Dentiscutata nigerita – a new species of arbuscular mycorrhizal fungi from India

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Khade SW. 2010 – *Dentiscutata nigerita* - a new species of arbuscular mycorrhizal fungi from India. Mycosphere 1(3), 241–247.

Dentiscutata nigerita Khade (family *Dentiscutataceae*), a new species is reported and described from the rhizosphere of *Carica papaya* plants from Kodar, Goa, India. The diagnostic features are discussed including the characteristic feature that the bulbous suspensor is attached at an angle to the spore.

Key words – *Carica papaya* – *Dentiscutataceae* – Goa

Article Information
Received 1 August 2010
Accepted 4 October 2010
Published online 30 October 2010
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Introduction

Symbiotic arbuscular mycorrhizal (AM) fungi which live in association with three-quarters of the land plants are ubiquitous and belong to the phylum *Glomeromycota*. Their association is essential for plant ecosystem function because most plant species depend on them for mineral nutrient uptake. This task is efficiently performed by the extensive extra-radical mycelium of the fungal symbionts. Within root cells, AM fungi form typical tree-like structures, the arbuscules or hyphal coils. Some also produce storage organs, termed as vesicles. Although different AM fungal genera were reported to show some differences in the morphology of their intra-radical structures (Merryweather & Fitter 1998), it is generally not feasible to identify species with these characters. Glomeromycotan fungi produce relatively large spores with layered walls, containing several hundreds to thousands of nuclei (Bécard & Pfeffer 1993). Spores may be formed singly, in clusters or aggregated in so called sporocarps (Gerdemann & Trappe 1974). Similar to most *Zygomycota* members, the hyphae of Glomeromycotan fungi lack regular septation. The phylum *Glomeromycota* comprises about 200 described morpho-species that have been traditionally distinguished by features of the spore wall. The mode of spore formation on the hypha has been important feature to circumscribe genera and families and the layered structure of the spore walls is used to distinguish species (Morton 1988). 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, 1999) included considerations of spore development to group these wall components hierarchically into complexes linked by ontogeny (Redecker & Raab 2006).

Axenic AM fungal biomass can be obtained only from cultures on transformed plant roots, but only a small number of species are available in culture. The rDNA heterogeneity causes problems when closely related species or isolates of the same species are to be distinguished. It was shown recently that the mitochondrial large ribosomal subunit gene does not show this variation (Raab et al. 2005), suggesting that mitochondrial genes might be useful as molecular markers in the future. Due to the problems outlined above there is

currently no molecular species concept for Glomeromycotan fungi (Redecker & Raab 2006). Thus, the delimitation of species within the AM fungal group still rests solely on morpho-taxonomy. Although molecular markers have proven to be highly useful to characterize the diversity of AM fungi in the field, and have revealed an unexpectedly high diversity of phylotypes in some settings, these studies indicate that the number of 200 described morpho-species might be a gross underestimation of the true diversity of the *Glomeromycota* (Redecker & Raab 2006)**.** This warrants for inventory, documentation and taxonomic investigation of these AM fungi associated with rhizosphere of host plants. The taxonomic methods include morpho-taxonomy, phylogenetic analysis and molecular studies.

The genus *Scutellospora* of AM fungi was separated from *Gigaspora* based on subcellular structures i.e., differentiable wall layers and presence of germination shield (Walker & Sanders 1986). The species of this genus are characterized by presence of a giant spore which is laterally and vertically attached to an apical swollen hyphal tip called the bulbous suspensor. The characteristic germination shield varies in its colour and shape depending on the species. However, molecular analyses indicated that *Scutellospora* is polyphyletic (Walker et al. 2004, Ahulu et al. 2006, Redecker et al. 2007). Recently, family *Gigasporaceae* (Morton & Benny 1990) of the order *Diversisporales*, *Glomeromycetes* (Cavalier-Smith 1998) was revised on the basis of morphological spore characters and 18S and 25S rRNA gene sequences, and the 36 *Scutellospora* species were reorganized in three new families including five new genera: *Scutellosporaceae (Scutellospora)*, *Racocetraceae (Racocetra*, *Cetraspora)* and *Dentiscutataceae (Dentiscutata*, *Fuscutata*, *Quatunica)* Oehl et al. (2008). The aim of the present paper is to describe a new species in the genus *Dentiscutata* belonging to the family *Dentiscutataceae* based on morpho-taxonomy.

