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Morpho-Molecular characterization of *Beltrania pseudorhombica* Crous & Y. Zhang: A new addition to mycoflora of India

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ABSTRACT

As a part of studying "Diversity of Terrestrial Litter Fungi of Northern Western Ghats of India", an interesting species of genus *Beltrania* was recorded during a field survey of protected areas of Nashik, Maharashtra. After morpho-molecular analysis, the species was identified as *Beltrania pseudorhombica* Crous & Y. Zhang 2014, which was found to be a new record to Indian mycoflora.

Key words: Asexual morph, ITS, Morpho-molecular, Maharashtra.

INTRODUCTION

The genus *Beltrania* was established with the type species *B. rhombica* found on *Citrus limonum* by Penzig (1882) in Italy. Subsequently, Saccardo (1886) established the tribe Beltranieae within the didymosporous Dematieae Fr. (or Dematiaceae Fr. sensu Saccardo 1889) to accommodate *Beltrania* Penzig, represented by two species, *B. rhombica* Penzig and *B. querna* Harkness. Nannizzi in 1934 established family Beltraniaceae to accommodate the single genus *Beltrania.* Later on, many genera such as *Beltrania*, *Beltraniella*, *Beltraniomyces*, *Beltraniopsis*, *Parapleurotheciopsis*,*Porobeltraniella*, *Pseudobeltrania* and *Subramaniomyces* (Maharachchikumbura et al. 2016), *Hemibeltrania* (Rajeshkumar *et al.* 2016), Subsessila (Lin *et al.* 2017) were added in family *Beltraniaceae.* The genus *Beltrania* is characterized by having pigmented, unbranched setae, and basal conidiophores that give rise to conidiogenous cells that proliferate sympodially by means of short protruding denticles, giving rise to separating cells and conidia that are brown, biconic, with an equatorial band of lighter pigment and a single apical appendage (Seifert et al. 2011). The members of the genus has very conspicuous having V- shaped or Kite shaped conidia which are with appendages while sometimes without appendage. This is saprophytic genera, found in dead leaves, other plant parts, air and soil. Very less members of this genus are known, because the members of this genera would like to grow under the leaf litter and collectors failed to notice them. Presently, there are 29 legitimate species of *Beltrania* are listed in Index Fungorum, 2022. During a project on studying diversity of Terrestrial Litter Fungi of Northern Western Ghats of India, an interesting fungus was collected, from Vaitarna Reservoir, Nashik, Maharashtra, India. Exhaustive morphological and molecular studies revealed that the new collection is *Beltrania pseudorhombica* Crous & Y. Zhang, 2014. Review of relevant works revealed that this is the first report of *B. pseudorhombica* from India.

MATERALS AND METHODS

Fungal isolation and morphological characterization

Fallen Leaf litter infested with the fungus were collected from Vaitarna Reservoir, Dhargaon Dist., Nashik, Maharashtra. After hand sections, the samples were subjected to Particle plating Method (Bills & Polishook (1994)), the fungal living culture SM-5 was

isolated and grown on Potato Dextrose Agar (PDA) medium. For microscopic details, the slides prepared in lactophenol-cotton blue were observed under OLYMPUS - CX41 microscope supported with digital camera and finally photomicrographs were captured.

