Mycol Progress (2016) 15:1203–1214
DOI 10.1007/s11557-016-1240-6

ORIGINAL ARTICLE

Eight novel *Bipolaris* species identified from John L. Alcorn's collections at the Queensland Plant Pathology Herbarium (BRIP)

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Received: 23 June 2016 / Revised: 8 October 2016 / Accepted: 10 October 2016 / Published online: 8 November 2016

Abstract Several unidentified specimens of *Bipolaris* deposited in the Queensland Plant Pathology Herbarium (BRIP) that were previously recognised by Dr. John L. Alcorn as taxonomically interesting were re-examined. The morphology of conidia and conidiophores, as well as phylogenetic inference from the analyses of three loci (ITS, *GAPDH* and *TEF1α*) supported the classification of eight novel *Bipolaris* species, which were originally isolated from leaf spots on grasses (Poaceae).

Keywords Helminthosporioid fungi · Novel species · Taxonomy

Introduction

The genus *Bipolaris* Shoemaker (1959) has traditionally been treated as part of the helminthosporioid complex, so-called because the conidia and conidiophores morphologically resemble species of *Helminthosporium* Link (1809). *Bipolaris* was originally established to accommodate species that formed fusoid conidia with two or more septa that exhibited bipolar germination, but also included some species with curved conidia and hyaline apical cells (Shoemaker 1959).

Until the late 1990s, the classification and identification of *Bipolaris* species was based entirely on morphological characteristics (Sivanesan 1987). This proved problematic, as conidia and conidiophores are highly variable within species. In recent years, the generic limits for the helminthosporioid fungi (including *Curvularia*, *Drechslera*, *Exserohilum*, *Johnalcornia* and *Porocercospora*) have been more clearly defined with the aid of molecular sequence data (Ahmadpour et al. 2012; da Cunha et al. 2012; Madrid et al. 2014; Manamgoda et al. 2012, 2014; Tan et al. 2014). Subsequent analyses of DNA sequence data have established the synonymy between *Bipolaris* (typified by *B. maydis*) and its sexual morph, *Cochliobolus* Drechsler (1934) (typified by *C. heterostrophus*) (Manamgoda et al. 2012; Rossman et al. 2013). The rules of nomenclature for fungi only allow one name for each genus, instead of different names for different morphs in the fungal life cycle (McNeill et al. 2012). Although *Cochliobolus* is the older name, *Bipolaris* is more frequently used by plant pathologists in disease reports and widely applied in the taxonomic literature. The name *Bipolaris* was subsequently proposed for conservation against the earlier name *Cochliobolus* (Rossman et al. 2013).

Species of *Bipolaris* are commonly associated with leaf spots, leaf blights and root rots on hosts in the Poaceae (Ellis 1971; Sivanesan 1987; Manamgoda et al. 2011, 2014). Some species that are considered serious pathogens are those on high-value commodity cereal crops, such as *B. maydis* on maize, *B. oryzae* on rice (Sunder et al. 2014) and *B. sorokiniana* on wheat (Acharya et al. 2011). Several species have multiple grass hosts, including other cereals and weeds, which presents additional problems related to crop rotation and disease management (Iftikhar et al. 2009; Strange and Scott 2005; Sunder et al. 2014). Furthermore, many

Section Editors: Roland Kirschner and Pedro W. Crous

This article is part of the Special Issue "Biodiversity of Hyphomycetes—Special Issue in honour of Dr. Subramanian".

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Bipolaris species are saprobes or pathogens on hosts in the families Anacardiaceae, Araceae, Euphorbiaceae, Fabaceae, Malvaceae, Rutaceae and Zingiberaceae (Ellis 1971; Manamgoda et al. 2011, 2014). There are approximately 47 species of *Bipolaris* (Manamgoda et al. 2014), of which 29 occur in Australia (Alcorn 1982, 1990, 1996; DAF Biological Collections 2016; Sivanesan 1985, 1987). Most of these species were associated with hosts in the Poaceae, with the exceptions of *B. cactivora* and *B. incurvata*, which were only recorded on hosts in the families Cactaceae and Arecaceae, respectively (DAF Biological Collections 2016; Forsberg 1985; Fröhlich et al. 1997; Shivas 1995).

Accurate identification of *Bipolaris* species based on DNA sequences is dependent on the availability of ex-type cultures. In recent years, many DNA sequences from ex-type or reference cultures of *Bipolaris* species have been made available in GenBank (Manamgoda et al. 2012, 2014, Tan et al. 2014). In this study, 13 unidentified isolates of *Bipolaris* held in the Queensland Plant Pathology Herbarium (BRIP) were examined by molecular and morphological methods, and compared with ex-type and reference isolates. Most of the fungi were collected and isolated by Dr. John L. Alcorn as curator of the BRIP from the early 1960s through to the late 1990s. Ten new species of *Bipolaris* were revealed from the combined data analyses and morphological studies, and are herein introduced and described.

Materials and methods

Isolates and morphology

All isolates examined are listed in Table 1. The unidentified isolates of *Bipolaris* were obtained from BRIP, which retains cultures in a metabolically inactive state at −80 °C in a sterile solution of 15 % v/v glycerol. In order to observe conidia and conidiophores, living cultures were grown on sterilised leaf pieces of *Zea mays* placed on modified Sachs agar or sterilised wheat straws on water agar, incubated at 23 °C for 4 weeks, and exposed to near ultraviolet light source on a 12-h light/dark diurnal cycle (Sivanesan 1987). Conidia and conidiophores were mounted on glass slides in lactic acid (100 % v/v) and images captured with a Leica DFC500 camera attached to a Leica DM5500 B compound microscope with Nomarski differential interference contrast illumination. The images presented in Fig. 3d–e were taken from dried cultures, and Figs. 2e, i and 3a were taken from dried herbarium specimens. Conidial widths were measured at the widest part of each conidium. Means and standard deviations (SDs) were calculated from at least 20 measurements. Ranges were expressed as (minimum value–) mean−SD – mean+SD (–maximum value), with values rounded to 0.5 μm. Images of the herbarium specimens were captured by an Epson Perfection V700 scanner at 300 dpi resolution.

DNA isolation, amplification and phylogenetic analyses

The isolates were grown on PDA for 7 days at 23 °C. Mycelia were scraped off the PDA cultures and macerated with 0.5-mm glass beads (Daintree Scientific) in a TissueLyser (Qiagen). Genomic DNA was extracted with the Gentra Puregene DNA Extraction Kit (Qiagen), according to the manufacturer's instructions.

The primers V9G (de Hoog and Gerits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the internal transcribed spacer (ITS) region of the nrDNA. The primers gpd1 and gpd2 (Berbee et al. 1999) were used to amplify part of the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene. A partial region of the translation elongation factor 1-α (*TEF1α*) locus was amplified using the primers EF1983/EF12218R (Schoch et al. 2009). All loci were amplified with the Phusion High-Fidelity PCR Master Mix (New England Biolabs). The polymerase chain reaction (PCR) products were purified and sequenced by Macrogen Incorporated (Seoul, Korea).

