



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

G. Bonthond¹, M. Sandoval-Denis^{1,2}, J.Z. Groenewald¹, P.W. Crous^{1,3,4}

Key words

appendage-bearing conidia
canker pathogen
Cupressus
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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 (Natrass & 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 Natrass 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*

¹ Westerdijk Fungal Biodiversity Institute, P.O. Box 85167, 3508 AD Utrecht, The Netherlands;
corresponding author e-mail: p.crous@westerdijkinstituut.nl.

² Faculty of Natural and Agricultural Sciences, Department of Plant Sciences, University of the Free State, P.O. Box 339, Bloemfontein 9300, South Africa.

³ Department of Microbiology and Plant Pathology, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, Pretoria, South Africa.

⁴ WUR, Laboratory of Phytopathology, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands.

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 re-examined 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. phyllicae* was described (Crous et al. 2012), which is an aggressive pathogen of *Phyllica 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 \pm 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 μ L 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	ITS	RPB2	TEF	TUB
<i>Seiridium camelliae</i>	MFLUCC 12-0647 ^T	<i>Camellia reticulata</i>	Theaceae	China	12	JQ683725	–	JQ683741	JQ683709
<i>S. cancrinum</i>	IMI 52256 [†] ; BRL 1119; CBS 226.55 CBS 907.85; CMW 320	<i>Cupressus macrocarpa</i> <i>Cupressus lusitana</i>	Cupressaceae Cupressaceae	Kenya South Africa	5	LT853089 LT853090	LT853137 LT853138	LT853186 LT853187	LT853236 LT853237
<i>S. cardinale</i>	CBS 123911 CBS 122612; CMW 2133 CBS 172.56	<i>Cupressus sempervirens</i> unknown unknown	Cupressaceae unknown unknown	Morocco Chile unknown	1	LT853057 LT853058 LT853059	LT853106 LT853107 LT853108	LT853154 LT853155 LT853156	LT853204 LT853205 LT853206
	CBS 280.93; CPC 23780 CBS 522.82; CPC 23784; NBRC 32683 CBS 523.82; CPC 23793 CBS 908.85; CPC 23779; CMW 616 CBS 909.85 ^R ; CMW 635 CBS 910.85; CPC 23781; CMW 600; CMW 638 CMW 1644	<i>Cupressus sp.</i> <i>Cupressus sempervirens</i> <i>Cupressocyparis sp.</i> <i>Cupressus lusitana</i> <i>Cupressus sempervirens</i> <i>Cupressus sp.</i>	Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae	Italy New Zealand New Zealand South Africa South Africa South Africa		LT853060 LT853061 LT853062 LT853063 LT853064 LT853065	LT853109 LT853108 LT853109 LT853110 LT853111 LT853112 LT853113 LT853114	LT853157 LT853158 LT853159 LT853160 LT853161 LT853162	LT853207 LT853208 LT853209 LT853210 LT853211 LT853212
	CMW 1645 CMW 18602 CMW 18603 CMW 18604 CMW 18605 CMW 18606 CMW 18794	<i>Cupressus sp.</i> <i>Cupressus sempervirens</i> <i>Cupressus macrocarpa</i> <i>Juniperus foetidissima</i> <i>Juniperus foetidissima</i> <i>Juniperus exelsa</i> <i>Cupressus sempervirens</i>	Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae	Italy Greece Greece Greece Greece Greece Greece		– – – – – – –	– – – – – – –	– – – – – – –	AF320497 AF320501 DQ926974 DQ926975 DQ926973 DQ926976 DQ926978 DQ926977
	CPC 23785 CPC 23787 CPC 23788 CPC 23790 CPC 23791 CPC 23792 CPC 23794 CPC 23795 CPC 23796 CPC 23797 CPC 23798 CPC 28286 CPC 28287	<i>Cupressus sempervirens</i> <i>Juniperus phoenicea</i> <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i> unknown unknown <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i>	Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae unknown unknown Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae	Greece Greece Italy Italy Italy Italy Greece Greece Italy Italy Italy Spain Spain		LT853066 LT853067 LT853068 LT853069 LT853070 LT853071 LT853072 LT853073 LT853074 LT853075 LT853076 LT853077 LT853078	LT853115 LT853116 LT853117 LT853118 LT853119 – LT853120 LT853121 LT853122 LT853123 LT853124 LT853125 LT853126	LT853163 LT853164 LT853165 LT853166 LT853167 – LT853168 LT853169 LT853170 LT853171 LT853172 LT853173 LT853174 LT853175	LT853213 LT853214 LT853215 LT853216 LT853217 LT853218 LT853219 LT853220 LT853221 LT853222 LT853223 LT853224 LT853225 DQ534043
<i>S. ceratoporum</i>	PHSI2001Pathcw07	<i>Vitis vinifera</i>	Vitaceae	China	16	AY687314	–	–	–
<i>S. cupressi</i>	CBS 122616; CMW 1646 IMI 52254 [†] ; BRL 1117; CBS 224.55 ^{ET} IMI 52255; BRL 1118; CBS 225.55 IMI 52258; BRL 1120; CBS 227.55 CBS 320.51 CMW 18607	<i>Cupressus sp.</i> <i>Cupressus macrocarpa</i> <i>Cupressus forbesii</i> <i>Cupressus macrocarpa</i> <i>Cupressus sp.</i> <i>Cupressus sempervirens</i>	Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae	Greece Kenya Kenya Uganda Kenya Greece	3	LT853082 LT853083 LT853084 LT853085 LT853086	LT853130 LT853131 LT853132 LT853133 LT853134	LT853179 LT853180 LT853181 LT853182 LT853183	LT853229 LT853230 LT853231 LT853232 LT853233
	CBS 343.97 ^{ET} ; CMW 5303 CBS 142629 [†] ; CPC 20183 IMI 52257 [†] ; BRL 1121; CBS 228.55 CBS 140403 ST CMW 420 CMW 5282; ATCC 48158 CBS 142625 [†] ; CPC 23786 CBS 142626; CPC 23789 CBS 142627; CPC 28351 VPRI 15696 VPRI 16083 VPRI 32740 VPRI 40658 VPRI 40665	<i>Eucalyptus delegatensis</i> <i>Eucalyptus cladocalyx</i> <i>Juniperus procera</i> <i>Rosa canina</i> <i>Cupressus macrocarpa</i> <i>Cupressus sp.</i> <i>Cupressus sempervirens</i> <i>Cupressus sempervirens</i> <i>Cupressus leylandii</i> <i>Cupressus sp.</i> <i>Cupressus sp.</i> <i>Cupressus sp.</i> <i>Cupressus sp.</i> <i>Cupressus sp.</i>	Myrtaceae Myrtaceae Rosaceae Rosaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae Cupressaceae	Australia Australia Kenya France New Zealand New Zealand Italy Italy Australia Australia Australia Australia Australia Australia	10 11 9 15 2	LT853099 LT853100 LT853098 KT949914 FJ430600 LT853079 LT853080 LT853081	LT853146 LT853147 LT853145 LT853149 – – LT853127 LT853128 LT853129	LT853196 LT853197 LT853195 LT853199 – – LT853176 LT853177 LT853178	LT853246 LT853247 LT853245 LT853249 – – LT853226 LT853227 LT853228

Table 1 (cont.)

