

Notes on two rare fungal isolates from Western Ghats, Goa India

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ABSTRACT

This paper describes isolation of two rare litter degrading anamorphic fungi from forests of Western Ghats of Goa, India. *Argopericonia indirae* is new record of genus to mainland of India and first report of molecular phylogeny of genus *Argopericonia* which is placed in family *Chaetothyriaceae*. *Pseudoxylomyces elegans* is a rare record of genus and species of aquatic fungus from India. Molecular phylogenetic placement of genus *Pseudoxylomyces* in *Pleosporales* supports its establishment to accommodate *Xylomyces elegans* Goh.

Keywords: Phylogeny of asexual fungi, aquatic fungi, fungal diversity.

INTRODUCTION

Study of litter degrading fungi of Western Ghats, India yielded several interesting isolates (Pratibha and Prabhugaonkar, 2015a; b; Pratibha *et al.*, 2014a and b). This study describes two such isolates namely *Argopericonia indirae* D' Souza, S.K. Singh & Bhat and *Pseudoxylomyces elegans* (Goh, W.H. Ho, K.D. Hyde & K.M. Tsui) Kaz. Tanaka & K. Hiray. Study also gives molecular phylogenetic data to strengthen genus concept of these anamorphic fungi and in order to move towards natural classification (Hyde *et al.*, 2011). Genus *Argopericonia* typified by *Argopericonia elegans* B. Sutton & Pascoe was described in 1987. D'Souza *et al.* (2002) added second species in genus, namely *A. indirae* isolated from Andaman Island. In current study *A. indirae* was isolated from dead twig of unidentified plant in Mhadei wild life sanctuary, Sonal, Sattari, Goa. This forms its first record from mainland of India. Identification of this fungus is supported with molecular phylogeny. This first report of molecular phylogeny of morphologically described genus *Argopericonia* shows it to be a member of family *Chaetothyriaceae*.

Pseudoxylomyces elegans (Goh, W.H. Ho, K.D. Hyde & K.M. Tsui) Kaz. Tanaka & K. Hiray. was established in 2015 to accommodate *Xylomyces elegans*, a species of aquatic fungus expelled from genus *Xylomyces* as the species belonged to order *Pleosporales*. Genus *Xylomyces* was observed to belong to order *Jahanulales* (Tanaka *et al.*, 2015). The rare fungus was re-isolated from India and molecular phylogenetic study was carried out which supported establishment of this new genus.

MATERIALS AND METHODS

Collection and culturing: Freshly collected litter samples were taken to the laboratory in sealed polythene bags. The samples were observed under stereomicroscope. Fungal material was picked with a fine-tipped needle and mounted on a slide containing a drop of lactophenol solution. This was examined under a light microscope for further details.

The cultures of *Argopericonia indirae* and *Pseudoxylomyces elegans* were obtained by single spore isolation. To begin with a drop of sterile distilled water was placed on a flame-sterilized slide. Then the sporulating fungal mass was aseptically transferred into the water droplet and teased apart

with flame-sterilized needle to obtain a spore suspension. The suspension was spread onto malt extract agar (MEA) plates containing antibiotics (20 mg/L each streptomycin and penicillin). Colonies developing from individual conidia were aseptically transferred into fresh plates (Bhat, 2010).

DNA isolation and PCR analysis: DNA isolation and sequencing work was done at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram. Fresh fungal mycelia (20 mg) was scraped from the growing culture incubated at 28°C for 7 days. DNA isolation and PCR analysis was done according to the methodology of Prabhugaonkar and Bhat (2011). The 5.8S nuclear ribosomal gene with the two flanking internal transcribed spacers (ITS) and 28S nrDNA sequence (LSU) genes were amplified and sequenced using the primer pairs ITS-1F + ITS-4R 4R (White *et al.*, 1990) and LR5 + LROR (Crous *et al.*, 2009), respectively. The sequence quality was checked using Sequence Scanner Software v.1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond *et al.*, 2010).

