KAVAKA 43: 64-69 (2014)

Lasiodiplodia indica -A new species of coelomycetous mitosporic fungus from India

Indu Bhushan Prasher* and Gargi Singh
Department of Botany, Mycology and Plant Pathology Laboratory
Panjab University, Chandigarh, India
** Corresponding author email: chromista@yahoo.co.in*
(Submitted in November, 2014; Accepted on December 20, 2014)

ABSTRACT

Lasiodiplodia indica sp. nov. is described as a new species based on morphological characteristics and DNA sequence data of ITS1 and ITS4. It differs from other species in the nature of the conidiomata, conidial septation, branching and septation of paraphyses. Detailed description, taxonomical remarks, and illustrations are provided.

Key words: *Coelomycetes*, conidiomata, ITS, phylogeny, taxonomy

INTRODUCTION

Lasiodiplodia species are widespread, most commonly found in tropical and subtropical regions where they cause variety of diseases (Punithalingam, 1980). However, only a single species viz: *L. theobromae* has been reported from different geographical regions of India (Bilgrami *et al*., 1991; Jamaluddin *et al*., 2004). The presence of pycnidial paraphyses and longitudinal striations on mature conidia are the typical characteristics of this genus that distinguishes it from other closely related genera.

This report is a part of the ongoing study to inventorize anamorphic and telomorphic fungi of North West India including Himalaya (Sohi and Prasher, 1981; Prasher and Sharma, 1997; Prasher *et al*., 2003; 2004; 2005; 2008; Prasher and Verma 2012a; b; Prasher and Ashok, 2013; Prasher and Lalita, 2013; Prasher and Singh, 2012; 2013; 2014; Ashok and Prasher, 2014a; b; Prasher and Sushma, 2014). An interesting coelomycetous fungus was isolated from fallen twigs near the trees of *Morus alba* L. collected from the Botanical Gardens, Department of Botany, Panjab University, Chandigarh, India. A thorough review of literature (Sutton, 1980; Abbas *et al*., 2004; Pavlic *et al*., 2004; 2008; Burgess *et al*., 2006; Damm *et al*., 2007; Alves *et al*., 2008; Abdollahzadeh *et al*., 2010; Begoude *et al*., 2010; Úrbez-Torres *et al*., 2011; Ismail *et al*., 2012; Phillips *et al*., 2013) and detailed examination using both morphological characteristics and DNA sequence data of the rDNA internal transcribed spacers, ITS1 and ITS4, revealed it to be an undescribed species of *Lasiodiplodia*.

MATERIAL AND METHODS

Fungal isolation: The fungus was isolated from fallen twigs collected from Botanical Gardens, Panjab University, Chandigarh. The isolations were made by directly plating out pieces of the fungal tissue after surface sterilization (2 min in 90% ethanol). The conidiomata were cut through horizontally and the contents were transferred on plates of PDA. The plates were incubated at 25 °C.

Morphology and cultural characteristics: To induce sporulation the isolates were transferred on 2% PDA (HiMedia) and incubated at 25 °C for 4-6 weeks in the dark. Culture colours (upper surface and reverse) were described using the colour charts of Rayner (1970). Morphological characters were studied from the isolates sporulating on PDA as well as from the fungal material on the host tissue. Cross-sections of conidiomata were made by hand, stained in Cotton blue (Cotton blue 0.01g+Lactic acid 100 ml) and mounted in glycerol to observe conidiophores and paraphyses morphology. Conidial masses were mounted in Amann's Lactophenol (Phenol-20 g, Lactic acid-20 g, Glycerol-40 g, Distilled water 20 ml). All digital images were recorded with Matrix VL-Z60 stereo triocular microscope and Matrix VRS-2f transmission microscope. Measurements were made using dgsoft ProMed software.

DNA extraction, amplification and sequencing: The molecular characterization of *Lasiodiplodia indica* was done by employing the technique of White *et al*. (1990) by amplifying the entire ribosomal internal transcribed spacer (ITS) using ITS1 (5'- TCCGTAGGTGAACCTGCGG-3') and ITS 4 (5'-TCCTCCGCTTATTGATATGC-3'). Mycelium was harvested from colonies on PDA grown at 25 °C for 7 days in the dark and total genomic DNA was extracted using HiPurA™ SP Fungal DNA mini kit (HiMedia) by following the manufacturer's instructions. DNA was stored at -20 °C for further use.

