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ORIGINAL ARTICLE

Debunking *Acroconidiella*

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Abstract

Acroconidiella was proposed to accommodate *Acroconidiella tropaeoli*, a fungal species causing leaf spots on *Tropaeolum majus*. At the time, it was recognized as deserving to be treated as a distinct genus because, although being somewhat similar to *Alternaria*, it did not present muriform conidia formed in chains. More recent observations of *A. tropaeoli* in culture forming acropetal conidial chains, and the recognition of several non-dictioconidial species as belonging to *Alternaria*, prompted a reappraisal of the genus, starting with the re-examination of the type species. Samples of *Acroconidiella tropaeoli*, and also of *Acroconidiella trisepta*, were recollected in Brazil, and a study involving an analysis of their morphology, under light microscopy and SEM, and a molecular phylogenenetic analysis was performed. A multi-gene phylogeny, including the large subunit of the nrDNA (nc LSU rDNA), internal transcribed spacer (ITS) region, translation elongation factor 1-α (*EF1*), and polymerase II second largest subunit (*RPB2*), placed *A. tropaeoli* within *Alternaria*, close to *A. sonchi* and *A. cinerariae*. The ITS and nc LSU rDNA phylogenetic study of *A. trisepta* placed it within *Dendryphiella*. The new combination *Dendryphiella trisepta* comb. nov is proposed to accommodate *A. trisepta.* Nevertheless, the new name *Alternaria obtusa* is proposed for *Acroconidiella tropaeoli* since it could not be recombined into *Alternaria tropaeoli* because this name is already in use for another valid (and distinct) species in this genus described from India. This study showed that *Acroconidiella* is an artificial genus which is now rejected, since its type species belongs to *Alternaria*—which has nomenclatural priority over *Acroconidiella*. Other species placed in *Acroconidiella*, given below, await reappraisal in order to determine their correct taxonomic affinity.

Keywords *Alternaria* · *Dendryphiella* · Multi-gene phylogeny · New taxa · Reappraisal · Taxonomy

Introduction

The genus *Acroconidiella* was proposed by Lindquist and Alippi (1964) to accommodate fungi with macronematous, mononematous, simple, or occasionally branched conidiophores with integrated, terminal, polytretic sympodial conidiogenous cells bearing solitary ellipsoidal, septate echinulate conidia (Ellis 1971). Baker (1947) made the first report of a disease in *Tropaeolum majus* (garden nasturtium—chagas, in Brazil) in California and considered that it was caused by a fungus belonging to *Heterosporium*, but without giving it a name. In the same year, Bond (1947) independently described the fungus on *Tropaeolum majus* in Ceylon (Sri Lanka) naming it *Heterosporium tropaeoli*. De Vries (1952), considered that *Heterosporium* should be regarded as analogous to *Cladosporium*. However, the fungus described as *Heterosporium tropaeoli* has morphological features which were recognized as clearly distinct from members of *Cladosporium*. The fungus on garden nasturtium has porospores instead of blastospores—typical of *Cladosporium*—and a scar morphology which is also distinct from those of *Cladosporium*. Lindquist mentioned a personal communication made to him by M. B. Ellis (Lindquist and Alippi 1964) "*H. tropaeoli* is closer to *Alternaria*, than to *Curvularia* and *Cladosporium*". However, this fungus did not present muriform conidia in chains, thought to be diagnostic for *Alternaria*, at the time, and the conidia did not have its central cells larger than the apical cells, accepted as key for *Curvularia* at the time [now much changed after the works of Berbee et al. 1999 and Manamgoda et al. 2012]. Based on Ellis' views, Lindquist and Alippi (1964) proposed a new genus *Acroconidiella* to accommodate this fungus and designated it *Acroconidiella tropaeoli*.

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Presently, there are five species in the genus *Acroconidiella*: *A. eschscholtziae*, *A. indicus*, *A. manoharacharii*, *A. tropaeoli*, and *A. trisepta* (Lindquist and Alippi 1964; Ellis 1971, 1976; Muchovej 1980; Prasher and Verma 2015).

The lack of molecular studies for any of the species in this genus and the observations made by Vieira and Barreto (2002), particularly the *in vitro* production of short acropetal conidial chains, prompted a reappraisal of *Acroconidiella*. This was based on freshly collected material of two members of *Acroconidiella*, namely *Acroconidiella tropaeoli* and *Acroconidiella trisepta*.

Materials and methods

Sample collection processing and observation of fungus morphology

Samples of diseased foliage of *Tropaeolum majus* and dead branches of *Glycine max* were collected, the former from the original host of *Acroconidiella tropaeoli*, but the latter both from the substrate plant species and exactly from the type locality (Viçosa, state of Minas Gerais, Brazil). These were screened under a stereomicroscope, and parts of the samples bearing sporulating colonies of the fungi were selected and dried in a plant press. Fungal structures were scraped from the sample surface with a scalpel and mounted in lactophenol and lactofuchsin. Observations were made with an Olympus BX53 adapted with differential contrast lighting and equipped with digital capture system (Olympus Q-Color 3™). Representative specimens were deposited in the local herbarium (Herbarium Universidade Federal de Viçosa, VIC).

Isolations were performed by aseptic transfer of conidia from the leaf surfaces onto potato dextrose-agar (PDA) plates with a sterile fine-pointed needle. Culture descriptions were based on the observation of 7-day-old (*A. tropaeoli*) and 14-day-old (*A. trisepta*) colonies formed in plates containing either potato dextrose-agar (PDA) or potato carrot-agar (PCA), maintained at 25 °C under a 12-h daily/light regime (light provided by two white and one near-UV lamps placed 35 cm above the plates). The color terminology followed Rayner (1970).

Samples of dried material containing fungal structures were mounted on stubs with double-sided adhesive tape and gold-coated using a Balzer's FDU 010 sputter coater. A Carl-Zeiss Model LEO VP 1430 scanning electron microscope (SEM) was used to analyze and generate images from the samples.

DNA isolation

Total genomic DNA was extracted from 7-day-old cultures formed on PDA by using Wizard® Genomic DNA Purification Kit (Promega Corporation, WI, USA) following the manufacturer's instructions and the steps described by Pinho et al. (2012).

PCR amplification

The large subunit of the nrDNA (nc LSU rDNA) and internal transcribed spacer (ITS) regions from each fungus included in the study were sequenced with the primers LSU1Fd (Crous et al. 2009) and LR5 (Vilgalys and Hester 1990) and IT5 + ITS4 (White et al. 1990), respectively. For *Acroconidiella tropaeoli*, two additional loci, polymerase II second largest subunit (*RPB2*) and translation elongation factor 1-α (*TEF1*), were amplified and sequenced with the primer pairs RPB2-5F2 (Sung et al. 2007) and fRPB2-7R (Liu et al. 1999) and EF1-728F + EF1-986R (Carbone and Kohn 1999). PCR amplifications were performed in a total volume of 12.5 μL containing 10–20 ng of template DNA, 1× PCR buffer, 0.63 μL DMSO (99.9%), 1.5 mM $MgCl_2$, 0.5 μM of each primer, 0.25 mM of each dNTP, and 1.0 U BioTaq DNA polymerase (Bioline GmbH Luckenwalde, Germany). Conditions for PCR amplification consisted of an initial denaturation step of 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 48 °C, and 90 s at 72 °C for nc LSU rDNA, ITS and 40 cycles of 30 s at 94 °C, 30s at 52 °C/59 °C and 45 s at 72 °C for *TEF1*, and a final elongation step of 7 min at 72 °C. The partial *RPB2* gene was obtained by using a touchdown PCR protocol of 5 cycles of 45 s at 94 °C, 45 s at 60 °C, and 2 min at 72 °C, followed by 5 cycles with a 58 °C annealing temperature and 30 cycles with a 54 °C annealing temperature. Amplicons were analyzed on 0.8% agarose electrophoresis gels stained with GelRed (InstantAgarose) in a 1× TAE buffer and visualized under UV light to check for amplification size and purity. PCR products were purified and sequenced by Macrogen Inc. (http://www.macrogen.com).

