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ORIGINAL ARTICLE

Coprinoid *Psathyrellaceae* species from Cyprus: three new sabulicolous taxa from sand dunes and a four-spored form of the fimicolous species *Parasola cuniculorum*

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Abstract

The island of Cyprus occupies a unique geographical position in the south-eastern region of the Mediterranean basin, with European, West Asian and North African elements present in its fungal diversity. Our investigations over the years have revealed considerable diversity of species belonging to the genera *Coprinellus*, *Tulosesus*, *Coprinopsis*, *Coprinus* and *Parasola* on the island. Nevertheless, a number of collections remain unnamed as their molecular, ecological and morphological profiles do not match any presently known taxa. In this first contribution of our study, two setulose *Tulosesus* taxa and one new *Parasola* species collected from coastal dunes and adjacent areas are proposed: *Tulosesus maritimus*, *Parasola litoralis* spp. nov. and *Tulosesus callinus* var. *miionis* var. nov. They are characterised by a combination of unique morphological features and significant genetic differences evidenced by phylogenetic analyses based on multiple DNA markers. Two additional lineages of *Tulosesus* putatively representing unnamed species are also identified. An ITS rDNA sequence from the type collection of the 2-spored species *Parasola cuniculorum* was obtained for the first time and unexpectedly revealed to be conspecific with a 4-spored collection from Cyprus. DNA data was obtained also from the type collections of *P. megasperma* and *P. nudiceps*, and compared with homologous sequences from *Parasola litoralis* and other *Parasola* species. Detailed descriptions and imagery of the newly described taxa are provided, as well as a comparative study of similar species in genera *Coprinellus*, *Tulosesus* and *Parasola*.

Keywords Coprinoid fungi · Mediterranean · *Parasola litoralis* · *Parasola* type sequences · *Tulosesus callinus* var. *miionis* · *Tulosesus maritimus*

Introduction

Until the turn of the last century, species formerly placed in the genus *Coprinus* Pers.: Fr. (Persoon 1797) were classified into different subgenera, sections and subsections on the basis of morphology (Gray 1821; Fries 1838; Karsten 1879; Lange 1915; Buller 1909, 1922, 1924, 1931; Kühner 1928; Lange 1952; Kühner and Romagnesi 1953; Lange and Smith 1953; Patrick 1977; Orton and Watling 1979; Uljé and Bas 1988, 1991; Uljé and Noordeloos 1993, 1997, 1999; Uljé 2005). However, the first phylogenetic analyses based on sequences of the 28S rDNA region (LSU) (Hopple and Vilgalys 1994, 1999; Moncalvo et al. 2000) revealed that *Coprinus comatus* (O.F. Müll.) Pers.: Fr. (type species of *Coprinus*) and a few other species such as *C. sterquilinus* (Fr.: Fr.) Fr. formed a monophyletic clade within the family *Agaricaceae* Chevall. (Redhead et al. 2001), isolated from most other coprinoid taxa, that were subsequently placed in three genera within

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the new family *Psathyrellaceae* Vilgalys, Moncalvo & Redhead: *Coprinellus* P. Karst., *Coprinopsis* P. Karst., and *Parasola* Redhead, Vilgalys & Hopple. Subsequent DNA-based phylogenetic works confirmed the monophyly of *Parasola*, with *Coprinellus* and *Coprinopsis* also broadly supported, but perhaps in need of further revision and with the closely allied *Psathyrella* (Fr.) Quél. still not entirely disentangled (Moncalvo et al. 2002; Walther et al. 2005; Vašutová et al. 2008; Larsson and Örstadius 2008; Padamsee et al. 2008). Schafer (2010) transferred nine subsections of *Coprinus sensu* Uljé (2005), as sections of *Parasola* (sections *Glabri* and *Auricomi*), *Coprinellus* (sections *Setulosi*, *Micacei* and *Domestici*) and *Coprinopsis* (sections *Atramentarii*, *Alachuani*, *Narcotici* and *Nivei*); the tenth subsection comprising species remaining in *Coprinus* s. str. However, the morphology-based classifications have been shown to require revision on the basis of molecular studies of the *Psathyrellaceae* (particularly Nagy et al. 2013 and Örstadius et al. 2015).

In an extensive revision of *Parasola*, Nagy et al. (2009, 2010a, 2010b) provided a phylogenetic backbone for the genus, linking morphological traits to evolutionary patterns, revising type material and clarifying a number of species concepts. Additional taxa were described from Britain (Schafer 2014) and Pakistan (Hussain et al. 2017, 2018), while Szarkándi et al. (2017) provided several new sequences and described three newly discovered species from grasslands of Central Europe. Nagy et al. (2012a, 2012b) studied also the evolutionary diversification and species delimitation in *Coprinellus*, particularly the setulose species, based on a multigene phylogenetic approach. Their analyses suggested that a number of setulose species were nested in clades together with non-setulose species. In a recent extensive, taxon-rich phylogenetic analysis, Wächter and Melzer (2020) proposed a substantial revision of the family *Psathyrellaceae*, adding two coprinoid (*Tulosesus* and *Narcissea*) and four other genera. Genetically supported monophyletic sections of these genera were also introduced, supplementing or replacing the earlier arrangements based on morphology, a classification endorsed in the present paper.

Coprinoid fungi are well-represented on the island of Cyprus, situated at the crossroads of three continents in the south-eastern borders of the Mediterranean basin. Although a number of coprinoid species have been reported in the past, genetic studies to confirm their identity are nonetheless lacking. In the first checklist of Cyprus fungi, Nattrass (1937) reported *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo [as "*Coprinus atramentarius* (Bull.) Fr"], a species yet to be confirmed on the island by later authors. Viney (2005) reported *Coprinellus domesticus* (Bolton) Vilgalys, Hopple & Jacq. Johnson [as "*Coprinus domesticus* (Bolton) Gray"], *Coprinopsis cinerea* (Schaeff.) Redhead, Vilgalys & Moncalvo [as "*Coprinus cinereus* (Schaeff.) Gray"], *Coprinopsis cothurnata* (Godey) Redhead, Vilgalys & Moncalvo (as "*Coprinus cothurnatus* Godey"), *Coprinopsis picacea* (Bull.) Redhead, Vilgalys & Moncalvo [as "*Coprinus picaceus* (Bull.) Gray"], *Coprinus comatus* (O.F. Müll.) Pers., *Coprinus ovatus* (Schaeff.) Fr., *Parasola megasperma* (P.D. Orton) Redhead, Vilgalys & Hopple (as "*Coprinus megaspermus* P.D. Orton"), and *Parasola plicatilis* (Curtis) Redhead, Vilgalys & Hopple [as "*Coprinus plicatilis* (Curtis) Fr."]. More recently, Loizides et al. (2011) reported *Coprinellus radians* (Fr.) Vilgalys, Hopple & Jacq. Johnson, *Coprinopsis romagnesiana* (Singer) Redhead, Vilgalys & Moncalvo, *Coprinus spadiceisporus* Bogart, and *Coprinus vosoustii* Pilát, to which *Coprinopsis cortinata* (J.E. Lange) Gminder and *Coprinellus disseminatus* (Pers.) J.E. Lange, were later added (Loizides 2016, 2021), bringing the total number of coprinoid taxa reported from the island to fifteen.

Our ongoing studies of coprinoid fungi in Cyprus suggest that considerable diversity remains to be explored. Over the past decade, several species of *Coprinellus*, *Coprinopsis*, *Coprinus*, *Parasola* and *Tulosesus* were collected and identified by some of the authors, with a number of samples remaining nevertheless unnamed, not conforming to any of the species concepts described in published literature. In one area of coastal dunes, a habitat from which very few coprinoid species have been previously described, two striking setulose taxa were collected and found to be morphologically distinct from all other *Coprinellus* or *Tulosesus* species known so far, including the sabulicolous species *Coprinellus sabulicola* L. Nagy et al. (now *Tulosesus sabulicola* (L. Nagy et al.) Wächter & A. Melzer) proposed by Nagy et al. (2012a) from Hungary, and the closely related *C. christianopolitanus* Örstadius & E. Larss., (now *Tulosesus christianopolitanus* (Örstadius & E. Larss.) Wächter & A. Melzer) proposed by Örstadius et al. (2015) from Sweden. A third species collected in the same area displayed affinities with *Parasola schroeteri* (P. Karst.) Redhead, Vilgalys & Hopple and to a lesser extent *P. megasperma* (P.D. Orton) Redhead, Vilgalys & Hopple, but differed in the shape and size of its spores. A combined molecular and morphological investigation to clarify the taxonomic status of these collections is presented below.

Materials and methods

Morphological studies

Specimens were photographed in situ and notes of the macroscopic characters, habitat, altitude, soil composition and nearby vegetation were taken. Collections were examined fresh, when possible, or dried soon after collection. Dried material was examined after soaking in either 10% aqueous ammonia or Congo Red solution, followed by washing off with 10%

aqueous ammonia. A Leica MZ APO dissecting microscope at magnifications from ×8 to ×80 was used to examine the pileus for the presence of setules, veil and other critical features. At least 30 spores mounted in ammonia were measured from each specimen. Slides were examined at up to ×1000 in oil in a Leica DMLS trinocular microscope with an Infinity plan achromatic objective, or a Leica BM E binocular microscope. Microscopic structures were photographed through the eyepiece. The scale in the microphotographs, where present, is 1 division = 1 μm at ×1000 and 1 division = 2.5 μm at ×400.

DNA extraction, amplification and sequencing

Total DNA was extracted from dry specimens employing a modified protocol based on Murray and Thompson (1980). PCR reactions (Mullis and Faloona 1987) included 35 cycles with an annealing temperature of 54 °C. Primers ITS1F and ITS4 (White et al. 1990; Gardes and Bruns 1993) were employed to amplify the ITS rDNA region, while LR0R and LR5 (Vilgalys and Hester 1990; Cubeta et al. 1991) were used for the 28S rDNA region, EF1-983F and EF1-1567R (Rehner and Buckley 2005) for the translation elongation factor 1a (*TEF1*) gene, and B36f_psa and B12r_psa (Thon and Royse 1999; Nagy et al. 2011) for the beta-tubulin gene (*TUB2*). PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

Alignments and phylogenetic analyses

BLAST (Altschul et al. 1990) was used to select the most closely related sequences from INSD public databases. Two different datasets were built, one including ITS rDNA, *TEF1* and *TUB2* sequences of *Tulosesus* and another including ITS rDNA, 28S rDNA (LSU), *TEF1* and *TUB2* sequences of *Parasola*. Sequences of *Tulosesus* came mainly from Nagy et al. (2011) and Örstadius et al. (2015), while those of *Parasola* came from Nagy et al. (2009), Hussain et al. (2018) and Szarkándi et al. (2017). Sequences (Table 1) first were aligned in MEGA 5.0 (Tamura et al. 2011) software with its Clustal W application and then corrected manually. In the *Tulosesus* dataset, the final alignment of ITS rDNA included 125/591 variable sites among 44 sequences, while that of *TEF1*-exons had 59/445 variable sites among 11 sequences, the *TEF1* intron had 43/53 variable sites among 11 sequences, the alignment of *TUB2* exon included 77/315 variable sites among 26 sequences, and that of *TUB2* intron had 56/64 variable sites in 26 sequences. In the *Parasola* dataset, the final alignment consisted of 256/617 variable sites among 78 ITS rDNA sequences, 217/1089 variable sites among 45 LSU rDNA sequences, 204/422 variable sites among 15 *TEF1* sequences, and 125/392 variable sites among 7 *TUB2* sequences. Each dataset was loaded in MrBayes 3.2.6 (Ronquist et al. 2012), where a Bayesian analysis was performed (partitions *Tulosesus*: ITS, *TEF1* exons, *TEF1* introns, *TUB2* exons, *TUB2* introns, partitions *Parasola*: ITS, LSU, *TEF1*, *TUB2*; two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 0.8 M (*Tulosesus*) and 0.62 M (*Parasola*) generations, standard deviation having fallen below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis 2014) using the standard search algorithm (2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP). Alignments as fasta files are available as supplementary information.

