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Torula goaensis, a new asexual ascomycetous fungus in *Torulaceae*

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ABSTRACT

A new species of asexual ascomycete, genus *Torula*, is described. *Torula goaensis* was found growing on decaying plant litter collected from Bondla, Goa, India and differs from previously known *Torula* species by having two unique types of conidia. Molecular phylogenetic analysis of internal transcribed spacers and large subunit regions shows its placement in the genus *Torula*, which is placed in the family Torulaceae in Pleosporales.

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Anamorph; litter fungi;
Torulaceae

Introduction

During a biodiversity survey of litter-degrading microfungi from the forests of Western Ghats in Goa, a novel anamorphic fungus with morphological characteristics similar to genus *Torula* Pers. and *Pseudotorula* Subram. (Subramanian 1958) was found on decaying twigs of an unidentified plant. The fungus differed from other species in genus *Torula* by having two types of conidia – namely phragmoconidia and scolecoconidia. Morphologically, the fungus was more similar to genus *Pseudotorula*, but this genus, typified by *Pseudotorula heterospora* Subramanian 1958, has no sequence data. The fungus showed cultural characters more similar to *Torula* and molecular phylogenetic analysis of internal transcribed spacers (ITS) and large subunit (LSU) regions confirmed its relation to the genus *Torula*, which is placed in the family Torulaceae in Pleosporales (Crane and Miller 2016); hence, a new species of genus *Torula* namely *Torula goaensis* is described and illustrated here.

Materials and Methods

Sampling

Freshly gathered litter samples from the Bondla forests of Goa were taken to the laboratory in polythene bags, incubated for 2–3 days and examined under a stereomicroscope. Fungal fruiting bodies were carefully picked up with a sterile needle, mounted in lactophenol and observed under a light microscope. The cultures were obtained by single spore isolation (Chomnunti et al. 2014). After confirming the purity and identity of the culture, molecular sequencing was done at the Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, Kerala,

India. Details of collection were added to Mycobank and Faces of Fungi (Jayasiri et al. 2015).

DNA isolation and polymerase chain reaction analysis

Fresh fungal mycelium (20 mg) was scraped from the growing culture incubated at 28°C for 7 days. DNA isolation and polymerase chain reaction analysis were carried out according to Prabhugaonkar and Bhat (2011). The 5.8S nuclear ribosomal gene with the two flanking ITS and 28S nrDNA sequence (LSU) genes were amplified and sequenced using the primer pairs ITS1F+ITS4 (White et al. 1990) and LROR+LR5 (Vilgalys and Hester 1990; Rehner and Samuels 1995), respectively. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 (Drummond et al. 2010).

Sequence alignment and phylogenetic analysis

The sequences were blasted in GenBank with BLASTn. The ITS and LSU data sets were analysed. Based on the BLAST analysis, further related sequences were assembled. The combined data matrix was aligned using MAFFT v.7 (<https://mafft.cbrc.jp/alignment/software>) and manually adjusted using MEGA 6.06 to allow maximum alignment and maximum sequence similarity. A phylogenetic analysis was conducted using maximum likelihood in MEGA 6.06 (Kumar et al. 2008) with 1000 bootstrap replicates. Forty-five taxa are included in the phylogenetic analysis (Table 1, Tree 1). A data set of 10 families of Pleosporales was assembled. *Mytilinidion*

Table 1. Sequence data used in combined internal transcribed spacers (ITS) and large subunit (LSU) analyses. Newly deposited sequences are in bold.

