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RESEARCH ARTICLE

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Reappraisal of the genus *Alternariaster* (*Dothideomycetes*)

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Key words

Alternaria
fungal pathogens
host-range
multi-gene phylogeny

Abstract *Alternariaster* was erected in 2007 to accommodate *Alternaria helianthi*, a fungal species known to cause leaf spots on *Helianthus annuus* (sunflower). It was segregated from *Alternaria* based on conidial morphology. Recently an unknown alternaria-like dematiaceous fungus was found associated with leaf spots on *Bidens sulphurea* (yellow cosmos) in Brazil. Based on a multi-gene phylogeny of parts of the ITS and LSU genes, this fungus was placed within the *Leptosphaeriaceae* with *Alternariaster helianthi* as its closest neighbour. Additional genes sequenced, RPB2 and GAPDH, confirmed this close relationship. The fungus on *B. sulphurea* has smaller conidia, 50–97.5 × 12.5–20 µm, compared to *Al. helianthi,* 80–160 × 18–30 µm, and lacks oblique or transverse septa which can be present in *Al. helianthi*. Pathogenicity studies on 18 plant species belonging to the *Compositae* showed that the *B. sulphurea* fungus only infected *B. sulphurea,* whereas *Al. helianthi* infected *H. annuus* and *Galinsoga quadriradiata,* a yet unreported host of *Al. helianthi*. The fungus causing disease on *B. sulphurea* is hence closely related but phylogenetically, morphologically and pathologically distinct from *Al. helianthi,* and therefore newly described as *Alternariaster bidentis.* The collection of a second species in the genus *Alternariaster* and the multigene phylogenetic analysis of these two species, confirmed *Alternariaster* to be a well-delimited genus in the *Leptosphaeriaceae* rather than the *Pleosporaceae*, to which *Alternaria* belongs.

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INTRODUCTION

The fungal genus *Alternariaster* was established by Simmons (2007) to accommodate *Alternaria helianthi*, a species known to cause leaf spots on *Helianthus annuus* (sunflower) worldwide (Alcorn & Pont 1972, Ribeiro et al. 1974, Leite et al. 2007). This monotypic genus was segregated from *Alternaria* based on several morphological differences. Conidia of *Alternariaster* are not formed in chains, are cylindrical, ellipsoid or broadly ovoid, subhyaline to greyish brown, and only rarely form longitudinal or oblique septa. A fungus bearing significant morphological similarity to *Alternariaster helianti* was found on *Bidens sulphurea* in Brazil during studies of the pathogenic mycobiota of ornamentals.

Bidens sulphurea (*Asteraceae*) (common name yellow cosmos; in Brazil, cosmos-amarelo, aster-do-méxico and others), is a plant that is both regarded as a minor ornamental and as a weed, and appears in Brazil on published lists of ornamentals (Lorenzi & Souza 2001) and weeds (Kissman & Groth 1999, Lorenzi 2000). It is an annual herb, native to Mexico, which produces abundant showy yellow or orange flowers, and was probably introduced to Brazil as an ornamental, but became naturalised and invades rural areas, pastures and vegetable gardens. In 2004, a population of *B. sulphurea* was observed in the locality of Cristais in Viçosa (state of Minas Gerais, Brazil) in a garden and a nearby pasture bearing leaf spots, which eventually led to extensive blight and premature plant death. Only one published record of a fungal disease attacking *B. sulphurea* is known from Brazil, namely grey mold caused by *Botrytis cinerea* (Guatimosin et al. 2011). The leaf spot disease observed on *B. sulphurea* in 2004 was clearly dissimilar from grey mold. Samples were collected and examined on several occasions, and an alternaria-like dematiaceous hyphomycete was found to be associated with the disease. Elucidating the identity of this fungus was of relevance for the clarification of the etiology of the disease, and for the potential use of the fungus as a biocontrol agent of *B. sulphurea*. This contribution includes a description of a new fungal species as well as observations on its phylogenetic relationships and host range, together with a reappraisal of the genus *Alternariaster*.

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MATERIALS AND METHODS

Samples and isolates

Representative samples of diseased specimens of *Bidens sulphurea* and *Helianthus annuus* were collected, dried in a plant press and deposited in the herbarium of the Universidade Federal de Viçosa (VIC). The fungi associated to the leaf spots on *B. sulphurea* and *H. annuus* were isolated in pure culture by direct transfer of spores onto plates containing vegetable broth-agar (VBA; Pereira et al. 2003) with a sterile fine pointed needle. Representative isolates of the fungi were deposited in the culture collection of the Universidade Federal de Viçosa (COAD) Brazil, and the CBS-KNAW Fungal Biodiversity Centre (CBS) the Netherlands (Table 1). The three *Alternariaster helianthi* strains present at the CBS, including the ex-type strain CBS 119672, were added to the study.

Phylogeny

For DNA extraction pure cultures of the respective taxa were grown on potato-carrot agar (PCA; Crous et al. 2009) for 7 d at 25 °C. Total genomic DNA of the isolates mentioned in Table 1 was extracted using an Ultraclean microbial DNA isolation kit (Mobio Laboratories, Carlsbad, CA, USA) according to the manufacturer's instructions. The primers V9G (de Hoog &

Table 1 Isolates used in this study and GenBank accession numbers for sequences. **Bold** accession numbers were generated in this study.