Fragment containing the region encoding ITS 1, 5.8 s rDNA and ITS 4 was amplified using primer pair ITS 1 and ITS 4 (White *et al*., 1990). DNA amplification was performed in a 25 µl reaction using 2 µl of template DNA (30 ng), 1U of Taq DNA polymerase (Genei, Bangalore India), 2.5 µl of 10 x Taq DNA polymerase buffer, 1µl of 10 pmol primer, H_2O (Sterile Ultra Pure Water Sigma) to make up volume 25 µl. For the amplification of ITS region following PCR condition were used: 3 min at 95 ºC, 1 min at 56 ºC, 1 min at 72 ºC and final 7 min extension step at 72 ºC. The PCR product was purified with an Axygen PCR cleanup kit (Axygen Scientific, CA, USA) and sequenced with the same primers using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, USA). The sequencing reactions were run on an ABI 3730/3730XL-1409-023 automated DNA sequence (Applied Biosystems, USA). The sequencing step was done by Xcelris genomics, An Abellon Company.

Phylogenetic analysis: ITS sequences of 19 isolates of *Lasiodiplodia* species, 2 isolates representing 2 species of *Diplodia* and *Botryosphaeria dothidea* were retrieved from GenBank **(Table 1)** and compared with *Lasiodiplodia indica*. Fungal sequences were aligned using the ClustalW multiple alignment program (Thompson *et al*., 1994). Manual adjustments of sequence

Table 1 Isolates of species considered in the phylogenetic study

Species	Isolate	Origin	Host	Collector	GenBank accession no.
Lasiodiplodia theobromae	CBS 164.96	New Guinea	Fruit	-	NR_111174
L. viticola	CMM4014	Brazil	*Mangifera indica*	-	JX464098
L. hormozganensis	CMM3987	Brazil	*M. indica*	-	JX464094
L. brasiliense	CMM2320	Brazil	*Carica papaya*	-	KC484814
L. egyptiacae	CBS130992	Egypt	*Mangifera indica*	A Ismail	NR_120002
L. mahajangana	CMW27801	Madagascar	*Terminalia catappa*	-	FJ900595
L. iraniensis	WAC13297	Australia	*Mangifera indica*	-	GU172379
L. missouriana	UCD2199MO	Missouri, USA	*Vitis* sp.	K Striegler & GM Leavitt	HQ288226
L. parva	CBS 456.78	Colombia	Cassava-field soil	O Rangel	NR_111265
L. pseudotheobromae	CBS 116459	Costa Rica	*Gmelina arborea*	J Carranza-Velásquez	NR_111264
L. gilanensis	IRAN1501C	Iran	-	J Abdollahzadeh & A Javadi	GU945352
L. plurivora	STE-U5803	South Africa	*Prunus salicina*	U Damm	EF445362
L. citricola	7E80	California, USA	-	-	KC357300
L. margaritacea	CBS122065	Australia	*Adansonia gibbosa*	TI Burgess	EU144051
L. rubropurpurea	WAC12538	Australia	*Eucalyptus grandis*	TI Burgess & G Pegg	DQ103556
L. venezuelensis	P1	Venezuela	-		JX545103
L. crassispora	WAC 12533	Australia	*Santalum album*	TI Burgess & B Dell	NR_111194
L. gonubiensis	CBS 115812	South Africa	*Syzygium cordatum*	D Pavlic	NR_111218
L. lignicola	MFLUCC 11-0435	Thailand	Wood	AD Ariyawansa	NR_111795
L. indica	IBP 01	India	Angiosperm wood	IB Prasher & G Singh	KM376151
Diplodia africana	STE-U5908	South Africa	*Prunus persica*	U Damm	NR_119635
D. mutila	B53	Italy	-	-	FJ481586
Botryosphaeria dothidea	CMW 8000	Switzerland	*Prunus* sp.	B Slippers	NR_111146

