Reappraisal of the genus Alternariaster (Dothideomycetes)

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Key words
Alternaria
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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 Compositeae showed that the B. sulphurea fungus only infected B. sulphurea, whereas Al. helianthi infected H. annuus and G. linsoga 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 helianthi 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 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.

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. | Other no. | Host, substrate        | Country | ITS     | LSU     | RPB2    | GAPDH    |
|-------------------------------|---------|-----------|------------------------|---------|---------|---------|---------|----------|
| Alternaria aster bidentis sp. nov. | CBS 134021 | VIC 31814; COAD 384 | Bidens sulphurea         | Brazil  | KC609333 | KC609341 | KC609347 | KC609325 |
| Alternaria helianthi          | CBS 37269 | IFO 9089   | Helianthus annuus       | Unknown | KC609335 | KC584349 | KC584494 | KC609327 |
|                               | CBS 199.86 | EGS 36.007 | Helianthus annuus       | Hungary | KC609336 | KC609343 | KC609349 | KC609328 |
|                               | CBS 119672 | IMI 9089   | Helianthus annuus       | Brazil  | KC609337 | KC584348 | KC584493 | KC609329 |
|                               | CBS 134020 | VIC 31926; COAD 1188 | Helianthus annuus     | Brazil  | KC609338 | KC609344 | KC609350 | KC609330 |
|                               | CBS 134020 | VIC 31527; COAD 1187 | Helianthus annuus     | Brazil  | KC609339 | KC609345 | KC609351 | KC609331 |
| Conidiobolus sp.              | CBS 105.91 | Quercus robur | Conidiobolus sp.       | Germany | JF740181 | GQ387599 |         |          |
| Conidiobolus dolichicollis    | CBS 124140 | IMI 217262 | Dolichos biflorus      | India   | JF740183 | GQ387611 |         |          |
| Conidiobolus globosum         | CBS 124141 | IMI 217262 | Conidiobolus globosum  | Zimbabwe| JF740185 | GQ387598 |         |          |
| Conidiobolus multiformis      | CBS 353.65 | IMI 113689; ATCC 16207 | Conidiobolus multiformis | India   | JF740187 | JF740268 |         |          |
| Conidiobolus palmarum         | CBS 400.71 | IMI 113689; ATCC 16207 | Conidiobolus palmarum  | Italy   | AY720708 | EU754153 |         |          |
| Conidiobolus telephi           | CBS 188.71 | IMI 113689; ATCC 16207 | Conidiobolus telephi   | Finland | JF740188 | GQ387599 |         |          |
| Cucurbitula berberidis        | CBS 363.93 | IMI 113689; ATCC 16207 | Cucurbitula berberidis | Netherlands | JF740191 | GQ387606 |         |          |
| Heterospora chenopodi         | CBS 448.68 | IMI 113689; ATCC 16207 | Heterospora chenopodi | Netherlands | FA47023 | EU754187 |         |          |
| Heterospora dimorphospora      | CBS 165.78 | IMI 113689; ATCC 16207 | Heterospora dimorphospora | Peru | JF740204 | JF740281 |         |          |
| Leptosphaeria conoidea         | CBS 616.75 | IMI 113689; ATCC 16207 | Leptosphaeria conoidea | Netherlands | JF740201 | JF740279 |         |          |
| Leptosphaeria doliolum         | CBS 541.66 | IMI 113689; ATCC 16207 | Leptosphaeria doliolum | Netherlands | JF740206 | JF740284 |         |          |
| Leptosphaeria errabunda        | CBS 617.75 | IMI 113689; ATCC 16207 | Leptosphaeria errabunda | Netherlands | JF740216 | JF740289 |         |          |
| Leptosphaeria eugeniae         | CBS 125980 | DAOM 216539; PD 95/1483 | Leptosphaeria eugeniae | Canada | JF740221 | JF740291 |         |          |
| Leptosphaeria macrocapita      | CBS 640.93 | IMI 113689; ATCC 16207 | Leptosphaeria macrocapita | Netherlands | JF740237 | JF740304 |         |          |
| Leptosphaeria pedicularis      | CBS 390.80 | PD 86/1186 | Leptosphaeria pedicularis | Switzerland | JF740224 | JF740294 |         |          |
| Leptosphaeria rubefaciens      | CBS 223.77 | PD 86/1186 | Leptosphaeria rubefaciens | Switzerland | JF740243 | JF740312 |         |          |
| Leptosphaeria sclerotiorum     | CBS 144.84 | CECT 20025; PD 82/1061 | Leptosphaeria sclerotiorum | Canada | JF740182 | JF740289 |         |          |
| Leptosphaeria solatrica        | CBS 389.80 | PD 79/171 | Leptosphaeria solatrica | Switzerland | JF740204 | JF740281 |         |          |
| Leptosphaeria rubidae          | CBS 385.80 | PD 74/171 | Leptosphaeria rubidae | Netherlands | JF740204 | JF740281 |         |          |
| Leptosphaeria velutinae        | CBS 145.84 | CECT 20059; PD 78/273 | Leptosphaeria velutinae | Netherlands | JF740254 | JF740320 |         |          |
| Pauleptosphaeria dryadicola    | CBS 643.86 | PD 86/1186 | Pauleptosphaeria dryadicola | Switzerland | JF740213 | GU018283 |         |          |
| Pauleptosphaeria macrospora    | CBS 114198 | PD 86/1186 | Pauleptosphaeria macrospora | Switzerland | JF740239 | JF740308 |         |          |
| Pauleptosphaeria rutschieki    | CBS 306.51 | IMI 113689; ATCC 16207 | Pauleptosphaeria rutschieki | Switzerland | JF740239 | JF740308 |         |          |
| Pauleptosphaeria orobancha     | CBS 101638 | PD 86/1186 | Pauleptosphaeria orobancha | Switzerland | JF740239 | JF740308 |         |          |
| Pauleptosphaeria praetemissula | CBS 114591 | PD 86/1186 | Pauleptosphaeria praetemissula | Switzerland | JF740239 | JF740308 |         |          |
| Phoma herbarum                | CBS 615.75 | PD 86/1186 | Phoma herbarum          | Portugal | JF740239 | JF740308 |         |          |
| Plenodomus agnitus             | CBS 121.89 | PD 82/903 | Plenodomus agnitus       | Portugal | JF740239 | JF740308 |         |          |
| Plenodomus biglobosus          | CBS 119051 | PD 82/903 | Plenodomus biglobosus    | Portugal | JF740239 | JF740308 |         |          |
| Plenodomus chrysanthemi        | CBS 639.63 | PD 82/903 | Plenodomus chrysanthemi  | Portugal | JF740239 | JF740308 |         |          |
| Plenodomus collinsoniae        | CBS 120227 | JCM 13073; MAFF 239583 | Plenodomus collinsoniae | Portugal | JF740239 | JF740308 |         |          |
| Plenodomus confluens           | CBS 375.64 | PD 82/903 | Plenodomus confluens     | Portugal | JF740239 | JF740308 |         |          |
| Plenodomus congostus           | CBS 244.64 | PD 82/903 | Plenodomus congostus     | Portugal | JF740239 | JF740308 |         |          |
| Plenodomus enterolocus         | CBS 142.84 | PD 82/903 | Plenodomus enterolocus   | Portugal | JF740239 | JF740308 |         |          |
| Plenodomus fallacioides        | CBS 414.62 | PD 82/903 | Plenodomus fallacioides  | Portugal | JF740239 | JF740308 |         |          |
| Species                        | Host                  | Collection Details                                      |
|-------------------------------|-----------------------|--------------------------------------------------------|
| *Plenodomus hendersoniae*     | *Pyrus malus*         | CBS 143.84, CECT 20064, PD 78/883                      |
| *Plenodomus influorescens*    | *Fraxinus excelsior*   | Netherlands JF740228, JF740297                         |
| *Plenodomus libanotidis*      | *Seseli libanotis*    | Sweden JF740231, JF740300                             |
| *Plenodomus lingam*           | *Brassica oleracea*   | Netherlands JF740235, JF740307                         |
| *Plenodomus lupini*           | *Lupinus mutabilis*   | Canada JF740233, JF740302                             |
| *Plenodomus pimpinellae*      | *Citrus limonia*      | Israel JF740249, Israel JF740250                      |
| *Plenodomus wasabiae*         | *Eutrema wasabi*      | Taiwan JF740257, JF740258                             |
| *Pyrenochaeta cava*           |                      | Germany JF740261, JF740262                            |
| *Pyrenochaeta nobilis*        | *Laurus nobilis*      | Italy EU930011, EU754206                              |
| *Pyrenochaetopsis leptospora* | *Secale cereale*      | Europe JF740262, GQ387627                             |
| *Pyrenochaetopsis pratorum*   | *Lolium perenne*      | New Zealand JF740263, GU23816                          |
| *Subplenodomus drobnjacensis* | *Eustoma exaltatum*   | Netherlands JF740211, JF740285                         |
| *Subplenodomus violicola*     | *Viola tricolor*      | Netherlands FJ427054, GU238156                        |