Phylogenetic analysis

DNA consensus sequences were generated and imported into MEGA v. 6 (Tamura et al. 2013) for initial alignment and the construction of sequence datasets. Sequences obtained from the GenBank (www.ncbi.nlm. nih.gov) and the novel sequences generated on this study were aligned and edited using DNA Dragon program (http://www.dna-dragon.com\index.php) (Table 1).

Bayesian inference analyses were conducted, and the best-fit evolutionary model was determined by comparing different evolutionary models via the Akaike information criterion using PAUP (version 4.0b10, Sinauer Associates) and MrModeltest 2.2 (Nylander 2004). Posterior probabilities were determined by Markov chain Monte Carlo sampling (MCMC) in MrBayes v. 3.2.1 (Ronquist et al. 2012). Six simultaneous Markov chains were run for 10,000,000 generations, and trees were sampled every 100th generation and

Table 1 Taxa and collections used for multi-gene phylogenetic analyses in this study

Species name	Strain number	GenBank accession numbers			
		nc LSU rDNA	*RPB2*	ITS	*TEF1*
Alternaria alternata	CBS 916.96*	DQ678082	KC584375	AF347031	KC584634
Alternaria alternantherae	CBS 124392	KC584251	KC584374	KC584179	KC584633
Alternaria anigozanthi	CBS 121920*	KC584252	KC584376	KC584180	KC584635
Alternaria argyranthemi	CBS 116530*	KC584254	KC584378	KC584181	KC584637
Alternaria armoraciae	CBS 118702*	KC584255	KC584379	KC584182	KC584638
Alternaria avenicola	CBS 121459*	KC584256	KC584380	KC584183	KC584639
Alternaria brassicae	CBS 116528	KC584258	KC584382	KC584185	KC584641
Alternaria brassicicola	CBS 118699	KC584259	KC584383	JX499031	KC584642
Alternaria calycipyricola	CBS 121545*	KC584260	KC584384	KC584186	KC584643
Alternaria capsici-annui	CBS 504.74	KC584261	KC584385	KC584187	KC584644
Alternaria chlamydospora	CBS 491.72*	KC584264	KC584388	KC584189	KC584647
Alternaria cinerariae	CBS 116495	KC584265	KC584389	KC584190	KC584648
Alternaria conjuncta	CBS 196.86*	KC584266	KC584390	FJ266475	KC584649
Alternaria cumini	CBS 121329*	KC584267	KC584391	KC584191	KC584650
Alternaria dianthicola	CBS 116491	KC584270	KC584394	KC584194	KC584653
Alternaria ellipsoidea	CBS 119674*	KC584272	KC584396	KC584196	KC584655
Alternaria eryngii	CBS 121339	KC584273	KC584397	JQ693661	KC584656
Alternaria ethzedia	CBS 197.86*	KC584274	KC584398	AF392987	KC584657
Alternaria gaisen	CBS 632.93	KC584275	KC584399	KC584197	KC584658
Alternaria geniostomatis	CBS 118701*	KC584276	KC584400	KC584198	KC584659
Alternaria gypsophilae	CBS 107.41*	KC584277	KC584401	KC584199	KC584660
Alternaria helianthiinficiens	CBS 117370	KC584278	KC584402	KC584200	KC584661
	CBS 208.86*	KC584279	KC584403	JX101649	EU130548
Alternaria infectoria	CBS 210.86*	KC584280	KC584404	DQ323697	KC584662
Alternaria japonica	CBS 118390	KC584281	KC584405	KC584201	KC584663
Alternaria leucanthemi	CBS 421.65*	KC584347	KC584472	KC584240	KC584732
	CBS 422.65	KC584348	KC584473	KC584241	KC584733
Alternaria limaciformis	CBS 481.81*	KC584283	KC584407	KC584203	KC584665
Alternaria longipes	CBS 540.94	KC584285	KC584409	AY278835	KC584667
Alternaria mimicula	CBS 118696*	KC584287	KC584411	FJ266477	KC584669
Alternaria molesta	CBS 548.81*	KC584288	KC584412	KC584205	KC584670
Alternaria mouchaccae	CBS 119671*	KC584289	KC584413	KC584206	KC584671
Alternaria nepalensis	CBS 118700*	KC584290	KC584414	KC584207	KC584672
Alternaria oregonensis	CBS 542.94*	KC584292	KC584416	FJ266478	KC584674
Alternaria panax	CBS 482.81	KC584293	KC584417	KC584209	KC584675
Alternaria petroselini	CBS 112.41*	KC584295	KC584419	KC584211	KC584677
Alternaria photistica	CBS 212.86*	KC584296	KC584420	KC584212	KC584678
Alternaria porri	CBS 116698	KC584297	KC584421	DQ323700	KC584679
Alternaria radicina	CBS 245.67	KC584299	KC584423	KC584213	KC584681
Alternaria selini	CBS 109382*	KC584302	KC584426	AF229455	KC584684
Alternaria septorioides	CBS 106.41*	KC584303	KC584427	KC584216	KC584685
Alternaria solani	CBS 116651	KC584306	KC584430	KC584217	KC584688
Alternaria soliaridae	CBS 118387*	KC584307	KC584431	KC584218	KC584689
Alternaria solidaccana	CBS 118698*	KC584308	KC584432	KC584219	KC584690
Alternaria sonchi	CBS 119675	KC584309	KC584433	KC584220	KC584691
Alternaria tenuissima	CBS 918.96	KC584311	KC584435	AF347032	KC584693
Alternaria thalictrigena	CBS 121712*	KC584312	KC584436	EU040211	KC584694

Table 1 (continued)