Species name	CBS no.[1]	Other no.[1]	Host, substrate	Country	GenBank accession no.			
					ITS	LSU	RPB2	GAPDH
Alternariaster bidentis sp. nov.	CBS 134021	VIC 31814; COAD 364	*Bidens sulphurea*	Brazil	**KC609333**	**KC609341**	**KC609347**	**KC609325**
	CBS 134185	VIC 31881; COAD 1191	*Bidens sulphurea*	Brazil	**KC609334**	**KC609342**	**KC609348**	**KC609326**
Alternariaster helianthi	CBS 327.69	IFO 9089	*Helianthus annuus*	Unknown	**KC609335**	KC584369	KC584494	**KC609327**
	CBS 199.86		*Helianthus annuus*	Hungary	**KC609336**	**KC609343**	**KC609349**	**KC609328**
	CBS 119672	EGS 36.007	*Helianthus* sp.	USA	**KC609337**	KC584368	KC584493	**KC609329**
	CBS 134018	VIC 31838; COAD 1190	*Helianthus annuus*	Brazil	**KC609338**	**KC609344**	**KC609350**	**KC609330**
	CBS 134019	VIC 31926; COAD 1188	*Helianthus annuus*	Brazil	**KC609339**	**KC609345**	**KC609351**	**KC609331**
	CBS 134020	VIC 31927; COAD 1187	*Helianthus annuus*	Brazil	**KC609340**	**KC609346**	**KC609352**	**KC609332**
Coniothyrium carteri	CBS 105.91		*Quercus robur*	Germany	JF740181	GQ387594		
Coniothyrium dolichi	CBS 124140	IMI 217262	*Dolichos biforus*	India	JF740183	GQ387611		
Coniothyrium glycines	CBS 124141		*Glycine max*	Zimbabwe	JF740185	GQ387598		
Coniothyrium multiporum	CBS 353.65	IMI 113689; ATCC 16207	Saline soil	India	JF740187	JF740268		
Coniothyrium palmarum	CBS 400.71		*Chamaerops humilis*	Italy	AY720708	EU754153		
Coniothyrium telephii	CBS 188.71		Air	Finland	JF740188	GQ387599		
	CBS 101636	PD 86/1186	*Glycine max*	Zimbabwe	JF740190	GQ387601		
Cucurbitaria berberidis	CBS 363.93		*Berberis vulgaris*	Netherlands	JF740191	GQ387606		
Heterospora chenopodii	CBS 448.68		*Chenopodium album*	Netherlands	FJ427023	EU754187		
Heterospora dimorphospora	CBS 165.78	PD 77/884	*Chenopodium quinoa*	Peru	JF740204	JF740281		
Leptosphaeria conoidea	CBS 616.75	IMI 199777; ATCC 32813; PD 74/56	*Lunaria annua*	Netherlands	JF740201	JF740279		
Leptosphaeria doliolum	CBS 541.66	PD 66/221	*Rudbeckia* sp.	Netherlands	JF740206	JF740284		
Leptosphaeria errabunda	CBS 617.75	IMI 199775; ATCC 32814; PD 74/201	*Solidago* sp.	Netherlands	JF740216	JF740289		
Leptosphaeria etheridgei	CBS 125980	DAOM 216539; PD 95/1483	*Populus tremuloides*	Canada	JF740221	JF740291		
Leptosphaeria macrocapsa	CBS 640.93	PD 78/139	*Mercurialis perennis*	Netherlands	JF740237	JF740304		
Leptosphaeria pedicularis	CBS 390.80	PD 77/711	*Pedicularis* sp.	Switzerland	JF740224	JF740294		
Leptosphaeria rubefaciens	CBS 223.77		*Quercus* sp.	Switzerland	JF740243	JF740312		
Leptosphaeria scleroitoides	CBS 144.84	CECT 20025; PD 82/1061	*Medicago sativa*	Canada	JF740192	JF740269		
Leptosphaeria slovacica	CBS 389.80	PD 79/171	*Balota nigra*	Netherlands	JF740247	JF740315		
Leptosphaeria sydowii	CBS 385.80	PD 74/477	*Senecio jacobaea*	UK	JF740244	JF740313		
Leptosphaeria veronicae	CBS 145.84	CECT 20059; PD 78/273	*Veronica chamaedryoides*	Netherlands	JF740254	JF740320		
Paraleptosphaeria dryadis	CBS 643.86		*Dryas octopetala*	Switzerland	JF740213	GU301828		
Paraleptosphaeria macrospora	CBS 114198	UPSC 2686	*Rumex domesticus*	Norway	JF740238	JF740305		
Paraleptosphaeria nitschkei	CBS 306.51		*Cirsium spinosissimum*	Switzerland	JF740239	JF740308		
Paraleptosphaeria orobanches	CBS 101638	PD 97/12070	*Epifagus virginiana*	USA	JF740230	JF740299		
Paraleptosphaeria praetermissa	CBS 114591		*Rubus idaeus*	Sweden	JF740241	JF740310		
Phoma herbarum	CBS 615.75		*Rosa multiflora*	Netherlands	FJ427022	EU754186		
Plenodomus agnitus	CBS 121.89	PD 82/903	*Eupatorium* sp.	Netherlands	JF740194	JF740271		
Plenodomus biglobosus	CBS 119951		*Brassica rapa*	Netherlands	JF740198	JF740274		
Plenodomus chrysanthemi	CBS 539.63		*Chrysanthemum* sp.	Greece	JF740253	GU238151		
Plenodomus collinsoniae	CBS 120227	JCM 13073; MAFF 239583	*Vitis coignetiae*	Japan	JF740200	JF740276		
Plenodomus confertus	CBS 375.64		*Anacyclus radiatus*	Spain	AF439459	JF740277		
Plenodomus congestus	CBS 244.64		*Erigeron canadensis*	Spain	AF439460	JF740278		
Plenodomus enteroleucus	CBS 142.84	CECT 20063; PD 81/654	*Catalpa bignonioides*	Netherlands	JF740214	JF740287		
Plenodomus fallaciosa	CBS 414.62	ETH 2961	*Satureja montana*	France	JF740222	JF740292		