Taxon	Accession no.	ITS	LSU
<i>Astrosphaeriella bakeriana</i>	MFLUCC11-0027	JN846716	JN846730
<i>Astrosphaeriella stellata</i>	MFLUCC10-0555	JN846719	JN846723
<i>Astrosphaeriella stellate</i>	MFLUCC10-0095	JN846709	JN846720
<i>Leptosphaerulina chartarum</i>	CBS 329.86	KJ796400	KJ796401
<i>Leptosphaerulina australis</i>	CBS 317.83	GU237829	GU301830
<i>Lindgomyces ingoldianus</i>	ATCC 200398	JF419898	AB521736
<i>Lophiostoma versicolor</i>	KH 110	AB918731	AB918732
<i>Lophiostomama crostomum</i>	KT 508	JN942961	AB619010
<i>Pseudotetraploa curviappendiculata</i>	KT 2558	AB524794	AB524610
<i>Quadricrura bicornis</i>	yone 153	AB524797	AB524613
<i>Tetraplosphaeria nagasakiensis</i>	KT 1682	AB524806	AB524630
<i>Triplosphaeria cylindrica</i>	KT 2550	AB524811	AB524636
<i>Triplosphaeria maxima</i>	KT 870	AB524812	AB524637
<i>Triplosphaeria yezoensis</i>	KT 1732	AB524814	AB524639
<i>Mytilinidion mytilinellum</i>	CBS:303.34	HM163570	FJ161184
<i>Cucurbitaria berberidis</i>	CBS 363.93	JF740191	GQ387606
<i>Berkleasium</i> sp.	BCC 17003	DQ280263	DQ280274
<i>Aquasubmersa mircensis</i>	MFLUCC11-0401	JX276954	JX276955
<i>Quadricrura septentrionalis</i>	HC 4983	AB524799	AB524615
<i>Lepidosphaeria nicotiae</i>	CBS 559.71	GQ203760	DQ384106
<i>Lindgomyces apiculatus</i>	KT1108	JF419892	JF419884
<i>Quadricrura meridionalis</i>	KT 2607	AB524798	AB524614
<i>Pseudotetraploa javanica</i>	HC 4934	AB524795	AB524611
<i>Westerdykella angulata</i>	IMI 090323	GQ203758	GQ203720
<i>Subplenodomus apiicola</i>	CBS 285.72	JF740196	GU238040
<i>Coniothyrium carteri</i>	CBS 105.91	KF251209	KF251712
<i>Coniothyrium multiporum</i>	CBS 501.91	JF740186	GU238109
<i>Arthopyrenia salicis</i>	CBS 368.94	KF443410	AY538339
<i>Rousoella percutanea</i>	CBS 868.95	KF322118	KF366449
<i>Rousoella percutanea</i>	CBS 128203	KF322117	KF366448
<i>Rousoella thailandica</i>	MFLUCC 11-0621	KJ474838	KJ474846
<i>Rousoella siamensis</i>	MFLUCC 11-0149	KJ474837	KJ474845
<i>Rousoella scabrispora</i>	MFLUCC 11-0624	KJ474836	KJ474844
<i>Rousoella nitidula</i>	MFLUCC 11-0182	KJ474835	KJ474843
<i>Rousoella neopustulans</i>	MFLUCC 11-0609	KJ474833	KJ474841
<i>Rousoella acaciae</i>	CBS:138873	KP004469	KP004497
<i>Rousoella hysteroioides</i>	CBS 546.94	KF443405	KF443381
<i>Neorousoella bambusae</i>	MFLUCC 11-0124	KJ474827	KJ474839
<i>Torula herbarum</i>	CBS 111855	KF443409	KF443386
<i>Torula herbarum</i>	CBS 595.96	KF443408	KF443385
<i>Torula caligans</i>	MMI00054	JQ246356	-
<i>Torula caligans</i>	KNU8	KJ921608	-
<i>Torula caligans</i>	CZ16	JN104523	-
<i>Torula herbarum</i>	CBS 220.69	KF443406	KF443384
<i>Torula goaensis</i>	MTCC 12620	KY440969	KY440969

mytilinellum from Mytilinidiales was selected as the outgroup taxon. The most suitable substitution models for the respective data sets were selected by using MEGA6.06. A Tamura Nei model with γ distribution was used in the analysis. Gaps were treated as a pairwise deletion and trees were viewed with MEGA6.06. All newly generated sequences used in this study are deposited in GenBank.