Species	Collection accession No. ¹	Host/Substrate	Host family	Location	Clade No. ²	ITS	RPB2	TEF	TUB
<i>S. papillatum</i>	CBS 340.97 [†] ; VPRI 20827; CMW 5302	<i>Eucalyptus delegatensis</i>	Myrtaceae	Australia	17	LT853102	LT853150	LT853200	LT853250
<i>S. phyllicae</i>	CBS 133587 [†] ; CPC 19964	<i>Phyllica arborea</i>	Rhamnaceae	Tristan da Cunha	6	LT853091	LT853139	LT853188	LT853238
	CPC 19962	<i>Phyllica arborea</i>	Rhamnaceae	Tristan da Cunha		LT853092	LT853140	LT853189	LT853239
	CPC 19965	<i>Phyllica arborea</i>	Rhamnaceae	Tristan da Cunha		LT853093	LT853141	LT853190	LT853240
	CPC 19970	<i>Phyllica arborea</i>	Rhamnaceae	Tristan da Cunha		LT853094	–	LT853191	LT853241
<i>S. podocarp</i>	CBS 137995 [†] ; CPC 23429	<i>Podocarpus latifolius</i>	Podocarpaceae	South Africa	13	LT853101	LT853148	LT853198	LT853248
<i>S. pseudocardinale</i>	MFLUCC 13-0525 [†]	<i>Cupressus arizonica</i>	Cupressaceae	Italy	8	KU848210	–	–	–
	CBS 122613; CMW 1648	<i>Cupressus</i> sp.	Cupressaceae	Portugal		LT853096	LT853143	LT853193	LT853243
	CBS 122614; CMW 1649	<i>Cupressus</i> sp.	Cupressaceae	Portugal		LT853097	LT853144	LT853194	LT853244
<i>S. spyridicola</i>	CBS 142628 [†] ; CPC 29108	<i>Spyridium globosum</i>	Rhamnaceae	Australia	7	LT853095	LT853142	LT853192	LT853242
<i>S. unicomre</i>	CBS 120306; CMW 5596	<i>Cupressus sempervirens</i>	Cupressaceae	South Africa	4	LT853087	LT853135	LT853184	LT853234
	CBS 538.82 [†] ; NBRC 32684; CMW 5443	<i>Cryptomeria japonica</i>	Cupressaceae	New Zealand		LT853088	LT853136	LT853185	LT853235
<i>S. venetum</i>	MFLU 15-0369 [†]	<i>Cornus mas</i>	Cornaceae	Italy	14	KT438836	–	–	KT438837
<i>Neopestalotopsis protearum</i>	CBS 114.178 [†]	<i>Leucospermum cuneiforme</i>	Proteaceae	Zimbabwe	Outgroup	LT853103	LT853151	LT853201	LT853251
<i>Bartalinia robillardoides</i>	CBS 122705 [†]	<i>Leptoglossus occidentalis</i>	Coreidae (Animalia)	Italy	Outgroup	LT853104	LT853152	LT853202	LT853252
<i>Salmatosporium rosae</i>	CBS 139823 [†] ; MFLUCC 14-0621	<i>Rosa</i> 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, CAB International, Egham, Surrey, England; MFLU: Mae Fah Luang University, Chiang Rai, Thailand; NBRC: National Institute of Technology and Evaluation, Kisarazu-shi, Chiba, Japan; PHST: from Liu et al. (2007); VPR: 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- α ; TUB: Beta-tubulin.

[†] type or ex-type; [‡] ex-epitype; [§] lectotype; [¶] 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'-GGGGWGAYCAGAAAGAAGGC-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 (Kato et al. 2002, Kato & 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 1000 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 1000 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 2863 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.

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 Tsoelas 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. phyllicae* (clade 6; Crous et al. 2012); CBS 142628, isolated from *Spyridium globosum* in Australia

