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Article

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New distribution record of *Cyathus stercoreus* (Schwein.) De Toni (Nidulariaceae) for India from Gujarat state

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Abstract

Cyathus stercoreus (Schwein.) De Toni (Nidulariaceae) was collected from Shoolpaneshwar Wildlife Sanctuary from the Narmada districts of Gujarat state, India. Present study reports *C. stercoreus* as a new distribution record from the Gujarat state. It was identified by using morphological features and further confirmation was carried out by using molecular methods with its DNA barcoding. Nucleotide sequence is submitted into NCBI and molecular data are submitted into BOLD data system for DNA barcoding.

Key words – *Cyathus* – DNA barcoding – Gujarat – Nidulariaceae

Introduction

The family Nidulariaceae have a gasteroid fruiting body (i.e. spores develop internally, as in an angiocarp) that are typically gregarious. It consists of five genera *viz. Crucibulum, Cyathus, Mycocalia, Nidula,* and *Nidularia.* All of members are distinguished from each other by their morphological characters and peridiole structure (Brodie 1975).

The genus *Cyathus* is easily distinguished from the other members of the family Nidulariaceae by the presence of grey to black peridioles with funicular cords and peridia composed with three layers of tissues (Brodie 1974, Brodie 1975, Brodie & Sharma 1980, Das & Zhao 2012, 2013, Lloyd 1906). The generic name was first introduced by Haller in 1768 and later it's adopted by Persoon (1801). Till date, available literature indicates that nearly 45 species of *Cyathus* are distributed worldwide (Brodie 1967, 1975, Kirk et al. 2008). On the other hand, studies carried out by Das et al. (2014), Sharma (2016) showed that nearly 17 species of *Cyathus* are distributed throughout India. Among them, *Cyathus stercoreus* is commonly known as the dung-loving bird's nest fungi and prefers growing on dung, or soil containing dung. According to Peric & Peric (2006) *C. stercoreus* is listed under the category of endangered species in a number of European countries.

The state Gujarat has different vegetation zone like moist deciduous forest (south Gujarat), dry deciduous to scrub forest in central and north Gujarat. Though the climatic conditions are favourable and unique for the luxurious growth of fungal flora; there are only few sporadic reports on fungal diversity for the state of Gujarat (Assudani et al. 2013, Bhavsar et al. 2012, Dhingani et al. 2013, Koyani et al. 2016, Nagadesi & Arya 2014, Rajput et al. 2015, Vasava et al 2015, 2017). As a part of fungal diversity documentation, present study reports *C. stercoreus* as a new distributional record from Shoolpaneshwar Wildlife Sanctuary (Sagai, Gujarat state).

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Materials & Methods

Collection

Fungal specimen was collected from Shoolpaneshwar Wildlife Sanctuary (Sagai forest, Gujarat), India). Fresh fruiting bodies were collected in sterile polyethylene bag for further taxonomic study. Field photographs were taken through Canon 1200 D. Morpho-taxonomical characters were observed for both fresh and dried sample. Macro-morphological characters of the fruiting bodies were observed under Leica stereo zoom Microscope. Distinguishing morphological and microscopical structures were noted using Leica DM 2000 trinocular research microscope. Mycelial characters as well distinctive features of fruiting body were studied and compared for identification with available literature (Das et al. 2014, Martin 1927, Sharma 2016, Thind 1977, Thind et al. 1984). Fruiting bodies were inoculated on PDA medium for mycelium development which will be further utilized for molecular identification of the species.

DNA isolation and PCR

Genomic DNA were extracted from fresh fruiting body and established pure culture of *Cyathus stercoreus* by using a Plant/Fungi DNA isolation kit (Sigma Cat# E5038). 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 universal primers ITS 1 and ITS 4 (White et al. 1990). PCR reactions were carried out using Veriti® thermal cycler (Applied BioSystems) under the following conditions: initial 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 product was visualized on 2% agarose gel and amplified PCR product was purified using PurelinkTM Quick PCR Purification kit (Cat# K310001).

DNA sequencing

Purified PCR product was sent for DNA sequencing to Eurrofins Genomics India Pvt. Ltd., Bangalore. The obtained sequences were compared with sequences available in the NCBI database using the Basic Local Alignment Search Tool. BOLD data system was used for generate DNA barcodes for nucleotides sequences.