Species name	Strain number	GenBank accession numbers			
		nc LSU rDNA	*RPB2*	ITS	*TEF1*
Alternaria triglochinicola	CBS 119676*	KC584313	KC584437	KC584222	KC584695
Alternaria obtusa	COAD 2389*	**MK277356**	**MK317924**	**MK278897**	**MK981886**
	COAD 2799	**MK968119**	**MK988439**	**MK968121**	**MK981887**
Alternaria vaccariae	CBS 116533	KC584314	KC584438	KC584223	KC584696
Aquaticheirospora lignicola	RK 2006a*	AY736378	–	AY864770	–
Dendryphiella eucalyptorum	CPC 22927*	KJ869196	–	KJ869139	–
Dendryphiella fasciculata	MFLUCC 17–1074*	MF399214	–	MF399213	–
Dendryphiella paravinosa	CPC 26176*	KX228309	–	KX228257	–
Dendryphiella trisepta	COAD 2388*	**MK277357**	–	**MK278898**	–
Dendryphiella vinosa	NBRC 32669		–	EU848590	–
Dictyocheirospora aquatica	KUMCC 15–0305*	KY320513	–	KY320508	–
Dictyocheirospora bannica	KH 332*	AB807513	–	LC014543	–
Dictyocheirospora garethjonesii	MFLUCC 16–0909*	KY320514	–	KY320509	–
Dictyocheirospora pseudomusae	KH 412	AB807516	–	LC014549	–
Dictyocheirospora pseudomusae	yone 234*	AB807520	–	LC014550	–
Dictyocheirospora rotunda	MFLUCC 14–0293*	KU179100	–	KU179099	–
Dictyosporium alatum	ATCC34953*	DQ018101	–	NR077171	–
Dictyosporium elegans	NBRC 32502*	DQ018100	–	DQ018087	–
Dictyosporium olivaceosporum	KH 375*	AB807514	–	LC014542	–
Dictyosporium sexualis	MFLUCC 10–0127*	KU179106	–	KU179105	–
Dictyosporium stellatum	CCFC 241241*	JF951177	–	JF951154	–
Dictyosporium thailandicum	MFLUCC 13–0773*	KP716707	–	KP716706	–
Digitodesmium bambusicola	CBS 110279*	DQ018103	–	DQ018091	–
Gregarithecium curvisporum	KT 922*	AB807547	–	AB809644	–
Jalapriya inflata	NTOU 3855	JQ267363	–	JQ267362	–
Jalapriya pulchra	MFLUCC 15–0348*	KU179109	–	KU179108	–
Jalapriya toruloides	CBS 209.65	DQ018104	–	DQ018093	–
Paradendryphiella arenariae	CBS 181.58*	KC793338	–	KF156010	–
Paradendryphiella salina	CBS 142.60	KC793339	–	DQ411540	–
Pseudocoleophoma calamagrostidis	KT 3284*	LC014609	–	LC014592	–
Pseudocoleophoma polygonicola	KT 731*	AB807546	–	AB809634	–
Pseudocoleophoma typhicola	MFLUCC 16–0123*	KX576656	–	KX576655	–
Pseudodictyosporium elegans	CBS 688.93*	DQ018106	–	DQ018099	–
Pseudodictyosporium wauense	NBRC 30078	DQ018105	–	DQ018098	–
Stemphylium herbarum	CBS 191.86	GU238160	KC584471	KC584239	KC584731
	BRIP 65181	–	KY009907	KY009903	KY009905
Stemphylium botryosum	CBS 714.68	–	AF107804	KC584238	KC584729

Sequences produced in the present study are in bold. The other sequences are from Woudenberg et al. (2013) and Liu et al. (2017) except those of *Stemphylium herbarum* and *Stemphylium botryosum* from Moslemi et al. (2017)

Ex-type strains are indicated with "*" after collection number. nc LSU rDNA = large subunit of the nrDNA, *RPB2* = polymerase II second largest subunit, ITS = internal transcribed spacer, *TEF1* = translation elongation factor 1-α

10,000 trees were obtained. The first 2000 trees representing the burn-in phase were discarded, whereas the remaining 8000 trees were used for calculating the posterior probabilities. Bayesian posterior probabilities are presented on the left of each node. The analysis was hosted by CIPRES Science Gateway portal at San Diego Supercomputer Center (Miller et al. 2010). Phylogenetic trees were visualized with the program FigTree v1.3.1 (Rambaut 2009).

For maximum parsimony (MP), analyses were conducted using PAUP v. 4.0b10 (Swofford 2003) with phylogenic

relationships estimated by heuristic searches with 1000 random stepwise addition sequences and tree bisection and reconstruction (TBR) branch swapping. Alignment gaps were treated as missing data, and all characters were weighted equally. Measures calculated for parsimony included tree length (TL), retention index (RI), consistency index (CI), rescaled consistency index (RC), and homoplasy index (HI). Statistical support for branch nodes in the most parsimonious trees was obtained by performing 1000 bootstrap replicates.

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 in MEGA. The branch 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 three methods (MP, ML, and BI) were then compared, and the phylogram was edited with CorelDRAW Graphics Suite 2017.

Sequences of *Stemphylium herbarum* (CBS 191.86 and BRIP 65181) and *Stemphylium botryosum* (CBS 714.68) were used as the outgroups in the *Alternaria* phylogeny, and sequences of *Paradendryphiella arenariae* (CBS 181.58) and *Paradendryphiella salina* (CBS 142.60) were used in the *Dendryphiella* phylogeny. Sequences derived in this study were lodged in GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1). The alignment and tree were deposited in TreeBASE (http://www.treebase.org) (study numbers S24915 and S24916) and taxonomic novelties in MycoBank (www.MycoBank.org).

Results

Phylogeny

Phylogenetic analysis using the ITS, nc LSU rDNA, *RPB2*, and *TEF1* regions were based on 51 *Alternaria* strains, 2 isolates of *Acroconidiella tropaeoli*, and 3 outgroup sequences (Fig. 1). The combined alignment was comprised of 2731 characters with gaps (625 for ITS, 852 for nc LSU rDNA, 865 for *RPB2*, and 389 for *TEF1*). The phylogenetic analyses generated by maximum parsimony, maximum likelihood (ML), and Bayesian analysis indicate that *Acroconidiella tropaeoli* grouped within the genus *Alternaria* and formed a monotypic lineage near sect. *Sonchi* with a well-supported clade (99%/100%/1.00, MP/ML/BI supports, respectively).

Another phylogenetic analysis was performed using the ITS and nc LSU rDNA loci to clarify the phylogenetic position of *Acroconidiella trisepta* (Fig. 2). The alignment of combined ITS and nc LSU rDNA sequence data comprised the total of 2168 characters with gaps (772 for ITS, 1396 for nc LSU rDNA). The dataset comprised 30 strains including 1 newly sequenced taxon and 2 outgroup sequences, *Paradendryphiella arenariae* and *P. salina* (Pleosporaceae). The phylogenetic analyses generated by maximum parsimony (MP), maximum likelihood (ML), and Bayesian analysis indicate that *Acroconidiella trisepta* belongs within the genus *Dendryphiella* with high support (100%/98%/1.00, respectively). The isolate of *Acroconidiella trisepta* clustered together with 2 other *Dendryphiella* species. Additionally, *Acroconidiella trisepta* formed a distinct lineage and was a sister to a strain of *D. eucalyptorum*.

Taxonomy

Alternaria Nees, Syst. Pilze (Würzburg): 72 (1816)

≡ Syn. nov. *Acroconidiella* J.C. Lindq. & Alippi, Darwiniana 13: 612, 1964

Alternaria obtusa **B.W. Ferreira & R.W. Barreto**, nom. nov. (Fig. 3).