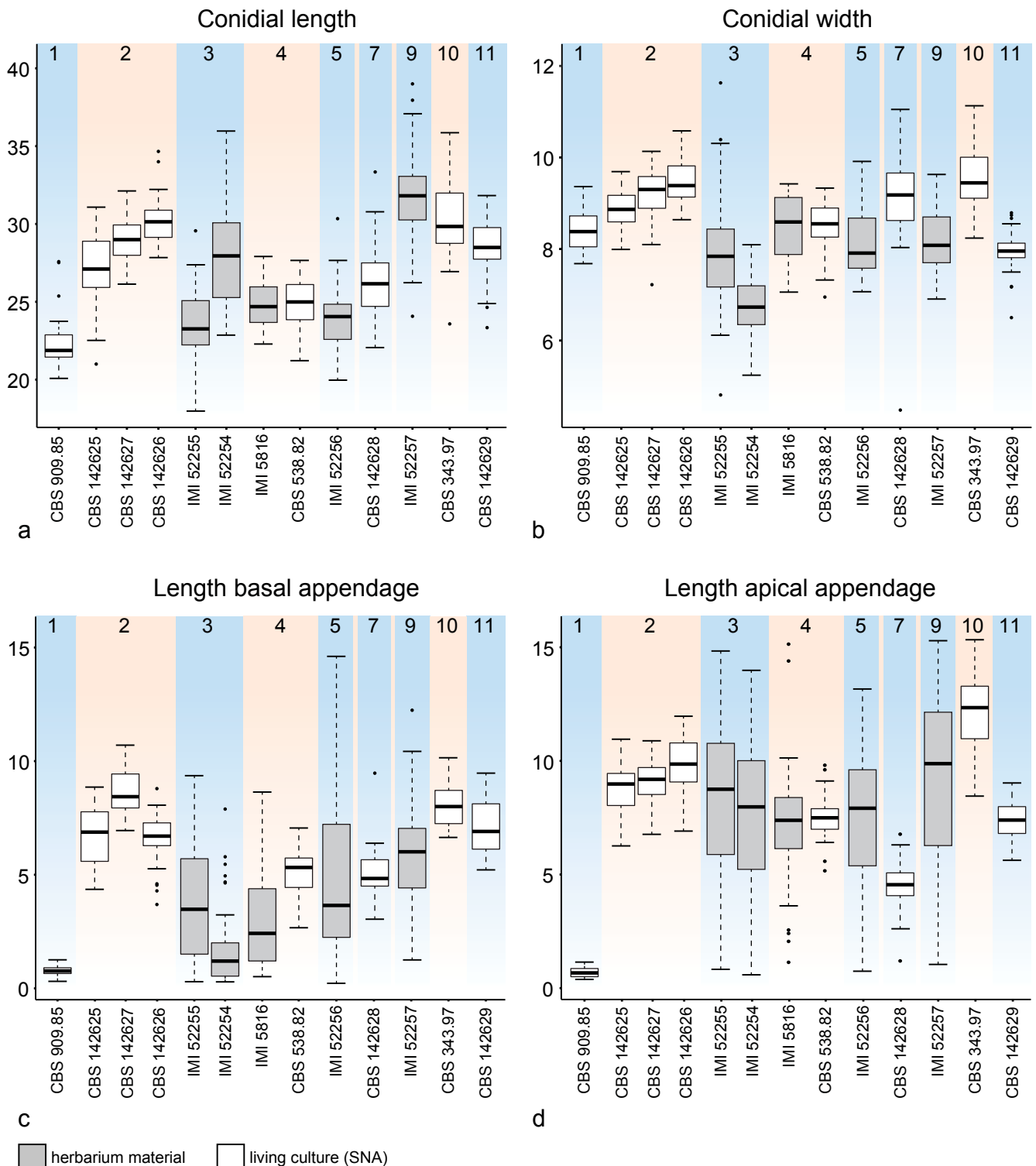


Fig. 3 Boxplots reflecting the distribution of variance in conidial measurements in micrometres as acquired from living culture material on SNA (in white) or from herbarium material (in grey). The boxes show the first and third quartiles. Lower and upper whiskers extend from the boxes to the extreme 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 pycnidoid, 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 doliform (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 \times 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) \times (7.5–)8–9(–9.5) μm , mean \pm SD = 22.3 \pm 1.8 \times 8.4 \pm 0.4 μm

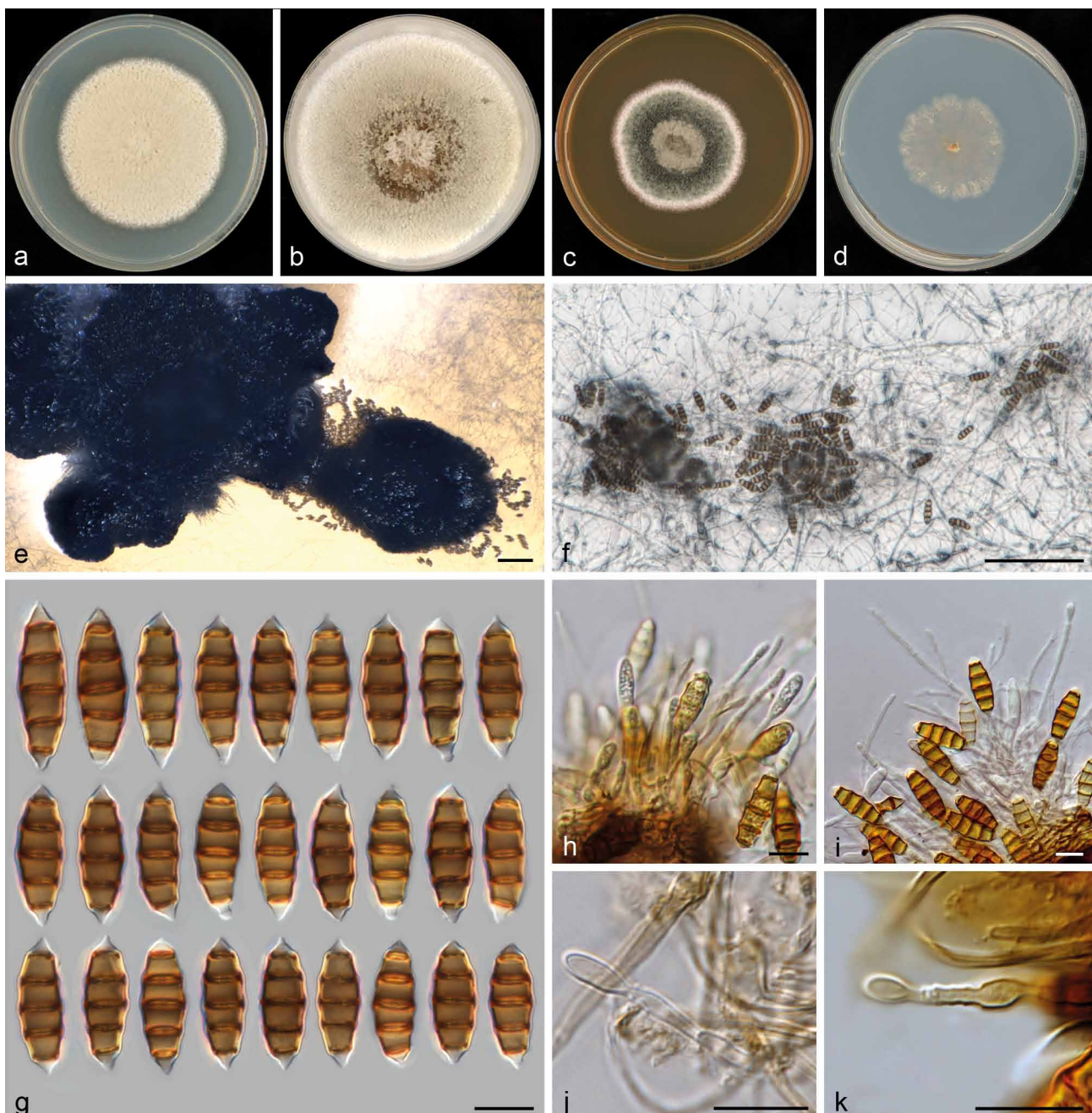


Fig. 5 *Seiridium cardinale* (CBS 909.85, reference strain). a–d. Colony morphology in 90 mm Petri dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e. conidioma on PDA partially immersed in agar; f. sporodochia on SNA immersed in agar; g. conidia; h–i. conidiophores; j–k. conidiogenous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

