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Seiridium (*Sporocadaceae*): an important genus of plant pathogenic fungi

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appendage-bearing conidia
canker pathogen
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Abstract The genus *Seiridium* includes multiple plant pathogenic fungi well-known as causal organisms of cankers on *Cupressaceae*. Taxonomically, the status of several species has been a topic of debate, as the phylogeny of the genus remains unresolved and authentic ex-type cultures are mostly absent. In the present study, a large collection of *Seiridium* cultures and specimens from the CBS and IMI collections was investigated morphologically and phylogenetically to resolve the taxonomy of the genus. These investigations included the type material of the most important *Cupressaceae* pathogens, *Seiridium cardinale*, *S. cupressi* and *S. unicorne*. We constructed a phylogeny of *Seiridium* based on four loci, namely the ITS rDNA region, and partial translation elongation factor 1-alpha (*TEF*), β-tubulin (*TUB*) and RNA polymerase II core subunit (*RPB2*). Based on these results we were able to confirm that *S. unicorne* and *S. cupressi* represent different species. In addition, five new *Seiridium* species were described, *S. cupressi* was lectotypified and epitypes were selected for *S. cupressi* and *S. eucalypti*.

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INTRODUCTION

The genus *Seiridium* (*Sordariomycetes*, *Xylariales*, *Sporocadaceae*) comprises a variety of mainly plant pathogenic fungi (Boesewinkel 1983, Graniti 1986, 1998, Barnes et al. 2001, Tsopelas et al. 2007). The genus was established based on *Seiridium marginatum* (Nees 1817), collected from rose stems in Germany and recently epitypified by Jaklitsch et al. (2016). The *Sporocadaceae* includes acervular asexual-morphs producing distinctive appendaged (pestalotioid) conidia (Jaklitsch et al. 2016), including other commonly known appendaged genera such as *Bartalinia*, *Pestalotiopsis* and *Seimatosporium.* Morphologically, *Seiridium* is distinguishable by its 5-septate conidia. Based on this characteristic, Maharachchikumbura et al. (2014) suggested that the monotypic genus *Pestalotia*, accommodating *P. pezizoides* (De Notaris 1841), might also be a synonym of *Seiridium*.

Seiridium is particularly known for its plant pathogenic species which have manifested profound economic damage globally. Presently, three *Seiridium* species (*S. cardinale*, *S. cupressi* and *S. unicorne*) are considered responsible for a pandemic of cypress canker disease, impacting plantations for wood production and ornamental tree cultivation (Boesewinkel 1983, Graniti 1986, 1998). Infection of *Cupressaceae* usually occurs secondary to tissue wounds produced by various agents, including wind, frost and insects, upon which the cankers appear as necrotic lesions on the tree bark. Once infected, cell necrosis of cypress tissue progresses steadily, reaching the cortical parenchyma, the phloem and the cambium, eventually causing the plant to die (Graniti 1998). Of these three species, *Seiridium cardinale* is the most aggressive and was first identified in California, from where the disease has since spread to other continents. Currently, the disease is particularly advanced in the Mediterranean region (Xenopoulos & Diamandis 1985, Graniti 1993, 1998, Della Rocca et al. 2011, 2013). The other two causal agents of cypress canker, *S. cupressi* and *S. unicorne*, are less destructive, but are considered responsible for disease in cypress plantations in East Africa and Japan, respectively (Nattrass & Ciccarone 1947, Jones 1953, 1954a, b, Tabata 1991). Whereas *S. cardinale* and *S. cupressi* appear to be restricted to *Cupressaceae*, *S. unicorne* has been reported from a range of plant families, including *Anacardiaceae*, *Caprifoliaceae*, *Cornaceae*, *Cupressaceae*, *Hamamelidaceae*, *Rosaceae* and *Vitaceae* (Guba 1961, Boesewinkel 1983, Cho & Shin 2004).

While *S. cardinale* is morphologically easily distinguishable from the other two putative *Cupressus* pathogens, *S. cupressi* and *S. unicorne* share more similarities and have, depending on the author, been treated as one or separate species (Guba 1961, Natrass et al. 1963, Swart 1973, Sutton 1975, Boesewinkel 1983, Graniti 1986, Nag Raj 1993, Viljoen et al. 1993). *Seiridium unicorne* (as *Pestalozzia unicornis*) was originally described from *Chamaecyparis thyoides* in New Jersey (Cooke & Ellis 1878). Guba (1961) introduced *S. cupressi* as *Cryptostictis cupressi* from multiple specimens of *Cupressus macrocarpa* collected in East Africa. Based on sequence data, both species are presently accepted (Barnes et al. 2001), and considered to be morphologically highly variable (Boesewinkel 1983, Graniti 1986, Chou 1989). Apart from intraspecific morphological variation, strains of the same species also vary in aggressiveness. Guba (1961) used both herbarium and culture material for the description of *S. cupressi.* The two cited herbarium materials were collected from *Cupressus macrocarpa* by Nattrass and the cultures by Jones (1953, 1954a) from *Cupressus* and *Juniperus*, who had studied their pathogenicity and, based on this feature, distinguished different strains. Although the cultures are still available, they have not been included in previously published phylogenetic analyses addressing the identity of *S. unicorne*

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and *S. cupressi* (Barnes et al. 2001, Jeewon et al. 2002, Cunnington 2007, Tsopelas et al. 2007, Maharachchikumbura et al. 2015). Moreover, since Guba (1961) did not designate a holotype specimen, a lectotype needs to be selected for *S. cupressi*. Furthermore, it is currently unclear whether these original specimens truly represent a single phylogenetic lineage. Similarly, no DNA sequence information has been generated thus far for the type of *S. unicorne.* However, a holotype specimen exists, and it can be epitypified if an appropriate culture is available. Previous studies using genetic markers to construct phylogenies (Barnes et al. 2001, Cunnington 2007, Tsopelas et al. 2007), as well as the restriction length polymorphism assay for *Seiridium* spp. identification (Krokene et al. 2004), did not include the type specimens and have relied on morphological identification of *S. unicorne* and *S. cupressi*. It remains therefore debatable if the clades identified in these phylogenies as *S. unicorne* and *S. cupressi* truly represent the lineages in which the type specimens would nest. To resolve this problem, the original materials of *S. unicorne* and *S. cupressi* need to be reexamined and if possible lecto- and epitypified in combination with analysis of DNA sequence data. In addition, to clarify the taxonomic identity of cultures and specimens from earlier studies (Barnes et al. 2001, Krokene et al. 2004, Cunnington 2007, Tsopelas et al. 2007), which are presently available in the public collections, a phylogenetic overview of the genus is required.

Seiridium is not solely comprised of *Cupressaceae* pathogens. Cankers inflicted by *Seiridium* spp. have been observed in *Eucalyptus* spp. (*Myrtaceae*) (Yuan & Old 1995, Yuan & Mohammed 1997, 1999, 2001), and recently, *S. phylicae* was described (Crous et al. 2012), which is an aggressive pathogen of *Phylica arborea* (*Rhamnaceae*), endemic to the archipelago Tristan da Cunha (Ryan et al. 2014). Furthermore, less destructive and perhaps more opportunistic pathogens are common to the genus, such as the generic type *S. marginatum*, for example.

The most well-studied sister genus of *Seiridium*, namely *Pestalotiopsis*, is known to include many endophytes (Maharachchikumbura et al. 2014). *Pestalotiopsis* has received particular interest due to the surprising high number of bioactive compounds produced by different species (Xu et al. 2010), and the record number of secondary metabolite clusters that was detected in the recently sequenced genome of *Pestalotiopsis fici* (Wang et al. 2015). Although poorly investigated, bioactive compounds have been isolated from *Seiridium* spp. as well, including phytotoxins (Ballio et al. 1991, Graniti et al. 1992, Evidente et al. 1993). Being perhaps the most aggressive genus of plant pathogens in the *Sporocadaceae*, *Seiridium* might be considered a unique evolutionary lineage within the family and consequently a unique lineage with regard to novel metabolite production.

In the present study we aimed to investigate the DNA phylogeny of the genus *Seiridium*, and to epitypify species for which no ex-type culture is currently available. Furthermore, we aimed to identify the clade or clades representing the important *Cupressaceae* pathogens *S. cardinale*, *S. cupressi* and *S. unicorne*, and to resolve the dubiety surrounding their taxonomy. To this end we obtained all *Seiridium* strains available in the CBS and CPC collections (housed at the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands) supplemented with additional collections, holotype materials and other herbarium specimens from Kew Royal Botanic Gardens (IMI, CABI Biosciences, Egham, Surrey, England). We constructed a multi-gene phylogeny, using the internal transcribed spacer (ITS) region and partial β-tubulin (*TUB*), which are the DNA markers commonly sequenced for *Seiridium*, supplemented with the translation elongation factor 1-alpha (*TEF*) and RNA polymerase II core subunit (*RPB2*) gene, including available *Seiridium* sequences from previously published phylogenies (Barnes et al. 2001, Cunnington 2007, Tsopelas et al. 2007, Maharachchikumbura et al. 2015).

MATERIALS AND METHODS

Isolates and specimens

All *Seiridium* isolates present in the CBS and CPC collections were included in the present study. The ex-epitypes of *Bartalinia robillardoides* (CBS 122705), *Neopestalotiopsis protearum* (CBS 114178) and *Seimatosporium rosae* (CBS 139823) were used as outgroups in the phylogenetic analyses (Table 1). Sequences from other strains, not examined here but published in previous phylogenetic studies of the genus (Barnes et al. 2001, Jeewon et al. 2002, Cunnington 2007, Tsopelas et al. 2007, Maharachchikumbura et al. 2015, Wijayawardene et al. 2016), were retrieved from GenBank (Table 1). In addition, herbarium material was requested from IMI (housed at Kew), including the holotypes of *S. cardinale* and *S. unicorne* and the authentic material Guba (1961) used to describe *S. cupressi.*

Culture preparation and morphology analysis

Lyophilized cultures from the CBS and CPC collections were reactivated on Petri dishes with 2 % malt extract agar (MEA) and isolates from the CBS collection stored in liquid nitrogen were revived on either MEA or oatmeal agar (OA). Culture characteristics were studied on Petri dishes containing cornmeal agar (CMA), MEA, potato dextrose agar (PDA), and synthetic nutrient-poor agar (SNA). All culture media were prepared according to recipes as in Crous et al. (2009). Cultures were grown at 22 °C with a 12 h daylight/darkness photoperiod for 2 wk. Colony colours were assessed using colour charts from Rayner (1970). Conidiomatal morphology was examined on both PDA and SNA and all other morphological analyses were conducted from colonies on SNA using Differential Interference Contrast (DIC) optics on a Nikon Eclipse 80i microscope and a Nikon AZ100 dissecting microscope, both equipped with Nikon DS-Ri2 high definition digital cameras. To visualize relevant morphological features of multiple conidia on composite photo plates, separate photographs of conidia were combined using Photoshop CS5.1. Measurements were made from microphotographs using the Nikon NIS-elements D v. 4.50 software. Morphology of conidiophores, conidiogenous cells and conidia was examined and measured from colonies grown on SNA. For the descriptions, a minimum of 20 measurements was taken for conidiophores and conidiogenous cells, and for cell dimensions of conidia a minimum of 30 measurements was taken per examined specimen. Length of the conidia was measured from the base of the basal end to the base of the apical appendage, and conidial width was measured at the widest point of the conidium. Each length and width values of conidia are reported as one standard deviation (SD) below the mean to one SD above, with the extreme measurements in parentheses followed by the mean ± SD. For other measurement values the full range is reported, from lowest to highest extreme.

PCR and sequencing

DNA was extracted from fungal mycelia grown on MEA with the Promega Genomic DNA purification kit (Fitchburg, Wisconsin, USA) according to the manufacturers' protocol. PCRs were facilitated in 12.5 µL solutions as in Crous et al. (2013). The ITS rDNA region was amplified with the primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3'; White et al. 1990) and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'; White et al. 1990), *TEF* gene with EF728 and EF2 (5'-CATYGAGAA-GTTCGAGAAGG-3' and 5'-GGARGTACCAGTSATCATGTT-3';

Table 1 Specimens and corresponding accession numbers used in the phylogenetic analysis.

Species	Collection accession No.[1]	Host/Substrate	Host family	Location	Clade No.[2]	GenBank accession No.[3]			
						ITS	*RPB2*	*TEF*	*TUB*
Seiridium camelliae	MFLUCC 12-0647[T]	*Camellia reticula*	*Theaceae*	China	12	JQ683725	–	JQ683741	JQ683709
S. cancrinum	IMI 52256[T]; BRL 1119; CBS 226.55	*Cupressus macrocarpa*	*Cupressaceae*	Kenya	5	**LT853089**	**LT853137**	**LT853186**	**LT853236**
	CBS 907.85; CMW 320	*Cupressus lustinaca*	*Cupressaceae*	South Africa		**LT853090**	**LT853138**	**LT853187**	**LT853237**
S. cardinale	CBS 123911	*Cupressus sempervirens*	*Cupressaceae*	Morocco	1	**LT853057**	**LT853106**	**LT853154**	**LT853204**
	CBS 122612; CMW 2133	*Cupressus* sp.	*Cupressaceae*	Chile		**LT853058**	**LT853107**	**LT853155**	**LT853205**
	CBS 172.56	unknown	unknown	unknown		**LT853059**	**LT853108**	**LT853156**	**LT853206**
	CBS 280.93; CPC 23780	*Cupressus* sp.	*Cupressaceae*	Italy		**LT853060**	**LT853109**	**LT853157**	**LT853207**
	CBS 522.82; CPC 23784; NBRC 32683	*Cupressus sempervirens*	*Cupressaceae*	New Zealand		**LT853061**	**LT853110**	**LT853158**	**LT853208**
	CBS 523.82; CPC 23793	*Cupressocyparis* sp.	*Cupressaceae*	New Zealand		**LT853062**	**LT853111**	**LT853159**	**LT853209**
	CBS 908.85; CPC 23779; CMW 616	*Cupressus lustinaca*	*Cupressaceae*	South Africa		**LT853063**	**LT853112**	**LT853160**	**LT853210**
	CBS 909.85[R]; CMW 635	*Cupressus lustinaca*	*Cupressaceae*	South Africa		**LT853064**	**LT853113**	**LT853161**	**LT853211**
	CBS 910.85; CPC 23781; CMW 600; CMW 638	*Cupressus sempervirens*	*Cupressaceae*	South Africa		**LT853065**	**LT853114**	**LT853162**	**LT853212**
	CMW 1644	*Cupressus* sp.	*Cupressaceae*	Italy		–	–	–	AF320497
	CMW 1645	*Cupressus* sp.	*Cupressaceae*	Italy		–	–	–	AF320501
	CMW 18602	*Cupressus sempervirens*	*Cupressaceae*	Greece		–	–	–	DQ926974
	CMW 18603	*Cupressus macrocarpa*	*Cupressaceae*	Greece		–	–	–	DQ926975
	CMW 18604	*Juniperus foetidissima*	*Cupressaceae*	Greece		–	–	–	DQ926973
	CMW 18605	*Juniperus foetidissima*	*Cupressaceae*	Greece		–	–	–	DQ926976
	CMW 18606	*Juniperus exelsa*	*Cupressaceae*	Greece		–	–	–	DQ926978
	CMW 18794	*Cupressus sempervirens*	*Cupressaceae*	Greece		–	–	–	DQ926977
	CPC 23785	*Cupressus sempervirens*	*Cupressaceae*	France		**LT853066**	**LT853115**	**LT853163**	**LT853213**
	CPC 23787	*Juniperus phoenica*	*Cupressaceae*	Greece		**LT853067**	**LT853116**	**LT853164**	**LT853214**
	CPC 23788	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853068**	**LT853117**	**LT853165**	**LT853215**
	CPC 23790	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853069**	**LT853118**	**LT853166**	**LT853216**
	CPC 23791	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853070**	**LT853119**	**LT853167**	**LT853217**
	CPC 23792	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853071**	–	**LT853168**	**LT853218**
	CPC 23794	unknown	unknown	Greece		**LT853072**	**LT853120**	**LT853169**	**LT853219**
	CPC 23795	unknown	unknown	Greece		**LT853073**	**LT853121**	**LT853170**	**LT853220**
	CPC 23796	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853074**	**LT853122**	**LT853171**	**LT853221**
	CPC 23797	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853075**	**LT853123**	**LT853172**	**LT853222**
	CPC 23798	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853076**	**LT853124**	**LT853173**	**LT853223**
	CPC 28286	*Cupressus sempervirens*	*Cupressaceae*	Spain		**LT853077**	**LT853125**	**LT853174**	**LT853224**
	CPC 28287	*Cupressus sempervirens*	*Cupressaceae*	Spain		**LT853078**	**LT853126**	**LT853175**	**LT853225**
S. ceratosporum	PHSI2001Pathcw07	*Vitis vinifera*	*Vitaceae*	China	16	AY687314	–	–	DQ534043
S. cupressi	CBS 122616; CMW 1646	*Cupressus* sp.	*Cupressaceae*	Greece	3	**LT853082**	**LT853130**	**LT853179**	**LT853229**
	IMI 52254[L]; BRL 1117; CBS 224.55[ET]	*Cupressus macrocarpa*	*Cupressaceae*	Kenya		**LT853083**	**LT853131**	**LT853180**	**LT853230**
	IMI 52255; BRL 1118; CBS 225.55	*Cupressus forbesii*	*Cupressaceae*	Kenya		**LT853084**	**LT853132**	**LT853181**	**LT853231**
	IMI 52258; BRL 1120; CBS 227.55	*Cupressus macrocarpa*	*Cupressaceae*	Uganda		**LT853085**	**LT853133**	**LT853182**	**LT853232**
	CBS 320.51	*Cupressus* sp.	*Cupressaceae*	Kenya		**LT853086**	**LT853134**	**LT853183**	**LT853233**
	CMW 18607	*Cupressus sempervirens*	*Cupressaceae*	Greece		–	–	–	DQ926979
S. eucalypti	CBS 343.97[ET]; CMW 5303	*Eucalyptus delegatensis*	*Myrtaceae*	Australia	10	**LT853099**	**LT853146**	**LT853196**	**LT853246**
S. kartense	CBS 142629[T]; CPC 20183	*Eucalyptus cladocalyx*	*Myrtaceae*	Australia	11	**LT853100**	**LT853147**	**LT853197**	**LT853247**
S. kenyanium	IMI 52257[T]; BRL 1121; CBS 228.55	*Juniperus procera*	*Cupressaceae*	Kenya	9	**LT853098**	**LT853145**	**LT853195**	**LT853245**
S. marginatum	CBS 140403[GT]	*Rosa canina*	*Rosaceae*	France	15	KT949914	**LT853149**	**LT853199**	**LT853249**
S. neocupressi	CMW 420	*Cupressus macrocarpa*	*Cupressaceae*	New Zealand	2	–	–	–	AF320487
	CMW 5282; ATCC 48158	*Cupressus* sp.	*Cupressaceae*	New Zealand		FJ430600	–	–	AF320489
	CBS 142625[T]; CPC 23786	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853079**	**LT853127**	**LT853176**	**LT853226**
	CBS 142626; CPC 23789	*Cupressus sempervirens*	*Cupressaceae*	Italy		**LT853080**	**LT853128**	**LT853177**	**LT853227**
	CBS 142627; CPC 28351	*Cupressus leylandii*	*Cupressaceae*	Australia		**LT853081**	**LT853129**	**LT853178**	**LT853228**
	VPRI 15696	*Cupressus* sp.	*Cupressaceae*	Australia		–	–	–	EF517787
	VPRI 16083	*Cupressus* sp.	*Cupressaceae*	Australia		–	–	–	EF517786
	VPRI 32740	*Cupressus* sp.	*Cupressaceae*	Australia		–	–	–	EF517789
	VPRI 40658	*Cupressus* sp.	*Cupressaceae*	Australia		–	–	–	EF517790
	VPRI 40665	*Cupressus* sp.	*Cupressaceae*	Australia		–	–	–	EF517788

