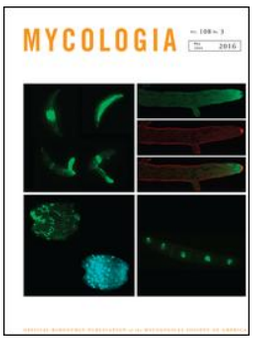

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The genus Parasola: Phylogeny and the description of three new species

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The genus *Parasola*: Phylogeny and the description of three new species

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ABSTRACT

Parasola represents an enigmatic lineage of veil-less, coprinoid fungi in Psathyrellaceae (Agaricales). The species-level taxonomy of the genus has been in a flux recently, resulting in the elimination of some long-established names and the description of new taxa. Here, we reconstruct the phylogeny of *Parasola* using two nuc rDNA loci, the internal transcribed spacers region (ITS1-5.8S-ITS2) and 28S and identify several putatively undescribed species, of which three are formally described here (*Parasola crataegi*, *P. ochracea* and *P. plicatilis-similis)* based on molecular and morphological data. Morphological descriptions for the new

species and an identification key to accepted *Parasola* species are given. We revise and discuss our current understanding of the phylogeny of *Parasola*.

KEY WORDS: coprinoid fungi, generic phylogeny, nuc rDNA, revision, species description

INTRODUCTION

Parasola Redhead, Vilgalys & Hopple is a genus of small, coprinoid mushrooms with colors ranging from ochre-orange to lilac with most being brownish, belonging to Psathyrellaceae Vilgalys, Moncalvo & Redhead (Redhead et al. 2001; Nagy et al. 2009). The species are common saprotrophic agarics found on lawns, along forest trails and in grassland habitats with a few species growing on dung, e.g. *Parasola misera* (P. Karst.) Redhead, Vilgalys & Hopple, *P. cuniculorum* D.J. Schaf. (Schafer 2014), or occasionally *P. conopilus* (Fr.) Örstadius & E. Larss. (Padamsee et al. 2008; Larsson & Örstadius 2008; Schafer 2010, 2014).

Parasola species have small and deeply grooved parasol or umbrella-like pilei, ranging from vivid orange-ochraceous to lilac but are mostly yellow-brown to tawny and paler colors. *Parasola* differs from the closely related *Coprinellus* P. Karst, *Coprinopsis* P. Karst. and *Psathyrella* (Fr.) Quél. in the complete absence of a veil structure and the lack of pileo- and caulocystidia, although *P. conopilus*, *P. auricoma* (Pat.) Redhead, Vilgalys & Hopple, *P. setulosa* (Berk. & Broome) Redhead, Vilgalys & Hopple, and *P. malakandensis* S. Hussain, N. Afshan & H. Ahmad possess sclerocystidia (Nagy et al. 2010a). Basidiocarps of *Parasola* are non-deliquescent, but show some level of tissue degradation (Buller 1931;

Redhead et al. 2001; Larsson & Örstadius 2008) referred to as incomplete deliquescence or collapse (Nagy et al. 2009, 2012). Basidiospores are dark, generally rounded triangular, heart-shaped or ovoid with a mostly eccentric germ pore, although earlier-diverging species tend to have ellipsoidal basidiospores. Molecular phylogenetic analyses recover the monophyly of *Parasola* with strong support (Padamsee et al. 2008; Nagy et al. 2009, 2010b, 2012).

The genus is divided into two sections by the presence or absence of thick-walled, brown sclerocystidia (setae) on their pilei (Schafer 2010). Section *Auricomi* (Singer) D.J. Schaf. has such setae and section *Parasola* does not. According to Index Fungorum (www.indexfungorum.org) the genus currently has 22 species, although recent taxonomic treatments report a lower number of recognized taxa (Nagy et al. 2009, 2010a; Schafer 2010, 2014). This number has fluctuated because of synonymizations, e.g. *P. leiocephala* (P.D. Orton) Redhead, Vilgalys & Hopple with *P. lactea* (A.H. Sm.) Redhead, Vilgalys & (Nagy et al. 2010a) and because of the discovery of new species. Recently, two new species were described, *Parasola cuniculorum*, differing from *P. misera* (P. Karst.) Redhead, Vilgalys & Hopple by having two-spored basidia (Schafer 2014), and *P. malakandensis*, closely related to *P. setulosa* (Berk. & Broome) Redhead, Vilgalys & Hopple (Hussain et al. 2017).

