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# NEW CHARACTERISTICS FOR MORPHOTAXONOMY OF *GIGASPORA* SPECIES BELONGING TO ARBUSCULAR MYCORRHIZAL FUNGI

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**Abstract:** New characteristics for morpho-taxonomy were devised to support the species concept in genus *Gigaspora* belonging to arbuscular mycorrhizal fungi. Three species viz. *G. margarita*, *G. decipiens* and unidentified *Gigaspora* sp. were studied for various characters viz. bulbous suspensor, sporophore, germ tube, presence of septum and presence or absence connecticle. The term "Connecticle" is newly introduced and is a region present between the base of bulbous suspensor and septum overlying the sporophore. The term "Germ pore" is also newly introduced. The present study reported that germ tube in *Gigaspora* is always attached to the spore through a pore which is now named as germ pore. In all, eight types of subtending hypha were recorded during the present study with variations in shape of bulbous suspensor and sporophore along with presence or absence connecticle. Presence of germ pore, septum and germ tube was common feature in *Gigaspora* species undertaken for the study. The location of septum was another new character devised for taxonomy in the present study. Thus the present study upholds the species concept in *Gigaspora* based on morpho-taxonomy.

**Key words:** arbuscular mycorrhizal fungi, bulbous suspensor, connecticle, germ pore, germ tube, *G. margarita*, *G. decipiens*, morpho-taxonomy, septum, sporophore, unidentified *Gigaspora* sp.

## Introduction

Traditionally, Glomeromycotan taxonomy of arbuscular mycorrhizal (AM) fungal group has been based on the morphology of the spores. The way the spore is formed on the hypha ("mode of spore formation") has been important to circumscribe genera and families, and the layered structure of the spore wall is used to distinguish species [WALKER, 1983; MORTON, 1988]. Glomeromycotan taxonomy is relatively young. Among the glomeromycotan fungi, the *Gigasporaceae* (*Scutellospora* and *Gigaspora*) members are distinguished by the formation of their spores on a "bulbous suspensor" and are well supported by molecular data. Recently, OEHL & al. (2008) revised family *Gigasporaceae* on the basis of morphological spore characters and 18S and 25S rRNA gene sequences. In the family *Gigasporaceae*, 36 *Scutellospora* species were reorganized in three new families including five new genera: *Scutellosporaceae* (*Scutellospora*), *Racocetraceae* (*Racocetra*, *Cetraspora*) and *Dentiscutataceae* (*Dentiscutata*, *Fuscutata*, *Quatunica*). The family *Gigasporaceae* now remains with a single genus *Gigaspora.*

The group *Gigaspora* is the smallest group since member species were transferred into erected *Scutellospora* Walkers & Sanders primarily on the basis of presence of sub-cellular structures associated with germination [WALKERS & SANDERS, 1986]. With this, species level differences in *Gigaspora* rested upon seemingly small morphological differences in spore colour, size and wall thickness [BENTIVENGA & MORTON, 1995].

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*Gigaspora* posses a problem since spore of all species differentiate only a spore wall and diverge in characteristics of that spore wall [BENTIVENGA & MORTON, 1995]. Research workers questioned the fact that, do small morphological differences within the genus constitute adequate criteria for delimitation of species? Advanced studies in taxonomy viz. ontogeny define relationships between the characters while a phylogenetic study provides information on relationship between *Gigaspora* and other fungi in *Glomeromycota* [BENTIVENGA & MORTON, 1995]. Thus the validity and importance of morphological characters in establishing taxonomic species are of considerable importance to construct workable system of identification. In view of the above, the present paper throws light on new characteristics to be incorporated to boost the traditional morpho-taxonomy in *Gigaspora* species.

## Material and methods

*Extraction of AM fungal spores*. Spores of AM fungi associated with *Carica papaya* L. plants from Goa, India were isolated directly from rhizosphere soil samples by wet sieving and decanting method [GERDEMANN & NICOLSON, 1963]. All the available healthy turgid spores were isolated from the rhizosphere soil. Repeated sampling were carried out in monsoons (rainy season) when the spores sporulated newly in the soil. The host plant was same for all the samplings. This plant was grown in monoculture and mulching practices carried out in the field and because of this there were no weeds. Therefore the samplings were carried out under same environmental conditions and from the rhizosphere soil of the replicates of single host plant viz. papaya.

