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ORIGINAL ARTICLE

Debunking *Duosporium*

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Abstract
Duosporium is a monotypic genus including only the type species *Duosporium yamadanum* which has been treated in the literature as "anamorphic Pezizomycotina." Nevertheless, this is just a conjecture since its true phylogenetic affinities remain unknown. This fungus is known to cause leaf spots on several members of *Cyperus* spp. It has been intensively investigated in the 1990s as a potential candidate for use as an inundative biocontrol agent against purple nutsedge—a major tropical weed. Its morphology was recognized, as somewhat close to those of *Curvularia* and *Bipolaris*. Nevertheless, it was kept in a separate genus based on two distinctive morphological features: production of two kinds of spores (as indicated by its generic name) and straight versicolored macroconidia. No molecular studies have ever been made to elucidate the placement of *Duosporium*. In a relatively recent publication, *Curvularia americana* and *C. chlamydospora* were found to produce macro- and microconidia, as in *Duosporium.* Besides, there are several species of *Curvularia* known to have predominantly straight conidia. Here, a multilocus phylogenetic analysis including the internal transcribed spacer regions and intervening 5.8S nrRNA gene (ITS) and translation elongation factor 1-α (*TEF1*) sequences placed *D. yamadanum* within *Curvularia*, close to *C. tuberculata*, *C. oryzae*, and *C. reesi*. This led to the proposal of the new combination *C. yamadana* and the synonymization of *Duosporium* with *Curvularia.*

Keywords *Curvularia* · Multilocus phylogeny · Reappraisal · Taxonomy

Introduction

Duosporium is a genus treated by Kirk et al. (2008) as "anamorphic Pezizomycotina." Only its asexual dematiaceous hyphomycete stage is known. Its correct taxonomic placement is uncertain since no molecular study has ever been made to elucidate its true phylogenetic affinities. *Duosporium* was proposed by Thind and Rawla (1961) based on a fungus found infecting the leaves of *Cyperus iria* in India. Since its proposal, no additions were made to the genus which remains monotypic and only contains *Duosporium yamadanum*. Tsuda and Ueyama (1982) in their literature survey found that well before *D. cyperi* was proposed by Thind and Rawla (1961), Matsuura (1931) had described a highly morphologically similar fungus from Japan (Honshu). This was found attacking *Cy. iwasakii.* Matsuura named it *Brachysporium yamadanum*. Tsuda and Ueyama (1982) concluded upon this observation that the name *D. cyperi* was inadequate since *B. yamadanum* had priority over *D. cyperi.* The new combination *D. yamadanum* having *B. yamadaeanum* as basionym was then proposed by Tsuda and Ueyama (1982).

As pointed out by Thind and Rawla (1961) and Tsuda and Ueyama (1982), *Duosporium* appeared to be related to either *Bipolaris* or *Curvularia*. However, in these earlier publications, it was recognized that conidiogenous cells leading to the formation of macroconidia of *Duosporium* were mostly monotretic whereas those where microconidia originated were often polytretic. Conversely, the conidiogenous cells on both *Bipolaris* and *Curvularia* are typically polytretic. Also, as emphasized in its generic name, *Duosporium* was found to typically produce two kinds of conidia: macro and microconidia. Such a feature was, at the time, unknown for members of *Bipolaris* and *Curvularia*. This justified the proposal of *Duosporium* to accommodate the fungus on *Cyperus* spp.

Duosporium yamadanum is only known in association with members of the genus *Cyperus*. Four species of

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Cyperus have been reported as hosts of this fungus: *Cyperus ferax* in Venezuela (Urtiaga 1986), *Cy. iria* in India and Venezuela (Ellis 1971), and *Cy. iwasakii* Matsuura (1931) in Japan and *Cy. rotundus* in Brazil (Barreto and Evans 1995). Additional records of *D. yamadanum* have been published but without a full identification of their *Cyperus* host. These records are from Cuba (Mercado Sierra 1984) and West Indies (Minter et al. 2001).

This fungus attracted great interest when it was first collected in association with severe leaf spots and blight of foliage of *Cy. rotundus* in Brazil during surveys for potential weed biocontrol agents (Barreto and Evans 1995). *Cyperus rotundus* is considered as one of the worst tropical weeds worldwide (Holm et al. 1991) and a challenging target for mechanical or chemical management (Edenfield et al. 2005). A series of intensive studies on the biology and management of *D. yamadanum* was initiated at the Universidade Federal de Viçosa (state of Minas Gerais, Brazil) by Pomella (1999) and later retaken by Macedo (2006). Results of the attempts at developing a *D. yamadanum*-based mycoherbicide produced inconsistent results and this project was interrupted. Observations made during these years of study led to speculation that the taxonomic treatment for *Duosporium* might be incorrect and that its affinity with the *Curvularia*/*Bipolaris* complex should be reevaluated. A study including the use of molecular tools was then conducted in order to better clarify the taxonomic status of *Duosporium* and results are presented herein.

