New record of *Megacapitula villosa* and *Paradictyoarthrinium diffractum* from India

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Two monotypic genera, *Megacapitula* and *Paradictyoarthrinium*, are reported for the first time from palm litter from India. ML analysis suggests that they have a close affinity with members of the order *Pleosporales*, *Dothideomycetes*.

Key words – Western Ghats – palm fungi – fungal diversity – taxonomy

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Introduction

During studies on palm-associated microfungi of the Western Ghats of India, a large number of fungi were collected. This paper illustrates two interesting species, namely, *Megacapitula villosa* J.L. Chen & Tzean and *Paradictyoarthrinium diffractum* Matsush., forming the first report of their occurrence in India. The species, which belong to monotypic genera, are described and illustrated with micro-photographs. The cultures obtained from single spore isolation were used to generate ITS/5.8S rRNA gene sequence-data. Maximum likelihood analysis based on this gene marker suggests that these fungi have a close affinity with members of the order *Pleosporales*, *Dothideomycetes*.

Methods

Isolates and morphology

Direct isolation from plant litter: Palm fronds and spathe were scanned under a stereomicroscope to locate the fungi. A small portion of any fungal material was picked up with a fine-tipped needle and placed in distilled water or lactophenol on a slide and examined under a microscope. Detailed morpho-taxonomic study of the fungi were done using a light-transmitted microscope. Photographs were taken on an Olympus microscope with a DP12 camera attached. The specimens were deposited in the Botany Herbarium of Goa University (GUBH). Pure cultures were obtained by aseptically transferring single spore on malt extract agar incorporated with a mixture of antibiotics (Bacitracin 0.02 g, Neomycin 0.02 g, Penicillin G 0.02 g, Polymixin 0.02 g, Streptomycin 0.02 g and Tetramycin 0.04 g dissolved in 10 ml of distilled water and added to 1 L of MEA medium). The cultures were deposited in Goa University fungal culture collection (GUFCC).

DNA Isolation and PCR conditions

Ten-days-old fungal cultures grown as above were used for DNA isolation. Using a sterile spatula, the fungal cells were scraped and transferred to a 1.5 mL centrifuge tube and resuspended in 500 µL extraction buffer (100 mM tris HCl, pH 8.0, 10 mM EDTA, 2% SDS, 100 ug/mL proteinase-k, 1% B-mercaptoethanol) and incubated for 20 min at 60°C. Salt concentration was adjusted to 1.4 M with 5 M

NaCl, 1/10th volume of 10% CTAB was added and samples incubated a further 10 min at 65°C, before 1 vol chloroform: isoamyl alcohol mixture was added and gently emulsified by inversion, and centrifuged for 10 min at 4°C at rpm max. Top phase was than transferred to fresh 1.5 mL microfuge tube, 1/2 vol 5M NH_4O-Ac was than added and mixed gently, and incubated on ice for 60 min; the sample was centrifuged at 4°C at rpm max. Supernatant was transferred to fresh tube and treated with RNase 10 mg/mL to a final concentration of 0.02 µg/µL. To this 0.55 vol isopropanol was added, and centrifuged 5–10 min at rpm max. to precipitate the DNA. Supernatant was aspirated off and the DNA pellet was washed twice with 70% ETOH and air dried for 20 min. The pellet was finally resuspended in 50 µL TE buffer.

PCR and DNA sequencing

The ITS/ 5.8S rRNA gene from the fungal cultures were amplified by Polymerase Chain Reaction (PCR) using ITS4-ITS5 primer-pair (White et al. 1990). This was done by M/S Royal Life Sciences, Secunderabad, attached with MIDI Labs. DNA sequencing was done using the above-mentioned primer pair in an Applied Biosystem MicroSeq System.

