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Article

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Morphology and phylogeny of root-endophytic fungus *Periconia igniaria*

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Abstract

Periconia igniaria, isolated as an endophytic fungus from roots of *Cheilocostus speciosus* from Calangute, Bardez, Goa, India was described in this manuscript. The identity of the endophytic isolate was based on asexual-morphs, cultural characteristics and phylogenetic analyses of internal transcribed spacer rDNA sequence data. To our knowledge, this is the first report of this taxon as root endophyte of *Cheilocostus speciosus* from Western Ghats forests, Goa, India.

Key words – *Cheilocostus speciosus* – Goa – Taxonomy – Western Ghats

Introduction

During our studies on endophytes associated with medicinal plants from the Western Ghats forests of Goa, India, a number of rare and interesting microfungi were isolated (D'Souza & Bhat 2002, 2012). In the present study, a culture was obtained as an endophyte *Cheilocostus speciosus* (J. Koenig) C.D. Specht. *Cheilocostus speciosus* is an important medicinal as well as ornamental plant cultivated in India belonging to Costaceae (Zingiberaceae). It is a perennial, succulent, herbaceous plant, thick creeping rhizomes with height 120-300 cm. It possesses numerous pharmacological activities like antibacterial, antifungal, anticholineesterase, antihelmentic, antioxidant, anti-inflammatory, analgesic, antipyretic, antihyperglycemic, antistress, larvicidal, diuretic and estrogenic (Pawar & Pawar 2014, Waisundara et al. 2015, Choudhury & Sarma 2016, Khayyat et al. 2017). On careful morphological examination, the species was identified as *Periconia igniaria* E.W. Mason & M.B. Ellis. Review of literature reveals that *P. igniaria* has already been reported from India as an anamorphic form of *Massarina igniaria* (C. Booth) Aptroot. However, earlier this taxon was described merely based on morphological and cultural characteristics (Booth 1968) and as plant pathogen.

Therefore, considering a rare taxonomic record from the region, the present taxon is re-described and taxonomy is re-validated based on morphological and cultural characteristics and ITS sequence-data along with its phylogenetic analysis.

Materials & Methods

Sample collection and isolation of endophytic fungi

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Periconia igniaria was isolated as an endophyte from fresh asymptomatic roots of *Cheilocostus speciosus* from the Calangute, Bardez, Goa (15.5311° N, 73.7625° E). The samples were collected, excess moisture was removed, then placed in sealed plastic bags and later stored at 4°C until isolation. The endophytic fungi were isolated using 'Three-step surface sterilization' technique which was described by Petrini (1986) and adapted from Doilom et al. (2018). Briefly, plant samples were surface-sterilized with 70% ethanol for 1 min, 4% sodium hypochlorite for 3 min and again with 70% ethanol for 30 sec, followed by thorough washing with sterile distilled water. After air-drying in a laminar-flow hood the root, stem and leaves were cut separately into pieces with sterile blade and transferred onto 2% MEA (malt extract agar) at pH 5.5, which was used for isolation of endophyte in pure cultures. The isolation medium was incorporated with a cocktail of antibiotics (0.02 g/l each of Penicillin G and Streptomycin sulphate) to inhibit bacterial growth. The effectiveness of sterilization was double checked by following method of Schulz et al. (1993). After inoculating, the plates were labeled, incubated at 28°C for 3–7 days to observe the emerging mycelial growth and the pure cultures of the fungal isolates were raised. The developing colonies, emerged from the margins of root pieces were aseptically transferred onto fresh MEA plates (Bhat 2010). The emerging endophytes were studied in details. A pure endophytic culture is deposited and accessioned in National Fungal Culture Collection of India (NFCCI 4748).