All sequences generated were assembled using Geneious v. 9.1.5 (Biomatters Ltd.) and deposited in GenBank (Table 1, in bold). These sequences were aligned with selected sequences of *Bipolaris* species obtained from GenBank (Table 1) using the MAFFT alignment algorithm (Katoh et al. 2009) in Geneious. *Curvularia lunata* CBS 730.96 was included as the outgroup (Table 1). The sequences of each locus were aligned separately and manually adjusted as necessary. Alignment gaps were treated as missing character states, and all characters were unordered and of equal weight. The Markov chain Monte Carlo (MCMC) algorithm was used to create a phylogenetic tree based on Bayesian probabilities using MrBayes v.3.2.1 (Huelsenbeck and Ronquist 2001; Ronquist and Huelsenbeck 2003) in Geneious. To remove the need for a priori model testing, the MCMC analysis was set to sample across the entire general time-reversible (GTR) model space with a gamma-distributed rate variation across the sites. Ten million random trees were generated using the MCMC procedure with four chains. The sample frequency was set at 100 and the temperature of the heated chain was 0.1. Burn-in was set at 25 %, after which the likelihood values were stationary. Maximum likelihood (ML) analysis was run using RAxML v.7.2.8 (Stamatakis and Alachiotis 2010) in Geneious and started from a random tree topology. The nucleotide substitution model used was GTR with a gamma-distributed rate variation. The concatenated alignment was deposited in TreeBASE (Study 19483). All novel sequences were deposited in GenBank (Table 1).

In order to determine the species limits, the criterion of genealogical concordance phylogenetic species recognition (GCPSR) was applied to the molecular data (Taylor et al. 2000). A combined analysis of three genes was used to determine the final species boundaries with the support of all single gene trees inferred. Unique fixed nucleotides are used to characterise genetic differences in the new species. For each

Table 1 *Bipolaris* isolates examined in this study

Species	Isolate no.[a]	Host	Location	GenBank accession numbers[b]			
				ITS	GAPDH	TEF	LSU
Bipolaris austrostipae sp. nov.	BRIP 12490*	*Austrostipa verticillata*	Australia	**KX452442**	**KX452408**	**KX452459**	**KX452425**
Bipolaris axonopicola sp. nov.	BRIP 11740*	*Axonopus fissifolius*	Australia	**KX452443**	**KX452409**	**KX452460**	**KX452426**
Bipolaris bamagaensis sp. nov.	BRIP 13577*	*Brachiaria subquadripara*	Australia	**KX452445**	**KX452411**	**KX452462**	**KX452428**
	BRIP 10711	*Dactyloctenium aegyptium*	Australia	**KX452444**	**KX452410**	**KX452461**	**KX452427**
	BRIP 14847	*Dactyloctenium aegyptium*	Australia	**KX452446**	**KX452412**	**KX452463**	**KX452429**
	BRIP 15934	*Dactyloctenium aegyptium*	Australia	**KX452447**	**KX452413**	**KX452464**	**KX452430**
Bipolaris bicolor	CBS 690.96	Unknown	Cuba	KJ909762	KM042893	KM243287	
Bipolaris chloridis	BRIP 10965*	*Chloris gayana*	Australia	KJ415523	KJ415423	KJ415472	
Bipolaris clavata	BRIP 12530*	*Dactyloctenium radulans*	Australia	KJ415524	KJ415422	KJ415471	
Bipolaris coffeana	BRIP 14845*	*Coffea arabica*	Kenya	KJ415525	KJ415421	KJ415470	
Bipolaris cookei	MAFF 51191	*Sorghum bicolor*	Japan	KJ922392	KM034834	KM093777	
Bipolaris crotonis	BRIP 14838*	*Croton* sp.	Samoa	KJ415526	KJ415420	KJ415469	
Bipolaris cynodontis	CBS 109894*	*Cynodon dactylon*	Hungary	KJ909767	KM034838	KM243288	
Bipolaris drechsleri	CBS 136207*	*Microstegium vimineum*	USA	KF500530	KF500533	KM093760	
Bipolaris gossypina	BRIP 14840*	*Gossypium* sp.	Kenya	KJ415528	KJ415418	KJ415467	
Bipolaris heliconiae	BRIP 17186*	*Heliconia psittacorum*	Australia	KJ415530	KJ415417	KJ415465	
Bipolaris heveae	CBS 241.92	*Hevea* sp.	Nigeria	KJ909763	KM034843	KM243294	
Bipolaris luttrellii	BRIP 14643*	*Dactyloctenium aegyptium*	Australia	AF071350	AF081402	KJ415464	
Bipolaris maydis	CBS 136.29*	*Zea mays*	Japan	HF934926	HG779086	KJ415463	
Bipolaris microlaenae	CBS 280.91*	*Microlaena stipoides*	Australia	JN600974	JN600974	JN601017	
Bipolaris microstegii	CBS 132550*	*Microstegium vimineum*	USA	JX089579	JX089575	JX100808	
Bipolaris oryzae	MFLUCC 10-0715*	*Oryza sativa*	Thailand	JX256416	JX276430	JX256384	
Bipolaris panici-miliacei	BRIP 12282*	*Panicum miliaceum*	Japan	KJ415531	KJ415415	KJ415462	
Bipolaris peregianensis	BRIP 12790*	*Cynodon dactylon*	Australia	JN601034	JN600977	JN601022	
Bipolaris pluriseptata	BRIP 14839*	*Eleusine coracana*	Zambia	KJ415532	KJ415414	KJ415461	
Bipolaris sacchari	ICMP 6227	*Oplismenus imbecilis*	New Zealand	KJ922386	KM034842	KM093785	
Bipolaris salviniae	BRIP 16571*	*Salvinia auriculata*	Brazil	KJ415535	KJ415411	KJ415457	
Bipolaris secalis	BRIP 14453*	*Secale cereale*	Argentina	KJ415537	KJ415409	KJ415455	
Bipolaris shoemakeri sp. nov.	BRIP 15806	*Ischaemum rugosum* var. *segetum*	Australia	**KX452452**	**KX452418**	**KX452469**	**KX452435**
	BRIP 15929*		Australia	**KX452453**	**KX452419**	**KX452470**	**KX452436**
Bipolaris simmondsii sp. nov.	BRIP 12030*	*Zoysia macrantha*	Australia	**KX452454**	**KX452420**	**KX452471**	**KX452437**
Bipolaris sivanesaniana sp. nov.	BRIP 15847*	*Paspalidium distans*	Australia	**KX452455**	**KX452421**	**KX452472**	**KX452438**
	BRIP 15822	*Setaria sphaecelata*	Australia	**KX452456**	**KX452422**	**KX452473**	**KX452439**
Bipolaris sorokiniana (=*B. multiformis*)	CBS 480.74*	*Tribulus terrestris*	South Africa	KJ909771	KM034827	KM243282	
Bipolaris subramanianii sp. nov.	BRIP 16226*	*Setaria sphacelata*	Australia	**KX452457**	**KX452423**	**KX452474**	**KX452440**
Bipolaris urochloae	ATCC 58317*	*Urochloa panicoides*	Australia	KJ922389	KM230396	KM093770	
Bipolaris victoriae	CBS 327.64*	*Avena sativa*	USA	KJ909778	KM034811	KM093748	
Bipolaris woodii sp. nov.	BRIP 12239*	*Paspalidium caespitosum*	Australia	**KX452458**	**KX452424**	**KX452475**	**KX452441**
Bipolaris yamadae	CBS 202.29	*Panicum miliaceum*	Japan	KJ909779	KM034830	KM243275	
Bipolaris zeae	BRIP 11512*	*Zea mays*	Australia	KJ415538	KJ415408	KJ415454	
Bipolaris zeicola	FIP532*	*Zea mays*	USA	KM230398	KM034815	KM093752	
Curvularia lunata (outgroup)	CBS 730.96*	Human lung biopsy	USA	JX256429	JX276441	JX266596	

