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A new endophytic species of *Chaetomium* from *Jatropha podagrica*

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Abstract — A new endophytic species of *Chaetomium* from fruits of *Jatropha podagrica* is described from Maharashtra State, India. A combined sequence dataset of the ITS region, LSU rDNA, and β-tubulin genes supports recognition of this fungus as a new species that is largely concordant with morphological characters. *Chaetomium jatrophae* is characterized by spirally coiled, predominantly unbranched, and coarsely roughened terminal ascomatal hairs and elongate-ellipsoidal ascospores with a subapical or lateral germ pore. It is a mesophilic *Chaetomium* species with a temperature maximum of 40±0.2°C.

Key words — *Chaetomiaceae*, *Euphorbiaceae*, phylogeny, taxonomy

Introduction

Chaetomium Kunze (Kunze 1817) (*Chaetomiaceae*) accommodates more than a hundred species. *Chaetomium* species are widespread and found on various substrates, most with remarkable cellulolytic activity. Some are pathogenic for humans (Barron et al. 2003, Al-Aidaroos et al. 2007, de Hoog et al. 2009, Hubka et al. 2011) and others are plant endophytes (Syed et al. 2009). *Chaetomium* is a widely studied genus (Ames 1969; von Arx et al. 1986; Gené & Guarro 1996; Decock & Hennebert 1997; Udagawa et al. 1997; Rodríguez et al. 2002; Wang & Zheng 2005a,b; Asgari & Zare 2011) that is mainly characterized by superficial ostiolate ascomata covered with hairs. During our biodiversity survey of endophytic fungi, we isolated 20 strains of fungi from *Jatropha podagrica* belonging to 13 species. Two strains belonged to *Chaetomium: C. bostrychodes* Zopf (MCC 1019), isolated from a leaf petiole, and a new species isolated from the fruit of the same plant. This new species is described here as *Chaetomium jatrophae* based on morphological and combined phylogenetic data of three loci.

Table 1. *Achaetomium* and *Chaetomium* isolates used in phylogenetic analysis.

Taxon	Strain	GenBank accession numbers		
		ITS	LSU	β-tubulin
A. strumarium	CBS 333.67 (T)	AY681204	AY681170	AY681238
C. acropullum	CBS 126783	HM365258	HM365258	HM365292
C. ancistrocladum	CBS 126784	HM365241	HM365241	HM365290
	CBS 126673	HM365242	HM365242	HM365291
C. atrobrunneum	CBS 379.66 (T)	HM365255	HM365255	HM365294
C. bostrychodes	IMI 325809	HM365261	HM365261	HM365296
C. carinthiacum	CBS 126669	HM365265	HM365265	HM365299
C. coarctatum	CBS 162.62 (T)	HM365260	HM365260	HM365281
C. crispatum	CBS 152.49	HM365267	HM365267	HM365293
C. cruentum	CBS 371.66 (T)	HM365266	HM365266	HM365270
C. elatum	CBS 126657	HM365236	HM365236	HM365282
	CBS 126656	HM365238	HM365238	HM365284
	CBS 910.70 (T of *C. ramipilosum*)	HM365237	HM365237	HM365283
C. funicola	IMI 300511	HM365262	HM365262	HM365300
C. globosum	CBS 148.51	HM365254	HM365254	HM365269
C. grande	CBS 126780 (T)	HM365253	HM365253	HM365273
C. indicum	CBS 126667	HM365248	HM365248	HM365301
	CBS 126668	HM365249	HM365249	HM365302
C. interruptum	CBS 126661	HM365245	HM365245	HM365276
	CBS 126662	HM365244	HM365244	HM365275
	CBS 126660 (T)	HM365246	HM365246	HM365277
C. iranianum	CBS 126670 (T)	HM365257	HM365257	HM365297
C. jatrophae	**MCC 1025, CBS 134263 (T)**	**JQ246354**	**HE981193**	**HE981190**
C. jodhpurense	CBS 280.79	HM365243	HM365243	HM365295
C. madrasense	CBS 126663	HM365252	HM365252	HM365274
C. megalocarpum	CBS 533.79	HM365259	HM365259	HM365271
	CBS 126666	HM365264	HM365264	HM365272
C. murorum	CBS 126785	HM365256	HM365256	HM365288
	CBS 636.83	HM365268	HM365268	HM365289
C. rectangulare	CBS 126659	HM365240	HM365240	HM365286
	CBS 126778 (T)	HM365239	HM365239	HM365285
C. subaffine	CBS 126777	HM365247	HM365247	HM365280
C. truncatulum	CBS 126782 (T)	HM365263	HM365263	HM365298
C. undulatulum	CBS 126776	HM365250	HM365250	HM365278
	CBS 126775 (T)	HM365251	HM365251	HM365279