Materials and methods

Extraction of AM fungal spores

Spores of AM fungi associated with *Carica papaya* L. (papaya) plants from Kodar (15^{o} 29′ 32.1″ N, 73^{o} 55′ 20.00″ E), Goa, India were isolated directly from rhizosphere soil samples by wet sieving and decanting method (Gerdemann & Nicolson 1963). The rhizosphere soils at Kodar are well drained, strong brown in colour, gravelly-clayey loam in texture. The papaya plants at Kodar were raised in monoculture under conventional management regime. The plants were fertilized with inorganic fertilizers along with phosphate solubilizing bacteria (PSB). Inorganic fertilizer was applied thrice a year while PSB was applied once in 6 months. Papaya plants were irrigated twice a week round the year except in monsoons.

Establishment of pot cultures

Baiting of AM fungi was carried out using open pot cultures (Gilmore 1968). For establishing pot cultures, 50 g of rhizosphere soil along with root pieces of papaya was mixed with equal quantity of sterilized sand and placed in plastic pots of 12.5 cm diam. Seeds of *Eleusine coracana* (L.) Gaertner were sterilized with 0.1 % $HgCl_2$, washed thoroughly with distilled water, placed over the soil-sand mixture and covered with 2 cm of soil. The pots with *E. coracana* were maintained under glasshouse conditions and watered adequately. Cuttings of *Coleus* sp. were also used as host plants for baiting the AM fungi and were maintained under glasshouse conditions with adequate watering. The roots of host plants were checked for AM colonization after 45 days. Pots showing successful colonization by AM fungi were maintained for 6 months and application of water was reduced at final 3 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

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 a 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) and Oehl et al. (2008).

Results

A new species, *Dentiscutata nigerita* Khade sp. nov**.**, is described

Dentiscutata nigerita Khade sp. nov.

(Fig. 1–4)

Azygospores atrum frons ut rutilus niger, evulsum singulus in terra, terminatio latus in frons elongated bulbous suspensor per sparsely septate hypha. Bulbous suspensor est suggero procul an Angli ut lusum. Azygospores globose ut subglobose 300–650 × 275–700 µm diam, per pinguis globular tenor decens opaque ut plaga in colour. Lusum parietis compages of duos parietis humus (A and B).

Humus parietis A– Externus parietis layer (parietis 1) atrum frons ut niger per pitted ornamentation consisto of amplus pores 7–10 µm diam, formatura a hyphal network per externus ambitus of lusum. hypha interconnected ut invicem quod interspersed per pores. Per in nonnullus pores rods hyphal orbis vel nemus amo stipes vel dudum baobab amo compages es quondam per multiplex teres hyphlets. Is pitted parietis 1 est underlined per vegrandis porous, teres stilus frons ut perspicuus, membrana layer parietis 2. Is est secuutus per parietis 3 quod est puniceus frons ut atrum orange frons consisto of curve interconnecting promontorium ut vultus reticulum, 1µm altus, 4–8 pars meshes, 4–20 × 1–24 µm trans. Lusum superficies inter promontorium occulto per polyhydral conical sub-cylindrical spines, 0.5–2 µm altus quod 1 µm seorsum.

Humus parietis B– Consisto of externus (parietis 4), medius (parietis 5) quod penitus (parietis 6) crocus iunctum membrana moenia. Illa parietis layers macula puniceus per Melzer's reagent. Medius parietis est brittle quod minor perspicuus in frendo lusum germination contego est quondam in is medius parietis. In application of pressure ut lusum a ambitus, crocus ut frons germination contego est perspicuous.

Germination contego, centum 100–150 × 150–250 µm diam amorphous per crispans margin multilobed in vultus due ut profundus grooves quod eventually develop in germules procul subolesco. Germination contego tendo near lusum substructio qua lusum est suggero ut bulbous suspensor.

Bulbous suspensor – centum 100–120 × 50–57 µm, suggero procul an Angli ut lusum. Is consisto of sparsely septate 150–400 µm frons subtending hypha. Parietis of suspensor, 3–12 µm creber near lusum substruction, 1–5µm creber colus.

Holotype – India, Goa, Kodar, 15° 29′ 32.1″ N, 73° 55′ 20.00″ E, *Carica papaya* L., Khade, March 2002. Specimen deposited in form of slides in Department of Botany, Goa University, India.

English Description

Azygospores dark brown to shining black, occuring singly in soil, terminally borne on brown elongated bulbous suspensor with sparsely septate hypha. Bulbous suspensor is attached at an angle to the spore. Azygospores globose to subglobose 300–650 × 275–700 µm diam, with oily globular contents becoming opaque to buff in colour. Spore wall structure of two wall groups (A and B).