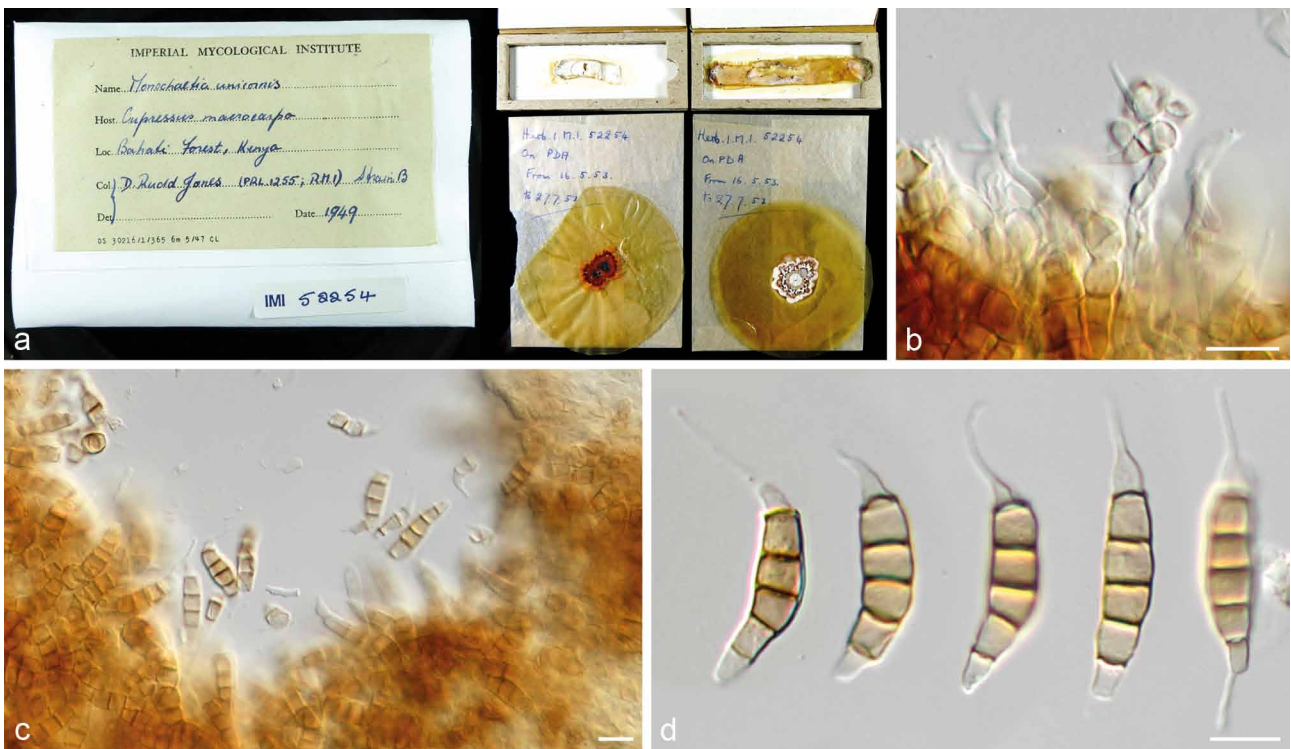


Fig. 7 *Seiridium cupressi* (IMI 52254, lectotype of *Cryptostictis cupressi*). a. Herbarium specimen; b–c. conidiogenous cells and conidia; d. conidia. — Scale bars: b–d = 10 µm.

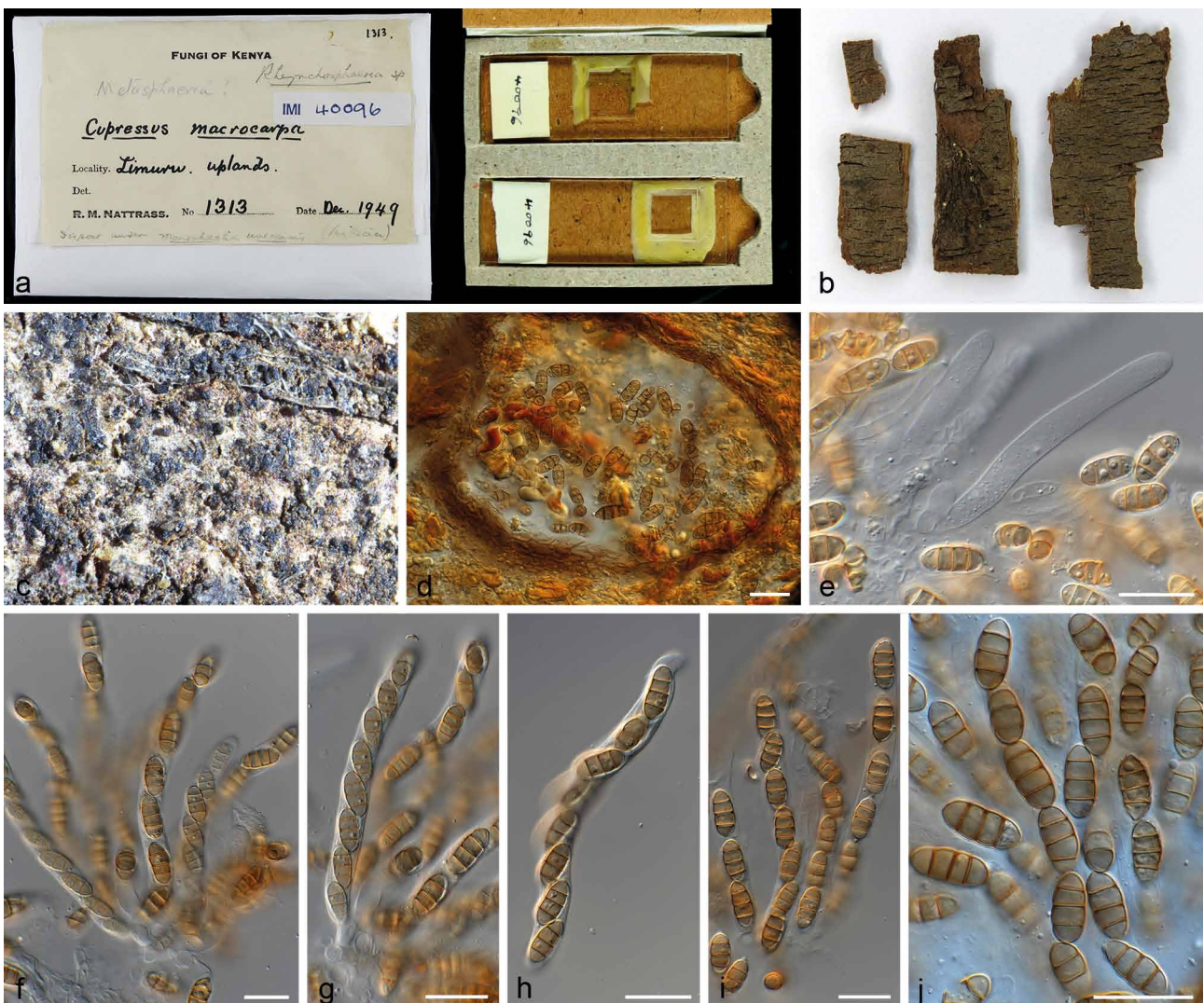


Fig. 8 *Rhynchosphaeria cupressi* (IMI 40096). a–b. Herbarium specimen; c. ascomata; d. ascoma with asci; e–j. paraphyses and asci. — Scale bars: d–j = 20 µm.