Plenodomus hendersoniae	CBS 139.78		*Pyrus malus*	Netherlands	JF740226	JF740296
Plenodomus influorescens	CBS 143.84	CECT 20064; PD 78/883	*Fraxinus excelsior*	Netherlands	JF740228	JF740297
Plenodomus libanotidis	CBS 113795	UPSC 2219	*Seseli libanotis*	Sweden	JF740231	JF740300
Plenodomus lindquistii	CBS 381.67		*Helianthus annuus*	Canada	JF740233	JF740302
Plenodomus lingam	CBS 260.94	PD 78/989	*Brassica oleracea*	Netherlands	JF740235	JF740307
Plenodomus lupini	CBS 248.92	PD 79/141	*Lupinus mutabilis*	Peru	JF740236	JF740303
Plenodomus pimpinellae	CBS 101637	PD 92/41	*Pimpenella anisum*	Israel	JF740240	JF740309
Plenodomus tracheiphilus	CBS 551.93	PD 81/782	*Citrus limonia*	Israel	JF740249	JF740317
Plenodomus visci	CBS 122783	PD 74/1021	*Viscum album*	France	JF740256	EU754195
Plenodomus wasabiae	CBS 120119	FAU 559	*Eutrema wasabi*	Taiwan	JF740257	JF740323
Pyrenochaeta cava	CBS 257.68	IMI 331911	Wheat field soil	Germany	JF740260	EU754199
Pyrenochaeta nobilis	CBS 407.76		*Laurus nobilis*	Italy	EU930011	EU754206
Pyrenochaetopsis leptospora	CBS 101635	PD 71/1027	*Secale cereale*	Europe	JF740262	GQ387627
Pyrenochaetopsis pratorum	CBS 445.81	PD 80/1254	*Lolium perenne*	New Zealand	JF740263	GU23816
Subplenodomus apiicola	CBS 285.72		*Apium graveolens* var. *rapaceum*	Germany	JF740196	GU238040
Subplenodomus drobnjacensis	CBS 269.92	PD 88/896	*Eustoma exaltatum*	Netherlands	JF740211	JF740285
Subplenodomus valerianae	CBS 630.68	PD 68/141	*Valeriana phu*	Netherlands	JF740251	GU238150
Subplenodomus violicola	CBS 306.68		*Viola tricolor*	Netherlands	FJ427054	GU238156

[1] ATCC: American Type Culture Collection, Virginia, USA; CBS: Culture collection of the Centraalbureau voor Schimmelcultures, Fungal Biodiversity Centre, Utrecht, The Netherlands; CECT: Colección Española de Cultivos Tipo, Valencia University, Spain; COAD: Culture collection of the Universidade Federal de Viçosa, Brasil; DAOM: Canadian Collection of Fungal Cultures, Ottawa, Canada; EGS: Personal collection of Dr. E.G. Simmons; ETH: Swiss Federal Institute of Technology, Switzerland; FAU: Personal collection of Francis A. Uecker; IFO: Institute for Fermentation Culture Collection, Osaka, Japan; IMI: Culture collection of CABI Europe UK Centre, Egham, UK; JCM: Japan Collection of Microorganisms, Riken Biosource Center, Japan; MAFF: MAFF GenBank Project, Ministry of Agriculture, Forestry and Fisheries, Tsukuba, Japan; PD: Plant Protection Service, Wageningen, The Netherlands; UPSC: Uppsala University Culture Collection, Sweden; VIC: herbarium of the Universidade Federal de Viçosa, Brasil.

Gerrits van den Ende 1998) and ITS4 (White et al. 1990) were used to amplify the ITS region, and LSU1fd (Crous et al. 2009) and LR5 (Vilgalys & Hester 1990) for the LSU region. The PCR conditions were as follows: 1 µL DNA, 1× PCR buffer (Bioline GmbH, Luckenwalde, Germany), 40 µM of each dNTP, 0.2 µM of each primer, 0.25 units Taq polymerase (Bioline) and 1 mM (ITS) or 2 mM (LSU) $MgCl_2$ in a final volume of 12.5 µL. The amplification reactions were performed on a 2720 thermal cycler (Applied Biosystems, Foster City, CA, USA). The initial denaturation step of 94 °C for 5 min was followed by 35 cycles of 94 °C (30 s), 48 °C (30 s), and 72 °C (60 s) and a final elongation step of 72 °C (7 min). The amplicons were sequenced in both directions using the same PCR primers and the BigDye® Terminator v. 1.1 cycle sequencing kit (Applied Biosystems) according to the manufacturer's recommendations. The products were analysed on an ABI Prism 3730 XL DNA Sequencer (Applied Biosystems). A consensus sequence was computed from the forward and reverse sequences using the Bionumerics v. 4.61 software package and deposited in GenBank (Table 1). The consensus regions of ITS and LSU were blasted against the NCBI Nucleotide collection database using Megablast to identify their closest neighbours. Hit sequences were downloaded and aligned using the multiple sequence alignment program MAFFT v. 6.864b (http://mafft.cbrc.jp/alignment/server/index.html), and adjusted by eye where necessary. A Bayesian analysis was performed with MrBayes v. 3.2.1 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003) using a GTR model with gamma distributed rate variation for the single and concatenated gene regions. Further settings included a temperature value of 0.05, sample frequency of 100, for 5 M generations or when the average standard deviation of split frequencies dropped below 0.01. The 50 % majority rule consensus tree was calculated where the first 25 % of sampled trees were discarded as 'burn-in'. The program Tracer v. 1.5.0 (Rambaut & Drummond 2009) was used to ensure the convergence of the chains. Phylogenetic trees were visualised with Treeview v. 1.6.6 (Page 1996) and deposited in TreeBASE (www.treebase.org). The RPB2 and GAPDH sequences of the strains mentioned in Table 1 were also obtained and deposited in GenBank to confirm the close but distinct relationship of *Alternariaster helianthi* and the isolate from *Bidens sulphurea*.