MycoBank: MB832351

Etymology: having blunt/rounded-ended conidia

≡ *Heterosporium tropaeoli* Bond, Ceylon Journal of Science 12: 185, 1947

≡ *Acroconidiella tropaeoli* (Bond) Lindquist & Alippi, Darwiniana 13: 613, 1964

On fruits or, most abundantly, on leaves forming irregular to subcircular brownish or purple amphigenous spots, up to 1 cm diameter coalescing and leading to the blight of large areas of the leaf with yellow periphery. Colonies predominantly hypophyllous. Mycelium immersed, branched, septate, hyaline, smooth, hyphae 3–7 μm diameter. Conidiophores arising singly or in small groups through stomata or breaking through epidermis, erect, flexuous, simple, or occasionally branched, up to 180 μm long, 5–10 μm diameter, septate, often geniculate, sometimes slightly swollen at the apex, pale to mid olivaceous brown, smooth, conidial scars similar to those formed in *Drechslera* and *Curvularia*. Conidiogenous cells polytretic, integrated, terminal, subcylindrical to cylindrical, sometimes geniculate with sympodial proliferation, 6–22 × 4–5 μm, with one locus on a broadly obtuse apex, with a pigmented alternarioid scar, 2–4 μm wide. Conidia tretic, solitary or in short (2–3) unbranched chains, ellipsoidal, 30–50 (av. 41) μm long, 15–27 (av. 21) μm wide in the broadest part, lacking longitudinal septa, (1–) 2 (−3) transverse septa, strongly constricted at the septa, olivaceous brown, thin-walled, verruculose.

In culture: Fast growing (4–7 cm diam after 7 days), umbonate, cottonose, either fimbriate (in PCA) or entire edged (in PDA), smoke gray becoming white, gray olivaceous with white edges reverse. No sporulation was observed after 7 days of incubation.

Material examined: Brazil: Rio de Janeiro, Nova Friburgo, Ponte da Saudade, on *Tropaeolum majus*, 23 May 2017, R. W. Barreto (VIC 44410 – neotype designated here, neotype culture COAD 2389, MBT 388771); Minas Gerais, Antônio

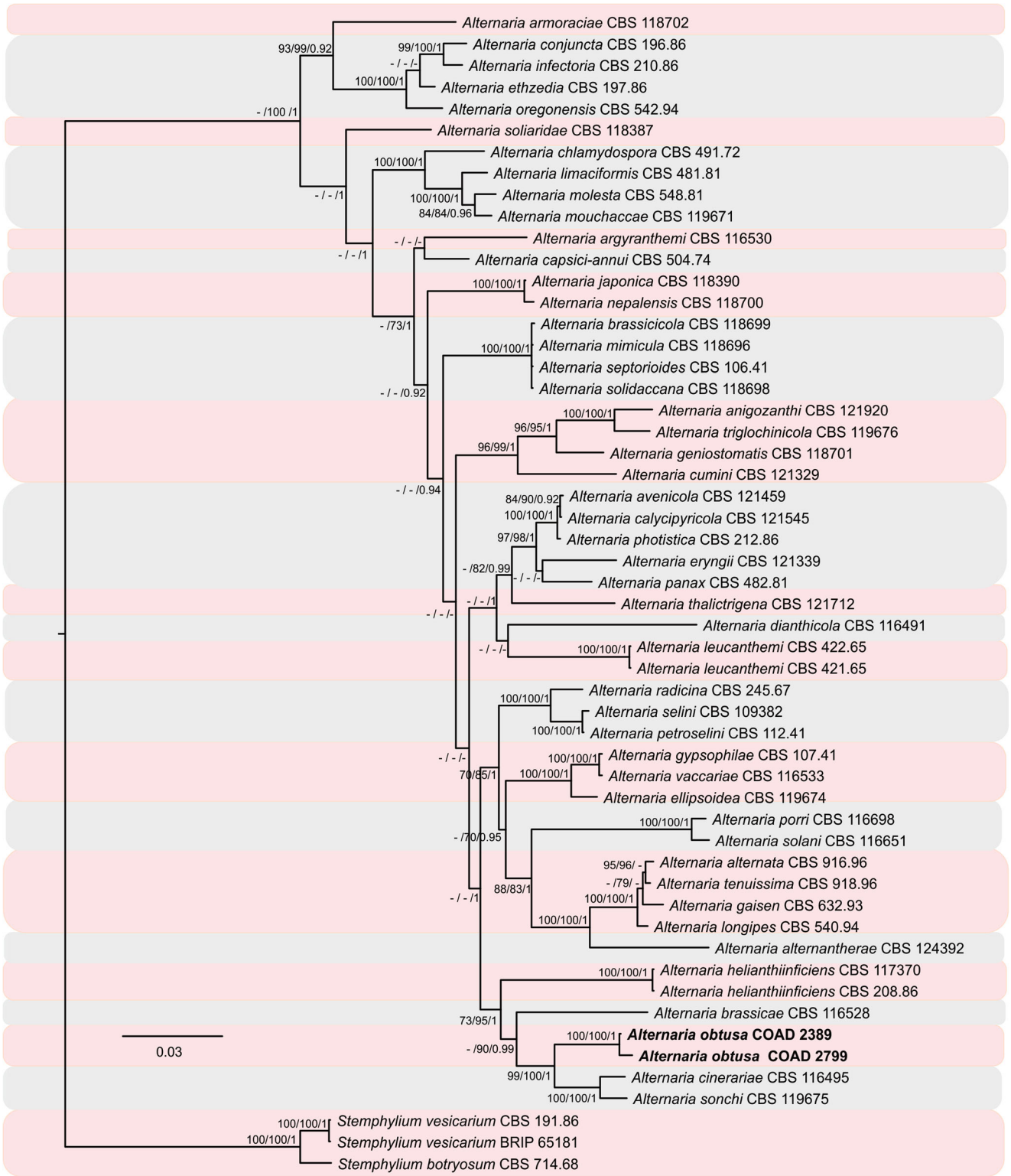

Fig. 1 Phylogenies based on combined nc LSU rDNA, ITS, *RPB2*, and *TEF1* showing the relationship of *Alternaria obtusa* with other closely related species within *Alternaria*. Bootstrap support values (MP and ML) or Bayesian posterior probabilities higher than 70% or 0.90 are indicated above or below the thickened branches ("–" indicates lack of support). Isolates from this study are indicated by bold text