Terminology, abbreviations and herbarium material

The term "deliquescence" (or "autodigestion") describes the process of sequential spore maturation and release, combined with autolysis of lamellae cells ("inaequii-hymeniferous development"), described in detail by Buller (1909, 1922, 1924, 1931), and referred to in subsequent works (Van de Bogart 1975, 1976; Reijnders 1979; Singer 1986). Species in *Parasola* are not strictly deliquescent, but their gill cystidia collapse and the gills lose turgor without substantial liquification, a process referred to as "partial" or "incomplete deliquescence" (Buller 1931; Redhead et al. 2001; Larsson and Örstadius 2008). The terminology of microscopic characters follows Uljé and Bas (1991) and Uljé (2005), applying the term "lentiform" to spores which are broader in face view than in side view. The term "setule" is applied to lageniform pileocystidia or caulocystidia embedded in and projecting out of the pileus or stipe. The term "transitional setules" (Schafer 2012) is applied to a proportion of the pileal veil cells that have an appendage of varying size, up to and resembling that of the pileocystidia, but are part of the velar structure and not embedded in the hymeniderm. The abbreviation "L" refers to the number of lamellae, "Qm" to the median spore quotient, "Q(B)" to the ratio of spore length to breadth (face view) and "Q(W)" to the ratio of spore length to width (side view). Exsiccata are deposited at K (Royal Botanic Gardens, Kew), with additional material in the private collections of the authors.

Results

The Cypriot samples of *Tulosesus* tested in this work represent at least four significantly distinct lineages by ML and Bayesian analyses (Fig. 1). Three samples (DJS20120213003, ML20110310001 and ML21131CM) nested inside the clade of *T. sabulicola* and *T.*

Table 1 Voucher numbers, geographic origins, GenBank accession numbers and dates of specimens included in this study. Bold type is used for specimens sequenced in this study. *Tulosesus* species were deposited in GenBank as *Coprinellus*

Species	Origin	Date	Identifier	Voucher number	ITS	LSU	*TUB2*	*TEF1*
Coprinellus domesticus			L. Nagy	SZMC-NL-2933	FN396109			
Coprinopsis pseudonivea	Hungary	2007	L. Nagy	SZMC-NL-2340	FM163181	FM160728	FN396288	FN430698
Parasola auricoma	Hungary	2005	L. Nagy	SZMC-NL-0087	JN943107	JQ045871	FN396252	FM897236
P. auricoma	Pakistan	20140810	S. Hussain	LAH-SHP-7	KY461721	KY461730		MG587083
P. conopilea	USA	20180320	A. Rockefeller	312390	MH125285			
P. conopilea	China			CZ429	FJ755216			
P. crataegi	Hungary	20081012	L. Nagy	SZMC-NL-4175	KY928603	KY928631		
P. crataegi	Germany	19980929	G. Schmidt-Stohn	SSt98-239	KY928604			
P. cuniculorum	**England**	**20110609**	**D.J. Schafer**	**K(M)191984[1]**	**OL630105**			
P. cuniculorum	**England**	**20130923**	**D.J. Schafer**	**DJS20130923001**	**OL630104**			
P. cuniculorum [2]	**Cyprus**	**20120211**	**D.J. Schafer**	**DJS20120211001**	**OL630103**			
P. galericuliformis	Hungary	2006	L. Nagy	SZMC-NL-6601	FM163187	FM160722		
P. galericuliformis	Hungary	2004	L. Nagy	SZMC-NL-0095	FM163188	FM160721		
P. glabra	Pakistan	20140815	S. Hussain	LAH-SHP-5[1]	KY461717	KY621806		
P. glabra	Pakistan	20150828	S. Hussain	LAH-SHP-23	KY461718	KY621805		
P. hercules	Italy	19981108	A. Gennari	MCVE:7878	JF907839			
P. hercules	Netherlands	19840810	C.B. Uljé	L146[1]	HQ847027	HQ847112		
P. hercules	Netherlands	1994	C.B. Uljé	L1269	FM163190	FM160719		
P. aff. hercules	Netherlands		J. Volders	L78	KY928630	KY928646		
P. aff. hercules				L25	KY928607	KY928632		
P. kuehneri	England		D.J. Schafer	Schafer 1602	KY928609			
P. kuehneri	Netherlands	19870531	C.B. Uljé	L: C.B. Uljé 31 V 1987[1]	KY928608	KY928633		
P. kuehneri	Netherlands		C.B. Uljé	Uljé 1241	HQ847026	HQ847111		
P. kuehneri	Netherlands	1997	L. Nagy	L: Uljé 904	FM163191	FM160718		
P. lactea	Hungary		L. Nagy	NL-0678	KY928612			
P. lactea	Hungary		L. Nagy	NL-0660b	KY928611			
P. lactea[3]	Hungary		L. Nagy	SZMC-NL-0466	FM163192	FM160717	FN396254	
P. leiocephala			L. Nagy	SZMC-NL-0283	JN943113	JQ045887	FN396248	
P. leiocephala	Latvia			KuP6.2.2.1	KP698198			
P. lilatincta[4]	Pakistan		S. Hussain	LAH-SHP-8	KY461722			KY461731
P. lilatincta[4]	Pakistan		S. Hussain	LAH-SHP-31	KY461723	KY461726		KY461732
P. lilatincta[4]	Pakistan		S. Hussain	LAH-SHP-12	KY461724	KY461727		
P. lilatincta	Hungary		L. Nagy	NL-2929	KY928615	KY928634		
P. lilatincta	Hungary	2005	L. Nagy	SZMC-NL-0660	FM163195	FM160714	FN396246	FM897230
P. lilatincta	Scotland	20040823	D.J. Schafer	Schafer 2382004	FM163201	FM160708		
P. lilatincta	Hungary	2006	L. Nagy	SZMC-NL-0683	FM163203	FM160706		
P. lilatincta	Pakistan	20130803	S. Hussain	HUP SH-4	KP886462			
P. lilatincta	Pakistan	20140810	S. Hussain	HUP SH-P2	KP886463			
P. lilatincta	Pakistan	20140814	S. Hussain	HUP SH-P9	KP886464			
P. aff. lilatincta	Hungary	2006	L. Nagy	SZMC-NL-0086	FM163204	FM160705		
P. aff. lilatincta	Hungary	2006	L. Nagy	SZMC-NL-0096	FM163205	FM160704		
P. littoralis	**Cyprus**	**20180126**	**M. Loizides**	**K(M):264814[1] (ML 81162CM)**	**OL630108**			
P. littoralis	**Cyprus**	**20130125**	**D.J. Schafer**	**DJS20130125001**	**OL630107**			
P. littoralis	**Cyprus**	**20120213**	**D.J. Schafer**	**DJS20120213004**	**OL630106**			
P. malakandensis	Pakistan	20140909	S. Hussain & A.N. Kahlid	LAH-SHP-17	KU599827	KU599830		KU599832
P. malakandensis	Pakistan	20140912	S. Hussain	LAH-SHP-13	KU599828	KU599829		KU599831
P. megasperma	**Cyprus**	**20041227**	**C. Hobart**	**CH20041227001**	**OL630109**			
P. megasperma[5]	**England**	**19711024**	**P.D. Orton**	**E:Orton 4132 [1]**	**OL630101**			**OL630935**
P. megasperma	Netherlands		C.B. Uljé	L: C.B. Uljé 1275	KY928618	KY928637		
P. megasperma	Czech Republic	20121120	J. Cervenka	JC20121120	KY928617			
P. megasperma	USA	20180126	A. Rockefeller	307614	MH158284			
P. megasperma	Spain	1998	G. Moreno & L. Nagy	AH 13089	FM163207			
P. megasperma	Denmark	1996	M. Klamer & L. Nagy	C 19683	FM163206			
P. misera	**Wales**	**2014**	**D.J. Schafer**	**DJS20141030001[6]**	**OL630110**			
P. misera	Hungary	2007	L. Nagy	SZMC-NL-0280[7]	FM163210	FM160699		
P. misera	Hungary		L. Nagy	NL-0462	KY928619	KY928638		

Table 1 (continued)

Species	Origin	Date	Identifier	Voucher number	ITS	LSU	*TUB2*	*TEF1*
P. misera	Hungary	2006	L. Nagy	SZMC-NL-0490	FM163209	FM160700		
P. nudiceps[8]	**Scotland**	**19710903**	**P.D. Orton**	**E:Orton 4133** [1]	**OL630102**			**OL630936**
P. nudiceps	Germany	19870911	H. Bender	HB19870911A	MK063783			
P. ochracea	Norway	20090905	L. Nagy	SZMC-NL-3621[1]	JN943134			
P. ochracea[4]			L. Nagy	SZMC-NL-3167	HQ847029	JQ045865		
P. ochracea[4]			L. Nagy	SZMC-NL-3167	JN943136			
P. ochracea	Sweden	20080910	L. Nagy & T. Knuttson	SZMC-NL-3623	KY928626	KY928644		
P. plicatilis	Hungary	2006	L. Nagy	SZMC-NL-0284[9]	FM163189	FM160720	FN396251	FM897235
P. plicatilis	Hungary	2007	L. Nagy	SZMC-NL-0075[10]	FM163214	NG_075167		
P. plicatilis	Hungary	2005	L. Nagy	SZMC-NL-0295	FM163216	FM160693		FM897242
P. plicatilis-similis	Sweden	20070928	L. Nagy & T. Knuttson	SZMC-NL-2125[1]	KY928620			
P. plicatilis-similis	Slovakia	20081003	L. Nagy	SZMC-NL-3980	KY928621	KY928639		
P. plicatilis-similis	Sweden	2006	L. Nagy	SZMC-NL-0287	FM163218[10]	FM160691	FN396245	
P. pseudolactea	Pakistan	20140709	S. Hussain	HUP-SU-413	KY461720	KY621800		KY461734
P. pseudolactea	Pakistan	20140709	S. Hussain	HUP-SU-412	KY461719	KY621799		KY461733
P. 'schroeterii' [11]	Germany		R.M. Dahnke	Dahnke 1502	KY928616	KY928635		
P. 'schroeterii' [11]	Netherlands		C.B. Uljé	L: Uljé 1067	KY928627			
P. 'schroeterii' [11]	Netherlands		C.B. Uljé	L: Uljé 1140	KY928628			
P. 'schroeterii' [11]	Netherlands	1999	L. Nagy	Brier 1051999	FM163219	FM160690		
P. 'schroeterii' [11]	Denmark	1998	L. Nagy	Klamer 061998	FM163217	FM160692		
P. 'schroeterii' [11]	Norway		L. Nagy	SZMC-NL-3624	JN943135	JQ045874		
P. setulosa	South Korea	20150812	H.J. Cho, H. Lee & Y.W.Lim	SFC20150812-15	MF445222			
P. setulosa	USA	20140307	D.L. Viess	MICH 232900	KR869775			
P. sp. 4[12]	Hungary	2006	L. Nagy	SZMC-NL-0472	FM163199	FM160710		
Tulosesus aff. amphithallus[13]	Netherlands	19840719	C.B. Uljé	Uljé 562[13]	JN159533			
T. angulatus	Portugal	2014		P2_D7_D16	KU325003			
T. angulatus			L. Nagy	SZMC-NL-0906	JN159535			
T. angulatus			L. Nagy	SZMC-NL-1934	HQ846994			
T. angulatus			C.B. Uljé	Arnolds99-22	JN159536			
T. bisporiger			L. Nagy	WU 7403	HQ846974			
T. callinus	Italy	19840423	G. Simonini	MCVE 15323	JF907841			
T. callinus			L. Nagy	SZMC-NL-1931	FN396105			
T. callinus			C.B. Uljé	Uljé 1204	JN159518			
T callinus	**England**	**20171111**	**D.J. Schafer**	**DJS20171111003**	**OL630112**			**OL630937**
T. callinus var. limicola[14]	Netherlands	19860820	C.B. Uljé	Uljé 1009b[1]	HQ847003			
T. callinus var. miionis	**Cyprus**	**20120113**	**M. Loizides**	**K(M):264813[1] (ML20120113201)**	**MG857121**		**MG859659**	**OL630939**
T. callinus var. miionis	**Cyprus**	**20120213**	**D.J. Schafer**	**DJS20120213002**	**MG857122**			
T. callinus var. miionis	**Cyprus**	**20130129**	**D.J. Schafer**	**DJS20130129003**	**MG857120**	**MG857117**	**MG859658**	**OL630938**
T. christianopolitanus	Sweden		L. Örstadius	LÖ141-08	KC992944			
T. eurysporus			L. Nagy	SZMC-NL-3418	JN943114			
T. aff. eurysporus[15]			L. Nagy	SZMC-NL-1761	JN159541			
T. aff. eurysporus[16]			C.B. Uljé	Uljé 1191	HQ846995			
T. hiascens			L. Nagy	SZMC-NL-0628	JN159530			
T. hiascens	Hungary		L. Nagy	SZMC-NL-1350	GU227720			
T. hiascens[17]			L. Nagy	SZMC-NL-2598	JN159526			
T. hiascens			L. Nagy	SZMC-NL-0770	JN159529			
T. hiascens			L. Nagy	SZMC-NL-1349[18]	JN159525			
T. aff. hiascens			C.B. Uljé	Uljé 935	HQ846971			
T. aff. hiascens	**Cyprus**	**20130216**	**M. Loizides**	**ML31216CC**	**OL630111**			
T. maritimus	**Cyprus**	**20120113**	**M. Loizides**	**ML21131CM**	**MG857119**	**MG857116**		**MG859660**
T. maritimus	**Cyprus**	**20110310**	**M. Loizides**	**ML20110310001**	**OL630114**			**OL630941**
T. maritimus	**Cyprus**	**20120213**	**M. Loizides, D.J. Schafer**	**K(M):264812[1] (DJS20120213003)**	**MG857118**	**MG857115**		
T. maritimus	**Cyprus**	**20180126**	**M. Loizides**	**ML81162CMA**	**OL630115**			
T. pallidus	Hungary	20080509	L. Nagy	SZMC-NL-0625	JN159521			