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 isoelectotype, 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-

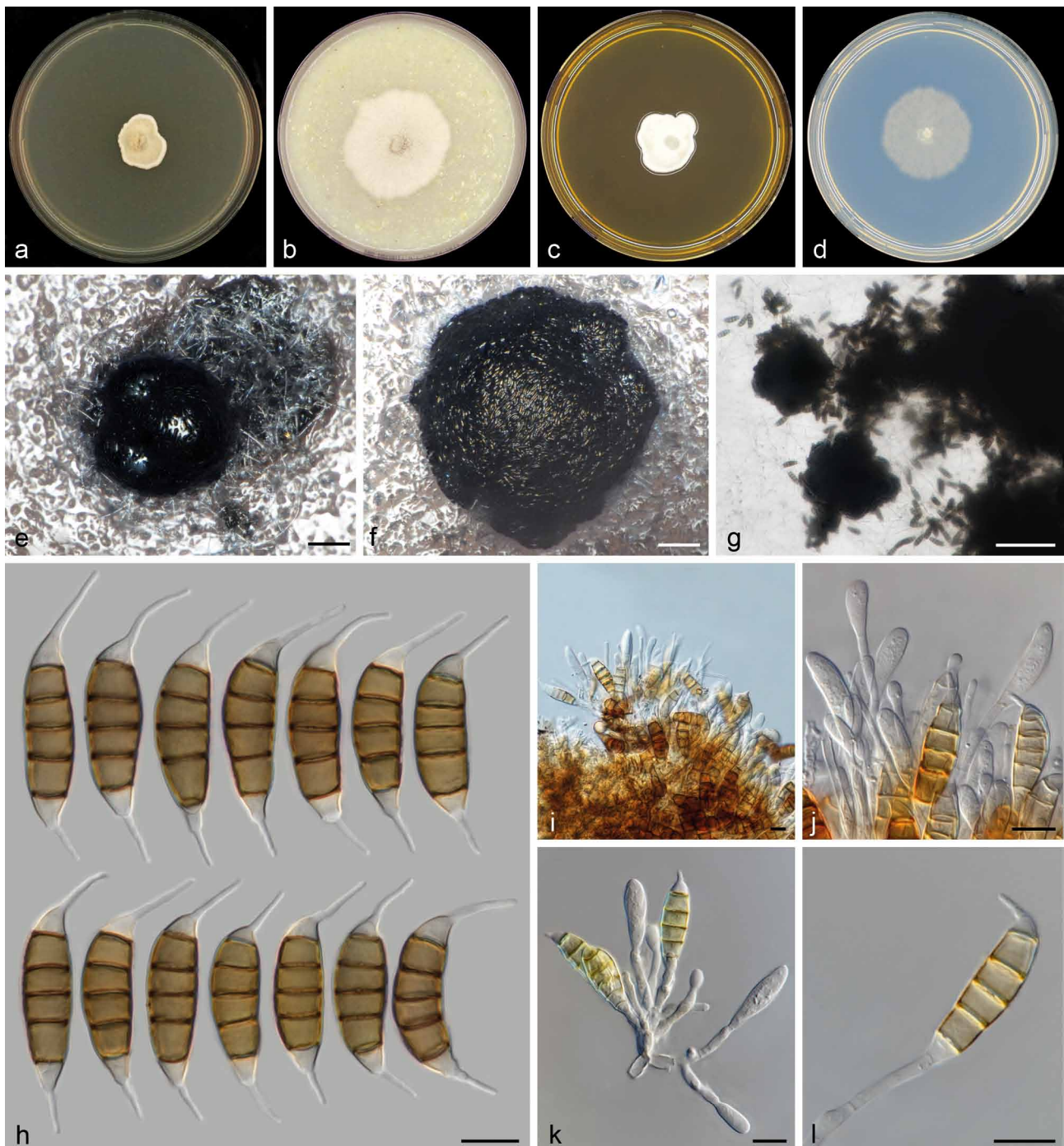


Fig. 9 *Seiridium eucalypti* (CBS 343.97, culture ex-epitype). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e–g. sporodochia on SNA; h. conidia; i–j. conidiophores; k. conidiophore; l. conidiogenous cell. — Scale bars: e–g = 100 µm; h–l = 10 µm.

dant aerial mycelium, sporulating poorly and not within 4 wk, pycnidoid 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. pseudo-cardinale* 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

