

*Gigaspora*  
(*GIGASPORACEAE*) from India, with  
morpho-taxonomic records

*Gigaspora*  
(*GIGASPORACEAE*) da Índia, com notas  
morfo-taxonomias

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The persisting taxonomic problem within genus *Gigaspora* (Gerd. & Trappe) emend. Walkers & Sanders (family *GIGASPORACEAE*) of arbuscular mycorrhizal (AM) fungi has been, the equivocal nature of the species even though members have been examined extensively in the study of the spore wall ultrastructure, germination phenomena, ecological dynamic, *in vitro* analysis and commercial use as inocula (BENTIVENGA & MORTON, 1995). Out of the total eight species documented by SCHENCK & PEREZ (1990), three species viz. *G. ramisporophora* Spain Sieverding & Schenck, *G. candida* Bhattacharjee, Mukerji, Tewarii & Skoropad and *G. tuberculata* Neeraj, Mukerji, Sharma & Varma were rejected and the remaining five species viz. *G. albida* Schenk & Smith, *G. decipiens* Hall & Abbott, *G. gigantea* (Nicol. & Gerd.) Gerd. &

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Trappe, *G. margarita* Becker & Hall and *G. rosea* Nicol. & Schenck are recognized. Out of this, five species, three species are recorded from Goa, India and they are studied for their morpho- taxonomy in the present paper.

## MATERIALS AND METHODS

### EXTRACTION OF AM FUNGAL SPORE

Spores of AM fungi associated with *Carica papaya* L. (papaya) plants from Old Goa (15° 29' 32.1 N, 73° 55' 00.00 E), Goa, India were isolated directly from rhizosphere soil samples by wet sieving and decanting method (GERDEMANN & NICOLSON, 1963). The rhizosphere soils at Old Goa are well drained, dark grey brown to dark brown gravelly sandy loam to sandy clay loam. The papaya host plants were fertilized with inorganic fertilizers. Inorganic fertilizer was applied thrice a year. Papaya plants were irrigated twice a week round the year except in monsoons.

### ESTABLISHMENT OF POT CULTURE

Baiting of native AM fungi were carried out by using open pot cultures (GILMORE, 1968). For establishing pot cultures, 50 g of rhizosphere soil along with root bits of papaya was mixed with equal quantity of sterilized sand and placed in 12.5 cm diameter pots. Seeds of *Eleusine coracana* (L.) Gartner were sterilized with 0.1 % HgCl<sub>2</sub> washed thoroughly with distilled water and placed over the soil sand mixture and covered with 2 cm of soil. The pots with *E. coracana* were maintained under glass house conditions and watered adequately. Cuttings of *Coleus* sp. were also used as host plants for baiting the native AM fungi. Similarly, *Coleus* plants were maintained under glass house conditions and watered adequately. The roots of host plants were checked for AM colonization after 45 days. Pots showing successful mycorrhization were maintained for period of six months and application of water was reduced at final three weeks to maximize spore production (MENGE, 1982). At the end of 6 months the plants were cut near the base, the cultures were air-dried and checked for the presence of spores. Spores isolated from pot cultures were used for identification of AM fungi.

#### 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). The specimens studied were deposited at Department of Botany Goa University, Goa, India.

#### RESULTS

Morpho-taxonomical analysis of the three species *G. decipiens* (Fig.1), *G. margarita* (Fig. 2) and *G. rosea* (Fig. 3) recorded in the present study from Goa, India is as follows.

#### *Gigaspora decipiens* (Fig. 1)

*Gigaspora decipiens* (Hall & Abbott) Bentivenga & Morton, *Mycologia* 87(5): 719-731, 1995.  
= *Gigaspora decipiens* Hall & Abbott, *Transactions of British Mycological Society* 83: 203-208, 1984.

INDIA, GOA: Old Goa, 15° 29' 32.1 N, 73° 55' 00.00 E, *C. papaya* fruiting, Khade, 8/19/2002 (Fig. 1)

Azygospore produced singly in soil, bright white to translucent golden yellow, globose to subglobose, (280) 320-400 (-490) µm in diam. Spore wall with three layers. Outer hyaline 2-4 µm thick sheared, a variable number of robust laminated middle wall, 20-35 µm thick in youth and 20-45 (-90) µm thick at maturity and an inner papillate layer formed prior to germination extending 1-5 µm into the inner lumen of the spores. Often the papillate layers folds when spores are mounted in PVLG. Multiple germ tube arises from areas where papillae are mostly concentrated usually near the sporogenous cell. Germination occurs directly through spore wall. Spore formed terminally on bulbous sporogenous cell, pale yellow to brownish yellow, 50-65 µm diam. with septate subtending hypha. Auxillary cells formed in soil, 33 -54 µm diam, spherical single or tight spherical clusters upto 20, each, attached to helically coiled hypha, 10µm wide. The surface of the vesicles consists of simple or forked spines upto 10µm high and 2 µm wide.