[a] *ATCC* American Type Culture Collection; *BRIP* Queensland Plant Pathology Herbarium, Brisbane, Queensland, Australia; *CBS* Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands; *ICMP* International Collection of Microorganisms from Plants, Auckland, New Zealand; *MAFF* MAFF Genebank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; *MFLUCC* Mae Fah Luang University Culture Collection, Chiang Rai, Thailand

[b] GenBank accessions derived from this study are shown in **bold**

*Indicates ex-type culture

species description, the closest phylogenetic neighbour was selected and these alignments were subject to single nucleotide polymorphism (SNP) analyses. These SNPs were determined for each aligned locus using the Find Variation/SNPs feature in Geneious. SNPs were determined based on a minimum variant frequency of 0.2. Taxonomic novelties were registered in MycoBank (http://www.mycobank.org; Crous et al. 2004).

Results

Phylogenetic analysis

On average, 860 bp of the ITS region, 551 bp of the *GAPDH* gene and 876 bp of the *TEF1α* gene were sequenced from the BRIP isolates. For the phylogenetic analyses, the ITS and GAPDH were trimmed to 474 and 445 bp, respectively. The

combined alignment deposited in TreeBASE is composed of 1733 characters from 46 isolates, of which 96 bp (20.3 %), 156 bp (35.1 %) and 99 bp (11.3 %) were variable for ITS, *GAPDH* and *TEF1α*, respectively. The ITS alignment was able to resolve 19 out of 38 *Bipolaris* species, including four of the new species (data not shown). Individually, both the *GAPDH* and *TEF1α* alignments were able to resolve 36 out of 38 *Bipolaris* species, including the ten new species described here (data not shown). None of the ITS, *GAPDH* or the *TEF1α* alignments were able to differentiate between the ex-holotype strain of *B. coffeana* and the recently designated ex-epitype strain of *B. cynodontis* (Manamgoda et al. 2014). A pairwise comparison of the unannotated sequences of *B. coffeana* and *B. cynodontis* showed 100 % identity in the ITS and *TEF1α* loci, and one SNP in the *GAPDH* locus, indicating a potential synonymy. Morphologically, *B. coffeana* can have conidiophores longer than *B. cynodontis* (up to 260 μm versus 170 μm), though the conidial dimensions of *B. coffeana* (32–75 × 11–14 μm) falls within the range described for *B. cynodontis* (30–75 × 10–16 μm). To avoid duplication, the novel taxa described below are, therefore, compared to *B. cynodontis*. The inferred phylogenetic tree based on the concatenated alignment resolved the 17 BRIP isolates into ten well-supported and unique clades, which are accepted in this study as novel species (Fig. 1).

Taxonomy

Bipolaris austrostipae Y.P. Tan & R.G. Shivas, sp. nov. (Fig. 2a–b)

MycoBank MB 817461

Etymology: Named after *Austrostipa*, the grass genus from which it was isolated.

Holotype: Australia, Queensland, Leyburn, from *Austrostipa verticillata* (Nees ex Spreng.) S.W.L. Jacobs & J. Everett, 11 May 1977, J.L. Alcorn, BRIP 12490 (includes ex-type culture).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, geniculate towards the apex, uniformly brown to dark brown, smooth, septate, up to 260 μm × 5–6 μm; basal cell swollen and darker than the other cells, up to 10 μm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic. *Conidiogenous nodes* darkening and becoming verruculose. *Conidia* fusiform, straight to slightly curved, (55–) 70–77 (–86) × (11–) 14–15.5 (–20) μm, brown to dark brown, 6–9-distoseptate. *Hilum* thick and darkened.

Notes: *Bipolaris austrostipae* is only known from the type specimen on *Austrostipa verticillata*, which is an Australian perennial grass found predominantly in Queensland and New South Wales (Simon and Alfonso 2011). *Bipolaris austrostipae* is phylogenetically close to *B. cynodontis* (Fig. 1), and its conidial size falls within the range given for *B. cynodontis* (30–75 × 10–16 μm) (Sivanesan 1987). *Bipolaris austrostipae* differs from the ex-type culture of *B. cynodontis* in two loci: *GAPDH* 98 % match (Identities 432/443, Gaps 0/443); *TEF1α* positions 225 (C), 266 (G) and 717 (T).

Bipolaris axonopicola Y.P. Tan & R.G. Shivas, sp. nov. (Fig. 2e–f)

MycoBank MB 817462

Etymology: Named after *Axonopus*, the grass genus from which it was isolated.