Sequences in bold font were generated in this study. (T) = ex-type strain.
CBS = Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands.
IMI = CABI BioScience, Egham, UK.

Materials & methods

Sampling, fungal isolation and growth

The fungus was isolated from a healthy fruit of *Jatropha podagrica*, an ornamental plant, collected from Pune (Pimpri), Maharashtra, India (18°37′07.04″N 73°48′13.43″E). Various plant parts (leaves, petioles, fruits, flowers, young stems) were used to isolate various endophytic fungi upon Potato Dextrose Agar (PDA) and Czapek Dox Agar (CDA). Healthy fruits were harvested with clean blades and immediately dipped in beakers containing water. The fruits were subsequently washed with tap water (10 min), cut into fragments, washed twice in sterile distilled water (5 min), 70% ethanol (2–3 min), and 100% ethanol (20–30 sec). The fungal strains were isolated on CDA at room temperature after 3 d of incubation. Colony growth rate and characteristics were recorded on 2% MEA (Hi-Media, India) at room temperature (28–30°C) after 7 d in dark. Growth rate was also measured at different temperatures (20–45°C, at intervals of 5±0.2°C) on five media including PDA, CDA (Hi-Media, India), PCA (Potato Carrot Agar), MEA (Malt Extract Agar) and OA (Oatmeal Agar) after 7 d.

Morphological observations and scanning electron microscopy (SEM)

The ascomata and ascospores were studied on slides mounted in water or lactophenol. The slides were observed under Nikon YS100 microscope (Nikon, Japan). Microphotographs were taken on Olympus BX53 (Olympus Corporation, Japan) fitted with ProgRes C5 camera (Jenoptik, USA). Measurements of morpho-taxonomic characters were recorded and compared with type descriptions of known species (Ames 1969, Tiwari et al. 1977, Wang & Zheng 2005a,b; Asgari & Zare 2011). Fifty readings were recorded for each measurement using Jenoptik software. Ascomatal hair ornamentation was determined by scanning electron microscopy (SEM). Dry ascomata were placed on black double-sided tape on a stub (1 cm diam.), coated with platinum in a Jeol sputter coater (Jeol-JFC 1600), and then visualized in SEM (Jeol-JSM 6360A) at 10 kV.

DNA extraction

Genomic DNA for amplification of internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) was extracted using QIAamp® DNA Mini Kit (Qiagen, Inc., Valencia CA). Genomic DNA for D1, D2 region of 28S large subunit (LSU) of ribosome and β-tubulin (BTUB) genes was extracted by crushing fungal mycelia in cetyl trimethylammonium bromide (CTAB) extraction buffer, pH 8 (Hi-Media, India). Thereafter 20 µl of proteinase K (20 mg/ml) was added and incubated at 65±0.2°C for 1 h (Baker & Mullin 1994), followed by addition of 20 µl RNase (10 mg/ml) and further incubation at 65±0.2°C for 15 min. Chloroform: isoamylalcohol (24:1) was added (500 µl) to the supernatant collected after centrifugation (8000 rpm, 10 min). The mixture was shaken for 5 min and centrifuged at 10,000 rpm, 4°C for 15 min. Two volumes of CTAB precipitation buffer (1% CTAB, 50 mM Tris, pH 8.0, 10 mM EDTA) was added to the supernatant and kept at room temperature for 1 h. The pellet collected after centrifugation was dissolved in 500 µl of 1.2 M NaCl and then 500 µl of chloroform: isoamylalcohol (24:1) was added. Two volumes of absolute alcohol were added to the aqueous phase to precipitate the DNA (Murray & Thompson 1980, Brandfass & Karlovsky 2008). The quantity of DNA was measured by the amount of absorbance of the sample at 260 nm on NanoDrop (ND-1000, Thermo Scientific, USA) and purity was checked on 0.8% agarose gel electrophoresis.