Sequence alignment and phylogenetic analysis

Sequences obtained from the respective primers were aligned in MEGA version 5.02 (Tamura et al. 2011) and the consensus sequences were deposited in NCBI-GeneBank with accession numbers JN128868 and JN128869. A dataset based on ITS/5.8S rRNA gene sequence data was prepared using MEGA. Phylogenetic analyses were conducted in MEGA. First 100 blast search results in NCBI-GenBank were considered to select the taxa and most of the sequences with repetitive names were ignored. Phylogenetic relationships of *Paradictyoarthrinium diffractum* GUFCC 15514 and *Megacapitula villosa* GUFCC 15515 with closely related members of the *Pleosporales*, *Dothideomycetes* were inferred based on Maximum Likelihood method and GTR model incorporated in MEGA. The tree with the highest log likelihood (-3249.0486) is shown in Fig. 15.

Taxonomy

Megacapitula villosa J.L. Chen & Tzean

Fig 1–7

Colonies on MEA growing up to 4 cm in 10 days, dark green to brown, cottony, reverse black, forming thick mat of mycelium. Mycelium brown, septate, 2–3 µm broad, often verruculose, forming mycelial cords from which conidia arise. Conidiogenous cells integrated or terminal on mycelial extensions. Conidia holoblastic, rounded when very young then elongating and forming a beak-like structure from which dense hairy appendages arise at maturity, mature conidia ovoid to obclavate, muriform having distinct outer wall which breaks and starts peeling off after mounting, 80–240 µm tall standing erect from mycelial mat, up to 20 to 45 µm broad at base, 60–180 µm broad at centre and 20–40 µm broad at tip from where numerous appendages arise; appendages few to many, grey to brown, smooth, up to 3 µm broad.

Known distribution – As per USDA fungal distribution database and other available literature, the fungus is so far known only from its type locality in Taiwan on fallen decayed petiole.

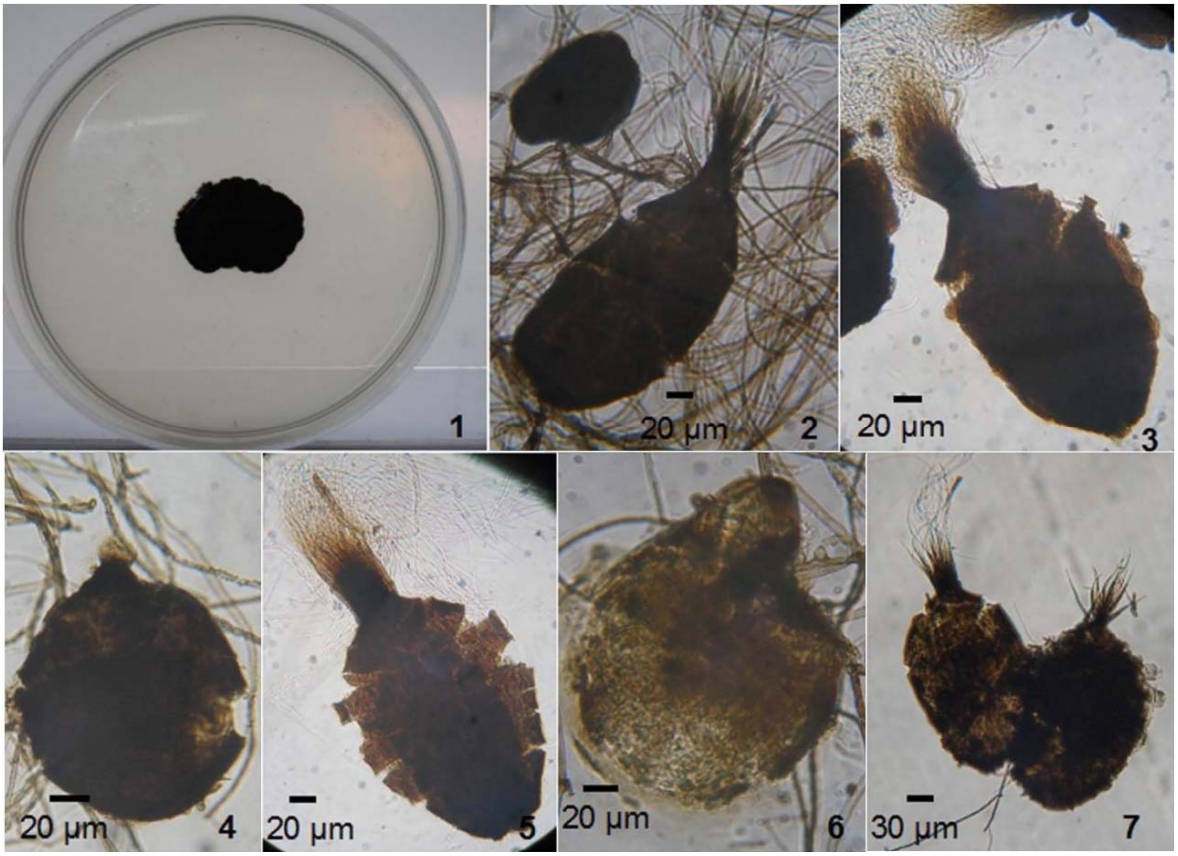
Material examined – India, Goa, near Bombolim beach, on dead and decaying fronds of *Caryota urens*, 3 December 2007, A. Prabhugaonkar Herb. No. GUBH 131AP24; culture no. GUFCC 15515.