Materials and method

Isolates and morphology

Samples deposited in the herbarium at the Universidade Federal de Viçosa (VIC) during the 1990s were re-examined under a dissecting microscope in the laboratory. Fungal structures growing externally on plant tissues were scraped from colonized plant surfaces and mounted in lactophenol or lactofuchsin. Observations of fungal structures were performed with an Olympus BX53 microscope adapted with differential interference contrast lighting and digital image capture system (Olympus Q-Color 3 ™). Biometric data was obtained from the measurement of at least 30 representative fungal structures.

Existing pure cultures from the studies performed in the 1990s and deposited in the culture collection of the Universidade Federal de Viçosa - Coleção Octávio de Almeida Drumond (COAD) in silica gel, as described in Dhingra and Sinclair (1995), were recovered by aseptically transferring fragments of filter paper carrying fungal structures onto Petri dishes containing potato carrot-agar (PCA) and maintained in a controlled temperature room at 25 °C under a 12-h daily light/12-h dark regime (light provided by two white and one near-UV lamps placed 35 cm above the plates).

Culture description

Colony descriptions were based on fungal growth on PDA and PCA, after 7 days. The fungus was grown either under a daily 12-h light regime as mentioned above, or in the dark (Petri dishes wrapped in aluminum foil). Color terminology followed Rayner (1970).

Molecular characterization and multilocus phylogenetic analysis

Genomic DNA was extracted from COAD 141, COAD 359, and COAD 375 grown in vegetable broth-agar (VBA)-medium described in Pereira et al. (2003) under a 12-h daily light regime for 2 weeks. Approximately 50 mg of mycelium was scraped from the surface of the colonized medium and placed inside sterile plastic tubes containing zirconium spheres and placed in a grinder (L-Beader-3, Loccus Biotecnologia). After 5-s grinding, the resulting suspension was drained into a sterile plastic tube and used for DNA extraction. This was performed with the Wizard Genomic DNA Purification Kit following the manufacturer's protocol. The PCR reaction was performed as described by Pinho et al. (2012). The primers ITS4 and ITS5 (White et al. 1990) were used to amplify the ITS region and the 5.8S rRNA gene. A partial region of *TEF1* was amplified using the primer pair EF1-983 and EF1-2218R (Schoch et al. 2009). PCR products were analyzed on GelRed ™ (Biotium Inc., Hayward, CA, E.U.A.) and visualized under UV light to verify the size and purity of amplification. The PCR products were sequenced by Macrogen Inc., South Korea (http://www.macrogen.com). The nucleotide sequences were edited with software SeqAssem ver. 07/2008 (Hepperle 2004). All sequences were manually verified and nucleotides with ambiguous positions were clarified using sequences from both directions.

The ITS and *TEF1* consensus sequences were compared with others deposited in the GenBank database using the MegaBLAST program. Sequences from GenBank and Tan et al. (2018) were aligned using MUSCLE (Edgar 2004) and built in MEGA v.6 (Tamura et al. 2011). All of the ambiguously aligned regions within the dataset were excluded from the analyses. Gaps (insertions/deletions) were treated as missing data.

Bayesian inference (BI) analyses employing a Markov Chain Monte Carlo method were performed with all sequences, first with each locus separately and then with the concatenated sequences. Before launching the BI, the best nucleotide substitution models were determined for each gene with MrMODELTEST 2.3 (Posada and Buckley 2004). Once

the likelihood scores were calculated, the models were selected according to the Akaike Information Criterion (AIC). The GTR + I + G model of evolution was used for ITS, whereas GTR + G was used for *TEF1*. One concatenated tree with ITS and *TEF1* was generated with Mesquite v. 3.1 (Maddison and Maddison 2011) and estimated on the CIPRES web portal using MrBayes on XSEDE 3.2.6 (Miller et al. 2010).

Additionally, a maximum likelihood (ML) tree was generated with the nearest-neighbor-interchange (NNI) ML heuristic method and the Tamura-Nei substitution model as tree inference options, using CIPRES web portal. The chain stabilities of the phylogenetic tree were assessed by using the bootstrap re-sampling strategy with 1000 bootstrap test replicates. The resulting tree topologies using the two methods (ML and BI) were then compared and the phylogram layout (BI tree) was edited with CorelDRAW Graphics Suite 2017.