*Identification of AM fungi*. Diagnostic slides containing intact and crushed spores of AM fungi were prepared in polyvinyl alcohol lactoglycerol [KOSKE & TESSIER, 1983]. Spore morphology and wall characteristics were considered for the identification of AM fungi and these characteristics were ascertained using compound microscope, Leica WILD MP 3 and Nikon E 800. Arbuscular mycorrhizal fungi were identified to species level using bibliographies provided by SCHENCK & PEREZ (1990), SCHÜßLER & al. (2001) and OEHL & al. (2008).

## Results

The genus *Gigaspora* consists of azygospore with subtending hyphae. This hypha is attached to spore through a pore (Fig. 1). The subtending hypha consists of terminal swollen sporogenous cell called bulbous suspensor and sporogenous hypha called sporophore (Fig. 1). A septum is present at the base of the swollen portion separating the bulbous suspensor from the sporophore (Fig. 1). In some cases, the subtending hypha at the base of the bulbous suspensor metamorphoses to produce various shaped structure. This part of the subtending hypha connecting the bulbous suspensor to the sporophore which is overlined by the presence septum is called a connecticle (Fig. 1). In the present study, the type of bulbous suspensor, sporophore and connecticle varied in *Gigaspora* species.

In all, eight types of subtending hypha were recorded during the present study (Table 1); (Fig. 2). 1) thin walled clavate bulbous suspensor with funnel shaped sporophore (Fig. 2a), 2) thin walled clavate bulbous suspensor with straight sporophore (Fig. 2b), 3) thick walled clavate bulbous suspensor with recurved sporophore (Fig. 2c), 4) thin walled clavate bulbous suspensor with recurved sporophore (Fig. 2d), 5) subglobose bulbous

suspensor with funnel shaped sporophore (Fig. 2e), 6) globose bulbous suspensor with funnel shaped connecticle and curved sporophore (Fig. 2f), 7) globose bulbous suspensor with vase shaped connecticle and curved sporophore (Fig. 2g), 8) globose bulbous suspensor with elongated connecticle and curved sporophore (Fig. 2h).

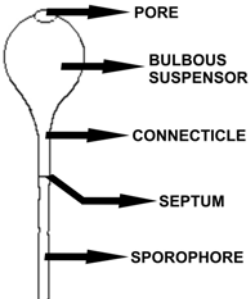


**Fig. 1.** Diagrammatic representation of characteristics of subtending hypha in *Gigaspora* species.

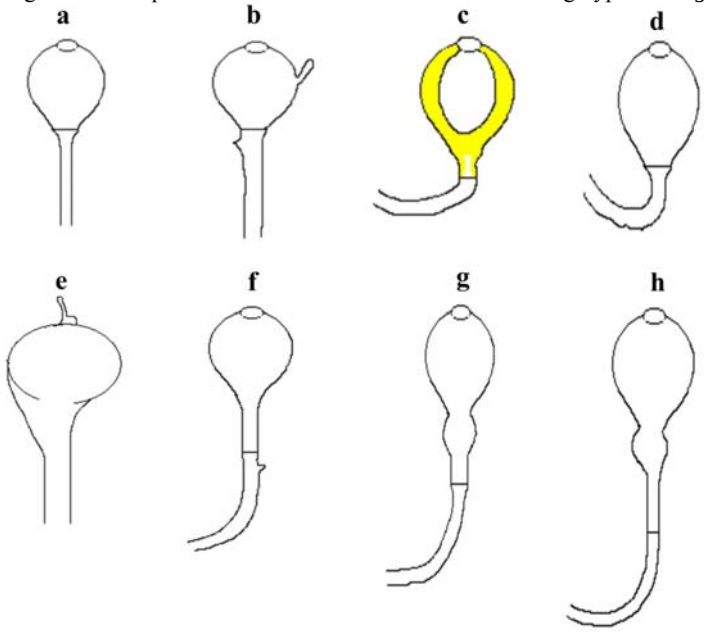


**Fig. 2.** Diagrammatic representation of variations in subtending hyphae of *Gigaspora* species.
a) Thin walled clavate bulbous suspensor with funnel shaped sporophore.
b) Thin walled clavate bulbous suspensor with straight sporophore.
c) Thick walled clavate bulbous suspensor with recurved sporophore.
d) Thin walled clavate bulbous suspensor with recurved sporophore.
e) Sub-globose bulbous suspensor with funnel shaped sporophore.
f) Globose bulbous suspensor with funnel shaped connecticle and curved sporophore.
g) Globose bulbous suspensor with vase shaped connecticle and curved sporophore.
h) Globose bulbous suspensor with elongated connecticle and curved sporophore.