Table 1 (continued)

Species	Origin	Date	Identifier	Voucher number	ITS	LSU	*TUB2*	*TEF1*
T. pallidus	Hungary	20060805	L. Nagy	SZMC-NL-1556[1]	HQ846989			
T. pallidus[19]	Hungary		L. Nagy	SZMC-NL-4218	JN159522			
T. sabulicola	Hungary	20080610	L. Nagy	SZMC-NL-1763	HQ847007			**MG859663**
T. sabulicola	Hungary			SZMC-NL-0825				**MG859661**
T. sabulicola				SZMC-NL-1027	JN159558			**MG859662**
T. sabulicola	Hungary	20080620	L. Nagy	SZMC-NL-2906	JN159559			
T. sclerocystidiosus	Sweden		L. Örstadius	LO407-05	KC992942			
T. sclerocystidiosus			L. Nagy	SZMC-NL-1444	JN159537			
T. sclerocystidiosus			L. Nagy	SZMC-NL-1022	JN159538			
T. sp.			L. Nagy	SZMC-NL-1751	HQ846980			
T. sp.[20]			L. Nagy	SZMC-NL-0195	JN159556			
T. sp.	Denmark[8]		S. Rønhede	CBS 118528	AJ890441			
T. sp.	Canada	20150816	C. Hay	BIOUG24046-H03	KT695352			
T. sp.			L. Nagy	SZMC-NL-1356	HQ846979			
T. sp.			P Höijer	Höijer 95067	HQ846990			
Tulosesus cf. subimpatiens	**Cyprus**	**20140528**	**M. Loizides**	**ML41582C5**	**OL630113**		**OL614103**	**OL630940**

Footnotes

[1] Holotype

[2] 4-spored *P. cuniculorum*

[3] Deposited in GenBank as *P. leiocephala*; *P. lactea* in Szarkandi et al. (2017)

[4] Deposited in GenBank as "P. schroeterii"

[5] As *Coprinus megaspermus* (E00204206; P.D. Orton 4132)

[6] Two collections (DJS20141030001 on cow dung & DJS20141111002 on rabbit dung) with identical sequences

[7] Neotype

[8] As *Coprinus nudiceps* (E00204198; P.D. Orton 4133)

[9] Deposited in GenBank as *P. hemerobia*; *P. plicatilis* in Szarkándi et al. (2017)

[10] Epitype

[11] *P. schroeteri*; deposited in GenBank as "*P. schroeterii*"

[12] Deposited in GenBank as *P. lilatincta*; *P. sp.4* in Szarkándi et al. (2017)

[13] Two collections identified as *C. amphithallus* by Uljé fall in different clades in Nagy et al. 2012a, b; this one has been labelled *aff. amphithallus* in that paper

[14] = *Coprinellus limicola* (Uljé) Doveri & Sorrocco

[15] Deposited in GenBank as *Coprinellus* sp.; *C. aff. eurysporus* in Nagy et al. (2012a, b)

[16] Deposited as *Coprinellus subimpatiens*; *C. aff. eurysporus* in Nagy et al. (2012a, b)

[17] Deposited in GenBank as *Coprinellus sp.*; *C. hiascens* in Nagy et al. (2012a, b)

[18] Deposited in GenBank as *Coprinellus* sp., SZMC-NL-1349; as *C. hiascens*, SZMC-NL-1349 in Nagy et al. (2012a, b); SZMC-NL-1439 in supplementary table

[19] Deposited in GenBank as *Coprinellus sp.*; *C. pallidus* in Nagy et al. (2012a, b)

[20] Deposited as *Coprinellus sp.* 13; listed as *C. limicola* in supplementary table to Nagy et al. (2012a, b) but as a second C. sp. 1 in the phylogram of that paper

[21] Soil isolate, strain GR177, see Rehner and Buckley (2005)

christianopolitanus, forming a significantly monophyletic clade sister to *T. christianopolitanus* in all analyses. No significant support could be obtained for *T. sabulicola*, whose samples were attached to the root of the clade, so a reciprocal monophyly between these taxa could not be proven. While all three species showed a very similar ITS rDNA (only 4–5/584 differences, 99.14–99.31% similar), they displayed a greater distance in *TEF1* exons (7/439 and 10/449 differences, 97.78% and 98.4%), and *TEF1* intron 4 (8–13/50 differences, 74–84%). No variability at all was found inside these clades in ITS rDNA or *TEF1* (either exons or introns), except for one mutation in the *TEF1* exon in one of the Cypriot samples not present in the others, although a number of polymorphic sites were present in *TEF1* intron 4 of two collections of *T. sabulicola* (but not in the other one) sequenced for the present study. On the contrary, several intraspecific mutations

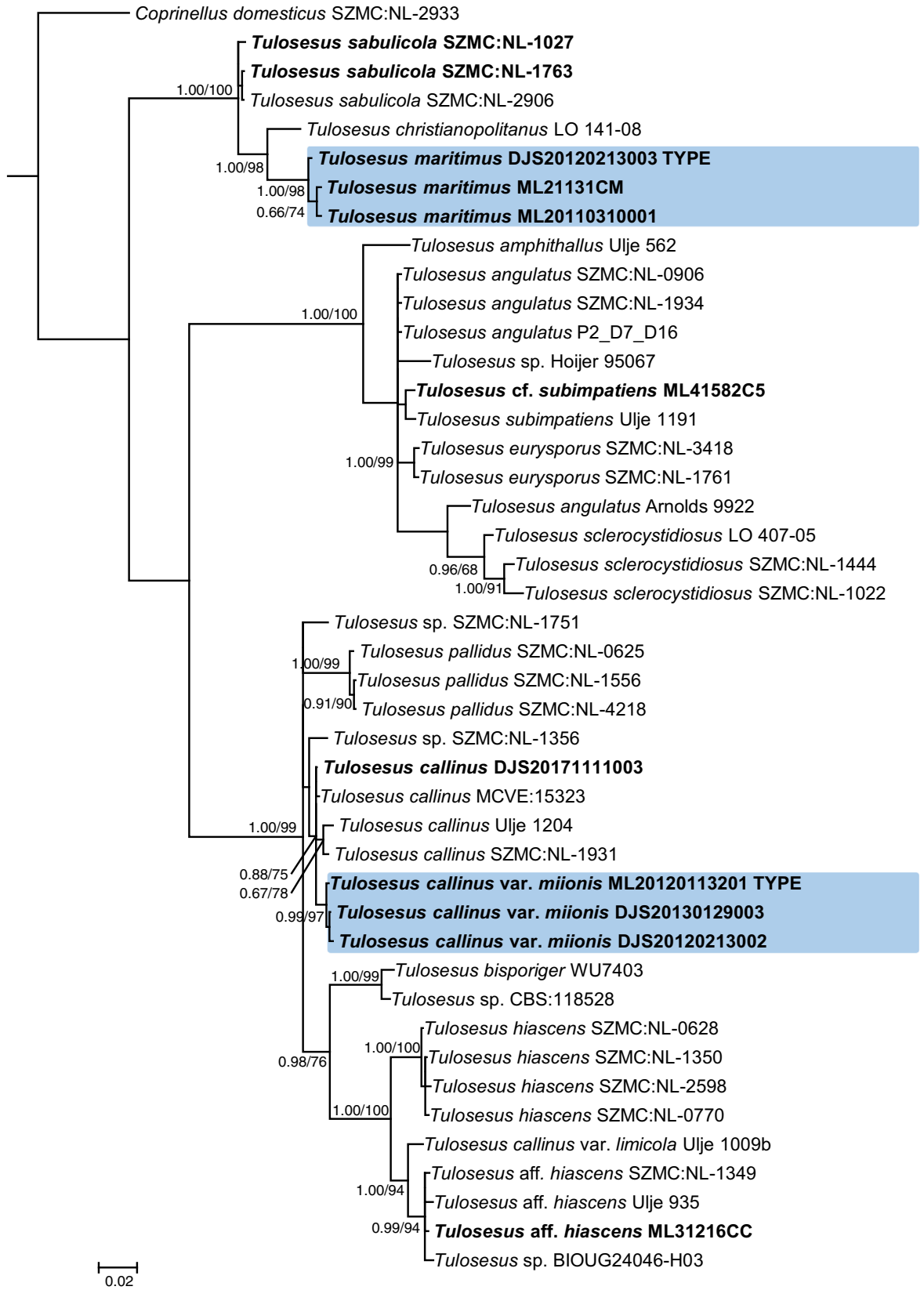

Fig. 1 A 50% majority rule ITS rDNA-*TEF1-TUB2* consensus phylogram of the samples of the cypriot samples of *Tulosesus* and the most similar sequences in databases (with *Coprinellus domesticus* as outgroup) obtained using MrBayes from 6000 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Sequences newly generated in this study are in bold

can be observed between *TUB2* sequences of *T. sabulicola*, maybe contributing to the loss of support for this species. Since the morphologically distinct Cypriot samples form a significantly monophyletic clade displaying a considerable genetic distance with the other species, it is here hypothesised they belong to a distinct species that is described below and accommodated under a new name.

Another Cypriot sample (ML41582C5) nested within the Eurysporoid clade (along with *T. eurysporus*, *T. sclerocystidiosus*, *T. angulatus* and *T. subimpatiens*), but did not show any significant similarity with any of the samples in it. While the clades of *T. eurysporus* and *T. sclerocystidiosus* received a significant statistical support, *T. angulatus* collapsed. While ITS rDNA of these species is very similar, a great intraspecific variability was observed in *TUB2*, and *TEF1* data is lacking from most collections. With these variable and incomplete genetic data, taxonomic conclusions are risky, and so this sample is provisionally identified as *Tulosesus* cf. *subimpatiens* because this is the taxon with the most similar *TUB2* sequence (98.07%), although this clade does not have a significant support (0.75 PP, 55 BP).

All other Cypriot collections were related with the core of "Setulosi" (Nagy et al. 2012a), one of them (ML31216CC) being significantly similar to some specimens identified as *T. hiascens* (Ulje935, SZMC-NL-1349), and another three (DJS20130129003, ML20120113201 and DJS20120213002) represented a significantly monophyletic clade related to *T. callinus* (0.88 PP, 75 BP). Samples of *T. callinus* did not form a reciprocally monophyletic clade, but collapsed. In this case, the genetic distance observed between samples of *T. callinus* and the Cypriot monophyletic clade is quite small, maybe because sample ML20120113201 shows polymorphic states at the ITS rDNA positions where the other collections have putatively apomorphic mutations. This maybe indicates that this collection has both types of ITS alleles, that of *T. callinus* and that of the other Cypriot samples, evidencing gene flow between both groups, and so preventing us from considering them isolated species. No evident synapomorphic mutation could be found in *TEF1* in either *T. callinus* or the Cypriot samples, and only 3/367 bp in *TUB2*, but the overall variability of this gene between both groups was too high (8/367 bp) to produce any significant statistical support. With these results, there is not enough genetic evidence to consider that the Cypriot samples belong to a different species other than *T. callinus*, and so we here prefer to accommodate them as a variety to reflect their morphological characters.

The analysis of ITS rDNA, LSU rDNA, *TEF1* and *TUB2* sequences of a *Parasola* (Fig. 2) suggested that three Cypriot samples (ML81162CM, DJS20130125001, DJS20120213004) form a monophyletic lineage related to samples identified as *P. lilatincta*, *P. schroeterii*, *P. kuehneri* and *P. ochracea*. Since most clades in this group are reciprocally monophyletic and display average distances between them, the lineage of the Cypriot samples studied in the present work is accommodated below under a new species name, *Parasola litoralis*. Finally, the ITS sequence of a four-spored fimicolous collection of *Parasola* from Cyprus (DJS20120211001), identified initially as *P. misera* (P. Karst.) Redhead, Vilgalys & Hopple, matched that of the holotype of the two-spored species *P. cuniculorum* D.J. Schaf., described from Britain (Schafer 2014), obtained in the present work. Several additional collections of the two-spored form from different regions of the UK were also genetically similar to the four-spored collection from Cyprus. ITS data from another two holotypes was produced in the present work, *P. nudiceps* and *P. megasperma*, helping to clarify the taxonomic identity of the clades in which they nest (Fig. 2). In the case of *P. nudiceps*, a synonymy with the more recent name *P. ochracea* is suggested.

