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Neopestalotiopsis eucalypticola (Pestalotiopsidaceae, Ascomycota): A new record to Indian mycoflora

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As a part of studying "Microfungi of Sanjay Gandhi National Park", an interesting species of genus *Neopestalotiopsis* was recorded during a field survey of Sanjay Gandhi National Park, Mumbai, Maharashtra. After morpho-molecular analysis, the species was identified as *Neopestalotiopsis eucalypticola* Maharachch., *et al* 2014, which was found to be a new record to Indian mycoflora. A detailed morphological description supported by a DNA sequence phylogram based on Maximum likelihood (ML) and Bayesian posterior probabilities (BPP) analysis, photo-illustrations are provided for this interesting species.

Key words: Asexual morph, ITS, Morpho-molecular, National Park

INTRODUCTION

Pestalotia (Pestalotiopsidaceae, Ascomycota) infect a wide range of typically leafy plants on which they are associated with many different disease symptoms. Steyaert (1949) splited *Pestalotia* into three genera, namely *Pestalotia, Pestalotiopsis* and *Truncatella* based on the conidial forms. *Pestalotiopsis* has been divided into additional sections, namely Monosetulatae, Bisetulatae, Trisetulatae and Multisetulatae, based on the number of apical appendages. Maharachchikumbura *et al.* (2014), based on combined morphological and molecular data of 91 *Pestalotiopsis* isolates segregated two novel genera, namely *Neopestalotiopsis* and *Pseudopestalotiopsis* with types *Neopestalotiopsis protearum* and *Pseudopestalotiopsis theae*, respectively under family Pestalotiopsidacea.

Neopestalotiopsis can easily be distinguished from *Pestalotiopsis* by its five-celled, fusiform conidia, with versicolorous median cells.Updated data of per Index fungorum 2022, reveals the existence of 72 species under the genus *Neopestalotiopsis*. During a study of microfungi of Sanjay Gandhi National Park, Mumbai, along with the collection and identification of other fungi, *Neopestalotiopsis eucalypticola* Maharachch., *et al.* 2014, was also described which was found to be a new record to Indian Mycoflora.

MATERIALS AND METHODS

Fungal isolation and morphological characterization

The fallen Palm fruit were collected from Sanjay Gandhi National Parkand were subjected to particle filtration method. The morphological features of each colony, viz. colour, growth rate, texture, and mycelial form were noted and recorded. Colonies on PDA reaching 6 cm diameter at 25°C were observed after six days with crenate edge, whitish, and some aerial mycelium on surface (Fig. 1). Pycnidial conidiomata, black, were embedded or semi-immersed, solitary or aggregated, globose up to 350 ìm diameter, exuding globose and black conidial masses. For microscopic details, the slides prepared in lactophenol-cotton blue were observed under OLYMPUS microscope CX41 supported with digital camera and finally photomicrographs were captured.

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DNA extraction, PCR amplification, and DNA sequencing

HiPurA Fungal DNA Purification Kit (Hi Media, India) was used for extraction of Genomic DNA from the growing mycelia as per manufacturer's instructions. 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 5.8S rRNA gene and flanking internal transcribed spacer regions (ITS) of a SM 1 was amplified using primer pair ITS4 and ITS5 for ITS regions using Simpli Amp Thermal cycler, Applied Biosystems, USA). 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 at 65 V and after staining with ethidium bromide (0.5 µg/ml) 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 submitted for sequencing to Avanira Biotech Pvt. Limited, Pune, India.

Phylogenetic analysis

The ITS sequence of the fungal isolate SM 1 was used to confirm the identification of fungal species. The Mega BLAST search algorithm were used to examine the sequence chromatograms and allied reference sequences of already known taxa of *Neopestalotiopsis* were recovered from National Center for Biotechnology Information (NCBI) for phylogenetic analysis. Resultant chromatograms were patterned with BioEdit v.5 to confirm sequence quality. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-parameter model (Kimura, 1980). *Amphisphaeria umbrina* (Fr.) De Not.1863 (NCBI accession no.AF009805.1) was selected as an outgroup taxon. The analysis involved 30 nucleotide sequences. All positions containing gaps and missing data were eliminated. There was a total of 354 positions in the final dataset. Evolutionary analyses were conducted in MEGA7 (Kumar *et al.* 2016). The concatenated file contained sequence data of 28 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 523 bp sequence from this study was deposited in NCBI GeneBank with no ON