Table 1 (cont.)

Species	Collection accession No.[1]	Host/Substrate	Host family	Location	Clade No.[2]	GenBank accession No.[3]			
						ITS	*RPB2*	*TEF*	*TUB*
S. papillatum	CBS 340.97[T]; VPRI 20827; CMW 5302	*Eucalyptus delegatensis*	*Myrtaceae*	Australia	17	**LT853102**	**LT853150**	**LT853200**	**LT853250**
S. phylicae	CBS 133587[T]; CPC 19964	*Phylica arborea*	*Rhamnaceae*	Tristan da Cunha	6	**LT853091**	**LT853139**	**LT853188**	**LT853238**
	CPC 19962	*Phylica arborea*	*Rhamnaceae*	Tristan da Cunha		**LT853092**	**LT853140**	**LT853189**	**LT853239**
	CPC 19965	*Phylica arborea*	*Rhamnaceae*	Tristan da Cunha		**LT853093**	**LT853141**	**LT853190**	**LT853240**
	CPC 19970	*Phylica arborea*	*Rhamnaceae*	Tristan da Cunha		**LT853094**	–	**LT853191**	**LT853241**
S. podocarpi	CBS 137995[T]; CPC 23429	*Podocarpus latifolus*	*Podocarpaceae*	South Africa	13	**LT853101**	**LT853148**	**LT853198**	**LT853248**
S. pseudocardinale	MFLUCC 13-0525[T]	*Cupressus arizonica*	*Cupressaceae*	Italy	8	KU848210	–	–	–
	CBS 122613; CMW 1648	*Cupressus* sp.	*Cupressaceae*	Portugal		**LT853096**	**LT853143**	**LT853193**	**LT853243**
	CBS 122614; CMW 1649	*Cupressus* sp.	*Cupressaceae*	Portugal		**LT853097**	**LT853144**	**LT853194**	**LT853244**
S. spyridicola	CBS 142628[T]; CPC 29108	*Spyridium globosum*	*Rhamnaceae*	Australia	7	**LT853095**	**LT853142**	**LT853192**	**LT853242**
S. unicorne	CBS 120306; CMW 5596	*Cupressus sempervirens*	*Cupressaceae*	South Africa	4	**LT853087**	**LT853135**	**LT853184**	**LT853234**
	CBS 538.82[R]; NBRC 32684; CMW 5443	*Cryptomeria japonica*	*Cupressaceae*	New Zealand		**LT853088**	**LT853136**	**LT853185**	**LT853235**
S. venetum	MFLU 15-0369[R]	*Cornus mas*	*Cornaceae*	Italy	14	KT438836	–	–	KT438837
Neopestalotiopsis protearum	CBS 114178[GT]	*Leucospermum cuneiforme*	*Proteaceae*	Zimbabwe	Outgroup	**LT853103**	**LT853151**	**LT853201**	**LT853251**
Bartalinia robillardoides	CBS 122705[GT]	*Leptoglossus occidentalis*	Coreidae (Animalia)	Italy	Outgroup	**LT853104**	**LT853152**	**LT853202**	**LT853252**
Seimatosporium rosae	CBS 139823[GT]; MFLUCC 14-0621	*Rosa* sp.	*Rosaceae*	Russia	Outgroup	**LT853105**	**LT853153**	**LT853203**	**LT853253**

[1] ATCC: American Type Culture Collection; BRL: Bristol City Library, Bristol, England, UK; CBS: Westerdijk Fungal Biodiversity Institute (formerly CBS-KNAW Fungal Biodiversity Centre), Utrecht, the Netherlands; CMW: Culture collection of the Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, South Africa; CPC: Culture collection of P.W. Crous, held at the Westerdijk Fungal Biodiversity Institute, Utrecht, the Netherlands; IMI: Kew Royal Botanic Gardens, CABI Biosciences, Egham, Surrey, England; MFLU and MFLUCC: Mae Fah Luang University, Chiang Rai, Thailand; NBRC: National Institute of Technology and Evaluation, Kisarazu-shi, Chiba, Japan; PHSI: from Liu et al. (2007); VPRI: Victorian Department of Environment and Primary Industries, Knoxfield, Victoria, Australia.

[2] Clade numbers correspond to the multi-gene phylogeny in Fig. 1 and single gene phylogenies in Fig. 2.

[3] ITS: Internal transcribed spacer regions 1 and 2 and 5.8S; *RPB2*: RNA polymerase II core subunit; *TEF*: Translation elongation factor 1-alpha; TUB: Beta-tubulin.

[T] type or ex-type; [ET] ex-epitype; [L] lectotype; [GT] ex-generic type; [R] reference strain. Accession numbers of sequences generated in this study are in **bold**.

O'Donnell et al. 1998), region 1 of *TUB* with T1 and bt2b (5'-AACATGCGTGAGATTGTAAGT-3' and 5'-ACCCTCAGTGTAGTGACCCTTGGC-3'; O'Donnell & Cigelnik 1997, Glass & Donaldson 1995) and the *RPB2* subunit with 5F2 and 7cR (5'-GGGGWGAYCAGAAGAAGGC-3' and 5'-CCCATRGCTTGYTTRCCCAT-3'; Liu et al. 1999, Sung et al. 2007). Except for the annealing temperature, the PCR programs for the different loci were identical: 7 min and 30 s of initial denaturation at 95 °C, 35 cycles of 30 s denaturation at 95 °C, 30 s annealing and 90 s elongation at 72 °C and a final elongation step of 7 min 30 s at 72 °C. For ITS the annealing step was at 55 °C and for *TEF*, *TUB* and *RPB2* annealing temperatures were 53 °C. The amplicons were sequenced with both forward and reverse primers, using an ABI Prism 3730XL Sequencer (Applied Biosystems). Sequences were quality checked and assembled using DNASTAR Lasergene SeqMan Pro v. 8.1.3 software, and deposited in GenBank (Table 1).

Phylogenetic analysis

Sequences acquired in this study were supplemented with those retrieved from GenBank (Barnes et al. 2001, Cunnington 2007, Tsopelas et al. 2007, Maharachchikumbura et al. 2015) and aligned using MAFFT v. 7 (Katoh et al. 2002, Katoh & Standley 2013). Alignments were checked and concatenated in MEGA v. 6.06 (Tamura et al. 2013). Maximum-likelihood (ML) analyses for both single-locus and concatenated alignments were performed with RAxML-HPC2 on XSEDE v. 8.2.10 (Stamatakis 2014) using a GTR+GAMMA substitution model with 1 000 bootstrap iterations. For the Bayesian inference (BI) analyses, the optimal substitution model for ITS, *TEF* and *TUB* was determined to be HKY+I+G and for *RPB2*, GTR+I+G, using MrModeltest software v. 2.2. (Nylander 2004). The BI analyses were computed with MrBayes v. 3.2.6 (Ronquist et al. 2012) with four simultaneous Markov Chain Monte Carlo chains from random trees over 5 M generations, ending the run automatically when standard deviation of split frequencies dropped below 0.01. Both RAxML and Bayesian analyses were run on the CIPRES Science Gateway portal (Miller et al. 2012). Maximum Parsimony (MP) analyses were conducted with PAUP v. 4.0b10 (Swofford 2002), inferring trees with the heuristic search option with TBR branch swapping and 1 000 random sequence additions. The robustness of equally parsimonious trees was evaluated by 1 000 bootstrap replications. Alignments and trees were deposited in TreeBASE (www.treebase.org; study 21661).

RESULTS

Phylogenetic analysis

The concatenated four-locus alignment contained 2 863 nucleotide positions including gaps (ITS: 616, *RPB2*: 803, *TEF*: 634 and *TUB*: 810), comprising 70 strains, including the three outgroup taxa. The phylogram of the best ML tree (lnL = -15056.777813) of the concatenated alignment is shown in Fig. 1. The BI analysis ran 1 345 000 generations before the average standard deviation for split frequencies reached below 0.01. After discarding the first 25 % of generations, 1 010 trees remained from which 50 % consensus trees and posterior probabilities were calculated. In the MP analysis 1 708 characters were identified to be constant, 437 variable characters as parsimony-uninformative and 718 (25.1 %) characters as parsimony-informative. After the heuristic search, 1 000 equally most parsimonious trees were saved (tree length = 408 steps, CI = 0.707, RI = 0.750, RC = 0.530, HI = 0.293). The topologies of the BI and MP trees were similar to the ML tree, which was used to visualize the combined topology (Fig. 1). A node separating the genus *Seiridium* from the three family members used as outgroup taxa received strong support (99/1/100). Single gene ML, BI and MP trees (Fig. 2) were computed from the same alignments to investigate the suitability of DNA markers used in this study to delineate species.

The largest clade (clade 1) comprised *S. cardinale* isolates that appeared to be genetically closely related and monophyletic. Isolates previously labelled as *S. unicorne* and *S. cupressi* were, however, polyphyletic. Previously labelled *S. unicorne* strains clustered in two different clades while labelled *S. cupressi* strains, including the cultures from the authentic materials (CBS 224.55, CBS 225.55, CBS 226.55, CBS 227.55 and CBS 228.55; Jones 1953, 1954a, Guba 1961) were scattered over five distinctive clades. Clade 2 comprised three yet unclassified specimens (CPC 23786 = CBS 142625, CPC 23789 = CBS 142626 and CPC 28351 = CBS 142627) and specimens identified in previous phylogenetic studies to be *S. cupressi* (CMW 5282, CMW 420 from Barnes et al. 2001; VPRI 40658, VPRI 32740, VPRI 15696, VPRI 16083 and VPRI 40665 from Cunnington 2007). The cultures from Jones (1953, 1954a, b), on which Guba (1961) based the description of *S. cupressi*, did not appear in this clade but were present in clades 3 (CBS 224.55 = IMI 52254, CBS 225.55 = IMI 52255 and CBS 227.55 = IMI 52258), 5 (CBS 226.55 = IMI 52256) and 9 (CBS 228.55 = IMI 52257).

Jones (1953, 1954a, b) distinguished different strains based on the aggressiveness of the canker pathogens. While Guba (1961) concluded these were different specimens of one

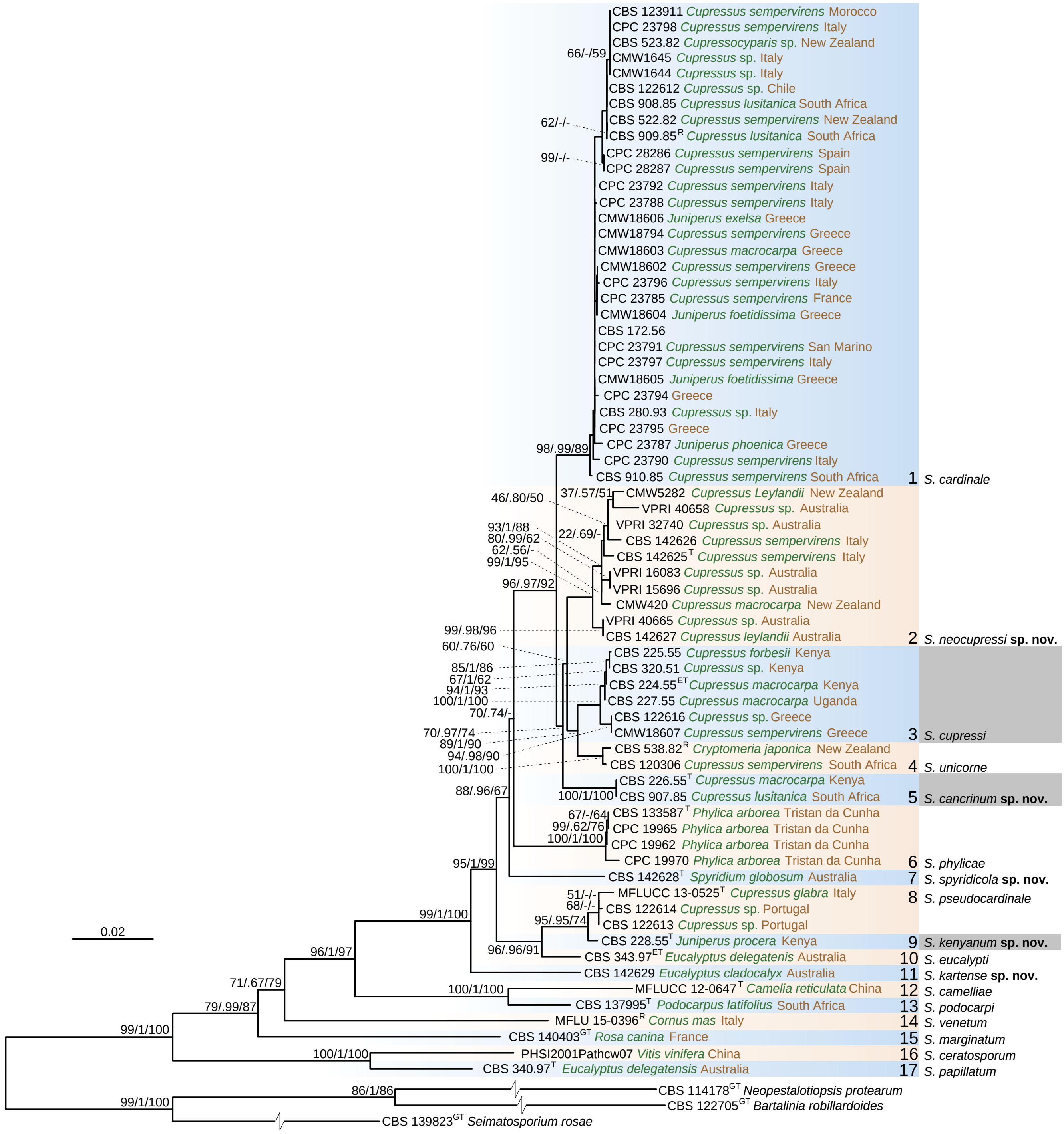

Fig. 1 The best Maximum Likelihood tree (lnL = -15056.777813) from the multi-gene alignment (with the 4 loci ITS, *RPB2*, *TUB* and *TEF*) for the *Seiridium* acquired and sequenced in this study. Nodes are labelled with bootstrap values from RAxML / Bayesian posterior probabilities / Parsimony bootstrap values. Nodes receiving below 50 bootstrap values and 0.5 probability values are not labelled. Grey highlighted names indicate groups containing specimens that were part of the original description of *S. cupressi* (Guba 1961). Clades 2, 5, 7, 9 and 11 represent new lineages described here as the novel species *Seiridium neocupressi*, *S. cancrinum*, *S. spyridicola*, *S. kenyanum* and *S. kartense*. Ex-type culture, ex-epitype culture, ex-generic type culture and reference strains are denoted behind strain numbers with [T], [ET], [GT] and [R], respectively.

