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A Combined Morpho-Molecular Approach towards Identification of *Chaetomium* Species of India

V. Chandra Sekhar, T. Prameeladevi and Deeba Kamil

Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi-110012, India.

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The *Chaetomium,* recently identified as a bio control fungal genus includes many species. The classification of the *Chaetomium* species based on morphology alone is very difficult as this genus has multiple species-specific characters in several species. Therefore, an attempt has been made to confirm the identity of *Chaetomium* species by integrating morphological and molecular (using Internal Transcribed Spacer region; ITS) characters of different species of *Chaetomium*. Sixty one isolates were morphologically characterized using perithecium, terminal hairs, lateral hairs, rhizoids and ascospores. The identity of these isolates was confirmed using ITS region based molecular characters. The taxonomy of nine valid species of the *Chaetomium* were described here.

Key words: *Chaetomium*, Morphological characterization, ITS region, Identification.

Many fungi have significant importance to human being. Some of them are harmful and some are beneficial. Harmful fungi generally cause diseases in animals, humans and plants. On the other hand beneficial fungi have many advantages such as; biodegradation, bioremediation, biocontrol activities. Recently *Chaetomium* and its species have drawn much attention to be used to control several economically important diseases. Some isolates of *C. globosum* produce antibiotics that can suppress damping-off of sugar beet caused by *Pythium ultimum* [1]. *C. cupreum* and *C. globosum* reduce leaf spot disease of corn caused by *Curvularia lunata*, rice blast caused by *Pyricularia oryzae,* sheath blight of rice caused by *Rhizoctonia oryzae* and tomato wilt caused by *Fusarium oxysporum* f.sp. *lycopersici* [2,3]

Chaetomium belongs to the division Ascomycota, and family Chaetomiaceae [4] and one of the largest genera of saprophytic fungi. It comprises more than 400 species worldwide [5]. This genus is characterized by superficial ascomata usually covered with hairs or setae [6]; membranaceous peridium, consisting of several pseudoparenchymatous layers; asci that are clavate or fusiform (with biseriately arranged ascospores) or sometimes cylindrical (with uniseriately arranged ascospores), thin-walled, evanescent and without apical structures; scarce paraphyses that disappear before ascocarps mature [7]; ascospores that are brown or gray-brown (never opaque or black), one celled, with one or sometimes two germ pores, and exuding as a dark, black, sticky mass [8].

Species identification based morphological characters alone is difficult to confirm certain closely related species such as C. *indicum* and *C. funicola; C. globosum* and *C. olivaceum; C. globosum* and *C. globosporum* etc [9]. Thus it is necessary to find an alternative method for accurate identification of the species of this genus. The advent of molecular tools for investigations in fungal identification has paved better way for easier and more accurate identification, if it is coupled with traditional methods such as morphological characters. ITS is

* To whom all correspondence should be addressed.
E-mail: vcsekhar123@gmail.com;
deebakamil@gmail.com

universal region for fungal identification [5,10,11]. Therefore the current research work was carried out by combining the molecular data (ITS based identification) with morphology for authentic identification of *Chaetomium* species.

MATERIALS AND METHODS

Collection of *Chaetomium* isolates

Chaetomium isolates were collected from different places and sources (Table 1). *Chaetomium* genus identified by using basic morphological features. Species identification was done based on key from Ames (1961).The same isolates were used for the molecular confirmation for ITS region amplification.

Optimization of cultural conditions

Single spore cultures were made through serial dilution technique. Growth conditions were standardized using different growth media (Potato dextrose Agar, Malt extract Agar, Oat meal Agar, and Potato carrot Agar) and temperature (20^0C, 25^0C, 30^0C, 35^0C, 40^0C and 45^0C).

Morphological characterization

The cultures of *Chaetomium* isolates were grown on malt extract agar medium in Petri dish at 35^0C and examined for size, shape and colour of the perithecium, ornamentation of terminal hairs, lateral hairs, rhizoids and size and shape of ascospores. For all photomicrographs Progres 2.7 version (Jenoptik, USA) camera was used. Measurements of perithecium, terminal hairs and ascospores were recorded and compared with type descriptions [12].

Genomic DNA Extraction

Genomic DNA was extracted from frozen mycelium of *Chaetomium* species based on Cetyl trimethyl ammonium bromide (CTAB) mini extraction method [13].The DNA concentration and purity of the samples was determined with Nano drop spectrophotometer (Thermo Scientific).

Amplification of the ITS region of *Chaetomium* spp.

DNA from 61 isolates was subjected to PCR amplification of ITS region by using universal primers (ITS1 (TCCGTAGGTGAACCTGCGG) and ITS4 (TCCTCCGCTTATTGATATGC) [14]). PCR reactions were carried out in 0.2 ml thin walled PCR tubes with a total reaction volume of 25µl containing 12.5µl of dream taq (master mix consisting of buffer, dNTP's, $MgCl_2$, Taq polymerase at appropriate concentrations and pre mix of loading dye), 1 µl of each forward and reverse primers, 1 µl of DNA sample and rest nuclease free water. The PCR amplification conditions were initial denaturation at 94^0C for 5 min followed by 35 cycles of denaturation at 94^0C for 1 min, primer annealing at 55^0C for 1min, primer extension at 72^0C for 2 min, and a final primer extension at 72^0C for 5 min.

