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Camarosporium uniseriatum nom. nov., from *Celtis occidentalis* in European Russia

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Abstract

Celtis occidentalis (American hackberry) is a deciduous tree widely distributed in northern America and introduced in many regions of Europe. In this study we collected *Cucurbitaria celtidis* from dead or dying twigs and branches of *C. occidentalis* (Cannabaceae) in the Rostov region (Southern European Russia), where this tree is a common ergasiophyte in artificial forests. The placement of this species in *Camarosporium sensu stricto* in Pleosporinae, Pleosporales is shown in a multi-locus tree based on combined LSU, SSU and ITS sequence data. *Camarosporium uniseriatum nom. nov.* is introduced based on morphological and phylogenetic analyses.

Keywords – *Cucurbitaria* – phylogeny – Pleosporales – sexual morph

Introduction

Camarosporium is a frequently accounted coelomycetous genus which was introduced by Schulzer (1870). Subsequently, during the past century, more than 500 epithets were recorded for this genus (Index Fungorum 2016). Recent studies based on multi-locus phylogeny showed that camarosporium-like species are polyphyletic in Pleosporales (Liu et al. 2015, Wijayawardene et al. 2014 a, b, 2015, 2016, Ariyawansa et al. 2015), and cucurbitaria-like sexual morphs have been reported for the genus (Wijayawardene et al. 2014b, Tibpromma et al. 2016). Wijayawardene et al. (2014b) established *Camarosporium sensu stricto* in Pleosporinae, Pleosporales with *C. quaternatum* Schulzer, the type of the genus, *C. Aloes* Crous & M.J. Wingf. and two other new species. Liu et al. (2015) introduced three new *Camarosporium* species in *Camarosporium sensu stricto*.

Camarosporium elongatum and *C. Arezzoensis* Tibpromma, et al. are the sexual morphs reported for the genus *Camarosporium* and they share similar morphological characters with *Cucurbitaria* species (Doilom et al. 2013, Wijayawardene et al. 2014a, Tibpromma et al. 2016). In this study, we collected *Cucurbitaria celtidis* Shear on dead or dying twigs and branches of *Celtis occidentalis* in Russia, Rostov region (Southern European Russia), where this tree is a common ergasiophyte in artificial forests in steppe zone of Eastern Europe. We used morphological

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characters plus multi-gene molecular analyses to resolve its correct placement according to the modern taxonomic concepts.

Materials and methods

Collection of samples, isolation and morphological examination

Fresh specimens were collected from dead or dying twigs and thin branches of *Celtis occidentalis* in the Botanical Garden of Southern Federal University (Russia, Rostov region, Rostov-on-Don City) and samples were grown on potato dextrose agar. Isolates were derived via single spore isolation following the protocols of Chomnunti et al. (2014). Germinating spores were transferred to PDA and incubated at 25°C in the dark. Cultural characteristics, such as mycelium colour, shape, texture and growth rate were determined. Type specimens are deposited in the herbarium of Mae Fah Luang University (MFLU), Chiang Rai, and Guizhou Academy of Agricultural Sciences (GZAAS), while cultures are deposited at the Mae Fah Luang University Culture Collection (MFLUCC) with duplicates in International Collection of Microorganisms from Plants (ICMP) Landcare Research, New Zealand.

The specimens were observed and examined with a Motic SMZ 168 stereomicroscope. Micro-morphological characters of the specimen were examined under a Nikon ECLIPSE 80i compound microscope and images were captured using a Nikon ECLIPSE 80i compound microscope with a Canon EOS 550D digital camera. Observations and photographs were made from material mounted in water and Indian ink was added to water mounts to show the presence of gelatinous sheaths around the ascospores. Measurements were made with the Tarosoft (R) Image Frame Work and images used for figures were processed with Adobe Photoshop CS3 Extended version 10.0 software. Faces of fungi numbers and Index Fungorum numbers are provided as detailed in Jayasiri et al. (2015) and Index Fungorum (2016).