alignment was done using BioEdit Sequence Alignment Editor Version 7.0.8. (©19972005 Tom Hall). Phylogenetic analyses of sequence data were done using PAUP* v.4.0b10 (Swofford, 2003) for Maximum-parsimony (MP) and Neighbour joining (NJ) analyses. The NJ analysis was performed using Kimura-2 parameter nucleotide substitution model (Kimura, 1980). All characters were unordered and of equal weight. Bootstrap values were obtained from 1000 NJ bootstrap replicates. Maximum-parsimony analysis was performed using the Tree-Bisection-Regrafting (TBR) algorithm (Nei and Kumar, 2000) with search level 1 in which the initial trees were obtained by the random addition of sequences (100 replicates). All characters were unordered and of equal weight and all positions containing gaps and missing data were eliminated. Branches of zero length were collapsed and all multiple, equally parsimonious trees were saved. The robustness of the most parsimonious trees was evaluated by 1000 bootstrap replications (Hillis and Bull, 1993). Other measures used were consistency index, retention index and composite index. Evolutionary analyses were conducted in MEGA6 (Tamura *et al.*, 2013). Fungal sequences were deposited in GenBank and the specimen (Holotype) was deposited in the Herbarium of Botany Department Panjab University, Chandigarh, India (PAN). Culture of the novel species described in this study was deposited in the culture collection of the Botany Department, Panjab University, Chandigarh (PAN).

RESULTS

PHYLOGENETIC ANALYSIS

The analysis involved 23 isolates compared on the basis of ITS sequences. There were a total of 424 positions in the final dataset. Maximum parsimony analysis of the final dataset resulted in 10 equal, most parsimonious trees

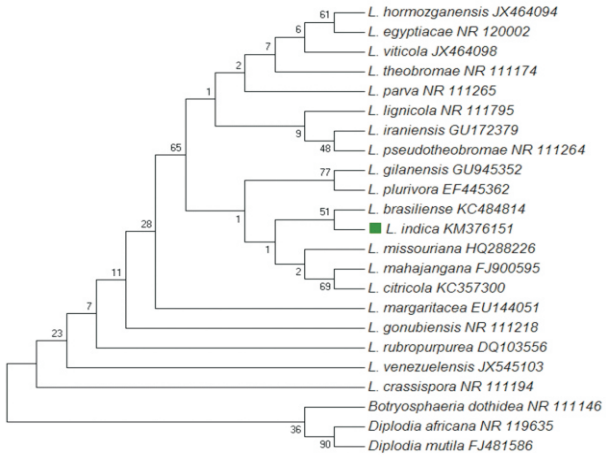

Fig. 1 Maximum Parsimony analysis of taxa. The evolutionary history was inferred using the Maximum Parsimony method. Tree #1 out of 10 most parsimonious trees (length = 90) is shown. The consistency index is (0.611111), the retention index is (0.686567), and the composite index is 0.526368 (0.419569) for all sites and parsimony-informative sites (in parentheses).

(consistency index = 0.611111; retention index = 0.686567 and composite index = 0.526368) each with the same topology. One of the 10 most parsimonious tree is presented in **Fig. 1**.

TAXONOMY

Lasiodiplodia indica I.B. Prasher and Gargi Singh sp. nov. **Figs. 2-4**

MycoBank MB810909

Conidiomata multilocular, with 1-2 ostioles; paraphyses hyaline, with fusoid pointed tip,

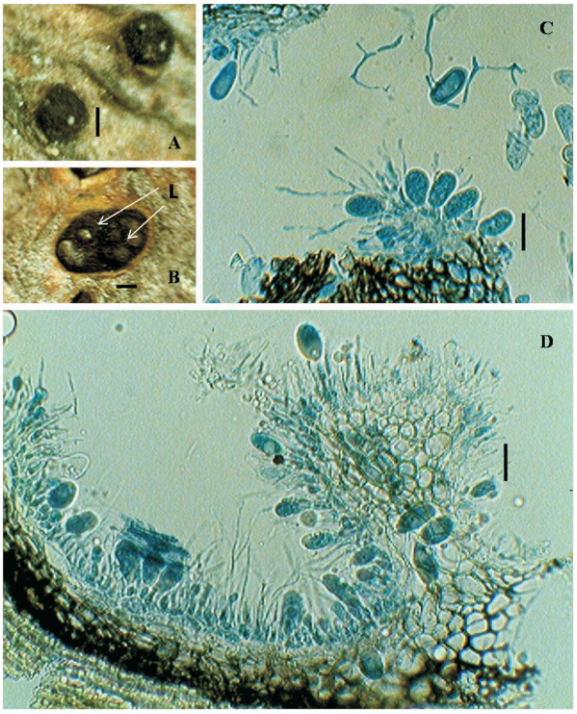

Fig. 2 ***Lasiodiplodia indica*** A-*Conidiomata* erumpent through the host bark, B-*Conidiomata* cut through horizontally showing locules (L), C & D-Cross section of *conidiomata* showing paraphyses, conidiogenous cells and conidia. Bars A, B= 200 µm; C, D=20 µm.