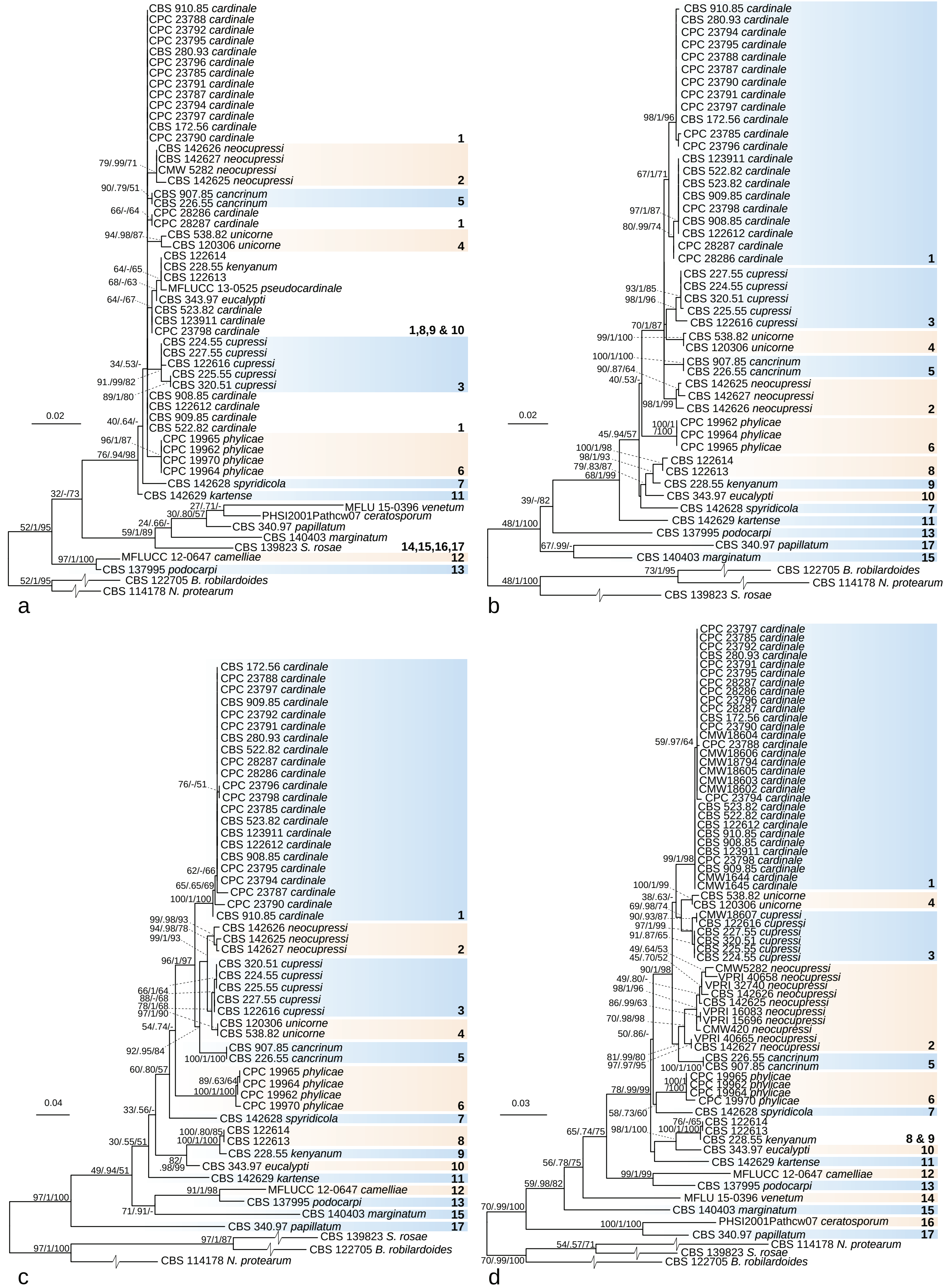

Fig. 2 The best Maximum Likelihood trees from single-locus alignments. Nodes are labelled with bootstrap values from RAxML/Bayesian posterior probabilities/Parsimony bootstrap values. Nodes receiving below 50 bootstrap values and 0.5 probability values are not labelled. Clade numbers and colours, indicating monophyletic lineages, correspond to the combined phylogeny in Fig. 1: a. The best Maximum Likelihood tree for the ITS alignment including 616 positions (lnL = -2040.052011); b. the best Maximum Likelihood tree for the *RPB2* alignment including 803 positions (lnL = -3938.132103); c. the best Maximum Likelihood tree for the *TEF* alignment including 634 positions (lnL = -3885.959255); d. the best Maximum Likelihood tree for the *TUB* alignment including 810 positions (lnL = -4789.464706).

species distinct from *S. unicorne*, the classification used by Jones (1953, 1954a, b) corresponds to the combined phylogeny in the present study, where strain B (IMI 52254 = CBS 224.55, IMI 52255 = CBS 225.55 and IMI 52258 = CBS 227.55) falls within clade 3, strain A (IMI 52256 = CBS 226.55) in clade 5 and strain D (IMI 52257 = CBS 228.55) in clade 9. Clade 3 also includes the specimens CBS 320.51, CBS 122616 (= CMW 1646 classified as *S. cupressi* in Barnes et al. 2001) and CMW 18607, classified as *S. cupressi* in Tsopelas et al. (2007). The fifth clade contains CBS 907.85 (not classified in previous studies) in addition to the strain A specimen from Jones (1953; CBS 226.55). *Seiridium cupressi* strain D (Jones 1953, Guba 1961; CBS 228.55) clustered together with CBS 122613, CBS 122614 (both classified as *S. unicorne* in Barnes et al. 2001) and the type of the recently described *S. pseudocardinale* (Wijayawardene et al. 2016). The fourth clade includes CBS 538.82 (= CMW 5443), which has also been classified as *S. cupressi* (Barnes et al. 2001).

Other clades included *S. phylicae* (clade 6; Crous et al. 2012); CBS 142628, isolated from *Spyridium globosum* in Australia

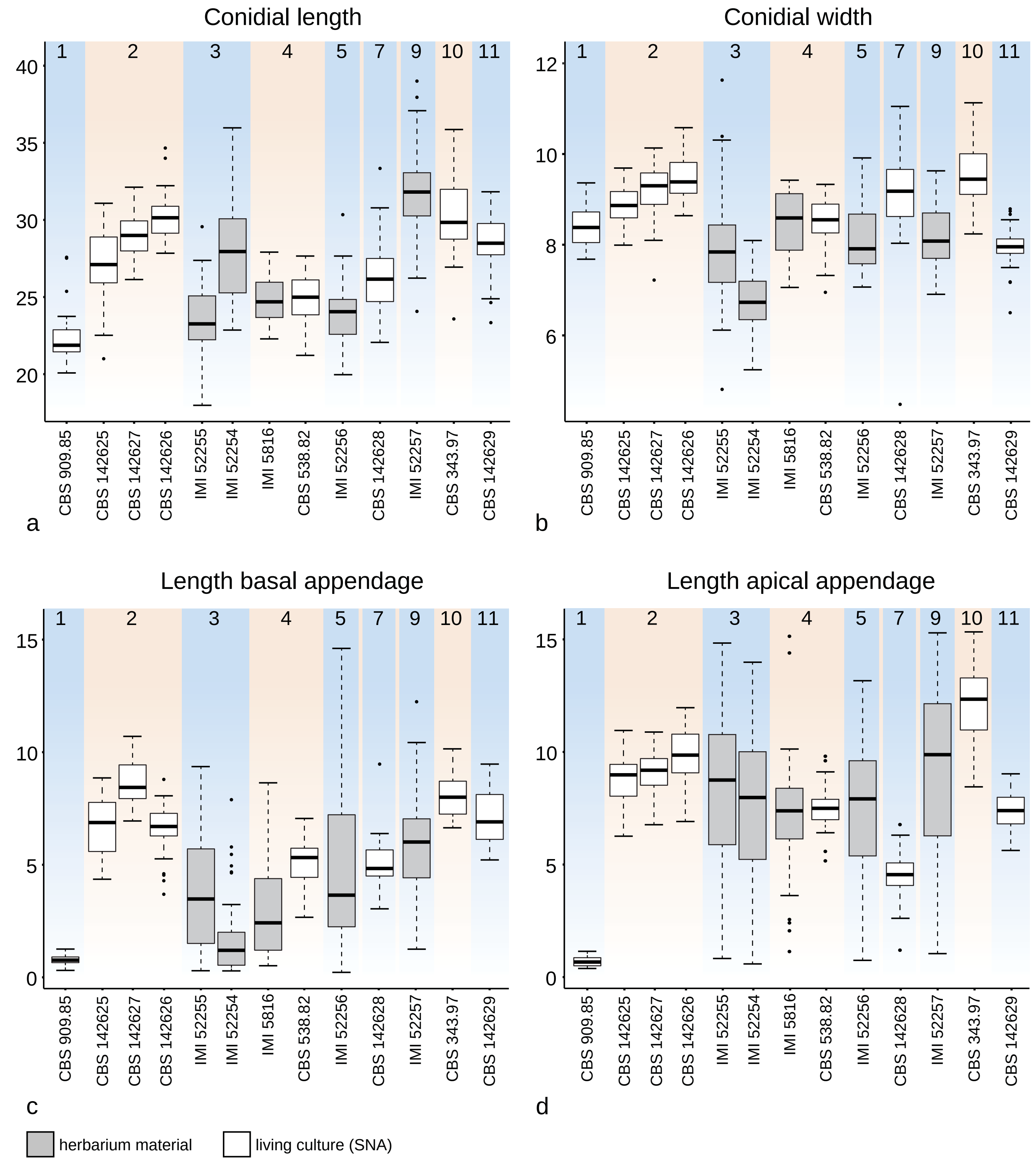

Fig. 3 Boxplots reflecting the distribution of variance in conidial measurements in micrometres as acquired from living culture material on SNA (in white) or from herbarium material (in grey). The boxes show the first and third quartiles. Lower and upper whiskers extend from the boxes to the extreme values or 1.5 times the inter quartile range when the extreme values are outside this range, in which case outlying values are indicated by black dots. Strains are ordered by clade as in Fig. 1 and in the background coloured accordingly to the clade colours in Fig. 1: a. Conidial length; b. conidial width; c. length of basal appendage; d. length of apical appendage.

(clade 7), a *Seiridium eucalypti* strain (CBS 343.97; clade 10) and CBS 142629, isolated from *Eucalyptus cladocalyx* in Australia (clade 11) and *S. marginatum*, the generic type (clade 15).

Taxonomy

Based on the results of the combined multi-gene phylogenies (Fig. 1), morphological observations, measurements of fungarium specimens, cultures (Fig. 3) and ecological data, five novel species of *Seiridium* are described, a lectotype is designated for *S. cupressi*, and epitypes are selected for *S. cupressi* and *S. eucalypti*. Overall, from four clades (3, 5, 7 and 8) all available cultures were sterile. However, DNA was extracted and sequenced from mycelia of these cultures and for three of the clades (3, 5 and 7) morphological characters were studied from the associated herbarium specimens.

Seiridium Nees, Syst. Pilze (Würzburg): 22. 1817

Synonyms. *Hyaloceras* Durieu & Mont., Expl. Sci. Algerie 1: 587. 1849. *Adea* Petr., Bot. Jahrb. Syst. 62: 144. 1928.

Type species. *Seiridium marginatum* Nees.

Ascomata perithecial, immersed to semi-erumpent, depressed, globose to pyriform, scattered or confluent; peridium dark brown, pseudoparenchymatous. *Ostioles* central, slightly papillate, black, periphysate. *Paraphyses* hyaline, smooth, filiform. *Asci* cylindrical, 8-spored, unitunicate, thin-walled, stipitate, with an apical amyloid ring. *Ascospores* cylindrical-oblong, euseptate, septa often thicker than the wall, yellow- to dark brown, guttulate. *Conidiomata* acervuloid to pycnidioid, semi-immersed to erumpent, uni- to plurilocular, brown or black, glabrous, dehiscing by irregular splits in the upper wall. *Conidiophores* lining the cavity of the conidioma, septate and sparsely branched at the base, or reduced to conidiogenous cells, hyaline, smooth. *Conidiogenous cells* discrete, integrated, ampulliform to lageniform or subcylindrical, hyaline, smooth, proliferating percurrently at the apex. *Conidia* fusiform, distoseptate (septal pores present or not), end cells hyaline, median cells dark brown to brown, wall thick, smooth or with striations, constricted at septa or not; apical cell with a single, cellular, unbranched or branched, appendage; basal cell with or without a centric, unbranched or sometimes branched appendage.

Notes — *Seiridium* includes coelomycetes producing versicolorous, 5-septate conidia with appendages and typically forming acervuli on the plant host. The original description of the generic type, *S. marginatum*, dates back over 200 years ago and was re-described by Shoemaker et al. (1966), Sutton (1980) and Nag Raj (1993). Recently, the generic type *S. marginatum* was epitypified (Jaklitsch et al. 2016) from which additional sequence data was generated in this study and included in the present phylogeny (Fig. 1).

Seiridium cancrinum Bonthond, Sandoval-Denis & Crous, *sp. nov.* — MycoBank MB823296; Fig. 4

Etymology. From the three strains that Jones (1953, 1954a, b) conducted pathogenic experiments on, this fungus (strain A) was the most aggressive. Therefore, the fungus is named after the canker formation it induces on *Cupressus*.

Conidia lunate to falcate, curved, 5-septate, occasionally 4-septate, not striate, bearing two appendages, euseptate with no visible pores, (20–)22–26(–30.5) × (7–)7.5–9(–10) µm, mean ± SD = 24.0 ± 2.1 × 8.2 ± 0.7 µm (n = 35); basal cell obconic with truncate base, hyaline, walls smooth, bearing marginal frills, 1.2–5.8 µm; four median cells, varying in colour ranging from pale to dark brown, smooth, cylindrical to doliiform (second cell from base 3–5.5 µm long; third cell 3–5 µm long; fourth cell 3.5–5.5 µm long; fifth cell 3.5–6.5 µm long); apical cell conical, hyaline, smooth, 2–4.5 µm long; apical appendage single, centric, 1–13 µm; basal appendage, single, cylindrical, centric, 0.5–14.5 µm.

Known distribution — Kenya, South Africa.

Materials examined. Kenya, from cankers in branches of *Cupressus macrocarpa*, 1950, *D.R. Jones* (holotype IMI 52256, isotype BRL 1119, culture ex-type CBS 226.55). – South Africa, from *Cupressus lusitanica*, unknown collection date, *M.J. Wingfield* (CBS 907.85 = CMW 320).

Notes — The pathogenicity of the strain IMI 52256 was studied by Jones (1953, 1954a, b), who classified the fungus as *Monochaetia unicornis* strain A. Jones identified strain A as

Fig. 4 *Seiridium cancrinum* sp. nov. (IMI 52256, holotype). a. Herbarium specimen; b. conidiogenous cells and conidia; c. conidia. — Scale bars: b–c = 10 µm.

the most aggressive of the three strains that would eventually be described as *S. cupressi* (Guba 1961). However, here we show that the three strains (A: IMI 52256 = CBS 226.55, B: IMI 52254 = CBS 224.55, IMI 52255 = CBS 225.55 and IMI 52258 = CBS 227.55 and D: IMI 52257 = CBS 228.55) are three different species, which in turn correlates with the observations originally made by Jones (1953, 1954a, b). Although both cultures (CBS 226.55 = IMI 52256 and CBS 907.85) remained sterile on culture media, conidia could be studied from herbarium material (IMI 52256). Based on conidial morphology, *S. cancrinum* is highly similar to *S. cupressi*, but on average has longer basal appendages. Conidia with basal appendages up to almost 15 µm can be observed, whereas conidia of *S. cupressi* bear basal appendages that do not exceed 10 µm in length.

Seiridium cardinale (W.W. Wagener) B. Sutton & I.A.S. Gibson, CMI Descriptions of Pathogenic Fungi and Bacteria: 36. 1972 — Fig. 5, 6

Basionym. Coryneum cardinale W.W. Wagener, J. Agric. Res. 58: 8. 1939.