Results

Phylogenetic analysis

The combined ITS and LSU data set contains 45 taxa with *Mytilinidion mytilinellum* CBS.303.34 selected as outgroup taxon. The evolutionary relation tree was constructed using the maximum likelihood method in Mega 6.06. The final tree (Fig. 1), represents the phylogenetic relationships of the new taxon with other genera in Torulaceae (Pleosporales). *Torula goaensis* forms a single clade clustering with the genus *Torula*.

Taxonomy

Torula goaensis Pratibha & Prabhugaonkar, **sp. nov.** Fig. 2

Mycobank MB819598, Faces of Fungi FoF 02876

Description

Colonies on natural substrate effuse, hairy, dark brown to black. Mycelium partly superficial, partly immersed in the host tissue, composed of brown, smooth, branched, 1.5–3 μm wide hyphae. Conidiophores mononematous, semi-macronematous, rarely branched, sub-hyaline to pale brown, verrucose, 31.5–42 \times 2–3.5 μm , composed of two to four cells in linear series. Conidiogenous cells terminal or intercalary, polyblastic, cupulate, verrucose, 5.5–6.5 μm wide. Conidia two types: shorter phragmoconidia and longer scolecoconidia. Phragmoconidia catenate, simple or sometimes in branched chains, broadly fusiform, straight, bi- or tri-septate, brown, 14.5–21 \times 5.5–6.5 μm . Scolecoconidia, wider than