DNA extraction, PCR amplification and sequencing

Fresh fungal mycelium was grown on PDA at 25°C for 14 days. Extraction of genomic DNA from mycelia and sequencing of PCR products were carried out following the method of Thambugala et al. (2015). Four partial gene portions were amplified in this study including LROR and LR5 (Vilgalys & Hester, 1990) for the nuclear ribosomal large subunit (LSU), ITS4 and ITS5 (White et al. 1990) for the internal transcribed spacer (ITS), EF1-983F and EF1-2218R (Carbone and Kohn 1999) for translation elongation factor 1-alpha (EF1-α) and NS1 and NS4 (White et al. 1990) for the nuclear ribosomal small sub unit (SSU). The amplifications were performed in 25 μL of PCR mixtures containing 9.5 μL ddH_2O, 12.5 μL 2×PCR Master Mix (TIANGEN Co., China), 1 μL of DNA template, 1 μL of each primer (10 μM). The amplification reactions were performed and analysed as described by Thambugala et al. (2015).

Phylogenetic analyses

The phylogeny of the new strain was determined based on a combined data set of LSU, SSU and ITS sequence data of 44 isolates belonging to the suborder Pleosporineae, Pleosporales (Table 1) with *Halojulella avicenniae* (BCC 20173) as the out group taxon. SeqMan v. 7.0.0 (DNASTAR, Madison, WI) was used to compute consensus sequences. The sequence data were aligned and combined using Bioedit (Hall 1999) and MEGA 5.0 (Tamura et al. 2011). Phylogenetic analyses were performed based on maximum likelihood (ML) criterion using RAxML-HPC BlackBox (8.2.4) (Stamatakis et al. 2008) in the CIPRES (Miller et al. 2010). The general time reversible model of evolution including estimation of invariable sites (GTRGAMMA + I) and assuming a discrete gamma distribution with four rate categories was used for the ML analysis. The best scoring tree (-13523.550799) was selected and visualized with MEGA v. 5 (Tamura et al. 2011). ML Bootstrap support (BS) (greater than or equal 50 %) are shown below or above each branch (Fig. 1). All the sequences newly generated in this study are deposited in GenBank.

Table 1 GenBank and culture collection accession numbers of species included in the phylogenetic study. Newly generated sequences are shown in bold.

Species	Isolate no.	GenBank Accession no			
		LSU	SSU	ITS	EF
Alternaria alternata	CBS 916.96	DQ678082	KC584507	FJ196306	
Alternaria murispora	MFLU 14-0758	KP334704	KP334724	KP334714	
Ascochyta pisi	CBS 126.54	DQ678070	DQ678018	GU237772	
Bipolaris melinidis	BRIP 12897	JN600994	_	JX256411	
Camarosporium aborescentis	MFLUCC 14-0604	KP711378	KP711379	KP711377	
Camarosporium aloes	CBS 136437	KF777198	_	KF777142	
Camarosporium arezzoensis	MFLUCC 14-0238	KP120927	KP120928	KP120926	
Camarosporium aureum	MFLUCC 14-0620	KP744478	KP753948	KP744436	
Camarosporium caraganicola	MFLUCC 14-0605	KP711381	KP711382	KP711380	
Camarosporium uniseriatum	**MFLUCC 15–0444**	**KU697614**	**KU697615**	**KU697613**	**KU697612**
Camarosporium clematidis	MFLUCC 13–0336	KJ562188	KJ589414	KJ562213	
Camarosporium quaternatum	CBS 483.95	GU301806	GU296141	_	
Camarosporium robiniicola	MFLUCC 13-0527	KJ589412	KJ589415	KJ562214	
Camarosporium spartii	MFLUCC 13-0548	KJ589413	KJ589416	KJ562215	
Comoclathris compressa	CBS 156.53	KC584372	KC584630	_	
Comoclathris sedi	MFLUCC 13-0817	KP334705	KP334725	KP334715	
Coniothyrium palmarum	CBS 758.73	EU754154	EU754055	_	
Coniothyrium palmarum	CBS 400.71	EU754153	EU754054	AY720708	
Cucurbitaria berberidis	CBS 363.93	GQ387606	GQ387545	JF740191	
Cucurbitaria berberidis	CBS 394.84	GQ387605	GQ387544	_	
Cucurbitaria elongata	MFLUCC 14-0260	KJ724249	_	_	
Curvularia lunata	CBS 730.96	JX256396	_	JX256429	
Didymella exigua	CBS 183.55	EU754155	GU296147	GU237794	
Dothidotthia aspera	CPC 12933	EU673276	EU673228	_	
Dothidotthia symphoricarpi	CPC 12929	EU673273	EU673224	_	
Halojulella avicenniae	BCC 20173	GU371822	GU371830	_	
Leptosphaeria doliolum	CBS 541.66	JF740284	_	JF740206	
Leptosphaeria doliolum	MFLUCC 13-0740	KP729445	_	KP729444	
Leptosphaeria etheridgei	CBS 125980	JF740291	_	JF740221	
Leptosphaerulina australis	CBS 317.83	EU754166	EU754067	GU237829	
Neocamarosporium betae	CBS 109410	EU754178	EU754079	_	
Neocamarosporium goegapense	CBS 138008	KJ869220	_	KJ869163	
Neophaeosphaeria agaves	CBS 136429	KF777227	_	KF777174	
Neophaeosphaeria filamentosa	CBS 102202	GQ387577	GQ387516	JF740259	
Neophaeosphaeria filamentosa	CBS 102203	JX681104	_	_	
Ophiosphaerella herpotricha	CBS 620.86	DQ678062	DQ678010	KF498728	
Paraphoma radicina	CBS 111.79	EU754191	EU754092	KF251172	
Phaeosphaeriaoryzae	CBS 110110	KF251689	KF251186	GQ387530	
Pleospora herbarum	CBS 191.86	GU238160	GU238232	NR_111243	
Pyrenochaeta cava	CBS 257.68	EU754199	EU754100	JF740260	
Pyrenochaeta nobilis	CBS 407.76	EU754206	EU754107	NR103598	
Pyrenochaetopsis leptospora	CBS 101635	GQ387627	GQ387566	JF740262	
Pyrenochaetopsis pratorum	CBS 445.81	GU238136	GU238228	JF740263	
Pyrenophor aphaeocomes	DAOM 222769	JN940093	JN940960	JN943649	