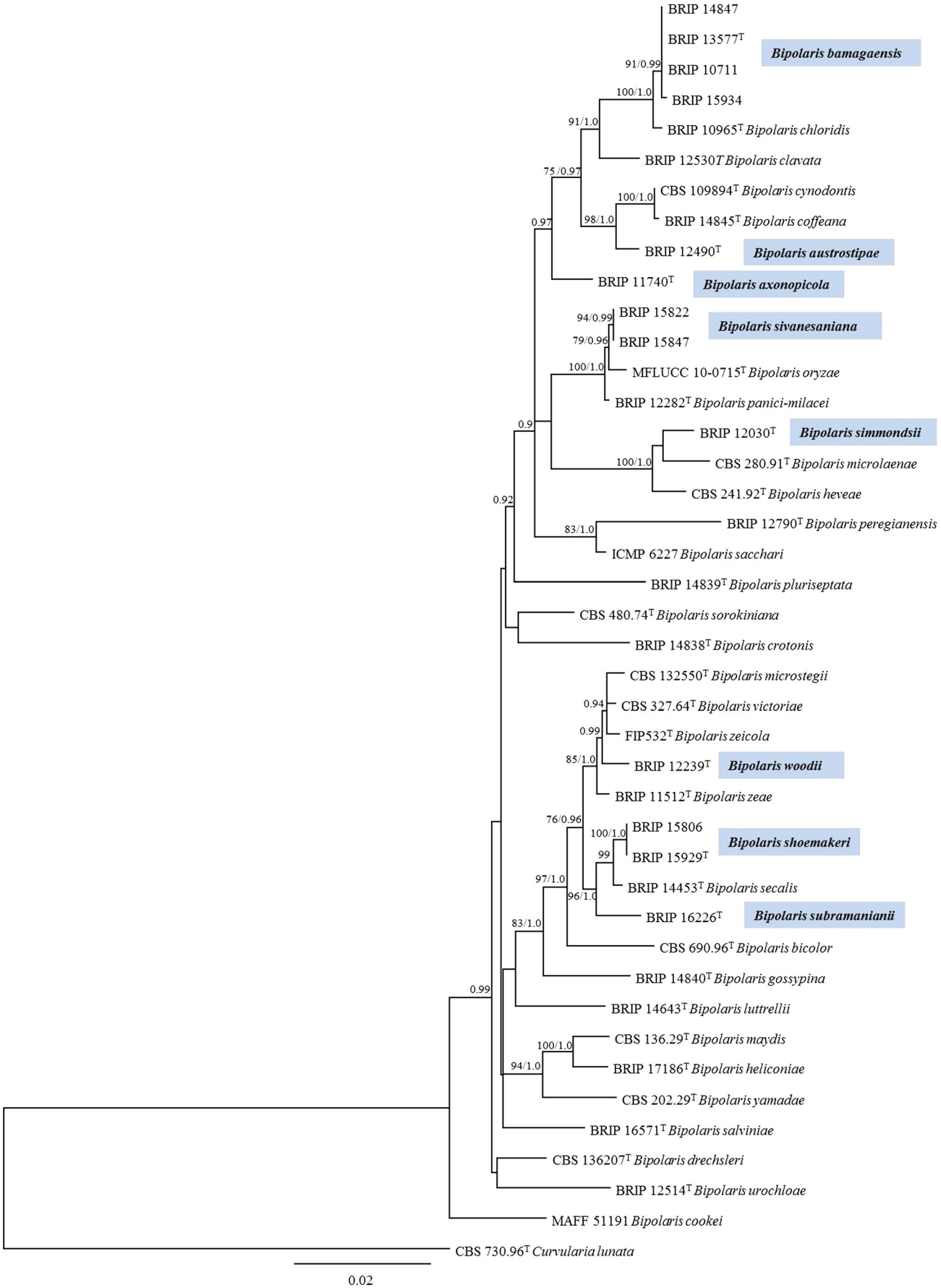
Holotype: Australia, Queensland, Peregian Beach, from leaf spot on *Axonopus fissifolius* (Raddi) Kuhlm., 6 June 1976, J.L. Alcorn, BRIP 11740 (includes ex-type culture).

Leaf spots on *Axonopus fissifolius*, narrowly ellipsoidal, up to 1 × 0.5 mm, reddish brown, larger spots with grey centres. *Conidiophores* mononematous, erect, straight to flexuous, rarely branched, geniculate towards the apex, uniformly pale brown to brown, smooth, septate, up to 250 μm × 5–9 μm; basal cell swollen and darker than the other cells, up to 18 μm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic with undarkened circular scars. *Conidiogenous nodes* distinct, slightly verruculose below the node. *Conidia* fusiform to subcylindrical or obclavate, (40–) 55–60 (–71) × (10–) 11.5–12.5 (–14) μm, pale brown with the end cells slightly paler than the central cells, smooth, 5–10-distoseptate, apex rounded, base obconically truncate or rounded. *Hilum* darkened and sometimes thickened. Germination bipolar.

Culture characteristics: Colonies cover the entire plate; surface grey olivaceous with smoky grey patches, velutinous with abundant aerial mycelium.

Notes: *Bipolaris axonopicola* is only known from a single specimen on *Axonopus fissifolius* in south-east Queensland. *Axonopus fissifolius* is native to the Americas and was introduced to Australia as a pasture grass (Simon and Alfonso 2011). The conidial dimensions of *B. axonopicola* overlap with those of *B. cynodontis* (30–75 × 10–16 μm). Marignoni (1909) described *Helminthosporium cynodontis* (synonym of *B. cynodontis*) as having conidia 60–75 μm long and also illustrated them as slightly curved. Subsequently, many morphologically similar isolates with slightly curved conidia have been assigned to *B. cynodontis* from a wide range of hosts

Fig. 1 Phylogenetic tree based on maximum likelihood analysis of the combined multilocus alignment. RAxML bootstrap values (bs) greater than 70 % and Bayesian posterior probabilities (pp) greater than 0.9 are given at the nodes (bs/pp). Novel species are in bold and highlighted in blue. Ex-type isolates are marked with a superscript ^{T}. The outgroup is *Curvularia lunata*


BRIP 14847
BRIP 13577T
Bipolaris bamagaensis
91/0.99
BRIP 10711
100/1.0
BRIP 15934
91/1.0
BRIP 10965T Bipolaris chloridis
BRIP 12530T Bipolaris clavata
75/0.97
CBS 109894T Bipolaris cynodontis
100/1.0
BRIP 14845T Bipolaris coffeana
0.97
98/1.0
BRIP 12490T
Bipolaris austrostipae
BRIP 11740T
Bipolaris axonopicola
94/0.99
BRIP 15822
Bipolaris sivanesaniana
79/0.96
BRIP 15847
100/1.0
MFLUCC 10-0715T Bipolaris oryzae
BRIP 12282T Bipolaris panici-milacei
0.9
BRIP 12030T
Bipolaris simmondsii
100/1.0
CBS 280.91T Bipolaris microlaenae
CBS 241.92T Bipolaris heveae
0.92
BRIP 12790T Bipolaris peregianensis
83/1.0
ICMP 6227 Bipolaris sacchari
BRIP 14839T Bipolaris pluriseptata
CBS 480.74T Bipolaris sorokiniana
BRIP 14838T Bipolaris crotonis
CBS 132550T Bipolaris microstegii
0.94
CBS 327.64T Bipolaris victoriae
0.99
FIP532T Bipolaris zeicola
85/1.0
BRIP 12239T
Bipolaris woodii
BRIP 11512T Bipolaris zeae
76/0.96
100/1.0
BRIP 15806
Bipolaris shoemakeri
99
BRIP 15929T
97/1.0
BRIP 14453T Bipolaris secalis
96/1.0
BRIP 16226T
Bipolaris subramanianii
83/1.0
CBS 690.96T Bipolaris bicolor
BRIP 14840T Bipolaris gossypina
0.99
BRIP 14643T Bipolaris luttrellii
CBS 136.29T Bipolaris maydis
100/1.0
94/1.0
BRIP 17186T Bipolaris heliconiae
CBS 202.29T Bipolaris yamadae
BRIP 16571T Bipolaris salviniae
CBS 136207T Bipolaris drechsleri
BRIP 12514T Bipolaris urochloae
MAFF 51191 Bipolaris cookei
CBS 730.96T Curvularia lunata
0.02