Taxonomy

Morphological characterisation of the isolates was done using fungal structures scraped from freshly infected leaves, and mounted in lactophenol or lactofuchsin on microscope slides and observed with an Olympus BX 51 light microscope fitted with a drawing tube and a digital camera (Olympus E330). Colony characteristics were noted after 14 d of growth on VBA and PCA at 25 °C, under a 12 h light regime. Colony colours were determined using the colour charts of Rayner (1970). Nomenclatural data were deposited in MycoBank (Crous et al. 2004).

Pathogenicity studies

Fungal isolates were transferred to VBA plates and incubated for 14 d at 25 °C under a 12 h light regime; light provided by two 40 W day-light fluorescent lamps and one 40 W NUV black-light lamp, placed 40 cm above the plates. After fungal colonies colonised the plates, 10 mL of sterile water was added to each plate and the surface of the plates was scraped with a rubber spatula. The resulting conidial suspension was adjusted to a concentration of 2×10^4 conidia/mL with a haemocytometer. Twenty-day-old *Bidens sulphurea* plants, cultivated in individual pots, were sprayed until runoff with this conidial suspension. Each plant was covered with a transparent plastic bag wetted internally and left for 48 h with the base of the pots immersed

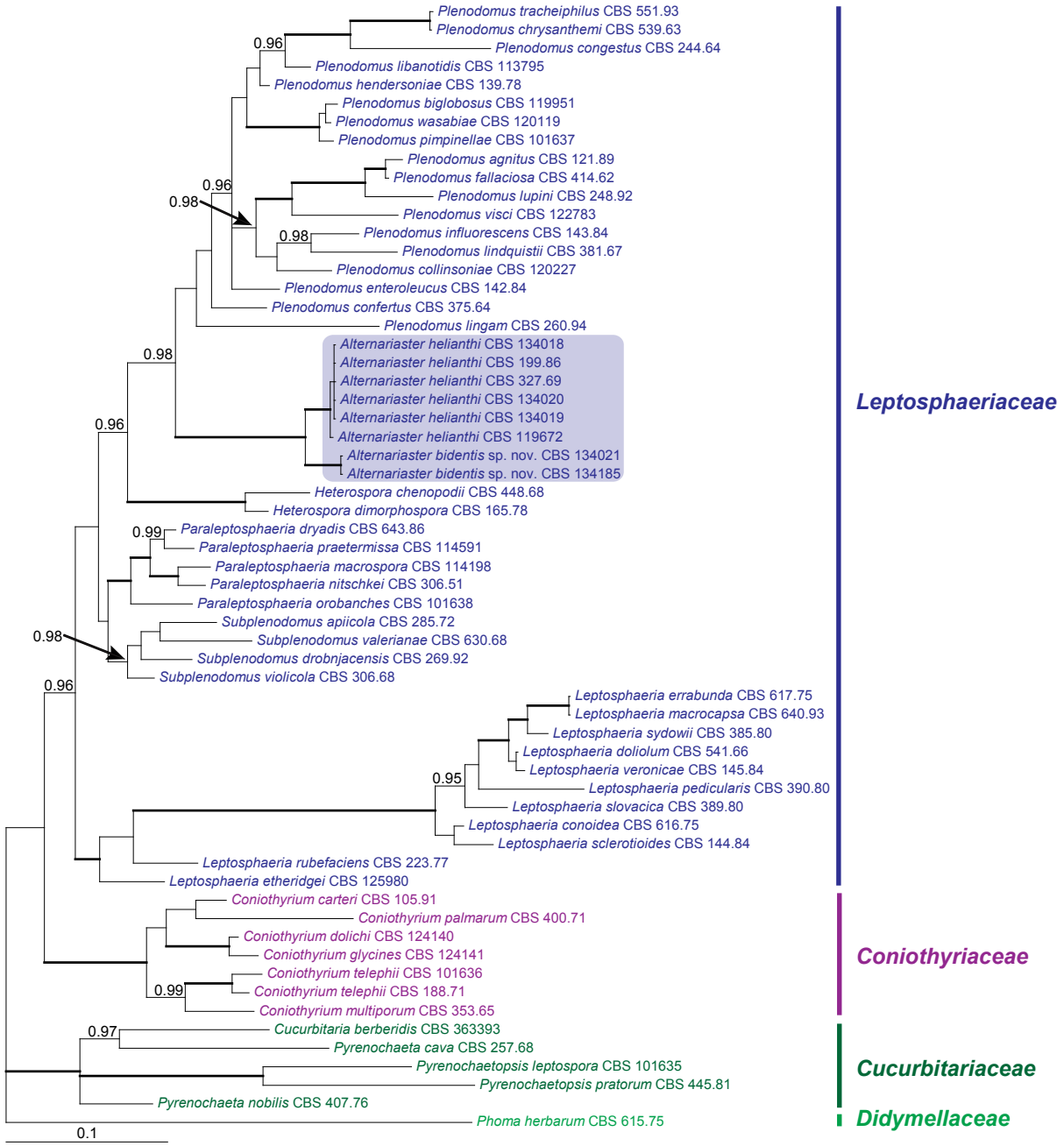

Fig. 1 Bayesian 50 % majority rule consensus tree based on the ITS and LSU sequences of 61 strains. The Bayesian posterior probabilities (PP) of 0.95 and above are given at the nodes. Thickened lines indicate a PP of 1.0. The tree was rooted using *Phoma herbarum* (CBS 615.75).

Table 2 Pathogenicity results of *Alternariaster bidentis* (CBS 134021) and *Al. helianthi* (CBS 134018) on 18 plants belonging to the *Asteraceae*.

Subfamily	Tribe	Species	*Al. bidentis*[1]	*Al. helianthi*[1]
Cichorioideae	*Cardueae*	*Cynara scolymus*	–	n
	Lactuceae	*Lactuca sativa*	–	n
		Sonchus oleraceus	–	–
		Vernonia polyanthes	–	n
	Mutisiae	*Gerbera jamesonii*	–	–
Asteroideae	*Astereae*	*Conyza canadensis*	–	–
	Anthemideae	*Crysantemum morifolium*	n	n
	Eupatorieae	*Mikania micrantha*	–	–
	Gnaphalieae	*Helichrysum italicum*	–	–
	Helenieae	*Tagetes minuta*	–	–
	Heliantheae	*Bidens subalternans*	–	–
		Bidens sulphurea	+	–
		Bidens pilosa	–	–
		Dalia pinnata	–	–
		Galinsoga quadriradiata	–	+
		Helianthus annuus	–	+
		Sphagneticola trilobata	–	–
		Zinnia elegans	–	–

[1] – = no symptoms; + = leaf spot symptoms; n = necrosis.