Sequences derived from this study were deposited in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1). The alignment and tree were deposited in TreeBASE (http://www.treebase.org) (study number S25966).

Results

Phylogenetic analyses

The alignment to construct phylogenetic trees included 52 strains (Table 1), representing many of the known *Curvularia* species, some isolates of *Bipolaris*, three isolates of *Duosporium yamadanum*, and the outgroup taxon (*Alternaria alternata*). The combined matrix consisted of 1911 characters including alignment gaps (ITS: 802 and *EF1*: 1109). The number of conserved sites was 1155 (ITS: 582 and *EF1*: 573). The number of variable and parsimony uninformative sites was 745 (ITS: 210 and *EF1*: 535) and 571 sites were variable and parsimony informative (ITS: 141 and *EF1*: 430). The trees obtained with ML and BI agreed on topology. The phylogenetic analyses inferred from the combined dataset (Fig. 1) indicated that the three strains of the fungus on *Cy. rotundus* (COAD 141, COAD 359, and COAD 375) clustered together with 100% (ML) and 1.0 (BI) support. These isolates of *D. yamadanum* clustered together with three *Curvularia* species: *C. oryzae*, *C. reesii*, and *C. tuberculata*. Additionally, *D. yamadanum* formed a distinct lineage within *Curvularia*. Based on the results of the molecular study, the status of *Duosporium* as a synonym of *Curvularia* became clear. The new combination *C. yamadana* was then proposed.

Taxonomy

Curvularia Boedijn, Bulletin du Jardin Botanique de Buitenzorg 13 (1): 123 (1933).

= *Duosporium* K.S. Thind & Rawla, American Journal of Botany 48 (10): 862 (1961). **Syn. nov.**

Curvularia yamadana (Matsuura) B.W. Ferreira and R.W. Barreto, **comb. nov.** (Fig. 2)

Basionym. Brachysporium yamadanum Matsuura, Byochu-gai Zasshi [J. Pl. Prot. Tokyo] 17: 419, 1931 (as "*yamadaeanum*"). = *Duosporium cyperi* Thind & Rawla, American Journal of Botany 48: 862, 1961.

≡ *Duosporium yamadanum* (Matsuura) Tsuda and Ueyama, Mycotaxon 14: 145, 1982.

Lesions on living leaves; starting as dark brown, linear necrosis at apex or in the middle of the midrib, expanding to cover large portions of leaves, causing the blight of leaves and, sometimes, to death of all aerial part of plants. Internal mycelium intracellular, 1–6-μm diam, branched, septate, constricted at septae, concentrated adaxially under the epidermis, hyaline. External mycelium absent. Stromata absent. Macroconidiophores arising through stomata, amphigenous, mostly in loose fascicles of few conidiophores, rarely solitary, cylindrical, 43–132 × 4–7 μm, straight to slightly curved, inflated at the base and apex, septate, unbranched, smoky brown, smooth. Conidiogenous cells terminal, integrated, monotretic or polytretic, proliferating sympodially, or with percurrent proliferation with enteroblastic regenerative growth, cylindrical, 10–45 × 7 μm, inflated apical portion up to 11 μm wide, smoky brown becoming paler towards the apices. Conidiogenous loci indistinct to darkened. Macroconidia dry, solitary, tretic, oblong, 29–43 × 15–25 μm, apex and base rounded, hilum mostly indistinct, but visible in some spores as a darkened basal area, 3–4 μm wide, 3-septate, eguttulate, central cells dark brown to brown, end cells hyaline to subhyaline, smooth. Microconidiophores only seen in culture, arising from macroconidia or vegetative hyphae, cylindrical, strongly geniculate, straight to flexuous, mononematous, macronematous, 11–86 × 4–5 μm, walls thicker than on vegetative hyphae, brown, paler towards apex. Conidiogenous cells integrated, terminal or intercalary, cylindrical, proliferating sympodially, 6–11 × 3–5 μm, pale brown to brown, smooth, mono- or polytretic. Microconidia globose to subglobose to ellipsoidal, 5–13 × 5–9 μm, aseptate, brown to dark brown, warted, warts prominent, 3 × 2.5 μm.