**Tab. 1.** Characteristics of subtending hypha in *Gigaspora* species

| *Type of subtending hypha | Occurrence in | Bulbous suspensor (Bs) | Connecticle | Sporophore (Sp) | Pore | Location of septum |
|---|---|---|---|---|---|---|
| Thin walled clavate Bs with funnel shaped Sp (Fig. 2a, Fig. 3) | *G. margarita* | 40–70 µm wide; 50–320 µm long; 2–10µm thick wall | Absent | 30µm wide at the base of Bs; 15–20µm wide, away from the base of Bs | Present | At the base of Bs |
| Thin walled clavate Bs with straight Sp (Fig. 2b, Fig. 8) | *G. decipiens* | 80–200 µm wide; 170–350 µm long; 2–10µm thick wall | Absent | 30–40µm wide at the base of Bs; 15–20µm wide, away from the base of Bs | Present | At the base of Bs |
| Thick walled clavate Bs with recurved Sp (Fig. 2c, Fig. 9) | *G. decipiens* | 200–350 µm wide; 270–400 µm long; 20–65µm thick wall | Absent | 30–60µm wide at the base of Bs; 20–30µm wide, away from the base of Bs | Present | At the base of Bs |
| Thin walled clavate Bs with recurved Sp (Fig. 2d, Fig. 10) | Unidentified *Gigaspora* species | 150–250 µm wide; 180–300 µm long; 2–10µm thick wall | Absent | 30–50µm wide at the base of Bs; 20–30µm wide, away from the base of Bs | Present | At the base of Bs |
| Subglobose Bs with funnel shaped Sp (Fig. 2e, Fig. 4) | *G. margarita* | 60–150 µm wide; 80–300 µm long; 20–50µm long lateral hypha; 2–10µm thick wall | Absent | 100–50 µm wide at the base of Bs; 20–40 µm wide, away from the base of Bs | Present | At the base of Bs |
| Globose Bs with funnel shaped connecticle and curved Sp (Fig. 2f, Fig. 5) | *G. margarita* | 60–140 µm diam. 2–10µm thick wall | 40–60 µm wide at the base of Bs; 20–30µm near septum 250–320 µm long | 20–30µm wide | Present | At the end of connecticle |
| Globose Bs with vase shaped connecticle and curved Sp (Fig. 2g, Fig. 6) | *G. margarita* | 60–130 µm diam. 2–10µm thick wall | 30–40 µm wide at the base of Bs, inflated at the centre, 40–60 µm wide, narrow near septum, 20–30 µm wide; 200–350 µm long | 20–30µm wide | Present | At the end of connecticle |
| Globose Bs with elongated connecticle and curved Sp (Fig. 2h, Fig.7) | *G. margarita* | 60–250 µm diam. 2–10µm thick wall | Constricted at the base, 30–40 µm wide, swollen below, 50–80µm wide, below the swollen portion straight 40–60 µm wide; 350–500 µm long | 30–40µm wide | Present | At the end of connecticle |

*Bs = Bulbous suspensor; *Sp = Sporophore

Three species of *Gigaspora* with eight types of subtending hypha were recorded during the present study (Tab. 1); (Fig. 3 – Fig. 10). In *G. margarita* Becker & Hall, five types of subtending hypha were observed viz. thin walled clavate bulbous suspensor with funnel shaped sporophore (Fig. 3), subglobose bulbous suspensor with funnel shaped sporophore (Fig. 4), globose bulbous suspensor with funnel shaped connecticle and curved sporophore (Fig. 5), globose bulbous suspensor with vase shaped connecticle and curved sporophore (Fig. 6) and globose bulbous suspensor with elongated connecticle and curved sporophore (Fig. 7). In *G. decipiens* Hall & Abbott, two types of subtending hyphae were observed viz. thin walled clavate bulbous suspensor with straight sporophore (Fig. 8) and thick walled clavate bulbous suspensor with recurved sporophore (Fig. 9). In unidentified *Gigaspora* species, one type of subtending hypha was observed viz. thin walled clavate bulbous suspensor with recurved sporophore (Fig. 10).