Taxonomy

Tulosesus maritimus D.J. Schaf., Loizides & P. Alvarado **sp. nov.**

Index Fungorum registration number IF559579 Figs. 3–4

Etymology: Maritime, from the Latin word *mare*, meaning sea

Diagnosis: Minute deliquescing agaric with reddish-brown to ochraceous-pink pileus; relatively short, mainly capitate to subcapitate pileocystidia often with thickened, yellow-brown encrusted base; mixed globose-vesiculose and lageniform, frequently bifurcate cheilocystidia sometimes with subcapitate apices; clamp connections present; and broadly ellipsoid spores [(9–)11.1–13.5(–14) × (6.5–)7.1–8.2(–9.5) µm], with distinct, up to 3 µm wide strongly eccentric germ pore. On coastal dunes under *Tetraena alba*.

Holotype: Cyprus. Lady's Mile, 0 m a.s.l., on embryonic shifting dunes under *Tetraena alba*, leg. M. Loizides & D.J. Schafer, 13-II-2012, K(M)264812.

Additional collections studied: Cyprus. Akrotiri, 1 m a.s.l., on embryonic shifting dunes under *Tetraena alba*, leg. M. Loizides, 13-II-2009. Lady's Mile, 0 m a.s.l., on embryonic shifting dunes under *T. alba*, leg. M. Loizides, 10-III-2011, *Ibidem*, M. Loizides, 13-I-2012, *Ibidem*, leg. M. Loizides, 10-III-2012, *Ibidem*, leg. M. Loizides, 26-I-2018

Fig. 2 A 50% majority rule ITS rDNA consensus phylogram of selected species of *Parasola* (with *Coprinopsis pseudonivea* as outgroup) obtained using MrBayes from 4650 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Nonsignificant support values are exceptionally represented inside parentheses. Sequences newly generated in this study are in bold

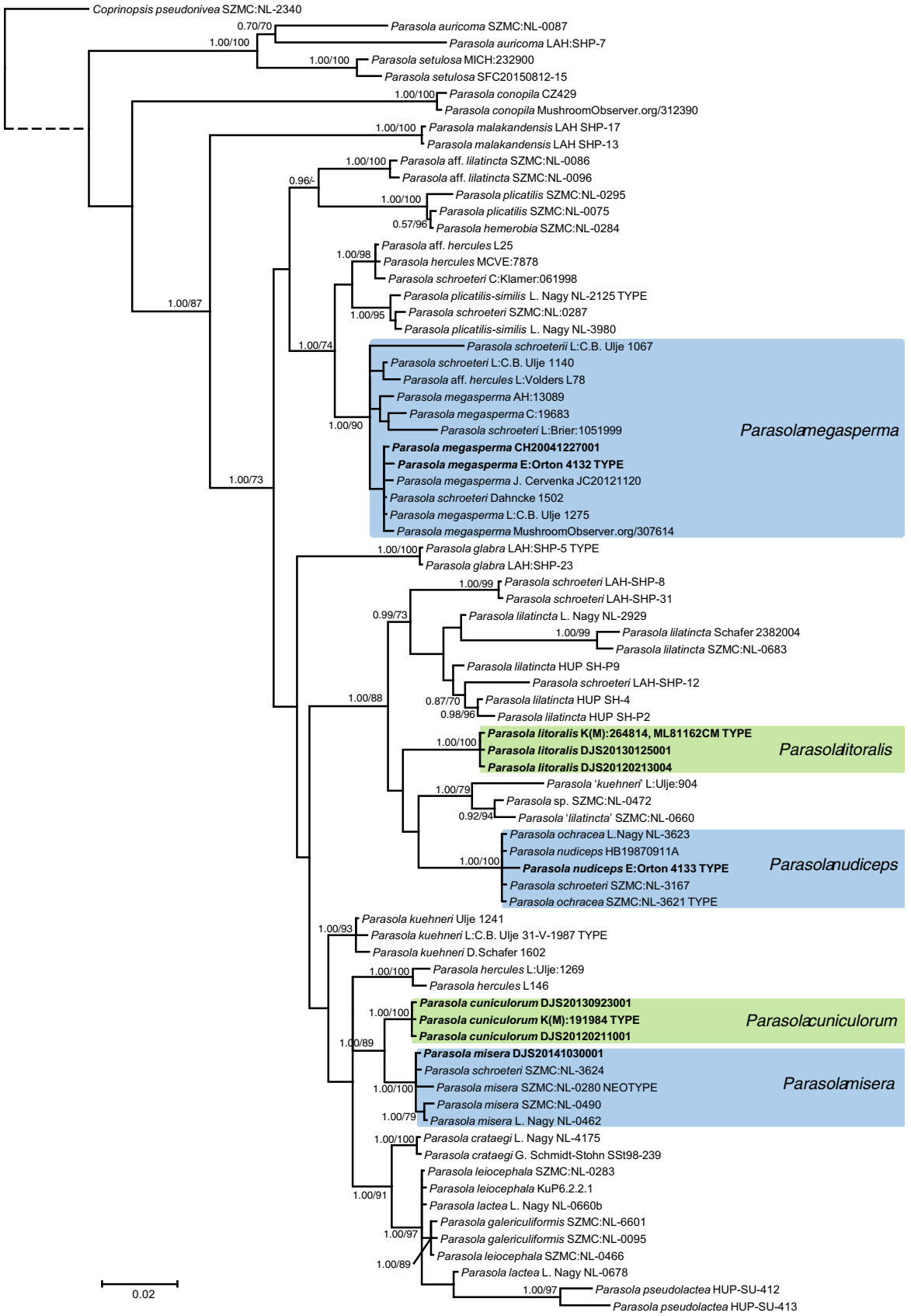
Coprinopsis pseudonivea SZMC:NL-2340
Parasola auricoma SZMC:NL-0087
Parasola auricoma LAH:SHP-7
Parasola setulosa MICH:232900
Parasola setulosa SFC20150812-15
Parasola conopila CZ429
Parasola conopila MushroomObserver.org/312390
Parasola malakandensis LAH SHP-17
Parasola malakandensis LAH SHP-13
Parasola aff. lilatincta SZMC:NL-0086
Parasola aff. lilatincta SZMC:NL-0096
Parasola plicatilis SZMC:NL-0295
Parasola plicatilis SZMC:NL-0075
Parasola hemerobia SZMC:NL-0284
Parasola aff. hercules L25
Parasola hercules MCVE:7878
Parasola schroeteri C:Klamer:061998
Parasola plicatilis-similis L. Nagy NL-2125 TYPE
Parasola schroeteri SZMC:NL:0287
Parasola plicatilis-similis L. Nagy NL-3980
Parasola schroeterii L:C.B. Ulje 1067
Parasola schroeteri L:C.B. Ulje 1140
Parasola aff. hercules L:Volders L78
Parasola megasperma AH:13089
Parasola megasperma C:19683
Parasola schroeteri L:Brier:1051999
Parasola megasperma CH20041227001
Parasola megasperma E:Orton 4132 TYPE
Parasola megasperma J. Cervenka JC20121120
Parasola schroeteri Dahncke 1502
Parasola megasperma L:C.B. Ulje 1275
Parasola megasperma MushroomObserver.org/307614
Parasola megasperma
Parasola glabra LAH:SHP-5 TYPE
Parasola glabra LAH:SHP-23
Parasola schroeteri LAH-SHP-8
Parasola schroeteri LAH-SHP-31
Parasola lilatincta L. Nagy NL-2929
Parasola lilatincta Schafer 2382004
Parasola lilatincta SZMC:NL-0683
Parasola lilatincta HUP SH-P9
Parasola schroeteri LAH-SHP-12
Parasola lilatincta HUP SH-4
Parasola lilatincta HUP SH-P2
Parasola litoralis K(M):264814, ML81162CM TYPE
Parasola litoralis DJS20130125001
Parasola litoralis DJS20120213004
Parasola litoralis
Parasola 'kuehneri' L:Ulje:904
Parasola sp. SZMC:NL-0472
Parasola 'lilatincta' SZMC:NL-0660
Parasola ochracea L.Nagy NL-3623
Parasola nudiceps HB19870911A
Parasola nudiceps E:Orton 4133 TYPE
Parasola schroeteri SZMC:NL-3167
Parasola ochracea SZMC:NL-3621 TYPE
Parasola nudiceps
Parasola kuehneri Ulje 1241
Parasola kuehneri L:C.B. Ulje 31-V-1987 TYPE
Parasola kuehneri D.Schafer 1602
Parasola hercules L:Ulje:1269
Parasola hercules L146
Parasola cuniculorum DJS20130923001
Parasola cuniculorum K(M):191984 TYPE
Parasola cuniculorum DJS20120211001
Parasola cuniculorum
Parasola misera DJS20141030001
Parasola schroeteri SZMC:NL-3624
Parasola misera SZMC:NL-0280 NEOTYPE
Parasola misera SZMC:NL-0490
Parasola misera L. Nagy NL-0462
Parasola misera
Parasola crataegi L. Nagy NL-4175
Parasola crataegi G. Schmidt-Stohn SSt98-239
Parasola leiocephala SZMC:NL-0283
Parasola leiocephala KuP6.2.2.1
Parasola lactea L. Nagy NL-0660b
Parasola galericuliformis SZMC:NL-6601
Parasola galericuliformis SZMC:NL-0095
Parasola leiocephala SZMC:NL-0466
Parasola lactea L. Nagy NL-0678
Parasola pseudolactea HUP-SU-412
Parasola pseudolactea HUP-SU-413
0.02

Fig. 3 Macro- and micromorphological features of *Tulosesus maritimus*: (**A, B**) basidiocarps in situ, scale bar = 5 mm; (**C**) transitional setules in Congo Red, scale bar = 10 μm; (**D**) pileocystidium in Congo Red, scale bar = 10 μm; (**E**) magnification of pileus surface and pileocystidia under dissecting microscope, scale bar = 100 μm; (**F**) magnification of lamellar edge and projecting cheilocystidia under dissecting microscope, scale bar = 100 μm; (**G**) basidiospores in water, scale bar = 10 μm, spores on right illuminated/lightened to show germ pore; (**H**) basidia in Congo Red, scale bar = 10 μm; (**I**) caulocystidia in Congo Red, scale bar = 10 μm; (**J, K**) cheilocystidia in Congo Red, scale bar = 10 μm. (**C, D, E, F, G** left, **J, K**) from holotype, (**A, B, G** on the right) 13/2/2009, (**H, I**) 10/3/2011

Macromorphological description: Closed pileus subglobose to ovoid, 3–5 mm high by 3–5 mm wide, expanding to hemispherical to broadly conical, rufous-brown to ochraceous-pink, dark reddish-orange at the centre, paler and plicate towards the margin, 6–9 mm wide when fully expanded, often with an uplifted margin; scattered brown dots from veil more or less evenly present over the entire surface; bristles on cap surface difficult to see, barely visible with a hand lens. Lamellae free to finely adnexed, narrow, fairly distant, whitish to greyish-buff at first but soon blackish, not