in water in a greenhouse where temperature varied between 25–30 °C. Two plants were sprayed with sterile water and served as controls. After the 2 d period in the humid chamber, the plants were transferred to a bench in a greenhouse and observed daily for the appearance of disease symptoms.

A pathogenicity test was performed by separately inoculating the two isolates (*B. sulphurea* isolate CBS 134021 and *Alternariaster helianthi* CBS 134018) in duplo on individuals belonging to 18 plant species representing two subfamilies and nine tribes of the *Asteraceae* (Table 2). Plants inoculated were 30–60-d-old and 30–40 cm high. Whenever disease symptoms appeared observations were made under a dissecting microscope for the appearance of fungal structures. If necrosis of tissues appeared but no fungal structures were observed on such necrotic tissues after repeated observations, then fragments of these seemingly diseased tissues were removed, surface sterilized with sodium hypochlorite and plated on VBA plates to allow for possible isolation of the fungus.

RESULTS

Phylogeny

The ITS and LSU consensus sequences obtained for the *B. sulphurea* isolates and *Alternariaster helianthi* isolates showed a high level of identity to *Plenodomus*, *Leptosphaeria* and *Paraleptosphaeria* isolates (*Leptosphaeriaceae*) present in the NCBI nucleotide database. The closest relatives of our isolates were delineated in a study by de Gruyter et al. (2012). The alignment of the latter study was therefore used to construct a phylogenetic tree (Fig. 1, Table 1). Isolates from four families were included, with *Phoma herbarum* (CBS 615.75, *Didymellaceae*) as outgroup. The final alignment consisted of 61 taxa and 1 425 characters (ITS 571, LSU 854), with 389 (ITS 288, LSU 101) unique site patterns. The Bayesian analysis resulted in 6 451 trees per run, from which the burn-in was discarded and the consensus tree and posterior probabilities were calculated on a total of 9 678 trees from two runs.

The eight *Alternariaster* isolates formed a well-supported clade (posterior probability of 1.0) between the genera *Plenodomus* and *Heterospora* within the *Leptosphaeriaceae*. The *Alternariaster* species formed two well-supported subclades within the *Alternariaster* clade. The RPB2 and GAPDH sequences showed 100 % identity within the species, and 97 % (881/908 nt) and 95 % (561/593 nt) identity between species, which confirmed *Al. helianthi* and *Al. bidentis* as distinct species within the genus.

Taxonomy

Alternariaster bidentis J.L. Alves & R.W. Barreto, *sp. nov.* — MycoBank MB800215; Fig. 2

Etymology. Name refers to its host genus, *Bidens*.