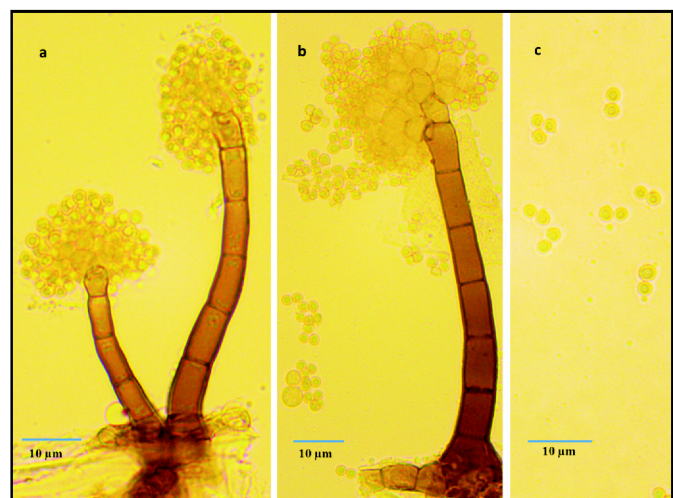


Fig. 1. *Argopericonia indirae*: a-b. Conidiophores and conidia, c. Conidia

Sequence alignment and phylogenetic analysis: The sequences were blasted in GenBank with Blastn. ITS and LSU data sets were analyzed. Based on the blasts, further

Table 1. Sequence data used in combined ITS and LSU analyses. Newly deposited sequences are in bold

Taxon	Accession no.	ITS	LSU
<i>Aliquandostipite khaoyaiensis</i>	SS3321	JN819278	-
<i>Argopericonia indirae</i>	GUFCC 18018	KY977981	KY977982
<i>Brachiosphaera atropicalis</i>	SS2523	FJ887923	JN819284
<i>Capnodium coffeae</i>	CBS 147.52	AJ244239	DQ247800
<i>Capronia pilosella</i>	AFTOL-ID 657	DQ826737	DQ823099
<i>C. carniolicum</i>	CBS 175.95	KC978733	KC455251
<i>Ceramothyrium podocarpi</i>	CPC:19826	KC005773	KC005795
<i>C. thailandicum</i>	MFLU(CC)10-0008	HQ895838	HQ895835
<i>Chaetothyrium agathis</i>	MFLUCC 12 C0113	KP744437	KP744480
<i>Cladophialophora boppii</i>	ATCC MYA -4778	JN882312	JN874491
<i>C. chaetospora</i>	CBS 514.63	KF928449	KF928513
<i>Cochliobolus heterostrophus</i>	ATCC 64121	JX094779	JX094789
<i>Coniothyrium multiporum</i>	CBS 353.65	JF740187	JF740268
<i>Cucurbitaria berberidis</i>	CBS 363.93	JF740191	GQ387606
<i>Eurotium herbariorum</i>	DAOM 221134	JN942870	JN938918
<i>Exophiala hongkongensis</i>	HKU32	JN625231	LC159284
<i>Jahnuia appendiculata</i>	BCC11400	JN819280	FJ743446
<i>J. appendiculata</i>	BCC11445	JN819279	FJ743445
<i>J. aquatica</i>	R68-1	JN942354	EF175655
<i>J. bipileata</i>	AF220-1	JN942352	EF175656
<i>J. bipolaris</i>	SS44	JN819281	EF175658
<i>J. sangamonensis</i>	F81-1	JN942351	EF175663
<i>Lepidosphaeria nicotiae</i>	CBS 559.71	GQ203760	DQ384106
<i>L. pedicularis</i>	CBS 390.80	JF740224	JF740294
<i>Lophiostoma macrostomum</i>	HHUF:27293	AB433276	AB433274
<i>Manglicola guatemalensis</i>	BCC20156	JN819283	FJ743448
<i>Montagnula aloes</i>	CPC 19671	JX069863	JX069847
<i>Phaeosaccardinula dendrocalami</i>	IFRDCC:2649	KF667242	KF667245
<i>P. ficus</i>	MFLU(CC)10-0009	HQ895840	HQ895837
<i>P. multiseptata</i>	IFRDCC:2639	KF667241	KF667244
<i>Phaeosphaeria vagans</i>	CBS 604.86	KF251193	KF251696
<i>Phialophora verrucosa</i>	BMU 03356	KF881928	KJ930100
<i>Pleodomus wasabiae</i>	CBS 120119	JF740257	JF740323
<i>Pleospora tarda</i>	CBS 714.68	KC584238	KC584345
<i>Polyposphaeria fusca</i>	KT 1640	AB524790	AB524605
<i>Preussia persica</i>	IRAN 844	GQ292750	GQ292752
<i>Pseudotetraploa longissima</i>	HC 4933	AB524796	AB524612
<i>Pseudoxylomyces elegans</i>	GUFCC 18014	KY977978	KY977983
<i>P. elegans</i>	KT2887	LC014593	AB807598
<i>P. elegans</i>	SS1077	FJ887920	-
<i>Pyrenophora seminiperda</i>	DAOM 213153	JN943665	JN940088
<i>Quadricrura septentrionalis</i>	HC 4984	AB524800	AB524616
<i>Setosphaeria rostrata</i>	P3006008	KC150019	KC150020
<i>Tetraplophaeria yakushimensis</i>	KT 1906	AB524808	AB524632
<i>Triplophaeria maxima</i>	KT 870	AB524812	AB524637
<i>Westerydella reniformis</i>	RKGE-35	JX235700	JX235704
<i>Xylomyces aquaticus</i>	BPF10.2012	KF280586	KF313075
<i>X. chlamydosporus</i>	SS2917	FJ887919	-