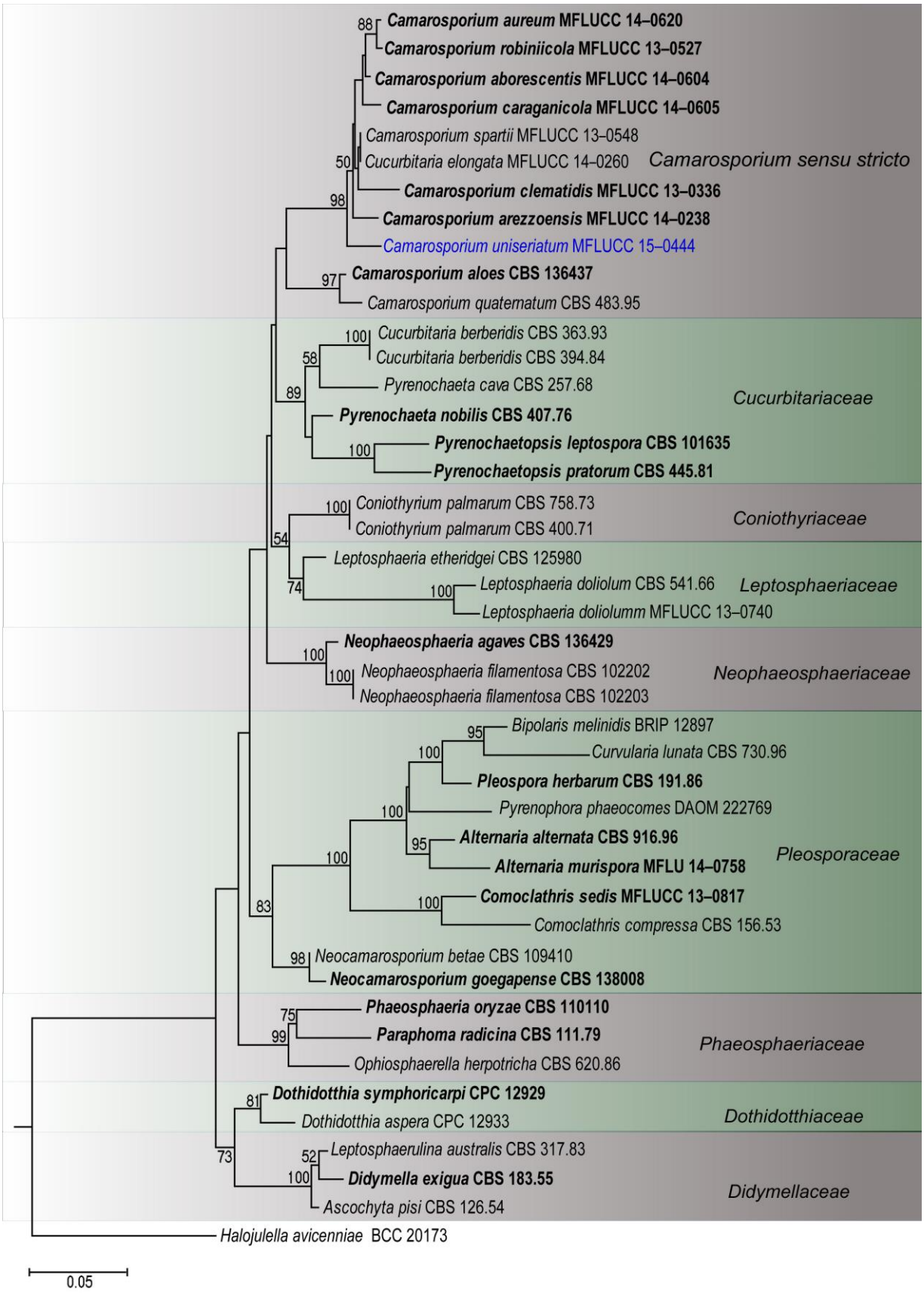

Fig. 1 – Maximum Likelihood (ML) tree from analysis of combined dataset of LSU, SSU and ITS sequence data of Pleosporinae, Pleosporales. Bootstrap support values equal or greater than 50% are given above and below the nodes. The tree is rooted to *Halojulella avicenniae*(BCC 20173). Newly generated sequences are in blue. Type strains are in bold.

Results

Phylogenetic analysis

The analysis was based on a combined LSU, SSU and ITS sequence data belonging to Pleosporinae, Pleosporales and the best scoring RAxML tree is shown in Fig. 1. The outgroup taxon, *Halojulella avicenniae* (BCC 20173) is clearly excluded from the other taxa. Species residing in Pleosporinae, Pleosporales were positioned on the tree, and represented *Camarosporium sensu stricto* and the families Coniothyriaceae, Cucurbitariaceae, Didymellaceae, Dothidotthiaceae, Leptosphaeriaceae, Neophaeosphaeriaceae, Phaeosphaeriaceae and Pleosporaceae. The new strain (MFLUCC 15–0444) clustered in a separate subclade in *Camarosporium sensu stricto*, with a strong bootstrap support (98%).

Taxonomy

Camarosporium uniseriatum Thambugala, Bulgakov& K.D. Hyde**, *nom. nov*.** Fig. 2

Index Fungorum number: IF551991

Facesoffungi number: FoF 01964

Replaced synonym –Cucurbitaria celtidis Shear, Bull. Torrey bot. Club 29: 451 (1902); non *Camarosporium celtidis* Ellis & Everh., nec Gucevič