Conidiomata on PDA sporodochial, pseudostromatic, globose or clavate, at the edge of the colony, dark brown to black; on SNA sporodochial, pseudostromatic, globose, scattered, irregular in outline, mostly immersed in agar. *Conidiophores* septate, cylindrical, irregularly branched, branch lengths variable (20–85 µm long), hyaline, thin- and smooth-walled, invested in mucus. *Conidiogenous cells* discrete, hyaline, subcylindrical to lageniform, thin- and smooth-walled, 4–15 × 1.5–2.5 µm, proliferating percurrently multiple times, with collarettes and minute periclinal thickenings. *Conidia* fusiform, straight to slightly curved, 5-septate, not striate, bearing two short appendages, euseptate with pores clearly visible, (20–)20.5–24(–27.5) × (7.5–)8–9(–9.5) µm, mean ± SD = 22.3 ± 1.8 × 8.4 ± 0.4 µm

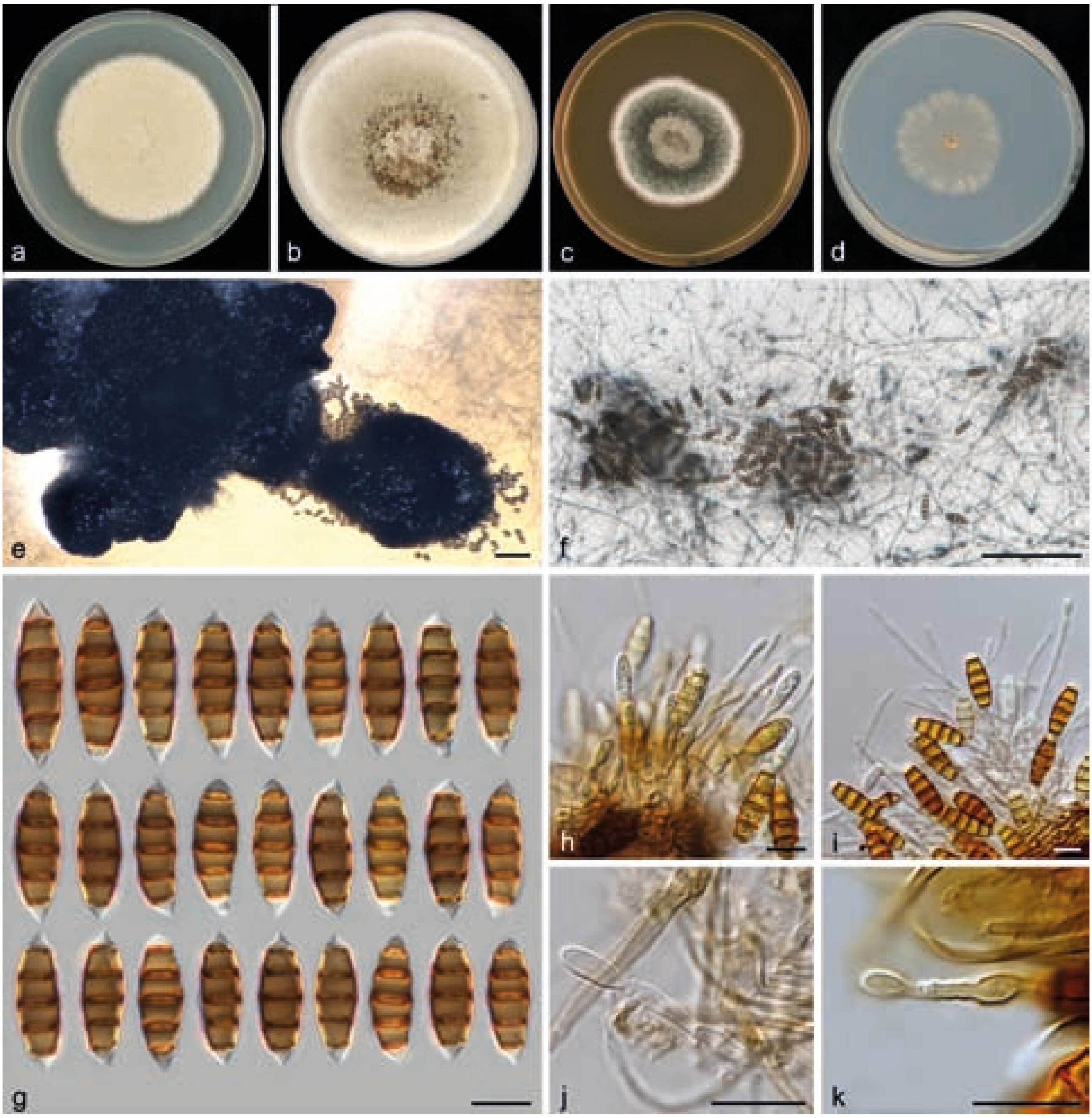

Fig. 5 *Seiridium cardinale* (CBS 909.85, reference strain). a–d. Colony morphology in 90 mm Petri dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e. conidioma on PDA partially immersed in agar; f. sporodochia on SNA immersed in agar; g. conidia; h–i. conidiophores; j–k. conidiogenous cells. — Scale bars: e–f = 100 µm; g–k = 10 µm.

Fig. 6 *Seiridium cardinale* (IMI 75045 isotype of *Coryneum cardinale*). a. Herbarium specimen; b–c. conidiomata and conidia *in vivo*; d. conidia *in vitro*. — Scale bars: b–c = 20 µm; d = 10 µm.

(n = 30); basal cell obconic with a truncate base, hyaline, walls smooth, bearing minute marginal frills, 2–3 µm; four median cells, smooth, cylindrical to doliiform, young conidia concave cylindrical to subcylindrical, pale brown to brown, septa darker than the rest of the cells (second cell from base 3.9–4.8 µm long; third cell 3.8–4.8 µm long; fourth cell 4–4.9 µm long; fifth cell 3.9–4.8 µm long); apical cell conical, hyaline, smooth, 2–3 µm long; apical appendage single, centric, short, < 1 µm; basal appendage, single when present, cylindrical, centric, < 1 µm.

Culture characteristics — Colonies on PDA circular, reaching 60–61 mm diam after 14 d at 22 °C, flat at centre and margin, white- to pale luteous-coloured, with aerial mycelium abundant on the surface, sporulating poorly at the margin of the colony and not within 2 wk with pycnidioid conidiomata. On CMA circular, reaching 79–81 mm diam after 14 d at 22 °C, flat at the centre and margin, white-, buff- to hazel-coloured in the centre, hazel-coloured at the inner margin to white at the outer margin, with aerial mycelium formed abundantly on the surface, sporulating poorly, after at least 4 wk with black spore masses produced in sporodochia. On MEA circular, reaching 45–49 mm diam after 14 d at 22 °C, hazel-coloured at the centre surrounded by a grey olivaceous to hazel ring and a white margin, flat at centre and margin, with aerial mycelium abundantly on the surface, sporulating poorly with few sporodochia producing black spore masses. On SNA circular to irregular, reaching 37–39 mm diam after 14 d at 22 °C, flat at centre and margins, with moderate aerial mycelium mostly at the margin, sporulation after approximately 2 wk, sporodochia scattered and immersed in agar.

Known distribution — Africa, Asia, Australia, Europe, New Zealand, North and South America.

Materials examined. New Zealand, Christchurch, 1981, from *Cupressocyparis* sp., *H.J. Boesewinkel* (CBS H-18011, culture CBS 523.82 = CPC 23793). – South Africa, East Transvaal, Mac Mac State Forest, 1985, *M.J. Wingfield* (CBS H-18015, culture CBS 908.85 = CMW 616); Algeria State forest, from *Cupressus lusitanica*, 1985, *M.J. Wingfield* (CBS H-18012 reference strain designated here, culture CBS 909.85 = CMW 635). – USA, California, Atherton, from *Cupressus macrocarpa*, 16 Mar. 1934, *W.W. Wagener* (isotype of *Coryneum cardinale* IMI 75045).

Notes — *Seiridium cardinale* (clade 1) forms a separate cluster in the combined phylogeny with four sister clades including specimens collected from *Cupressaceae*: *S. neocupressi* (clade 2), *S. cupressi* (clade 3), *S. unicorne* (clade 4) and *S. cancrinum* (clade 5). Morphologically, *S. cardinale* is clearly distinct from members of these sister clades by its reduced basal and apical appendages. Since a culture from the correct locality is currently not available, we designated CBS 909.85 as a reference strain, which matches the characteristics of the isotype (IMI 75045). The isotype consists of two slides. One of the slides includes conidia but lacks conidiophores and conidiogenous cells. The second slide is better preserved and contains sections of the fungus in the host, forming acervuli (Fig. 6). By designating a reference strain, we aim to provide a specimen of *S. cardinale* that contains both a detailed morphological description and is represented by multiple loci in GenBank to promote consistent use in future studies until an appropriate culture is collected from *Cupressus macrocarpa* in California that can be designated as epitype to supplement the current materials.

Seiridium cupressi (Guba) Boesew., Trans. Brit. Mycol. Soc. 80: 545. 1983 — Fig. 7, 8

Basionym. Cryptostictis cupressi Guba, Monograph of *Monochaetia* and *Pestalotia*: 47. 1961.

Synonyms. *Rhynchosphaeria cupressi* Nattrass et al., Trans. Brit. Mycol. Soc. 46: 103. 1963.

Lepteutypa cupressi (Nattrass et al.) H.J. Swart, Trans. Brit. Mycol. Soc. 61: 79. 1973.

Conidia on dried PDA cultures from Jones (1953) lunate to falcate, curved, 5-septate, not striate, bearing two appendages, euseptate, (18–)22–29.5(–36) × (5–)6–8.5(–11.5) µm, mean ± SD = 25.8 ± 3.6 × 7.4 ± 1.2 µm (n = 61); basal cell obconic with a truncate base, hyaline, 1.5–6 µm; four median cells, smooth, cylindrical to doliiform, pale to dark brown (second cell from base 3–6.5 µm long; third cell 2.5–5.5 µm long; fourth cell 2.5–6 µm long; fifth cell 3–7.5 µm long); apical cell conical, hyaline, 1.5–7 µm long; appendages cylindrical, attenuated;

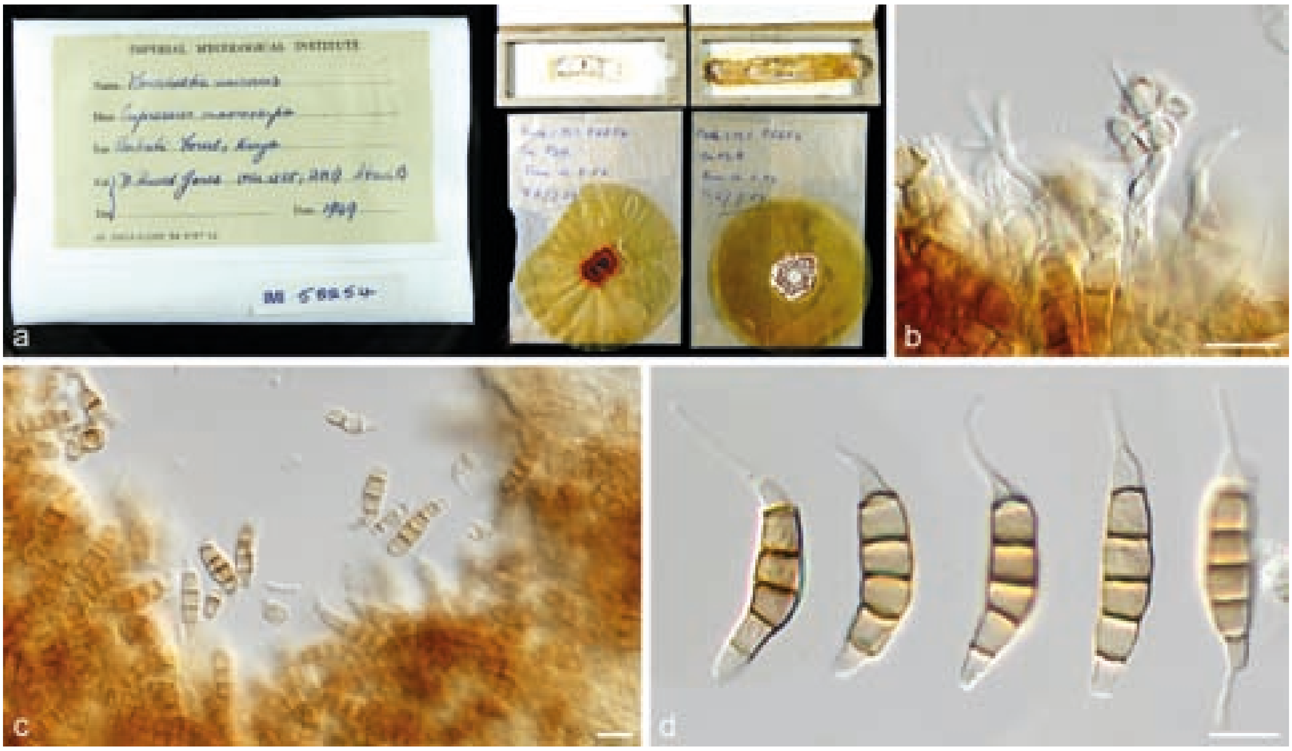

Fig. 7 *Seiridium cupressi* (IMI 52254, lectotype of *Cryptostictis cupressi*). a. Herbarium specimen; b–c. conidiogenous cells and conidia; d. conidia. — Scale bars: b–d = 10 µm.

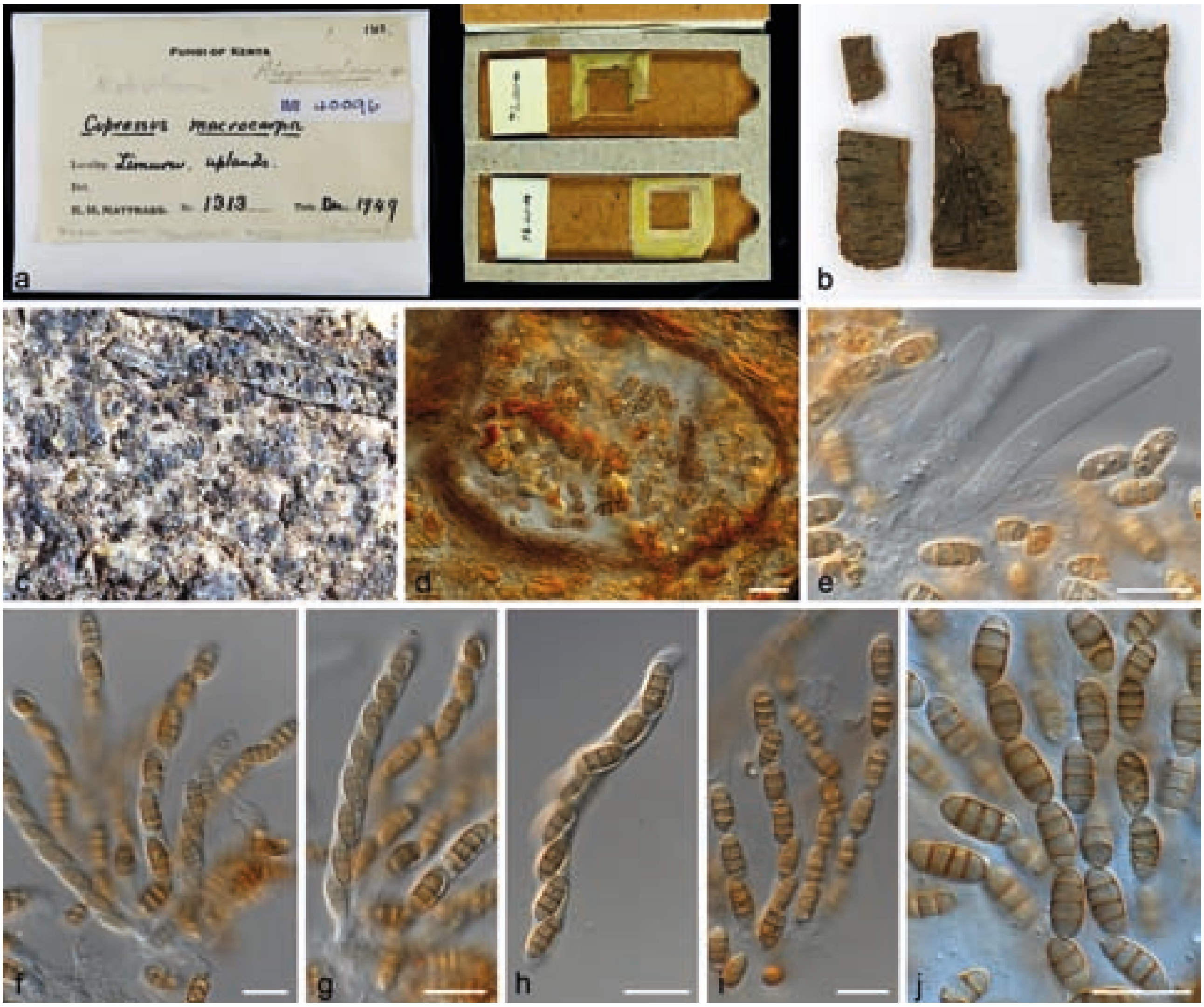

Fig. 8 *Rhynchosphaeria cupressi* (IMI 40096). a–b. Herbarium specimen; c. ascomata; d. ascoma with asci; e–j. paraphyses and asci. — Scale bars: d–j = 20 µm.

apical appendage single, centric, 0.5–9.5 µm long, occasionally branched near the tip; basal appendage consistently present, single, cylindrical, excentric, 0.5–15 µm long.

Known distribution — Greece, Kenya, Uganda.

Materials examined. Greece, from *Cupressus* sp., *A. Graniti* (CBS 122616 = CMW 1646). – Kenya, non pathogenic isolate from *Cupressus* sp., collection data unknown (CBS 320.51); on *Cupressus macrocarpa*, July 1948, *R.M. Nattrass* (IMI 37158 holotype of *Rhynchosphaeria cupressi*); on *Cupressus macrocarpa*, Dec. 1949, *R.M. Nattrass* (IMI 40096); from cankers in branches of *Cupressus macrocarpa*, 1953, *D.R. Jones* (IMI 52254 lectotype of *Cryptostictis cupressi* designated here (MBT379187), BRL 1117 isolectotype, CBS 224.55 epitype designated here (MBT379208), metabolically inactive culture, culture ex-epitype CBS 224.55); from cankers in branches of *Cupressus forbesii*, 1949, *D.R. Jones* (IMI 52255 dried culture = BRL 1118, living culture CBS 225.55).