d sequences were assembled for each fungus (**Table 1**). The combined data matrix was aligned using MAFFT v.7 (<http://mafft.cbrc.jp/alignment/server/index.html>) and manually adjusted using MEGA 6.06 to allow maximum alignment and maximum sequence similarity. Phylogenetic analysis was conducted using maximum likelihood (ML) in MEGA6.06 (Kumar *et al.*, 2008) with 1,000 bootstrap replicates. The most suitable substitution model Kimura-2-parameter model with Gamma distributed with Invariant sites (G+I) was selected by using MEGA6.06. Gaps were treated as a pair wise deletion and tree was viewed with MEGA6.06. Newly generated ITS and LSU sequences used are deposited in GenBank.

TAXONOMY

1. *Argopericonia indirae* D'Souza, S.K. Singh & Bhat [as '*indiraei*'], *Mycotaxon* **82**: 135 (2002) (**Fig. 1**)

Colonies effuse, hairy, dark brown. Mycelium partly superficial, partly immersed, composed of light brown, smooth, septate, 2-3 μm wide hyphae. Conidiophores macronematous, mononematous, erect, straight to flexuous, unbranched, dark brown at the base, paler towards the apex, smooth, branched at the tip, 45-110 \times 2.5-5 μm . Conidiogenous cells polyblastic, discrete, terminal, hyaline, 3.5-4.5 \times 2-3 μm . Conidia solitary, sometimes catenate, spherical, aseptate, smooth, hyaline, formed in slimy mass, 2-4 μm in diam.

Specimen examined: On dead twig, unidentified plant, Valpoi, Sattari Goa, 26/01/2013, coll. Pratibha Jalmi, Herb No. VTL-12. GUFCC 18018.

Genus *Argopericonia* B. Sutton & Pascoe was published in 1987. Another species *Argopericonia indirae* was added in