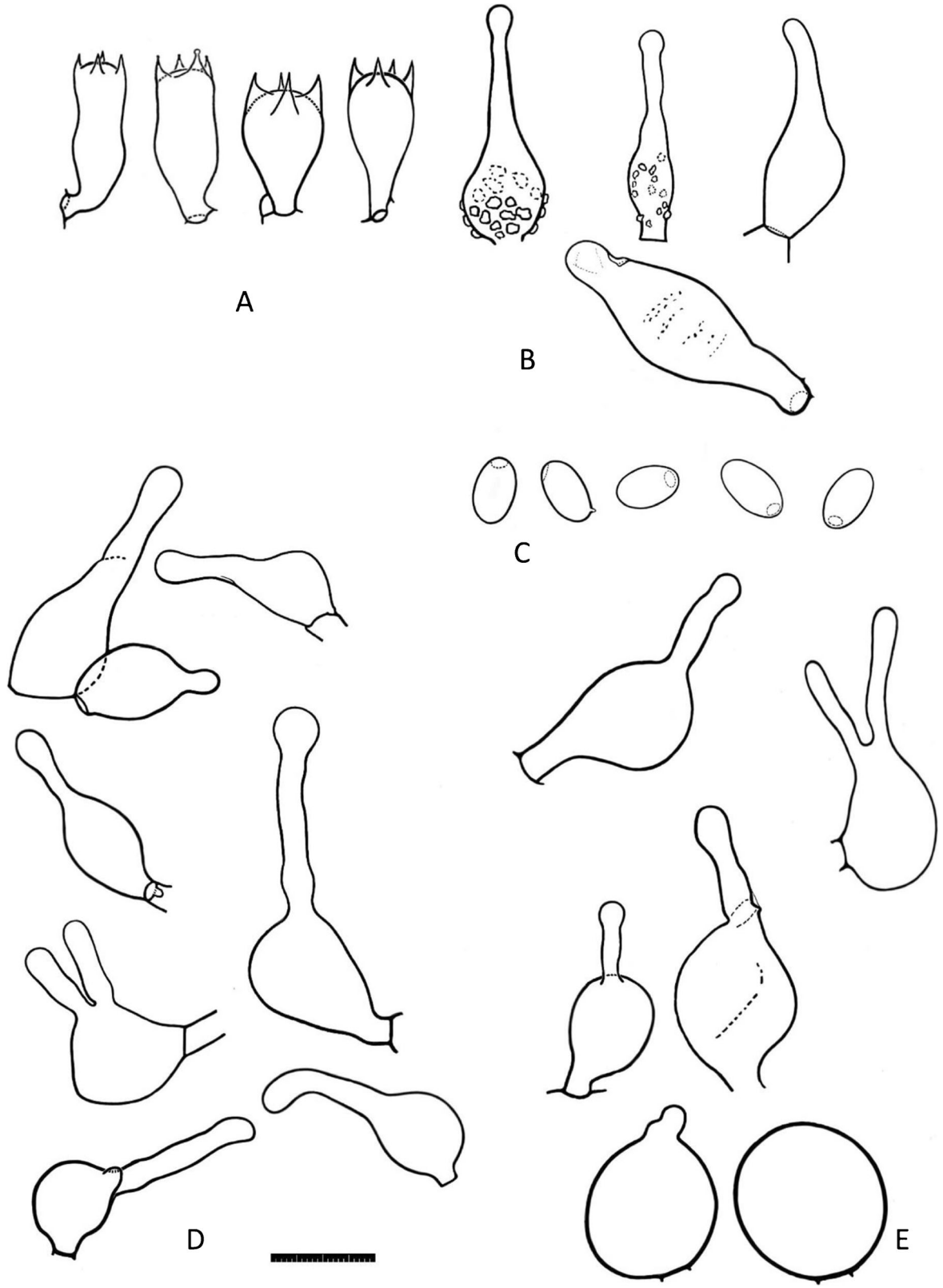

Fig. 4 *Tulosesus maritimus* line drawings of microscopic features: (**A**) basidia; (**B**) setules on pileus; (**C**) basidiospores; (**D**) setules (caulocystidia) on the stipe; (**E**) cheilocystidia; scale bar = 20 μm. (figured from different collections)

fully deliquescing; L. 12–18. Stipe at first squat and fully submerged into the sand, white, exannulate, soon surfacing and expanding 18–30 mm long × 1 mm wide.

Micromorphological description: Spores in face view (9–)11.1–13.5(–14) × (6.5–)7.1–8.2(–9.5) μm, on average 12.3 × 7.6 μm, Qm = 1.6, broadly ellipsoid with large, strongly eccentric germ pore up to 3 μm wide, slightly lentiform, Qm in side view = 1.7. Basidia uniformly 4-spored, bimorphic: predominantly clavate 24–32 × 12–15μm, 4–5.5 μm at base; some with a constricted middle 29–33 × 10–12 μm (9–10 μm at middle), 4 μm wide at the base. Cheilocystidia of two kinds, consisting of globose to vesiculose cells measuring 15–32 × 13–31 μm, but also lageniform, measuring overall 36–56 × 17–25 μm with a more or less cylindrical, frequently bifurcate appendage measuring 16–25 × 3.5–5 μm, narrowing towards the apex and at the base; apex slightly enlarged to subcapitate, up to 6 μm wide. Pleurocystidia absent. Pileocystidia mainly lageniform, measuring overall 27–54 × 12–19 μm; thick-walled, heavily encrusted and yellow-brown at the base, tapering and thinner-walled upwards; apex swollen, or more usually distinctly capitate to subcapitate, 4–7 μm wide. Veil present, composed of thick-walled yellow-brown, broadly filamentous cells, intermixed with some thick-walled rounded cells and transitional setules; all velar elements covered in thick resinous encrustation. Caulocystidia lageniform with a cylindrical appendage and swollen to subcapitate apex, sometimes bifurcate, measuring overall 28–55 × 14–19 μm (including a 16–41 × 3.5–5 μm appendage and a 5–8 μm wide apex).

Ecology & distribution: Found in mid- to late winter between January and February, fruiting in small groups on coastal embryonic dunes and open sands with scattered halophytic vegetation, usually in very close proximity to the shore, under or near *Tetraena alba* (L.f.) Beier & Tulin; its tiny fruitbodies often deeply submerged into the substrate and difficult to see. So far only known from Cyprus.

Remarks: *Tulosesus maritimus* is a morphologically well-defined species. It nests in a monophyletic clade, also including *T. sabulicola*, which Nagy et al. (2012a) considered possibly basal to other related lineages. *Tulosesus christianopolitanus*, described from a grassy substrate in Sweden (Örstadius et al. 2015), also nests in this clade, and it is probably closest to *T. maritimus* (as suggested by phylogenetic results reported above). Both species have partially lageniform or globose to sphaeropedunculate cheilocystidia. However, *T. christianopolitanus* has larger spores with an indistinct germ pore, measuring 13–16.5 × 8–9 μm in the type collection, pileocystidia which are not encrusted at the base and lacks bifurcate cheilo- and caulocystidia. In addition, the cheilocystidia and pileocystidia of *T. christianopolitanus* are reported to turn yellow or green in 10% solution of ammonia, a reaction not observed in any of the *T. maritimus* collections studied so far. The apparent absence of a veil in the type material of *T. christianopolitanus* might be an additional discriminating feature between the two species, but this character needs to be verified from further collections of the latter. The other species in this clade, *T. sabulicola*, is also described from sand dunes. Although it and *C. maritimus* share several common features, notably the short, capitate pileocystidia with encrusted base, they are easily separated microscopically: *T. sabulicola* has much larger spores measuring 15–21.8 × 10–13 μm, strictly two-spored monomorphic basidia and lacks lageniform cheilocystidia (Nagy et al. 2012a).

Another morphologically similar though phylogenetically distinct species is *Coprinellus curtus* (Kalchbr.) Vilgalys, Hopple & Jacq. Johnson. It shares with *T. maritimus* pileocystidia with a capitate apex and similarly shaped spores with a strongly eccentric germ pore, while it also has strongly encrusted, thick-walled yellow-brown veil on the pileus (Buller 1931; Uljé and Bas 1991; Doveri 2004; Uljé 2005). It differs from *T. maritimus* in its coprophilous ecology, absence of clamp connections and monomorphic rounded cheilocystidia. Furthermore, the veil in *T. maritimus* is thick walled, yellow-brown and encrusted, but with only a small proportion of small rounded cells, rather than the mainly rounded cells seen in *C. curtus.* Many of the pileocystidia in *T. maritimus* have a thickened yellow-brown base, arising within the veil cells and therefore are transitional to the pileocystidia found in most other setulose *Coprinellus* and *Tulosesus* species. This feature is also found in *T. cinnamomeotinctus* (P.D. Orton) Wächter & A. Melzer, but to a lesser extent also in *C. micaceus* (Bull.: Fr.) Vilgalys, Hopple & Jacq. Johnson (see Schafer 2012), and in *C. hepthemerus* (M. Lange & A.H. Sm.) Vilgalys, Hopple & Jacq. Johnson, a species morphologically close to *C. curtus* (Lange and Smith 1953).

Tulosesus cinereopallidus (L. Nagy et al.) Wächter & A. Melzer, reported from mossy clayish soil or leaf litter, is also rather similar to *T. maritimus*, having capitate pileocystidia and cheilocystidia, as well as veil on the pileus. It lacks the thick-walled encrusted base to the cystidia and the encrusted veil, and has narrower spores (< 7 μm) with a smaller germ pore, in addition to a paler coloured pileus (Nagy et al. 2012a). Finally, *T. callinus* var. *miionis*, sometimes co-occurring in the same habitat as *T. maritimus* and also described here for the first time, differs in its slightly larger basidiomata, longer, broadly tapering thin-walled pileocystidia, two-spored bimorphic basidia, and larger spores (11.5–15.3 × 7.0–10.0 μm) which have only a moderately eccentric germ pore.

Tulosesus callinus* var. *miionis D.J. Schaf., Loizides & P. Alvarado **var. nov.**

Index Fungorum registration number IF559581 Figs. 5–6

Etymology: From the ancient Greek name of Cyprus, *Μῐονίς*.

Fig. 5 Macro- and micromorphological features of *Tulosesus callinus var. miionis*: (**A, B**) basidiocarps in situ, scale bar = 5 mm; (**C**) magnification of pileus surface and pileocystidia under dissecting microscope, scale bar = 100 μm; (**D**) pileocystidia in Congo Red, scale bar = 10 μm; (**E**) basidia in Congo Red, scale bar = 10 μm; (**F**) basidiospores in water, scale bar = 10 μm; (**G**) caulocystidia in Congo Red, scale bar = 10 μm; (**H**) cheilocystidia in Congo Red, scale bar = 10 μm. (**A, B, E**) from holotype, (**C, F, G, H**) 13/2/2012, (**D**) 29/1/2013

Diagnosis: Small deliquescent agaric with reddish-brown to ochraceous-grey pileus, tapering pileocystidia, slender bimorphic 2-spored basidia, filamentous veil, clamp connections and ellipsoid lentiform spores (11.5–15.3 × 7.0–10.0 µm), with a moderately eccentric germ pore up to 2.3 µm wide. On coastal and inland dunes.

Holotype: Cyprus. Lady's Mile, 0 m a.s.l., on embryonic shifting dunes among plant litter, leg. D.J. Schafer, 13-I-2012, K(M)264813.

Additional collections studied: Cyprus. Lady's Mile, 0 m a.s.l., on embryonic shifting dunes among plant litter, *Ibidem*, leg. D.J. Schafer, 13-II-2012, DJS20120213001 and

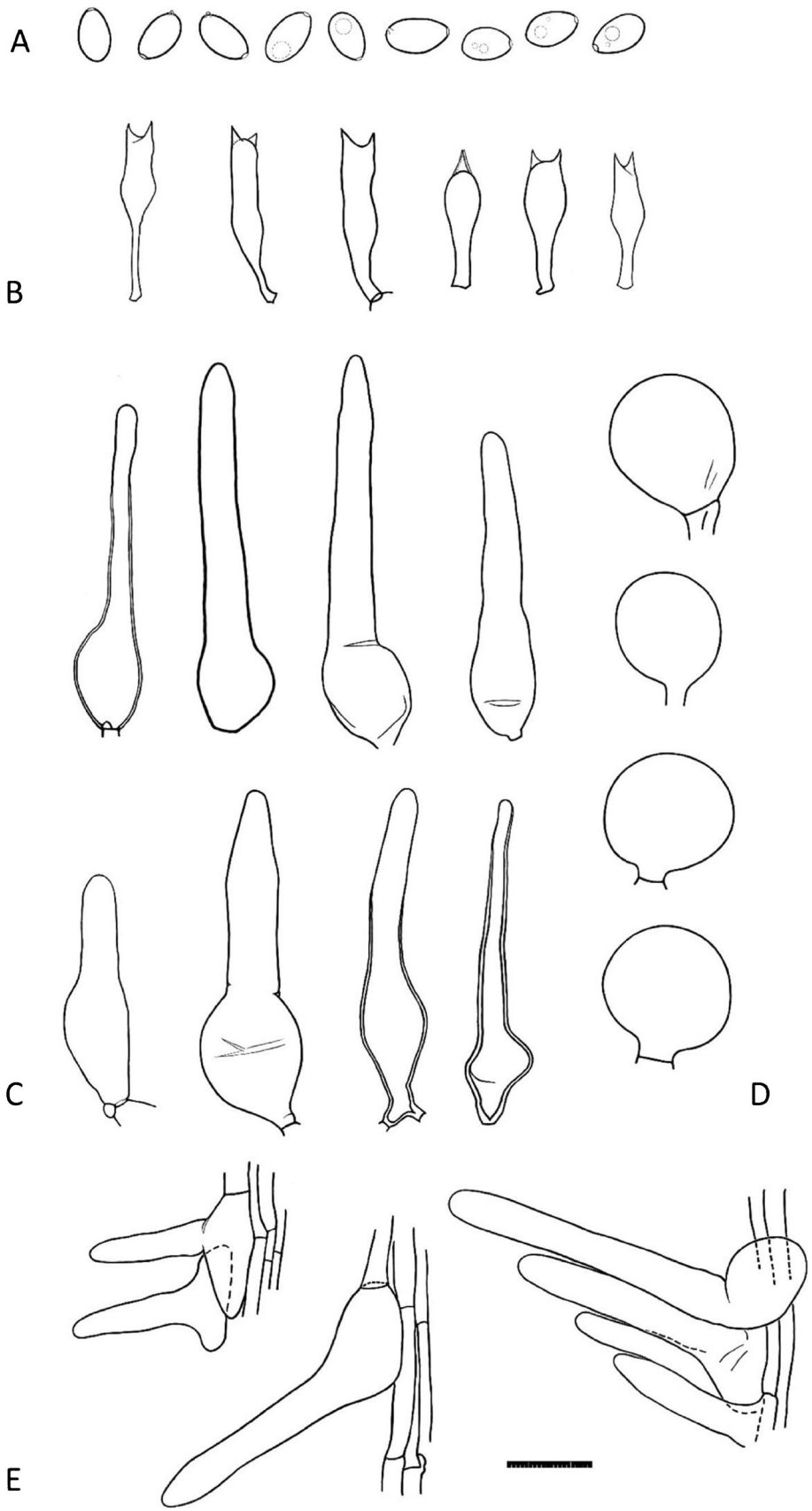

Fig. 6 *Tulosesus callinus var. miionis* line drawings of microscopic features: (**A**) basidiospores; (**B**) basidia; (**C**) setules on pileus; (**D**) cheilocystidia; (**E**) setules (caulocystidia) on the stipe; scale bar = 20 μm. (figured from different collections)

DJS20120213002; Pentakomo 90 m a.s.l., on sandy roadside bank among plant litter leg. D.J. Schafer, 29-I-2013, DJS20130129003.