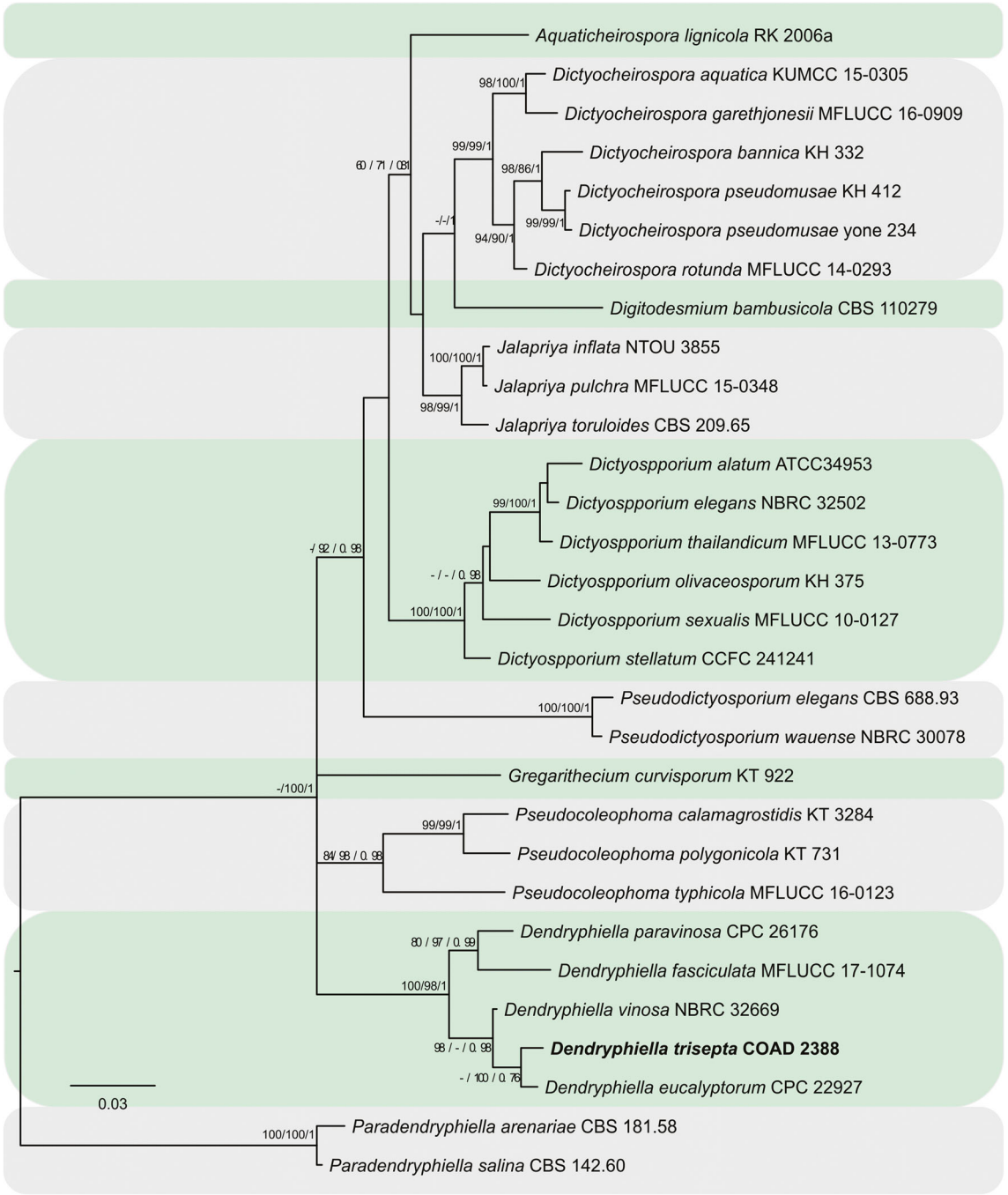

Fig. 2 Phylogenies based on combined nc LSU rDNA and ITS showing the relationship of *Dendryphiella trisepta* with other closely related genera from family Dictyosporiaceae. Bootstrap support values (MP and ML) or Bayesian posterior probabilities higher than 70% or 0.90 are indicated above or below the thickened branches ("–" indicates lack of support). Isolates from this study are indicated by bold text

Carlos, Sítio São Jorge, on *Tropaeolum majus*, 20 May 2019, B. W. Ferreira (VIC 47206, culture COAD 2799).

Notes: *Heterosporium tropaeoli* was originally described from a specimen collected from a non-designated site in Sri Lanka on living leaves of *T. majus*. No herbarium specimen is known for this specimen, and no ex-type culture was designated. It was hence considered necessary to designate a neotype and ex-neotype culture here. It might be argued that this should be obtained from Sri Lanka. Nevertheless, *A. tropaeoli* is a pathogen known only from *T. majus*, which is a plant native to South America. The fungus has been reported from many countries ranging from Australasia to Africa and the Americas, but not from Eurasia (Farr and Rossman 2019). In South America, it has been first reported