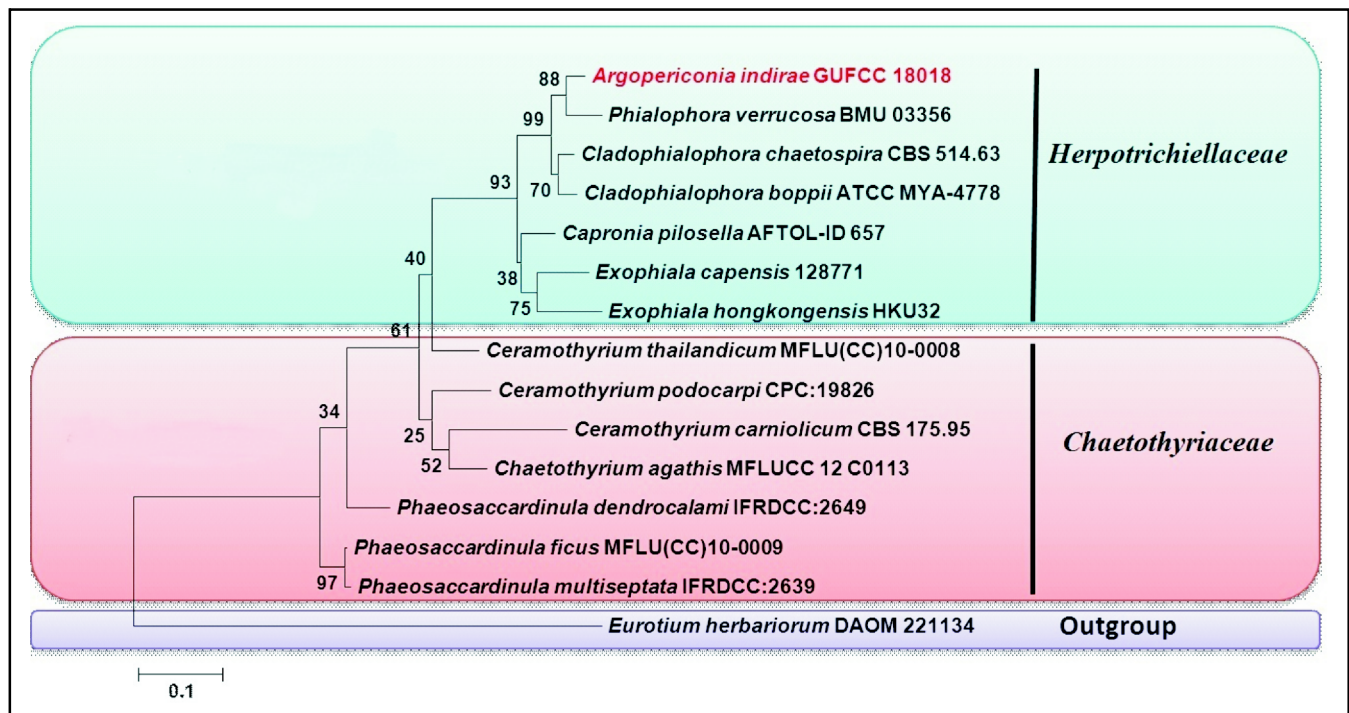


Fig. 2. Maximum likelihood (ML) tree inferred from ITS and LSU showing the relationship of *Argopericonia indirae* with *Chaetothyrium* and other members from *Chaetothyriaceae/Herpotrichiaceae* in *Chaetothyriales*. Species described in the present study is in red

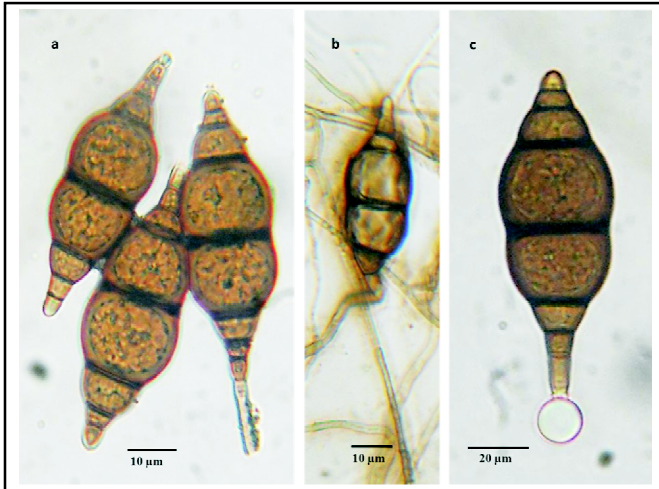


Fig. 3. *Pseudoxylomyces elegans*: a. Chlamydospores, b-c. Chlamydospores in culture

2002 from Andaman, India (D'Souza *et al.*, 2002). Fungus was placed *Incertae sedis* in *Peziromycotina* without any molecular phylogenetic studies (Index Fungorum, 2017) and any morphological evidence of its teleomorph. In current

study this fungus was isolated from unidentified litter from Goa. This forms first report of this fungus to Indian main land. Molecular phylogeny using ITS and LSU gene regions suggests relationship of *Argopericonia indirae* with *Chaetothyrium* and other members from *Chaetothyriaceae*/*Herpotrichiaceae* in order *Chaetothyriales* with good bootstrap support (**Fig. 2**).

2. *Pseudoxylomyces elegans* (Goh, W.H. Ho, K.D. Hyde & K.M. Tsui) Kaz. Tanaka & K. Hiray., *Stud. Mycol.* **82**: 126 (2015) (**Fig. 3**)

Colonies effuse, dark brown, shiny. Mycelium partly immersed, partly superficial, composed of light brown, smooth, branched, septate, 2-4.5 µm wide, hyphae. Conidiophores and conidia not developed. Chlamydospores solitary, intercalary, broadly fusiform, dark brown, smooth, multiseptate, constricted at the septa, 60-100 x 25-37.5 µm.

Specimen examined: On dead twig in fresh water stream, unidentified plant, Netravali, 3/01/2013, coll. Pratibha Jalmi, Herb No.NS-14. GUFCC 18014.