PCR amplification and DNA sequencing

The ITS-LSU rDNA region was amplified using the 5.8S (CGCTGCGTTCTTCATCG) and LR7 (TACTACCACCAAGATCT) primers (http://www.botany.duke.edu/fungi/mycolab/primers.htm). The reaction mixture (25 µl) containing genomic DNA (100 ng), 1X PCR buffer, 0.2 mM dNTPs, 10–20 pmoles of each primer, and 1 unit of Taq DNA polymerase was amplified with Gene Amplifier PCR System 9700 (Perkin Elmer, USA) using the following protocol: initial denaturation at 95°C for 3 min, 35 cycles of 94°C for 1 min, 57°C for 30 sec, 72°C for 2 min, and final extension at 72°C for 10 min. The BTUB gene was amplified using primers T1 (AACATGCGTGAGATTGTAAGT) and Bt2b (ACCCTCAGTG TAGTGACCCTTGGC) (O'Donnell & Cigelnik 1997) using the same reaction mixture and equipment as for the ITS-LSU region using the following protocol: initial denaturation at 95°C for 3 min, 35 cycles of 94°C for 30 sec, 52°C for 30 sec, 72°C for 30 sec, and final extension at 72°C for 10 min. Five µL of the PCR amplified product was run on 1.0% agarose gel in 1X TBE buffer (54 g Tris base, 27.5 g boric acid, 20 ml EDTA 0.5 M, pH 8). Electrophoresis was carried out for about 90 min at 80 V. The gel was stained in 1% ethidium bromide for 15 min and observed under UV illumination. The PCR products were purified according to Sambrook et al. (1989). The sequencing reactions were carried out using the 'ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit' (Perkin Elmer Applied Biosystems Division, Foster City, CA) according to the manufacturer's protocol. DNA sequencing was carried out on ABI 3730xl Automated Sequencer.

Phylogenetic analysis

An NCBI and DDBJ BLASTn search for ITS region (555 bp) was conducted for sequence similarity (Zhang et al. 2000). The phylogenetic relationships of *C. jatrophae* and other *Chaetomium* species were examined using ITS, LSU, and BTUB gene sequences. Thirty-three sequences corresponding to 23 *Chaetomium* species were retrieved from GenBank along with the outgroup, *Achaetomium strumarium* (Table 1). The sequences generated in this study were manually edited using ChromasPro version 1.34. The ITS region, rDNA LSU, and BTUB gene sequences were analyzed individually and together in MEGA 5.0. All sequences were aligned by multiple alignments using MUSCLE program in MEGA 5.0 (Edgar 2004, Tamura et al. 2011). Sequences were analyzed by maximum parsimony (MP) statistical method using bootstrap method of phylogeny test (1000 replicates and 10 initial trees) (Tamura et al. 2011). Gaps and missing data were deleted. MP tree was obtained by close-neighbor-interchange (CNI) on random trees algorithm (Nei & Kumar 2000). Bootstrap values below 50% were removed from tree. Sequences were also analyzed by neighbour- joining (NJ) and maximum likelihood (ML).

Results

Taxonomy

Chaetomium jatrophae Rohit Sharma, **sp. nov.** Plates 1–2

MycoBank 563940

Differs from *Chaetomium atrobrunneum* by its larger ascomata with spiraling coarsely roughened terminal hairs, its peridium of texture intricata, and its ascospores with a sub-apical to lateral germ pore.