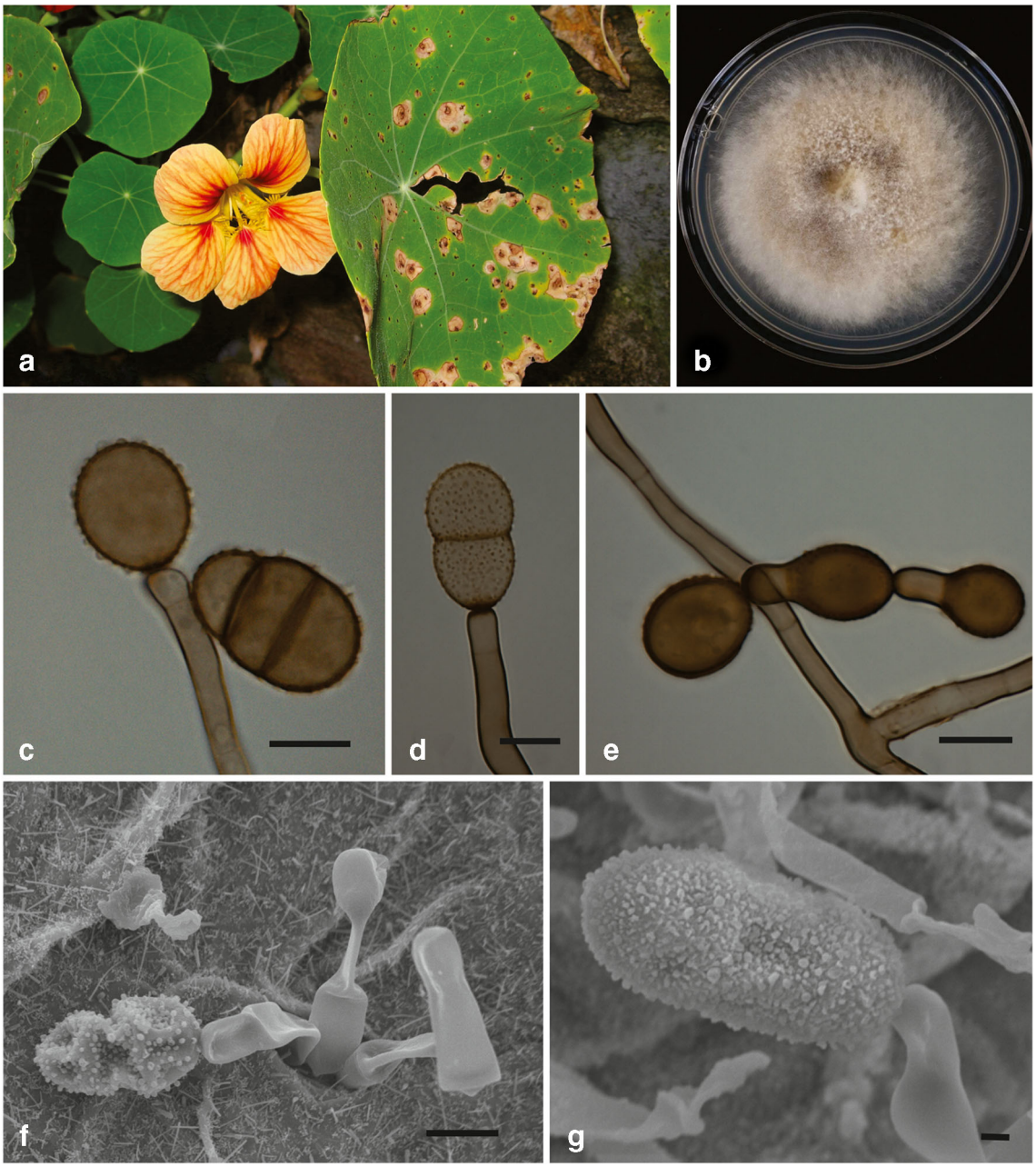

Fig. 3 *Alternaria obtusa* (VIC 44410). **a** Leaf spots on *Tropaeolum majus*. **b** Colony on PDA after 7 days (after incubation at 25 °C in 12 h light/dark cycle). **c** Detail of tretic conidiogenous cell bearing attached immature conidium and one mature conidium (note constrictions at septa of mature conidium) and verrucose surface on both immature and mature conidia. **d** *Ibid* one-septate immature conidium. **e** Acropetal conidial chain. **f** SEM image showing smooth conidiophores arising through the stoma and bearing one verrucose conidium (collapsed). **g** SEM image of one verrucose conidium. Bars = 10 μm except **g** = 2 μm

by Vieira and Barreto (2002), in Brazil. It is likely that its original occurrence and original description from Sri Lanka is fortuitous, and the fungus is actually native to South America which spread to other regions of the globe in planting material of its host but without being noticed in its center of origin until recently.

The new name *Alternaria obtusa* is proposed above for *Acroconidiella tropaeoli*. It could not be recombined into *Alternaria tropaeoli* because this name is already in use for another valid (and distinct) species in this genus described from India on *T. majus*.

***Dendryphiella trisepta* (Muchovej) B.W. Ferreira & R.W. Barreto, comb. nov.** (Fig. 4)

MycoBank: MB832343

Basionym. *Acroconidiella trisepta* J.J. Muchovej, Mycologia 72: 1045 (1980)

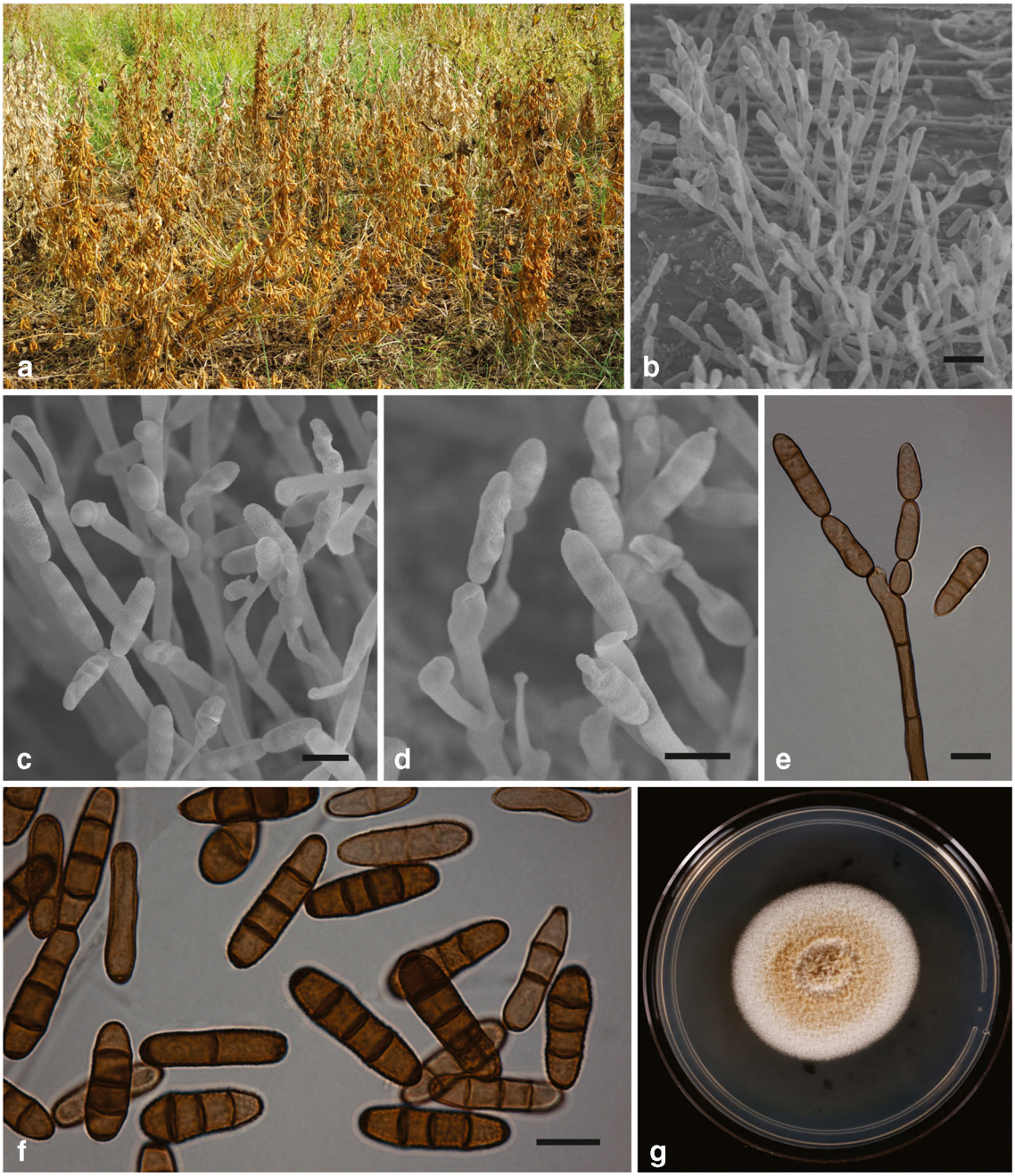

Fig. 4 *Dendryphiella trisepta* (VIC 44409). **a** *Glycine max* debris at the type locality (experimental area of the campus of the Universidade Federal de Viçosa, Viçosa, state of Minas Gerais, Brazil). **b** SEM images of *D. trisepta* colony on the surface of dead soybean stems. **c**, **d** SEM images of conidial chains. **e** Conidial chains under light microscopy. **f** Group of typical predominantly triseptate conidia (note: thickened and darkened conidial scars). **g** Colony on PDA after 15 days (incubation at 25 °C in 12-h light/dark cycle). Bars = 10 μm except **b** = 20 μm

Colonies bursting through the cuticle and forming dark spots amidst colonies of a range of other saprophytic fungi on rotting stems of *Glycine max*. Conidiophores erect, macronemateous, forming loose tufts, cylindrical, flexuous, geniculate, up to 140 μm, 5–6.5 μm diam, reddish-brown, smooth. Conidiogenous cells polytretic, cylindrical, terminal, proliferating sympodially, slightly swollen apically, 5–13 μm in width. Conidia tretic, cylindrical, straight, 22–27(–33) × 8–10(–12) μm, apex obtuse to rounded, base rounded, mostly 3-septate, in acropetal unbranched chains, pale to mid reddish-brown, echinulate, hila thickened and darkened, 1 μm diameter.