Goh *et al.* (1997) described *X. elegans* from submerged wood. In current study fungus was isolated from similar habitat. Result of molecular phylogenetic study show that the fungus

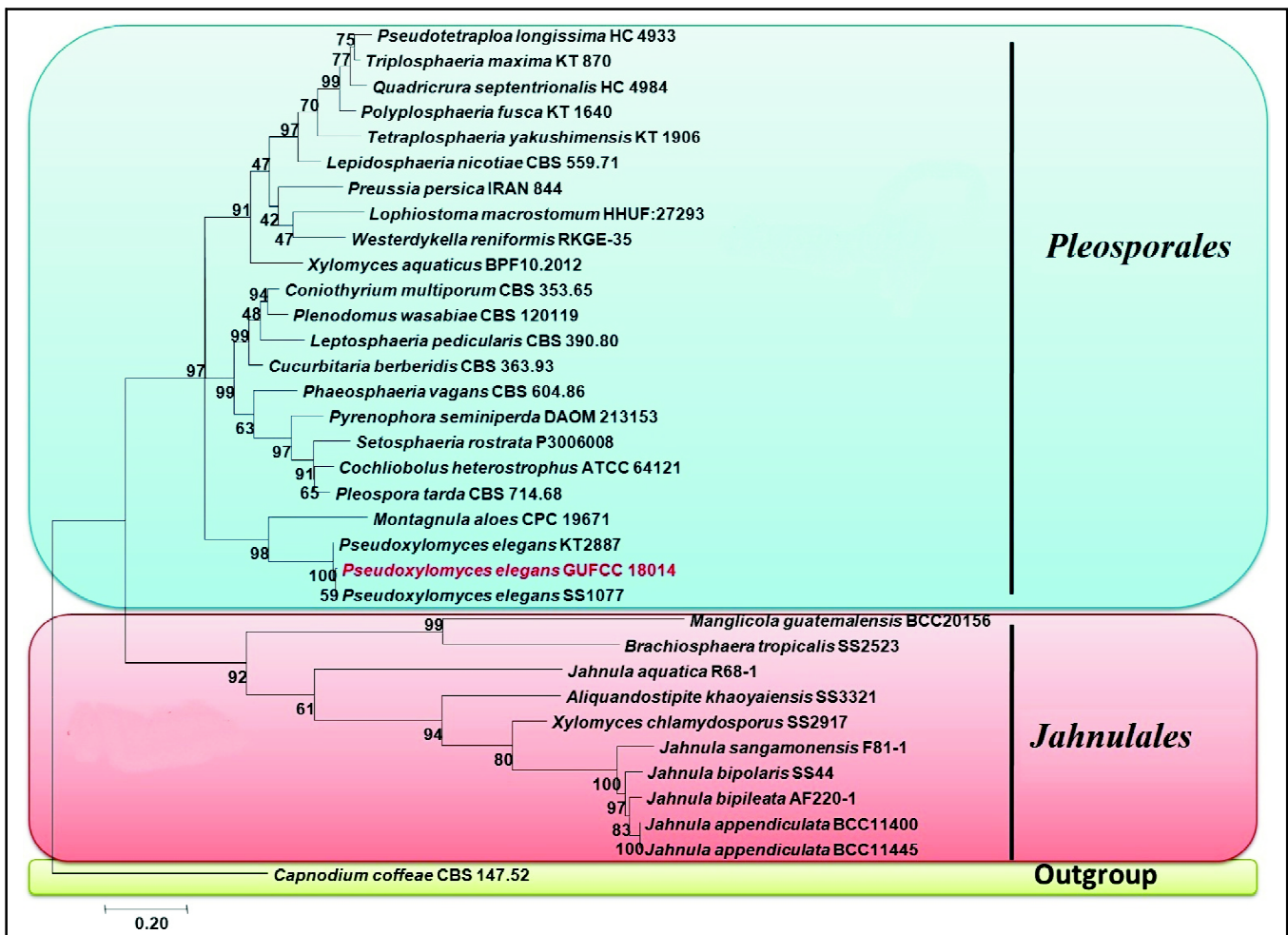


Fig. 4. Maximum likelihood (ML) tree inferred from ITS and LSU showing the relationship of *Pseudoxylomyces elegans* with *Pleosporales*. Species described in the present study is in red

belongs to *Pleosporales* (Fig. 4). Tanaka *et al.* (2015) with similar observations established new genus *Pseudoxylomyces* to accommodate this fungus as type species of the genus *Xylomyces* was placed in order *Jahnulales*. This report supports new genus *Pseudoxylomyces* placed *Incertae sedis* in order *Pleosporales*. Borse *et al.* (2014) described *X. elegans* from India and this forms another report of this rare fungal isolate.

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