In this study, we describe three new species in *Parasola* based on an extended sampling of specimens for phylogenetic analyses. Species descriptions are based on morphological characters and molecular phylogenetic analyses of the internal transcribed spacer regions (ITS1-5.8S-ITS2 = ITS) and the D1-D2 domains of the 28S gene of the nuc rDNA (28S). We provide an identification key for currently accepted species of *Parasola,* including the three newly described species and other putatively new species.

MATERIALS AND METHODS

Taxon sampling, laboratory protocols.—We sampled collections of 15 currently accepted species in *Parasola*, and collections of 7 additional apparently undescribed species, selected on the basis of morphological observations and preliminary sequence data. We extracted genomic DNA from dried basidiomes using the DNeasy Plant Mini Kit (Qiagen, Redwood City, California, USA) and amplified the nuclear ribosomal loci using primers LR0R and LR7 for the 28S gene, and ITS1 and ITS4 for the ITS region (White et al. 1990). Standard PCR protocols were used for the amplification using DreamTaq polymerase (Thermo Scientific, Waltham, Massachusetts, USA) (White et al. 1990; Gardes & Bruns 1993). PCR products were purified and sequenced by LGC Genomics Ltd (Berlin, Germany) in both directions and reads were assembled into contigs by using CodonCode Aligner (CodonCode Corporation, Centerville, Massachusetts, USA) and DNA Baser (Heracle BioSoft SRL, Lilienthal, Germany). All sequence accession numbers produced for this study (KY928603–KY928647) are shown in Supplementary Table 1. Contigs were evaluated by BLAST against NCBI's nucleotide database (Altschul et al. 1990). Additional 28S and ITS sequences were obtained from GenBank for outgroup species (Supplementary Table 1).

Alignments and phylogenetic reconstruction.—We aligned 28S and ITS sequences independently using PRANK 091016 (Löytynoja & Goldman 2005) with default settings and corrected the alignments manually after visual inspection in JALVIEW 2.4.0 (Waterhouse et al. 2009). During inspection we discarded sequences shorter than 1000 bps for 28S and 400 bps for ITS. We concatenated the ITS and 28S alignments into a supermatrix. Indels in the ITS region were recoded as presence/absence characters using the simple indel coding algorithm (Simmons & Ochoterena 2000) as implemented in gapcode.py (version 2.1, http://www.bioinformatics.org/~rick/software.html). For Bayesian analyses this matrix was

added to the concatenated alignment. We also analyzed the ITS and 28S alignments independently. We chose *Coprinopsis lagopus* (Fr.) Redhead, Vilgalys & Moncalvo, *C. picacea* (Bull.) Redhead, Vilgalys & Moncalvo, *C. pseudonivea* (Bender & Uljé) Redhead, Vilgalys & Moncalvo and *C. marcescibilis* (Britzelm.) Örstadius & E. Larss. as outgroups.

Phylogenetic inference was conducted using Bayesian and Maximum Likelihood (ML) methods for both the concatenated and the independent ITS and 28S datasets. For Bayesian inference, we used MRBAYES 3.2.1 (Ronquist et al. 2012) with a partitioned GTR+ Γ model, a chain length of 10 million generations, sampling frequency 100, two independent replicates with 3 heated and one cold chains per replicate. We partitioned the concatenated alignment into ITS, 28S and indel matrices and the parameters of the evolutionary model were estimated separately for the individual partitions. Indels were modeled by a 2-state Markov model for restriction sites implemented in MRBAYES. We established burn-in by inspecting the convergence of likelihood values in Tracer 1.6 (Rambaut et al. 2014). Post burn-in trees were used to compute a 50% majority rule consensus tree using the SUMTREES script of the DENDROPY package (Sukumaran & Holder 2010). Maximum Likelihood analyses were run in RAXML 7.0.4 (Stamatakis 2006) under the GTR+ Γ model and two partitions (ITS and 28S). Branch support was calculated by 500 non-parametric bootstrap replicates and mapped onto the ML tree using SUMTREES. Nodes were considered strongly supported when bootstrap values (BS) were ≥70% and posterior probability values (PP) were ≥0.95.