Paradictyoarthrinium diffractum Matsush.

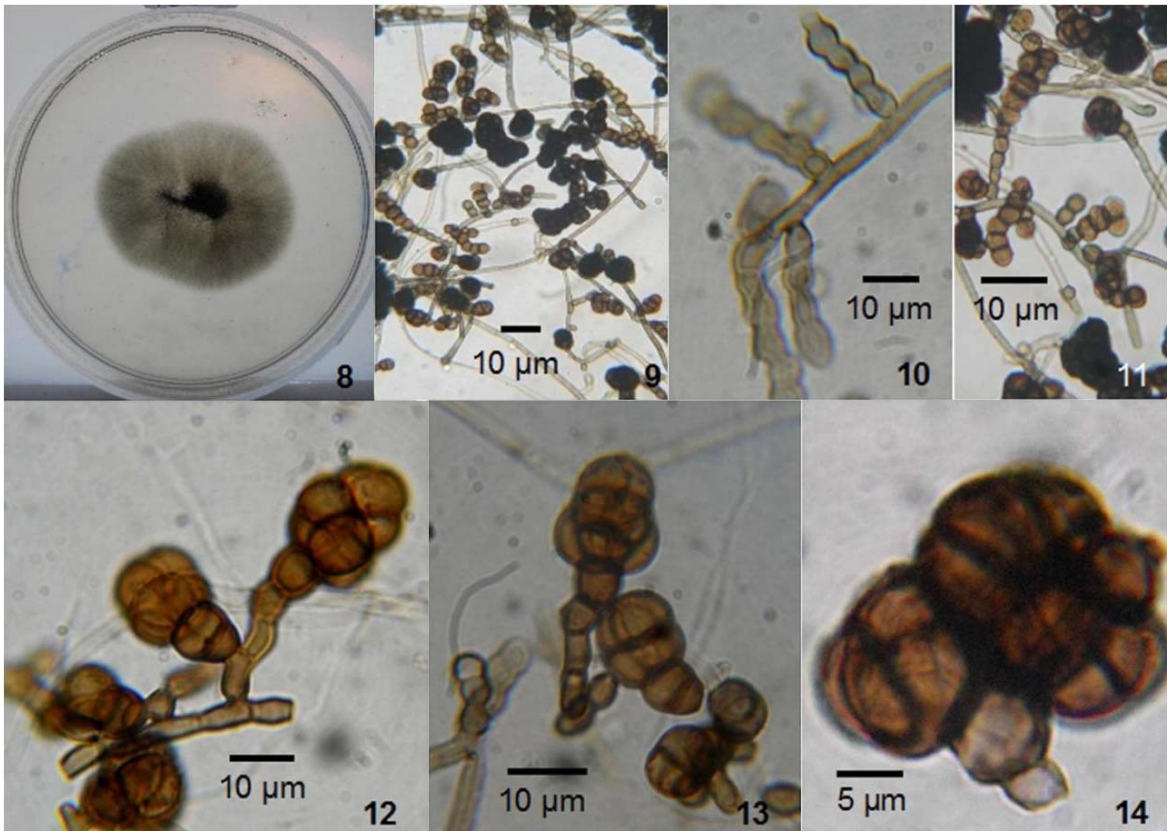
Fig 8–14

Colonies on MEA growing up to 6 cm in 10 days, off-white to brown, becoming dark brown to black after sporulation. Mycelium 1–3 µm broad. Conidiophores macronematous, rarely micronematous, short, when macronematous showing much varied morphology, unbranched to much branched, uneven, constricted at septa, turning brown to dark brown on maturity, 10–75×4–5 µm, part of mature conidiophores often released along with conidia. Conidiogenous cell blastic, mostly terminal, 4–10×4–5 µm. Conidia 2 to many unevenly dictyoseptate, circular to irregular, dark brown to black on maturity, 8–33×10–30 µm.

Known distribution – As per USDA fungal distribution database and other available



Figs 1–7 – *Megacapitula villosa*. **1** Colony on MEA, **2** Mycelia and attached conidia, **3–7** Mature and young conidia.



Figs 8–14 – *Paradictyoarthrinium diffractum*, **8** Colony on MEA, **9** Conidiophores and conidia, **10** Conidiophores, **11–13** Conidiophores and conidia, **14** Single conidium.