Microscopic observations and photomicrography

Examination of the inoculated samples to locate and subsequently to isolate growing colonies was done using an Olympus SZX7 stereomicroscope. Fungal fruiting bodies were mounted in lactophenol-cotton blue and observed using a light microscope. Micromorphology was studied using an Olympus CX21iFS1 compound microscope and photomicrographs were taken with a Microscopic Digital Camera MagCam Series DC 10 fitted to the microscope. Both microscopic and cultural characters of the fungal isolates were recorded. Morphology based identification was done using standard literature/taxonomic keys (Ellis 1971, 1976, Matsushima 1971, 1975 Seifert et al. 2011).

DNA extraction and polymerase chain reaction (PCR)

DNA isolation, PCR amplification and sequencing were carried out at Agharkar Research Institute Pune, Maharashtra. Genomic DNA was isolated from pure colony grown on PDA Petri-plate incubated for seven days following DNA extraction protocol using FastPrep®24 tissue homogenizer (MP Biomedicals GmbH, Germany) (Aamir et al. 2015).

The amplification of internal transcribed spacer region 1, 5.8 ribosomal RNA gene and internal transcribed spacer region 2 was achieved using the primers ITS 4 and ITS 5 (White et al. 1990) using Applied Biosystems ProFlex PCR System. PCR was performed in a 25 µl reaction using 2 µl template DNA (10–20 ng), 0.5 U Taq DNA polymerase (Genei, Bangalore, India), 2.5 µl 10X Taq DNA polymerase buffer, 0.5 µl 200 µM of each dNTP (Genei, Bangalore, India), 1µl of 10 pmol primer, H_2O (Sterile Ultra Pure Water, Sigma) qsp 25 µl. The thermo-cycling conditions involved an initial denaturation at 94°C for 4 min, followed by 35 cycles of 1 min at 94°C, 30 sec at 52°C, 1 min at 72°C and a final extension at 72°C for 8 min. The PCR amplicons were purified using FavorPrep™ PCR purification Kit as per manufacturer's instructions. Purified PCR products of these marker genes were checked on 1.2% agarose electrophoresis gels stained with ethidium bromide and were subjected to direct sequencing using BigDye®Terminator v3.1 Cycle sequencing Kit and ABI 3100 DNA analyzer (Perkin Elmer, Applied Biosystems, Foster City, CA, USA). Sequences were submitted in NCBI GenBank (accession numbers MN417116 (ITS)).

Molecular phylogeny

The sequences were analyzed using Megablast search algorithm and sequences of related strains were retrieved from NCBI and aligned along with sequences of *P. igniaria* using MAFFT (Katoh et al. 2009). *Suillus americanus* (TJB-7683) was selected as the outgroup taxon. A maximum likelihood tree based on multiple sequence alignment of ITS gene region was

constructed using MEGA 7 with 1000 bootstrap replications (Kumar et al. 2016). A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.2970)). The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model (Kimura 1980) (Fig. 2).

Results

Taxonomy

Periconia igniaria E.W. Mason & M.B. Ellis, Mycol. Pap. 56: 104. (1953) Fig. 1

= *Didymosphaeria igniaria* C. Booth, Trans. Br. mycol. Soc. 51(5): 803 (1968)

= *Lophiostoma igniarium* (C. Booth) Aptroot & K.D. Hyde, in Hyde, Wong & Aptroot, Fungal Diversity Res. Ser. 7: 107 (2002)

= *Massarina igniaria* (C. Booth) Aptroot, Nova Hedwigia 66: 117 (1998)

Colonies on MEA effuse, generally woolly producing a typical rose-madder or vinaceous pigment in centre periphery white with irregular margin attaining a diam., 6.5 cm. in 7 days at 28°C. *Mycelium* 1.5–2 µm wide, partly immersed and superficial, composed of hyaline, smooth, branched, thin-walled hyphae. *Hyphae* swollen, brownish, very coarsely warted or encrusted at conidiophore base. *Conidiophores* macronematous, up to 550 µm long, 9–13 µm wide at the base, 6–10 µm wide, smooth, 4–8-septate, immediately below the head bearing shorter branches or stipes. *Stipes* erect, stout, branched, pale brown, smooth-walled, showing a bead like appearance. *Conidiogenous cells* arising mostly directly from the stipe, spherical or subspherical, pale to mid brown, smooth-walled, bearing simple or branched chains of conidia, which form loose heads, borne apically and laterally on the stipes. *Conidia* 7–11 µm diam., blastic, develop acropetally, verruculose to echinulate, spherical, dark brown to black, one-celled, thick-walled.