Constraint analyses were performed using the CONSEL package (Shimodaira & Hasegawa 2001) on the concatenated dataset. Topological constraints were designed by hand in Mesquite 2.75 (Maddison & Maddison 2011), and used in RAXML to estimate site-wise likelihoods. In RAXML we inferred ten constrained and 10 unconstrained trees, which were then compared using the approximately unbiased test (at $p<0.05$). Datasets were submitted to

TreeBase (TB2:S20867).

Morphological descriptions.— Line drawings of microscopic characters are based on microphotographs; basidiospores were partly drawn with a microscope-adapter. Measurements were made at 1000× magnification with a calibrated optical micrometer. Basidiospore measurements are based on at least 20 basidiospores from each collection; numbers in square brackets refer to the number of basidiospores measured, the number of basidiocarps and the collections they originate from, respectively. Spore measurements are given as follows: length range × breadth range × width range. Q_1 and Q_2 values were calculated as follows: Q_1 = length divided by breadth; Q_2 = length divided by width. Measurements of basidia included sterigmata. The calculation of the proportion of 4-, 3- or 2-spored basidia was made according to the methods of C. Bas (pers. comm.) and Schmidt-Stohn (2012): an even, ripe piece of lamella is placed on a piece of moist paper tissue on a microscope slide. The basidia are then observed on the face of the lamella with 40× or 400× magnification and the number of basidiospores per basidium is counted. Pleurocystidia and cheilocystidia were observed and measured by cutting the lamellae edge from the rest of lamella to avoid blending of the two types of cystidia.

RESULTS

Phylogenetic analyses.—We generated 28 new sequences from 12 species. The concatenated alignment comprised 77 specimens with 2166 nucleic acid sites (ITS: 785 sites, 28S: 1381 sites) and 167 binary characters coded from indels in the ITS alignment. The ITS alignment comprised 77 specimens with 785 nucleic acid sites and the 28S alignment consisted of 58 specimens with 1381 nucleic acid sites. Phylogenetic trees reconstructed using ML and Bayesian methods were largely congruent with each other and reflected current views on the

phylogeny of the genus (FIG. 1., SUPPLEMENTARY FIGS 1–4. Nagy et al. 2009). Briefly, both inference strategies recover *Parasola conopilus*, *P. auricoma*, *P. setulosa* and *P. malakandensis* as basal groups with strong support and grouping species of section *Parasola* in a 'crown' *Parasola* clade. *Parasola malakandensis* (Hussain et al. 2017) grouped closely to the crown-group of *Parasola* (1.00/81) occupying an intermediate position between species of section *Auricomi* and those of section *Parasola*. The phylogeny indicated three species-level groups that could not be assigned to known species, and are described here as *P. crataegi*, *P. ochracea* and *P. plicatilis-similis*. *Parasola crataegi* was inferred basal to a clade formed by *P. misera* and *P. lactea*. *Parasola plicatilis-similis* was inferred sister to the clade including *P. megasperma* (P.D. Orton) Redhead, Vilgalys & Hopple and *P. schroeteri* (P. Karst.) Redhead, Vilgalys & Hopple (1.00/75). Specimens of the third species, *P. ochracea* nested within *P. lilatincta* complex (0.96/68). Although its position within *P. lilatincta* was strongly supported by Bayesian analyses, constraint analyses indicated that a monophyletic *P. lilatincta* with *P. ochracea* as a sister species cannot be rejected (p = 0.102 – 0.103, au-test). Our analyses indicate the existence of further four species that we tentatively named *Parasola* sp. 1–4. *Parasola* sp. 1 forms a monophyletic group with *P. plicatilis* with strong support (1.00/97). *Parasola* sp. 2 groups together with *P.* cf. *lilatincta* in a weakly supported clade (0.67/47) close to the clade which contains the true *lilatincta*-group. *Parasola* sp. 3 forms a clade with *P. lilatincta* and *P. ochracea* but this is only supported in ML analyses (-/99). *Parasola* sp. 4 forms a monophyletic group with *P. misera* in both the Bayesian and ML analyses (1.00/98).