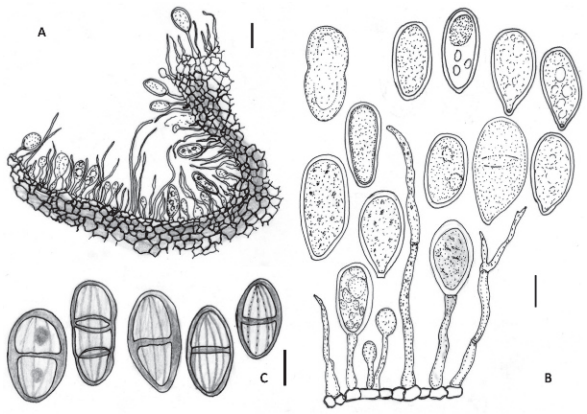

Fig. 4 ***Lasiodiplodia indica*** *(*Line drawings) A-Cross-section of conidiomata showing paraphyses, conidiogenous cells and conidia, B-Conidiogenous cells, paraphyses and hyaline conidia, C-Mature septate conidia with striations. Bars A= 20 µm; B, C=10 µm.

septate and occasionally branched; conidia initially hyaline, unicellular, later developing one to two septa, with dark brown pigmentation and longitudinal striations from apex to base.

Etymology: After the name of the country of origin.

Mycelium semi-immersed, branched, septate, dark brown. *Conidiomata* eustromatic, semi-immersed, globose, dark brown, multilocular, up to 1 mm, with 1-2 ostioles; wall dark brown, thick-walled, texura angularis, paler and

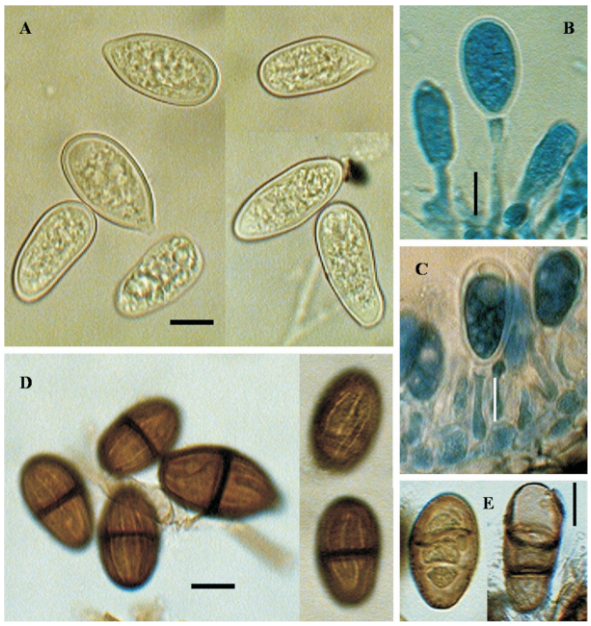

Fig. 3 ***Lasiodiplodia indica*** A-Hyaline conidia, B & C-Conidia attached to conidiogenous cell, D-Brown conidia with one septum and striations, E-Brown conidia with two septa. Bars A-E = 10 µm.

thinner towards the conidiogenous region, often with dark brown superficial hyphae over the surface. Paraphyses hyaline, with fusoid pointed tip, septate and occasionally branched, up to 120 × 1.5-3.5 µm. Conidiogenous cells holoblastic, determinate, discrete, cylindrical, hyaline, smooth, formed from cells lining the inner pycnidial walls, 8.5-15(17.5) × 1.5-3.5(4) µm. Conidia acrogenous, initially hyaline, unicellular, ellipsoid to obovoid, thick walled, guttulate, rounded at apex, truncate at the base, later developing one to two septa, dark brown pigmentation and longitudinal striations from apex to base, 20-38 × 11-20.5 µm.

Collection examined: India, Chandigarh 321 m, Botanical gardens, Panjab University, 21.03.2011, on fallen twig of an angiospermous tree, I. B. Prasher and Gargi Singh (Holotype: PAN 30202).

Culture characters: The fungus was isolated on 2% PDA (HiMedia) at 25 °C in the dark. The fungus produced aerial white mycelia initially, turning paler after 7 days and becoming olivaceous black within 20-25 days, reverse side of the colony dark slate-blue, producing pycnidia after 45 days. Optimum temperature for growth 25-30 °C.