DNA sequencing of the amplified fragments

The amplified products were separated on 1.2% agarose gel at 80 volts for 45 minutes using 1 x TAE buffer pH 8.0 containing ethidium bromide (0.5 µg/ml). The amplified fragment of DNA were compared with ladder of 1000 bp and the bands of DNA fragments were shown approximately 550 to 650 bp indicating the amplification of ITS region. The gels were photographed using gel documentation system (Fig.3). Sequencing of all the samples with distinct band were done through outsourcing.

Phylogenetic analysis

Molecular identification of *Chaetomium* spp. using ITS region sequences was done through NCBI BLAST (http://blast.ncbi.nlm.nih.gov) for the species identification. ITS sequences were aligned using CLUSTALW multiple sequence alignment [15]. Phylogenetic analysis was carried out using MEGA 5 program [16] and a maximum parsimony tree was constructed. Confidence values were assessed from 1000 bootstrap replicates of the original data.

RESULTS

Optimization of cultural conditions

From the observations (Fig.1, Table 2 & Table 3) it is inferred that the best medium for the good sporulation of the *Chaetomium* was found on malt extract agar medium and the optimum temperature is ranged between 35-40^0C. Although the oat meal agar have showed more growth rate than the any other media, the sporulation obtained was meager. Thus malt extract agar media was selected as best media for further studies.

Morphological identification

Morphological observations were made from the cultures grown on malt extract agar at 35-40^0C. *Chaetomium* species identification was done using following characters *viz*., size and shape of

Table 1. Details of isolates of *Chaetomium* species used in present study

Isolate No:	Source	Place
C-1	Soil	New Delhi
C-2	Soil	New Delhi
C-3	Soil	New Delhi
C-4	Soil	New Delhi
C-5	Wheat leaves	New Delhi
C-6	Soil	New Delhi
C-7	Soil	Hyderabad
C-8	Soil	Bhavnagar
C-9	Soil	New Delhi
C-10	Compost	New Delhi
C-11	Soil	Vadodara
C-12	*Cassia fistula*	Vadodara
C-13	*Tamarindus indica*	Vadodara
C-14	*Capsicum annuum* seed	Bhavnagar
C-15	Bottle brush tree	New Delhi
C-16	Soil	Pune
C-17	Soil	Jaipur
C-18	Cotton	Nagpur
C-19	Coprophilous	New Delhi
C-20	Wheat grain	New Delhi
C-21	Soil	Hyderabad
C-22	*Capsicum annuum* seed	Kamal
C-23	Soil	New Delhi
C-24	Mushroom compost	Solan
C-25	Soybean seeds	Shillong
C-26	Cotton	Nagpur
C-27	Compost	Chambaghat
C-28	Soil	New Delhi
C-29	Wheat	New Delhi
C-30	Wheat	Pune
C-31	Wheat	New Delhi
C-32	Wheat	Jaipur
C-33	Wheat leaves	New Delhi
C-34	Wheat grain	New Delhi
C-35	*Elettaria cardamomum*	Dapoli
C-36	Soil	Hyderabad
C-37	Soil	New Delhi
C-38	Soil	New Delhi
C-39	Soil	Vadodara
C-40	Soil	New Delhi
C-41	Mushroom compost	Solan
C-42	Neem bark	New Delhi
C-43	Soil	New Delhi
C-44	Bottle Palm	Junagadh
C-45	*Capsicum annuum* seed	Karnal
C-46	Soil	New Delhi
C-47	Decaying basket of *Mangifera indica*	New Delhi
C-48	Wheat grain	New Delhi
C-49	Soil	Ujjain
C-50	Funnel seed	New Delhi
C-51	Coriander seed	New Delhi
C-52	Black pepper seed	New Delhi
C-53	Sorghum seed	New Delhi
C-54	Sorghum seed	New Delhi
C-55	Ajwain	New Delhi
C-56	Cardamom	New Delhi
C-57	Funnel seed	New Delhi
C-58	Paper	New Delhi
C-59	Wood	New Delhi
C-60	Funnel seed	New Delhi
C-61	Methi seed	New Delhi

the perithecium ; branching pattern, surface texture, septation and density of terminal hairs; presence of lateral hairs and rhizoids; size, shape and colour of the ascospores. Predominantly the isolates were identified based on the terminal hairs and size and shape of ascospores as these are very distinct from species to species.

Taxonomy description of genus *Chaetomium*

Pertithecia are opaque, ostiolate, with blunt, narrow or beaked apex and its wall membranous, fragile, brittle with age, distinctly cellular, ornamented with appendages in the form

Table 2. Growth rate of *Chaetomium* isolate (C-35) on different media

	Growth in diameter * in cm on 15th day				
Media	R1	R2	R3	R4	R5
PDA	8.4	8.2	8.2	8.3	8.2
MEA	8.4	8.3	8.5	8.0	8.4
OAM	9.2	8.9	9.0	9.2	8.6
CPA	8.2	8.9	8.6	8.5	9.0
CD @ 5%	0.28				