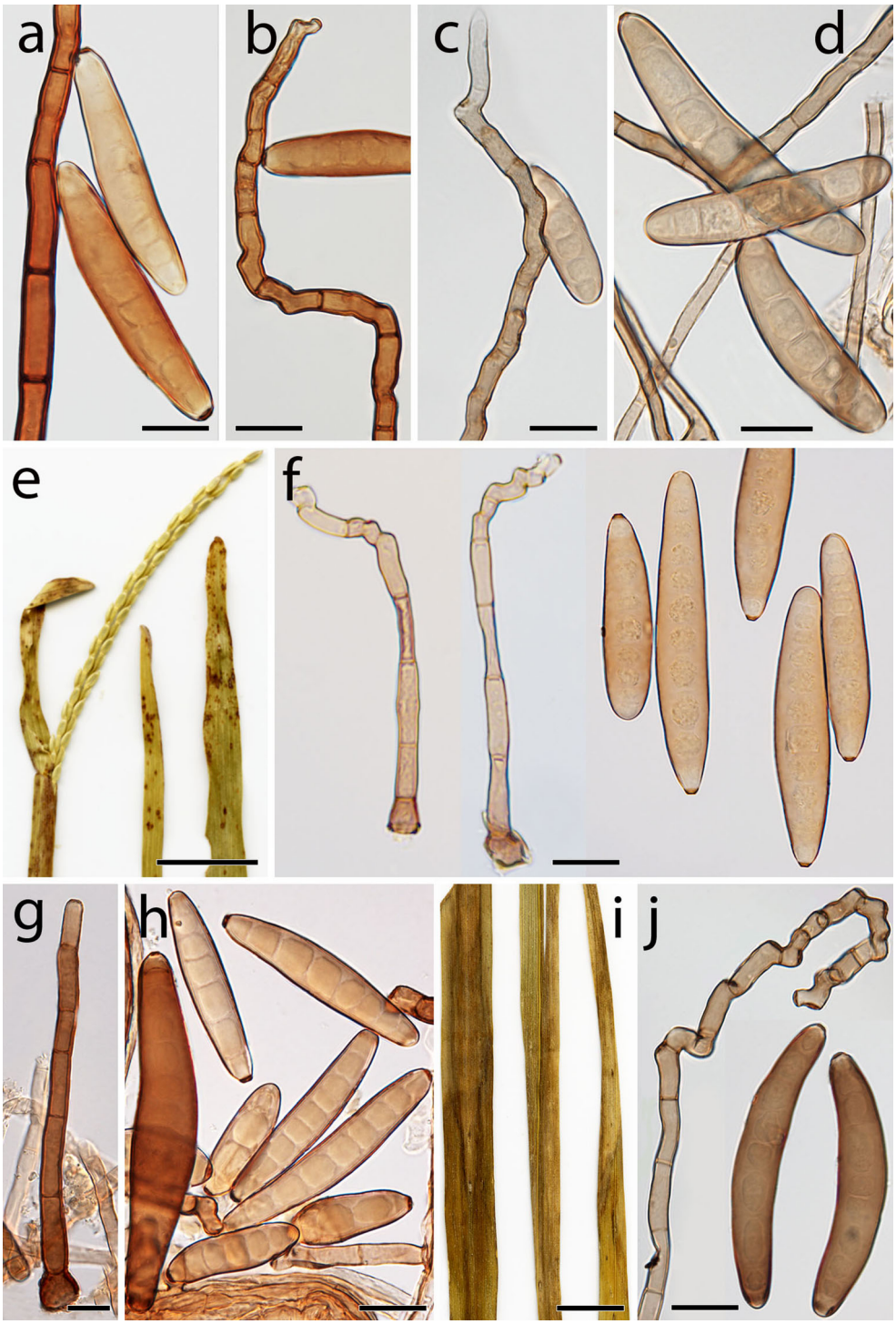


Fig. 2 *Bipolaris austrostipae* (ex-holotype BRIP 12490): **a** conidiophore with conidia; **b** conidiophore with conidium. *Bipolaris shoemakeri* (ex-holotype BRIP 15929): **c** conidiophore with a conidium; **d** conidia. *Bipolaris axonopicola* (ex-holotype BRIP 11740): **e** leaf spots on *A. fissifolius*; **f** conidiophores and conidia. *Bipolaris subramanianii* (ex-holotype BRIP 16226): **g** conidiophore, **h** conidia, **i** leaf spots on *S. sphacelata*. *Bipolaris woodii* (ex-holotype 12239): **j** conidiophore and conidia. Scale bars: **e**, **i** = 1 cm; **a**–**d**, **f**, **h**, **j** = 20 μm; **g** = 10 μm

(Manamgoda et al. 2014), including *A. fissifolius* (Sivanesan 1987). *Bipolaris axonopicola* has straight conidia, which distinguishes it from *B. cynodontis*.

Bipolaris axonopicola is phylogenetically close to *B. cynodontis* and *B. austrostipae* (Fig. 1). *Bipolaris axonopicola* differs from *B. cynodontis* in three loci: ITS 99 % match (Identities 451/457, Gaps 2/457); *GAPDH* 97 % match (Identities 427/441, Gaps 0/441); and *TEF1α* 99 % match (Identities 865/873, Gaps 0/873). The straight conidia of *B. axonopicola* distinguishes it from the slightly curved conidia of *B. austrostipae*, in addition to differences in three loci: ITS 98 % match (Identities 450/457, Gaps 2/457); *GAPDH* 98 % match (Identities 431/441, Gaps 0/441); and *TEF1α* 99 % match (Identities 866/875, Gaps 0/875).

Fig. 3 *Bipolaris bamagaensis* (ex-holotype BRIP 16634): **a** necrotic leaves from *U. subquadripara*; **b** conidiophore; **c** conidia. *Bipolaris simmondsii* (ex-holotype BRIP 12030): **d** conidiophore; **e** conidia. *Bipolaris sivanesaniana* (ex-holotype BRIP 15847): **f** conidiophores; **g** conidia. Scale bars: **a** = 1 cm; **b**–**g** = 10 μm

Bipolaris bamagaensis Y.P. Tan & R.G. Shivas, sp. nov. (Fig. 3a–c)

MycoBank MB 817463

Etymology: Named after the locality, Bamaga, from where it was collected.