Material examined – India, Goa, Calangute, Bardez taluka, from fresh asymptomatic roots of *Cheilocostus speciosus*, 8 November 2018, Maria A. D'Souza, (living culture NFCCI 4748, new host record).

Distribution and ecology – The anamorphic form, *Periconia igniaria*, is known from a variety of substrata including soil, leaf litter and grasses. It is reported from British Isles, Great Britain on scorched leaves of *Phalaris arundinacea* (Gramineae) (holotype IMI 9758), Upington, Northern Cape Province, South Africa from seed of *Medicago sativa* (Fabaceae) and Taman, Russia from diseased yellow starthistle (YST; *Centaurea solstitialis*) (Mason & Ellis 1953, Marasas & Van der Westhuizen 1971, Kolomiets et al. 2008). Moreover, other specimens reported contain anamorph (including living culture CBS 845.96 from Papua New Guinea and numerous dried cultures in IMI) (Tanaka et al. 2015)

Phylogenetic analyses

The ITS sequence alignment was used to confirm species resolution for the present isolate. The evolutionary history was inferred by using the maximum likelihood method based on the Kimura 2-parameter model (Kimura 1980). The tree with the highest log likelihood (-2843.2998) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches (Fig. 2). The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 25 taxa (Table 1). There was a total of 497 positions in the final dataset (Fig. 2). Phylogenetic analysis placed the present isolate in a position allied to *Periconia iginaria* in phylogenetic tree, within genus *Periconia*, Class *Pleosporales* and Family *Periconiaceae*. A dataset of two families *Periconiaceae* and *Massarinaceae* from order *Pleosporales* was used in the study. *Suillus americanus* (Boletales) from *Suillaceae* family was selected as an outgroup taxon. Upon analysis of the sequence data, it was observed that the present isolate is similar to *P. igniaria* (CBS 282.67) and *P. igniaria* (EU367468) with 87% bootstrap support, thus confirming its identity. This forms an interesting new host record from Goa India.

However, *P igniaria* was earlier reported as a plant pathogen from British Isles (Mason & Ellis 1953), while the present isolate is obtained as root endophyte of *Cheilocostus speciosus.*