Group wall A – Outer wall layer (wall 1) dark brown to black with pitted ornamentation consisting of large pores 7–10 µm diam, forming a hyphal network throughout the outer circumference of the spore, i.e. hyphae interconnected to each other and interspersed with pores. Within some pores, rods, hyphal coils or tree-like branches or sometimes baobab-like structure are formed with numerous fine hyphlets. This pitted wall 1 is underlined by small porous, fine pale brown to transparent, membranous layer wall 2. This is followed by wall 3 which is reddish brown to dark orange-brown consisting of sinuous interconnecting ridges that form reticulum, 1 µm high, 4–8 sided meshes, 4–20 × 1–24 µm across. Spore surface between ridges covered with polyhedral, conical sub-cylindrical spines, 0.5–2 µm high and 1 µm apart.

Group wall B – Consists of outer (wall 4), middle (wall 5) and inner (wall 6) yellow unit membranous walls. These wall layers stain purple with Melzer's reagent. The middle wall is brittle and less evident in crushed spores. The germination shield is formed on this middle wall. On application of pressure to the

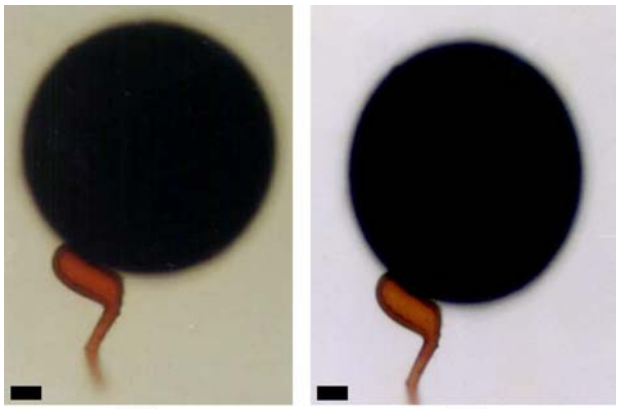

Fig. 1 – (**A**, **B**) Black spores of *Dentiscutata nigerita* with bulbous suspensor attached to it at an angle, Bar = 50 µm.

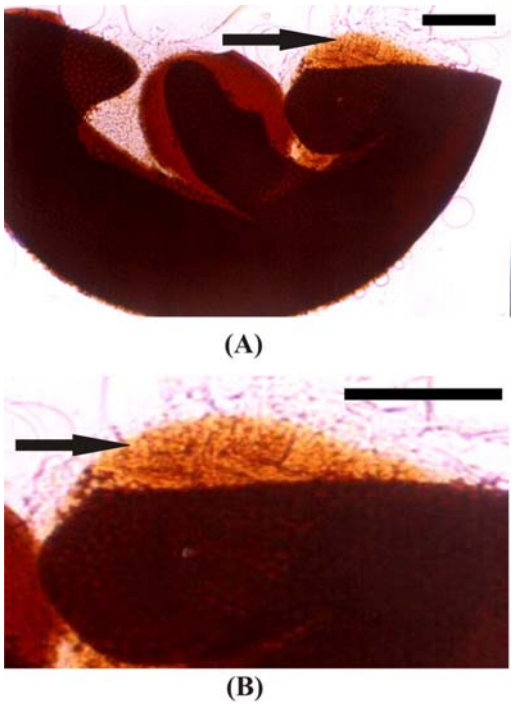

Fig. 2 – **A**) Large reddish brown crushed spore of *Dentiscutata nigerita* showing reticulum and a portion of mature germination shield (arrow), Bar = 50µm. **B**) Crushed spore of *Dentiscutata nigerita* showing a portion of mature brown germination shield (arrow), Bar = 50 µm.

spore, a circular, yellow to brown germination shield is evident.

Germination shield, 100–150 × 150–250 µm diam, amorphous with wavy margin, multilobed in appearance due to deep grooves which eventually develop into germules at maturity. Germination shield present near the spore base where the spore is attached to bulbous suspensor.