Holotype: Australia, Queensland, Bamaga, from necrotic leaf on *Urochloa subquadripara* (Trin.) R.D. Webster, 28 May 1981, J.L. Alcorn, BRIP 13577 (includes ex-type culture).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, geniculate towards the apex, pale brown to brown to subhyaline at the apex, smooth, septate, up to 370 μm × 4 μm, base sometimes swollen (7–9 μm). *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic with undarkened circular scars. *Conidiogenous nodes* dark, distinct and slightly verruculose. *Conidia* ellipsoidal, fusiform, straight to slightly curved, (40–) 50–55 (–70) × (10–) 12–13 (–17) μm, uniformly pale brown to brown, smooth, 3–7 (usually 5)-distoseptate. *Hilum* darkened and sometimes thickened.

Additional specimens examined. Australia, Queensland, Bamaga, from leaf on *Dactyloctenium aegyptium* (L.) Willd., 29 May 1981, J.L. Alcorn, BRIP 10711; Queensland, culture formed in vitro by crossing isolates BRIP 13577 and BRIP 10711, 26 June 1985, J.L. Alcorn, BRIP 14847; Queensland, on Yarrabah Road, Mackey Creek (near Gordonvale), from leaf blight on *D. aegyptium*, 1 May 1987, J.L. Alcorn, BRIP 15879; Queensland, culture formed in vitro by single-spored isolates of BRIP 15897, June 1987, J.L. Alcorn, BRIP 15934.

Notes: *Bipolaris bamagaensis* is known from specimens on *Dactyloctenium aegyptium* and *Urochloa subquadripara* with leaf necrosis. Although both grass hosts are found across Australia, *B. bamagaensis* has only been found in northern Queensland. Many *Bipolaris* species have been associated with *Dactyloctenium*, including *B. clavata*, *B. cynodontis*, *B. luttrellii* and *B. maydis* (Sivanesan 1987; Manamgoda et al. 2014), while only one species, *B. urochloae*, has been recorded on *Urochloa* (Manamgoda et al. 2014; Sivanesan 1987). There may be other records in the literature of *Bipolaris* species on *Urochloa*, as many *Brachiaria* species were transferred to *Urochloa* (Webster 1987).

Bipolaris bamagaensis formed its sexual morph in culture (BRIP 14847) when single-spored isolates from different cultures (ex-holotype BRIP 13577 and BRIP 10711), as well as from the same culture (BRIP 15879), were crossed (J.L. Alcorn herbarium notes). The sexual morph was not observed during this study, and, therefore, a description could not be provided. Morphologically, the conidiophores of *B. bamagaensis* in culture are much shorter than that observed for *B. chloridis* (up to 1.2 mm long), and the dimensions of the typically straight to slightly curved conidia fall within the range described for the mostly curved conidia of *B. chloridis* (30–100 × 10–20 μm). *Bipolaris bamagaensis* differs from *B. chloridis* in two loci: *GAPDH* positions 20 (C) and 62 (T); *TEF1α* positions 307 (A) and 312 (G).

Bipolaris shoemakeri Y.P. Tan & R.G. Shivas, sp. nov. (Fig. 2c–d)

MycoBank MB 817466

Etymology. Named after Professor Robert Alan Shoemaker, an internationally respected mycologist and plant pathologist, who established *Bipolaris* for helminthosporioid species with fusoid conidia and bipolar germination, thereby differentiating it from *Drechslera* and *Helminthosporium* (Shoemaker 1959).

Holotype: Australia, Queensland, Mount Molloy, from leaf spot on *Ischaemum rugosum* var. *segetum* (Trin.) Hack., culture formed in vitro by crossing single-spored isolates, June 1987, J.L. Alcorn, BRIP 15929 (includes ex-type culture).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, uniformly pale brown to brown, smooth, septate, up to 1.8 mm × 6 μm. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale to subhyaline, smooth, mono- or polytretic with undarkened circular scars. *Conidiogenous nodes* distinct and slightly verruculose. *Conidia* fusiform, straight to slightly curved, (60–) 70–80 (–100) × (10–) 13.5–15 (–19) μm, pale brown to brown, smooth, 4–10 (usually 8)-distoseptate. *Hilum* darkened.

Additional specimen examined: Australia, Queensland, Mount Molloy, from leaf spot on *Ischaemum rugosum* var. *segetum*, 30 Apr. 1987, J.L. Alcorn, BRIP 15806.

Notes: *Bipolaris shoemakeri* was isolated from *Ischaemum rugosum* var. *segetum*, which is found mainly in the northern coastal region of Australia, and extends from India to Taiwan (Simon and Alfonso 2011). The ex-holotype culture (BRIP 15929) produced ascospores and was derived in vitro from self-crossed single-spored isolates of BRIP 15806 (J.L. Alcorn herbarium notes). The sexual morph was not observed during this study, and, therefore, a description could not be provided. Other species recorded on *I. rugosum* are *B. cynodontis*, *B. oryzae* and *B. setariae* (Sivanesan 1987; Manamgoda et al. 2014; Farr and Rossman 2016; Herbarium Catalogue 2016). *Bipolaris shoemakeri* has longer conidiophores (up to 1.8 mm) than *B. cynodontis* (up to 170 μm), *B. oryzae* (up to 600 μm) and *B. setariae* (200 μm). *Bipolaris shoemakeri* is phylogenetically close to *B. secalis* (Fig. 1). Morphologically, the very long, straight to flexuous conidiophores of *B. shoemakeri* differ from the shorter (up to 300 μm) and apically geniculate conidiophores of *B. secalis*. *Bipolaris shoemakeri* differs from *B. secalis* in three loci: ITS positions 103 (G) and 339 (indel); *GAPDH* positions 209 (T) and 446 (C); *TEF1α* positions 453 (C) and 816 (T).

Bipolaris simmondsii Y.P. Tan & R.G. Shivas, sp. nov. (Fig. 3d–e)

MycoBank MB 817467

Etymology: Named after the Australian plant pathologist Dr. John Howard (Jack) Simmonds MBE, who listed the first helminthosporioid fungi found in Queensland (Simmonds 1966).

Holotype: Australia, Queensland, Peregian Beach, on leaf spot on *Zoysia macrantha* Desv., 14 Nov. 1976, J.L. Alcorn, BRIP 12030 (includes ex-type culture).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, sometimes geniculate towards the apex, uniformly yellowish brown, paler at the apex, smooth, septate, up to 240 μm × 8 μm, basal cell swollen, up to 18 μm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic with circular scars. *Conidiogenous nodes* distinct and darkened. *Conidia* fusiform, straight or slightly curved, (70–) 78–116 (–130) × (12–) 13–17 (–18) μm, widest at the middle, yellowish brown to pale yellowish brown, paler at the ends, 7–10-distoseptate. *Hilum* darkened.