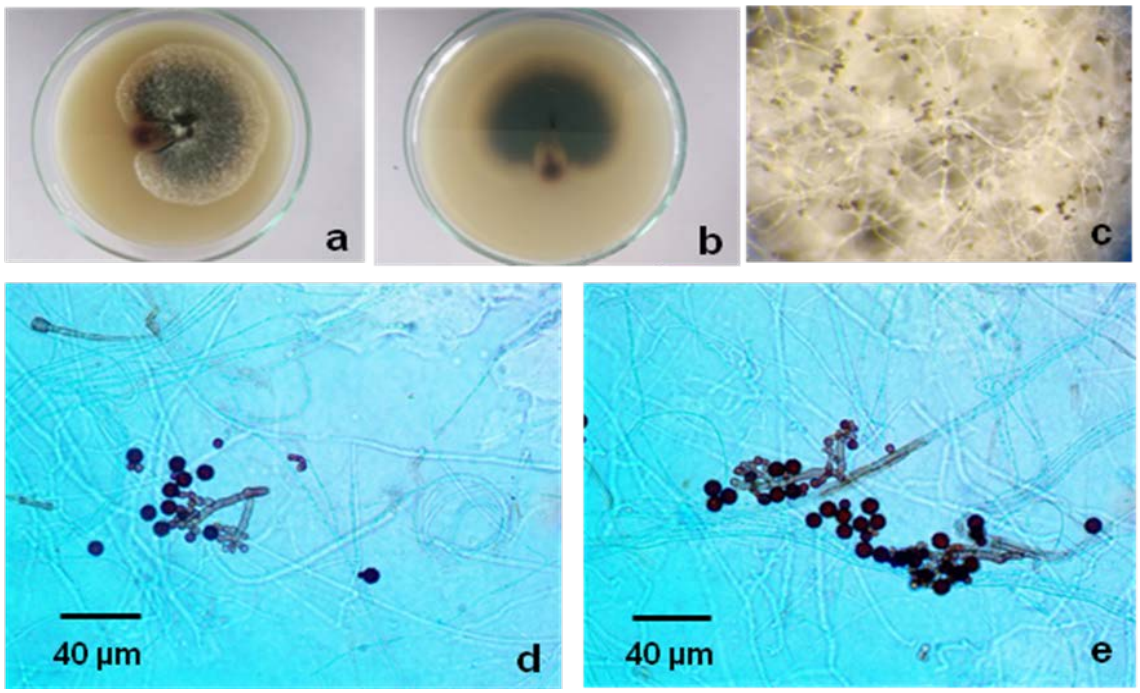

Fig. 1 – *Periconia igniaria* (NFCCI 4748). a Front view of endophytic culture on MEA. b Reverse view of endophytic culture. c Fruitings in culture. d Conidiophore aggregates with conidia. e Conidiogenous cells and conidia.