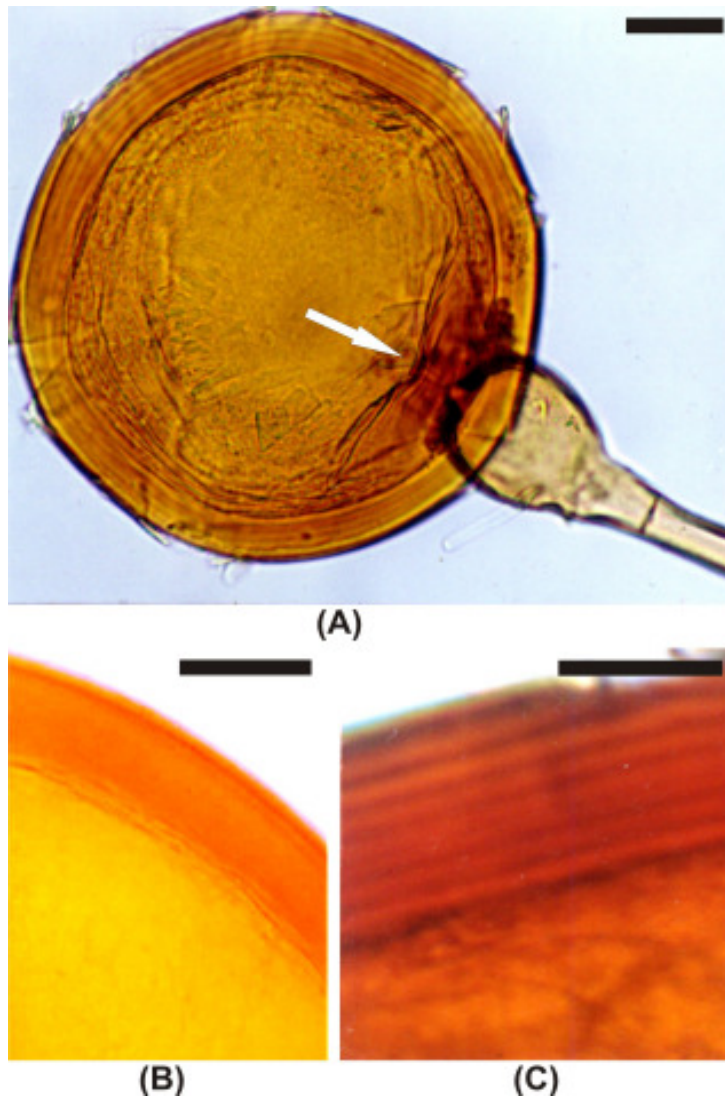


Fig 1. Characteristics of *G. decipiens*. A, single globose spore of *G. decipiens*, borne terminally on a bulbous suspensor with septate subtending hypha and thick laminated spore wall with outer layer sheared (Bar =100 $\mu$ m) [\*Note the papillate layer (arrow) formed prior to germination extending 1-5  $\mu$ m into the lumen of the spores]; B, portion of spore wall of *G. decipiens* showing the thick wall darker than the spore (Bar =30 $\mu$ m); C, magnified portion of matured spore wall of *G. decipiens* showing the numerous laminations (Bar =30 $\mu$ m).

*Gigaspora margarita*  
(Fig. 2)

*Gigaspora margarita* (Becker & Hall), Bentivenga & Morton *Mycologia*  
87(5): 719-731, 1995.  
= *Gigaspora margarita* Becker & Hall, *Mycotaxon* 4: 155-156, 1976.

INDIA, GOA: Old Goa, 15° 29' 32.1 N, 73° 55' 00.00 E, *C. papaya*  
fruiting, Khade, 8/19/2002 (Fig 2.)

