Mycorrhizal fossil fungi from the Miocene sediments of Mizoram, Northeast India

The fossil fungal hyphae, auxiliary cells, chlamydospores and a sporocarp belonging to Glomales – an order specialized in endomycorrhizal association on the various types of plants are reported from the Miocene sediments of Mizoram.

Mycorrhiza is a symbiotic association between certain fungal hyphae and the roots of plants. In this cohabitation, the fungi is provided with the carbohydrates and vitamins by the plants and in return the fungi enhance the active area of the root system and supply water and nutrients; they also increase the tolerance capacity for drought and protect the plant from infection from other fungi and nematodes[1–4]. The association is divided into ecto-, endo-, ectoendo-, ericoid- and monotropoid types[5]. Among these, ecto- and endomycorrhizal types are most common.

In ectomycorrhizal type, the fungal hyphae grow in intercellular fashion and the infected root tip is covered by a sheath of hyphae and Hartig net. This type of infection is generally caused by the discomycetes and basidiomycetes. About 10% of the world flora has this type of fungal association and is most common in Pinaceae, Betulaceae, Fagaceae, etc.[4].

The endomycorrhizae are devoid of external outgrowths and grow in intracellular position. The hyphae generally form vesicles and arbuscules – the minute branches within the cells of the host. For this reason they are also called vesicular–arbuscular mycorrhizae (VAM)[5]. This type of mycorrhizae occurs in 70% of all the plant families, including some algae[6]. Such fungal hyphae, vesicles and spores were recovered from the famous Devonian Rhynie chert, which led to speculation that the pioneer land plants could invade the barren land due to symbiotic association[7]. This hypothesis was subsequently opposed by others[8,9]. The DNA sequence information on this type of fungi, however, estimated an age of 353–462 million years (m.y.) and that of *Glomus*-like fungus, 415 m.y., which strikingly coincides with the age of the Rhynie chert[9,10].

The fungal hyphae, auxiliary cells and chlamydospores of the supposed endomycorrhizae were recovered from the Bhuban Formation of Miocene age from a section near Tlangsam village (lat. 23°28′ and long. 93°25′) just at the India–Myanmar border, about 50 km east of Champhai (Figure 1). The Bhuban Formation in Mizoram is more than 7000 m thick, and is predominantly argillaceous at the base and arenaceous at the top. The geology of the area has been worked out by a number of workers, mostly belonging to government and semi-government organizations[11–13]. The sampled horizons, lithological details and position of productive samples have been shown in Figure 1.

The samples were macerated with commercial nitric acid (40%) followed by a wash with potassium hydroxide solution (5%), and subsequently slides were prepared for study. The palynology of the Miocene sediments in Mizoram has been investigated[14–16] and fungal and pteridophytic spores, angiospermic and gymnospermic pollen have been reported. However, symbiotic fungi from these sediments have not been reported so far.

The samples are rich in fungal elements, pteridophytic spores and pollen of angiosperms and gymnosperms. The presence of *Striatriletes susannae*, *Pinuspollenites crestus*, *Abiespollenites cognatus*, *Hibisceaepollenites splendus* and *Palaeomalvaceaepollis mammilatus* in the samples indicates Miocene age. The fungal fossils are

