



NEW DISTRIBUTION RECORD OF *CLATHRUS DELICATUS* BERK. & BROOME (PHALLACEAE) FROM GUJARAT

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Clathrus delicatus Berk. & Broome, popularly known as cage fungus (Phallaceae) was collected from the Dangs, Ahwa and Vansada forest for the first time in August 2014. Subsequently it was also collected from Wilson hill, Dharampur forest and Shoolpaneshwar Wildlife Sanctuary (Sagai forest) in 2015, 2016 and 2017 respectively during the field visit. Present study reports new distribution record for the *Clathrus delicatus* from Gujarat state and DNA barcoding data is submitted for the first time in BOLD data system (ID KSRF-0015). It was identified by morphological feature and further confirmation was carried out by using molecular methods with its DNA barcoding.

Key words: Cage fungus, *Clathrus*, DNA barcoding, Gujarat, Phallaceae

Members of the Phallaceae popularly known as stinkhorn mushrooms are unique group of fungi belonging to the order Phallales. Foul-smelling, sticky spore masses, or gleba, borne on the end of a stalk called the receptaculum are the characteristic features of stinkhorn (Dring 1980). Various chemicals like hydrogen sulphide and methyl mercaptan are responsible for their distinctive odour that attracts insects (List and Freund 1968) and help them to disperse their spores. The characteristic fruiting-body structure, a single, unbranched receptaculum with an externally attached gleba on the upper part, distinguishes the Phallaceae from other families in the order Phallales (Dring 1980). Although there is great diversity in body structure and shape among the various genera, all the species in this order originate their fruiting body as oval or round structures known as "myco eggs" often within few hours (Lloyd 1906, Pegler *et al.* 1995). According to Kirk *et al.*, (2008) family Phallaceae contains 21 genera and 77 species and there is no published information on the occurrence of its members from the Gujarat state (Rajput *et al.* 2015).

Gujarat state is well known for its varying climatic conditions starting from moist deciduous forest (south Gujarat), dry deciduous to scrub forest in central and north Gujarat while rann of Kuchh is known for typical desert conditions. In spite of all such

climatic conditions, fungal diversity of the state is poorly investigated (Rajput *et al.* 2015; Vasava *et al.* 2015). However, no special efforts are done in the state for the documentation of the fungal diversity.

Our study on the documentation of fungal diversity of Gujarat initiated in 2013-14 as a part of Gujarat Biodiversity Board activity for the documentation of fungal biodiversity. During that period we collected an interesting cage fungus that is identified as *Clathrus delicatus* and presented here for the first time as a new distribution record for the western part of India.

MATERIALS AND METHODS

Collection

Fungal specimens (*Clathrus delicatus* Berk. & Broome) were collected for the first time from Wilson Hills, Dharampur, Gujarat, India (20°29'39.70" N, 73°21'51.20" E). Subsequently, it was collected from Vansada National Park (20°45'45.14" N, 73°28'45.08" E) and Shoolpaneshwar Wildlife Sanctuary, Sagai (21°39'41.06" N, 73°48'31.81" E). Fresh specimens i.e. various stages of fruiting body development and fully grown fruiting bodies were collected in sterile polyethylene bag for further study in laboratory. Various stages of fruiting body development were micro-

photographed in its natural habitat. No relevant literature related with this species was available with us; therefore, molecular identification was carried out first and then characteristics of our specimen were compared with available literature.

DNA isolation and PCR

Genomic DNA was extracted using fresh fruiting body of *Clathrus delicatus* Berk. and Broome. Extraction of DNA was carried out using a Plant/Fungi DNA isolation kit (Sigma Cat# E5038) and subsequently PCR reactions were carried out using Veriti® thermal cycler (Applied BioSystems) under the following conditions: 94 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 55 °C for 30 sec and extension at 72 °C for 1:30 min., with a final extension at 72°C for 10 min. The PCR reactions were performed in a 20µl volume containing 1x final concentration of DreamTaq Green PCR

Master mix (Cat# K1081), 50 ng of genomic DNA and 10 pmol of both primers ITS 1 and ITS 4 (White *et al.* 1990). The PCR products were visualized on 2% agarose gel and amplified product was purified using Purelink™ Quick PCR Purification kit (Cat# K310001) and sent for sequencing to Eurofins Genomics India Pvt. Ltd., Bangalore.

Taxonomy of the species: Immature fruiting bodies *i.e.* 'myco-eggs' arise from the thick whitish mycelial mat (Fig. 1A) growing on the dead wood debris of un-identified wood (Fig. 1A-F) and dead bamboos. Volva is pale brown, extremely thin, delicate, enclosing the stipe or lower part of the receptacle, arising from the white hyphal strands (Fig. 1C, D). Fruiting body is small to medium size, soft, smooth, cream-white, initially spherical, oval to elliptical/globose 0.2-0.5 cm (Fig: 1A-C). Mature fruiting body is shuttle cock-shaped, white, without cap and gills with small stipe having thick rhizomorphs (Fig: 2A-C).

Material examined: Ahwa, Dangs, Dharampur, Wilson hill, Shoolpaneshwar and Vansda.