*Average of five replications

Table 3. Growth rate of *Chaetomium* isolate (C-35) at different temperatures

	Growth in diameter * in cm on 14th day					
Temperature	R1	R2	R3	R4	R5	R6
20^{0}C	8.5	6.6	7.3	8.2	7.5	8.0
25^{0}C	7.0	8.1	7.1	7.4	8.0	8.0
30^{0}C	7.0	8.6	8.3	8.3	8.5	8.6
35^{0}C	8.9	9.0	8.4	8.5	8.5	8.8
40^{0}C	9.0	9.0	9.0	9.0	9.0	9.0
45^{0}C	3.6	3.7	3.5	3.6	3.4	3.4
CD @ 5%	0.33					

*Average of six replications

Table 4. Integrated approach (morphological and molecular) for the identification of *Chaetomium* species

Isolate No.	Identified Species based on Morphology	ITS Region identification based on NCBI Data
C-1	*C.elatum*	*C. elatum*
C-2	*C.atrobrunneum*	*C.atrobrunneum*
C-3	*C.globosum*	*C.globosum*
C-4	*C.brasilense*	*C.brasiliense*
C-5	*C.cochliodes*	*C.globosum*
C-6	*C.cochliodes*	*C.globosum*
C-7	*C.globosum*	*C.globosum*
C-8	*C.funicola*	*C.funicola*
C-9	*C.funicola*	*C.funicola*
C-10	*C.funicola*	*C.funicola*
C-11	*C.atrobrunneum*	*C.atrobrunneum*
C-12	*C.atrobrunneum*	*C.atrobrunneum*
C-13	*C.atrobrunneum*	*C.atrobrunneum*
C-14	*C.megalocarpum*	*C.megalocarpum*
C-15	*C.globosum*	*C.globosum*
C-16	*C.globosum*	*C.globosum*
C-17	*C.globosum*	*C.globosum*
C-18	*C.cochliodes*	*C.globosum*
C-19	*C.globosum*	*C.globosum*
C-20	*C.globosum*	*C.globosum*
C-21	*C.globosum*	*C.globosum*
C-22	*C.globosum*	*C.globosum*
C-23	*C.globosum*	*C.globosum*
C-24	*C.globosum*	*C.globosum*
C-25	*C.globosum*	*C.globosum*
C-26	*C.globosum*	*C.globosum*
C-27	*C.globosum*	*C.globosum*
C-28	*C.globosum*	*C.globosum*
C-29	*C.globosum*	*C.globosum*
C-30	*C.globosum*	*C.globosum*
C-31	*C.globosum*	*C.globosum*
C-32	*C.globosum*	*C.globosum*
C-33	*C.globosum*	*C.globosum*
C-34	*C.brasilense*	*C.brasiliense*
C-35	*C.brasilense*	*C.brasiliense*
C-36	*C.funicola*	*C.funicola*
C-37	*C.megalocarpum*	*C.megalocarpum*
C-38	*C.globosum*	*C.globosum*
C-39	*C.brasilense*	*C.brasiliense*
C-40	*C.cochliodes*	*C.globosum*
C-41	*C.globosum*	*C.globosum*
C-42	*C.globosum*	*C.globosum*
C-43	*C.globosum*	*C.globosum*
C-44	*C.nigricolor*	*C.nigricolor*
C-45	*C.globosum*	*C.globosum*
C-46	*C.globosum*	*C.globosum*
C-47	*C.globosum*	*C.globosum*
C-48	*C.globosum*	*C.globosum*
C-49	*C.globosum*	*C.globosum*
C-50	*C.megalocarpum*	*C.megalocarpum*
C-51	*C.globosum*	*C.globosum*
C-52	*C.globosum*	*C.globosum*
C-53	*C.megalocarpum*	*C.megalocarpum*
C-54	*C.globosum*	*C.globosum*
C-55	*C.perlucidum*	*C.perlucidum*
C-56	*C.globosum*	*C.globosum*
C-57	*C.megalocarpum*	*C.megalocarpum*
C-58	*C.atrobrunneum*	*Catrobrunneum*
C-59	*C.globosum*	*C.globosum*
C-60	*C.funicola*	*C.funicola*
C-61	*C.perlucidum*	*C.perlucidum*