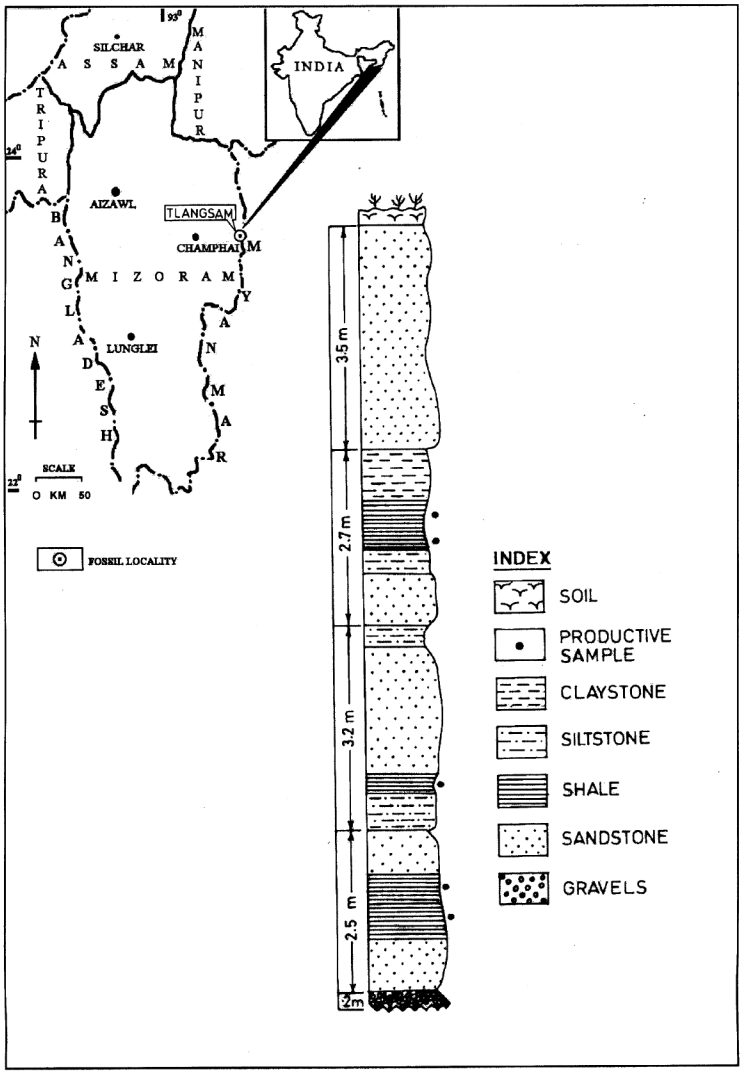

Figure 1. Locality and litholog from where samples were collected.

about 20% of the total elements and consist of hyphae, spores and other forms, e.g. *Colletotrichum, Cucurbitariaceites*, etc.

The endomycorrhizal fungi are quite common in the samples and are represented by hyphae, auxiliary cells, sporocarps, azygospores and chlamydospores belonging to the order Glomales. This order is divided into three families, viz. Gigasporaceae, Acaulosporaceae and Glomaceae. It is difficult to distinguish the different families in the dispersed condition because the hyphae, arbuscules and vesicles produced by the members of the different families are more or less similar, except, perhaps the wall structures[5,17]. However, Gigasporaceae, having the genera *Gigaspora* and *Scutellospora*, produces auxiliary cells, while the other families do not. These are specialized cells produced by the members of the family in the soil along with balloon-shaped structures called azygospores. These genera also produce arbuscules in the roots of their hosts.

Three types of auxiliary cells are observed in the samples. In the most common form, the hyphae branch laterally to produce a solitary cell at the end (Figure 2 *e*, *m–o*, *q*). These are oval in shape and the wall is roughly 1 µm thick. In the second type, the hyphae divide dichotomously and produce two auxiliary cells of more or less the same size. The shape of this type is subcircular – broadly oval, 10–22 × 8–12 µm in size, the wall is psilate and about 2 µm thick (Figure 2 *f*, *g*, *l*). In the third type, the hyphae branch out many times more or less in the same plane and bear many cells mostly in whorls. The cells are elongated, 18–37 × 12–20 µm, the side of attachment is slightly narrower than the other end, the wall is laevigate and 1–2 µm thick (Figure 2 *j*, *k*, *p*).