TAXONOMY

Parasola crataegi Schmidt-Stohn, sp. nov. FIGS. 2A, B, 3A, 4A, B

MycoBank: MB817192

Typification: GERMANY, SCHLESWIG-HOLSTEIN, Lübeck-Travemünde, Dummersdorfer Ufer (nature reserve), on grazed pasture under *Crataegus monogyna*, 29 Oct 2008, *G. Schmidt-Stohn*, *SSt08-154* (**holotype**, M 0280274). GenBank: KY928605.

Etymology: The epithet refers to the main habitat under *Crataegus*.

Pileus 4–6 × 2–3 mm when still closed, ellipsoidal to cylindrical, then convex to hemispherical and plano-convex to applanate when expanded, up to 15 mm wide, gray-brown with an ochraceous-brown center when young and moist, grayish to grayish white when mature, sulcate-striate almost up to the centre. Lamellae free, up to 30 reaching the stipe at a distance of 0.5–1 mm, additionally 15–20 lamellules, crowded, ventricose, < 2 mm broad, whitish to grayish, finally blackish, with an indistinct fimbriate edge. Stipe 15–40 × 0.5–1 mm, cylindrical, fistulose, translucent grayish, slightly flocculose when young, later glabrous; smell and taste not distinctive.

Basidiospores [41, 2, 2] 6.5–8.5 × 5.5–7.5 × 4–5.5 µm, ave 7.4 × 6.5 × 4.8 µm, Q_1 = 1.00–1.35, Q_1av = 1.15, Q_2 = 1.35–1.77, Q_2av = 1.49, rounded triangular to heart-shaped and also ovoid, flattened, ellipsoidal in lateral view, with eccentric germ-pore; Basidia in the majority (ca. 80%) 4-spored, but some (10–20%) also 2- or 3-spored, clavate, 31–48 × 10–14 µm. Cheilocystidia fairly densely packed, oblong, ellipsoidal, narrowly to broadly utriform, some also spheropedunculate, 23–40 × 10–17 µm. Pleurocystidia scattered, predominantly

near the edge of lamellae, not easily detected, utriform, 48–62 × 17–24 µm. Pileipellis a hymeniderm, pileo- and sclerocystidia lacking.

Habitat: Grazed grasslands, almost always in close vicinity to single *Crataegus monogyna* or in scrubs of this species, basidiocarps solitary.

Distribution: Germany, Hungary.

Other specimens examined: GERMANY, SCHLESWIG-HOLSTEIN, Lübeck-Travemünde, Dummersdorfer Ufer (nature reserve) on grazed pasture under *Crataegus monogyna*, 21 Oct 1995, *G. Schmidt-Stohn SSt95-113* (M 0280273); same location, 24 Oct 2009, *G. Schmidt-Stohn SSt09-105* (M 0280275); SACHSEN-ANHALT, Huy, north of Halberstadt, Paulskopfwarte, on naked soil under *Corylus* and *Crataegus*, 29 Sep 1998, *G. Schmidt-Stohn SSt98-239*; NIEDERSACHSEN, Heeseberg west of Jerxheim on grazed pasture under *Crataegus monogyna*, 17 Jul 2000 *SSt00-018a*. – HUNGARY, BÁCS-KISKUN, Fülöpháza (Kiskunság National Park), *Robinia pseudoacacia* plantation with *Crataegus* and *Ailanthus*, on leaf litter, 12 Oct 2008, *L.G. Nagy*, herb. *NL-4175*.

Notes: Before the description of *P. crataegi,* the only known species in *Parasola* with such small basidiospores was *P. kuehneri*. Therefore our new species was usually identified as *P. kuehneri* using existing keys. Considering other morphological and ecological characters, confusion of the two species is impossible: *P. kuehneri* has a distinctly darker pileus of < 3.5 cm diam and usually grows in forests and shrubs along trails on naked, mineral-, alkaline soil rather than in poorly manured grasslands like *P. crataegi*. Moreover, the basidiospores of *P. kuehneri* average 9.4 µm long, while those of *P. crataegi* are only 7.4 µm. Genetically, *P. crataegi* is closest to *P. lactea* (formerly *P. leiocephala*), forming two well separated clades. However, both the basidiospores of *P. lactea* (ave 10.7 × 8.8 × 6.7 µm) and the basidiocarps are much larger than those of *P. crataegi*.