Culture characters—slow-growing (3-cm diam on PDA and 3.4-cm diam on PCA after 7 days), flat to raised centrally with depressed marginal ring, undulate, edges entire, felty, center pale mouse gray becoming olivaceous gray, margin white, reverse fuscous black and white margin (PDA); center pale mouse gray, scarlet near the edge, border orange, reverse bay with orange margin (PCA). Not sporulating except on PCA in the dark.

Known distribution—Brazil, Cuba, India, Japan, Venezuela, West Indies.

Material examined: Brazil: Bahia, Itabuna, CEPLAC Brasil, on *Cyperus rotundus*, 11 Apr 2000, A. W. V. Pomella

Table 1 DNA sequences used for the phylogenetic tree

Species	Isolate	Host	Genbank ITS	*TEF1*	Reference
Alternaria alternata	EGS 34.016	*Arachis hypogaea*	AF347031	KC584634	Woudenberg et al. (2013)
Bipolaris bamagaensis	BRIP 13577*	*Brachiaria subquadripara*	KX452445	KX452462	Tan et al. (2016)
	BRIP 10711	*Dactyloctenium aegyptium*	KX452444	KX452461	Tan et al. (2016)
	BRIP 14847	*Dactyloctenium aegyptium*	KX452446	KX452463	Tan et al. (2016)
B. cookei	MAFF 51191	*Sorghum bicolor*	KJ922392	KM093777	Tan et al. (2016)
B. drechsleri	CBS 136207*	*Microstegium vimineum*	KF500530	KM093760	Tan et al. (2016)
B. maydis	CBS 137271/C5	*Zea mays*	AF071325	KM093794	Manamgoda et al. (2014)
	CBS 136.29*	*Zea mays*	HF934926	KJ415463	Manamgoda et al. 2014
B. shoemakeri	BRIP 15806	*Ischaemum rugosum var. segetum*	KX452452	KX452469	Tan et al. (2016)
	BRIP 15929*		KX452453	KX452470	Tan et al. (2016)
B. sivanesaniana	BRIP 15847*	*Paspalidium distans*	KX452455	KX452472	Tan et al. (2016)
	BRIP 15822	*Setaria sphaecelata*	KX452456	KX452473	Tan et al. (2016)
Curvularia aeria	CBS 294.61*	Air	HF934910	–	Tan et al. (2016)
C. americana	UTHSC 08-3414*	*Homo sapiens*	HE861833	–	Tan et al. (2018)
C. beasleyi	BRIP 10972*	*Chloris gayana*	MH414892	MH433654	Tan et al. (2018)
	BRIP 15854	*Leersia hexandra*	MH414893	MH433655	Tan et al. (2018)
C. boeremae	IMI 164633*	*Portulaca oleracea*	MH414911	–	Tan et al. (2018)
C. buchloës	CBS 246.49*	*Buchloë dactyloides*	KJ909765	KM196588	Tan et al. (2018)
C. carica-papayae	CBS 135941*	*Carica papaya*	HG778984	–	Tan et al. (2018)
C. chlamydospora	UTHSC 07-2764*	*Homo sapiens*	HG779021	–	Tan et al. (2018)
C. clavata	BRIP 61680b	*Oryza rufipogon*	KU552205	KU552159	Tan et al. (2018)
C. colbranii	BRIP 13066*	*Crinum zeylanicum*	MH414898	MH433660	Tan et al. (2018)
C. dactyloctenii	BRIP 12846*	*Dactyloctenium radulans*	KJ415545	KJ415447	Tan et al. (2018)
C. eragrostidis	CBS 189.48	*Sorghum* sp.	HG778986	–	Tan et al. (2018)
C. harveyi	BRIP 57412*	*Triticum aestivum*	KJ415546	KJ415446	Raza et al. (2019)
C. hawaiiensis	BRIP 11987*	*Oryza sativa*	KJ415547	KJ415445	Tan et al. (2018)
C. homomorpha	CBS 156.60*	Air	JN192380	JN601014	Tan et al. (2018)
C. kusanoi	CBS 137.29	*Eragrostis major*	JN192381	JN601016	Tan et al. (2018)
C. lamingtonensis	BRIP 12259*	*Microlaena stipoides*	MH414901	MH433663	Tan et al. (2018)
C. mebaldsii	BRIP 12900*	*Cynodon transvaalensis*	MH414902	MH433664	Tan et al. (2018)
	BRIP 13983	*Cynodon dactylon x transvaalensis*	MH414903	MH433665	Tan et al. (2018)
C. muehlenbeckiae	CBS 144.63*	*Muehlenbeckia sp.*	HG779002	–	Raza et al. (2019)
	LC11988	*Saccharum officinarum*	MN215681	MN263975	Raza et al. (2019)
	LC11989	*Saccharum officinarum*	MN215682	MN263976	Raza et al. (2019)
C. neoindica	IMI 129790*	*Brassica nigra*	MH414910	MH433667	Tan et al. (2018)
C. nicotiae	BRIP 11983*	Soil	KJ415551	KJ415442	Tan et al. (2018)
C. nodulosa	CBS 160.58	*Eleusine indica*	JN601033	JN601019	Tan et al. (2018)
C. oryzae	CBS 169.53*	*Oryza sativa*	KP400650	KM196590	Tan et al. (2018)
C. pallescens	CBS 156.35*	Air	KJ922380	KM196570	Tan et al. (2018)
C. portulacae	BRIP 14541*	*Portulaca oleracea*	KJ415553	KJ415440	Tan et al. (2018)
C. prasadii	CBS 143.64*	*Jasminum sambac*	KJ922373	KM230408	Tan et al. (2018)
C. pseudolunata	UTHSC 09-2092*	*Homo sapiens*	HE861842	–	Tan et al. (2018)
C. reesii	BRIP 4358*	Air	MH414907	MH433670	Tan et al. (2018)
C. spicifera	CBS 274.52	Soil	JN192387	JN601023	Tan et al. (2018)