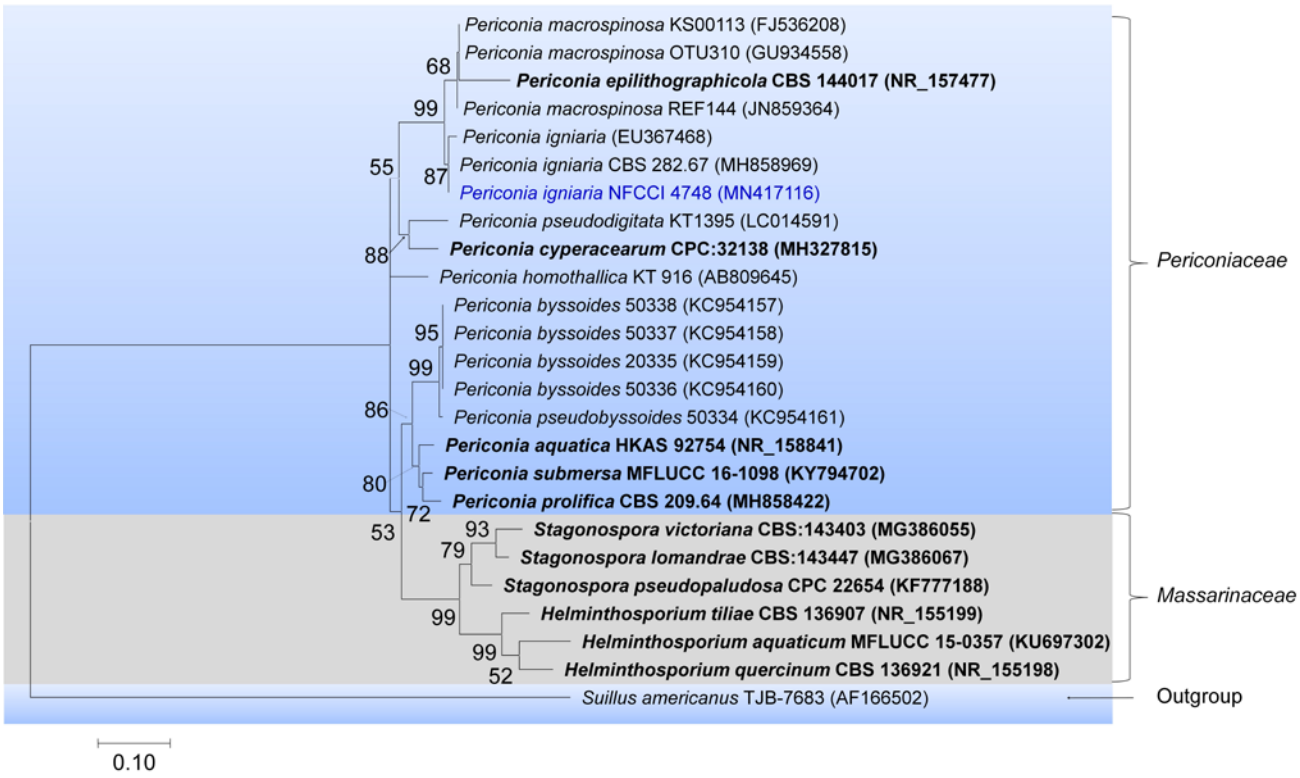

Fig. 2 – Molecular phylogenetic analysis by maximum-likelihood (ML) method tree of *Periconia igniaria* based on ITS sequence data. Our isolate is shown in blue bold. Type strains are in bold. GenBank accession numbers are provided in the brackets.

Table 1 Sequence data used in ITS analyses. Newly deposited sequence is in bold.

Taxon	**Accession no.**	**ITS**
Periconia macrospinosa	KS00113	FJ536208
Periconia macrospinosa	OTU310	GU934558
Periconia macrospinosa	*REF144*	JN859364
Periconia epilithographicola	CBS144017	NR157477
Periconia igniaria	-	EU367468
Periconia igniaria	CBS282.67	MH858969
Periconia igniaria	**NFCCI 4748**	**MN417116**
Periconia pseudodigitata	KT1395	LC014591
Periconia cyperacearum	CPC32138	MH327815
Periconia homothallica	KT916	AB809645
Periconia byssoides	50338	KC954157
Periconia byssoides	50337	KC954158
Periconia byssoides	50335	KC954159
Periconia byssoides	50336	KC954160
Periconia pseudobyssoides	50334	KC954161
Periconia aquatica	HKAS92754	NR_158841
Periconia submersa	MFLUCC16-1098	KY794702
Periconia prolifica	CBS209.64	MH858422
Stagonospora victoriana	CBS:143403	MG386055
Stagonospora lomandrae	CBS:143447	MG386067
Stagonospora pseudopaludosa	CPC 22654	KF777188
Helminthosporium tiliae	CBS 136907	NR_155199
Helminthosporium aquaticum	MFLUCC 15-0357	KU697302
Helminthosporium quercinum	CBS 136921	NR_155198
Suillus americanus	TJB-7683	AF166502

Discussion

The genus *Periconia* was described by Tode in 1791 and has 199 recorded species till date (Index fungorum 2019). Of which, *Periconia igniaria* was first reported on *Phalaris arundinacea* (Gramineae) from British Isles which caused scorch or prematurely killed the plant by burning (Mason & Ellis 1953). They also recorded this species on eight different host plants in England and on *Borassus flabellifer* var. *aethiopica* in Gold Coast (Ghana). *Periconia igniaria* has also been isolated from soil by Stenton at Wicken Fen. Cambridgeshire (Mason & Ellis 1953) and from the surface layer of a sand dune at Sandwich, Kent by Brown (1958). Later on, Booth (1968) studied a conidial culture of unknown origin from Lucknow (India) and described the perithecial state of *P. igniaria* as *Didymosphaeria igniaria* (IMI 128479, holotype). He reported the species as homothallic which produced uniloculate ascostromata when cultures were grown on potato dextrose agar with pieces of wheat straw and subjected to near ultraviolet light. Based on this findings Booth (1968) suggested that the spores of *P. igniaria* are very resistant to heat and chemical treatment, probably because of the thick epispore. Later the same fungus was isolated from Upington, Northern Cape Province, South Africa by Marasas & Van der Westhuizen (1971) from surface sterilization seed of *Medicago sativa* L. seeds. Kolomiets et al. (2008) reported leaf spot caused by *P. igniaria* on several hundred diseased yellow starthistle (YST; *Centaurea solstitialis*) plants found near Taman, Russia (Table 2).