Sexual morph unknown. *Lesions* on living leaves starting as broad, punctiform depressions on leaf blades and veins, becoming subcircular, yellowish brown and greyish centrally, up to 1 mm diam, surrounded by a halo of dark green tissue with a somewhat soaked appearance followed by a faint, yellow outer circular area; on leaf veins lesions elliptical to elongate, pale brown to purple; at later stages lesions coalescing and becoming flecked, subcircular up to 15 mm diam, leading to leaf blight and premature plant death. *External mycelium* indistinct. *Internal mycelium* composed of branched, septate, pale brown to greyish brown hyphae, 1.5–2.0 µm diam. *Conidiophores* hypophyllous, solitary or in groups of up to three, straight to slightly sinuous, 147.5–320 × 10–12.5 µm, simple to occasionally branched, 3–6-septate, chestnut-brown at base, becoming yellowish brown at apex, smooth. *Conidiogenous cells* tretic, integrated, terminal to intercalary, sympodial, cylindrical, 25–165 × 10–15 µm; pale brown to yellowish. *Conidiogenous loci* conspicuous, 1–3 per cell, protuberant, up to 5 µm diam, thickened and darkened. *Conidia* dry, solitary, cylindrical or subcylindrical, 50–97.5 × 12.5–20 µm, apex and base obtusely rounded, 2–9 transversely septate (longitudinal or oblique septa absent), often deeply constricted at septa and larviform (in turgid freshly collected samples), eguttulate, subhyaline to greyish, smooth, hilum thickened and darkened, germinating both through apical and basal cells, occasionally also medially. Germ tubes oriented perpendicularly to the main axis of the conidium.

Culture characteristics — Relatively slow-growing (35–54 mm diam after 14 d), colony raised centrally, cottony, white, with dark grey or brown outer zone (where sporulation is concentrated) and having a wide periphery of flat, sparse, greyish to brown mycelium, followed by an irregular dark grey rim. *Spermogonia* produced either with or without exposure to light, pycnidial, subglobose, 55–90 × 50–80 µm, walls of thick *textura angularis*. *Spermatia* subcylindrical, 6–12 × 1–2 µm, hyaline, smooth, germination not observed.

Specimens examined. Brazil, Minas Gerais, Viçosa, on living leaves of *Bidens sulphurea*, 21 Apr. 2004, *R.W. Barreto* (VIC 31814 – holotype, culture ex-type CBS 134021, COAD 364); Rio de Janeiro, Murineli, Duas Barras, on living leaves of *B. sulphurea*, 30 July 2011, *R.W. Barreto* (VIC 31883); Rio de Janeiro, Duas Barras, on living leaves of *B. sulphurea*, 4 Nov. 2011, *R.W. Barreto* (VIC 31884); Minas Gerais, Itabirito, São Gonçalo do Bação, on living leaves of *B. sulphurea*, 27 Jan. 2012, *E. Guatimosim* (CBS 134185, COAD 1191, VIC 31881); Minas Gerais, Itabirito, São Gonçalo do Bação, on living leaves of *B. sulphurea*, 7 Apr. 2012, *E. Guatimosim* (VIC 31882).

Alternariaster helianthi (Hansf.) E.G. Simmons, CBS Biodiversity Ser. (Utrecht) 6: 667. 2007. — MycoBank MB505050; Fig. 3

Basionym. *Helminthosporium helianthi* Hansf., Proc. Linn. Soc. London 49. 1943 (1942–1943).

= *Alternaria helianthi* (Hansf.) Tubaki & Nishih., Trans. Brit. Mycol. Soc. 53: 148. 1969.

Sexual morph unknown. *Lesions* on living leaves starting as dispersed punctiform spots, occurring throughout the leaf blade, becoming subcircular to irregular in shape, yellowish, 3–11 × 2–9 mm, surrounded by a halo of dark green tissue, at later stages lesions coalesce, resulting in leaf blight and premature plant death. *Conidiophores* hypophyllous, solitary or in small groups, straight to slightly sinuous, 100–225 × 7.5–10 µm, simple, 3–6-septate, pale to chestnut-brown, smooth. *Conidiogenous cells* tretic, integrated, terminal to intercalary, sympodial, cylindrical, 25–100 × 5–7.5 µm, yellowish to pale brown. *Conidiogenous loci* conspicuous, 1–2 per cell, protuberant, up to 5 µm diam, thickened and darkened. *Conidia* dry, solitary, cylindrical to subcylindrical, occasionally with cells of different size, 60–115 × 11–29 µm, apex and base rounded, transversally 5–9 septate (1–2 longitudinal or oblique septa), often deeply constricted at septa, eguttulate, subhyaline to pale brown, smooth, hilum thickened and darkened. Germ tubes orientated perpendicularly to the main axis of the conidium, and also polar.

Culture characteristics — On PCA and VBA, very slow-growing (8–11 mm diam after 14 d). On PCA colony raised centrally, aerial mycelium felted, white, having a wide periphery of flat, sparse, olivaceous-buff to greenish glaucous mycelium, with irregular margins. On VBA colonies of dense cottony to velvety aerial mycelium, grey-olivaceous alternating with smoke-grey zones. In reverse olivaceous-buff centrally, and olivaceous at the edges on PCA, and grey-olivaceous alternating with olivaceous-black zones on VBA. Sporulation abundant. Spermagonia not observed.

Fig. 2 *Alternariaster bidentis*. a. Flowering healthy plants of *Bidens sulphurea*; b. leaves with leaf spot and necrosis; c. extensive blight; d–h. conidia attached to conidiogenous cells; i. spermogonium on SNA. — Scale bars = 10 µm, except i = 100 µm.