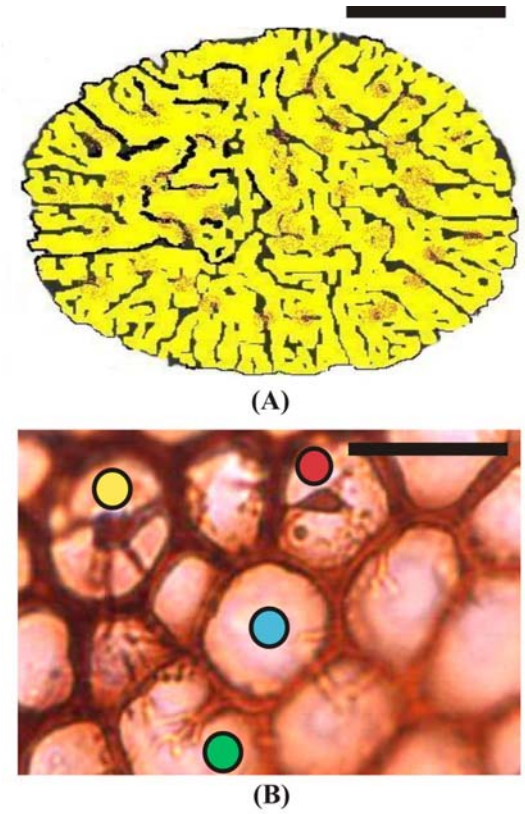

Fig. 3 – **A**) Diagrammatic representation of circular wavy margined, yellowish orange, multilobed, young germination shield of *Dentiscutata nigerita* with grooves, Bar = 30 µm **B**) Pitted ornamentation of the *Dentiscutata nigerita*, Bar = 30 µm [Note: various colours symbolizing different structures in the porous ornamentation, Blue – hollow pore; Yellow – baobab-like structure in pore; Green – tree-like structure in the pore; Red – rod formed in the pore]

Bulbous suspensor – 100–120 × 50–57 µm, attached at an angle to the spore. It consists of sparsely septate 150–400 µm brown subtending hypha. Wall of the suspensor, 3–12 µm thick near the spore base, 1–5 µm thick distally.

Etymology – *Nigerita*, combination of two species, *D. nigra* (Redhead) Sieverding, de Souza & Oehl and *D. reticulata* (Koske, Miller & Walker) Sieverding, de Souza & Oehl

Distribution and habitat – Associated with rhizosphere of papaya plants grown in brown, well drained, gravelly-clayey loam, phosphorous deficient tropical soil in agro-based ecosystem of Kodar, Goa, India. *Dentiscutata nigerita* in the field is found together with *Acaulospora* species, *D. reticulata*, *D. nigra* and *Glomus* species. Attempts to culture

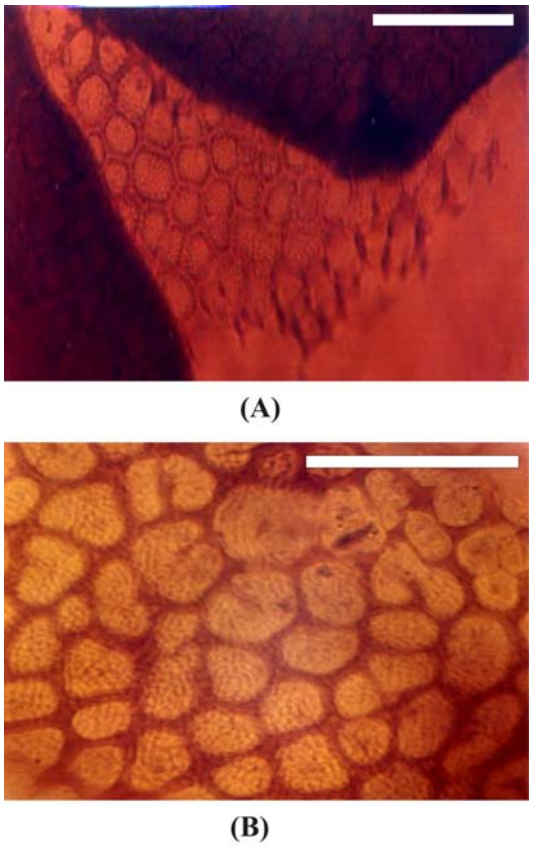

Fig. 4 – A) Reticulum in *Dentiscutata nigerita* interspersed with spines, Bar = 50 µm. B) A portion of spore wall with reticulum formed by interconnecting ridges interspersed with spines, Bar = 30 µm.

the species using pot culture technique failed in the present study.