**Types of subtending hypha in *Gigaspora margarita***

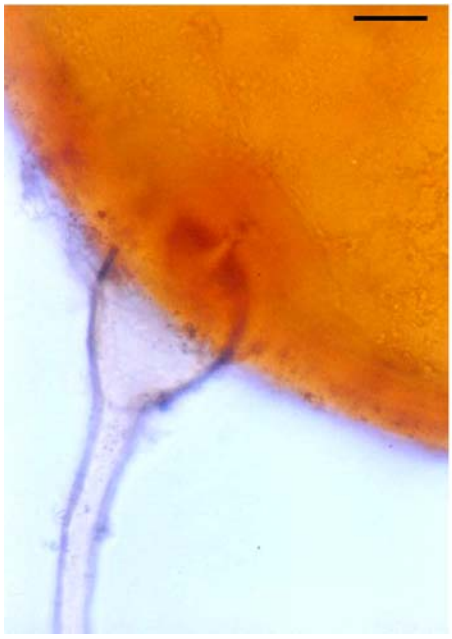

**Fig. 3.** Thin walled clavate bulbous suspensor with funnel shaped sporophore (Bar = 30µm).

**Fig. 4.** Sub-globose bulbous suspensor with funnel shaped sporophore (Bar = 20µm).

**Types of subtending hypha in *Gigaspora margarita***

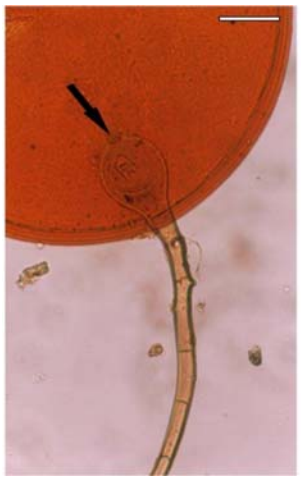

**Fig. 5.** Globose bulbous suspensor with funnel shaped connecticle and curved sporophore (Bar = 100µm).

**Fig. 6.** Globose bulbous suspensor with vase shaped connecticle and curved sporophore (Bar = 100µm).

**Fig. 7.** Globose bulbous suspensor with elongated connecticle and curved sporophore (Bar = 50 µm).

**[* Note the presence of pore (arrow) in Fig. 5-7]**

**Types of subtending hypha in *Gigaspora* species**

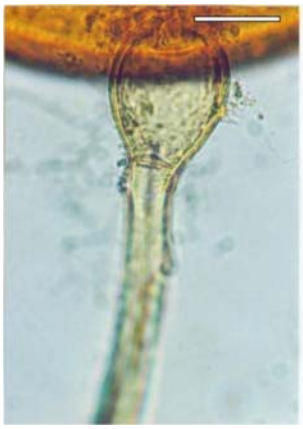

**Fig. 8.** Thin walled clavate bulbous suspensor with straight sporophore in *Gigaspora decipiens* (Bar = 20µm).

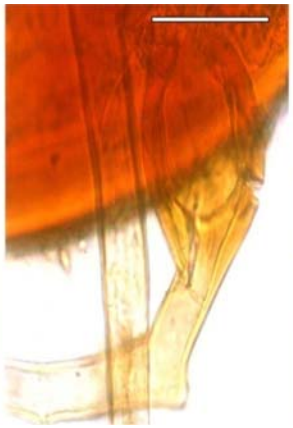

**Fig. 9.** Thick walled clavate bulbous suspensor with recurved sporophore in *Gigaspora decipiens* (Bar = 100µm).

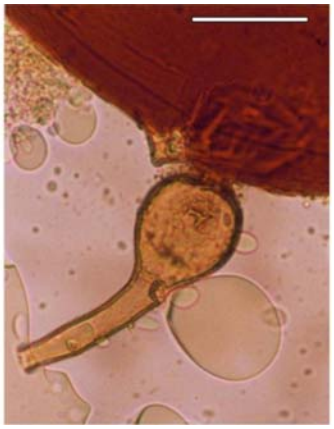

**Fig. 10.** Thin walled clavate bulbous suspensor with recurved sporophore in unidentified *Gigaspora* sp. (Bar = 100µm).