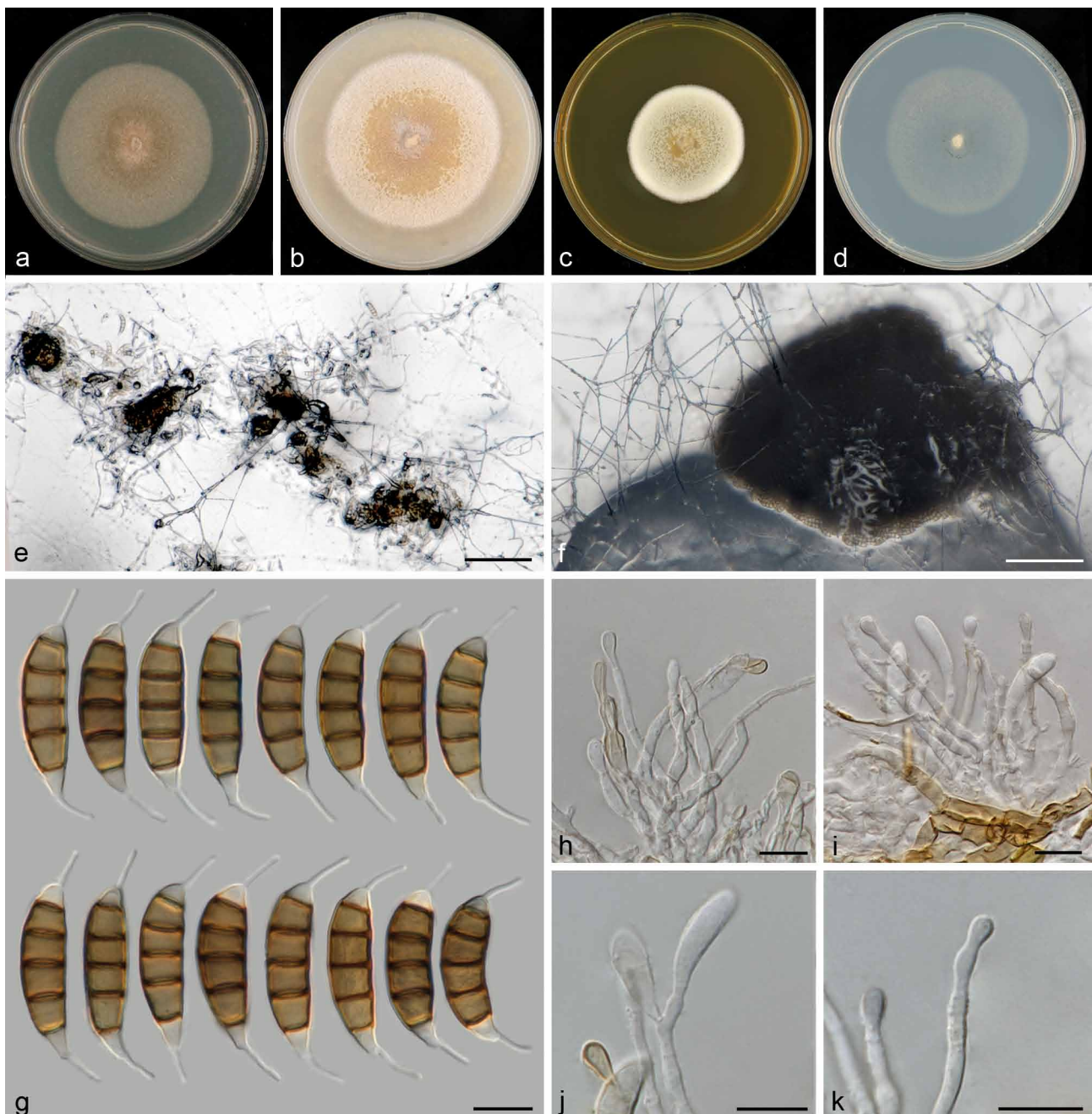


Fig. 10 *Seiridium kartense* sp. nov. (CBS 142629, culture ex-holotype). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e. sporodochia on SNA immersed in agar; f. sporodochia on SNA erumpent from agar; g. conidia; h–i. conidiophores; j. polyblastic conidiogenesis;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

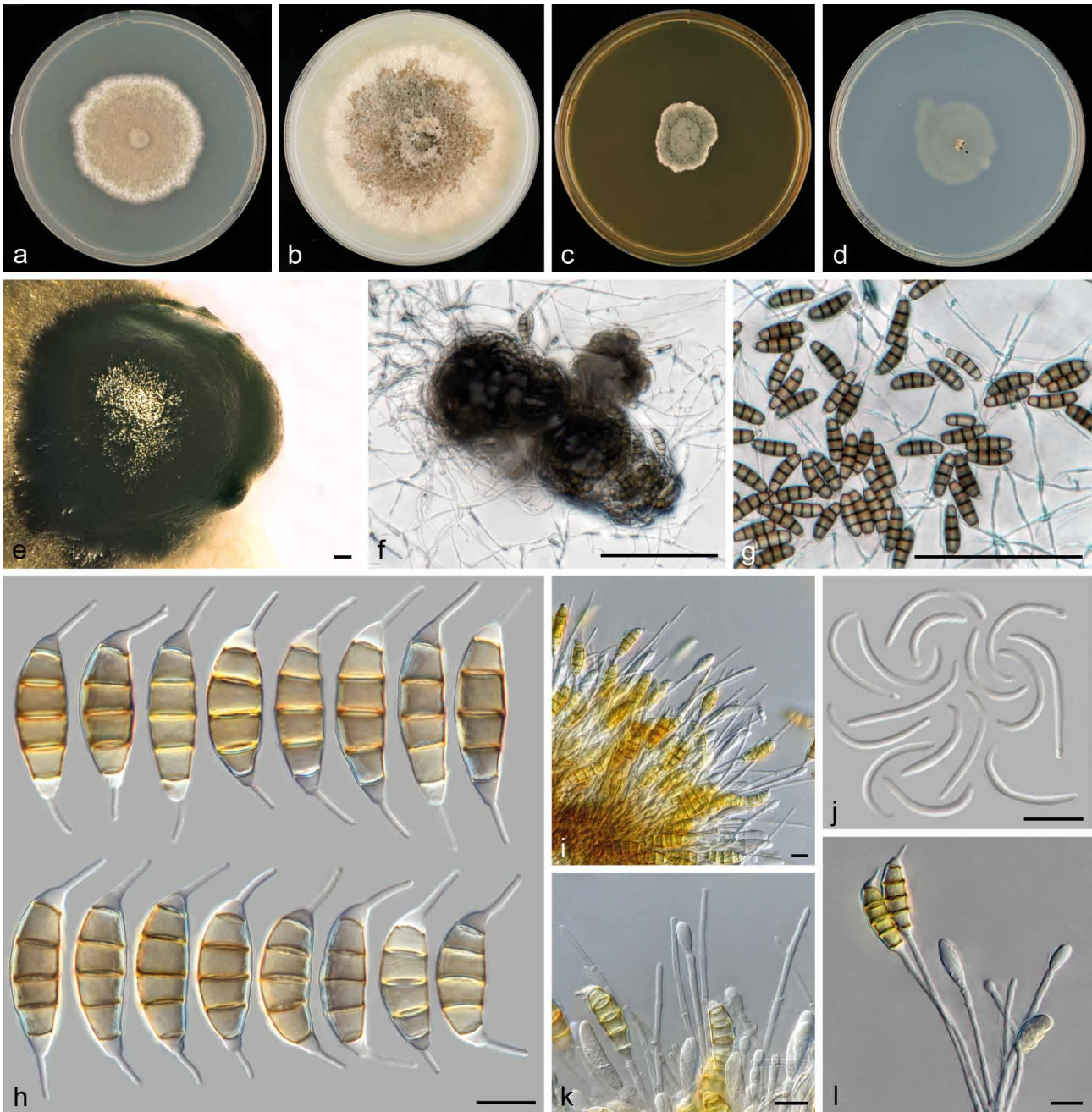


Fig. 12 *Seiridium neocupressi* sp. nov. (CBS 142625, culture ex-holotype). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e. conidioma on PDA; f–g. sporodochia on SNA immersed in agar; h. conidia; i. conidiophores and paraphyses-like structures; j. microconidia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