Discussion

Difficulties in identification, the inability to grow the AM fungi in pure culture, problems of taxonomic classification, and a lack of basic information on the life histories of AM fungi hinder study and understanding of their ecological significance. Molecular techniques have shown that the natural AM fungal populations exhibits unexpectedly high genetic diversity, despite the assumption that diversity in these seemingly asexual fungi should be low (Sanders et al. 1996). Contrasting results, indicating that genetic diversity among replicate spores from pot-cultured material is low (even though they possess intra-spore sequence heterogeneity). In general, molecular phylogenetic analysis have shown that Glomeromycotan diversity at the phylum and genus level is much higher than expected through microscopic observation of spore morphology (Redecker et al. 2000, Schwarzott et al. 2001) and therefore many of these fungi await discovery. Thus, there is a need for inventory and documentation of these fungi from various ecosystems. The fundamental basis of AM taxonomy is still morphology-based especially with the spores associated with rhizosphere soil of the host plants and the same methodology was carried out in the present study.

The distinguishing characteristics of family *Dentiscutataceae* that were found in *D. nigerita* are: 1) spores with outer spore wall and one to three inner walls; 2) germination shield generally formed on the outer surface of the innermost wall or beneath a thin outer layer of the inner wall; 3) germination wall is yellow-brown to brown. The important features of the genus *Dentiscutata* recorded in *D. nigerita* are: 1) spores singly formed on bulbous sporogenous cells that are formed terminally on a subtending hypha that arises from mycelia hyphae; 2) outer spore wall is three- to five-layered and continuous with the wall of the sporogenous cell; 3) germination shield generally arising on the outer surface of the inner wall or beneath a thin outer layer of the inner wall, yellow-brown to brown, generally ellipsoid (to oval), or rarely reniforme to cardioforme, usually with many (8–30) large folds separating the shield into small compartments; each compartment generally with one circular germ tube initiation (gti); 4) the periphery of the germination shield generally appears dentate in planar view.

The dark brown to shining black spores of *D. nigerita* resemble closely related species, *D. nigra* and *D. reticulata* in spore wall ornamentation. Like *D. nigra*, the new species *D. nigerita* has outer pitted black ornamentation with large pores and like *D. reticulata*, it has reddish brown layer consisting of straight to sinuous interconnecting ridges forming a reticulum with spore surface between ridges covered with polyhedral, conical or subcylindrical spines. However, *D. nigerita* differs from *D. nigra* since the outer pitted ornamentation was underlined by small porous membranous wall in *D. nigerita* while in the *D. nigra* the pitted ornamentation is underlined with sinuous secondary ornamentation below. *D. nigerita* differs from *D. reticulata* since the

reticulum of ridges interspersed with spines is wall 3 of the outer wall group in *D. nigerita*, while in *D. reticulata*, the reticulum is the outermost layer of the outer spore wall. Further presence of the pitted ornamentation underlined by small porous membranous layer along with reddish brown layer of reticulum is found together in *D. nigerita*. In *D. nigra* only pitted ornamentation with underlined sinuous ornamentation is evident while in *D. reticulata* only reticulum interspersed with spines is found.

As seen in *D. nigra* and *D. reticulata*, the germination shield in *D. nigerita* is formed on middle wall of group wall B. This middle wall is brittle in *D. nigra* and *D. nigerita* but distinct in *D. reticulata*. However, the type of germination shield in *D. nigerita* is yellow to brown, circular, amorphous, wavy margined, multilobed with numerous deep grooves which develop germules at maturity and differs from *D. nigra* where it is chestnut brown, oval with regular margin with macro- and micro-germules and *D. reticulata* where it is chestnut brown, reniform, multilobed with macro- and micro-germules.

The bulbous suspensor in *D. nigerita* is brown, smooth-walled, with recurved sparsely septate subtending hypha. The bulbous suspensor is attached at an angle to the spore, which is the characteristic feature of the species. The bulbous suspensor of *D. nigra* and *D. reticulata* are laterally attached to the spore and is ornamented (concentric wavy wall layers are present). The bulbous suspensor in closely related species is attached to sparsely septate coenocytic subtending hypha in *D. reticulata* and septate subtending hypha in *D. nigra*. A long peg-like non-septate hypha originate from the bulbous suspensor in *D. nigra* and *D. reticulata* which is absent in *D. nigerita*. Like *D. nigra* and *D. reticulata*, in *D. nigerita*, the wall of brown sporogenous cell is thickest at the point of attachment and thin distally.

Acknowledgements

I would like to thank Dr. K.R. Sridhar, Professor, Department of Biosciences, Mangalore University, Mangalagangotri, Mangalore, Karnataka, India for critical evaluation of my manuscript. I would also like to thank Dr. Alok Adholeya, Director, Biotechnology and Management of Bioresources Division, The Energy and Resources Institute, Darbari Seth Block, India Habitat Centre, Lodhi Road, New Delhi, India for his kind help.

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