DNA extraction, PCR amplification, and DNA sequencing

Fungal species were fully-fledged grown for 15 days in dark at 25 °C on PDA medium. The purity of fungi was guaranteed before scheduled for DNA isolation. The fungal mycelia/mass was collected into micro centrifuge tube by scrapping it using sterile blade under laminar air flow. The weighed fungal tissue was powdered by using a mortar and pestle in liquid nitrogen. HiPurA Fungal DNA Purification Kit (HiMedia, India) was used for extraction of Genomic DNA from the growing mycelia as per manufacturer's instructions. After DNA isolation, PCR amplification was conducted (using SimpliAmp Thermal cycler, Applied Biosystems, USA) with primer pair ITS4 and ITS5 to amplify the 5.8S rRNA gene and flanking internal transcribed spacer regions (ITS) (White et al. 1990). Polymerase Chain Reaction (PCR) was conducted in a 40 µl reaction mixture for 30 consecutive cycles. Finally, the amplified product is exposed to 1% Agarose gel electrophoresis. The amplified PCR products were studied by electrophoresis in 0.8 % (W/V) agarose gel in 1X TAE (Tris-acetate-EDTA) buffer (0.4 M Tris, 10 mM EDTA, 50 mM NaOAc, pH 7.8) at 65 V and after staining with ethidium bromide (0.5 µg ml–1) it was visualized under UV light using E-Gel Doc Molecular Imager (Thermo Fischer Scientific, UK). Later on Hi-PurA PCR Product Purification Kit (HiMedia, India) was used to purify the amplified PCR products as per manufacturer's instructions. The purified PCR products were rechecked using agarose gel electrophoresis and was submitted for sequencing to Avanira Biotech Pvt. Limited, Pune, India.

Phylogeny

The ITS sequence of the fungal isolate SM5 was used to confirm the identification of fungal species. The MegaBLAST search algorithm were used to analyse the sequence chromatograms and allied reference sequences of already known taxa of *Beltrania* were recovered from National Center for Biotechnology Information (NCBI) for phylogenetic analysis. *Polyscytalum vaccinii* CBS 148446 and *Kirstenboschia diospyri* CBS 134911 were selected as an outgroup taxa. All the sequence generated in this study was aligned using muscle and manually adjusted using BioEdit v.5 wherever necessary. The concatenated file contained sequence data of 29 taxa. Model K2+G (Kimura 2 parameter + Gamma distribution) resulted as a best-fit model out of 32 models tested and was preferred on the basis of the Bayesian information criterion (BIC). The maximum likelihood method was used to infer the phylogeny. Tree branches were tested based on 1000 ultrafast bootstrap (UFBoot) support replicates as well as with SH-like approximate likelihood ratio test (SH-like aLRT) with 1000 replicates. The newly formed 496 bp genomic sequence from this study was deposited in NCBI GeneBank with no ON668274.1.

RESULTS

Phylogenetic analysis

Using MegaBLAST search on NCBI GenBank nucleotide database by means of the ITS gene sequence, the closest hit was with NR_148074.1:89-581 *Beltrania pseudorhombica* CBS 138003 (Accession NR_148074.1) showing 99 % identity query cover. Thus, phylogenetic analysis using the ITS region (Fig. 1) showed the similarities between the study sample *Beltrania pseudorhombica* isolate SM 5 (Accession no. ON668274.1) and *Beltrania pseudorhombica* CBS 138003 (Accession NR_148074.1).

TAXONOMY

***Beltrania pseudorhombica* Crous & Y. Zhang**

Colonies reaching 70 mm diam after 2 wk at 22 °C. On MEA spreading, with fluffy aerial mycelium and lobate margins; surface and reverse dirty white. setae dark brown, erect, thick-walled, indistinctly septate, straight to somewhat flexuous, tapering to an acute apex, up to 5-septate, 145–225 × 4–5 µm, with lobed basal cell, 9–12 µm diam. Conidiophores smooth, 2–3-septate, medium brown, erect, unbranched, 30–50 × 4–5 µm. Conidiogenous cells terminal, pale brown, smooth, polyblastic with several denticles, 1.5–2 µm. Separating cells pale brown, finely roughened, 7–12 × 5–6 µm, with several apical, flat-tipped denticles, 1 µm diam. Conidia solitary, biconic, pale brown, aseptate, with a distinct median transverse band of lighter pigment, 22–25× 8–9 µm, apical appendage 7–11 × 1 µm, tapering to an acutely rounded tip (Fig.2).