Macromorphological description: Closed pileus subglobose to cylindrical or ovoid, 6–10 mm high by 3–5 mm wide, ochraceous-brown, darker ochraceous-orange to reddish-orange at the disk, soon expanding to campanulate or broadly conical, plicate and brown under dry conditions, up to 10–15 mm wide when fully expanded and grey from the margin inwards in moist conditions; margin often uplifted at full maturity. Pileus surface covered in easily seen bristles. Lamellae free to finely adnexed, whitish becoming blackish and deliquescing with age, narrow, moderately crowded; L 20– 28. Stipe white to ochraceous, minutely pubescent, exannulate, 18–30 mm long by 1.5–2 mm wide.

Micromorphological description: Spores in face view 11.5–15.3 × 7.0–10 μm, on average 13.3 × 8.3 μm, Qm = 1.6, broadly ellipsoid to ovoid or slightly rounded-rhomboid; lentiform, in side view ellipsoid with slightly flattened side, on average measuring 12.8 × 7.4 μm, Qm = 1.73, with a moderately eccentric germ pore measuring 2.2–2.3 μm wide. Basidia uniformly 2-spored, ranging from short-clavate and measuring 15 × 10 μm, 3.5 μm at base, to elongate and constricted at the middle, measuring 37 × 8 μm, narrowing to less than 2 μm towards the base. Cheilocystidia consisting mainly of globose cells measuring 18–32 μm in diameter. Pleurocystidia absent. Pileocystidia 70–93 × 15–24 μm, lageniform with a cylindrical or tapering appendage and rounded or bluntly conical apex. Sclerocystidia present. Caulocystidia 35–85 μm long with swollen base 25–29 × 12–17 μm, lageniform with a cylindrical or weakly tapering appendage, 7–9 μm wide with rounded or bluntly conical apex. Clamp connections present.

Ecology & distribution: Appearing in mid- to late winter in sand dunes and open sands with scattered vegetation; also found on wooden debris and among plant litter by roadsides and loamy banks, on sandy ground. So far only known from Cyprus.

Remarks: This morphologically distinct taxon differs from *Tulosesus callinus* by strictly 2-spored basidia and larger spores, but also by pileocystidia with a somewhat wider appendage and a rather distinct broadly tapering apex. *T. callinus* is described as a strictly 4-spored species, has smaller spores measuring 9.3–13.1 × 5.7–7.4 μm and commonly occurs in association with woody litter. Our multigene phylogenetic analysis has provided insufficient support for their segregation from the core clade of *T. callinus* (see "Results" above). However, the collections are well defined morphologically and therefore they could maybe represent a partially isolated lineage, or even a recently isolated species. Moreover, the analysis of additional collections and sequences from other markers could eventually produce significant reciprocal support for *T. callinus* and '*T. miionis*'. On the basis of the current data, however, we cautiously consider these collections a variety and propose the name *T. callinus* var. *miionis* to accommodate them.

This variant was first collected on coastal dunes together with *T. maritimus*, to which it is macromorphologically very similar. However, *T. maritimus* is a smaller species, further distinguished by the presence of 4-spored basidia and smaller spores with a strongly eccentric germ pore, measuring (9–)11.1–13.5(–14) × (6.5–)7.1–8.2(–9.5) μm, as well as capitate or subcapitate pileocystidia. The recently described *T. sabulicola*, a bisporic species nesting in the same clade as *T. maritimus* and *T. chrisitanopolitanus*, is also found on dunes and sandy habitats, but is distinguished by the presence of short pileocystidia with a small capitate or mucronate extension, monomorphic basidia and larger spores, measuring 15–21.8 × 10–13 μm (Nagy et al. 2012a).

Another similar russet to cinnamon-brown bisporic species is *Tulosesus sassii* (M. Lange & A.H. Sm.) Wächter & A. Melzer, a poorly known taxon originally described by Sass (1929) as *Coprinellus ephemerus* f. *bisporus* J.E. Sass, but later redescribed as a distinct species by Lange and Smith (1953). This species also features dimorphic 2-spored basidia, as well as similarly shaped pileocystidia and sclerocystidia. However, it is reported from horse-dung, manured and rotten straw substrates and has very large spores, measuring 13–20 × 8–11 μm, in addition to larger, globose to vesiculose cheilocystidia and pleurocystidia, measuring 50–90 × 15–55 μm (Sass 1929; Lange and Smith 1953; Doveri et al. 2005; Vesterholt 2008).

Tulosesus amphithallus (M. Lange & A.H. Sm.) Wächter & A. Melzer, a species reported from sandy roadsides and grassy habitats, features 2-spored basidia and has somewhat larger, similarly shaped spores to *T. callinus* var. *miionis*, but is distinguished by its markedly lageniform cheilocystidia and absence of sclerocystidia (Lange and Smith 1953; Uljé and Bas 1991; Vesterholt 2008). Somewhat similar to the latter is *T. pseudoamphithallus* (Uljé) Wächter & A. Melzer, which differs from *T. callinus* var. *miionis* in its distinctly slender and elongated subcylindrical spores, measuring 9–12.7 (–14.8) × 4.7–5.7 μm, occasionally 1-spored basidia, lageniform cheilocystidia and an absence of clamp connections (Uljé and Noordeloos 2003). *Tulosesus bisporus* (J.E. Lange) Wächter & A. Melzer, another setulose bisporic species, has considerably smaller spores measuring 9.7–13.7 × 6.1–8.4 μm, lacks clamp connections and sclerocystidia, and has a fimicolous ecology (Lange 1915; Uljé and Bas 1991; Uljé 2005). All of the above species have predominantly ochraceous-brown to ochraceous-cream colours, lacking rufus-orange tinges on the pileus (Orton and Watling 1979; Uljé and Bas 1991; Uljé and Noordeloos 2003; Uljé 2005).

Parasola litoralis Loizides, D.J. Schaf. & P. Alvarado **sp. nov.**

Index Fungorum registration number IF559580 Figs. 7–8

Etymology: Coastal, from Latin word *litore*, meaning coast

Fig. 7 Macro- and micromorphological features of *Parasola litoralis*: (**A**–**C**) basidiocarps in situ, scale bar = 5 mm; (**D**) hymenodermal pileipellis in Congo Red, scale bar = 20 μm; (**E**) hymeniderm cells with thickened yellow-brown pedicel in KOH, scale bar = 20 μm; (**F**) pleurocystidia in Congo Red, scale bar = 20 μm; (**G**) basidiospores in water, scale bar = 10 μm; (**H**) cheilocystidia in Congo Red, scale bar = 20 μm; (**I, J**) basidia in Congo Red, scale bar = 10 μm. (**A**, **F**, **G**, **I**, **J**) from holotype, (**B**) 13/2/2012, (**C**, **D**, **E**, **H**) 26/1/2017

Diagnosis: Small, partially deliquescent agaric with reddish-orange to ochraceous-grey pileus, 4-spored basidia, irregularly cylindrical, clavate, vesiculose, spheropedunculate or utriform cheilocystidia, broadly utriform or clavate pleurocystidia and lentiform spores, broadly ovate, rounded or with very rounded angles and tapering conically or with a slight protrusion towards the distinctly eccentric germ pore in face view, ellipsoid in side view [(13–)15–18(–19) × (9.5–)10–13(–14) µm × 7.5–9.5 µm; Q(B) 1.40. On coastal dunes.

Holotype: Cyprus. Lady's Mile, 0 m a.s.l., on embryonic shifting dunes among plant litter, leg. M. Loizides, 26-I-2018, K(M)264814.

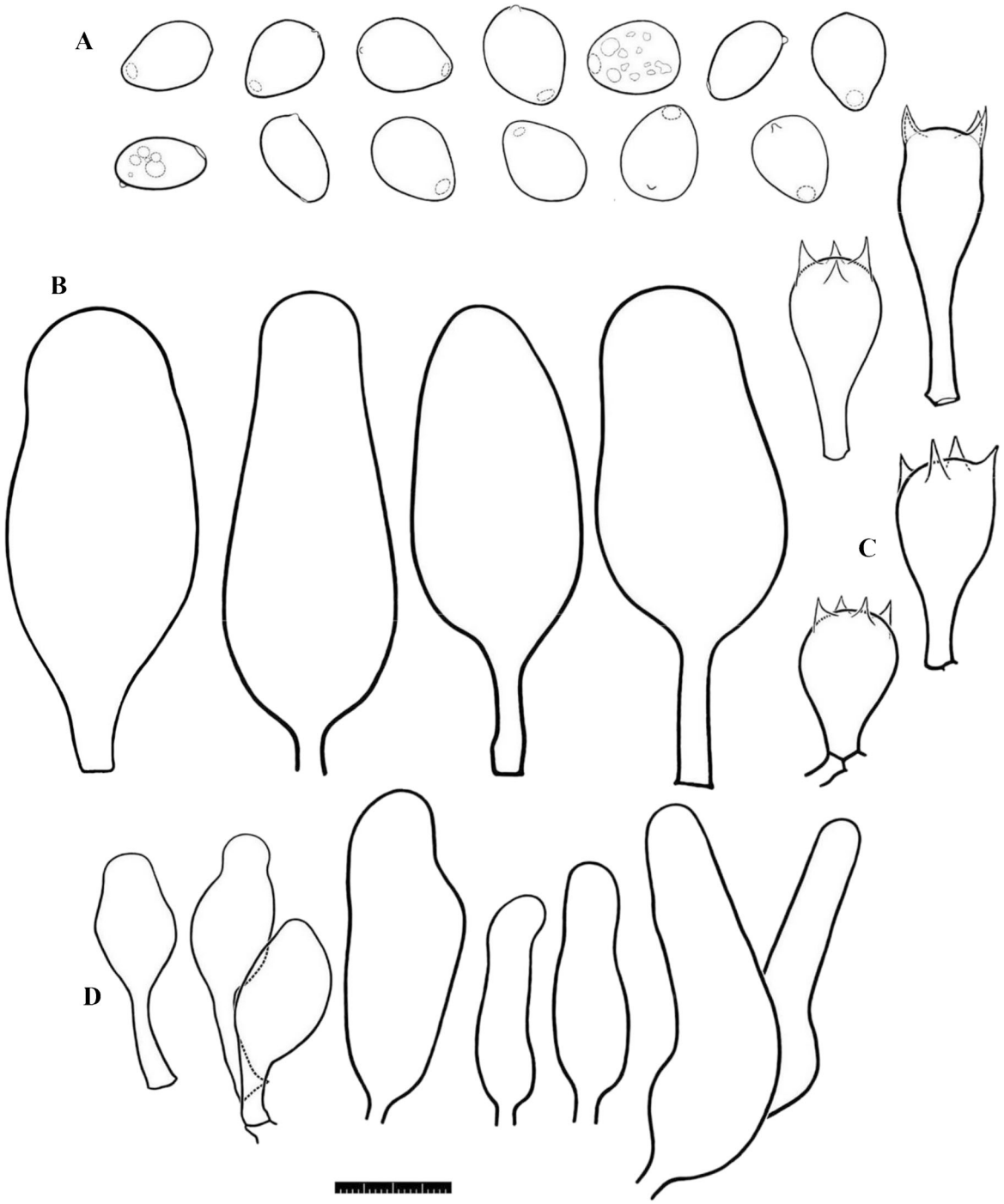

Fig. 8 *Parasola litoralis* line drawings of microscopic features: (**A**) basidiospores; (**B**) pleurocystidia; (**C**) basidia; (**D**) cheilocystidia; scale bar = 20 μm. (figured from different collections)

Additional collections studied: Cyprus. Lady's Mile, 0 m a.s.l., on embryonic shifting dunes among plant litter, leg. D.J. Schafer 13-II-2012, DJS20120213004; *Ibidem* D.J. Schafer, 25-I-2013, DJS20130125001; *Ibidem*, leg. D.J. Schafer, 26-I-2017, DJS20170126001.