Table 1 (continued)

Species	Isolate	Host	Genbank ITS	*TEF1*	Reference
C. tsudae	ATCC 44764*	*Chloris gayana*	KC424596	KC503940	Tan et al. (2018)
C. tuberculata	CBS 146.63*	*Zea mays*	JX256433	JX266599	Tan et al. (2018)
C. variabilis	CPC 28815*	*Chloris barbata*	MF490822	MF490865	Tan et al. (2018)
C. verruculosa	CBS 150.63	*Punica granatum*	KP400652	KP735695	Tan et al. (2018)
C. warraberensis	BRIP 14817*	*Dactyloctenium aegyptium*	MH414909	MH433672	Tan et al. (2018)
C. yamadana	COAD 375	*Cyperus rotundus*	MN954704	MT008259	This study
	COAD 359	*Cyperus rotundus*	MN954705	MT008260	This study
	COAD 141	*Cyperus rotundus*	MN954706	MT008261	This study

Ex-type strains are indicated in asterisk after collection number

(VIC 27784F–culture COAD 141, MBT390712); Minas Gerais, Viçosa, Chácara Cristal, on *Cyperus rotundus*, 11 Apr 1998, R. W. Barreto, (culture COAD 375); Rio de Janeiro, Carmo, Fazenda São José, on *Cyperus rotundus*, 20 Jan 1998, R. W. Barreto, (culture COAD 359).

Notes: *Duosporium* was originaly described from a specimen collected in Punjab, India, on living leaves of *Cyperus iria*, but collected earlier in Honshu, Japan, on leaves of *Cyperus iwasakii* and mistakenly placed in *Brachysporium*, as *B. yamadanum*. No herbarium specimen or ex-type culture were designated by these authors.

The multilocus phylogenetic analyses indicated *C. yamadana* to be close to *C. tuberculata*, *C. oryzae*, and *C. reesii*. *Curvularia reesii* was recently described from colonies obtained from an air sample (Tan et al. 2018) and has not known to have *Cyperus* spp. as a substrate. Nevertheless, Farr and Rossman (2020) lists *C. tuberculata* and *C. oryzae* on *Cyperus* spp. *C. oryzae* was reported on *Cy. rotundus* from West Indies (Minter et al. 2001) and *C. tuberculata* was reported on *Cy. malaccensis* from Taiwan (Matsushima 1980). *Curvularia yamadana* has conidia similar in size to *C. tuberculata* (23–52 × 13–20 μm), *C. oryzae* (24–40 × 12–22 μm) and *C. reesii* (31–35 × 12–13 μm). However, conidia of *C. tuberculata* are sometimes curved, have 3–8-distoseptae and these are tuberculate at maturity. Conidia in *C. oryzae* and *C. reesii* are obclavate to ellipsoidal, whereas in *C. yamadana* are oblong having a rounded apex and base. Two other *Curvularia* species have been listed by Farr and Rossman (2020) on *Cyperus* spp. *Curvularia aeria* on *Cy. rotundus* from West Indies (Minter et al. 2001) and *Curvularia pallescens* on *Cy. antillanus* from Cuba (Mercado Sierra 1984). Both are morphologically rather different from *C. yamadana*. *Curvularia aeria* has conidia which are straight or curved, ellipsoidal, obovoid or clavate, 18–32 × 8–16. *Curvularia pallescens* has ellipsoidal to fusiform conidia, usually slightly curved, 17–32 × 7–12 μm. In the phylogenetic tree, both *C. aeria* and *C. pallescens* were phylogenetically distant from *C. yamadana*.