Etymology – In reference to single row arrangement of the ascospores in the asci

Necrotrophic or saprobic on twigs and thin branches of *Celtis* species. **Sexual morph** – *Ascomata* 300–475 μm wide × 200–400 μm high ($\bar{x}$ = 390 × 338 μm, n = 10), black, semi-immersed, becoming erumpent, scattered, solitary to gregarious, globose to subglobose, coriaceous, rough or hairy, ostiolate. *Ostiole* central, short, ostiolar canal filled with hyaline to lightly pigmented pseudoparenchymatous cells. *Peridium* 30–60 μm, ($\bar{x}$ = 45 μm, n = 15) wide, comprising several layers, outer layers heavily pigmented, thick-walled, comprising blackish to dark brown cells of *textura angularis*, inner layers composed of hyaline, thin-walled cells of *texturaangularis*. *Hamathecium* comprising 1–3 μm wide, numerous, filamentous, septate, pseudoparaphyses. *Asci* 120–160 × 12–15 μm ($\bar{x}$ = 140 × 13 μm, n = 15), 8-spored, bitunicate, fissitunicate, cylindrical, short-pedicellate, apex rounded, with an ocular chamber. *Ascospores* (13.5–)15–26.5 × 7–8.8(–10) μm ($\bar{x}$ = 19.9 × 7.9 μm, n = 40), uniseriate, slightly overlapping, initially hyaline, becoming dark brown at maturity, ellipsoid, oblong to fusoid, straight, muriform, with 3–5 transverse septa, and 1–2(–3) longitudinal septa, deeply constricted at the central septum, with rounded or acute ends, smooth-walled, without a mucilaginous sheath. **Asexual morph** – Undetermined.

