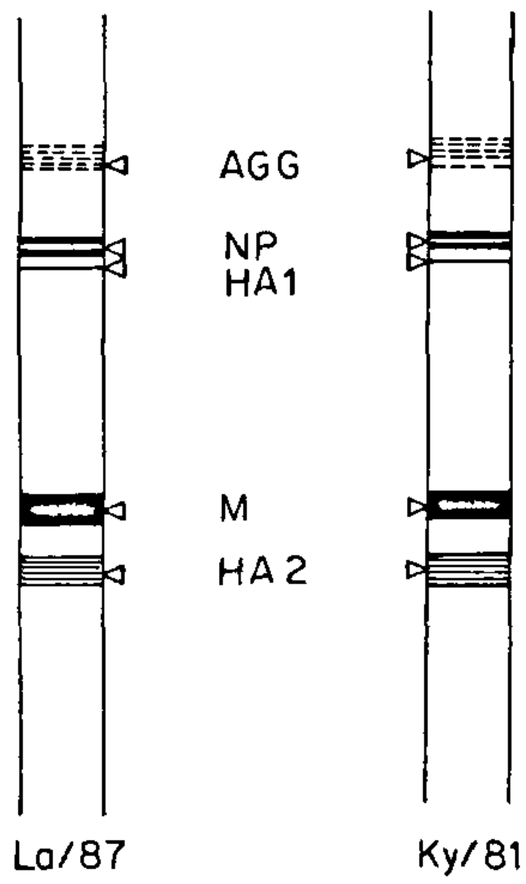

Figure 1. PAGE analysis of Ludhiana/87 (La/87) and Kentucky (KY/181) equine influenza virus A/Equine-2 (H3N8) isolates showing nucleo-protein (NP), matrix (M), haemagglutinin (HA1, HA2) polypeptide bands A high molecular weight aggregate is shown on the top (AGG)

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