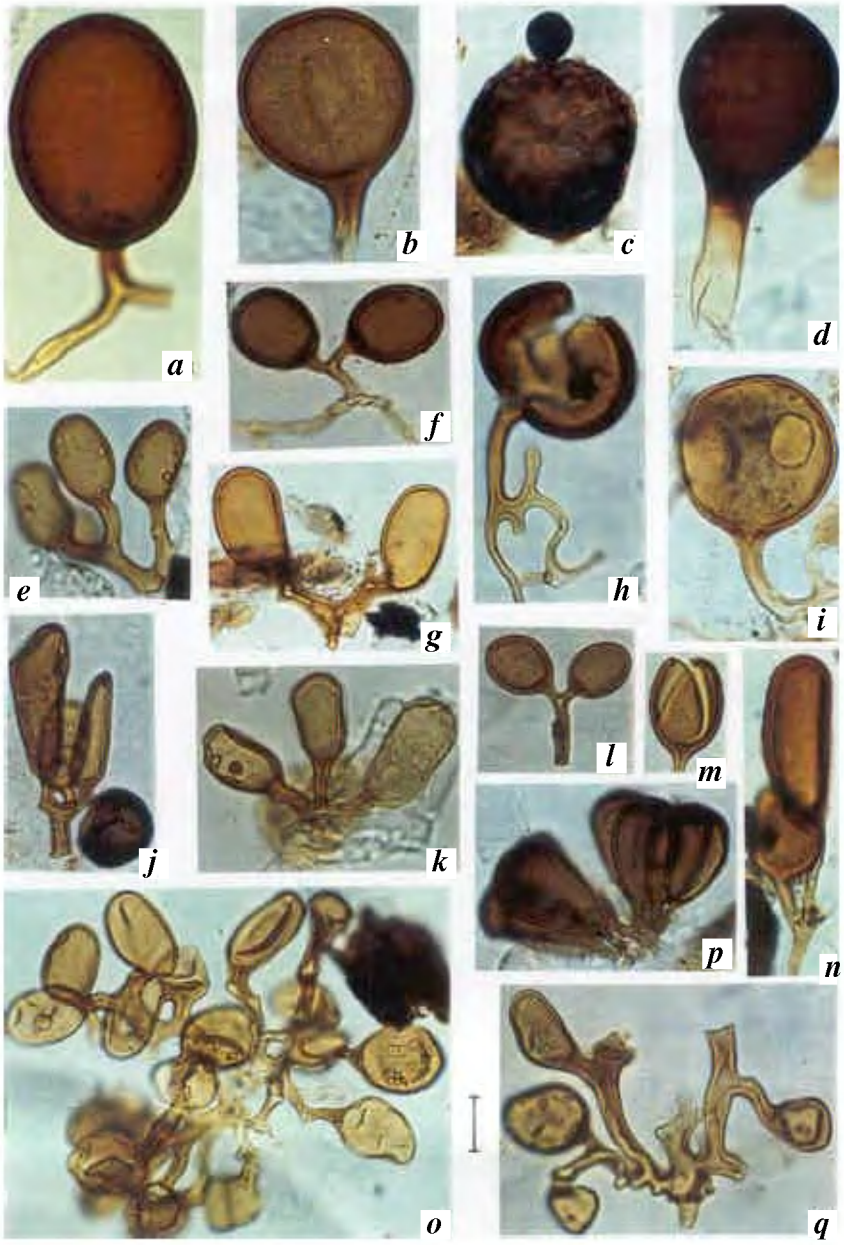

Figure 2. ***a***, ***b***, ***d***, ***h***, ***i***, Chlamydospores of family Glomaceae; ***c***, Sporocarp with a naked chlamydospore of Glomaceae; ***e***, ***m***, ***n***, ***o***, ***q***, Auxiliary cells produced by laterally branched hyphae; ***f***, ***g***, ***l***, Auxiliary cells produced by dichotomously branched hyphae; ***j***, ***k***, ***p***, Auxiliary cells in whorls. Bar – 10 µm.

Besides the auxiliary cells, many chlamydospores are also observed. These are found at the terminal end of the hyphae-like balloons (Figure 2 *a*, *b*, *d*, *h*, *i*). These are circular–subcircular in shape and often dark brown in colour, 25–52 × 20–49 µm in size, the wall is without any ornamentation and about 2 µm thick. The families Acaulosporaceae and Glomaceae produce this type of chlamydospores. However, in *Acaulospora* and *Entrophospora* of Acaulosporaceae, the chlamydospores are borne laterally and within the neck of the hyphae respectively, and are thus separated from the present specimens[5]. *Glomus* and *Sclerocystis* of the family Glomaceae, bear chlamydospores at the apex on the fertile hyphae and are produced singly in the soil. The chlamydospores detailed here, most probably, belong to these genera (Figure 2 *a*, *b*, *d*, *h*, *i*).