Discussion

The genus *Curvularia* includes species associated with plant diseases worldwide (Sivanesan 1987; Manamgoda et al. 2012a, b; da Cunha et al. 2013; Hyde et al. 2014; Manamgoda et al. 2015). It is characterized by the production of sympodial conidiophores with tretic, terminal, and intercalary conidiogenous cells and elongate, transversely septate conidia with a dark basal scar. Conidia are often curved because of it having asymmetrically swollen intermediate cells, (Sivanesan 1987; Manamgoda et al. 2015). For a long time, curvature of conidia was used as the key feature to differentiate fungi in the genus *Curvularia* from species belonging to related genera. However, it is now known that some *Curvularia* species such as *C. cymbopogonis*, *C. oryzae-sativae*, *C. protuberata*, and *C. ryleyi* have predominantly straight conidia (Manamgoda et al. 2015). Species delimitation in *Bipolaris* and *Curvularia* remained problematic for taxonomists for very long due to the overlapping morphological characters among many species (Manamgoda et al. 2014, 2015; Sivanesan 1987).

Such a subjectivity of the morphological distinction of taxa in *Bipolaris* and *Curvularia* was only resolved through the use of molecular data. In addition to ITS, other loci were found to be of high informative value in the phylogenetic analyses of sequence data from species belonging to these two genera. Particularly, the protein-coding loci of glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*), *TEF1*, and RNA polymerase II second largest subunit (*RPB2*) which became critical for such analyses (Hernández-Restrepo et al. 2018; Manamgoda et al. 2014; Marin-Felix et al. 2017).