Parasola crataegi is so far known from three sites in Germany, mostly found repeatedly in different years, and one in Hungary. The habitat is almost always in grasslands/open landscape and usually in close proximity to *Crataegus monogyna,* as emphasized with the epithet ”crataegi”. Nevertheless, we suspect that *P. crataegi* is more widespreadbecause of confusion with *P. kuehneri* in the past. The only previous reference to our new species is from Bender (1989, also pers. comm.), who when commenting on *Coprinus kuehneri* mentioned “a deviating form under *Crataegus monogyna* with smaller and ± ovoid basidiospores”. His collection may be our *P. crataegi.*

Parasola ochracea L. Nagy, Szarkándi & Dima, sp. nov. FIGS. 2/D-E, 3/B, 4/C

MycoBank: MB817193

Typification: NORWAY, NORD-TRØNDELAG, Steinkjer, Skrattåsen, on cow dung, 5 Sep 2009, *L.G. Nagy* & *T. Knuttson*, *NL-3621* (**holotype**, BP). GenBank JN943134.

Etymology: *ochracea* refers to the orange to ochraceous color of the basidiocarp.

Pileus 4–20 × 3–9 mm when still closed, ellipsoidal to cylindrical, campanulate to flat with an umbo when expanded, up to 35(40) mm wide, vivid orange to ochraceous, grayish orange to brownish when mature, sulcate and grooved up to the centre. Lamellae free, crowded, ventricose, < 3 mm broad, whitish to grayish, finally blackish, with a fimbriate edge. Stipe 30–90 × 0.5–2 mm, cylindrical, fistulose, pure white, slightly silky when young, later glabrous, smell and taste not distinctive.

Basidiospores [40, 1, 1] 10–11 × 6–8.5 µm, ave 10.8 × 7.4 µm, Q = 1.25–1.58, Qav = 1.46, rounded triangular to ovoid with rounded angles, sometimes almost hexagonal, lentiform, ellipsoid in lateral view, with eccentric germ-pore. Basidia 4-spored, clavate, 35–47 × 11–13.5 µm. Cheilocystidia densely packed, clavate, ellipsoidal, ovoid to balloon-

shaped, some utriform, 42–75 × 25–38 µm. Pileipellis hymeniderm, pileo- and sclerocystidia lacking.

Habitat: On cow dung or dung mixed with straw in wet grazed meadows.

Distribution: Norway, Sweden.

Other specimens examined: SWEDEN, ÖLAND, Möckleby/Gardstorp, on cow dung and dung mixed with straw, 10 Sep 2008, *L.G. Nagy & T. Knuttson*, *NL-3623*; ÖLAND, Ned-Västerstad, on cow dung, 10 Sep 2008, *L.G. Nagy & T. Knuttson*, *NL-3167*.

Notes: Parasola ochracea is morphologically close to *P. schroeteri*, *P. misera* and *P. cuniculorum*, with which it shares the habitat and spore shape. However, *P. schroeteri* has darker colored basidiocarps and pleurocystidia, whereas *P. misera* and *P. cuniculorum* have much smaller basidiocarps and smaller basidiospores.

Parasola plicatilis-similis L. Nagy, Szarkándi & Dima, sp. nov. FIGS. 2/F, 3/C, 4/D-E

MycoBank: MB817194

Typification: SWEDEN, ÖLAND, Mörbylanga parish, Södra Barspunkten, north of the road, in alvar vegetation, 28 Sep 2007, *L.G. Nagy & T. Knuttson*, *NL-2125* (**holotype**, BP). GenBank KY928620.

Etymology: *similis* (Latin) = like, referring to the similarity of the new species to *Parasola plicatilis*.

Pileus 6–20 × 4–10 mm when still closed, cylindrical to ellipsoidal or ovoid, expanding to campanulate then flat, 15–45 mm when expanded, honey-colored, ochre-brown when young, becoming sordid, grayish upon ageing, with a pale ochre-brown button when

mature, sulcate-striate up to the centre; Lamellae free, crowded, ventricose, up to 4 mm broad, at first whitish, then grayish and black. Stipe 40–90 × 1–2 mm, slender, fistulose, glabrous, pale ochre-brownish, slightly strigose at the base. Smell and taste not distinctive.