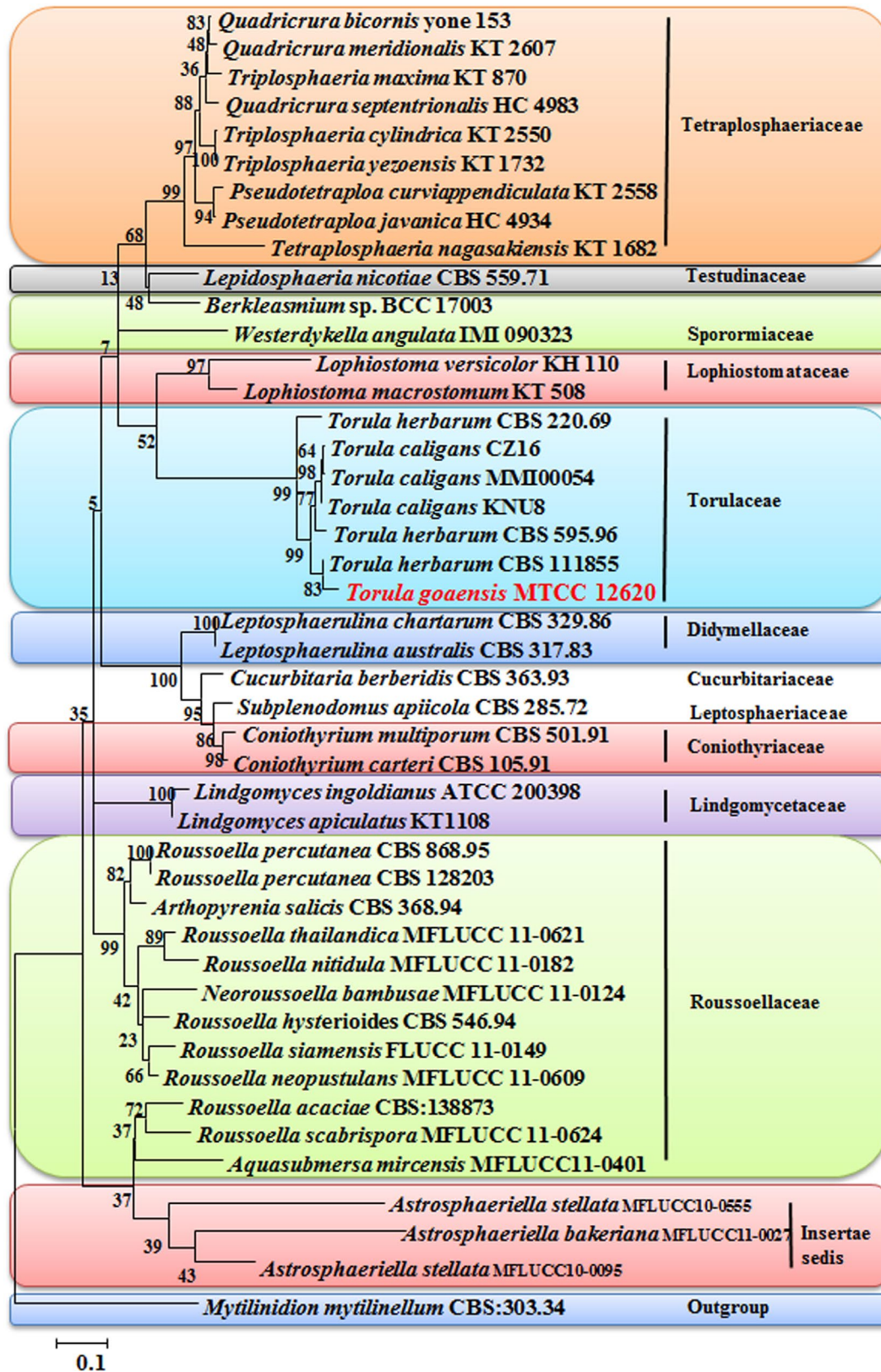


Figure 1. Maximum likelihood tree inferred from internal transcribed spacers (ITS) and large subunit (LSU) showing the relationship of *Torula goensis* with genus *Torula* in Torulaceae. Species described in the present paper is in red.

phragmoconidia, straight to flexuous, brown, 7- to 20-septate, constricted at septa, 42–105 × 6–8.5 μm.

Type: India, Goa, Bondla, 2 July 2013, J. Pratibha, on decomposing litter of unidentified tree, (Holotype HCIO 52050), ex-type culture MTCC 12620, NFCC 4040.

Etymology

Place of isolation, i.e. Goa, India.

Distribution

Known only from type locality.