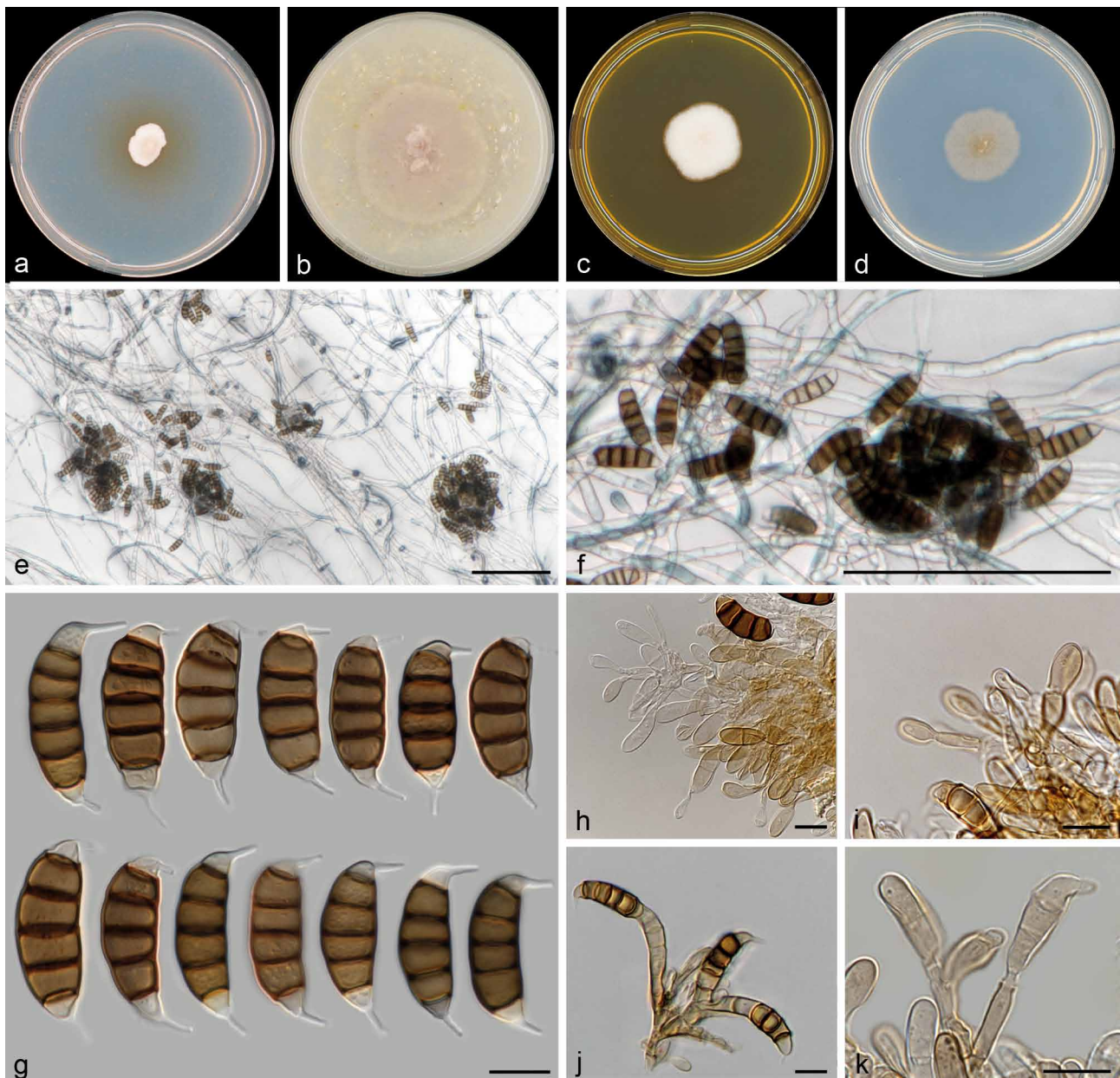


Fig. 13 *Seiridium spyridicola* sp. nov. (CBS 142628, culture ex-holotype). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e–f. sporodochia on SNA immersed in agar; g. conidia; h–j. conidiophores; k. conidiogenous cell. — Scale bars: e–f = 100 µm; g–k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