Basidiospores [38, 2, 1] 10.5–13.5 × 8–11.5 µm, ave 11.8 × 9.7 µm, Q = 1.1–1.4, Qav = 1.2, broadly ellipsoidal to broadly hexagonal, sometimes ovoid or almost rounded triangular, lentiform, ellipsoid in lateral view, with eccentric germ-pore. Basidia 4-spored, clavate, 30–45 × 10–14 µm. Cheilocystidia utriform, clavate, sometimes broadly lageniform, 35–80 × 12–20 µm, pleurocystidia utri- to lageniform, often quite narrow and slender 75–90 × 24–35 µm. Pileipellis hymeniderm, pileo- and sclerocystidia lacking.

Habitat: Grazed meadows and pastures, once found in alvar vegetation.

Distribution: Sweden, Slovakia.

Other specimens examined: SLOVAKIA, RIMAVSKÁ SOBOTA, Drňa , on grazed pasture with *Festuca*, 3 Oct 2008, *L.G. Nagy*, *NL-3980*. – SWEDEN, ÖLAND, Langlöt parish, 500 m NNW Astad, on grazed meadow, 22 Sept 2006, *L.G. Nagy* & *T. Knuttson*, *NL-0287*.

Notes: The species is highly similar to *P. plicatilis*, differing mostly in spore shape and to a smaller extent by the presence of many narrow, lageniform pleurocystidia as opposed to mostly utriform-ellipsoidal in *P. plicatilis*. The basidiospores of *P. plicatilis-similis* are mostly broadly ellipsoidal, whereas those of *P. plicatilis* are mostly ellipsoidal or hexagonal (Q = 1.15–1.5 vs. 1.25–1.6). The spore shape of *P. plicatilis-similis* is transitional between that of earlier diverging ellipsoid-spored species (*P. plicatilis*, *P. auricoma*) and more derived species with rounded triangular basidiospores (e.g. *P. schroeteri*, *P. lactea*). Accordingly, the species present a continuum in spore shape and their identification should

rely on the shape of the majority of basidiospores and measurements of 10-20 individual basidiospores.

KEY TO DESCRIBED AND UNDESCRIBED SPECIES OF PARASOLA.

1. Pileus with long brown, thick-walled hairs ("sclerocystidia") 2

1.′ Pileus without sclerocystidia 5

2. Basidiocarp psathyrelloid, pileus not sulcate, not deliquescent, does not collapse when mature .....***P. conopilus***

2.′ Basidiocarp collapses when mature 3

3a Basidiospores ellipsoidal to oblong .....***P. auricoma***

3.′ Basidiospores rounded triangular 4

4. Basidiospores subglobose to broadly ovoid, 8–11 µm long ..... ***P. setulosa***

4.′ Basidiospores broadly ellipsoidal to subglobose, 11–18 µm long ..... ***P. malakandensis***

5. Pleurocystidia absent, exclusively coprophilous 6

5.′ Pleurocystidia present, can occur on dung 8

6. Basidiocarps large, pileus >20 mm when expanded ..... ***P. ochracea***

6.′ Basidiocarps smaller 7

7. Basidia 4-spored .....***P. misera***

7.′ Basidia 2-spored .....***P. cuniculorum***

8. Average spore length 7–9.5 µm 9

8.′ Average spore length longer 10

9. Average spore length 9.5 µm, pileus dark brown to red-brown, expanded up to 35 mm wide, in forests or along trails .....***P. kuehneri***

9.′Average spore length 7.5 µm, pileus gray-brown, grayish to grayish white, expanded up to 15 mm wide, in grasslands mostly under *Crataegus* ..... ***P. crataegi***

10. Basidiospores ave 9–13 µm long 11

10.′ Basidiospores ave longer than 12.5 µm 15

11. Basidiospores with germ pore, central or eccentric to the adaxial side, basidiospores often broader than long ..... ***Parasola* sp. 2**

11.′ Germ pore eccentric to the opposite side of the hilum (abaxial side) 12

12. Basidiospores narrowly ovoid to ellipsoidal, sometimes ovoid, Qav = 1.15–1.60 13

12.′ Basidiospores ovoid, rounded triangular or subglobose 14

13. Basidiospores mostly ellipsoidal to hexagonal, Qav = 1.15–1.5, pleurocystidia mostly utriform…...…….. ***P. plicatilis***