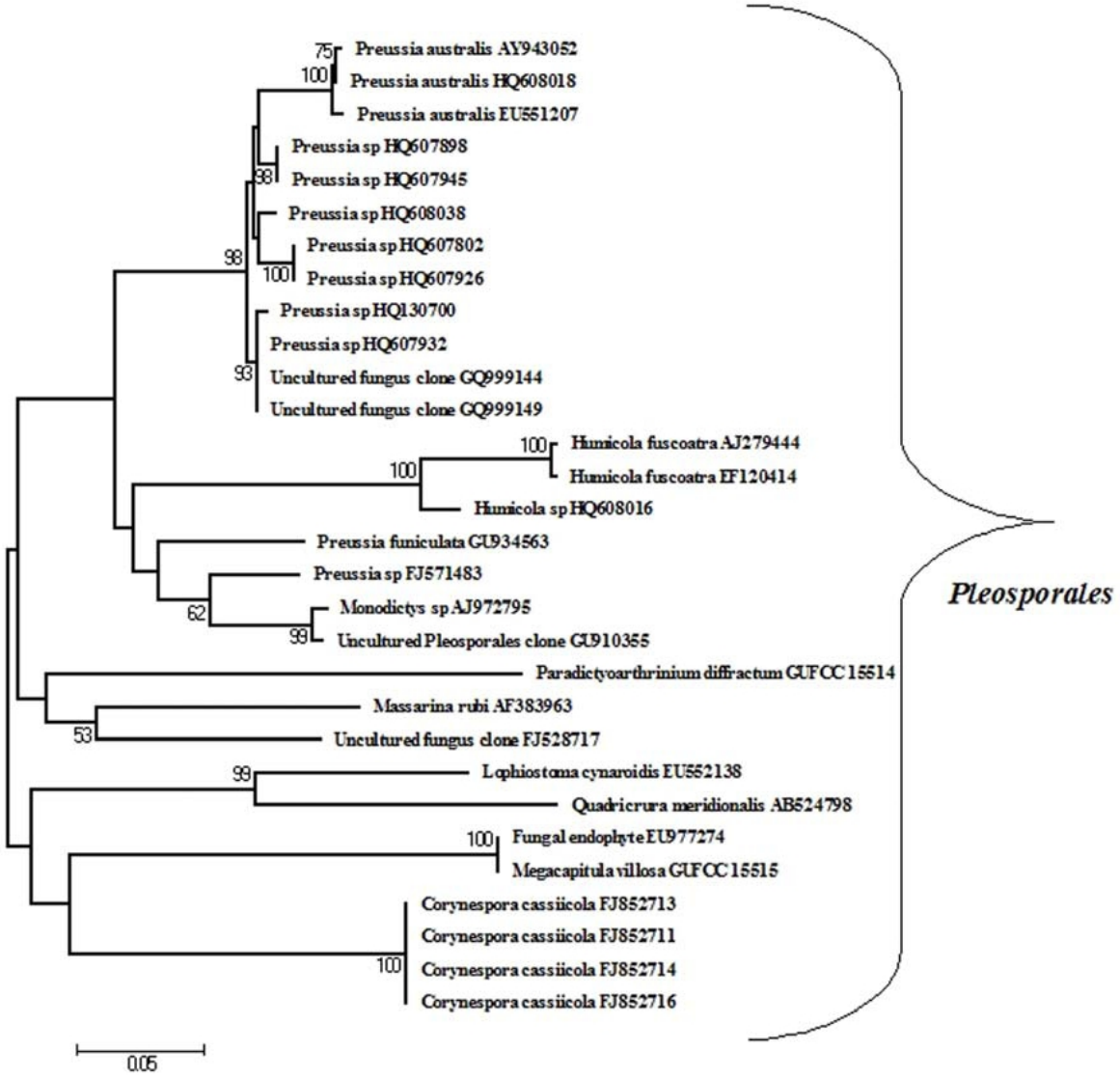


Fig. 15 – Phylogentic relationships of *Paradictyoarthrinium diffractum* GUFCC 15514 and *Megacapitula villosa* GUFCC 15515 with closely related members of the *Pleosporales*, *Dothideomycetes*. The tree with the highest log likelihood (-3248.7706) is shown. The persentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically as follows: Whan the number ofcommon sites was used; otherwise BIONJ method with MCL distance matrix was used (Tamura et al 2011). A Discrete Gamma distribution was used to model evolutionary rate differences among site [5 categories (+G, Parameter = 0.4775)]. The rate wariation model allowed for some sites to be evolutionarily invariable [(+I), 28.1848% sites]. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 30 nucleotide sequences. There were a total of 589 positions in the final dataset.

literature the fungus is so far known only from its type locality at Rustenbusrg in South Africa from dead plant material collected from a rivulet.

Material examined – India, Goa, Canacona, Mashem, on dead decaying spathe of *Cocos nucifera*, 2 January 2007, A. Prabhugaonkar, Herb. No. GUBH 136AP25; culture no. GUFCC 15514

Phylogenetic analyses

ML analysis suggests that *Megacapitula villosa* and *Paradictyoarthrinium diffractum* have a close affinity with the members of the order *Pleosporales*, *Dothideomycetes*. Multigene phylogenetic analysis with an extended dataset and the relevant type strains are required to resolve their placement within suitable fungal families.

Discussion

Chen & Tzean (1993) described *Megacapitula villosa* from fallen, decayed, unidentified petiole in Taiwan. In the present study, the fungus was collected on fronds of palm *Caryota urens* from Goa, India. The sequence of *Megacapitula villosa* showed much similarity with an unidentified fungal endophyte sequence (GenBank No. EU977274) isolated from an unidentified plant in Peru (Smith et al. 2008). *Paradictyoarthrinium diffractum* Matsush. was described by Matsushima (1996) from dead plant material collected from a rivulet at Rustenbusrg in South Africa. In the present study, the fungus was collected on dead decaying spathe of *Cocos nucifera* from Goa, India. On perusal of literature on fungi of India (Mukerji et al. 1974, Sarbhoy et al. 1986, 1996, Jamaluddin et al. 2004, Farr et al. 2011), both these collections formed new records to fungal flora of India.

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