Fig. 1. Growth rate of Chaetomium (C-35) isolate in different media

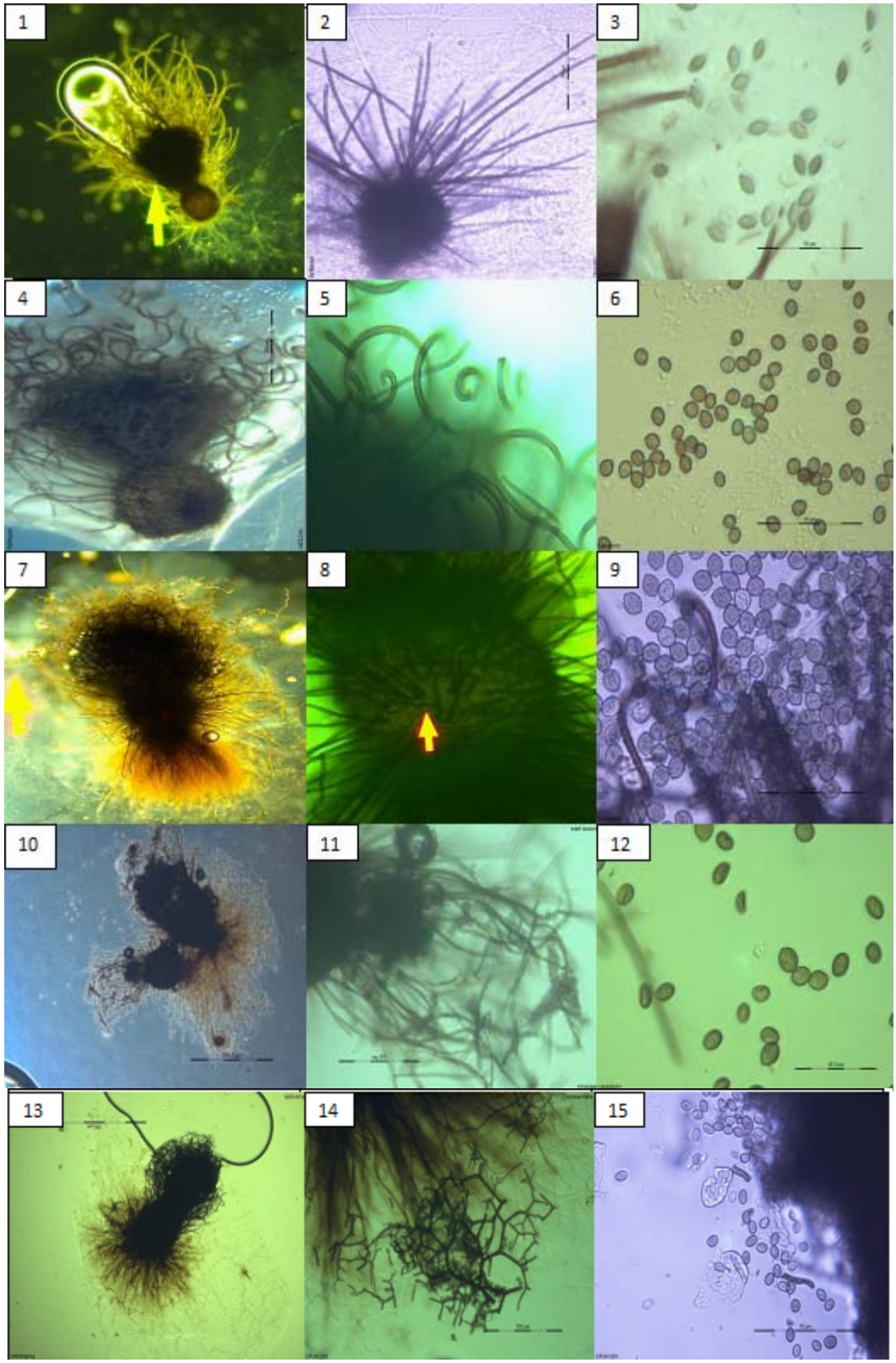
of diversely modified and variously coloured hairs called terminal hairs. Asci gelatinous–walled, delicate, stalked, club shaped, linear, cylindrical, evanescent, deliquescing before the ascospores are mature or sometimes persisting to their maturity before deliquescing, with 8 ascospores. Ascospores single celled, slightly or darkly coloured, most frequently olive-brown, occasionally bright coloured, typically lemon shaped but occurring in a variety of shapes, sizes and colours.

Comparison of morphological traits for species differentiation

1. Terminal hairs dichotomously branched. Ascospores ovoid, apiculate.

i. Terminal hairs uniformly branched with sharp tips, Ascospores size 11-13 x 8.5-9.5 µm.....*C. elatum*

ii. Branched hairs acutely angled, often inflated between septa, combined with long unbranched stiff, sharp-pointed hairs, Ascospores size 5.5-6.5 x 3.75-5 µm.*C .funicola*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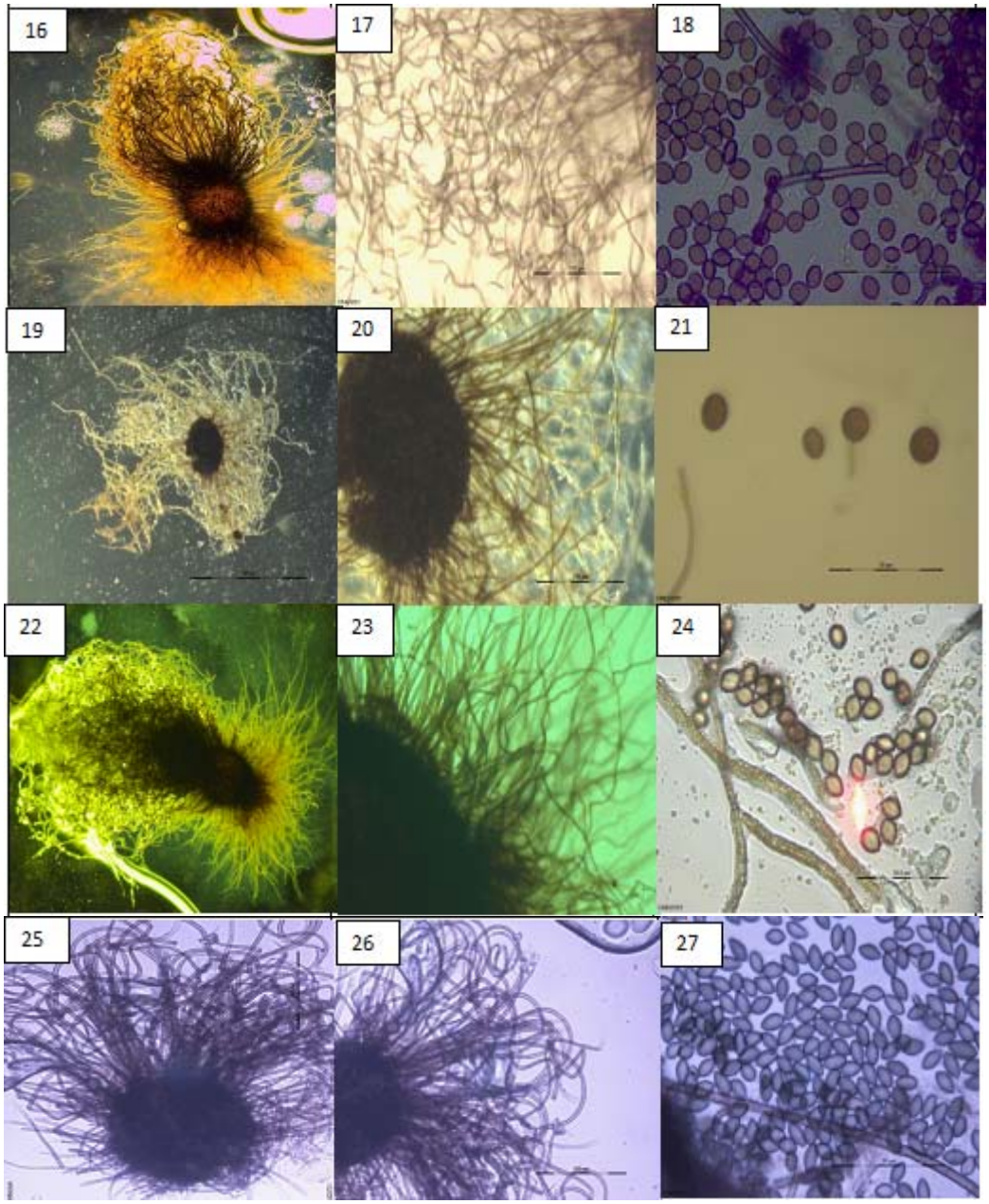