Material examined – Russia, Rostov Region, Rostov-on-Don City, Botanical Garden of Southern Federal University, Higher Park, on twigs and branches of *Celtis occidentalis* L. (Cannabaceae) 5 March 2014, T. Bulgakov T 37 (MFLU 16–0469, **reference specimen designated here**), living culture MFLUCC 15–0444, ICMP.

Culture characteristics – Ascospores germinated on PDA at 25 °C within 18 h and germ tubes produced from one or several septa. Colonies on PDA reaching 18 mm diam. after 4 days at 25 °C, flat, circular, surface initially white, becoming olivaceous brown starting from the centre, after 2 weeks, reverse dark grey to black, surface smooth, with edge entire to slightly undulate.

Notes – *Cucurbitaria celtidis* was introduced by Shear (1902) from *Celtis occidentalis* in the USA. Our collection from Russia on the same host fits with the original description of *C. celtidis* (Shear 1902), but we observed 1–2(–3) longitudinal septa and a slightly size variation of ascospores, compared to those of in the original description (Table 2). We believe this is because the different geographical distributions as these two collections were made in different continents. Our collection of *Cucurbitaria celtidis* (MFLUCC 15–0444) forms a distant clade in *Camarosporium sensu stricto*, with a strong bootstrap support (98%, Fig. 1). Therefore, we introduce *Camarosporium uniseriatum* to accommodate *Cucurbitaria celtidis* and we use the species epithet *uniseriatum* as this epithet celtidis and celtidicola are already used in the genus