Macromorphological description: Closed pileus ovoid to cylindrical, up to 8–12 mm high by 1 mm wide, deep orange to ochraceous-orange, somewhat darker rufous-orange and glabrous at the disk, soon expanding to campanulate or broadly conical, up to 14–20 mm wide when fully expanded, plicate and grey from the margin inwards, but remaining distinctly orange or ochraceous-orange at the disk. Lamellae free, whitish, becoming purplish-black with age, not or only partially deliquescing, narrow, moderately crowded; L up to 22–28. Stipe white to ochraceous, more or less glabrous, exannulate, up to 25–52 mm long by 1.5–2 mm wide.

Micromorphological description: Spores in face view (13–)14–18(–19) × (10–)10–13(–14) μm, on average 16.3 × 11.7 μm, Qm = 1.39, lentiform, broadly ovate, rounded or with very rounded angles and tapering conically or with a slight protrusion towards the distinctly eccentric germ pore, ellipsoid in side view, on average measuring 16.1 × 9.2 μm, Qm = 1.75, the eccentric germ pore visible in profile on the opposite side to the apiculus and through the spore in high illumination in face view, up to 2.5 μm across. Basidia 4-spored, bimorphic, clavate to pyriform or slightly constricted at the centre, thick-walled, measuring 28–48 × 12–20 μm, narrowing towards the clamped base; sterigmata 4–5 μm long. Cheilocystidia polymorphic, mostly irregularly cylindric, clavate, vesiculose, spheropedunculate or utriform, measuring 33–55 × 13–28 μm. Pleurocystidia broadly utriform or clavate, measuring 80–96 × 28–35 μm. Pileipellis a hymeniderm terminating in markedly inflated, clavate or spheropedunculate cells, many with a thick-walled, yellow-brown pedicel, measuring 35–50 × 17–27 μm. Stipitipellis a cutis, consisting of periclinal, cylindrical, thick-walled and occasionally constricted at the septa hyphae up to 10 μm wide; caulocystidia absent. Clamp connections present.

Ecology & distribution: So far known only from the island of Cyprus, where it fruits during the winter months in coastal embryonic dunes with scattered halophytic vegetation.

Remarks: *Parasola litoralis* belongs to sect. *Parasola* (previously referred to as sect. *Glabri*) and is morphologically close to *Parasola schroeteri*, *P. nudiceps*, *P. megasperma* and *P. hercules* (Uljé & Bas) Redhead, Vilgalys & Hopple. It differs from all these species in its sabulicolous ecology and spore size and shape. *Parasola litoralis* spores are large, in face view very rounded at the apiculus end (more or less hemi-spherical in most spores) and taper uniformly, or with a slight protrusion towards the germ pore end, with a Qm of 1.4. The *P. schroeteri* holotype was found by Uljé and Bender (1997) to correspond in all respects with the *P. nudiceps* holotype (see "Discussion" below). The spores differ from those of *P. litoralis*, in face view shorter on average (14 × 12 μm versus 16 × 12 μm) with a Qm of 1.2 and more shield-shaped, i.e., less rounded at the apiculus end with rounded shoulder angles and a more obtuse tapering towards the germ pore end. The spores of *P. hercules* (Uljé and Bas 1985) are similar to those of *P. schroeteri* and *P. nudiceps* but slightly larger, particularly broader with a Qm less than 1.2. The spores of *P. megasperma* (see below) in face view are of similar length but narrower (17 × 10.5 μm vs. 16.5 × 12 μm for *P. litoralis*) and are more or less ellipsoid with an average Qm of 1.6 and a centre of gravity at the mid-point or towards the germ pore end, in contrast to the other species where it is located towards the apiculus end. This species further differs by having more crowded lamellae (L = 30–40) (Uljé and Bas 1988; Uljé 2005; Schafer 2014). A collection from Cyprus (CH20041227001) previously reported as "*Coprinus megaspermus*" by Viney (2005) has been sequenced as part of this study and matches the type sequence.

Parasola megasperma and *Parasola nudiceps* holotypes

Reexamination of these two type collections was consistent with the earlier detailed type studies (Uljé and Bender 1997; Nagy et al. 2010a, 2010b) and focussed mainly on spore characteristics and DNA sequencing.

Parasola megasperma (P.D. Orton) Redhead, Vilgalys & Hopple

Coprinus megaspermus (Orton) *Notes R. bot. Gdn Edinb.* 32(1): 141 (1972).

Holotype: UK, Norfolk, Hedenham Wood 24/10/1971. Orton 4132 (E), E00204206.

From type description: "*Spores* ellipsoid or ellipsoid-ovoid, sometimes lentiform, 15–18/8½-9½/10–11 μm."

From Nagy 2010: "Basidiospores [26,1,1] 15–18.7 × 10–12 × 7.7–9 μm, on average 16.5 × 10.66 × 8.5 μm, Q_1 = 1.40–1.78, Q_2 =1.83–1.95 strongly lentiform, in the frontal view ellipsoid, broadly ellipsoid, rarely ovoid, in the lateral view ellipsoid or subamygdaliform, germ pore slightly eccentric, 2–2.3 μm wide, colour very dark reddish brown, subopaque, smooth, with moderately thick wall."

This study: Spores slightly to strongly lentiform. In face view broadly ellipsoid, occasionally somewhat ovoid or with rounded angles towards the germ pore end; germ pore slightly to distinctly eccentric and then visible through the spore in high illumination; in side view ellipsoid, sometimes slightly flattened on the apiculus side, rounded end with slightly to distinctly eccentric germ pore on the opposite side to the apiculus. Length 14.9–19.0 μm; breadth 9.4–12.4 μm; Q(B) 1.44–1.81; width 8.1–9.6 μm; Q(W) 1.81–1.93; average 16.9 × 10.4 × 9.1 μm; germ pore 2.0–4.0 μm, average 2.9 μm; average Q(B) 1.62; average Q(W) 1.86.

Figure 9: 1, 2 and 3 depicts three individual spores from the holotype in several different orientations, the remaining spores, 4, are 12 different spores. Although predominantly broadly ellipsoid, some spores have a slight, rounded angularity in face view; spores of *P. plicatilis* may be similar but are smaller, generally more angular and protrude at the germ pore end. *P. schroeteri*, *P. nudiceps and P.litoralis* have a more rounded triangular to shield shape in face view with a centre of gravity shifted more towards the apiculus end and a smaller Q value.

Parasola nudiceps (P.D. Orton) Redhead, Vilgalys & Hopple

Coprinus nudiceps (Orton) *Notes R. bot. Gdn Edinb.* 32(1): 142 (1972). Holotype: UK, Scotland, Tomich 03/09/1971. Orton 4133 (E) E00204198.

From type description: "Spores lentiform, ellipsoid in side view, 13-15½/8½-9½/10-12 μm, germ-pore central."

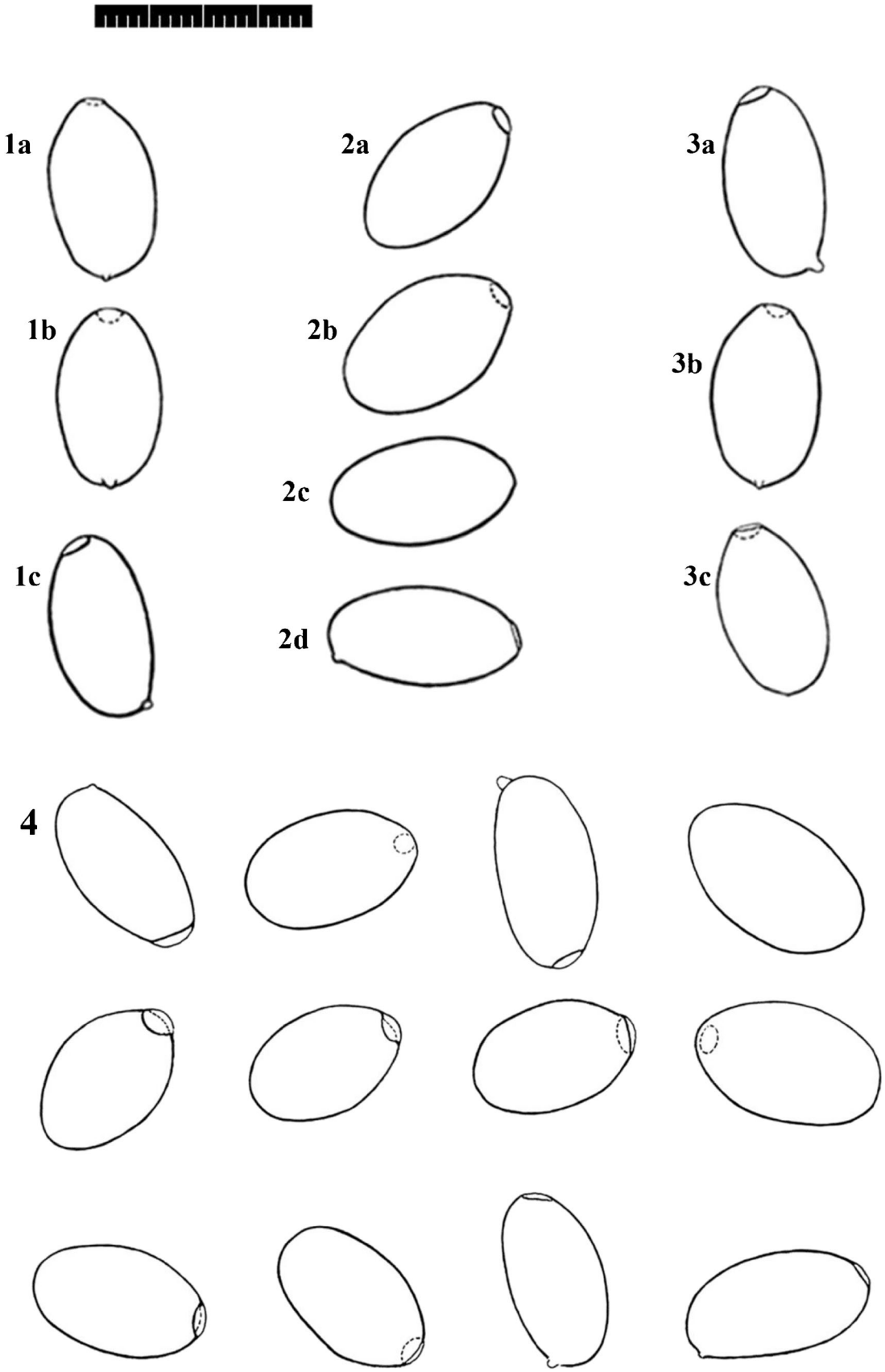

Fig. 9 *Parasola megasperma*: *Coprinus megaspermus* holotype E00204206 P D Orton 4132 Hedenham Wood, England 24/10/1971: (1a to c, 2a to d and 3a to c) individual spores 1, 2 and 3 in different orientations; (4) single orientations of 12 different spores; scale bar = 20 μm

From Uljé and Bender 1997: "Spores [40, 1, 1] 11.6–14.6 × 10.6–12.4 μm; Q = 1.05–1.20, av. Q = 1.15; av. L = 13.3, av. B = 11.6 μm."

From Nagy 2010: "Basidiospores [22,1,1] 11.8–16 × 11–13 × 8.2–8.7 μm, on average 13.94 × 11.84 × 8.45 μm, Q_1 = 1.07–1.37, Q_2 = 1.6–1.68 strongly lentiform, in the frontal view broadly ovoid to rounded triangular, some ovoid, in the lateral view ellipsoid or slightly amygdaliform, wall moderately thickened, with a strongly eccentric ca. 2 μm wide germ-pore, smooth, with a moderately thick wall."

This study: Spores strongly lentiform. In face view broadly ovoid, rounded triangular or shield-shaped, tapering mostly obtusely towards the distinctly eccentric germ pore, visible in high illumination through the body of the spore. In side view ellipsoid or somewhat flattened on the apiculus side with germ pore visible in profile on the opposite side to the apiculus. Length 12.8–16.0 μm; breadth 10.6–14.0 μm; Q(B) 1.03–1.31; width 6.7–9.1 μm; Q(W) 1.57–2.01; average 14.4 × 12.3 × 8.3 μm; germ pore 2.0–4.0 μm, average 3.0 μm; average Q(B) 1.18; average Q(W) 1.75.