Fig. 2. (1-3) *Chaetomium atrobrunnem,* (4-6) *Cheatomium brasiliense,* (7-9) *Chaetomium cochliodes,*(10-12) *Chaetomium elatum,* (13-15) *Chaetomium funicola,* (16-18) *Chaetomium globosum,* (19-21) *Chaetomium megalocarpum,* (22-24) *Chaetomium nigricolor,* (25-27) *Chaetomium perlucidum*

2. Terminal hairs dark brown, branched at right angles or nearly so, Ascospores almond-shaped or ellipsoid, 10-12 x 5.5-7.5 µm ……..…….....*C. atrobrunneum*

3. Terminal hairs branched, delicate and terminating into flexuous ends –

i. Ascospores very dark, irregularly spherical 13-16.5 x 12-14 µm…...*C. megalocarpum*

ii. Ascospores dilute brown, pale green when young, 5-6 x 4-5 µm ……*C. nigricolor*

4. Terminal, hairs straight, slightly curved or wavy ; Ascospores smaller to medium, size ranges between 9-13 x 6-9.5 µm, apiculate ….……...…….....*C.globosum*

5. Terminal hairs coiled, unbranched but intermingled with other hair types –

i. Terminal hairs with 4-7 close spirals accompanied with a few long, flexuous hairs, forming a relatively compact head,Ascospore size 6-7.5 x 5.5-6.5 µm….*C.brasiliense*

ii. Terminal hairs kinky-wavy, mildly distorted or loosely coiled, Ascospores fusiform, collapsing with a long furrow, 8-13.5 x 5.6-6 µm ……..*C.perlucidum*

iii. Terminal hairs forming a dense head with stout, spiraled with 3-4 convolutions, Ascospores lemon-shaped, apiculate on both ends, 7-10.5 x 6.5-8.5 µm long ……*C cochliodes*

Morphological characters of different species of *Chaetomium*

***Chaetomium atrobrunneum* Ames Fig. 2** [1-3]

Synonymy: *C. fusisporale / C. rectopillium.*

Perithecia dark brown to black, small, ostiolate, globose, subglobose to oval with size of 80-150 × 80-130 µm. Base of perithecia round and firmly attached to the substrate by short, thin, light brown rhizoids. Terminal hair long, dark brown straight, erect, unbranched /branched right angled with smooth to slightly rough tapering to narrow blunt tip. Lateral hairs dark brown colour, straight slightly rough, septate with blunt tips. Asci clup shaped, evanescent, 8 spored. Ascospores dark-brown, ellipsoid to almond shaped 8-13 × 4-7 µm.

Material examined: C-2, C-11, C-12, C-13 and C-58.