RESULTS AND DISCUSSION

Neopestalotiopsis eucalypticola

Maharachch., K.D. Hyde &Crous 2014

On PDA, conidiomata pycnidia in culture, globose, solitary or aggregate, partially immersed and totally immersed, black, 200–500 ìm in diameter; exuding viscous black and globose conidial masses; indistinct conidiophores, often reduced to conidiogenous cells; conidiogenous cells single, discrete, ampulliform to lageniform, hyaline, thin-walled, 2–4.5 × 9.0–20.5 µm; conidiumfusoid, ellipsoid, straight to slightly curved, 5 cells (4 septa); 19–29 × 6.5–8.0 µm, basal cell conic to obconic with a truncate base, hyaline, rough and thin-walled, 2.0–3.5 × 4.2– 6.05 µm in length; three medial cells light brown, septum darker than the rest of the cell (second cell from the base, 4.5–5.0 × 4.2 – 5.0 µm in length; third cell: 5.0 – 5.5 × 5.0–6.0 µm in length, fourth cell: 3.0–5.0 × 3.0–5.0 µm in length); apical cell 6.0–3.0 × 3.0–5.5 µm in length, hyaline, sub-cylindrical to obconical, thin-walled; with 2–4 apical tubular appendages, arising from the apical crest, unbranched, filiform, flexuose, 12–27 µm in length; basal appendix single, tubular, unbranched, centered, 3–7 µm in length. Conidiophores reduced to conidiogenous cells. Conidiogenous cells discrete, ampulliform to lageniform, hyaline, smooth, thin-walled, simple, proliferating up to several times percurrently, 3–10 × 2–8 µm, opening 2–6 µm diam. Conidia fusoid, ellipsoid, straight to slightly curved, 4-septate, 18.5 –25 × 6.5–7.0 µm, basal cell conic to obconic with a truncate base, hyaline, rugose and thin walled, 5–9 µm long; three median cells doliiform, 16–19.0 µm long, wall rugose, versicolored, septa darker than the rest of the cell (second cell from the base pale brown, 5–7 µm long; third cell darker brown, 4.5–6.5 µm long; fourth cell darker brown, 4.9–5