Results and Discussion

Studies on fungal diversity of Gujarat state are rare, though it has several applications in wellbeing of the human being such as: source of various enzymes, bioremediation of xenobiotic compounds, in recycling of carbon stored in the form of cell wall constituents of plants (Wikclow et al.1980, Halsall 1993, Akin et al.1995, Kang et al.2007, 2008, Bhatt et al. 2016). Based on the various enzymatic studies, *C. stercoreus* is considered to be efficiently capable for the production of lignin and cellulose degrading enzymes (Halsall 1993, Akin et al. 1995, Sethuraman et al. 1999); antioxidant compounds (Kang et al. 2007, 2008); and also reported as a folk medicine (Bo & Bu 1980).

Present study documents occurrence of *C. stercoreus* for the first time from Gujarat state. Its pure culture was successfully established on PDA medium (Fig. 1A, B) and maintained at 4°C for further study. Morpho-taxonomic features of the species is as follows:

Cyathus stercoreus (Schwein.) De Toni

Nest up to 1.5 cm tall and 4–9 mm wide, cone-shaped or goblet-shaped, outer surface brownish, shaggy and hairy (Fig. 2A–C), inner surface lead-grey to black, smooth, bald, shiny, containing tiny blackish eggs (Figs. 1C–F, 2A–C).

Peridioles 1–2 mm in diam., lens-shaped, dark grey to black, attached to the nest by funicular cords (Fig. 1C). Funicular cord is attached at one end to the peridiole and at the other end to an entangled mass of hyphae i.e. hapteron (Fig. 1E, F). The spores are smooth, thick walled globose to oval and hyaline in outline.

Edibility – Inedible.

Occurrence and substrate – July to October, grows on dung of herbivores, humus rich soil and wood chips.