In culture: Slow-growing (4.5–4.8 cm diameter after 15 days), umbonate, felty, edges entire, either entirely white

or buff centrally with white edges, honey reverse with white edges. No sporulation was observed after 15 days of incubation.

Type material: Brazil: Minas Gerais: Viçosa, Universidade Federal de Viçosa, on dead branches *Glycine max*, 8 Jul 1979, J. J. Muchovej, Herbarium Universidade Federal de Viçosa (holotype lost); Minas Gerais: Viçosa, Universidade Federal de Viçosa, on dead branches *Glycine max*, 9 May 2017, R. W. Barreto (VIC 44409 – neotype designated here, ex-neotype culture COAD 2388, MBT 388772).

Notes: *Dendryphiella trisepta* was only known from soybean debris at the type locality. Colonies of the fungus were readily found in this substrate at the type locality in the first attempt of recollecting the fungus. As the original type material has been lost, and also because no ex-type culture was designated by Muchovej, a neotype and ex-neotype culture are designated here.

Species included in *Acroconidiella*

Acroconidiella eschscholziae (Harkn.) M.B. Ellis, nom. dub. More dematiaceous hyphomycetes. 407 (1976)

Basionym. Heterosporium escholtziae Harkn., Bull. Calif. Acad. Sci. 1: 38 (1884)

Colonies effuse, dark olivaceous. Mycelium immersed. Conidiophores solitary or in small groups developing between the epidermal cells, erect, flexuous, simple or occasionally branched, up to 85 μm long, 5–8 μm wide, often geniculate, pale olivaceous or golden-brown, smooth, with several dark-brown scars. Conidiogenous cells polytretic, sympodial. Conidia solitary or occasionally in short chains, mostly cylindrical, 28–90 × 9–19 μm, with 1–7 transverse and occasionally 1–2 longitudinal or oblique septa, often constricted at the septa, pale to mid-golden-brown, echinulate—as described in Ellis (1976).

Type material: USA: California, San Francisco, on leaves of *Eschscholtzia californica*, Jan 1884, Harkness (holotype destroyed)

Note: The presence of longitudinal septa in *A. eschscholziae* strongly suggests it to belong to *Alternaria.*

Acroconidiella indica I. B. Prasher & R. K. Verma, nom. dub. Journal on New Biological Reports 4: 111 (2015)

Colonies on natural substrate effuse, superficial on the substratum forming large stromatoid masses, made up of mycelium 3.2–6.4 μm wide, dark-brown, thick-walled, slightly roughened, extensively branched (branches close), short celled, bearing erect vertical conidiophores. Conidiophores 8–126 × 3.2–11.2 μm, brown, short or elongate, cylindrical, straight or slightly curved, septate, thick-walled, pigmented opaque, with a swollen basal cell, bearing conidiogenous cells. Conidiogenous cells rachiform, pale-brown to colorless, straight or flexuous, geniculated, geniculations thickened and minute, few (1–2), poroid. Conidia phaeo-, ceteri-, phragmo-, porosporous, acrogenous, 3–33.5 × 1.5–10.5 μm, brown to dark-brown, thick-walled, oval to elliptical or elongate, cylindrical, straight or slightly curved, dry, (1–6 celled), with transverse septa only, smooth, constricted at the septum: septa thick, distinct, apical cells rounded or sometimes pointed; basal cells more or less triangular and narrowed towards the hilum; hilum protruding, thickened. Germination of the conidia starts in situ or on the substrate by short germ tube which bears secondary conidia on them—as described in Prasher and Verma (2015).

Holotype: India: Himachal Pradesh: Solan, on dead twigs of unidentified tree, 10 Feb 2009, I. B. Prasher (PAN 30076)

Note: Prasher and Verma (2015) did not provide cultural or molecular data for *A. indica* and the morphology described for this fungus might place it in a range of similar genera such as: *Dendryphiella*, *Paradendryphiella*, or *Alternariaster.*

Acroconidiella manoharacharii I. B. Prasher & R. K. Verma, nom. dub. Journal on New Biological Reports 4: 113(2015)

Colonies on natural substratum black, minute, velvety, distributed throughout the substrate forming a scum. Mycelium immersed in the substrate, composed of branched, septate, brown, smooth-walled hyphae. Conidiophores 7–51 × 3–9.5 μm, branched, brown, short, cylindrical, straight or slightly curved, septate, with a swollen basal cell, pigmented opaque, thick-walled, bearing an apical conidiogenous cell. Conidiogenous cell rachiform, pale-brown, straight or flexuous, geniculated, geniculations thickened and minute, few (1–2) poroid. Conidia phragmo-, ceteri-, phaeo-, porosporous, borne singly, 8–20 × 3–8 μm, brown, thick-walled, oval to elliptic, straight or slightly curved, dry, (1–3 celled) with transverse septa, smooth, constricted at the septum; septa thick-walled, distinct; apical cell round or occasionally pointed; basal cell more or less triangular and narrowed towards the hilum; hilum protruding and thickened—as described in Prasher and Verma (2015).

Holotype: India: Himachal Pradesh: Shimla, Tara Devi, on angiospermous sticks, 23 Sep 2010, I. B. Prasher (PAN 30077)

Notes: There are no DNA sequences available in public databases for *A. manoharacharii* or any other fungi described in *Acroconidiella* except for those in this publication. It was not possible to examine specimens of *A. manoharacharii* deposited in PAN herbarium, and it appears that no culture of this fungus was obtained by the authors, since no description of cultural features is provided in Prasher and Verma (2015). As for *A. indica*, morphological features alone would allow its placement in a range of dematiaceous genera. It is hence better to leave the two species as *nomen dubium* until the fungus is recollected allowing for a proper re-evaluation including molecular typing.