***Chaetomium brasiliense* Batista & Pontual (Ames) Fig. 2** [4-6]

Synonymy: *C. fuscum / C. albo-arenulum.*

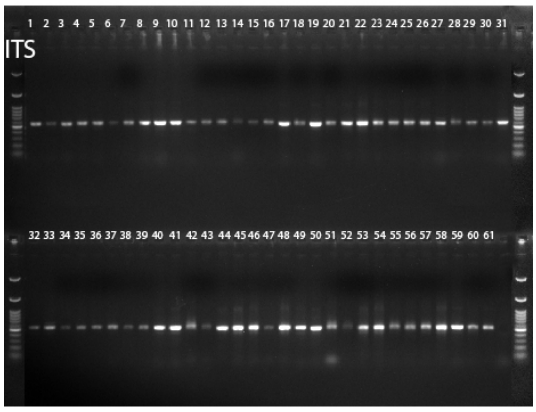

Fig. 3. Banding pattern of ITS of the 61 isolates of Chaetornium

Perithecia brown to black, ostiolate, small, subglobose or ovate, basal oval or pointed 100-130 x 90-110 µm, attached to substratum with black rhizoids. Terminal hairs flexed bellow, spirally coiled above with four to several coils, septate, smooth or finely rough with rounded tips. Lateral hairs slender, straight or flexed septate, finely roughened at base. Asci cylindrical linear. Ascospores light olive- brown, broadly ovate, 5-6 x 3-5 µm, sub apiculate at one end rounded at other end.

Material examined: C-4, C-35, C-34 and C-39.

***Chaetomium cochliodes* Palliser Fig. 2** [7-9]

Synonymy: *C. globosum / C. kunzeanum / C. olivaceum / C. spirale / C. barbatum / C. subterraneum / C. ochraceum / C. fibripilium / C. molliplium / C. lustanicum / C. subglobosum / C. coarctatum / C. rectum / C. spiculipilum.*

Perithecia olive green to brown, ostiolate, globose to subglobose 260-360 x 260-320 µm in size anchor to the substrate by rhizoids. Terminal hairs numerous interwoven, forming thick mass with two types of hairs (a) convolution beyond the dense head; (b) slender, twisted undulate with 3-5 coils. Lateral hairs numerous, septate, finely roughened. Asci irregularly club shaped. Ascosproes olive-brown, lemon-shaped, 8-12 x 6-9 µm apiculate at both ends.

Material examined: C-5, C-6, C-18 and C-40.

***Chaetomium elatum* Kunze ex Fr. Fig. 2** [10-12]

Synonymy: *C. pannosum / C. tenuissimum / C. virgicephalum / C. ramipilosum.*

Perithecia black, ostiolate, large, subglobose or ovate, 360-450 x 270-350 µm attached to substratum by black rhizoids. Terminal hairs olive brown, coarsely roughened with spines, stout, dichotomously branched. Lateral hairs dark olive brown, unbranched, straight or slightly flexed, long slender, roughened. Asci club shaped, 8-spored. Ascospores olive- brown, lemon shaped, 10-15 x 7-9 µm, apiculate at both ends.

Material examined: C-1.

***Chaetomium funicola* Cooke Fig. 2** [13-15]

Synonymy: *C. horrida / C. dolichotrichum.*

Perithecia dark brown, globose to sub globose, 90-160 x 90-180 µm, terminal hairs dark olive brown, of two kinds (a) unbranched, long and straight, gradually tapering to blunt tips and (b) shorter, dichototmously branched at acute angles, tips rounded, both hairs coarsely roughened. Lateral hairs dark olive brown with