Some members of *Glomus* and *Sclerocystis* also produce sporocarps. These are zygospores produced by conjugation and generally covered by sterile hyphae. Some of the azygospores or chlamydospores may also look like zygospores, but these are not formed by sexual conjugation. One sporocarp containing a naked chlamydospore on the external hyphae was recovered (Figure 2 *c*). This closely resembles the sporocarp of *Glomus mosseae* illustrated by Webster[18]. The sporocarp is subcircular in shape, 36 × 32 µm in size, dark brown and 1 µm thick, a few hyphae are found entangled on the surface.

The endomycorrhizal fungi mentioned here were previously placed in the order Endogonales; but now the genera *Endogone* and *Sclerogone* which are saprobic, are only included in it and the rest are accommodated in the order Glomales[5]. Most of the genera of this order are found

in the roots of plants of economic importance as symbionts, arousing the interest of many a worker and subsequent discovery of new species[19–21]. In the dispersed state, as has already been stated, it is difficult to delimit them into generic level because the auxiliary cells, vesicles and chlamydospores are monotonously uniform. The Glomales is associated with plants since its transmigration on land more than four hundred million years ago; still the morphology of the vesicles has not changed indicating its utmost stability against mutation and other evolutionary processes. Perhaps the urge and initiative for further development were lost due to the availability of readymade food from the hosts and external protection.

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Occurrence and intensity of some parasites in five sturgeon species (Chondrostei: Acipenseridae) southwest of Caspian Sea

Sturgeons (Chondrostei: Acipenseridae) are evolutionary relicts with a wide distribution in the northern hemisphere. Their status as basal actinopterygian fishes, their unique benthic specializations, and variation in their basic diadromous life history make sturgeons interesting biological and biogeographical subjects. Extensive studies on *Eurasian* sturgeons indicate that they are also unique among fishes, in possessing a markedly diverse assemblage of host-specific parasites.

The parasites of sturgeons have been studied by several authors[1–5]. However, there are only a few reports about such parasites in the southern part of the Caspian Sea. Mokhayer[6] studied parasites of three sturgeon species, including *Acipenser stellatus* ($n = 72$), *A. gueldenstaedti* ($n = 95$) and *Huso huso* ($n = 4$) and reported 17 parasite species from all of them. Gorogi[7] studied parasites of *A. persicus* ($n = 604$) and reported three parasite species. In another study, Gorogi[8] reported six parasite species from *H. huso*. Hence an attempt was made to determine the parasite fauna of all sturgeon species southwest of the Caspian Sea and also the status of the parasite communities.

A total of 542 samples of five sturgeon species (including *A. stellatus*, *A. persicus*, *A. gueldenstaedti*, *A. nudiventris* and *H. huso*) were collected from April 1999 to February 2001. The samples included sturgeons which were caught in fisheries sections 1 (location 1) and 2 (location 2) along with a shore area of more than 200 km and also the broodstocks of a hatchery adjacent to the Sefid-Rud River (location 3; Figure 1).

As the sampling in this study was restricted by the governmental fishing programme (including the induced spawning of broodstocks and then exporting their flesh), age determination of the sturgeons (by removing pectoral fin ray) was not possible. After recording biometric characteristics (Table 1), standard necropsy and parasitology methods[9] were used. After removal, all viscera were examined for parasites; sections of the spleen and liver were squashed and major ducts in the liver were dissected and examined. Mucus from the first part of the intestine was removed and examined between glass plates for protozoans. Live trematodes and acanthocephalans were relaxed in distilled water at 4°C for 1 h and fixed in 10% hot buffered formalin. Live nematodes were fixed in hot 70% ethanol and cleared in hot lactophenol. Frozen specimens were thawed in water, fixed with 10% formalin (trematodes and acanthocephalans) or 70% ethanol (nematodes). All specimens fixed in 10% formalin were stained with aqueous acetocarmine, dehydrated and