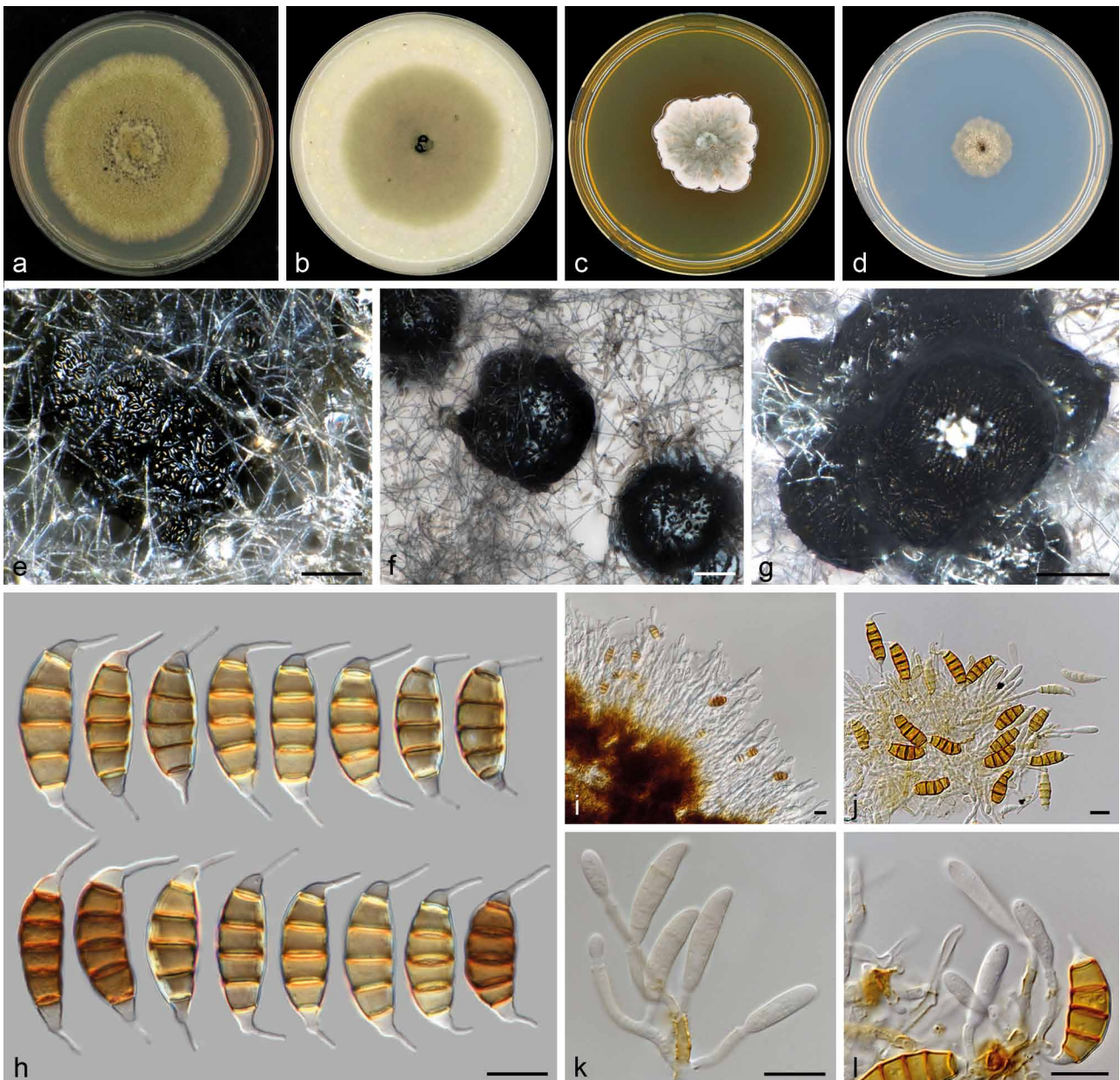


Fig. 14 *Seiridium unicorn* (CBS 538.82, reference strain). a–d. Colony morphology in 90 mm dishes after 2 wk at 22 °C on PDA, CMA, MEA, SNA, respectively; e. conidioma on PDA erumpent from agar partially covered with mycelium; f–g. sporodochia on SNA erumpent from agar; h. conidia; i–j. conidiophores 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. phyllicae* (Crous et al. 2012), which was isolated from *Phyllica 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.

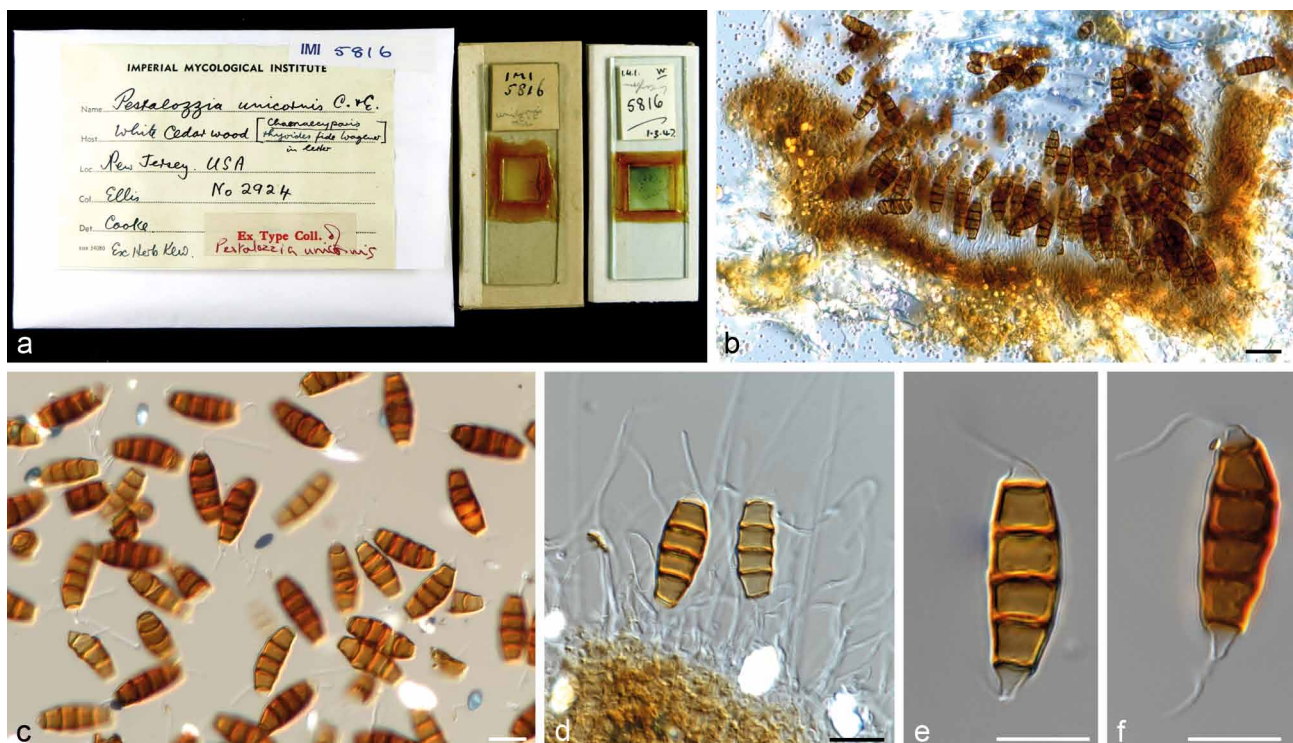


Fig. 15 *Seiridium unicorne* (IMI 5816, holotype of *Pestalozzia unicornis*). a. Herbarium specimen; b. conidiomata and conidia *in vivo*; c–f. conidiogenous cells and conidia *in vitro*. — Scale bars: b = 20 µm; c–f = 10 µm.

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 thuyoides*, 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. unicornis* (IMI 5816) consists of two slides. One of the slides includes conidia, but conidiphores 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 \pm 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. unicornis*, 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. unicornis* 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. podocarpus*, which was isolated from *Podocarpus latifolius* (*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 se-

quence 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 Raj 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.

Bligiascospora 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 & Mohammed (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 podocarpus Crous & A.R. Wood, Persoonia 32: 251. 2014

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

Known distribution — South Africa.

Notes — *Seiridium podocarpus* 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. unicolorne* 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. unicolorne* 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. unicolorne* 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. unicolorne*, 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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