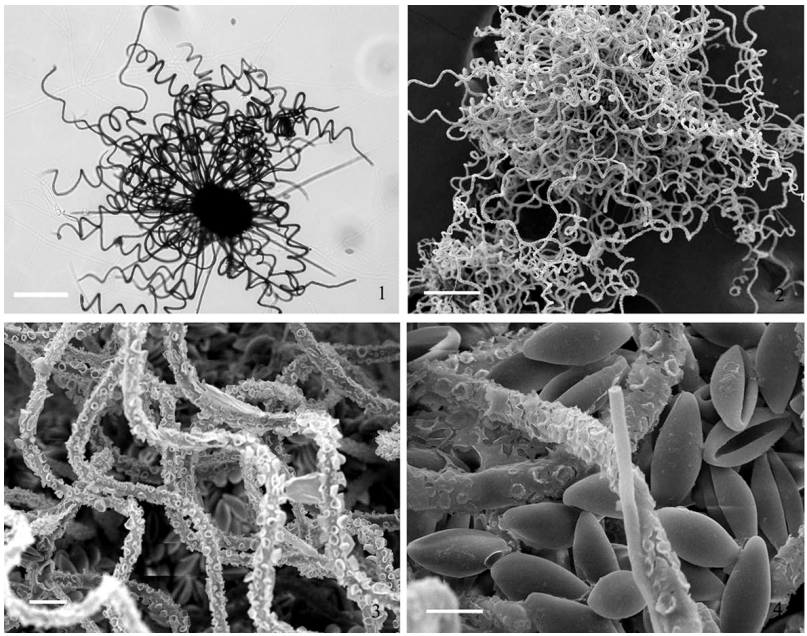

Plate 1. *Chaetomium jatrophae* (MCC 1025). Fig. 1: Mature ascoma, showing terminal coiled terminal hairs. Fig. 2, 3: Ascomatal hairs (SEM). Fig. 4: Ascospores (SEM). Scale bars: 1 = 100 µm; 2, 3 = 10 µm; 4 = 5 µm.

Type: India, Maharashtra State, Pune, Pimpri, 18°37′07.04″N 73°48′13.43″E, endophytic in fruit of *Jatropha podagrica* Hook. (*Euphorbiaceae*), 25.IV.2011, Rohit Sharma (**Holotype**, AMH 9558; ex-type culture, MCC 1025, NFCCI 2596, CBS 134263).

Etymology: Refers to the host genus *Jatropha*.

Colonies reaching 60 mm diam. on MEA after 7 d at room temperature (28–30°C), olivaceous-buff to grayish-sepia with sparse aerial mycelium; reverse similar in color to colony surface. Mycelium composed of hyaline to subhyaline, septate, smooth hyphae, 9.0–9.4 µm diam. Ascomata superficial to immersed, scattered, ostiolate, globose to subglobose, 187.3 (161.1–193.8) × 151.6 (129.2–161.1) µm, brown to dark brown, maturing in 20 d, loosely attached to mycelium with brown to olivaceous brown rhizoids. Peridium brown-vinaceous to brown-olivaceous, membranaceous with textura intricata. Terminal hairs of different length, 312.5 (250.0–375.0) µm long, numerous, pale yellowish-brown to dark brown, straight below and spirally coiled above, 8–11 coils (generally 7–8), 3.9 (3.2–4.8) µm wide at the base gradually tapering and paling towards the apex, hairs forming dense tuft around ostiole, unbranched, rarely branched, thick walled, coarsely roughened, septate. Lateral hairs straight, short, coarsely roughened, 56.0 (32.3–83.9) µm in length and 3.5 (3.2–4.0) µm

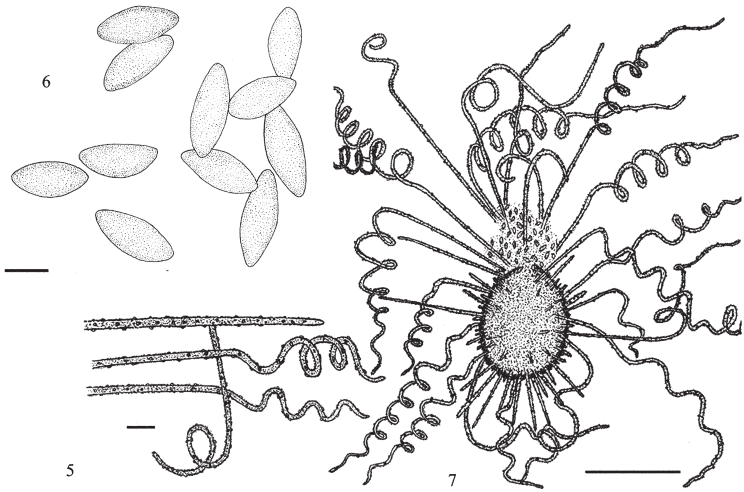

Plate 2. *Chaetomium jatrophae* (MCC 1025). Fig. 5: Ascomatal hairs. Fig. 6: Ascospores. Fig. 7: Mature ascomata. Scale bars: 5, 6 = 5 µm, 7 = 150 µm.