DISCUSSION

The presently examined collection was identified as a species of *Lasiodiplodia* based on the typical characteristics of the genus which is the presence of pycnidial paraphyses and longitudinal striations on mature conidia. It can be distinguished morphologically and phylogenetically from the previously described species. The species is differentiated from the rest of the species of the genus described till-to-date by the multilocular nature of the conidiomata. In the septation of conidia it resembles *L. gonubiensis* to some extent. However, the conidia in *L. gonubiensis* are 1-3 septate as compared to 1-2 septate in

Table 2 Conidial and paraphyses dimensions of *Lasiodiplodia* spp.

Species	Conidia (µm)	L/W ratio	No. of septa	Conidiogenous cell (µm)	Paraphyses Size (µm)	Septation and branching
Lasiodiplodia theobromae	21-31× 13-15.5	1.9	1	5-15 × 3	Up to 55 × 3-4	Septate, occasionally branched
L. fiorii	24-26 × 12-15	-	1	-	-	-
L. ricinii	16-19 × 10-11	-	1	-	25-35 × 2	-
L. thomasiana	28-30 × 11-12	-	1	-	Up to 90 × 1.5	-
L. gonubiensis	(28)32-36(39) × (14)16-18.5(21)	1.9	1-3	(6.5)10-15(18) × 1(2)-4(4.5)	(14)26.5-47(65) × (1.5)2-2.5(3)	Aseptate, unbranched
L. undulata	20 × 12	-	1	5-15 × 1.5-3	-	Septate, unbranched
L. crassispora	27-30(33) × 14-17	1.8	1	(6)8-16(19) × 3-7	(21)30-62(66) × 2-3.5(4)	Septate, unbranched
L. rubropurpurea	24-33 × 13-17	1.9	1	7-13(15) × 3-5	(30)32-52(58) × 1.5-3.5	Aseptate, unbranched
L. venezuelensis	26-33 × 12-15	2.1	1	(5)7-14(15) × 3-4.5(5)	(12)16-41(45) × (1.5)2-5	Septate, unbranched
L. pseudotheobromae	23.5-32 × 14-18	1.7	1	-	Up to 58 × 3-4	Aseptate, occasionally branched
L. parva	16-23.5 × 10.5-13	1.8	1	-	Up to 105 × 3-4	Septate, unbranched
L. plurivora	(22)26.5-32.5(35) × (13)14.5-17(18.5)	1.9	1	8-13 × 4-7	Up to 130 × 2-5	Septate, occasionally branched
L. margaritacea	(12)14-17(19) × (10)11-12(12.5)	1.3	1	(6)10-11(19.5) × (2)3-4 (4.5)	(19)28-46(54) × (1.5)2-2.5(3)	Septate, unbranched
L. citricola	(20)22-27(31) × (10.9)12-17(19)	1.6	1	11-16 × 3-5	Up to 125 × 3-4	Septate, rarely branched
L. gilanensis	(25.2)28-35(38.8) × (14.4)15-18(19)	1.9	1	11-18 × 3-5	Up to 95 × 2-4	Septate, rarely branched
L. hormozganensis	(15.3)18-24(25.2) × 11-14	1.7	1	9-15 × 3-5	Up to 83 × 2-4	Septate, rarely branched
L. iraniensis	(15.3)17-23(29.7) × 11-14	1.6	1	9-16 × 3-5	Up to 127 × 2-4	Septate, rarely branched
L. mahajangana	(13.5)15.5-19(21.5) × (10)11.5-13(14)	1.4	1	(10)10.5-18(26) × (3)3.5-5.5(6)	(27.5)33.5-52.5(66) × (2)2.5-3.5(5)	Aseptate, unbranched
L. viticola	(16.5-)18-20.5(-23) × (8-)9-10.1(-10.5)	2.05	1	-	Up to 60 × 2-3	Aseptate, unbranched
L. lignicola	(15-)16-17.5× (8)8.5-10.5(-11)	1.7	1	10-15 × 2.5-3.5	Up to 15	Aseptate
L. missouriana	(16-)17.5-19.5(-21) × (8-)9-10.5(-11.5)	1.9	1	-	Up to 55 × 2-3	Aseptate, unbranched
L. egyptiacae	(17–)20–24(-27) × 11–12(-13)	2	1	5-11 × 3-5	Up to 57 × 2-3	Aseptate
L. indica	20-38 × 11-20.5	1.8	1-2	8.5-15(17.5) × 1.5-3.5(4)	Up to 120 × 1.5-3.5	Septate, occasionally branched

L. indica. The size of conidia as well as that of conidiogenous cells and paraphyses are different in the two species **(Table 2)**. The paraphyses in *L. indica* are septate and branched where as in *L. gonubiensis* these are non septate and unbranched. It differs from rest of the species in which conidia are only single septate. On the basis of above characters it is proposed as a new species.