Figure 10: 1 and 2 depicts two individual spores from the holotype in several different orientations, the remaining spores, 3, are 12 different spores. The shape matches previously published studies of Orton (1972), Uljé and Bender (1997) and Nagy et al. (2010b) and, on the basis of similar morphology, it seems likely that *P. nudiceps*, as proposed by Uljé and Bender (1997) and confirmed by Nagy et al. (2010b), is a later synonym of *P. schroeteri*. However, in the absence of a sequence of the holotype of the latter, this conclusion deserves further study.

Discussion

In the present work, three coprinoid taxa from the sand dunes of Cyprus are described as new to science. *Parasola litoralis* is strongly supported in our phylogenetic analyses, nesting in a clade sister to three other well defined lineages. One of these groups comprises collections all identified as *P. lilatincta*, three of which were initially deposited in GenBank as *P. schroeteri*, a designation subsequently revised by the depositors to *P. lilatincta*. The second clade includes the type of *P. nudiceps* (E:Orton 4133) along with one collection identified as *P. nudiceps* and three collections identified as *P. ochracea*, including its holotype (SZMC:NL-3621) and two (SZMC:NL-3167 and L.Nagy NL-3623) that were initially deposited as *P. schroeteri* but revised to *P. ochracea* by the depositors. This provides molecular evidence that *P. ochracea* is a later synonym of *P. nudiceps*. The third clade includes samples identified as *P. lilatincta* (SZMC:NL-0660), *P. kuehneri* (L:Ulje:904), or identified to genus only (SZMC:NL-0472).

The holotype of *Parasola megasperma* from England (E:Orton 4132) nests in a distinct clade well separated from that of *P. nudiceps* along with collections from Cyprus, The Netherlands, Czechia, USA (California), Spain and Denmark, all identified as *P. megasperma*, and four collections from the Netherlands and one from Germany identified as either *P. schroeteri* or *P. aff. hercules*. The holotypes of *P. megasperma* and *P. nudiceps* are very distinct morphologically and also well separated in the phylogram. The present study therefore provides molecular and morphological evidence that *P. megasperma* and *P. nudiceps* are distinct species. Uljé and Bender (1997) studied the types of *P. nudiceps* and *P. schroeteri* and concluded, on morphological evidence, that they were synonyms, a conclusion confirmed by Nagy et al. (2010b) after studying the same two holotypes. It therefore seems unlikely that Karsten's *Coprinus schroeteri* would fall in the clade of *P. megasperma*, despite this being the clade labelled as "/schroeteri" in Wächter and Melzer (2020). The holotype of *Parasola* (as "*Coprinus*") *schroeteri* dates back to 1878 and has not been a subject of the present study. Since collections identified as *P. schroeteri* have been found to nest in a number of different clades in our phylogenetic analyses and/or have been revised in their identification, this species is still lacking a fixed molecular profile. Successful sequencing of the 1878 holotype would allow *P. schroeteri* to be allocated an appropriate molecular identity, possibly confirming *P. nudiceps* as a later synonym, but there are several other clades currently available as plausible alternatives.

Overall, the results presented in Fig. 2 provide clear molecular evidence for 20 distinct *Parasola* species, three unnamed and ten of the clades also including either the holotype (*P. plicatilis-similis*, *P. megasperma*, *P. glabra*, *P. kuehneri*, *P. cuniculorum*, *P. hercules*, *P. litoralis* and *P. nudiceps*), neotype (*P. misera*) or epitype (*P. plicatilis*) of a Linnean binomial. The presence of collections in these clades with different names, particularly *P. schroeteri*, makes the future study of correlation between morphology and molecular phylogeny a continuing urgent requirement in the genus. This may also be exacerbated by surprising morphological discordance, as discussed below in relation to the morphological disparity between UK and Cyprus collections of *P. cuniculorum*.

Tulosesus maritimus received strong phylogenetic support and showed a relatively long distance with its putative sister taxa, *T. sabulicola* and *T. christinopolitanus*. Although the reciprocal monophyly between these clades could not be demonstrated, *T. maritimus* is here considered an independent species because of the partial genetic support and its diagnostic phenotype. In contrast, *T. callinus* var. *miionis* is not interpreted as an independent species despite the remarkable phenotypic apomorphies of the Cypriot collections, because the present phylogenetic analysis did not show either a

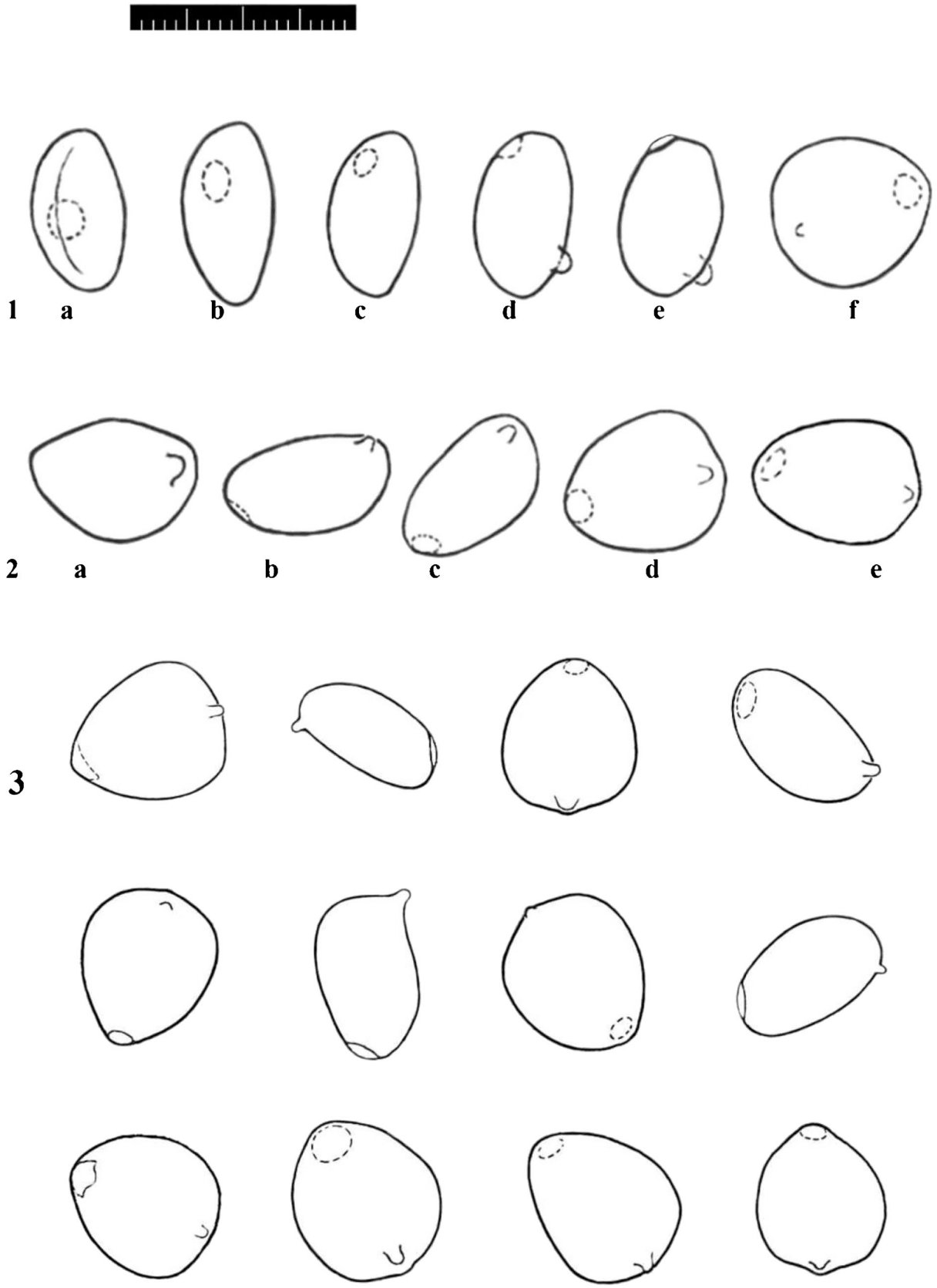

Fig. 10 *Parasola nudiceps*: *Coprinus nudiceps* holotype E00204198 Orton 4133 Tomich, Scotland 03/09/1971: (1 a to f and 2 a to e) individual spores 1 and 2 in different orientations; (3) single orientations of 12 different spores; scale bar = 20μm

reciprocal monophyly or a sufficient genetic distance with *T. callinus* var. *callinus*. The lack of *TEF1* sequences from *T. callinus* and related lineages (or other suitable markers), as well as the high intraspecific variability of ITS rDNA and *TUB2* sequences between these samples, was probably the cause of the low support values.

Tulosesus callinus has long been regarded as an aggregate and difficult to distinguish species. Lange (1952) identified two intersterile populations from Denmark, while Uljé and Bas (1991) discussed the difficulty of distinguishing it from closely related species. Uljé and Noordeloos (2003) described the variant *C. callinus* var. *limicola*, but collections identified as this taxon fall in a clade with *T. hiascens* and not *T. callinus* (Nagy et al. 2012a, 2012b). This morphological discordance might be due to a recent split of some of these lineages and is consistent with the hypothesis of an adaptive radiation in the genus (as postulated by Nagy et al. 2012a). A third lineage (SZMC-NL-1356) with 2-spored, bi- to trimorphic basidia, pileocystidia with a tapering acute appendage and a sand dune ecology is included in the key provided by Nagy et al. (2012a), but not formally named. Sequencing samples collected from a wider geographical range and analysis of additional markers will probably be necessary to resolve the most suitable taxonomic status of these clades, especially if new lineages are found to be related to them. The taxonomic position of two additional *Tulosesus* collections (ML41582C5 and ML31216CC) sequenced in this work is currently uncertain and these may represent additional undescribed lineages, to be further investigated in future studies (Fig. 1).

Two-spored versus four-spored taxa in the coprinoid Psathyrellaceae

In many genera, different collections of species may be found with varying proportions of two- and four-spored basidia in their fruitbodies, the character often being regarded as a variable phenotypic feature unrelated with reproductive isolation, justifying distinction only as a form or, occasionally, variety, depending on the different concepts of these ranks. In the coprinoid *Psathyrellaceae*, in contrast, taxa are essentially exclusively four-spored or have basidia with fewer sterigmata but not four. The distinction between four-spored and other otherwise similar taxa is also reflected in extensive laboratory mating studies (Lange 1952; Kemp 1970, 1975). Kemp (1975) reported that two-spored taxa were never found to be interfertile with four-spored taxa in his studies and described a form of speciation as a process in which incompatibility occurred at the hyphal level, morphological and other changes taking place later as a consequence. This and similar considerations were reflected in the four-spored versus two (or three)-spored character being regarded as justifying distinction at the species level along with other morphological features in the coprinoid genera.

Unexpectedly, a 4-spored collection from Cyprus (DJS20120211001) morphologically identified as the widely distributed *Parasola misera*, nested in a clade with the type collection of *P. cuniculorum*, whose ITS rDNA sequence is published for the first time in the present work (Fig. 2). *Parasola cuniculorum* was recently reported from Britain (Schafer 2014) on the basis of its occurrence on rabbit dung and the presence of strictly 2-spored basidia. These characters were not reflected in the collection from Cyprus (DJS20120211001), which interestingly featured 4-spored basidia and occurred on goat dung. A second 2-spored collection from a different geographical location in Britain (DJS20130923001) also had the same sequence as the *P. cuniculorum* type collection but *Parasola misera* DJS20141030001, a collection from Britain of the much more common four-spored taxon, nests with other published sequences of *P. misera*, including the neotype (Fig. 2). A wider sampling of both *P. misera* and *P. cuniculorum* will therefore be necessary, to establish which morphological features, if any, can be useful in discriminating between the two sister-species and whether there is any geographical pattern to the distribution of the two phylogenetically distinct species or the presence of two- or four-spored basidia.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-022-01803-2.

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Author contribution All authors contributed to the studies described in the paper and read and approved the final manuscript.

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Availability of data and materials DNA sequence data generated or used in the current study are deposited at GenBank as set out in Table 1 of the present paper.

Declarations

Ethics approval and consent to participate All authors confirm that no research involving humans or animals was involved in the current study, that there are no issues relating to animal welfare relating to the current study and that they have approval to participate in the current study.

Consent for publication All authors have given explicit consent to the submitted paper and to the inclusion of their data in it.

Competing interests The authors declare no competing interests.

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