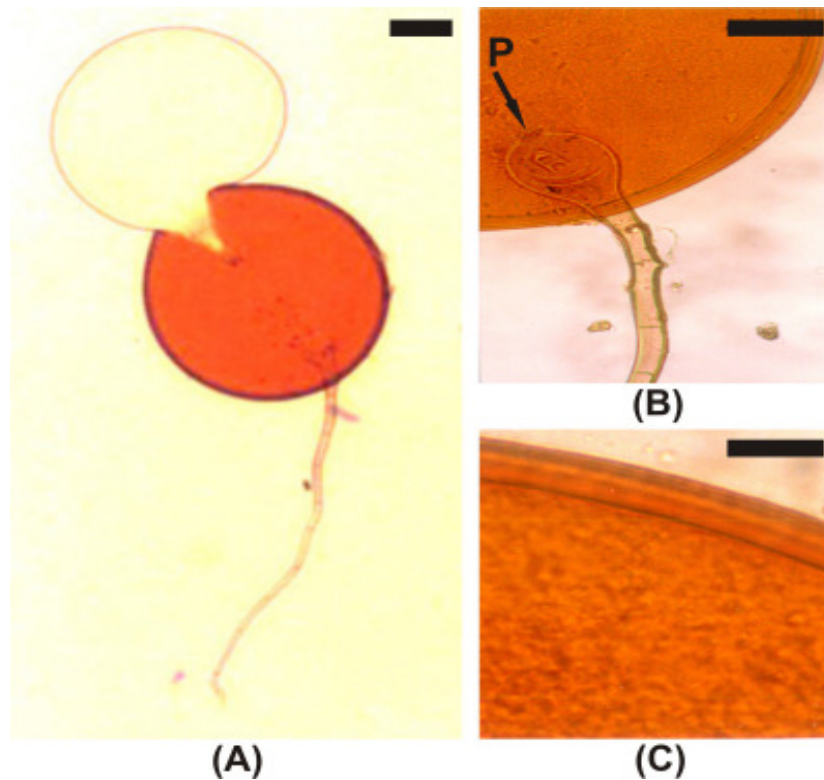


Fig 2. Characteristics of *G. margarita*. A, crushed spore of *G. margarita* terminally borne on bulbous suspensor with septate subtending hypha and showing oily contents (Bar =100µm); B, portion of spore of *G. margarita* borne on bulbous suspensor and septate subtending hypha (Bar =100µm). [\* Note the presence of pore ( P ) (arrow) at the point of attachment of bulbous suspensor to the spore]; C, portion of spore wall of *G. margarita* showing the wall laminations (Bar =100µm).

Azygospore produced singly in soil, pearly white, occasionally yellow, rarely creamy yellow in youth and generally orange to reddish brown at maturity, globose to subglobose, (200) 220-400 (-420)  $\mu\text{m}$  in diam. Spore wall smooth with three layers. Outer hyaline < 1.0- 2.5  $\mu\text{m}$  thick non sheared, middle laminated wall 5-24 (-29)  $\mu\text{m}$  thick at maturity, each lamination 1.5-4  $\mu\text{m}$  thick and inner most layer papillate, formed prior to germination, extending 1-5  $\mu\text{m}$  into the lumen of the spores. Inner most lamina on which the papillae are formed often folds when spores are mounted in PVLG. Contents of the spore white composed of small oil droplets which tend to coalesce at age. Multiple germ tube arises from areas where papillae are most concentrated usually near the sporogenous cell. Spore germination occurs directly through spore wall near the base. Spore formed terminally on bulbous sporogenous cell, (27-) 30-45(-60)  $\mu\text{m}$  diam. Walls 1-5 $\mu\text{m}$  thick with septate subtending hypha. Auxillary cells formed on coiled hyphae, singly or in tight clusters of upto 20, 22-35 $\mu\text{m}$  diam, thin walled and white when young turning light brown with age and covered with warty projections upto 4  $\mu\text{m}$  high and 5  $\mu\text{m}$  wide.

### *Gigaspora rosea*

(Fig. 3)

*Gigaspora rosea* (Nicol. & Schenck) Bentivenga & Morton, *Mycologia* 87(5): 719-731, 1995.

= *Gigaspora rosea* Nicolson & Schenck, *Mycologia*, 71: 178-198, 1979.

= *Gigaspora candida* Bhattacharjee, Mukerji, Tewarii & Skoropad, *Transactions of British Mycological Society* 78: 184-188, 1982.

INDIA, GOA: Old Goa, 15° 29' 32.1 N, 73° 55' 00.00 E, *C. papaya* fruiting, Khade, 8/19/2002 (Fig 3.)