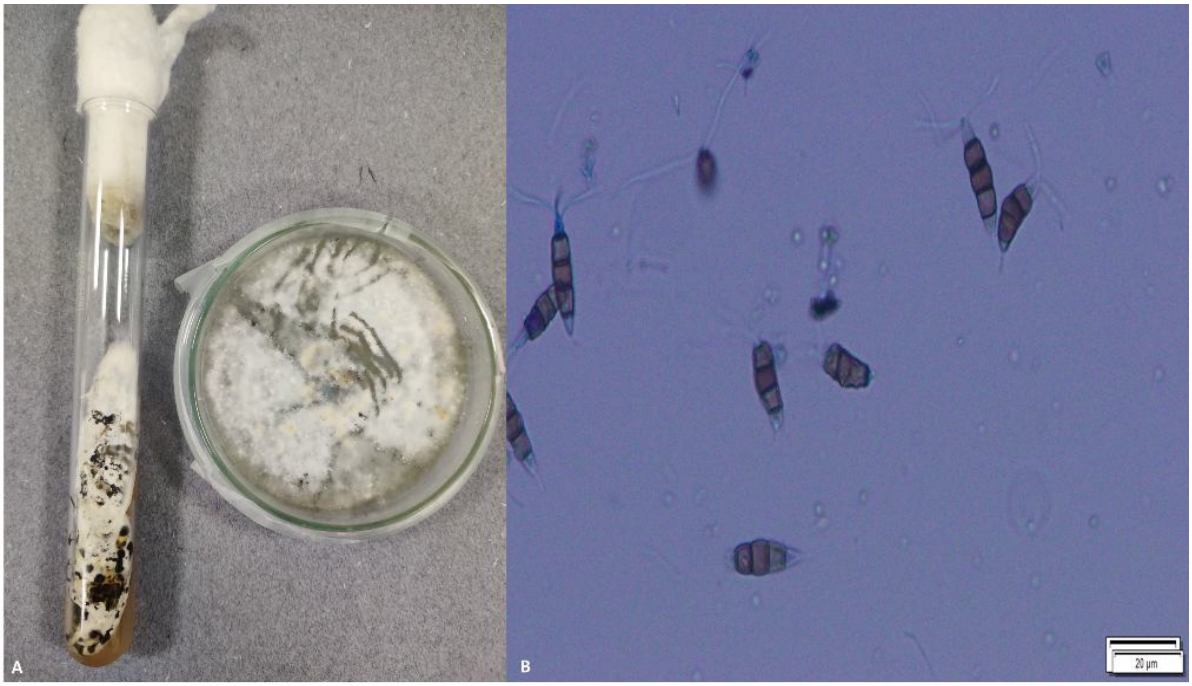

Fig.1 *Neopestalotiopsis eucalypticola* A. Fungal colonies on PDA; B. Conidia and conidiophores (Scale bar- B =20 µm)