**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).

**Pathogenicity studies**

Fungal isolates were transferred to VBA plates and incubated for 14 d at 25 °C under a 12 h light regime. 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).
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                   |
|           | Mutisieae   | Gerbera jamesonii   | –                  | –                   |
| Astroideae | Astereae    | Coryza canadensis    | –                  | –                   |
|           | Anthemideae | Crysantemum morifolium | n                  | n                   |
|           | Eupatorieae | Mikania micrantha   | –                  | –                   |
|           | Gnaphalieae | Helichrysum italicum | –                  | –                   |
|           | Heleniumeae | Tagetes minuta      | –                  | –                   |
|           | Heliantheae | Bidens subalternans  | –                  | –                   |
|           |             | Bidens sulphurea     | +                  | –                   |
|           |             | Bidens pliosa        | –                  | –                   |
|           |             | Dalla pinnata        | –                  | –                   |
|           |             | Galinsoga quadriradiata | –            | +                   |
|           |             | Helianthus annuus    | –                  | –                   |
|           |             | Sphagnicola 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 Asterales (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 and Alternariaster helianthi isolates showed a high level of identity to Plenodomus, Leptosphaeria and Para- leptosphaeria 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 1425 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 RP2B 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. Conidigenous cells tretic, integrated, terminal to intercalary, sympodial, cylindrical, 25–165 × 10–15 µm; pale brown to yellowish. Conidigenous 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 medi ally. 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. Spermagonia produced either with or without exposure to light, pycnidial, subglobose, 55–90 × 50–80 µm, walls of thick textura angularis. Spermatacia subcylindrical, 6–12 × 1–2 µm, hyaline, smooth, germination not observed.

Specimens examined. BRAZIL, Minas Gerais, Vícosa, 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 (CBS 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 heli a n th i (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. Conidigenous cells tretic, integrated, terminal to intercalary, sympodial, cylindrical, 25–100 × 5–7.5 µm, yellowish to pale brown. Conidigenous 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.
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
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

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).
and Vernonia polyanthes. As in the case of the inoculation of Alternaria helianthi on Chrysanthemum morifolium, it is likely that such necroses were a result of susceptibility of those hosts to one or more toxins produced by Alternaria helianthi. The delineation of a new Alternariaster species based on molecular, morphological and pathogenity 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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