Azygospore produced singly in soil, predominantly globose occasionally subglobose, 160 -280 (-305)  $\mu\text{m}$  in diam, white to cream in colour with rose pink tint. Pink colour variable from barely rose pink to distinct rose pink. Spore wall with three layers. Outer hyaline < 2.4-7.5  $\mu\text{m}$  thick with two to five inseparable layers, 1-2  $\mu\text{m}$  thick. Outer wall smooth, sheared at maturity. Middle wall with variable laminations 5-20 (-29)  $\mu\text{m}$  thick and a inner papillate layer formed prior to germination extending 1-5  $\mu\text{m}$  into the lumen of the spores. Often the papillate layers folds when spores are mounted in PVLG. Multiple germ tube arises from areas where papillae are most concentrated usually near the sporogenous cell. Germination occurs

directly through spore wall. Spore formed terminally on bulbous sporogenous cell, pale yellow to brownish yellow, 30-45(60)  $\mu\text{m}$  diam. with septate subtending hypha. Auxillary cells formed in clusters of 5 to 12 on coiled hyphae, individual vesicles 19-32  $\mu\text{m}$  wide, echinulate with spines upto 5  $\mu\text{m}$  long and 2.5  $\mu\text{m}$  wide.

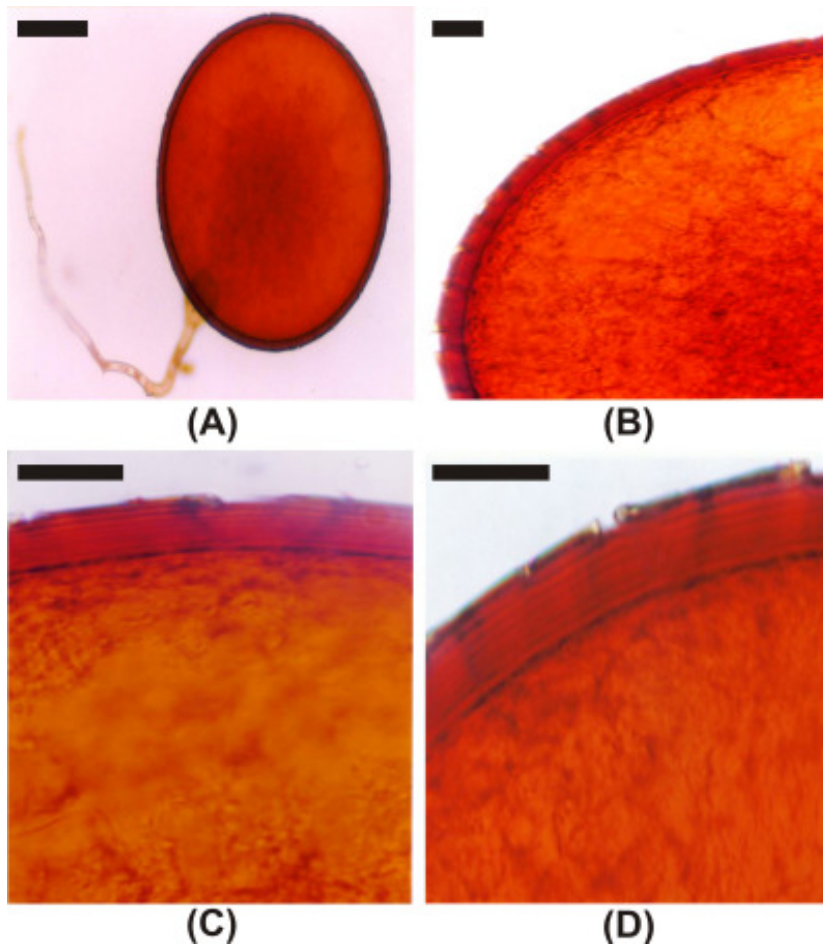


Fig 3. Characteristics of *G. rosea*. A, single spore of *G. rosea*, and globose, borne terminally on a bulbous suspensor with septate subtending hypha and with spore and wall darker than the spore (Bar =50 $\mu\text{m}$ ); B, portion of spore wall of *G. rosea* comprising of laminated wall with outer wall sheared (Bar =50 $\mu\text{m}$ ); C, D, portion of spore wall of *G. rosea* comprising of laminated wall with outer wall sheared (Bar =20 $\mu\text{m}$ ).