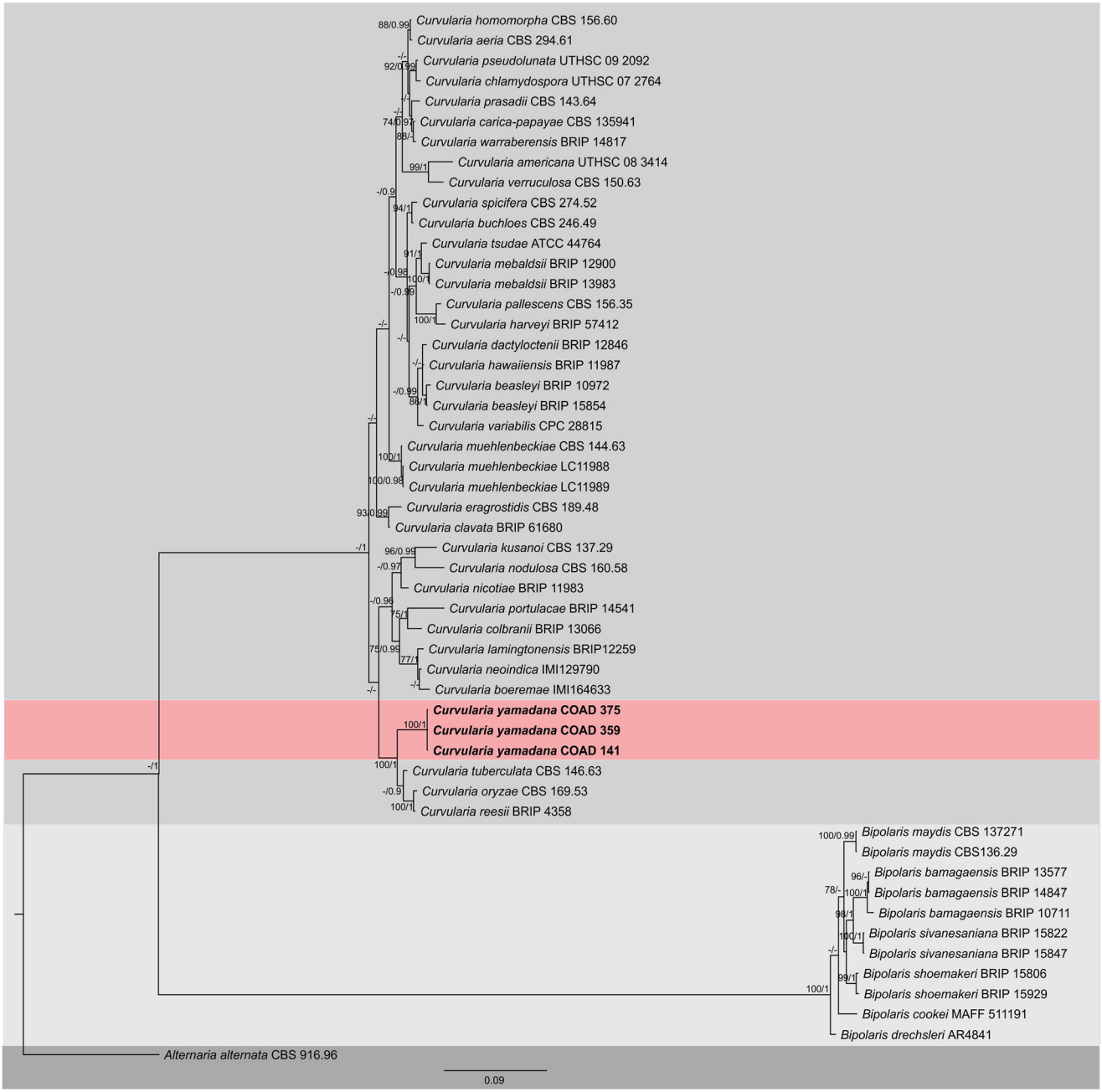

Fig. 1 Phylogeny based on Bayesian inference inferred of combined ITS and *TEF1* showing the relationship of *Curvularia yamadana* with other species within *Curvularia* and *Bipolaris*. Bootstrap support values (ML) or Bayesian posterior probabilities higher than 70% or 0.90 are indicated above or below thickened branches (– indicates lack of support). Isolates from this study are indicated by bold text

According to Thind and Rawla (1961) and Tsuda and Ueyama (1982), *Duosporium* was considered distinct from *Curvularia*, because conidiogenous cells generating macroconidia of *Duosporium* are mostly monotretic and those generating microconidia are polytretic whereas conidiogenous cells of *Curvularia* are, predominantly, polytretic. However, *C. muehlenbeckiae*, *C. beerburrumensis*, *C. boeremae*, *C. coatesiae*, *C. colbranii*, *C. kenpeggii*, *C. mebaldsii*, *C. petersonii*, *C. platzii*, *C. reesii*, and *C. warraberensis*, along with other species, were recognized to have either mono- or polytretic conidiogenous cells (Tan et al. 2018). *Duosporium* was also occasionally seen to produce polytretic macroconidiophores, as mentioned above and in the published description of Barreto and Evans (1995).

Another feature which led *Duosporium* to be treated as distinct from *Curvularia*, is the production of microconidia in *Duosporium*. This remains a partially valid difference, since it is now known that some species of *Curvularia* do in fact produce secondary conidia. Among them are *C. americana* and *C. chlamydospora* (Madrid et al. 2014). Nevertheless,