Notes: *Bipolaris simmondsii* is only known from the type specimen on *Zoysia macrantha*, an endemic temperate Australian grass. The ex-type isolate was sterile under the conditions it was grown. Fortunately, dried culture specimens from the original collection in 1976 had conidiophores and conidia that allowed morphological descriptions to be made. *Bipolaris simmondsii* is phylogenetically close to *B. heveae*, which has been associated with leaf spots on *Zoysia japonica* in Japan (Tsukiboshi et al. 2005). *Bipolaris heveae* has conidia that sometimes have a slightly protuberant hilum (3–4 μm), while *B. simmondsii* has an inconspicuous hilum. *Bipolaris simmondsii* differs from *B. heveae* in three loci: ITS positions 452 (indel), 453 (C) and 456 (T); *GAPDH* 98 % match (Identities 435/443, Gaps 0/443); *TEF1α* 9 (T), 102 (C), 307 (G), 453 (C), 655 (G), 735 (C) and 771 (C).

Bipolaris sivanesaniana Y.P. Tan & R.G. Shivas, sp. nov. (Fig. 3f–g)

MycoBank MB 817468

Etymology: Named after Dr. Asaipillai Sivanesan, in recognition of his contributions to mycology and plant pathology, especially his seminal monograph on graminicolous helminthosporioid fungi (Sivanesan 1987).

Holotype: Australia, Queensland, Atherton, from *Paspalidium distans* (Trin.) Hughes, 1 May 1987, J.L. Alcorn, BRIP 15847 (includes ex-type culture).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, uniformly pale brown to brown, smooth, septate, up to 600 μm × 4–6 μm; basal cell swollen and darker than the other cells, up to 18 μm diam. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to subhyaline, smooth, mono- or polytretic with undarkened circular scars. *Conidiogenous nodes* distinct and swollen. *Conidia* fusiform, straight to slightly curved, (60–) 70–77 (–86) × (11–) 14–15.5 (–20) μm, pale brown to brown, 5–8-distoseptate. *Hilum* darkened and sometimes thickened.

Additional specimen examined: Australia, Queensland, Julatten, from *Setaria sphacelata* (Schumach.) Stapf & C.E. Hubb., 30 Apr. 1987, J.L. Alcorn, BRIP 15822.

Notes: *Bipolaris sivanesaniana* is known from *Paspalidium distans* and *Setaria sphacelata* in Queensland. This hints at a co-evolutionary relationship as the grass hosts, *Setaria* and *Paspalidium*, are closely related (Kellogg et al. 2009; Morrone et al. 2012). *Bipolaris sivanesaniana* is the only species described on *P. distans*, a native Australian perennial grass found in temperate and tropical regions of Asia and the Pacific. One other species, *B. setariae*, has been recorded on *P. flavidum* (Farr and Rossman 2016). *Bipolaris sivanesaniana* has longer conidiophores (up to 600 μm) than *B. setariae* (up to 200 μm long). Molecular phylogenetic comparison with *B. setariae* cannot be reliably made, as there are no available sequences for a type or authentic strain. Other *Bipolaris* species recorded on *Setaria* are *B. bicolor*, *B. cynodontis*, *B. leersiae*, *B. maydis*, *B. oryzae*, *B. panici-milacei*, *B. sacchari*, *B. salviniae*, *B. setariae*, *B. sorokiniana*, *B. victoriae*, *B. yamadae* and *B. zeicola* (Sivanesan 1987; Manamgoda et al. 2014; Farr and Rossman 2016; Herbarium Catalogue 2016), although some of these identifications have not been verified by DNA sequencing analyses.

Bipolaris sivanesaniana is phylogenetically close to *B. oryzae* and *B. panici-milacei* (Fig. 1). Morphologically, *Bipolaris sivanesaniana* has shorter conidia (60–86 μm) than *B. oryzae* (63–153 μm), and fewer septa (up to 8 versus 14). *Bipolaris sivanesaniana* has longer conidiophores than *B. panici-milacei* (up to 255 μm long). *Bipolaris sivanesaniana* differs from *B. oryzae* in two loci: ITS position 97 (C); *TEF1α* position 381 (C). *Bipolaris sivanesaniana* differs from *B. panici-milacei* in three loci: ITS position 97 (C); *GAPDH* position 182 (A); *TEF1α* position 342 (C).

Bipolaris subramanianii Y.P. Tan & R.G. Shivas, sp. nov. (Fig. 2g–i)

MycoBank MB 817469

Etymology: Named after Professor C.V. Subramanian, in recognition of his contributions to mycology and plant pathology, especially his widely referenced monograph on hyphomycetes (Subramanian 1983).

Holotype: Australia, Queensland, Maclean Bridge, from leaf spot on *Setaria sphacelata*, 17 Mar. 1988, J.L. Alcorn, BRIP 16226 (includes ex-type culture).

Leaf spots on *Setaria sphacelata*, narrowly ellipsoidal, grey spots with brown margins, at first 1 × 0.5 mm, then expanding up to 5 cm in length with water-soaked appearance. *Conidiophores* mononematous, erect, straight to flexuous, never branched, uniformly brown to pale brown at the apex, smooth, septate, up to 830 μm × 5 μm; basal cell swollen and darker than the other cells, up to 13 μm diam. *Conidiogenous nodes* distinct and slightly swollen. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic with undarkened circular scars. *Conidia* straight to fusiform to subcylindrical, (70–) 90–99 (–130) × (9–) 11–12.5 (–15) μm, uniformly pale brown to subhyaline, smooth, 5–8-distoseptate, apex rounded, base obconically truncate. *Hilum* distinct and protuberant.

Notes: *Bipolaris subramanianii* is only known from the type specimen on *Setaria sphacelata*, which is a perennial African grass that has a worldwide distribution (Simon and Alfonso 2011). Other species recorded on *S. sphaecelata* are *B. cynodontis*, *B. maydis* and *B. zeicola* (DAF Biological Collections 2016; Farr and Rossman 2016; Herbarium Catalogue 2016); however, some of these records require verification by molecular methods. *Bipolaris subramanianii* has longer conidiophores (up to 830 μm) than *B. cynodontis* (up to 170 μm) and *B. zeicola* (up to 250 μm). *Bipolaris subramanianii* has longer conidia (70–130 μm) than *B. cynodontis* (30–75 μm). The conidia of *B. subramanianii* are typically straight to subcylindrical, whereas *B. cynodontis* and *B. zeicola* have slightly curved conidia that are broadest in the middle and taper towards the rounded ends. *Bipolaris maydis* has conidia that are distinctly curved.

Bipolaris subramanianii is phylogenetically close to *B. shoemakeri* and *B. secalis* (Fig. 1). The conidiophores of *B. subramanianii* are shorter than *B. shoemakeri* (up to 1.8 mm), but longer than *B. secalis* (up to 300 μm). The typically straight conidia of *B. subramanianii* are slightly longer and thinner than the slightly curved conidia of *B. shoemakeri* (70–80 × 13.5–15 μm). The conidia of *B. subramanianii* are uniformly paler in colour and have fewer septa than the conidia of *B. secalis*, which are mostly 10-distoseptate. *Bipolaris subramanianii* differs from *B. shoemakeri* by three loci: ITS 98 % match (Identities 452/461, Gaps 3/461); *GAPDH* positions 26 (T), 55 (T), 77 (G) and 209 (C); *TEF1α* positions 30 (G), 255 (C), 266 (G), 450 (A), 816 (C) and 843 (C). *Bipolaris subramanianii* differs from *B. secalis* by three loci: ITS 98 % match (Identities 453/460, Gaps 2/460); *GAPDH* positions 26 (T), 55 (T), 77 (G) and 446 (T); *TEF1α* positions 30 (G), 255 (C), 266 (G), 450 (A), 453 (C) and 843 (C).