So far isolates of *Periconia igniaria* are known from scorched leaves, surface layer of the sand dune, surface sterilized seeds and as causing leaf spots while the present collection is now re-isolated from India as an endophyte. Phylogenetic study confirms its identity. Repeated efforts of the current study did not reveal the sexual morph of *P. igniaria.* The second report of this taxon extends its distribution to the Western Ghats region in Goa, India from its first report from Lucknow, India in 1967 (which was not sequenced). To our knowledge, *P. igniaria* is being

reported for the first time as root endophyte of the asymptomatic roots of a medicinal plant *Cheilocostus speciosus* and, the first record of the occurrence of this species in Western Ghats, Goa, India.

Table 2 Details of morphological characteristics of *Periconia igniaria* reported from various hosts/substrates.

Author	Host/Substrate	Place/Locality of the Host	Growth medium	Mycelium	Conidiophores	Conidia
Mason & Ellis (1953)	*Phalaris arundinacea*	British Isles, Great Britain	Oatmeal agar	Woolly, rose madder in the centre, surrounded by a ferruginous zone, edged with buff and grey tinge	Pale to dark brown, warted at base, 3–6 septate, 140–340 µm long, 6–9 µm thick at the base, 4–6 µm in diam. below the head.	Formed in chains spherical, brown, 7–10 µm diam., more strongly echinulate with spines approximately l µm long.
Booth (1968)	Wheat straw	Lucknow, India	Potato dextrose agar	Pale vinaceous to vinaceous coloration	Long, narrower, short branches, 550 x 9–13 µm, thick at the base, 6–10µm immediately below the head.	Spherical, brown, 7–10 µm diam. with much more strongly echinulate, spines approximately l µm long.
Marasas & Van der Westhuizen (1971)	Surface sterilized seed of *Medicago sativa*	Upington, Northern Cape Province, South Africa	Potato dextrose agar	Initially smoky grey at centre later rose-madder with white margin	Singly, or in dense clusters with erect, stout, unbranched, brown, smooth or verruculose stipes, 225–540 x 5–7.5 µm	Spherical, dark brown, one-celled, 7–11 µm in diam., thick-walled, covered by 1 µm long spinose spines.
Kolomiets et al. (2008)	Parasitic causing leaf spots of *Centaurea solstitialis*	Taman, Russia	Potato glucose nutrition medium	Fluffy to pressed, colorless at the beginning violet purple to wine colored	Dark with short, swollen branched stipes, 550 x 9–13 µm	Spherical, dark brown, 7–9 µm diam., in short twisted chains, covered by 1 µm long spines.
This study	Endophyte on root of *Cheilocostus speciosus*	Calangute, Bardez, Goa, India	Malt extract agar	Fluffy white later violet purple to wine coloured to black	Up to 550 µm long, 9–13 µm thick at the base, bearing shorter branches or stipes erect, stout, branched, pale brown, smooth, showing a bead like appearance.	Spherical, dark brown to black, one-celled, thick-walled, verruculose, 7–11 µm diam.

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References

Aamir S, Sutar S, Singh SK, Baghela A. 2015 – A rapid and efficient method of fungal genomic DNA extraction, suitable for PCR based molecular methods. Plant Pathology & Quarantine 5(2), 74–81.

Bhat DJ. 2010 – Fascinating Microfungi (Hyphomycetes) of Western Ghats, India. Broadway Publishers, Panaji, Goa, pp. 219.

Booth BC. 1968 – *Didymosphaeria igniaria* sp. nov., the perfect state of *Periconia igniaria*. Transactions of the British Mycological Society 51(5), 803–805.