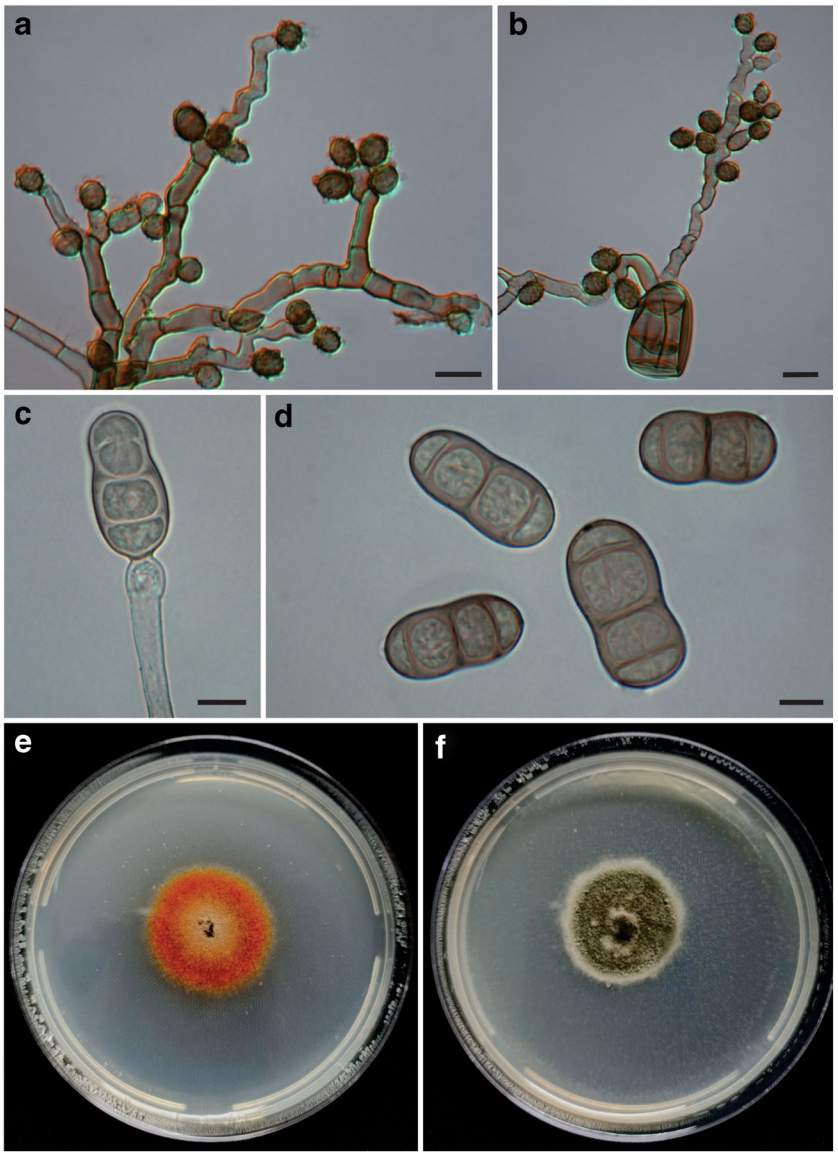

Fig. 2 *Curvularia yamadana* (COAD 141). **a** Microconidiophores producing warted microconidia. **b** Collapsed macroconidium germinating to produce microconidia. **c** Macroconidium attached to the conidiogenous cell. **d** Mature macroconidia. **e** Colony on PCA after 7 days (incubation at 25 °C in 12-h light/dark cycle). **f** Colony on PDA after 7 days (incubation at 25 °C in 12-h light/dark cycle). Bars = 10 μm

such secondary conidia in those two species are only formed directly from macroconidia and not from conidiophores as seen regularly in vitro for *C. yamadana.* In *C. americana*, microconidia are aseptate, pale brown, globose, and 5–6 μm wide. *C. chlamydospora* produce microconidia which are 1–2 celled, pale brown, globose to subglobose, and 4–6 μm diam. On the other hand, in *C. yamadana*, microconidia are aseptate, warted, brown to dark brown, globose to subglobose to ellipsoidal, and 5–13 × 5–9 μm. Microconidia remain a puzzle in the life cycle of *C. yamadana*. These are produced in large quantities in vitro in older cultures, but it was never seen in nature. Only a very small proportion of the microconidia were found to germinate, even after a series of treatments were attempted to break their dormancy (Pomella 1999). This led to the speculation that these structures would function as resting spores for *C. yamadana*, a hypothesis which remains to be better investigated.

The multilocus phylogenetic analyses clearly indicated that the fungus on *Cyperus* spp. belongs to *Curvularia*. *Curvularia yamadana* is close to *C. tuberculata*, *C. oryzae*, and *C. reesii*. Some species of *Curvularia* have some morphological resemblance to *C. yamadana* but the combination of oblong, straight versicolored 3-septate conidia and production of warted microconidia in culture is exclusive to the species formerly placed in *Duosporium.*

The need for epitipification of plant pathogenic fungi was emphasized by Cai et al. (2011) as the way forward towards clarification of the taxonomy and phylogeny of such taxa and towards the stability in the application of names. A recent case in point is that of the dematiaceous hyphomycete *Acroconidiella*, a genus which we recently "debunked" (Ferreira and Barreto 2019) showing its type species to be an "unusually shaped" *Alternaria.* Here, we provided a small contribution to this formidable task by showing that

Duosporium is an artificial genus which needs to be recognized as a late synonym of *Curvularia*. Nevertheless, the designation of an epitype, ideally a specimen from the type locality (Honshy, Japan) on *Cy. iwasakii*, would be of great value for a confirmation of our understanding on the taxonomy of *C. yamadana*.

The taxonomic placement of the fungus on *Cyperus* spp. has, hopefully, been finally resolved, but an important pending challenge remains. That of determining its true potential as a practical tool to be deployed against the "tropical scourge" *Cy. rotundus*, as placed by William (1976). Although the theme has been shelved long ago, improvements in tools for the mass production, formulation, and application of biological control agents have occurred along the years and may justify revisiting *C. yamadana* as an "ecologically benign" weed biocontrol product (mycoherbicide).

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