Notes — Clade 3 of the multi-gene phylogeny (Fig. 1) is in the present study identified as *S. cupressi* based on morphological comparison of the authentic material listed in the protologue (Guba 1961). These materials include one specimen with the sexual morph on *Cupressus macrocarpa*, collected in Dec. 1949 in the 'Uplands', Kenya. Within the IMI fungarium only IMI 40096, *Lepteutypa cupressi*, carries exactly this label. The other herbarium specimen Guba (1961) cited is from the same host, collector and date, but from Nairobi instead of the Uplands, Kenya and is without mention of a sexual morph. Therefore, we conclude that IMI 40096 is authentic and the specimen from Nairobi was either not preserved, or represents the same strain (Nairobi and the Uplands are only ~ 30 km apart). In addition, Guba (1961) used dried cultures from cankers on *Cupressus* and *Juniperus*, collected by Jones in Kenya and Uganda, including the strains A, B, C and D. The IMI fungarium contains five dried cultures that were deposited by Jones (IMI 52254 from *Cupressus macrocarpa*, IMI 52255 from *C. forbesii*, IMI 52256 from *C. macrocarpa*, IMI 52257 from *Juniperus procera* and IMI 52258 from *C. macrocarpa*). Together with IMI 40096, these specimens represent the authentic material of *S. cupressi.* Cultures of the same strains were also deposited in the CBS collection and the dried materials are thus linked to the living cultures CBS 224.55, CBS 225.55, CBS 226.55, CBS 228.55 and CBS 227.55, respectively. Jones (1953, 1954a, b) studied the pathogenic characteristics of the fungus prior to the formal description of *S. cupressi* by Guba (1961), and classified the fungi in four different strain categories, based on their aggressiveness. Whereas strain A was the most aggressive pathogen to different *Cupressus* hosts and strain B a mild pathogen, strain D was only pathogenic on *Juniperus* and appeared to be saprophytic on *Cupressus*. Strain C comprised mutated clones that had arisen in culture and was never deposited in the IMI or the CBS (see Jones 1954a). To re-evaluate the species and select a lectotype, we examined the herbarium specimen collected by Nattrass (IMI 40096) as well as the dried cultures from Jones (1953, 1954a), with the exception of the specimen from Uganda (IMI 52258 = CBS 227.55). In addition, all loci used in this study were sequenced from the living cultures, which are linked to the authentic dried cultures in the IMI collection. None of the living cultures were fertile, nor were any other cultures examined in this clade (CBS 320.51 and CBS 122616). Despite thorough examination of the material from IMI 40096 only the sexual-morph was observed (Fig. 8), making it impossible to compare this strain to the other authentic material or the original description itself. Therefore, IMI 40096 could not be used for the re-evaluation of *S. cupressi.* The same applied to the holotype of *Rhynchosphaeria cupressi* (Nattrass 1963; IMI 37158), in which also no asexual-morph was found, impeding us to link the sexual-morph to the other examined specimens. Furthermore, phylogenetic analysis revealed that the remaining five specimens (the dried cultures from Jones 1953, 1954a) were distributed over three distinct clades (clades 3, 5 and 7; Fig. 1), thus constituting three different species. Morphological comparison (see Fig. 3) showed the specimens in clade 3 (CBS 224.55 = IMI 52254 and CBS 225.55 = IMI 52255) to be similar to the original description. This clade also includes the Ugandan specimen (CBS 227.55 = IMI 52258) and thus the majority of the authentic material. We therefore designate IMI 52254 as lectotype of *Cryptostictis cupressi* (basionym of *S. cupressi*), while CBS 224.55, a strain derived from the same collection is designated as epitype in order to provide a stable platform for DNA data comparisons. Because the work of Jones (1953, 1954a, b) preceded the formal description of *Seiridium cupressi*, each of the dried cultures carries the label '*Monochaetia unicornis*', including the here designated lectotype IMI 52254. The lectotype material consists of four parts; two dried slant cultures on CMA and two dried plate cultures on PDA. Similar to IMI 52255 the material is in relatively poor condition, but the features of the conidia are clearly recognizable as *S. cupressi* (Fig. 7). Cultures derived from the other two specimens, CBS 226.55 (= IMI 52256) and CBS 228.55 (= IMI 52257), clustered in clades 5 (*S. cancrinum*) and 9 (*S. kenyanum*), respectively. Furthermore, the pathogenic differences that Jones (1953, 1954a, b) identified between the strains (A, B and D) are congruent with the topology of the multi-gene phylogeny (Fig. 1), indicating the three species (*S. cancrinum*, *S. cupressi and S. kenyanum*) have different pathology. Morphologically, IMI 52257 deviates most from the other specimens and the original description by bearing considerably larger conidia. This strain was also the only isolate retrieved from *Juniperus* sp. Instead of *Cupressus* sp. Specimen IMI 52256 (*S. cancrinum*; clade 5) is morphologically more similar to the fungarium specimens of *S. cupressi* (IMI 52254 and IMI 52255; clade 3) and the original description. However, conidia generally have a longer basal appendage (mean ± SD = 5.8 ± 2.4) compared to IMI 52254 and IMI 52255 (mean ± SD = 2.0 ± 2.0 and 3.9 ± 2.6, respectively). Since the living cultures presently available are sterile, future collections from Kenya are required to add more morphological details of this fungus in culture.

Seiridium eucalypti Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia (Ontario): 862. 1993 — Fig. 9

Conidiomata on PDA sterile. On SNA erumpent sporodochia, mostly globose, solitary and compact, producing large black spore masses, sporodochia immersed in agar more aggregated and amorphous. *Conidiophores* relatively long, varying in length, scattered, septate, cylindrical, irregularly branched, hyaline, smooth- and thin-walled, occasionally reduced to conidiogenous cells, 13–85 µm long. *Conidiogenous cells* discrete, hyaline, subcylindrical to cylindrical, smooth- and thin-walled, 5–18 × 1.5–3.5 µm, percurrent proliferation rarely observed, with minute periclinal thickenings. *Conidia* lunate to falcate, straight to curved, 5-septate, not striate, bearing two appendages, euseptate with visible pores, (23.5–)27.5–33(–36) × (8–)9–10.5(–11) µm, mean ± SD = 30.3 ± 2.7 × 9.6 ± 0.7 µm (n = 32); basal cell obconic with a truncate base, hyaline, walls smooth, 4–6 µm; four median cells, smooth, cylindrical to doliiform, brown to dark brown, and septa darker than the rest of the cells (second cell from base 4.5–8 µm long; third cell 4–6.5 µm long; fourth cell 3.5–6 µm long; fifth cell 4.5–7 µm long); apical cell conical, hyaline, smooth, 2.5–6 µm long; apical appendage single, centric, 8.5–15.5 µm long; basal appendage, single, cylindrical, centric and excentric, 6.5–10 µm long.

Culture characteristics — Colonies on PDA irregular, reaching 15–20 mm diam after 14 d at 22 °C, flat at centre, white to pale luteous-coloured at the centre, slightly raised at the margin, white-coloured, with abundant aerial mycelium, not sporulating. On CMA almost circular, reaching 37–42 mm diam after 14 d at 22 °C, flat at centre and margin, white-coloured, with abun-

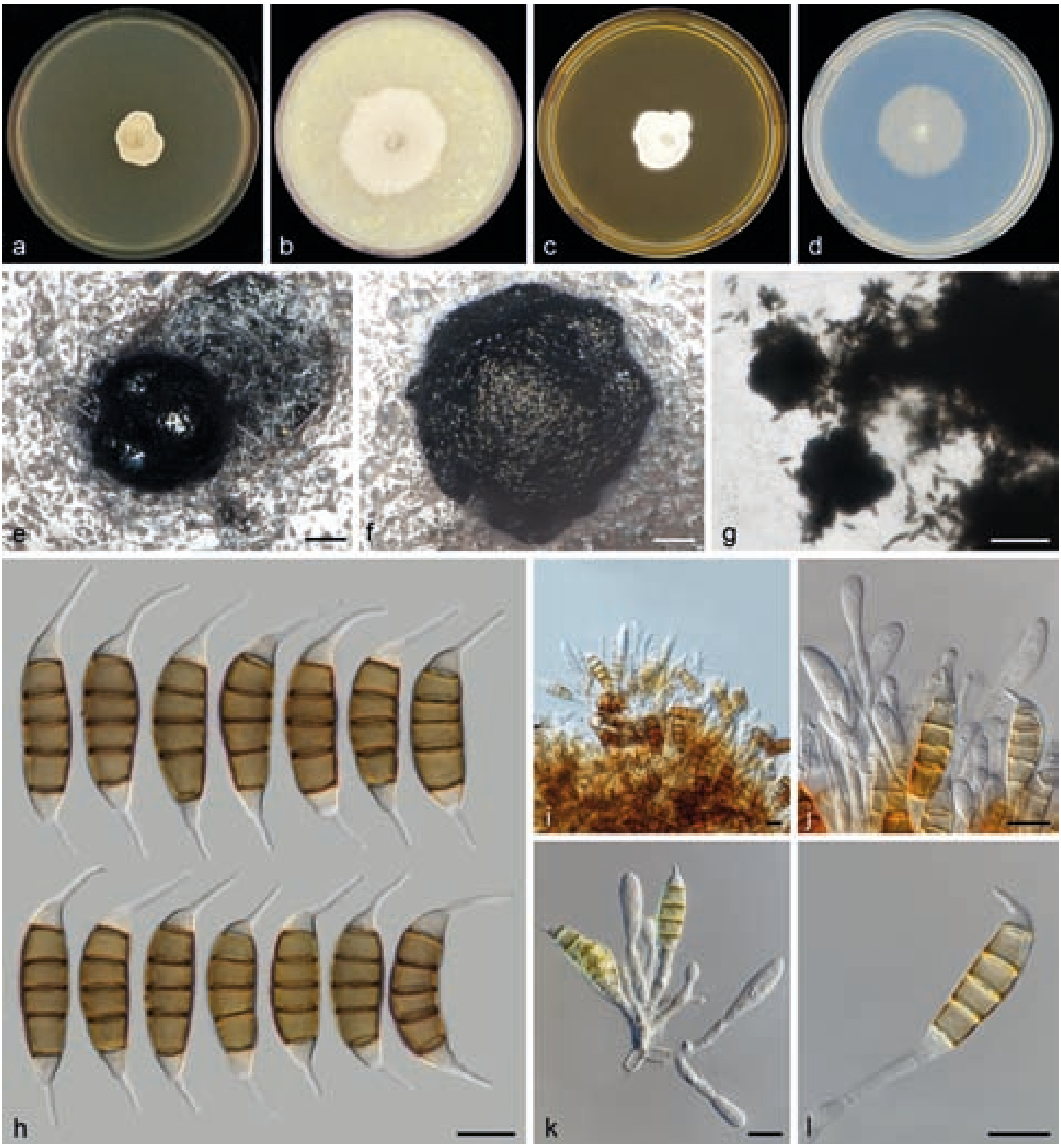

Fig. 9 *Seiridium eucalypti* (CBS 343.97, culture ex-epitype). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e–g. sporodochia on SNA; h. conidia; i–j. conidiophores; k. conidiophore; l. conidiogenous cell. — Scale bars: e–g = 100 µm; h–l = 10 µm.

dant aerial mycelium, sporulating poorly and not within 4 wk, pycnidioid sporodochia producing black spore masses. On MEA irregular, reaching 21–25 mm diam after 14 d at 22 °C, flat at centre and margin, white-coloured, with abundant aerial mycelium, no sporulation. On SNA circular, reaching 33–35 mm diam after 14 d at 22 °C, flat at centre and margin, white-coloured, abundant aerial mycelium, sporulation after 2 wk with scattered compact sporodochia.

Known distribution — Continental Australia, Tasmania.

Materials examined. Australia, South Australia, Adelaide, Mt Lofty Summit, on leaves of *Eucalyptus* sp., 16 Oct. 1979, *B. Kendrick* (holotype DAOM 215255); Tasmania, on *Eucalyptus delegatensis*, 13 Sept. 1996, *Z.Q. Yuan* (epitype designated here CBS H-23145 (MBT379188), ex-epitype culture CBS 343.97).

Notes — *Seiridium eucalypti* (clade 10 in Fig. 1) forms a separate clade in the combined phylogeny, sister to *S. pseudocardinale* and *S. kenyanum* (clades 8 and 9, respectively). Morphologically, *S. eucalypti* is characterised by conidia with distinctively long appendages, in particular the apical appendage (Fig. 3). Other *Seiridium* spp. isolated from *Eucalyptus* spp. (including *S. kartense* sp. nov. and *S. papillatum*) are not monophyletic in the combined phylogeny, nor in any of the single-locus phylogenies (Fig. 2). *Seiridium eucalypti* is known to inflict lesions on a wide range of *Eucalyptus* spp. Its pathogenicity to members of *Eucalyptus* was studied in detail by Yuan & Old (1995) and Yuan & Mohammed (1997, 1999, 2001). The strain examined in this work (CBS 343.97) matches the protologue of *S. eucalypti* (Nag Raj 1993), and is therefore designated as epitype.

Seiridium kartense Bonthond, Sandoval-Denis & Crous, *sp. nov.* — MycoBank MB823297; Fig. 10

Etymology. Named after the location where the fungus was collected which is known as 'Kangaroo Island' and by aboriginals from the mainland of Australia as *Karta*, meaning 'Island of the dead'.

Conidiomata on SNA sporodochial, globose to amorphous, solitary, immersed, 160–300 µm diam. *Conidiophores* short and compact, septate, cylindrical, irregularly branched, hyaline, smooth- and thin-walled, 27–44 µm long. *Conidiogenous cells* discrete, hyaline, cylindrical to globose, smooth- and thin-walled, 5.4–11.5 × 2.1–3.5 µm, proliferating multiple times percurrently, with minute periclinal thickenings and collarets visible, occasionally polyblastic. *Conidia* lunate to falcate, curved, 5-septate, not striate, bearing two appendages, euseptate with visible pores, (23.5–)27.5–30.5(–32) × (6.5–)7.5–8.5(–9) µm, mean ± SD = 28.5 ± 1.8 × 8.0 ± 0.4 µm (n = 38); basal cell obconic with a truncate base, hyaline, walls smooth, 4–5.5 µm; four median cells, smooth, cylindrical to doliiform, dark brown (second cell from base 5.5–6.5 µm long; third cell 5–5.5 µm long; fourth cell 4.5–5 µm long; fifth cell 4.5–5.5 µm long); apical cell conical, hyaline, smooth, 3–4 µm long; appendages cylindrical, attenuated, unbranched, slightly spathulate; apical appendage single, centric, 6.5–8.5 µm long; basal appendage consistently present, single, cylindrical, excentric, 6–8.5 µm long.