wide at the base. Paraphyses absent. Asci clavate, hyaline, evanescent. Ascospores one-celled, brown when mature, fusiform or elongate-ellipsoidal, symmetrical or sometimes asymmetrical, 12.3 (6.5–16.2) × 7.6 (6.5–9.7) µm, thick-walled, with a distinct sub-apical or lateral germ pore. Anamorph absent.

Cultural characteristics

The optimum growth of *C. jatrophae* was determined as 30±0.2°C (mesophilic in nature) and its temperature maximum at 40±0.2°C (Fig. 8). The temperature

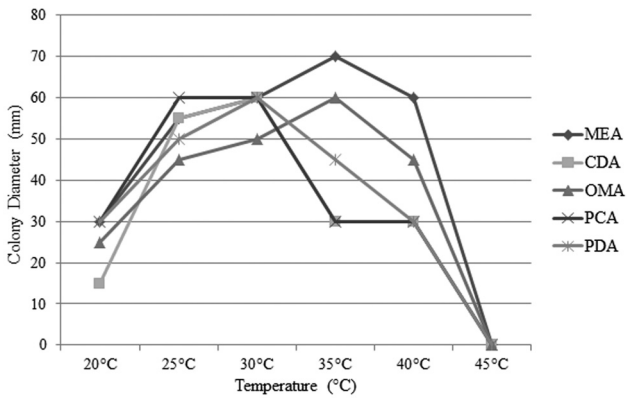

Fig. 8. Temperature-growth relationship of three *Chaetomium jatrophae* stains on Malt Extract Agar (MEA), Czapek Dox Agar (CDA), Oatmeal Agar (OA), Potato Dextrose Agar (PDA), and Potato Carrot Agar (PCA) (mm diam. of 7 d old colonies).