ACKNOWLEDGEMENTS

One of us (G. S.) is thankful for financial assistance under BSR scheme to the SAP assisted Departments. The authors are thankful to UGC (DRS-III) for the infrastructural support and to the Chairperson, Department of Botany for laboratory facilities.

REFERENCES

Abbas, S.Q., Sutton, B.C., Ghaffar, A. and Abbas, A. 2004. Reassessment of *Sphaeropsis undulata* Berk. & Curt. *Pakistan J. Bot.* **36**: 209-218.

Abdollahzadeh, J., Javadi, A., Mohammadi, G.E., Zare, R. and Phillips, A.J.L. 2010. Phylogeny and morphology of four new species of *Lasiodiplodia* from Iran. *Persoonia* **25**: 1-10.

Alves, A., Crous, P.W., Correia, A., and Phillips, A.J.L. 2008. Morphological and molecular data reveal cryptic speciation in *Lasiodiplodia theobromae*. *Fungal Divers*. **28**:1 -13.

Ashok, D. and Prasher, I.B. 2014a. Wood rotting non-gilled agaricomycetes new to India. *J. new Biol. Rep.* **3**: 04-08.

Ashok, D. and Prasher, I.B. 2014b. Some interesting wood rotting non-gilled *Agaricomycetes* new to India. *J. new Biol. Rep.* **3**: 155-158.

Begoude, B.A.D., Slippers, B., Wingfield, M.J. and Roux, J. 2010. *Botryosphaeriaceae* associated with *Terminalia catappa* in Cameroon, South Africa and Madagascar. *Mycol. Progr.* **9**:101 -123.

Bilgrami, K.S., Jamaluddin and Rizwi, M.A. 1991. *The Fungi of India* (List and Reference). Today and Tomorrow's Printers and Publishers. New Delhi, India.

Burgess, T.I., Barber, P.A., Mohali, S., Pegg, G., Beer, W. de and Wingfield, M.J. 2006. Three new *Lasiodiplodia* spp. from the tropics, recognized based on DNA sequence comparisons and morphology. *Mycologia* **98**: 423-435.

Damm, U., Crous, P.W. and Fourie, P.H. 2007. *Botryosphaeriaceae* as potential pathogens of *Prunus* species in South Africa, with descriptions of *Diplodia africana* and *Lasiodiplodia plurivora* sp. nov. *Mycologia* **99**:664-680.

Hillis, D.M. and Bull, J.J. 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* **42**: 182-192.

Ismail, A.M., Cirvilleri, G., Polizzi, G., Crous, P.W., Groenewald, J.Z. and Lombard, L. 2012. *Lasiodiplodia* species associated with dieback disease of mango (*Mangifera indica*) in Egypt. Australas Plant Path. **41**: 649-660.

Jamaluddin, Goswami, M.G. and Ojha, B.M. 2004. *Fungi of India*. Scientific Publishers, India,

Kimura, M. 1980. A simple method for estimating evolutionary rate of base substitution through comparative studies of nucleotide sequences. *J. Mol. Evol.* **16**: 111-120.

Nei, M., and Kumar, S. 2000. *Molecular Evolution and Phylogenetics*. Oxford University Press, New York.

Pavlic, D., Slippers, B., Coutinho, T.A., Gryzenhout, M. and Wingfield, M.J. 2004. *Lasiodiplodia gonubiensis* sp. nov., a new *Botryosphaeria* anamorph from native *Syzygium cordatum* in South Africa. *Stud. Mycol.* **50**: 313-322.

Pavlic, D., Wingfield, M.J., Barber, P., Slippers, B., Hardy, G.E.S.J. and Burgess, T.I. 2008. Seven new species of the *Botryosphaeriaceae* from baobab and other native trees in Western Australia. *Mycologia* **100**: 851-866.

Phillips, A.J.L., Alves, A., Abdollahzadeh, J., Slippers, B., Wingfield, M.J., Groenewald, J.Z. and Crous, P.W. 2013. The *Botryosphaeriaceae*: genera and species known from culture. *Stud. Mycol*. **76**: 51-167.