Material examined – India, Maharashtra, Vaitarna Reservoir, Dhargaon Dist., Nashik, Maharashtra, 19°47'42.0"N 73°31'20.3"E, Leaf litter, 02.7.2019, Rashmi Dubey, BSI-NW-2, living culture SM-5, GenBank number Accession no (ITS)– ON668274.1

Notes: The strain reported in this study is an asexual morph. The review of literature (Ellis 1971, Crous *et al.* 2014, Pirozynski 1970) reveals that this is the first report of *Beltrania pseudorhombica* from India and is a new addition to the mycoflora of India.

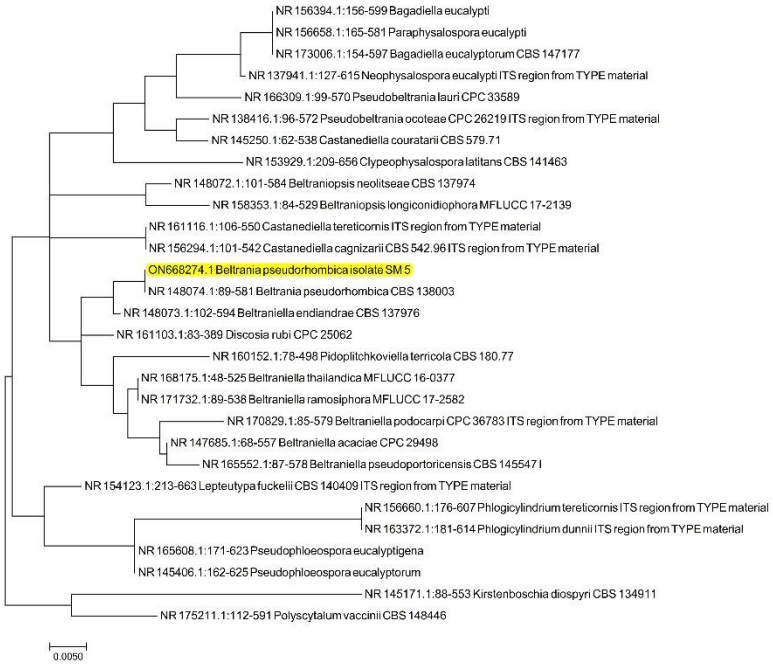

Figure.1 Molecular Phylogenetic analysis by Maximum Likelihood method

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model. The tree with the highest log likelihood (-738.14) is shown. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 29 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 235 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar et al. 2016).

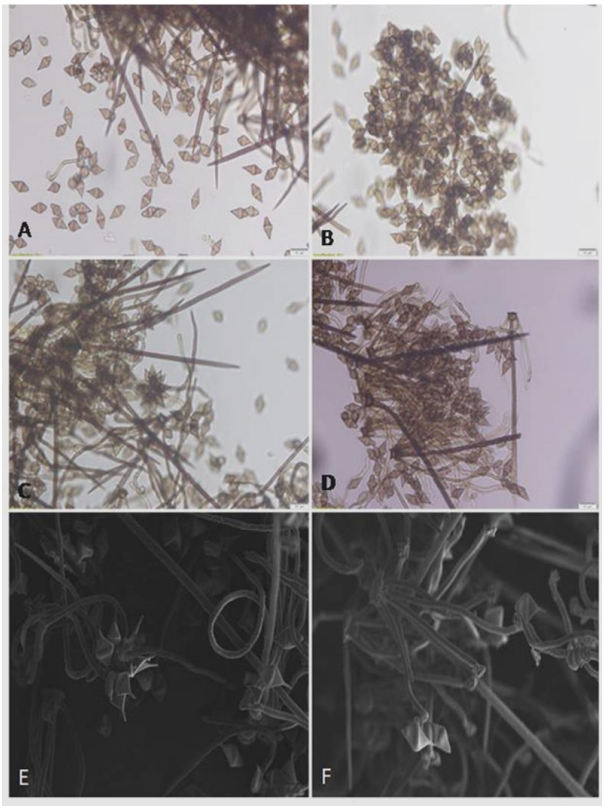

Fig. 2: *Beltrania pseudorhombica*: A-D Conidia and Conidiophores with long setae ; E-F: SEM Images (Scale bar A,B,C,D=20 µm

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