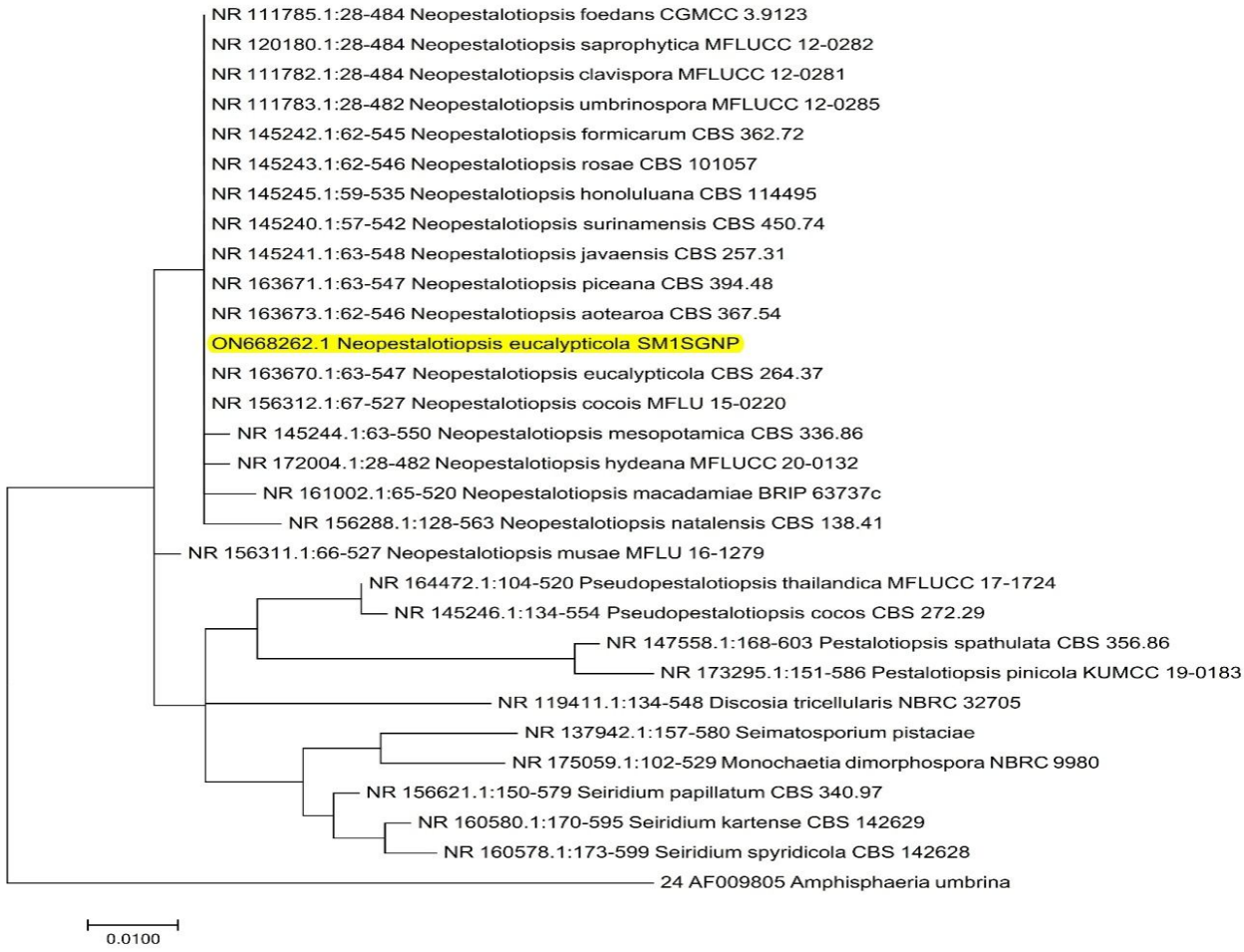

Fig. 2 : Molecular Phylogenetic analysis by Maximum Likelihood method

ìm long; apical cell 4.5–7.5 μm long, hyaline, cylindrical to subcylindrical, rugose and thin-walled; with 1–2 tubular apical appendages, arising as an extension of the apical cell, unbranched, attenuated, flexuous, 32–40 μm; long, basal appendage single, tubular, unbranched, centric, 6–9 μm long.

Phylogenetic analysis

Using Mega BLAST search on NCBI GenBank nucleotide database by means of the ITS gene sequence, the closest hit was with *Neopestalotiopsis eucalypticola* CBS 264.37 (Gene Bank Acession no.NR_163670.1) showing 100 % identity query cover. Thus, phylogenetic analysis using the ITS region (Fig. 2) showed the similarities between the study sample *Neopestalotiopsis eucalypticola* CBS 264.37. Based on these morphological and molecular criteria the fungus was identified as *Neopestalotiopsis eucalypticola* Maharachch., K.D. Hyde &Crous, 2014.

Material examined –On Palm fruit litter, Nagla Block, North of Vasai Creek, Yeoor Range [North], Sanjay Gandhi National Park, Palghar Dist., Maharashtra, India, date 10/09/2016, RD, 210611 BSI (WC), Accession no. BSI-F674, living culture SM 1. GenBank number ITS– ON628262.1

Notes: The isolate (SM 1, GenBank Accession no. ITS: ON628262.1) from this study clustered with the ex-type of *Neopestalotiopsis eucalypticola* CBS 264.37 (Gene Bank Accession no.NR_163670.1 (Fig. 2). The strain reported in this study is an asexual morph. A comparison of the ITS sequences of the 30 nucleotide sequences shows 100 closest similarities with *Neopestalotiopsis eucalypticola* CBS 264.37 (Gene Bank Acession no.NR_163670.1).

The review of literature (Bilgrami *et al.* 1979, 1981, 1991; Jamaluddin, 2004; Manoharachary *et al*. 2022) reveals that this is the first report of *Neopestalotiopsis eucalypticola f*rom India and is a new addition to the mycoflora of India.

REFERENCES

Bilgrami, K.S., Jamaluddin, Rizwi, M.A.1979. *The Fungi of India. Part I* (List and References). Today and tomorrow's printers and Publishers, New Delhi. 467pp.

Bilgrami, K.S., Jamaluddin, Rizwi, M.A. 1981. *The Fungi of India. Part II* (Host Index and Addenda). Today and tomorrow's Printer and Publishers. New Delhi. 128pp.

Bilgrami, K.S., Jamaluddin, Rizwi, M.A. 1991. The Fungi of India. Part III (List and References). Today and Tomorrow's Printer and Publishers, New Delhi. 798pp.

Jamaluddin, N.G., Goswami, M.G., Ojha, B.M. 2004. *Fungi of India 1989-2001*, Jodhpur, Scientific publishers- VII.326 pp.

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**:111-120.

Kumar, S., Stecher, G., Tamura K. 2016 . MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **33**:1870-1874.

Maharachchikumbura, S.S.N., Hyde, K.D., Groenewald, J.Z., Xu, J., Crous, P.W. 2014. *Pestalotiopsis* revisited. *Studies in Mycol.* **79**: 121-186.

Manoharachary, C., Atri, N.S., Prameela Devi, T., Kamil, D., Singh. S.K .Singh, AP. 2022. Bilgrami's Fungi of India List and References (1988-2020) ISBN 13: 9789391734411.