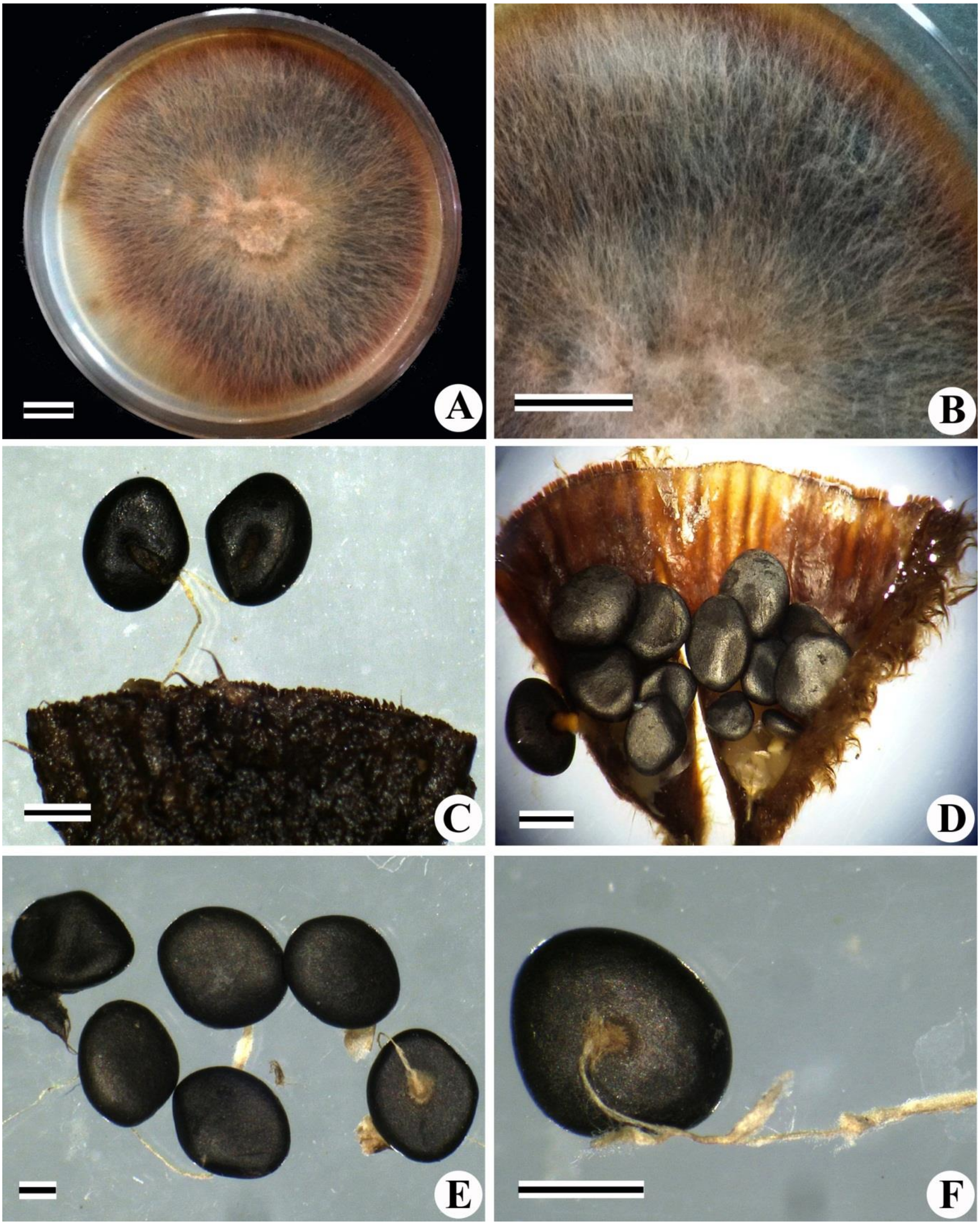

Fig. 1 – *Cyathus stercoreus*. A, B Mycelia cultures on PDA plate. C, D Section through longitudinal line of cup showing attached peridioles. E, F Peridioles with funicular cords Scale bars: A, B = 1 cm, C, D, E, F = 1 mm.

Distribution – India, West Indies, Europe, New Zealand, Hawaii, Hawaiian Islands, United States and Canada. Although it is widespread in the tropics as well as in the temperate zones (Ahmad 1942, Brodie 1975, Das et al. 2014, Kirk et al. 2008, Peric & Peric 2006).

Material examined – INDIA: Gujarat, Narmada districts, Shoolpaneshwar Wildlife Sanctuary (21°41'28.24" N & 73°46'32.30" E), 30-Aug-2014, Kishore Rajput, Ravi Patel, Ajit Vasava, voucher number: KSR0002.

Fig. 2 – *Cyathus stercoreus*. A, B, C Morphology of fruiting bodies growing on dung. Scale bars: A, B, C = 5 mm.

Molecular Data

The generated nucleotide sequence was used for BLAST search in the GenBank database (www.ncbi.nlm.nih.gov) for further confirmation of the species. Identification was done on the basis of 99% base pair match of the sequence obtained with the maximum scores for identity and Query coverage of the reference sequences from the NCBI database. Isolate KSRF-0002 of present study covered the maximum scores with 96% of identity and 95% of query coverage with *Cyathus stercoreus* (Accession no. KY706156) submitted by CRJ Hay 2017 as well as sequences deposited by the other authors *viz.* accession no FJ478125, KT365517, EU784192, EU784193 etc. Along with these mentioned sequences submitted by the other authors for *C. stercoreous*; When compared with above mentioned sequences, sequence generated in the present investigation showed 93 % base pair match with *C. striatus.* Therefore, macro-morphological and microscopic features were compared with available literature (Martin 1927, Sharma 1982, 2016, Thind 1977, Thind 2005, Thind et al. 1984) for the accurate identification.

After the analysis of morpho-taxonomic features, it was observed that present specimens differ from *C. striatus* by presence of dark black peridiole; length wise fruiting body was not plicate, while size of the basidiospores much larger in *C. strecoreus*. All these morphological features are characteristic of the species *C. strecoreus.* Molecular confirmation also showed up to 95 to 96 percent match with *C. stercoreus*. Therefore, we conclude that present specimen is *C. stercoreus* and not *C. striatus*. Nucleotide sequence of *C. stercoreus* generated in the present study are submitted to NCBI database (accession no. MF506822, MH543350) and to BOLD Data system to generate DNA barcode for *C. stercoreus* (BOLD ID KSRF-0002).

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