Bipolaris woodii Y.P. Tan & R.G. Shivas, sp. nov. (Fig. 2j)

MycoBank MB 817470

Etymology: Named after Dr. Peter Wood, in recognition of his mentorship of microbiologists at the Queensland University of Technology, including the lead author.

Holotype: Australia, Queensland, Goondiwindi, from *Paspalidium caespitosum* C.E. Hubb., 25 Apr. 1977, J. Brouwer, BRIP 12239 (includes ex-type culture).

Conidiophores mononematous, erect, straight to flexuous, rarely branched, geniculate towards the apex, uniformly pale brown to brown, smooth, septate, up to 250 μm × 5–10 μm. *Conidiogenous cells* integrated, terminal or intercalary, with sympodial proliferation, pale brown to brown, smooth, mono- or polytretic with darkened circular scars. *Conidiogenous nodes* distinct, darkened and verruculose. *Conidia* fusiform, straight to slightly curved, (60–) 69–76 (–86) × (10–) 12.5–13.5 (–15) μm, brown, smooth, 7–10-distoseptate. *Hilum* darkened and sometimes thickened.

Notes: *Bipolaris woodii* is only known from a single specimen on *Paspalidium caespitosum*. This grass is a native species widely distributed across inland regions of eastern Australia (Simon and Alfonso 2011). Two other species recorded on *Paspalidium* are *B. setariae* on *P. flavidum* (Farr and Rossman 2016) and *B. sivanesaniana* described in this study from *P. distans*. *Bipolaris woodii* has shorter conidiophores (up to 250 μm) than *B. sivanesaniana* (up to 600 μm). Molecular phylogenetic comparison with *B. setariae* cannot be reliably made at this point in time as there are no available sequences for an ex-type or authentic strain of *B. setariae*.

Bipolaris woodii is phylogenetically close to *B. microstegii*, *B. victoriae* and *B. zeicola* (Fig. 1). *Bipolaris woodii* differs from *B. microstegii* in three loci: ITS 98 % match (Identities 452/461, Gap 4/461); *GAPDH* positions 83 (T), 111 (T) and 383 (T); *TEF1α* positions 138 (T), 265 (T) and 572 (C). *Bipolaris woodii* also differs in morphology, with shorter conidiophores than *B. microstegii* (up to 750 μm). *Bipolaris woodii* differs from *B. victoriae* in three loci: ITS 98 % match (Identities 454/461, Gaps 4/461); *GAPDH* positions 83 (T), 98 (T), 111 (T) and 383 (T); *TEF1α* positions 333 (C) and 573 (C). *Bipolaris woodii* has slightly smaller and darker conidia than *B. victoriae* (40–120 × 12–19 μm) (Sivanesan 1987). *Bipolaris woodii* differs from *B. zeicola* in three loci: ITS 98 % match (Identities 452/462, Gaps 5/462); *GAPDH* positions 83 (T), 111 (T), 383 (T) and 425 (C); *TEF1α* position 573 (C). *Bipolaris woodii* has a darkened and conspicuous hilum, and, thereby, differs from *B. zeicola*, which has an inconspicuous hilum.

Discussion

Phylogenetic analyses based on ITS and *GAPDH* sequences, either individually or concatenated, provided sufficient resolution for delimiting taxa within *Bipolaris* (Berbee et al. 1999; Manamgoda et al. 2012, 2014; Tan et al. 2014). Further, a four-locus dataset (ITS, *GAPDH*, LSU and *TEF1α*) provided stronger support for the description of new helminthosporioid species (Manamgoda et al. 2012; Tan et al. 2014). In this study, 13 isolates from the BRIP collection, recognised by Dr. John L. Alcorn as taxonomically interesting and potentially distinct, were analysed against reference sequences of cultures available from currently accepted *Bipolaris* species based on three loci, ITS, *GAPDH* and *TEF1α*. Analyses with LSU were omitted in the dataset as they provided little information to warrant inclusion. Nonetheless, LSU sequences have been deposited in GenBank to facilitate future studies (Table 1). The phylogenetic analyses of the combined three-locus dataset resolved the 13 BRIP isolates into eight novel *Bipolaris* species. It is not known whether the species are pathogens, endophytes or saprobes. The description of these species provides a foundation upon which additional sampling and accumulation of molecular data will improve knowledge of their host ranges and ecological roles.

The ITS locus is the universal barcode marker for fungi (Schoch et al. 2012). The ITS alignment used in this study was able to resolve 19 out of 36 *Bipolaris* species, including four of the new species. However, some studies have used only ITS to identify and describe *Bipolaris* species (Ahmadpour et al. 2012; da Cunha et al. 2012). Most recently, taxonomists have accepted that a secondary locus is essential for the accurate identification of many taxa (Madrid et al. 2014; Manamgoda et al. 2012, 2015; Tan et al. 2014; Stielow et al. 2015). The protein-coding loci of *GAPDH*, *TEF1α* and RNA polymerase II second largest subunit (*RPB2*) have been reported to be phylogenetically informative in the analyses of helminthosporioid species, and complement species identification and classification studies (Crous et al. 2012, 2013; Madrid et al. 2014; Manamgoda et al. 2012, 2014, 2015; Tan et al. 2014). The *GAPDH* and *TEF1α* alignments used in this study were able to resolve 34 out of 36 *Bipolaris* species, including the eight new species described here. None of the ITS, *GAPDH* or the *TEF1α* alignments were able to differentiate between the ex-holotype strain of *B. coffeana* and the recently designated ex-epitype strain of *B. cynodontis* (Manamgoda et al. 2014). A comparison of the sequences of *B. coffeana* and *B. cynodontis* indicates a potential synonymy, which is supported by shared conidial characteristics. *GAPDH* and *TEF1α* were determined to be the most suitable single locus marker for species-level identification within *Bipolaris*. Madrid et al. (2014) found *RPB2*, followed by *GAPDH*, to be the most informative loci for helminthosporioid phylogeny. Analyses with *RPB2* could not be included in this study as sequences were only available for ex-type isolates of three *Bipolaris* species. It is strongly suggested that the classification of new taxa in *Bipolaris* be accompanied by the official fungal barcode, ITS and a secondary locus, *GAPDH*, *TEF1α* or *RPB2*.

Acknowledgements The authors wish to acknowledge Dr. John L. Alcorn (former curator of the BRIP) for his support, as well as his foresight in preserving all the cultures examined in this study.

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