13.′ Basidiospores broadly ellipsoidal, often ovoid to broadly hexagonal, Qav = 1.25–1.6, pleurocystidia often lageniform ..... ***P. plicatilis-similis***

14. Average length of basidiospores under 10.5–11 µm, basidiospores often remain immature ……..... ***P. lactea* (= *P. leiocephala*)**

14.′ Average length of basidiospores >11.5 µm, as usual fully mature, dark blackish brown, pileipellis and basidia filled with strongly refringent, yellowish granules .... ***P. lilatincta* s.l.**

(if granules absent and basidiospores ave >11 µm long: see ***P. schroeteri***)

15. Basidiospores narrowly ovoid to ellipsoidal, 14–19 µm long .....***P. megasperma***

(if basidiospores smaller, 12–14 µm, see ***P. plicatilis***)

15.′ Basidiospores ovoid, rounded triangular to subglobose 16

16. Basidiospores 12–14 µm long .....***P. schroeteri***

16.′ Basidiospores 13–18 µm long .....***P. hercules***

(plus ***P.* aff. *hercules*** and other undescribed species)

DISCUSSION

In this study we describe three new species, *Parasola crataegi*, *P. ochracea* and *P. plicatilis-similis*. All were collected in central Europe (Germany, Hungary) or northern Europe (Norway, Sweden), but wider geographical distributions are highly likely given their relationships to well-known species with broader documented distribution. All three species prefer grassland habitats, especially lawns or marginal grass patches close to forest trails.

Parasola crataegi is morphologically similar to *P. kuehneri*, but has significantly smaller basidiospores and basidiocarps, a different habitat and is genetically more closely related to *P. lactea*.

Parasola ochracea is phylogenetically closest to *P. lilatincta*, although morphologically it is more similar to *P. schroeteri* and *P. misera*. While all three species are more or less coprophilous, the conspicuous ochraceous to orange color of the pileus distinguishes *P. ochracea* from *P. schroeteri*, while *P. misera* has significantly smaller basidiocarps and differently shaped basidiospores. In the ML and Bayesian analyses *P. ochracea* formed a species-level lineage nested within a species complex around *P. lilatincta*. However, both morphological characters and constraint analyses suggest that *P. ochracea* is distinct from *P. lilatincta*.

Parasola plicatilis-similis is morphologically similar to *P. plicatilis* and forms a monophyletic group with the *P. schroeteri-P. megasperma* clade, whereas *P. plicatilis* branches off earlier in the crown-group *Parasola*. *Parasola plicatilis-similis* differs from *P. plicatilis* in having wider and differently shaped basidiospores and the habitat on dung. It is noteworthy that a significant intraspecific variability in stipe coloration was observed in *P. plicatilis*. We consistently encounter specimens with lilaceous to brown stipes, which

generally have larger basidiocarps than typical *P. plicatilis* specimens, although no evidence for the genetic separation of these specimens from typical *P. plicatilis* specimens was found in the genes used in this study.

We also found evidence for further undescribed species, but these lack sufficient specimen information and/or molecular data to allow them to be described in this study (*Parasola* sp. 1–4). These results and other recent reports of new species i.e. *Parasola cuniculorum* (Schafer 2014) and *P. malakandensis* (Hussain et al. 2017), suggest a considerable undescribed diversity in *Parasola* and probably also in other coprinoid lineages of Psathyrellaceae.

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LITERATURE CITED

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. Journal of Molecular Biology 215: 403–410.

Buller AHR. 1931. Researches on the Fungi IV: Further observations on the Coprini together with some investigations on social organization and sex in the 'Hymenomycetes'. London, New York: Longmans Green & Co. 329 p.

Bender H. 1989. Gattung *Coprinus* Sektion *Pseudocoprinus*. Beschreibung und Gegenüberstellung der Arten *Coprinus leiocephalus* und *Coprinus kuehneri.* APN. Mitteilungsblatt der Arbeitsgemeinschaft Pilzkunde Niederrhein 7: 36–45.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Molecular Ecology 2: 113–118.