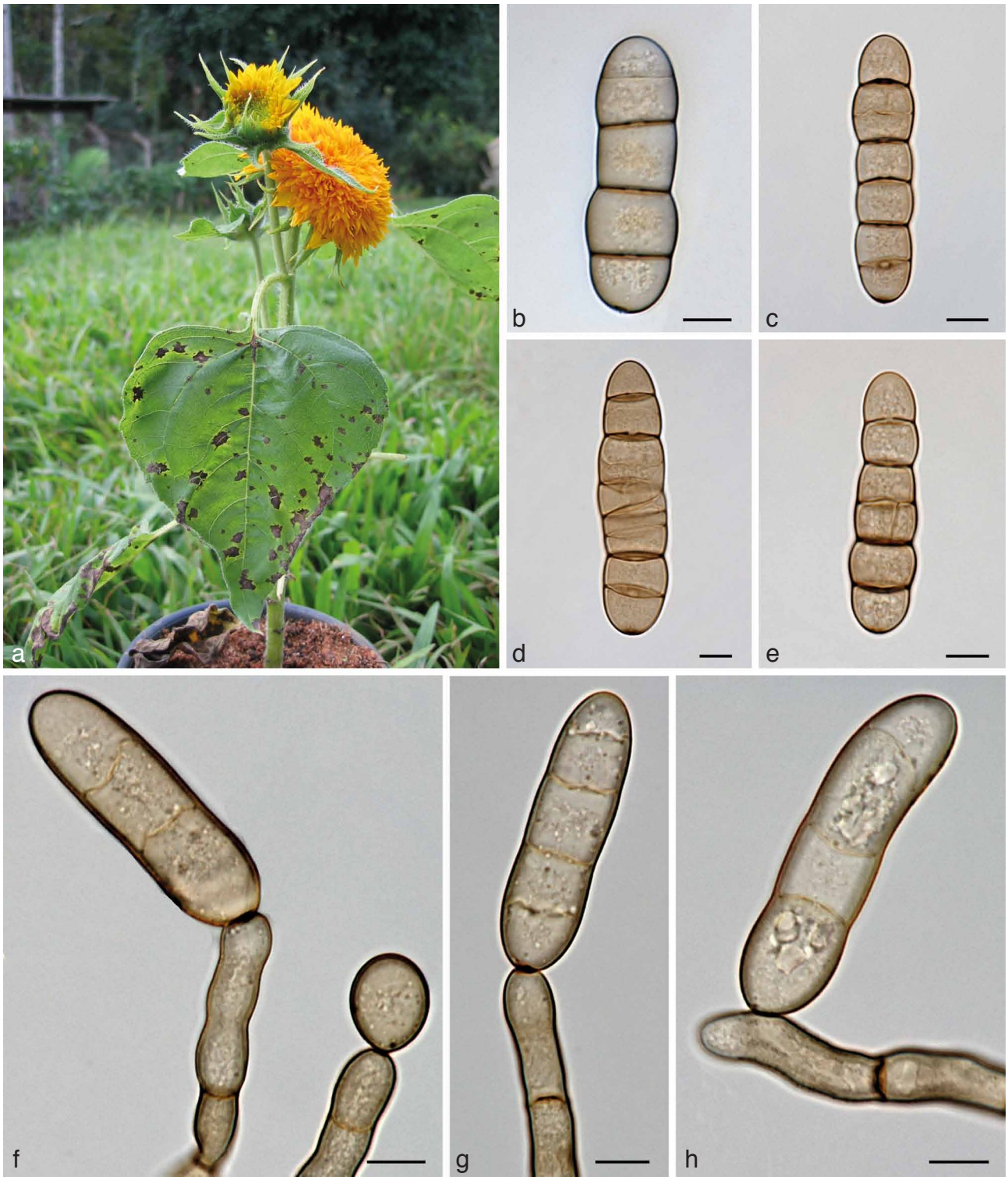

Fig. 3 *Alternariaster helianthi.* a. *Helianthus annuus* with leaf spot and necrosis; b–e. conidia; f–h. conidia attached to conidiogenous cells. — Scale bars = 10 µm.

Specimens examined. Brazil, Minas Gerais, Viçosa, on living leaves of *Helianthus annuus*, 30 May 2004 (COAD 302); Minas Gerais, Viçosa, on living leaves of *H. annuus*, 29 June 2010, *J.L. Alves* (CBS 134018, COAD 1190, VIC 31838); Minas Gerais, Belo Horizonte, on living leaves of *H. annuus*, 22 May 2012, *J.L. Alves* (CBS 134019, COAD 1188, VIC 31926); Minas Gerais, Viçosa, on living leaves of *H. annuus*, 25 May 2012, *J.L. Alves* (CBS 134020, COAD 1187, VIC 31927).

Pathogenicity studies

The *Al. bidentis* isolate (CBS 134021) produced leaf spots only on *B. sulphurea,* whereas *Al. helianthi* (CBS 134018) produced leaf spots on *H. annuus* and also on *Galinsoga quadriradiata* (Table 2). Leaf necrosis appeared on four other species inoculated with *Al. helianthi* and one species when inoculated with *Al. bidentis* (Table 2), but no sporulation was observed on such necrotic tissues, and no fungal colonies were obtained from fragments of such tissues when plated on culture media.