Culture characteristics — Colonies on PDA circular, reaching 56–59 mm diam after 14 d at 22 °C, flat, from centre to margin; salmon- to umber- to white-coloured, with moderate aerial mycelium, no sporulation. On CMA circular, reaching 65–68 mm diam after 14 d at 22 °C, flat to slightly crateriform, at the centre salmon-coloured, at the margin white-coloured, with abundant aerial mycelium, no sporulation. On MEA circular, reaching 42–44 mm diam after 14 d at 22 °C, raised, ochreous- and honey-coloured at the centre and white-coloured at the margin, with dense aerial mycelium, no sporulation. On

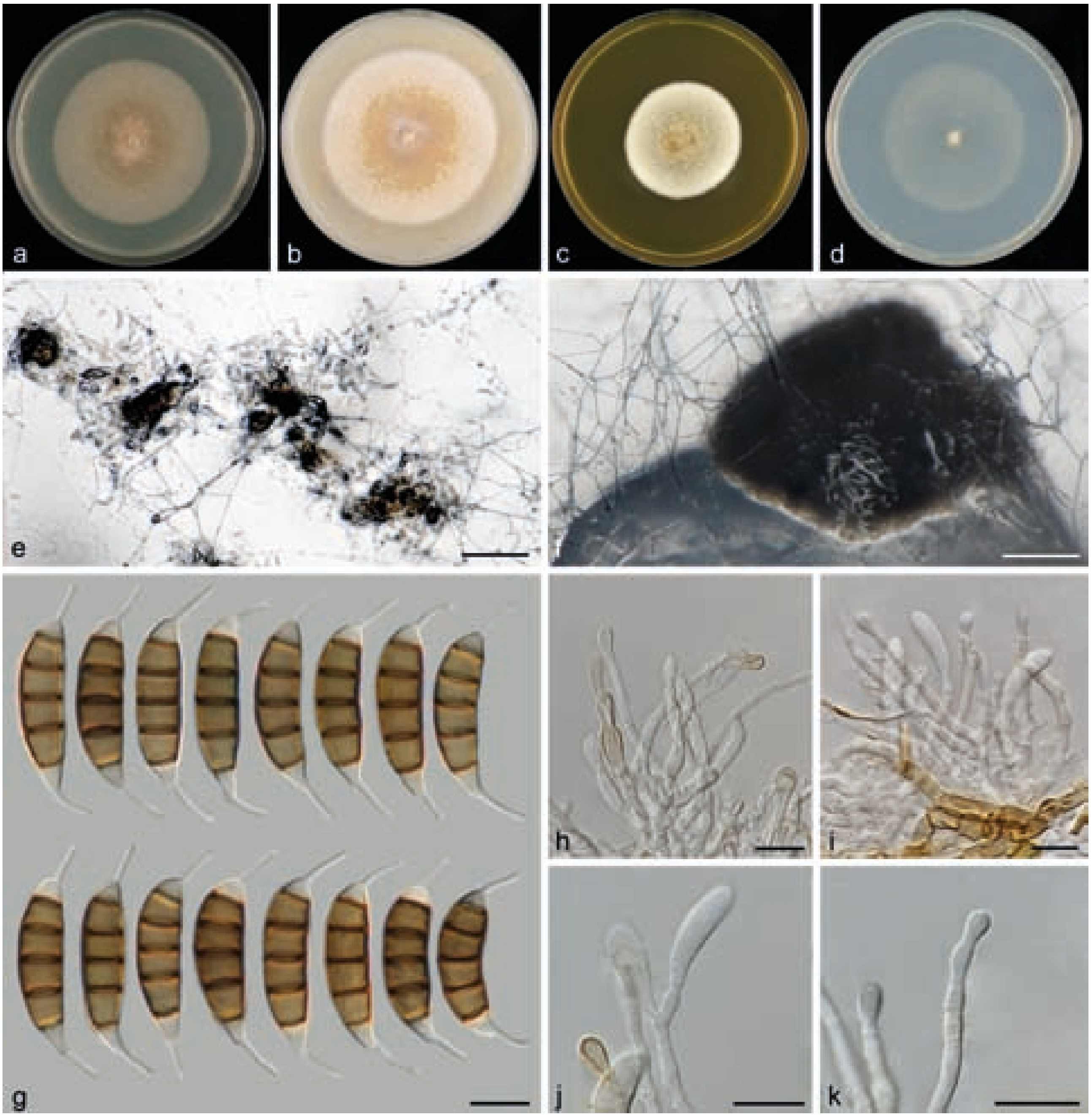

Fig. 10 *Seiridium kartense* sp. nov. (CBS 142629, culture ex-holotype). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e. sporodochia on SNA immersed in agar; f. sporodochia on SNA erumpent from agar; g. conidia; h–i. conidiophores; j. polyblastic conidiogenesis; k. conidiogenous cell proliferating percurrently showing multiple collarettes. — Scale bars: e–f = 100 µm; g–k = 10 µm.

SNA circular, reaching 51–54 mm diam after 14 d at 22 °C, flat, white-coloured, with moderate areal mycelium more densely produced at the margin, sporulation after 2 wk with scattered compact erumpent sporodochia in the centre.

Known distribution — Kangaroo Island, Australia.

Material examined. Australia, Kangaroo Island, on leaves of *Eucalyptus cladocalyx*, 15 Dec. 2012, *W. Quaedvlieg* (holotype CBS H-23146, ex-type culture CBS 142629 = CPC 20183).

Notes — *Seiridium kartense* forms a monotypic clade (clade 11 in Fig. 1), and was collected from Kangaroo Island (Australia) on *Eucalyptus cladocalyx*. *Seiridium eucalypti* (Nag Raj 1993; clade 10 in Fig. 1) and *S. papillatum* (Yuan & Mohammed 1997; clade 17 in Fig. 1) have both also been collected from Australia on *Eucalyptus* spp. Morphologically, *S. kartense* differs from *S. eucalypti* based on its conidial length and length of its apical appendage (Fig. 2), which are in both cases shorter than those of *S. eucalypti*. *Seiridium papillatum* (see Yuan & Mohammed 1997) is different from *S. kartense* by its shorter appendages.

Seiridium kenyanum Bonthond, Sandoval-Denis & Crous, *sp. nov.* — MycoBank MB823301; Fig. 11

Etymology. Named after the country where it was isolated, Kenya.

Conidia lunate to falcate, curved, 5-septate, not striate, bearing two appendages, euseptate with no visible pores, (20–)22–26(–30.5) × (7–)7.5–9(–10) µm, mean ± SD = 24.0 ± 2.1 × 8.2 ± 0.7 µm (n = 33); basal cell obconic with truncate base, hyaline, walls smooth, bearing marginal frills, 1–6 µm; four median cells, varying in colour ranging from pale to dark brown, smooth, cylindrical to doliiform (second cell from base 3–5.5 µm long; third cell 3–5 µm long; fourth cell 3.5–5.5 µm long; fifth cell 3.5–6.5 µm long); apical cell conical, hyaline, smooth, 2–4.5 µm long; apical appendage single, centric, 0.5–13 µm; basal appendage, single, cylindrical, centric and excentric, 0.5–14.5 µm.

Materials examined. Kenya, from cankers in branches of *Juniperus procera*, 1951, *D.R. Jones* (holotype IMI 52257, culture ex-type CBS 228.55); BRL 1121 isotype.

Notes — Phylogenetic analysis and morphological examination of IMI 52257 (= CBS 228.55) revealed this strain to be different from *S. cupressi* (see notes on *S. cupressi*). Jones (1953, 1954a, b) classified this fungus as *Monochaetia unicornis* strain D and already determined that it was ecologically different from *Seiridium cupressi* and *S. cancrinum* (*Monochaetia unicornis* strain B and *M. unicornis* strain A, respectively) by being non-pathogenic to *Cupressus* spp. The source of this fungus was *Juniperus procera*, on which it was a pathogen and induced cankers. Furthermore, its conidia deviate from *S. cupressi* and *S. cancrinum* by being considerably larger.

The fungus appears to be genetically highly similar to the sterile cultures CBS 122613 and CBS 122614, as well as to the type of *S. pseudocardinale.* However, the conidia bear long appendages, and are morphologically clearly different from the latter taxon, as this fungus is characterised by lacking or having reduced appendages (Wijayawardene et al. 2016). Since only an ITS sequence is available for *S. pseudocardinale*, it is likely that the short distance in the phylogenetic tree between the two species is a result of the limited ability of the ITS rDNA region to delineate species within *Seiridium* (Fig. 2a but see also Viljoen et al. 1993, Barnes et al. 2001, Maharachchikumbura et al. 2015).

Seiridium neocupressi Bonthond, Sandoval-Denis & Crous, *sp. nov.* — MycoBank MB823299; Fig. 12

Etymology. neo- cupressi denotes the fact that this phylogenetic clade has been repeatedly incorrectly assumed to represent *S. cupressi*.

Conidiomata on PDA pycnidioid sporodochial, globose to amorphous, in some strains exuding hyaline mucus, solitary, immersed. On SNA sporodochial, amorphous, immersed in agar. *Conidiophores* compact, septate, cylindrical, irregularly branched, hyaline, thin-walled, smooth, 13–160 µm long, in some strains conidiophores are intermingled with unbranched, hyaline, paraphyses-like hyphae, up to 115 µm long. *Conidiogenous cells* discrete, hyaline, cylindrical to globose, sometimes elongated, smooth- and thin-walled, (4–)3.5–24(–65) × (1.5–)2–3(–4) µm, with numerous percurrent proliferations. *Conidia* lunate to falcate, curved, 5-septate, not striate, bearing

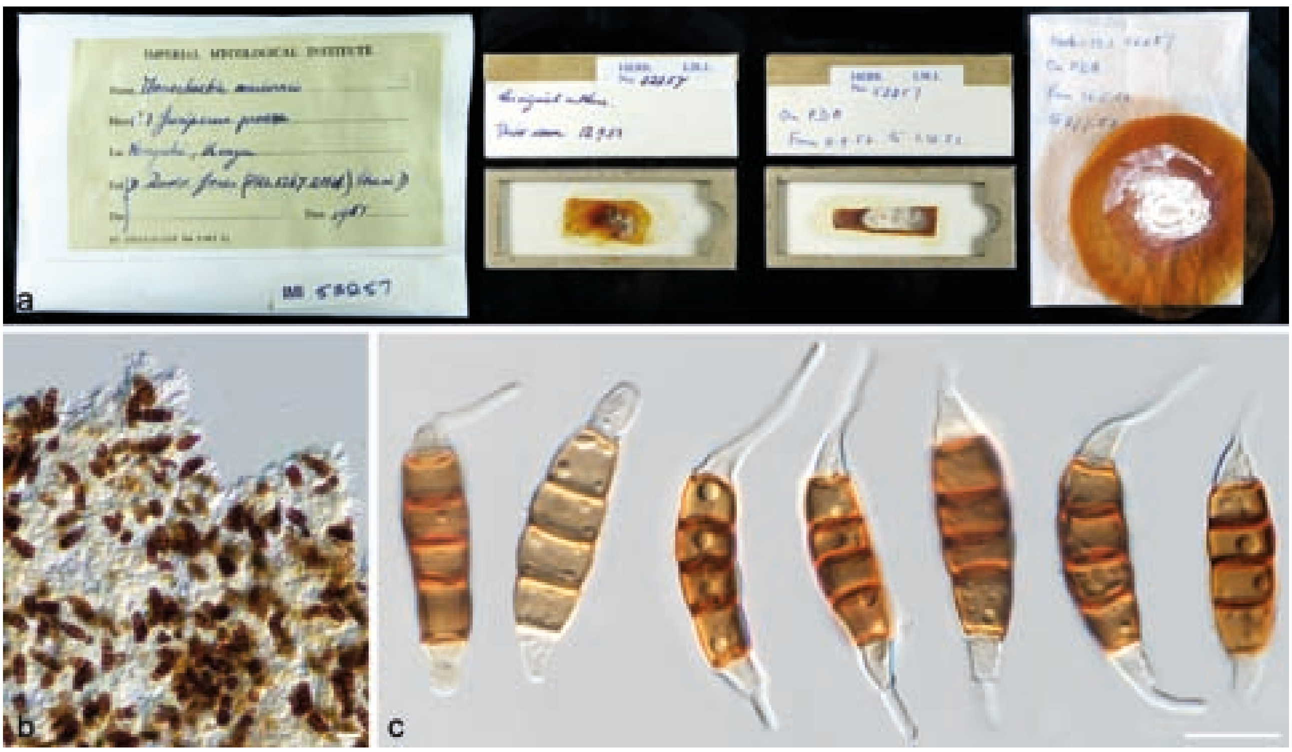

Fig. 11 *Seiridium kenyanum* (IMI 52257, holotype). a. Herbarium specimen; b–c. conidia. — Scale bars: b–c = 10 µm.

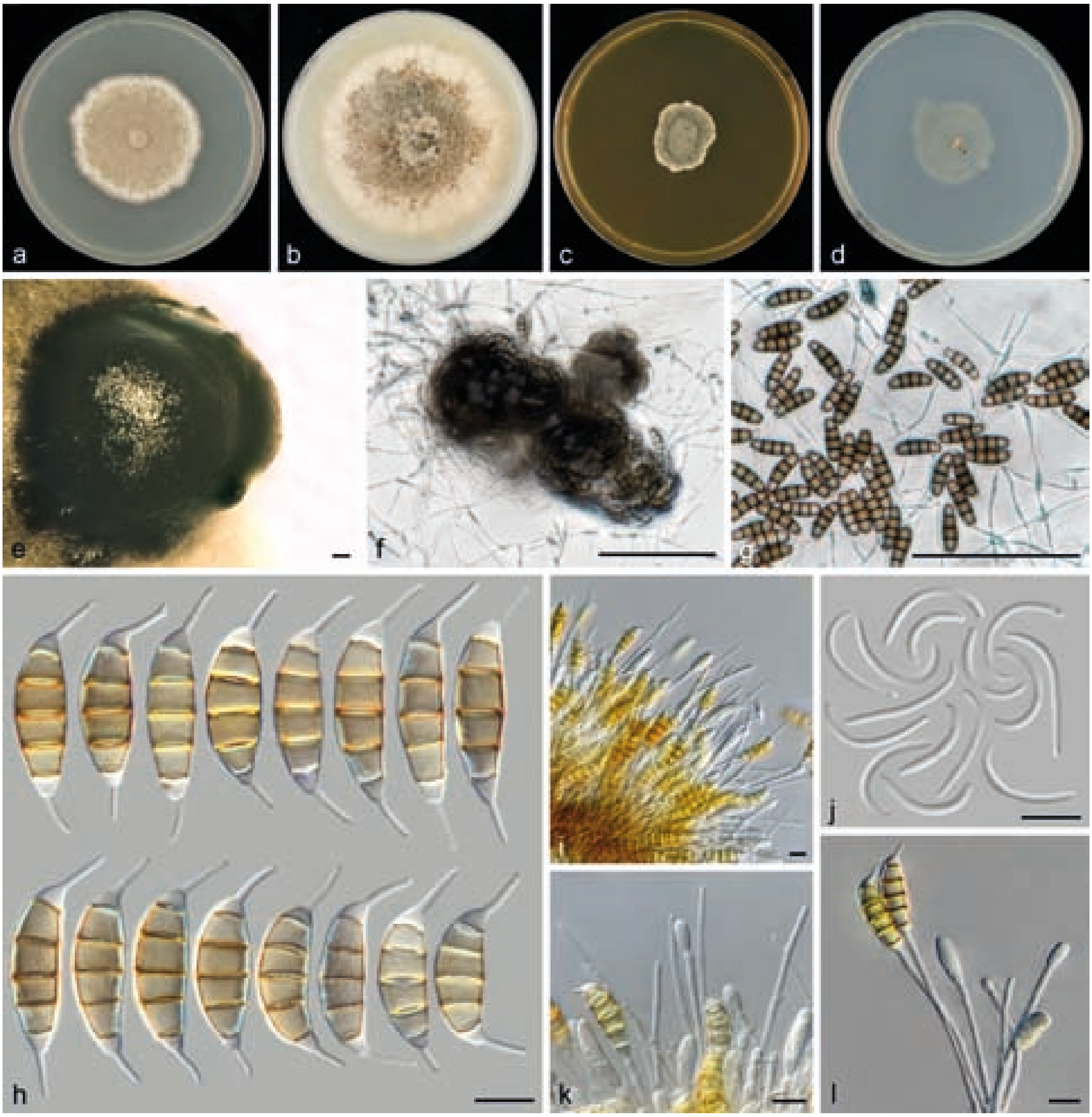

Fig. 12 *Seiridium neocupressi* sp. nov. (CBS 142625, culture ex-holotype). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e. conidioma on PDA; f–g. sporodochia on SNA immersed in agar; h. conidia; i. conidiophores and paraphyses-like structures; j. microconidia (spermatia) on PDA; k–l. conidiophores. — Scale bars: e–g = 100 µm; h–l = 10 µm.

two appendages, euseptate without visible pores, (21–)26.5–31(–35.5) × (7–)8.5–10(–10.5) µm, mean ± SD = 28.7 ± 2.3 × 9.2 ± 0.6 µm (n = 94); basal cell obconic with a truncate base, hyaline, 2.5–5 µm; four median cells, smooth, cylindrical to doliiform, pale to brown (second cell from base 2.5–7 µm long; third cell 4–6.5 µm long; fourth cell 4–6.5 µm long; fifth cell 3.5–6.5 µm long); apical cell conical, hyaline, 2.5–5 µm long; appendages cylindrical, attenuated; apical appendage single, centric, 6.5–12 µm long, occasionally branched near the tip; basal appendage consistently present, single, cylindrical, centric and excentric, 3.5–10.5 µm long. *Microconidia* or *spermatia*, when produced only on CMA and PDA, cylindrical, hyaline, on PDA (14.5–)18.5–24(–26) µm long.

Culture characteristics — Colonies on PDA circular to irregular, reaching 43–55 mm diam after 14 d at 22 °C, flat at the centre and flat to elevated at the margin, white to pale luteous, sometimes with a white outer ring, with abundant aerial mycelium, sporulating poorly and not within 2 wk with black, scattered pycnidioid conidiomata. On CMA circular, reaching 51–68 mm diam after 14 d at 22 °C, flat at centre and margin to slightly crateriform, buff to saffron with hazel coloured patches in the centre, buff to white coloured at the margin, with moderate aerial mycelium, sporulating after at least 4 wk with black, pycnidioid conidiomata. On MEA irregular, reaching 19–30 mm diam after 14 d at 22 °C, elevated at the centre, flat at the margin, buff to pale green, with aerial mycelium on the surface, no sporulation. On SNA circular to rhizoid, reaching 19–49 mm diam after 14 d at 22 °C, flat, with moderate aerial mycelium, sporulation after a few days with black sporodochial conidiomata.

Known distribution — Australia, Italy.

Materials examined. Australia, Victoria, Torquay, from *Cupressus leylandi*, 18 Dec. 2006, *A. Hoffert* (CBS H-23149, culture CBS 142627 = CPC 28351 = VPRI 40665). – Italy, Bari, from *Cupressus sempervirens*, 23 Nov. 2007, *unknown collector* (CBS H-23148, culture CBS 142626 = CPC 23789); Bari, from *Cupressus sempervirens*, 23 Nov. 2007, *unknown collector* (holotype CBS H-23147, ex-type culture CBS 142625 = CPC 23786).