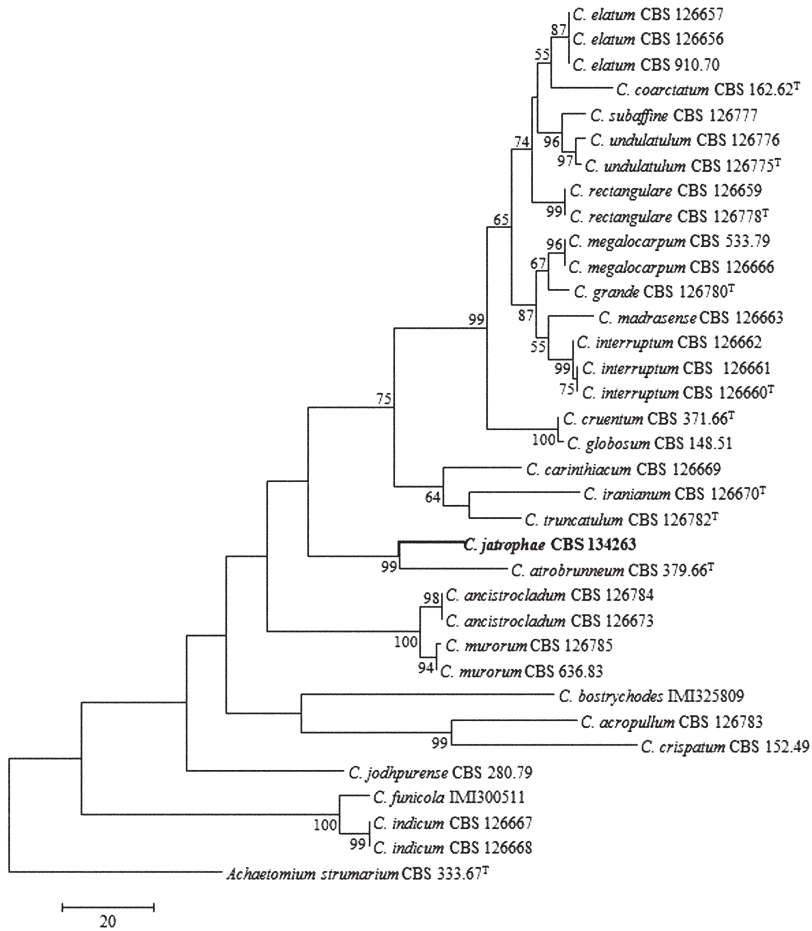

Fig. 9. Maximum parsimony phylogram of *Chaetomium jatrophae* (MCC 1025) based on combined dataset of ITS–LSU rDNA–BTUB gene sequences, with *Achaetomium strumarium* as outgroup. Bootstrap values > 50% (1000 replicates) are shown; CI = 0.531860, RI = 0.705642, RCI = 0.375302; gaps and missing data deleted. *C.* = *Chaetomium*, (T) = ex-type strain.

maximum is an important physiological character for species differentiation (Li et al. 2012). The maximum fungal growth occurred primarily on MEA followed by OA. Ascomata developed after 25–30 d of incubation on MEA.

Molecular analyses

The phylogenetic analysis of 24 taxa of *Chaetomium* was conducted using ITS and LSU (D1, D2 region) of rDNA and BTUB gene and sequence data

analyzed individually (not shown) and in combination. The ITS alignment consisted of 576 bases (438 conserved, 131 variable, 91 parsimony informative, 40 singleton). The maximum parsimony tree had CI = 0.687500 RI = 0.805654 and RCI = 0.553887 (for all sites). The LSU alignment consisted of 386 bases (361 conserved, 25 variable, 22 parsimony informative, 3 singleton), and the BTUB gene alignment consisted of 425 bases (209 were conserved, 215 variable, 193 parsimony informative, 22 singleton). The combined data set had 1392 sites (1008 conserved, 371 variable, 306 parsimony informative, 65 singleton). One most parsimonious tree is shown in Fig. 9. All phylogenetic analyses (NJ, MP, ML) supported the novelty of this species.

Discussion

The newly described species, *C. jatrophae*, is morphologically and molecularly differentiated from related species. Maximum parsimony analysis of combined dataset of ITS-LSU rDNA-BTUB gene (Fig. 9) groups *C. jatrophae* in a clade with *C. atrobrunneum* L.M. Ames with 97% bootstrap support. Although the LSU and BTUB sequence data is unavailable for *C. jabalpurense* D.P. Tiwari et al., the ITS sequence analysis (not shown) distinctly separates *C. jatrophae* from the morphologically similar *C. jabalpurense* and *C. perlucidum* Sergeeva. *Chaetomium jatrophae* resembles *C. jabalpurense*, *C. atrobrunneum,* and *C. perlucidum* mainly by ascospore shape and germ pore. Nevertheless, *C. jabalpurense* (Tiwari et al. 1977) can also be distinguished from *C. jatrophae* by its larger ascomata (154.7–304.5 × 150.3–297.2 µm), narrower (3.2 µm) terminal hairs, spirally coiled lateral hairs, and narrower (5.3–6.2 µm diam.) ascospores. *Chaetomium atrobrunneum* (Ames 1949), also close to *C. jatrophae*, is distinguished mainly by smaller ascomata (75–120 µm), peridium of textura angularis, straight seta-like mostly smooth-walled ascomatal hairs, and fusiform or elongate pyriform ascospores with a slightly subapical germ pore. *Chaetomium perlucidum* (Ames 1969) is differentiated from *C. jatrophae* by its olive-yellow to pale-brown smaller (105–135 × 100–130 µm) ascomata and narrower (5.6–6 µm diam.) ascospores.

The fact that *Chaetomium jatrophae* was isolated from *Jatropha podagrica,* a xerophytic latex-producing plant may help fungi to survive better than in outside environment. The occurrence of this *Chaetomium* species in fruits suggests that further research is needed to better understand its biology as well as its obligatory or facultative endophytic nature, and its role as an endophyte in *J. podagrica.* However, Syed et al. (2009) pointed that endophytic *Chaetomium* species are similar to *Chaetomium* strains obtained from other substrates (e.g., scat, seed, soil) both physiologically (utilizing the same carbon source) and genetically (similar ITS rDNA sequence data).

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