## DISCUSSION

The species *G. rosea* is new record to Goa, India. The species *G. rosea* is rare in occurrence. The common features of the three species viz. *G. decipiens*, *G. margarita* and *G. rosea* are as follows. 1) Spores are azygospores and are produced singly in soil. 2) Spores pearly white in youth, mostly globose, rarely subglobose and are produced terminally on the pale yellow to yellowish brown bulbous suspensor. 4) Spore consists of three wall layers, outer thin, middle laminated and inner germinal. 5) Spore wall always darker than the spore colour. 6) Innermost germinal layer, papillate and get folded when mounted in PVLG. 7) Germ tubes are initiated from areas where papillae are mostly concentrated. 8) Germ tubes are directly produced through spore wall. 9) Bulbous suspensor mostly septate with one or two laterally attached hypha. 10) Presence of auxillary cells or soil borne vesicles with projections.

The differences in three species viz. *G. decipiens*, *G. margarita* and *G. rosea* are as follows. 1) SPORE SIZE — The range of spore size is large in *G. decipiens* followed by *G. margarita* and *G. rosea*. 2) SPORE COLOUR AT MATURITY — a) in *G. decipiens*, the spore shows yellowish greenish tint to golden yellow colour; b) in *G. margarita*, the spore colour is orangish yellow to reddish brown; c) in *G. rosea*, the spore colour is barley pink to distinct rose pink. 3) VARIATIONS IN THE THICKNESS OF THE MIDDLE LAMINATED WALL — a) in *G. decipiens*, 20-35  $\mu\text{m}$  in youth and 20-45  $\mu\text{m}$  at maturity; b) in *G. margarita*, (-5) 14-25  $\mu\text{m}$  thick c) in *G. rosea*, 5-20(-29) thick. 4) DIMENSIONS OF BULBOUS SUSPENSOR — a) 50-60  $\mu\text{m}$  diam in *G. decipiens*; b) (27- ) 30-45 (-58)  $\mu\text{m}$  diam in *G. margarita*, c) 30-45(-60)  $\mu\text{m}$  in diam in *G. rosea*. 5) AUXILLARY CELLS — are large in *G. decipiens* followed by *G. margarita* and *G. rosea* respectively. 6) AUXILLARY CELLS type AND DIMENSIONS — a) in *G. decipiens*, presence of simple or forked spines, 10  $\mu\text{m}$  high and 2 $\mu\text{m}$  wide; b) in *G. margarita*, presence of warty projections, 4  $\mu\text{m}$  high and 5 $\mu\text{m}$  wide; c) in *G. rosea*, presence of echinulate vesicles with spines upto 5  $\mu\text{m}$  long and 2.5 $\mu\text{m}$  wide.

## SUMMARY

Three species of *Gigaspora* (Gerd. & Trappe) emend. Walkers & Sanders viz. *G. decipiens* Hall & Abbott, *G. margarita* Becker & Hall and *G. rosea* Nicol. & Schenck were recorded from Goa, India. The species *G. rosea* is new record to Goa, India. This species is rare in occurrence. Morpho-taxonomical analysis reveals that the range of spore size is large in *G. decipiens* followed by *G. margarita* and *G. rosea*. The laminated spore wall is thickest in *G. decipiens*, followed by *G. rosea* and *G. margarita* respectively. Similarly, auxillary cells are



large in *G. decipiens* followed by *G. margarita* and *G. rosea* respectively. The three species are similar to each other in size, shape, number of wall layers and presence of bulbous suspensor with septate subtending hyphae. The species are different from each other in colour, number of laminations and thickness of the laminated middle spore wall.

KEY WORDS: *Gigaspora-decipiens*; *Gigaspora-margarita*; *Gigaspora-rosea*; morpho-taxonomy.

### RESUMO

Três espécies de *Gigaspora* (Gerd. & Trappe) emend. Walkers & Sanders viz. *G. decipiens* Hall & Abbott, *G. margarita* Becker & Hall and *G. rosea* Nicol. & Schenck foram registradas em Goa, Índia. A espécie *G. rosea* é um registro novo para Goa, Índia. Esta espécie é de ocorrência rara. A análise morfo-taxonomica revela que a amplitude do tamanho do esporo é grande em *G. decipiens*, seguida, respectivamente, por *G. margarita* e *G. rosea*. Similarmente, as células auxiliares são grandes in *G. decipiens* seguida, respectivamente, por *G. margarita* e *G. rosea*. As três espécies são similares uma às outras em tamanho, forma, número de camadas da parede de hifas septadas. As espécies são diferentes uma das outras em cor, número de laminações e a espessura da parede laminada mediana do esporo.

PALAVRAS CHAVE: *Gigaspora-decipiens*; *Gigaspora-margarita*; *Gigaspora-rosea*; morfo-taxonomia.

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