DISCUSSION

The genus *Alternariaster* was first described by Simmons (2007) with *Alternariaster helianthi* (formerly *Alternaria helianthi* and *Helminthosporium helianthi*) as type, and has hitherto been monotypic. The present phylogenetic analysis confirms Simmons's segregation of *Alternariaster* from *Alternaria*, by showing

Fig. 4 a, b. *Alternariaster bidentis* sp. nov. (CBS 134021) on *Bidens sulphurea*: a. Pathogenicity test evaluated at 14 d after inoculation (control left, inoculated right); b. detail of necrosis. — c. *Alternariaster helianthi* (CBS 134018) on *Bidens sulphurea,* no observed injury (control left, inoculated right). — d, e. *Alternariaster helianthi* (CBS 134018) on *H. annuus*: d. Pathogenicity test evaluated at 4 d after inoculation (control left, inoculated right); e. detail of necrosis. — f. *Alternariaster bidentis* sp. nov. (CBS 134021) on *H. annuus*, no observed injury (control left, inoculated right).

that *Alternariaster* is a well-delimited taxon belonging to the *Leptosphaeriaceae* (Fig. 1), instead of the *Pleosporaceae* to which *Alternaria* belongs (Schoch et al. 2009).

Initial attempts at identifying *Alternariaster bidentis* to the generic level based on morphological characters alone was challenging. Initially the fungus was regarded as a potential species of *Alternaria.* Nevertheless, as the fungus did not produce conidial chains, had conidia that appeared hyaline when young and when directly observed on leaves, were distinctly constricted at septa (having a larviform appearance) and were never found to have longitudinal or oblique septa. This combination of features suggested that it might be inadequately placed in *Alternaria*. However, the genus *Alternaria* contains some taxa noted for the absence of oblique and transverse septa, namely: *A. chrysanthemi, A. thalictrina, A. thalictricola,* and *A. thalictrigena* (Schubert et al. 2007). Additionally, significant changes in conidial morphology were also observed when the fungus was grown in culture, particularly in older cultures where conidia became chestnut-brown and the formation of distosepta was observed at times. These features suggested that the species might belong to one of the genera segregated from *Helminthosporium* (Alcorn 1988), particularly *Drechslera* or *Bipolaris*. Alcorn (1991) separated *Bipolaris*, *Drechslera* and *Exserohilum* based on conidial germination patterns, septum ontogeny and their associated sexual morphs. Ironically, while the authors were trying to unravel the puzzle of the fungus occurring on *Bidens sulphurea*, the monograph on the genus *Alternaria* was published (Simmons 2007). In this monograph the genus *Alternariaster* was erected to accommodate *Alternaria helianthi*, a fungal species known to cause a serious disease of sunflower worldwide (Alcorn & Pont 1972, Ribeiro et al. 1974, Leite et al. 2007). *Alternariaster* was segregated from *Alternaria* based on it being morphologically distinct by having cylindrical, ellipsoid or broad-ovoid in shape, subhyaline to greyish brown conidia not formed in chains and only rarely exhibiting longitudinal or oblique septa.

The morphology of *Al. bidentis* fits well into the concept proposed by Simmons for *Alternariaster*. However, this newly proposed species can be readily distinguished from *Al. helianthi* based on its conidial characters. *Alternariaster bidentis* has smaller conidia, 50–97.5 × 12.5–20 µm, compared to *Al. helianthi,* 80–160 × 18–30 µm, without oblique or transverse septa, which though rare, could occur in *Al. helianthi.* Additionally spermogonia and spermatia were formed in cultures of *Al. bidentis* (but not in cultures of *Al. helianthi*) and were described here for the first time. Inoculations with *Al. bidentis* only resulted in leaf spots equivalent to those observed in the field on plants of *B. sulphurea*. Although necrosis appeared on leaves of *Chrysanthemum morifolium*, spots were limited to places where inoculum was deposited, and did not progress, nor could the fungus be re-isolated from such necrotic tissues. Necrosis was likely to be caused by one or more toxins produced by the fungus for which chrysanthemum was sensitive but not the other test plants. No leaf spot or necrosis of any kind appeared on *Helianthus annuus* inoculated with *Al. bidentis* or on *B. sulphurea* inoculated with *Al. helianthi* (Fig. 4). This is regarded as a complementary indication that *Al. helianthi* and *Al. bidentis* are distinct taxa. Inoculations of *Al. helianthi* (CBS 134018) led to typical *Alternariaster* leaf spots on *H. annuus* and *Galinsoga quadriradiata* after 5 d. Conidiophores and conidia could be identified as *Al. helianthi* on leaf spots on these two hosts after 7 d. *Galinsoga quadriradiata* is a new host for *Al. helianthi. Alternariaster helianthi* was previously reported to only infect *H. annuus* and *Rudbeckia bicolor* (Black-Eyed Susan) (Cho & Shin 2004). Tissue necrosis was observed in *Cynara scolymus*, *Chrysanthemum morifolium*, *Lactuca sativa*

and *Vernonia polyanthes*. As in the case of the inoculation of *Al. bidentis* on *Chrysanthemum morifolium,* it is likely that such necroses were a result of susceptibility of those hosts to one or more toxins produced by *Al. helianthi.* The delineation of a new *Alternariaster* species based on molecular, morphological and pathogenicity tests led to a reappraisal of the genus, with the conclusion that *Alternariaster* is a well-delimited genus belonging to the *Leptosphaeriaceae,* rather than to the *Pleosporaceae*, to which *Alternaria* belongs. The finding of this new taxon also confirmed a fortunate choice of name for the genus by Simmons, as this is also a fungus morphologically similar to *Alternaria* attacking a member of the *Asteraceae*.

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