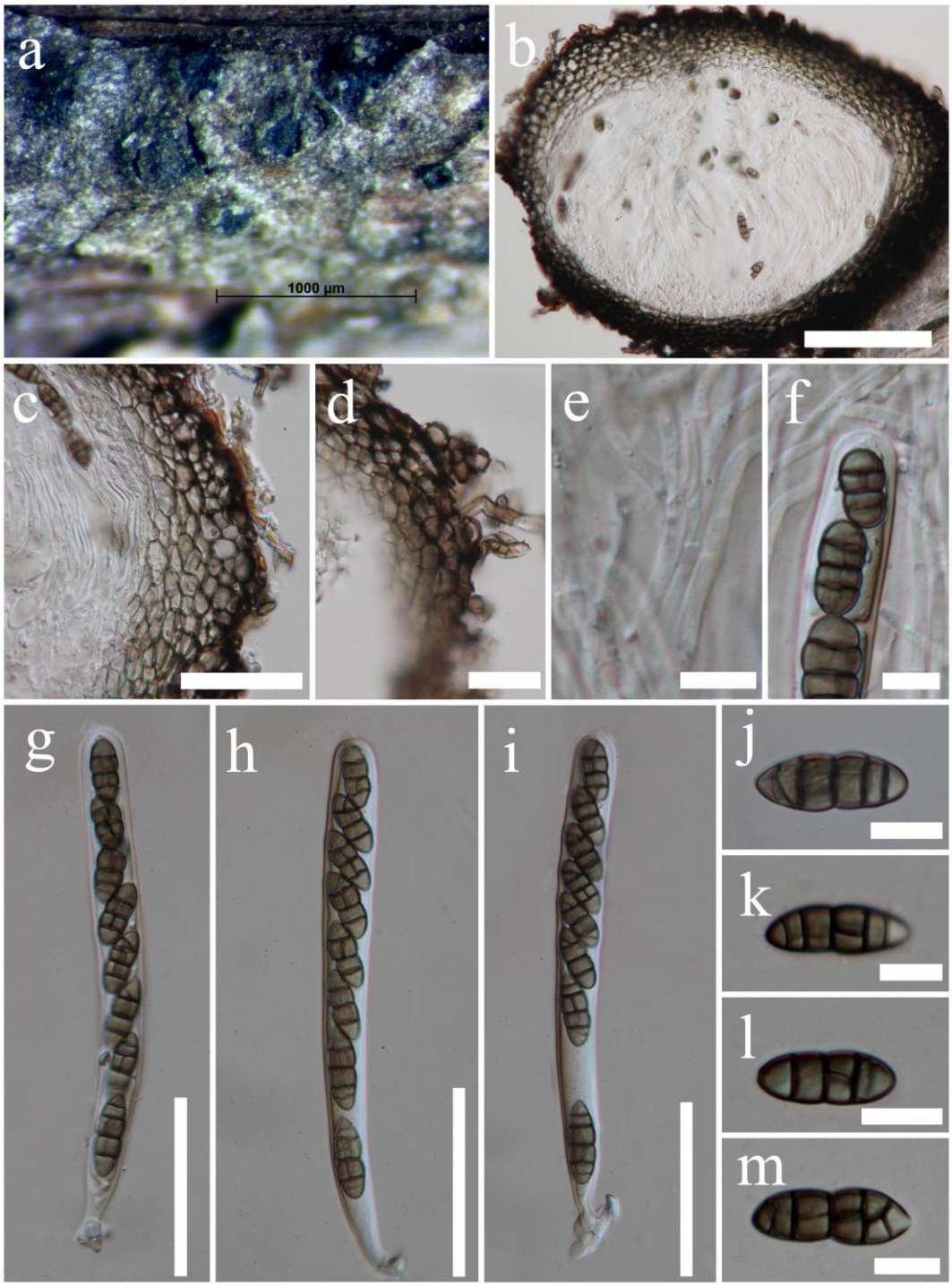

Fig. 2 – *Camarosporium uniseriatum* (MFLU 16–0469). a Appearance of ascomata on host surface. b Vertical section through ascoma. c, d Section through peridium (note light brown hyphae in d). E Pseudoparaphyses. f Apex of ascus g–i Asci. j–m Ascospores. Scale bars: b = 100 µm, c, g-i = 50 µm, d = 25 µm, e-f, j-m = 10 µm.

Camarosporium (*C.celtidis* Ellis &Everh. and *C. Celtidicola* Gucevič). *Camarosporium elongatum* and *C. arezzoensis*, the sexual morphs reported for *Camarosporium sensu stricto* also differs from *C. uniseriatum* in having ellipsoidal ascospores with different number of longitudinal and transverse septa (Mirza 1968, Hyde et al. 2013, Tibpromma et al. 2016). Only an asexual morph was reported for *Camarosporium celtidis* and *C. Celtidicola* Gucevič (Ellis and Everhart 1894, Saccardo 1895, Gucevič 1959), therefore, these species need recollection and sequencing to confirm their placements.

Table 2 Comparison of *Camarosporium uniseriatum* (*Cucurbitaria celtidis*) and collections in Shear (1902) from *Celtis* spp.

	Ascomata (µm)	Asci (µm)	Ascospores (µm)	Host and locality
This study	300–475 wide × 200–400 high ($\bar{x}$ = 390 × 338)	120–160 × 12–15 ($\bar{x}$ = 140 × 13)	(13.5–)15–26.5 × 7–8.8(–10) ($\bar{x}$ = 19.9 × 7.9), 3–5 transverse septa and 1–2(–3) longitudinal septa	*C. occidentalis* L., Russia
Shear (1902)	350–500	140–180 × 16	24–27 × 8 µm, 3–5 transverse septa and 1 longitudinal septum	*C. occidentalis* L., USA

Discussion

The genus *Camarosporium* was treated in different families by various authors and has been reported as the asexual morph of Cucurbitariaceae, Phaeosphaeriaceae and Botryosphaeriales (Kirk et al. 2008, Wijayawardene et al. 2012, Zhang et al. 2012, Hyde et al. 2013). Wijayawardene et al. (2014b) established *Camarosporium sensustricto* in Pleosporinae, Pleosporales, based on phylogenetic analyses.In this paper, we showed *Cucurbitaria celtidis* to group in *Camarosporium sensu stricto* in Pleosporinae, Pleosporales and describe it as a novel sexual member in *Camarosporium*. However, the sequences of *C. quaternatum* in GenBank (CBS 483.95) are not from the type strain. There are no sequences data available for a number of *Camarosporium* and *Cucurbitaria* species. Therefore, re-collection, epitypification or reference specimens (Ariyawansa et al. 2014) with molecular data are essential to establish their placement according to the modern taxonomic concepts.

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