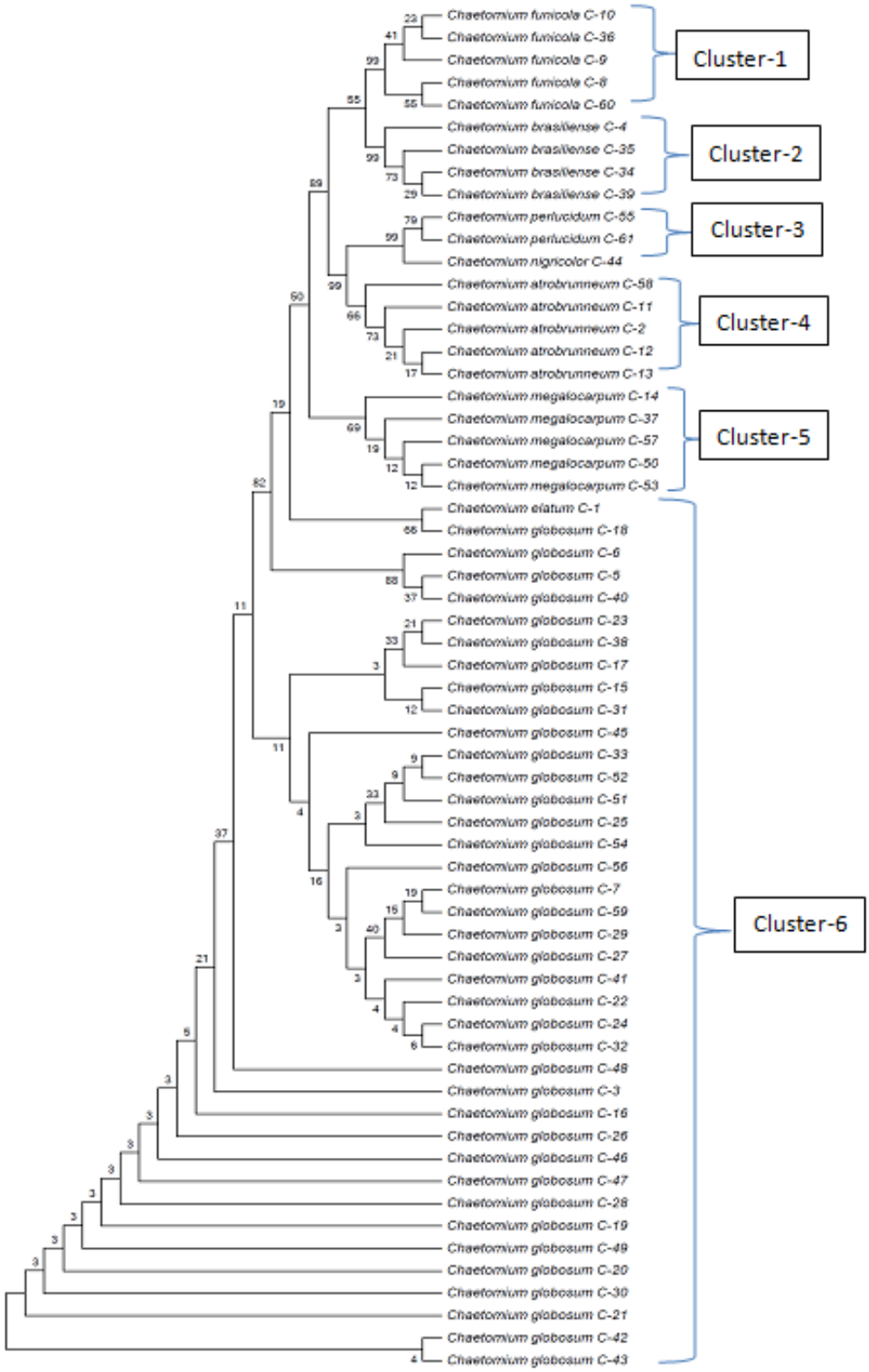

Fig. 4. Phylogenetic relationships of 61 isolates of *Chaetomium* spp. inferred by analysis of ITS1 sequences obtained by using maximum parsimony analysis

hyaline tips. Asci club shaped, eight- spored. Acsospores olive brown, ovate to ellipsoid 5-8 x 3-5 µm in size.

Material examined: C-8, C-9, C-10, C-36 and C-60.

***Chaetomium globosum* Kunze ex Fr Fig. 2** [16-18]

Synonymy: *C. kunzeanum / C. olivaceum / C. spirale / C. barbatum / C. subterraneum / C. ochraceum / C. fibripilium / C. molliplium / C. lustanicum / C. subglobosum / C. coarctatum / C. rectum / C. spiculipilum.*

Perithecia black, ostiolate, globose to subglobose or broadly ovate, 170-380 x 180-310 µm, in size. Terminal hairs numerous, slender, straight or slightly flexed, septate, asci club shaped, eight-spored. Ascospores dark olive-brown, shapes varies from broadly ovate to lemon shape 8-12 x 6-9 µm in size with ends apiculate.

Material examined: C-3, C- 5, C-6, C- 7, C- 15, C-16, C-17, C-18, C-19, C-20, C-21, C-22, C-23, C-24, C-25, C-26, C-27, C-28, C-29, C-30, C-31, C-32, C-33, C-38, C-40, C-41, C-42, C-43, C-45, C-46, C-47, C-48, C-49, C-51, C-52, C-54, C-56 and C-59.

***Chaetomium megalocarpum* Bainier Fig. 2** [19-21]

Synonymy: *C. atrosporum.*

Perithecia olive green in colour, subglobose to ovoid, without distinct neck, 300-330 x 240-290 µm in size. Terminal hairs long, slightly tapering to blunt tips, minutely roughened, non septate, occasionally branching at wide angles. Lateral hairs light yellow, long, nonseptate. Asci club shaped, 8-spored. Ascospores globose, subglobose or ovoid, 14-17 x15-16 µm, dark brown, often depressed on one side.

Material examined: C-14, C-37, C-50, C-53 and C-57.

***Chaetomium nigricolor* Ames Fig. 2** [22-24]

Synonymy: *C. abuense / C. vitis / C. verricichaeta / C. psuedoerraticum.*

Perithecia brown to black, ostiolate, globose to subglobose, 200-285 x 190-260 µm, attached to the substratum with brown rhizoids, the bushy-haired heads with a ragged appearance. Terminal hairs numerous, black, regularly and thickly covered with little black, projections, appearing at random, undulating or with occasional spiral coils, frequently branching, at base 3-4µ in diameter, tapering to faded tips. Ascospores when mature brown almond-shaped, slightly apiculate at both ends, 5-6 x 4-5µm.

Material examined: C-44.

Perithecia is olive- yellow to light brown, globose, 105- 135 x 100-130 µm, with rhizoids at the base, Terminal hairs forming a comparatively unbranched, septate, the lower third is straight, coiled above into a very elongated, somewhat conical spiral 30-45 µm in diameter at the base, with sparse septations. Lateral hairs are straight with septation, with tapering tips. Asci short, clavate, 39-45 x 12 µm, 8-spored. Ascospores spindle – shaped, with blunt, round ends, 12-13.5 x 5.6-6 µm, of a muddy brownish-yellow colour.

Material examined: C-55 and C-61.

Molecular Identification of different *Chaetomium* species using ITS region

ITS sequences of all the 61 isolates of *Chaetomium* species were interfaced with the NCBI genebank database using basic alignment search tool to identify as respective species (Table.4). Evolutionary analyses were conducted using the Maximum Parsimony method. The analysis involved 61 ITS1 nucleotide sequences. Tree 1, out of 4 most parsimonious trees (length = 147) is shown. The consistency index is 0.62, the retention index is 0.91, and the composite index is 0.62 (0.57) for all sites and parsimony-informative sites (in parentheses) were calculated. All positions containing gaps and missing data were eliminated. There were a total of 437 positions in the final dataset. It is depicted from the dendrogram (Fig.4) that the different species of *Chaetomium* clustered into six major groups.