Brown JC. 1958 – Soil fungi of some British sand dunes in relation to soil type and succession. Journal of Ecology 46, 641–664

Choudhury J, Sarma BP. 2016 – A review on anti-diabetic properties of *Costus speciosus* 4, 603.

D'Souza MA, Bhat DJ. 2012 – A new species of *Pleurophragmium* from India. Mycotaxon 119, 477–482. http://dx.doi.org/10.5248/119.477.

D'Souza MA, Bhat DJ. 2002 – *Didymobotryum spirillum*, a new synnematous hyphomycete from India. Mycologia 94(3), 535–538.

Doilom M, Manawasinghe IS, Jeewon R, Jayawardena RS et al. 2017 – Can ITS sequence data identify fungal endophytes from cultures? A case study from *Rhizophora apiculata*. Mycosphere 8, 1869–1892.

Ellis MB. 1971 – Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

Ellis MB. 1976 – More Dematiaceous Hyphomycetes. Commonwealth Mycological Institute, Kew, Surrey, England.

Katoh K, Asimenos G, Toh H. 2009 – Multiple alignment of DNA sequences with MAFFT. Methods in Molecular Biology 537, 39–64.

Khayyat S, Othman M, Al-Kattan. 2017 – Phytochemical screening and antimicrobial activities of *Costus speciosus* and Sea Qust. Biomedical Research 28, 1.

Kimura M. 1980 – A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16,111–120.

Kolomiets T, Pankratova L, Mukhina Z, Kassanelly D et al. 2008 – First report of leaf spot caused by *Periconia igniaria* on yellow starthistle in Russia. Plant Disease 92(6), 983.

Kumar S, Stecher G, Tamura K. 2016 – MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Molecular Biology and Evolution 33, 1870–1874.

Marasas WFO, Van der Westhuizen GCA. 1971 – New and Interesting Records of South African Fungi, Part VII. Bothalia 10(3), 411–416.

Mason EW, Ellis MB. 1953 – British species of Periconia. Mycological Papers 56, 1–127.

Matsushima T. 1971 – Microfungi of Solomon Islands and Papua-New Guinea. Matsushima. Kobe, Japan.

Matsushima T. 1975 – Icones Microfungorum A Matsushima Lectorum. Matsushima. Kobe, Japan.

Pawar VA, Pawar PR. 2014 – *Costus speciosus*: An Important Medicinal Plant. International Journal of Science and Research 3(7), 28–33.

Petrini O. 1986 – Taxonomy of endophytic fungi of aerial plant tissues. I: *Microbiology of the Phyllosphere.* (Eds. Fokkema, N.J. and van den Heuvel J.). Cambridge University Press, Cambridge, pp. 175–187.

Schulz U, Wanke S, Draeger H, Aust J. 1993 – Endophytes from herbaceous plants and shrubs: effectiveness of surface sterilization methods. Mycological Research 97(12), 1447–1450.

Seifert K, Morgan-Jones G, Gams W, Kendrick B. 2011 – The Genera of Hyphomycetes. CBS-KNAW Fungal Biodiversity Centre, Utrecht, Netherlands.

Tanaka K, Hirayama K, Yonezawa H, Sato G et al. 2015 – Revision of the Massarineae (Pleosporales, Dothideomycetes). Studies in Mycology 82, 75–136.

Tode HJ. 1791 – Fungi Mecklenburgenses Selecti. 2:1-64. J.F.G. Lemke, Luneburg.

Waisundara VY, Watawana MI, Jayawardena N. 2015 – *Costus speciosus* and *Coccinia grandis*: Traditional medicinal remedies for diabetes. South African Journal of Botany 98, 1–5. https://doi.org/10.1016/j.sajb.2015.01.012

White TJ, Bruns T, Lee S, Taylor J. 1990 – Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (ed) PCR protocols: a guide to methods and application. Academic Press, San Diego pp 315–22.