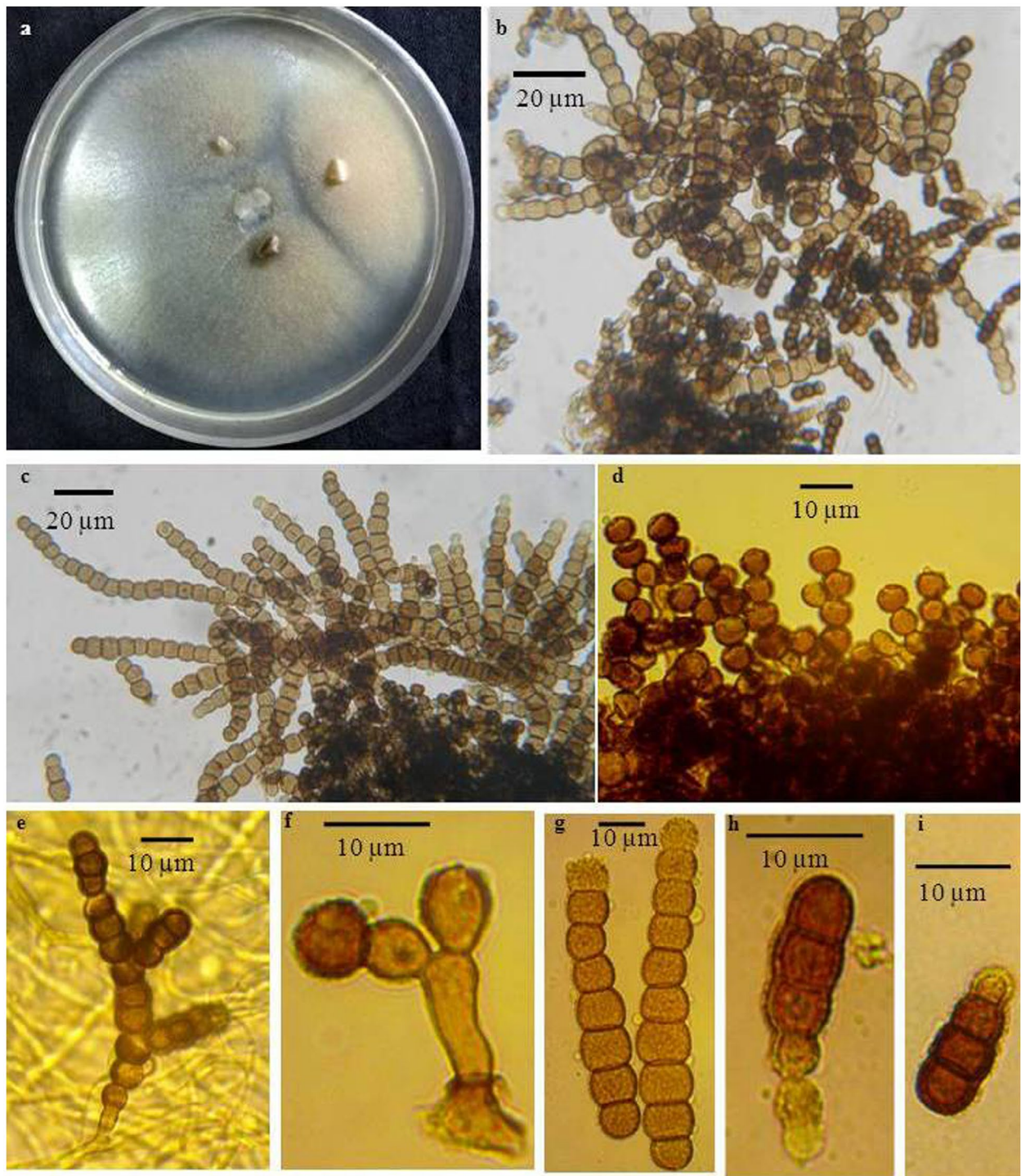


Figure 2. *Torula goaensis*. (a) Culture. (b,c) Crowded conidiogenous cells with conidia. (d-f) Conidiogenous cells on short conidiophores. (g) Scoleococonidia. (h,i) Phragmoconidia

Note

Fungus differs from other species in genus *Torula* by having two types of conidia. This placement is also supported by molecular phylogenetic data.

Discussion

Crous et al. (2015) revised the family Torulaceae with the genera *Dendryphon* and *Torula*; other genera were not included because of the lack of molecular phylogenetic data, though they were morphologically similar.

Another genus *Neotorula* was added to the family by Su et al. (2016). The result of our phylogenetic analysis showed that *T. goaensis* is placed within genus *Torula* in family Torulaceae in Pleosporales (Crous et al. 2015). In a recent review, Crane and Miller (2016) clearly delimited *Torula* and allied genera such as *Bahusaganda*, *Bahusandhika*, *Simmonsella* and *Pseudotorula* with a key to species in each genera. Genus *Pseudotorula* Subram. (Subramanian 1958) is morphologically more similar to *T. goaensis* but does not have sequence data. Our collection with details of cultural characters and

phylogenetic placement is well placed in genus *Torula* and it will be interesting to observe phylogenetic placement of genus *Pseudotorula*. *Torula goaensis* differs from species of genus *Pseudotorula*, i.e. *Pseudotorula heterospora* and *Pseudotorula sundara*, by the size of the scoleococonidia. The scoleococonidia in *P. heterospora* are 125–450 µm long and in *P. sundara* are 135–230 µm long, whereas in *P. goaensis* the scoleococonidia are 42–105 µm long. *Torula goaensis* differs from *Pseudotorula helica* in having straight scoleococonidia and more septa. Scoleococonidia in *P. helica* are terminally coiled and are 45-septate, whereas in *T. goaensis* scoleococonidia are straight and 7- to 20-septate. This supports the distinctiveness of the current species even though at a later date it was observed that genus *Pseudotorula* is a synonym of genus *Torula* on molecular phylogenetic bases.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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