Notes — The morphology of *S. neocupressi* bears similarity to the original description of *S. cupressi* and to the authentic strains that Jones (1953, 1954a) designated as 'strain B'. Strains CMW 420, CMW 5282, VPRI 15696, VPRI 16083, VPRI 32740, VPRI 40658 and VPRI 40665 were for this reason originally identified as *S. cupressi* (Barnes et al. 2001, Cunnington 2007, Tsopelas et al. 2007). However, although in the combined phylogenetic analysis the strains A, B and D from which *S. cupressi* was described clustered in genetically different clades (3, 5 and 9 in Fig. 1), none clustered within clade 2, and thus this clade is distinct from isolates representing *S. cupressi.* Morphologically, conidia are slightly longer and wider and bear longer basal appendages in comparison to *S. cancrinum* and *S. cupressi.*

Seiridium phylicae Crous & M.J. Wingf., Persoonia 29: 187. 2012

Description and illustration — Crous et al. (2012).

Known distribution — UK, Overseas territory of Saint Helena, Ascension, Tristan da Cunha islands.

Material examined. UK, Saint Helena, Ascension and Tristan da Cunha, Inaccessible Island, Blenden Hall, from stems of *Phylica arborea*, Sept. 2011, *P.G. Ryan* (holotype CBS H-21089, ex-type cultures CBS 133587 = CPC 19964, CPC 19962, CPC 19965, CPC 19970).

Notes — *Seiridium phylicae* was described and treated in detail in Crous et al. (2012). Similar to *S. spyridicola*, this fungus was isolated from a plant host in the *Rhamnaceae*. Given the sole isolation of this fungus from the Tristan da Cunha archipelago, it seems to be geographically isolated. The species is a problematic pathogen to the Tristan da Cunha endemic *Phyilica arborea* (Ryan et al. 2014).

Seiridium pseudocardinale Wijayaw. et al., Fung. Diversity 77: 248. 2016

Description and illustration — Wijayawardene et al. (2016).

Known distribution — Italy, Portugal.

Materials examined. Portugal, from *Cupressus* sp, collection date unknown, *A. Graniti* (CBS 122613 = CMW 1648); from *Cupressus* sp., collection date unknown, *A. Graniti* (CBS 122614 = CMW 1649).

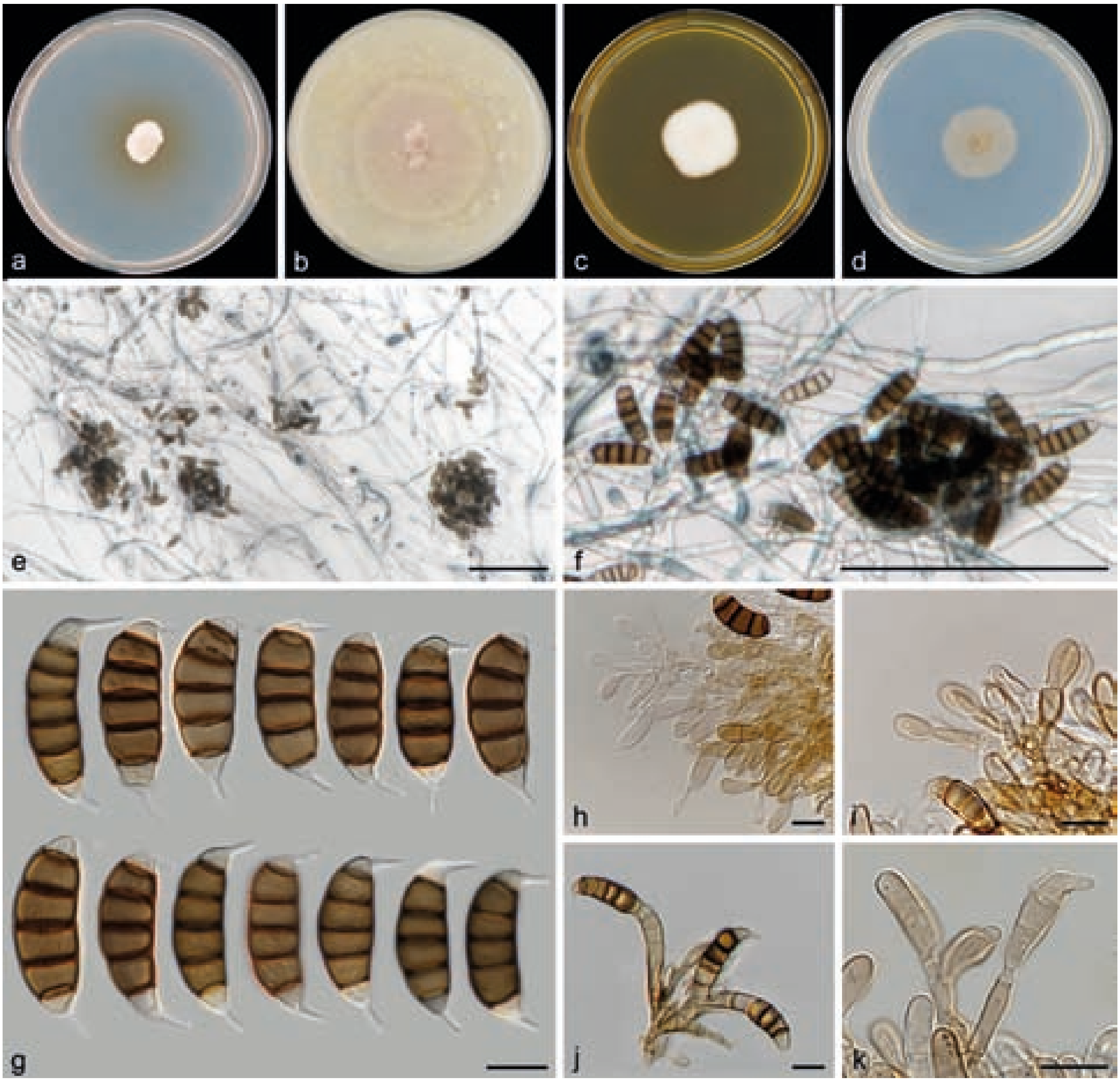

Fig. 13 *Seiridium spyridicola* sp. nov. (CBS 142628, culture ex-holotype). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e–f. sporodochia on SNA immersed in agar; g. conidia; h–j. conidiophores; k. conidiogenous cell. — Scale bars: e–f = 100 µm; g–k= 10 µm.

Notes — *Seiridium pseudocardinale* was recently described by Wijayawardene et al. (2016). In GenBank, only an ITS sequence is available for the type specimen (MFLUCC 13-0525) and thus in the combined phylogeny (Fig. 1) the other loci are missing data for this species. Given the limited resolution on the ITS region alone (Fig. 2a), its phylogenetic placement should thus be interpreted with care. This fungus was isolated from *Cupressus glabra* in Italy and based on ITS sequence seems to be closely related to cultures CBS 122612 and CBS 122613, which were isolated from *Cupressus* species in Portugal, and CBS 228.55 (= IMI 52257; *S. kenyanum* sp. nov.). However, morphologically, the fungus is different from IMI 52257 by the lack of conidial appendages and the poor delineation from CBS 228.55 is likely a result of the absence of informative loci. Since none of the two cultures from Portugal sporulated, we were unable to investigate this clade morphologically and compare it with the description in the protologue. To resolve the phylogenetic relation of *S. pseudocardinale* to *S. kenyanum* and the sterile cultures CBS 122613 and CBS 122614, sequence data of at least *TUB*, but preferably *RPB2* and *TEF* are required from the type (MFLUCC 13-0525).

Seiridium spyridicola Bonthond, Sandoval-Denis & Crous, *sp. nov.* — MycoBank MB823300; Fig. 13

Etymology. Named after the host genus, *Spyridium*, from which it was isolated.

Conidiomata on PDA sporodochial, solitary or aggregated, immersed in agar, dark brown to black. *Conidiophores* septate, cylindrical, irregularly branched, hyaline, smooth- and thin-walled, 9–52 µm long. *Conidiogenous cells* discrete, hyaline, subcylindrical to globose, smooth- and thin-walled, 5.5–12 × 2–4 µm, proliferating multiple times percurrently, with minute periclinal thickenings. *Conidia* straight to falcate, straight to slightly curved, 5-septate, sporadically 6-septate, not striate, bearing two appendages, euseptate, pores visible, (22–)24–28.5(–33.5) × (4.5–)8–10.5(–11) µm, mean ± SD = 26.4 ± 2.3 × 9.1 ± 1.2 µm (n = 31); basal cell obconic with a truncate base, hyaline, walls smooth, bearing minute marginal frills, 3–4.5 µm long; four median cells, smooth, doliiform, brown to dark brown, septa darker than the rest of the cells (second cell from base 5–6 µm long; third cell 4.5–5.5 µm long; fourth cell 4.5–5.5 µm long; fifth cell 4.5–5.5 µm long); apical cell conical, hyaline,

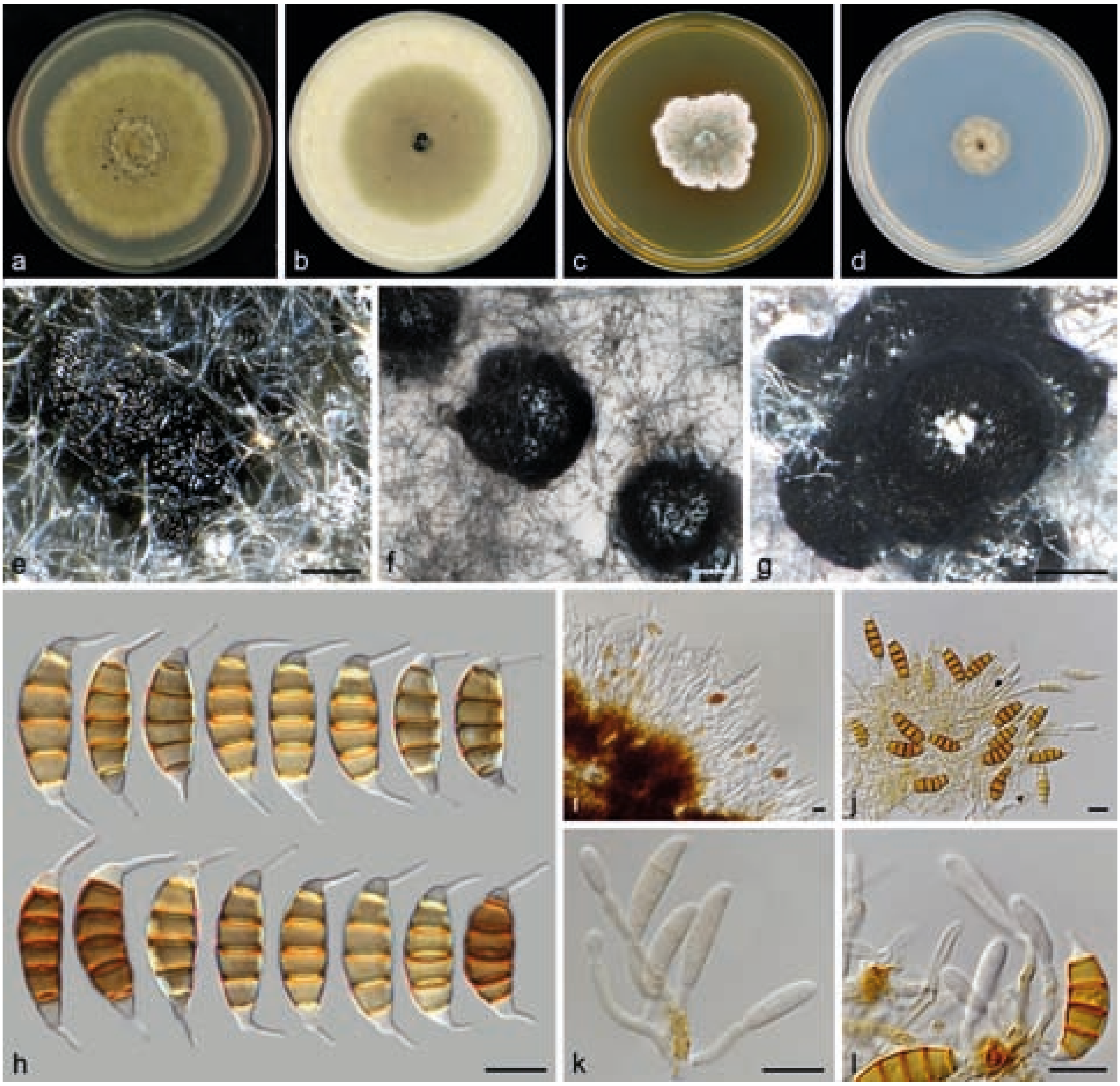

Fig. 14 *Seiridium unicorne* (CBS 538.82, reference strain). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e. conidioma on PDA erumpent from agar partially covered with mycelium; f–g. sporodochia on SNA erumpent from agar; h. conidia; i–j. conidiophores produced from sporodochia; k. conidiophore; l. conidiogenous cells — Scale bars: e–g = 100 µm; h–l = 10 µm.

smooth, 2.5–4 µm long; apical appendage single, excentric and typically oriented perpendicular to conidium, cylindrical, often spatulate, 4–6.5 µm; basal appendage single, cylindrical, often spatulate, mostly centric, 3.5–5.5 µm.

Culture characteristics — Colonies on PDA irregular, reaching 12–16 mm diam after 14 d at 22 °C, flat, from centre to margin, white-coloured, with dense aerial mycelium, sterile. On CMA circular, reaching 46–50 mm diam after 14 d at 22 °C, flat to slightly umbonate, white-coloured, with abundant aerial mycelium on the surface in the centre, mycelium at the margin immersed in agar, sterile. On MEA circular to irregular, reaching 27–29 mm diam after 14 d at 22 °C, flat, white-coloured, with dense aerial mycelium, sterile. On SNA circular, reaching 22–27 mm diam after 14 d at 22 °C, slightly umbonate, cinnamon-coloured at the centre, cinnamon- to white-coloured at the margin, with moderate areal mycelium, sporulation within 2 wk with scattered sporodochia around the centre.

Known distribution — Australia.

Material examined. Australia, Western Australia, from *Spyridium globosum*, 19 Sept. 2015, *P.W. Crous* (holotype CBS H-23150, ex-type culture CBS 142628 = CPC 29108).

Notes — This species was isolated from *Spyridium globosum* (*Rhamnaceae*) and in the combined phylogeny forms a basal lineage to clades 1 to 6, including most of the *Cupressaceae* pathogens as well as *S. phylicae* (Crous et al. 2012), which was isolated from *Phylica arborea*, a host also belonging to the *Rhamnaceae*. Conidia are, in comparison to the other studied *Seiridium* spp. of average length, but the appendages are notably shorter and the apical appendage is typically oriented perpendicular to the long axis of the conidium.

Seiridium unicorne (Cooke & Ellis) B. Sutton, Mycol. Pap. 138: 74. 1975 — Fig. 14, 15

Basionym. Pestalotia unicornis Cooke & Ellis, Grevillea 7: 6. 1878.
Synonym. *Monochaetia unicornis* (Cooke & Ellis) Sacc. & D. Sacc., Syll. Fung. 18: 485. 1906.

Conidiomata on PDA pycnidial to sporodochial, globose or clavate, mostly solitary, erumpent from agar, partially immersed in mycelium, producing large black spore masses. On SNA, sporodochial, mostly aggregated, erumpent from agar, producing large black spore masses. *Conidiophores* septate, cylindrical, relatively long and irregularly branched, brown or colourless, thin-walled, 29–68 µm long. *Conidiogenous cells* discrete, hyaline, cylindrical, smooth- and thin-walled, 3.5–29.5 × 1.5–3 µm, proliferating percurrently, with visible collarettes and minute periclinal thickenings. *Conidia* lunate to falcate, curved, 5-septate, rarely 4- or 6-septate, not striate, bearing two appendages, euseptate with no visible pores, (21.5–)23.5–26.5(–27.5) × (7–)8–9(–9.5) µm, mean ± SD = 24.9 ± 1.6 × 8.5 ± 0.6 µm (n = 31); basal cell obconic with a truncate base, hyaline, walls smooth, bearing minute marginal frills, 3–5.5 µm; four median cells, varying in colour from pale to dark brown, smooth, cylindrical to doliiform (second cell from base 3.5–5.5 µm long; third cell 3.5–5 µm long; fourth cell 4–5.5 µm long; fifth cell 4–5.5 µm long); apical cell conical, hyaline, smooth, 2–5 µm long; apical appendage single, mostly centric, 5–10 µm; basal appendage, single, cylindrical, mostly excentric, 4–6 µm long.

Culture characteristics — Colonies on PDA circular, reaching 65–68 mm diam after 14 d at 22 °C, slightly umbonate, citrine to olivaceous coloured, with aerial mycelium abundant on the surface, sporulating in between centre and margin of the colony within 2 wk. On CMA circular, reaching 58–59 mm diam after 14 d at 22 °C, flat at the centre and margin, citrine to olivaceous coloured, with moderate aerial mycelium on the surface, sporulating in the centre within 2 wk. On MEA irregular, reaching 35–40 mm diam after 14 d at 22 °C, flat to crateriform, slightly sunk into the agar, malachite green coloured at the centre becoming white at the margin with saffron spots or areas in between centre and margin, with dense mycelium on the surface, not sporulating. On SNA circular, reaching 20–21 mm diam after 14 d at 22 °C, umbonate, with aerial mycelium at the surface, sporulation within 2 wk.

Known distribution — New Zealand, South Africa, USA.