Five isolates of *C. funicola* (C-8, C-9, C-10, C-36, C-60) , four isolates of *C. brasiliense* (C-4, C-35, C-34, C-39) , five isolates of *C. atrobrunneum*, (C-2, C-11, C-12, C-13, C-58) and five isolates of *C. megalocarpum*, (C-14, C-37, C-50, C-53, C-57) and were grouped together in Clusters 1, 2, 4 and 5, respectively. C-55, C-61 and C-44 isolates of two species *viz*., *C. perlucidum* and *C. nigricolor* were grouped in Cluster 3 with 74-95 % similarity. Thirty eight isolates of *C.globosum* were grouped into Cluster 6 along with one isolate of *C.elatum* (C-1) which has showed similarity index of 77-92%. More than 33 isolates of *C.globosum* have showed 87-92% similarity

index with the *C. elatum* indicating it could have evolved from the *C. globosum*. Further, the species identified through the morphology were compared with the data obtained through molecular approach and found very much complemented (Table 4).

DISCUSSION

Chaetomium genus gaining more importance in agriculture as this can be used for bio-control to combat various economically important diseases [17, 18, 19]. More than 400 species have been described in *Chaetomium* according to the statistics of Index Fungorum. In this perspective it has become mandate for exact species identification. Terminal hairs have formed one of the important character to identify these species of this genus [12, 20, 21,22,23,24]. Other major characters for species differentiation are morphology of asci and ascospores [7, 8.25]. Present investigation was carried out based on integrated approach of morphological and molecular characters of different species of *Chaetomium*. Many characters of morphology were considered for species differentiation and all the species were clearly discussed in the area of taxonomy description. Since from the discovery of this fungus by Kunze 1817, only few taxonomists worked on morphology of this fungus [12, 26, 27, 28]. In the present study different species of *Chaetomium viz*., *C. globosum, C. atrobrunneum, C. brasiliense, C. elatum, C. cochliodes, C. funicola, C. nigicolor, C. megalocarpum* and *C. perlucidum* were defined based on morphological characters. The morphological data obtained in the study was supplemented earlier studies [7, 26, 29, 30, 31, 32, 33, 34], providing an authentic identification of these species. An exception has observed in combined approach through morphology and ITS based identification, a species of *Chaetomium* was identified as *C.cochliodes* (C-5, C-6, C-18 and C-40) through morphology. But these isolates were identified as *C.globosum* through ITS based identification. Both the species are reported as synonyms [35] and taxonomical description for *C.cochliodes* was mentioned to have two different types of terminal hairs, coiled and undulating kind. In our study we got only coiled type of terminal hairs which was very different from *C.globosum* morphology, the other type of terminal hairs which is similar to *C.globosum* morphology was not observed. Therefore we have kept *C.cochliodes* same intact in morphological identification. These observations further inferring that above species are most commonly occurring in India.

Morphological characteristics are alone not enough to define the identity of *Chaetomium* species as this genus has multiple species-specific characters in several species; therefore, it was decided to define the morphology along with ITS sequence analysis. The maximum parsimony analysis resulted into formation of six clusters showing different species of *Chaetomium*. The clustering of different species matches with the earlier findings of Asgari and Zare (2011).According to them a combined sequence dataset of the ITS region, partial LSU rDNA, and â-tubulin gene sufficiently resolved five species groups of *Chaetomium* and the molecular data largely concordant with combined features of morphology of the *Chaetomium* species. Analysis of the entire fingerprint profile using the unweighted pair-group method with arithmetic averages (UPGMA) clearly differentiated *C. globosum* isolates from *C. perlucidum* and *C. reflexum* [36]. Aggarwal *et al*. (2013) have done the molecular characterization of 18 *Chaetomium* isolates collected from India based on the ITS region of the rRNA gene sequences. Results indicate that, different isolates of the *Chaetomium* spp. may have different specialized life strategies in surviving diverse climates [10]. Wang *et al*., 2014 have done multigene phylogenetic analyses with ribosomal ITS, partial ribosomal large subunits (28S rDNA), â-tubulin, the translation elongation factor 1á (TEF1-á), and the largest subunit of RNA polymerase [22] (RPB1) and recognized eight well-supported lineages within the monophyletic *C. indicum* group [5]. All these data sufficiently supplements the resolution of species of *Chaetomium* by using molecular data.

The present paper describes the typical characters and identification of nine species of *Chaetomium* based on morphology which was complemented with ITS region sequence analysis. Thus morphological identification supplemented with the molecular data would result in to more accurate identification of the *Chaetomium* species.

CONCLUSION

From the data it can be suggested that the most commonly occurring species of *Chaetomium* in India include *C. atrobrunneum, C. brasilense, C. cochliodes, C. elatum, C. funicola, C. globosum, C. megalocarpum, C. nigricolor* and *C. perlucidum*. Combined morphological and molecular approach for identification of *Chaetomium* well fit for species identification. Molecular data can complement the morphological data for better identification.

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