Discussion

Alternaria was originally described by Nees von Esenbeck (1816), based on *A. tenuis*. The genus grew in number of species and included an assemblage of dematiaceous hyphomycetes producing dark-colored phaeodictyospores ended in a tapering beak and usually forming conidial chains (Ellis 1976). *Alternaria* was monographed by Simmons (2007) who, based on morphological and cultural features, recognized 273 *Alternaria* species and segregated several similar genera such as *Aternariaster*, *Chalastospora*, *Prathoda*, and *Teretispora*. A modern re-evaluation of *Alternaria* and related genera is presently under way (Lawrence et al. 2013; Woudenberg et al. 2013) and is producing major changes in the delimitation of this important group of fungi. Our results provide yet another contribution by modern-day mycologists and clarified that *Acroconidiella* is not a valid genus since its type species is a member of the genus *Alternaria.*

Lindquist and Alippi (1964) treated the genus *Acroconidiella* as distinct from *Alternaria* because it did not produce conidial chains or longitudinal or oblique septa. This combination of features suggested that it might be inadequately placed in *Alternaria*. This appeared a logical decision, based on morphology, at the time. Nevertheless, modern studies of *Alternaria* have indicated that the absence of longitudinal or oblique septae alone is not sufficient for excluding a species from *Alternaria.* Some examples of species of *Alternaria* having solely transverse septa are as follows: *A. leucanthemi*, *A. thalictrina*, *A. thalictricola*, *A. thalictrigena*, and members of sect. *Nimbya* (Schubert et al. 2007; Lawrence et al. 2012; Woudenberg et al. 2013). As for the presence or absence of conidial chains, a morphological feature which had taxonomic weight for erecting *Acroconidiella*, Vieira and Barreto (2002) had documented the presence of short conidial chains in *A. trapaeoli*—also observed and illustrated in the present study and also confirmed to be a dominant feature in *A. trisepta*, as already observed in the original description by Muchovej (1980). *Acroconidiella eschscholziae* described by Ellis (1976) presents conidia with longitudinal or oblique septa (as for the majority of the members of *Alternaria*) which are occasionally produced in short chains. It is very likely that *A. eschscholziae* is yet another species to be recombined into *Alternaria. Acroconidiella tropaeoli* was found here to be phylogenetically close to *Alternaria brassicae*, *Alternaria cinerariae*, and *Alternaria sonchi*. None of these taxa is morphologically similar to *A. obtusa.* Although sharing the feature of the absence of longitudinal/oblique septae with *A. obtusa* neither *A. leucanthemi* nor *A. thalictrigena* grouped with *A. obtusa.* There are no *A. thalictrina* or *A. thalictricola* sequences in GenBank available for comparison with the fungus on *T. majus*. However, these latter taxa have clear morphological distinctions separating them from *A. obtusa.* Both have longer, wider, rostrate conidia (Schubert et al. 2007). According to Lawrence et al. (2013), section *Sonchi* includes *Alternaria cinerariae* and *Alternaria sonchi* and is characterized morphologically by producing large subcylindrical, broadly ovoid, broadly ellipsoid or obclavate, formed singly or in short chains, with multiple transverse and few longitudinal septa, slightly constricted at the septa, with a blunt taper which can form secondary conidiophores. Lawrence et al. (2013) included *A. brassicae* as the basal lineage in sect. *Sonchi*, which was supported by a monotypic lineage in the analysis of Woudenberg et al. (2013). *Alternaria brassicae* has conidia which are straight or slightly curved, obclavate, rostrate, with transverse septa and longitudinal or oblique septa. Conversely, conidia of *A. obtusa* are ellipsoidal with rounded tips and lack longitudinal septa. Additionally, two species of *Alternaria*, *A. tropaeoli* (Deshpande and Rajderkar 1964) and *A. tropaeolicola* (Zhang 2000), have been described on *Tropaeolus majus* from India and China, respectively. Although there are no DNA sequences in public databases for these two species, they are clearly morphologically distinct from *Alternaria obtusa*. Both *A. tropaeoli* and *A. tropaeolicola* have rostrate, non-verrucous conidia which have transverse and longitudinal septae (Deshpande and Rajderkar 1964; Simmons 2007).

The fungus recollected from soybean debris was found here to be misplaced by Muchovej (1980). *Acroconidella trisepta* was found to clearly fit phylogenetically within the genus *Dendryphiella*. *Dendryphiella* was established by Ranojevic (1914) with the type species *D. interseminata* (Berk. & Ravenel) Bubák. Morphological characteristics used for delimitation of this genus included macronematous conidiophores with polytretic, integrated conidiogenous cells formed at the swollen tip and at intercalary swellings of conidiophores from which catenate or solitary conidia are formed (Ellis 1971; Matsushima 1971; Rai and Kamal 1986; Guo and Zhang 1999; Crous et al. 2014, 2016). Boonmee et al. (2016) performed phylogenetic analyses of *Dendryphiella* based on DNA sequence data for three loci (SSU, nc LSU rDNA, *TEF1*). The results showed that *Dendryphiella* represents a distinct genus within the Dictyosporiaceae. Different from other asexual morphs, genera belonging to this family—which form blastic conidiogenous cells—members of *Dendryphiella* have tretic conidiogenous cells.

Dendryphiella trisepta, as recombined in the present publication, has a distinct morphology from that of other species of *Dendryphiella.* Its conidiophores are shorter than those of *D. eucaliptorum* and *D. aspera* (up to 140 μm in length vs. up to 500 μm and 136–544 μm, respectively), and its conidia are larger [22–27 (–33) × 8–10 (–12) μm vs. (19–) 20–23 (–25) × 5 (–7) μm and 10–22 × 4–6 μm respectively]. In *D. indica*, *D. paravinosa*, *D. eucalypti*, *D. vinosa*, *D. uniseptata*, *D. infuscans*, and *D. dregeae*, conidiophores are mostly

solitary, whereas in *D. trisepta*, these are formed in tufts. *Dendryphiella broussonetiae* and *D. lycopersicifolia* have conidiophores which are longer than those of *D. trisepta* (129–303 μm and 100–500 μm vs. 140 μm). *Dendryphiella fasciculata* has longer conidiophores (170–250 μm) and narrower conidia (4.3–7.4 μm) than those of *D. trisepta.*

Nuclear DNA of *A. tropaeoli*, the type species of *Acroconidiella*, was extracted and supported a phylogenetic evaluation of *Acroconidiella* performed here, for the first time. It demonstrated that the morphological delimitation utilized by the scientists who dealt with this genus in the past does not mirror the true phylogenetic placement of this taxon. As it has happened with other genera in the past, *Acroconidiella* became a "dumping ground" for somewhat morphologically similar taxa of uncertain placement, a fact demonstrated here by *D. trisepta*. By transferring *A. tropaeoli* to *Alternaria*, the generic name *Acroconidiella* can no longer be applied to the other species placed in the genus. There are doubts as for the correct taxonomic placement of *A. eschscholziae*, *A. indicus*, and *A. manoharacharii*. For *D. trisepta*, samples needed for re-evaluating the taxon were within the type locality, in the campus of the Universidade Federal de Viçosa where the work was performed. Nevertheless, it was surprisingly easy to recollect (in the first attempt) the material of *A. trisepta* from old soybean stems in the field, nearly forty years after it was collected and described by Muchovej (1980), suggesting that a stable population of this saprophytic fungus exists at this locality (an experimental area belonging to the Departamento de Fitotecnia of the Univesidade Federal de Viçosa). Although no other record exists of this fungus outside this area, it is likely that this fungus is present in soybean debris in other fields in Brazil and perhaps other parts of the world. Although it appears that this is a saprophytic species, no studies on its relationship with soybean or other plant species have ever been conducted (Baird et al. 1997, 2003; Almeida et al. 2001).

Recollecting, isolating and re-evaluating the other species in the former genus *Acroconidiella* is a pending challenge, among so many others posed to mycologists worldwide of recollecting, neotypifying, or epitipifying the fungal taxa of the past (Hyde and Zhang 2008). Until then, these species are to be regarded as *Incertae sedis.*

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