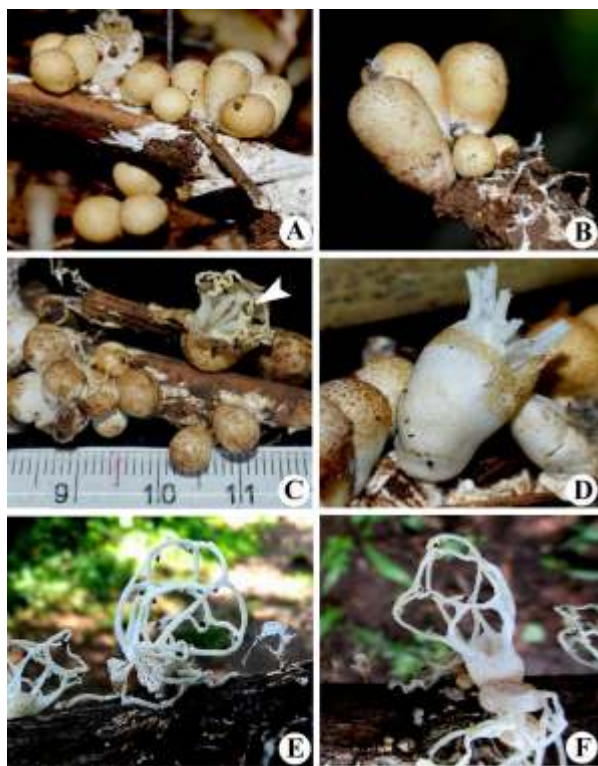


Figure 1(A-F): Various stages of fruiting body development of *Clathrus delicatus*. **A:** Developing fruiting bodies ('myco-eggs' stage) arising from thick whitish mycelial mat. **B:** Fully grown cluster of myco-egg ready to develop into mature fruiting body. **C:** Cluster of myco-eggs and volva (arrowhead) **D:** Volva **E, F:** Expanded receptacle.



Figure 2(A-C): Mature fruiting bodies (receptacles) of *Clathrus delicatus*.

Material was collected by: Ravi Patel, Ajit Vasava, and Kishore Rajput

Edibility: Edibility is not known

Habitat: Dead wood or wood debris of unidentified decaying wood and on the dead bamboo. Fungus shows thick creamy white rhizomorphs on the substrate.

Distribution: Sri Lanka, India

DISCUSSION

Fungi form an indispensable component of forest ecosystem in recycling of organic compounds along with the carbon stored in plant material (Fazio *et al.* 2010). They are adapted to grow on various kinds of substrates such as plant debris rich in lignin, cellulose, hemicellulose, humus soil and even on hairs (Marchisio 2000). During this course of adaptation to grow on different substrates, their fruiting bodies also evolved variously and may survive for a short period (most of the gill fungi) or may be annual or perennial like many of the polypore and phomes. On the basis of their external appearance, they are also classified as earth-tongue, grainy clubs, mushrooms, pimples, cup fungi, false morels (i.e. sponge mushrooms), lorchels, jelly fungi, coral fungi, hydnums (tooth fungi), boletes (fleshy pore fungi), wood crusts, ground warts, gill fungi, stinkhorns, bird nests and so on (McKnight and McKnight 1998).

In the present study we reported one of the stinkhorn, a unique and the most beautiful fungi popularly known as cage-fungi from different forests (*viz.* Ahwa, Dangs, Dharampur, Wilson hill, Shoolpaneshwar and Vansada) of Gujarat state. It is regularly collected from last three years from the above said forest regions and is identified as *Clathrus delicatus* Berk. and Broome from the family Phallaceae. Since the studies on fungal diversity of Gujarat state are meagre (Rajput *et al.* 2015, Vasava *et al.* 2015, 2017; Koyani *et al.* 2016); and the present genus stands as a new distribution record for the Gujarat state. Occurrence of *C. delicatus* has been reported

earlier from Mysore by Narasimahan (1932, Dring 1980). Thereafter, it has been reported by Apte (2005) from Sanjay Gandhi National park, Borivali. Subsequently, it was reported from Bhadra Wildlife Sanctuary of Karnataka state by Swapna *et al.* (2010). Recently, its presence is also documented from Hollongapar Gibbon Wildlife Sanctuary, Jorhat (Assam) by Gogoi and Prakash (2014) and from Arboretum and Botanical Garden of Mangalore University by Pavithra *et al.* (2017). These sporadic reports from India indicate its rare occurrence in the country. Our specimen was compared with all above mentioned Indian reports for the confirmation of the identity.

The generated nucleotide sequence was used for BLAST search in the GenBank database (www.ncbi.nlm.nih.gov) for identification of the fungal species. Identification was done by 99% base pair match of the sequence obtained to the closest available reference sequences (Leung *et al.* 1991). After molecular identification, morpho-taxonomic characteristics of the identified fungal species were compared with the literature (Dring 1980; Apte 2005, Swapna *et al.* 2010, Gogoi and Prakash 2014; Pavithra *et al.* 2017) for the further confirmation of the identity of fungi. Nucleotide sequence of *Clathrus delicatus* was submitted to NCBI database using BankIt sequence submission tool (accession number MF506820). The nucleotide sequence was also submitted to BOLD system to generate DNA barcode (BOLD ID KSRF-0015).

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