In *Gigaspora* the innermost wall known as the germinal wall in the close proximity of bulbous suspensor, produces germ tube at the time of spore germination. This germ tube is attached to the spore through germ pore (Fig. 11-14). No variation was observed in the type of germ pore. However, in the present study the type of germ tube varied in different *Gigaspora* species. In *G. margarita*, numerous "warts" or "papillae" were present on the inner surface of germinal layer and they were especially concentrated in regions where germ tube originated (in close proximity to the suspensor cell) (Fig. 7, Fig. 11). In this species, the germ tube was curved and germ pore was present at the point of attachment to the spore wall (Fig. 11). In *G. decipiens*, the warts were absent on the germinal wall in the vicinity of bulbous suspensor and here the germ tube was straight and attached to the spore through germ pore (Fig. 12). In unidentified *Gigaspora* sp., the germinal wall produced several centimeters long coiled germ tube with presence of germ pore at the point of attachment to the spore wall (Fig. 13, Fig. 14).

**Germ tubes in *Gigaspora* species**

**Fig. 11.** Curved germ tube in *Gigaspora margarita* (Bar = 25µm)

**Fig. 12.** Straight germ tube in *Gigaspora decipiens* (Bar = 25µm)

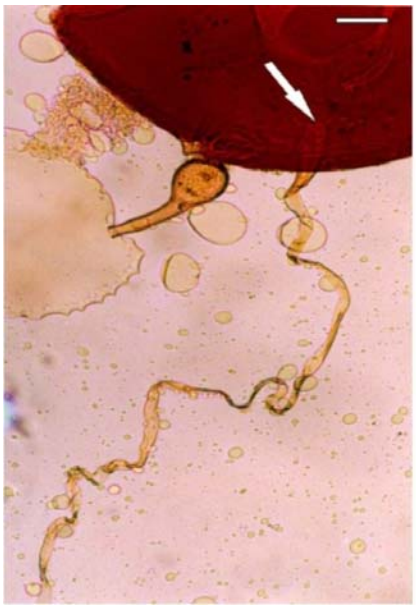

**Fig. 13.** Coiled germ tube in unidentified *Gigaspora* sp. (Bar = 100µm).

**Fig. 14.** Coiled germ tube in unidentified *Gigaspora* sp. (Bar = 50µm)

**[* Note the presence of Germ pore (arrow) in Fig. 11-14]**

## Discussions

Now, the phylum *Glomeromycota* comprises about 200 described morpho-species that traditionally have been distinguished by features of the spore wall. WALKER (1983) established the concept of "murographs" to describe and compare the layered structure of the spore walls more easily. MORTON (1995) and STÜRMER & MORTON (1997) and STÜRMER & MORTON (1999) included considerations of the spore development to group these wall components hierarchically into complexes linked by ontogeny [REDECKER & RAAB, 2006]. Therefore in the present study various characteristics of spore especially the spore attachment of *Gigaspora* viz. bulbous suspensor and sporophore was studied in detail to support the species concept.

Till date, eight species exists under genus *Gigaspora* of the family *Gigasporaceae*. They are as follows: *G. albida* Schenck & Smith, *G. alboaurantiaca* Chou, *G. candida* Bhattacharjee, Mukerji, Tewarii & Skoropad, *G. decipiens* Hall & Abbott, *G. gigantea* (Nicol. & Gerd.) Gerd. & Trappe, *G. margarita* Becker & Hall, *G. ramisporophora* Spain, Sieverding & Schenck, *G. rosea* Nicol. & Schenck [OEHL & al. 2008]**.** The synonymy of *G. ramisporophora* with *G. margarita* [BENTIVENGA & MORTON, 1995] is not accepted based on molecular [DE SOUZA & al. 2004] and morphological [SPAIN & al. 1989] differences. *Gigaspora rosea* and *G. candida* are also treated as separate species. *Gigaspora tuberculata* Neeraj, Mukerji, Sharma & Varma earlier reported under genus *Gigaspora* [SCHENCK & PEREZ, 1990] was later reported as synonym of *Scutellospora persica* (Koske & Walker) Walker & Sanders [BENTIVENGA & MORTON, 1995].