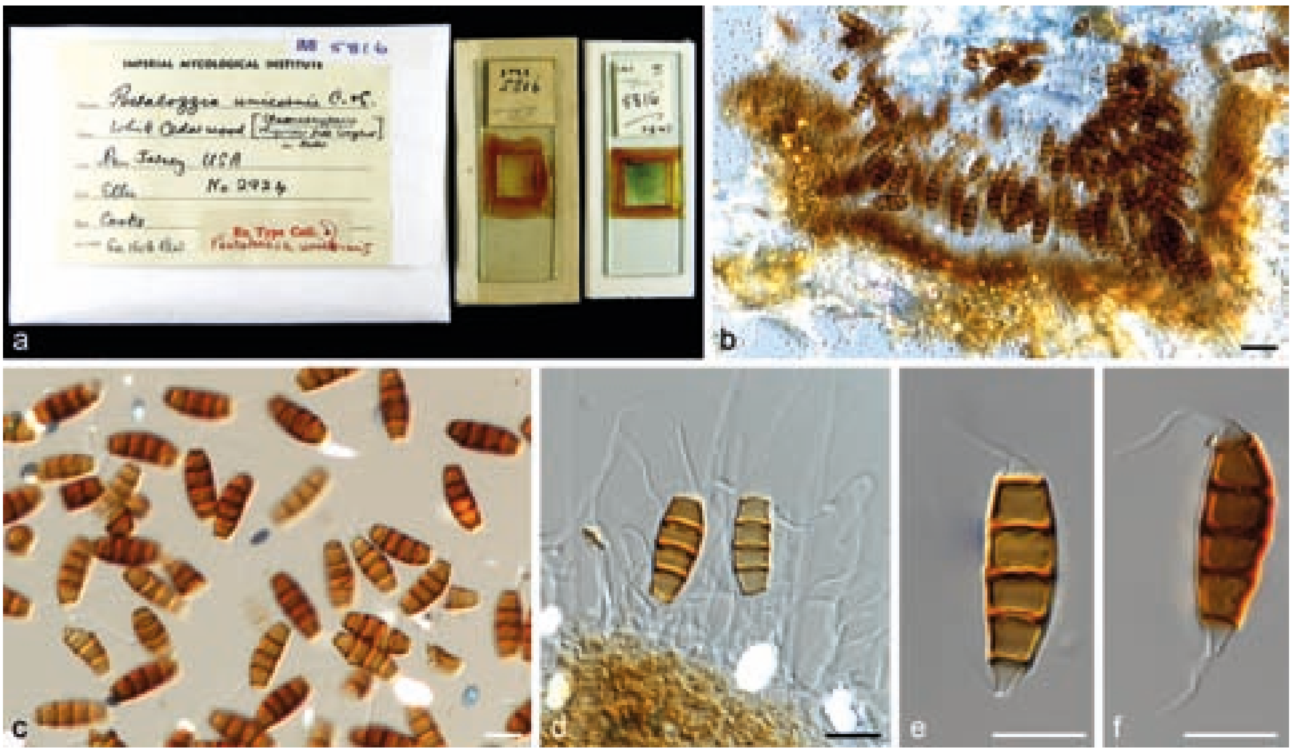

Fig. 15 *Seiridium unicorne* (IMI 5816, holotype of *Pestalozzia unicornis*). a. Herbarium specimen; b. conidiomata and conidia *in vivo*; c–f. conidiogenous cells and conidia *in vitro*. — Scale bars: b = 20 µm; c–f = 10 µm.

Materials examined. New Zealand, from *Cryptomeria japonica*, 1981, *H.J. Boesewinkel* (CBS H-23151 reference strain designated here, culture CBS 538.82 = CPC 23783 = IFO 32684). – South Africa, from *Cupressus sempervirens*, 1999, *I. Barnes* (culture CBS 120306 = CMW 5596). – USA, New Jersey, associated with *Chamaecyparis thyoides*, 1878, *J.B. Ellis* (IMI 5816 holotype of *Pestalotia unicornis*).

Notes — Culture CBS 120306 remained sterile on all media tested. The holotype material of *S. unicorne* (IMI 5816) consists of two slides. One of the slides includes conidia, but conidiophores and conidiogenous cells are not discernible, while the second slide contains sections of the fungus in host tissue (Fig. 15). Conidia were produced by CBS 538.82 which were morphologically, apart from slightly longer basal appendages (mean ± SD = 5.35 ± 1.14 opposed to 2.75 ± 2.10), highly similar to the examined holotype material from *P. unicornis* (see Fig. 3). Despite the highly similar morphological characters, both host and geographic origin of CBS 538.82 (*Cryptomeria japonica* and South Africa) are different from the holotype which was collected from *Cupressus sempervirens* in New Jersey. We therefore designate CBS 538.82 as reference strain of *S. unicorne*, providing a new description of the species and linking DNA sequence data to the morphological description. *Pestalozzia unicornis* (Cooke & Ellis 1878) was placed in *Seiridium* by Sutton (1980) and the typically broad host range has traditionally been regarded as an important character of the species. As described by Guba (1961), *S. unicorne* was found on the *Cupressaceae* genera *Chamaecyparis*, *Cupressus* and *Juniperus*, but also on *Anacardiaceae* (*Rhus*), *Caprifoliaceae* (*Lonicera*), *Hamamelidaceae* (*Hamamelis*), *Rosaceae* (*Crataegus*, *Malus*, *Rosa*) and *Vitaceae* (*Vitis*). Given that these observations preceded the use of DNA sequence data, it cannot be ruled out that these observations included additional cryptic species.

SPECIES NOT EXAMINED

Seiridium camelliae Maharachch. & K.D. Hyde, Mycol. Progr. 14. 85. 2015

Description and illustration — Maharachchikumbura et al. (2015).

Known distribution — China.

Notes — *Seiridium camelliae* was isolated from *Camellia reticulata* (*Theaceae*) and was described and treated in detail in Maharachchikumbura et al. (2015). In the phylogeny of the concatenated alignment (Fig. 1) as well as the single-locus phylogenies of ITS, *TEF and TUB* (Fig. 2) *S. camelliae* resolved as a distinct lineage, closely related to *S. podocarpi*, which was isolated from *Podocarpus latifolus* (*Podocarpaceae*).

Seiridium ceratosporum (De Not.) Nag Raj, Coelomycetous anamorphs with appendage-bearing conidia (Ontario): 859 (1993)

Basionym. Stilbospora ceratospora De Not., Mem. Reale Accad. Sci. Torino Ser. 2, 3: 67. 1841.

Synonyms. *Monochaetia ceratospora* Guba, Monograph of *Monochaetia* and *Pestalotia*: 50. 1961.

Pestalotia ceratospora (De Not.) Arx, The genera of fungi sporulating in pure culture: 226. 1981.

Description and illustration — Nag Raj (1993).

Known distribution — China.

Notes — *Seiridium ceratosporum* was first described as *Stilbospora ceratospora* by De Notaris (1841). Nag Raj (1993) examined holotype material and transferred it to *Seiridium*, providing a re-description of the fungus. Presently, DNA sequence data of the type is not available and the strain included in our analyses (PHSI2001Pathcw07) represents the only strain labelled *S. ceratosporum* for which multiple sequences are available (including ITS and *TUB*) and is from Liu et al. (2007) who did not examine morphological characters. Similarly, the strain was included in the ITS phylogeny in Maharachchikumbura et al. (2015) without morphological examination. PHSI2001Pathcw07 was isolated from *Vitis vinifera*, in contrast to the type which is from *Pyrus malus.* Therefore, it remains uncertain whether, and perhaps even unlikely, this strain truly is *S. ceratosporum*. Nonetheless, both single-locus and combined phylogenies indicate it is most closely related to *S. papillatum*, but separate and could thus be a yet undescribed species in *Seiridium* as well. Morphological comparison of available or newly collected strains to the protologue (Nag Rag 1993) is required to confirm the identity of this clade as *S. ceratosporum*.

Seiridium marginatum Nees, Syst. Pilze (Würzburg): 23. 1817

Synonyms. Coryneum marginatum (Nees) Fr., Syst. Mycol. 3: 473. 1832.

Massaria marginata Fuckel, Jahrb. Nassauischen Vereins Naturk. 27–28: 28. 1874.

Blogiascospora marginata (Fuckel) Shoemaker, E. Müll. & Morgan-Jones, Canad. J. Bot. 44: 248. 1966.

Description and illustration — Jaklitsch et al. (2016).

Known distribution — Austria, France, Germany, Switzerland.

Notes — *Seiridium marginatum* was recently epitypified and treated in detail by Jaklitsch et al. (2016).

Seiridium papillatum Z.Q. Yuan, Austral. Syst. Bot. 10: 70. 1997

Description and illustration — Yuan & Mohammed (1997).

Known distribution — Australia.

Notes — *Seiridium papillatum* was described and treated in detail by Yuan & Mohammend (1997). Sequence data for LSU, *TUB* and *HIS* was generated from ex-type material by Barnes et al. (2001). Additional sequence data was acquired in the present work (ITS, *RPB2* and *TEF*).

Seiridium podocarpi Crous & A.R. Wood, Persoonia 32: 251. 2014

Description and illustration — Crous et al. (2014).

Known distribution — South Africa.

Notes — *Seiridium podocarpi* was described and treated in detail by Crous et al. (2014).

Seiridium venetum (Sacc.) Nag Raj, Mycotaxon 35: 293. 1989

Basionym. *Pestalotia veneta* Sacc., Michelia 1: 92. 1877.

Synonyms. *Pestalotia corni* Allesch., Bot. Zbl. 42: 106. 1890.

Monochaetia veneta (Sacc.) Sacc. & D. Sacc., Syll. Fung. (Abellini) 18: 485. 1906.

Seiridium corni (Allesch.) B. Sutton, Canad. J. Bot. 47: 2091. 1969.

Descriptions and illustrations — Nag Raj (1989), Maharachchikumbura et al. (2015).

Known distribution — Italy.

Notes — *Seiridium venetum* was re-described and transferred to *Seiridium* by Nag Raj (1989). Maharachchikumbura et al. (2015) examined the holotype and designated a reference strain (MFLU 15-0396), of which *ITS* and *TUB* sequences are included in the combined phylogeny (Fig. 1).

DISCUSSION

The primary character distinguishing *Seiridium* (Nees 1817) from other pestalotioid genera in the *Sporocadaceae* (Corda 1842) is its five-septate conidia. For instance the four-septate *Pestalotiopsis*, and six-septate *Truncatella*, have in the past been accommodated together with *Seiridium* in the genus *Pestalotia* (De Notaris 1841), which underwent many taxonomic rearrangements (Steyaert 1949, Guba 1956, 1961, Sutton 1969, 1980), and now remains a monotypic genus with the type species *P. pezizoides* from *Vitis vinifera*. Similar to *Seiridium*, *P. pezizoides* produces five-septate conidia and might be a synonym of *Seiridium* (Maharachchikumbura et al. 2014). Conidial appendages of *P. pezizoides* are branched, which is also typical for *S. corni*, *S. indicum* and *S. venetum* (Nag Raj 1993), and the host is not unique, as *Seiridium* spp. have also been isolated from *Vitis* (Guba 1961). New collections of *P. pezizoides* are required to select an epitype and generate DNA sequence data to investigate whether *Pestalotia* is indeed a synonym of *Seiridium.*

Guba (1961) synonymized *Seiridium* and *Monochaetia* and argued to conserve *Monochaetia* over the older name *Seiridium*, as the name had been applied more commonly. Shoemaker (1966), however, provided evidence that multiple morphological characters distinguished the two genera from each other. The two names have subsequently been treated as separate genera (Sutton 1980, Nag Raj 1993). Species in *Monochaetia* share the character of six-celled conidia with *Seiridium* but typically only bear a single apical appendage. Recent phylogenetic analyses including the species *Monochaetia kansensis* support the separate identity of the genus (Maharachchikumbura et al. 2014, 2015). However, DNA sequence data linked to the type *M. monochaeta* has so far not been generated and is essential to provide conclusive evidence on the validity of *Monochaetia* and its relation to *Seiridium* and other genera in the *Sporocadaceae*.

Previous studies have provided phylogenies of *Seiridium* using ITS, *TUB* and histone protein H3 (*HIS*) sequences (Viljoen et al. 1993, Barnes et al. 2001, Cunnington 2007, Tsopelas et al. 2007, Maharachchikumbura et al. 2015, Wijayawardene et al. 2016). As evident from those studies that used solely ITS as phylogenetic marker and in the ITS tree presented in this study (Fig. 2a) this locus provides insufficient information for species delineation within *Seiridium.* Besides an overall lack of bootstrap support in the phylogeny of this locus alone, *S. cardinale* does not group under a single node and certain strains (CBS 123911, CBS 523.82 and CPC 23789) cluster with *S. eucalypti*, *S. kenyanum* and *S. pseudocardinale*. In addition to the limited delineation at the species level, separation at the generic level is also problematic, which is reflected in the ITS phylogeny where the outgroup strain *Seimatosporium rosae* (CBS 139823) clusters within *Seiridium*. Similarly, in the ITS phylogeny of Maharachchikumbura et al. (2015) *Seiridium* was not monophyletic. Barnes et al. (2001) showed regions of *TUB* and *HIS* to successfully separate *Seiridium* species. In subsequent studies (Cunnington 2007, Tsopelas et al. 2007) the use of *TUB* as a species marker was continued and as a result, *TUB* is at the moment the best available locus in GenBank and has sufficient resolution for delineating species in *Seiridium* currently known by sequence data. For sequence based species identification of *Seiridium* isolates this locus is therefore recommended. In addition to ITS and *TUB*, we sequenced *TEF* and *RPB2*, of which the phylogenies (Fig. 2a, b) show these loci are also informative for delineation of *Seiridium* at the species level. The phylogeny produced from the concatenated alignment, represents the currently most complete phylogeny of *Seiridium*. However, for many species DNA sequence data have not yet been generated. Our analysis comprises 12 of the 39 names recorded in Index Fungorum and MycoBank for which sequence data are presently available (17 of 44 names, including the novel species).

Apart from re-describing and designating a reference strain for *S. cardinale* (CBS 909.85 from *Cupressus lusitanica*), we provide a new description of *S. unicorne* with reference strain (CBS 538.82 from *Cryptomeria japonica*). Both species still require an epitype. However, by selecting a reference strain, descriptions of *S. cardinale* and *S. unicorne* are linked to DNA sequences in GenBank. From examination of the authentic materials in combination with the multi-gene phylogeny (Fig. 1), we have concluded that the original concept of *Cryptosticis cupressi* (basionym of *S. cupressi*) was based on three distinct *Seiridium* species. One of those species retains the name *S. cupressi* and the application of this name is here fixed by selection of both a lectotype and epitype. The other two species are introduced as *S. cancrinum* and *S. kenyanum*. These three species match the different pathogenic strains (strains A, B and D) Jones (1953, 1954a, b) identified, of which *S. cancrinum* is the most aggressive, and *S. cupressi* mildly aggressive. *Seiridium kenyanum* is only pathogenic to *Juniperus* (Jones 1953, 1954a, b).

Interestingly, *S. kenyanum* is more distantly related to the other *Cupressaceae* pathogens and seems to be related to the recently described *S. pseudocardinale* (Wijayawardene et al. 2016). This fungus, isolated from *Cupressus glabra* in Italy, was named after *S. cardinale* because of the morphological similarity, having reduced to absent conidial appendages. Morphologically, this is somewhat surprising given the large appendages observed for *S. kenyanum.* However, since only an ITS sequence is available for *S. pseudocardinale*, the poor delineation of *S. kenyanum* from *S. pseudocardinale* is most likely a result of the limited genetic resolution based on the ITS marker (Viljoen et al. 1993). Sequence data of at least *TUB*, but ideally also *TEF* and *RPB2* of *S. pseudocardinale* are needed to resolve its phylogenetic position in the genus and relation to *S. kenyanum*.

In addition to *S. cancrinum*, *S. cupressi* and *S. kenyanum* we introduce *S. neocupressi*, which represents a lineage previously classified as *S. cupressi* (Barnes et al. 2001, Cunnington 2007, Tsopelas et al. 2007). Morphologically, conidia and appendages of *S. neocupressi* are longer compared to the genetically related *S. cupressi*, *S. unicorne* and *S. cancrinum*. *Seiridium neocupressi* includes canker pathogens primarily from Australia and New Zealand (Barnes et al. 2001, Cunnington 2007, Tsopelas et al. 2007), and most likely represents the most important causal agent of cypress canker in this region.

In summary, the present work provides a re-evaluation of the genus *Seiridium*. We re-described both *S. cardinale* and *S. unicorne*, designating a reference strain for each. Importantly, we examined the authentic material of *S. cupressi* and sequenced four DNA loci of culture material linked to the holotypes. This resulted in the selection of a lecto- and epitype for *S. cupressi* and the introduction of two novel species, *S. cancrinum* and *S. kenyanum*. Therewith, this work resolves the longstanding confusion surrounding the species *S. cupressi*. Our analyses included four loci and provide a phylogenetic basis for the genus, including 17 of 44 currently accepted species in *Seiridium.* We show that in contrast to the ITS region, *RPB2*, *TEF* and *TUB* provide sufficient information for species delineation and recommend future work identifying or introducing species in *Seiridium* to prioritize the use of *TUB* over that of the ITS region.

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