Prashar, I.B., Sharma, S. and Khullar, S.P. 2005. Mycorrhizal associates of some ferns from Kangra district (Himachal Pradesh). *Indian Fern J.* **22**:81-86.

Prasher, I.B. and Ashok, D. 2013. A checklist of wood rotting fungi (non-gilled *Agaricomycotina*) of Himachal Pradesh. *J. new Biol. Rep*. **2**: 71-98.

Prasher, I.B., Baghla, A. and Khullar, S.P. 2004. VAM association in some ferns from Chail, Himachal Pradesh, NW Himalaya. *Indian Fern J.* **21**: 144-149.

Prasher, I.B. and Lalita 2013. A checklist of wood rotting fungi (non-gilled *Agaricomycotina*) of Uttarakhand. *J. new Biol. Rep.* **2**: 108-123.

Prasher, I.B., Manoharachary, C., Kunwar, I.K. and Agarwal, D.K. 2008. New species of *Dicranidion* Harkn. from India. *Indian Phytopath*. **61**: 367-378.

Prasher, I.B. and Singh, G. 2012. *Monodictys* spp. (Anamorphic fungi): New to North India. *Plant Sciences Feed*. **2**: 135-137.

Prasher, I.B. and Singh, G. 2013. Two hyphomycetes new to India. *J. new Biol. Rep*. **2**: 231-233.

Prasher, I.B. and Singh, G. 2014. Anamorphic fungi new to Shiwaliks- Northwest India. *J. new Biol. Rep.* **3**: 141-145.

Prasher, I.B. and Sushma 2014. *Hermatomyces indicus* sp. nov. (*Hyphomycetes*) from India. *Nova Hedwigia* **99**: 551-556.

Prasher, I.B. and Verma, R.K. 2012a. Two *Hyphomycetes* New to Himalayas. *Plant Sciences Feed* **2**: 122-124.

Prasher, I.B. and Verma, R.K. 2012b. *Periconia* species new to North-Western Himalayas. *J. new Biol. Rep.* **1**: 01-02.

Prasher, I.B. and Sharma, R. 1997. *Geoglossum* Pers. *Geoglossaceae*, *Leotiales* in Eastern Himalayas. In: *Achievements and Prospects in Mycology and Plant Pathology.* (Eds. Chahal, S.S., Prasher, I.B., Randhawa, H.S. and Arya, S.). Dehra Dun, India: International book distributors, pp. 12-19.

Prasher, I.B., Sharma, M.P. and Sharma, R. 2003. Diversity in the genus *Niptera* Fr. with particular reference to the Himalayan taxa. *Phytomorphology* **53**: 249-256.

Punithalingam, E. 1980. *Plant diseases attributed to Botryodiplodia theobromae* Pat. Vaduz, Cramer.

Rayner, R.W. 1970. *A mycological colour chart*. Kew, Surrey, U.K. CMI and British Mycological Society.

Sohi, H.S. and Prasher, I.B. 1981. A new leaf spot disease of *Bilbergia nutans* H. Wandel caused by *Phoma jolyana* Piroz. & Morg. *Curr. Sci.* **50**: 324-325.

Sutton, B.C. 1980. *The Coelomycetes, Fungi Imperfecti with Pycnidia, Acervuli and Stromata*. Kew, Surrey, England, Commonwealth Mycological Institute.

Swofford, D.L. 2003. PAUP*. *Phylogenetic analysis using parsimony* (*and other methods) Version 4. Sinauer Associates, Sunderland, Massachusetts.

Tamura, K., Stecher, G., Peterson, D., Filipski, A. and Kumar, S. 2013. MEGA6: Molecular evolutionary genetic analysis version 6.0. *Mol. Biol. Evol.* **30**: 2725-2729.

Thompson, J.D., Higgins, D.G. and Gibson, T.J. 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**:4673 -4680

Úrbez-Torres, J.R., Peduto, F., Striegler, R.K., Urrea-Romero, K.E., Rupe, J.C., Cartwright, R.D. and Gubler, W.D. 2011. Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. *Fungal Divers*. **52**:169-189.

White, T.J., Bruns, T., Lee, S. and Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A guide to Methods and Applications.* (Eds. Innis, M.A., Gelfand, D.H., Sninsky, J.J. and White T.J.). San Diego, U.S.A: Academic Press, pp. 315-322.