It is reported that spore size and colour are stable distinct characters to support species concept in *Gigaspora* [BENTIVENGA & MORTON, 1995]. In contrast to this,

development of spore with two permanent layers is shared with all *Gigasporaceae* members. Also the variation of spores in *Gigaspora* is more limited than *Scutellospora* that exhibit wide range in colour and are often ornamented. This is due to strong genetic and developmental constraints which appear to limit the expression of variation in *Gigaspora* [BENTIVENGA & MORTON, 1995]. However, in the present study large variation was seen in the subtending hypha of *Gigaspora* species. Five different types of subtending hypha were recorded in *G. margarita*. The species recorded three different types of bulbous suspensor viz. clavate, subglobose and globose. The connecticle were of three types viz. funnel shaped, vase shaped and elongated while the sporophores were also of three types viz. straight, funnel shaped and curved. Lateral hyphal projection was present in subglobose bulbous suspensor. In *G. decipiens*, the bulbous suspensor was clavate and of two types, thin walled and thick walled. Here the sporophores were also of two types, straight and recurved. In unidentified species of *Gigaspora*, the bulbous suspensor was clavate with recurved sporophore.

Another keen observation recorded in the present study was that septum delimiting the bulbous suspensor from the sporophore was present immediately at the base of all clavate bulbous suspensors and even at the base of subglobose bulbous suspensor of *Gigaspora* species. However, at the base of all globose bulbous suspensors, connecticles were present followed by septum delimiting the sporophore from it. These connecticles varied in shape and size and were associated only with globose bulbous suspensors of *G. margarita*.

In *Gigaspora* species, a vital life history function is germination of spore. This germination of spore is always associated with the spore wall [BENTIVENGA & MORTON, 1995]. The present study upholds the view of several workers [MAIA & al. 1993; SWARD, 1978; SWARD, 1981] who reported that germination takes place through the formation of germ tube which always arises from inner papillate layer and pushes through the spore wall. However, the present study brought out the fact that its development is like that of bulbous suspensor which is attached to the spore through a pore and in case of germ tube the pore is designated as germ pore. No feature of this germ pore, described in the present study, distinguishes any of the *Gigaspora* species compared in this study. However, through the present study, it is confirmed the germ tube is always associated with germ pore. Further, in the present study, the type of germ tube varied within the species of *Gigaspora*. It was curved in *G. margarita*, straight in *G. decipiens* and coiled in unidentified *Gigaspora* species. Presence of germ pore was recorded in all the three species.

## Conclusions

Glomeromycotan fungi are of great interest to ecologists because of its potential influence on ecosystem processes, its role in determining plant diversity in natural communities and the ability of the fungi to induce a wide variety of growth responses in coexisting plant species. Difficulties in identification, the inability to grow the fungi in pure culture, problems of taxonomic classification and a lack of basic information on the life histories of AM fungi hinder studies of the ecological significance of diversity of AM fungi. Nucleic acid based techniques have the potential to fill this gap in our knowledge by offering better means of identification and the opportunity to study links between the genetic diversity of AM fungi and functional and morphological diversity. The application of genus specific molecular markers has shown that different genera of AM fungi coexist in plant roots and that this is a common occurrence [SANDERS & al. 1996]. However the speciation concept still rests on the morpho-taxonomy of the spore. In *Gigaspora*, where there are relatively few species with small number of taxonomic characters, the present paper gives additional information on characters of taxonomic relevance.

New characters viz. bulbous suspensor, sporophore, germ tube, presence of septum and presence or absence connecticle are incorporated to differentiate *Gigaspora* members to species level. Even presence or absence of septum at the base of bulbous suspensor or the distance at which it is present from the base of bulbous suspensor is another distinguishing character. Further, the presence or absence of connecticle in *Gigaspora* species is newly introduced to carry out taxonomic studies. Additionally, the term germ pore used to designate the point of attachment of germ tube to the spore is also introduced for taxonomy of *Gigaspora* species. Further, my study contradicts the earlier reports that variations in *Gigaspora* are limited. My study brings out the facts that even the isolates of same species which was earlier distinguished on the basis of spore size and colour show large variations in the morphology of its subtending hypha and this aspect is newly studied in detail and documented in the present study.

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