Hussain S, Afshan NS, Ahmad H, Khalid AN, Niazi AR. 2017. *Parasola malakandensis* sp. nov. (Psathyrellaceae; Basidiomycota) from Malakand, Pakistan. Mycoscience 58: 69–76.

Larsson E, Örstadius L. 2008. Fourteen coprophilous species of *Psathyrella* identified in the Nordic countries using morphology and nuclear rDNA sequence data. Mycological Research 112: 1165–1185.

Löytynoja A, Goldman N. 2005. An algorithm for progressive multiple alignment of sequences with insertions. Proceedings of the National Academy of Sciences USA 102: 10557–10562.

Maddison WP, Maddison DR. 2011. Mesquite: a modular system for evolutionary analysis. version 2.75. http://mesquiteproject.org.

Nagy LG, Kocsubé S, Papp T, Vágvölgyi, C. 2009. Phylogeny and character evolution of the coprinoid mushroom genus *Parasola* as inferred from LSU and ITS nrDNA sequence data. Persoonia 22: 28–37

Nagy LG, Vágvölgyi C, Papp T. 2010a. Type studies and nomenclatural revisions in *Parasola* (Psathyrellaceae) and related taxa. Mycotaxon 112: 103–141.

Nagy LG, Urban A, Örstadius L, Papp T, Larsson E, Vágvölgyi C. 2010b. The evolution of autodigestion in the mushroom family Psathyrellaceae (Agaricales) inferred from Maximum Likelihood and Bayesian methods. Molecular Phylogenetics and Evolution 57: 1037–1048.

Nagy LG, Vágvölgyi C, Papp T. 2012. Morphological characterization of clades of the Psathyrellaceae (Agaricales) inferred from a multigene phylogeny. Mycological Progress 12: 505–517.

Padamsee M, Matheny PB, Dentinger BTM, Mclaughlin DJ. 2008. The mushroom family Psathyrellaceae: evidence for large-scale polyphyly of the genus *Psathyrella*. Molecular Phylogenetics and Evolution 46: 415–429.

Rambaut A, Suchard MA, Xie D, Drummond AJ. 2014. Tracer v 1.6. Tracer available online at http://beast.bio.ed.ac.uk/Tracer

Redhead SA, Vilgalys R, Moncalvo JM, Johnson J, Hopple JS Jr. 2001. *Coprinus* Persoon and the disposition of *Coprinus* species sensu lato. Taxon 50: 203–241.

Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. MrBayes 3.2: Efficient Bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542.

Schafer DJ. 2010. Keys to sections of *Parasola*, *Coprinellus*, *Coprinopsis* and *Coprinus* in Britain. Field Mycology 11: 44–51.

Schafer DJ. 2014. The genus *Parasola* in Britain, including *Parasola cuniculorum* sp. nov. Field Mycology 15: 77–99.

Schmidt-Stohn G. 2012. Drei seltene Tintlinge – Untersuchungsmethoden, Merkmale, taxonomische Stellung und Verbreitung in Europa. Zeitschrift für Mykologie 78: 137–153.

Shimodaira H, Hasegawa M. 2001. CONSEL: for assessing the confidence of phylogenetic tree selection. Bioinformatics 17: 1246–1247.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49: 369–382.

Stamatakis A. 2006. Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690.

Sukumaran J, Holder MT. (2010) DendroPy: A Python library for phylogenetic computing. Bioinformatics 26: 1569–1571.

Waterhouse AM, Procter JB, Martin DMA, Clamp M, Barton GJ. 2009. Jalview Version 2-a multiple sequence alignment editor and analysis workbench. Bioinformatics 25: 1189–1191.

White TJ, Bruns T, Lee S, Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR Protocols: a guide to methods and applications. New York, NY: Academic Press. p. 315–322.

Figure 1. 50% majority rule phylogram constructed from post-burn-in trees of the Bayesian analyses of 28S+ITS data. Numbers on the branches represent Bayesian posterior probabilities followed by ML bootstrap values. An asterisk (*) indicates 100% bootstrap or 1.00 posterior probablities while a minus sign (–) indicates support values lower than 50% boostrap or 0.70 posterior probability. Sequences of the newly described species highlighted in boldface. Bar indicates 0.01 expected change per site per branch.

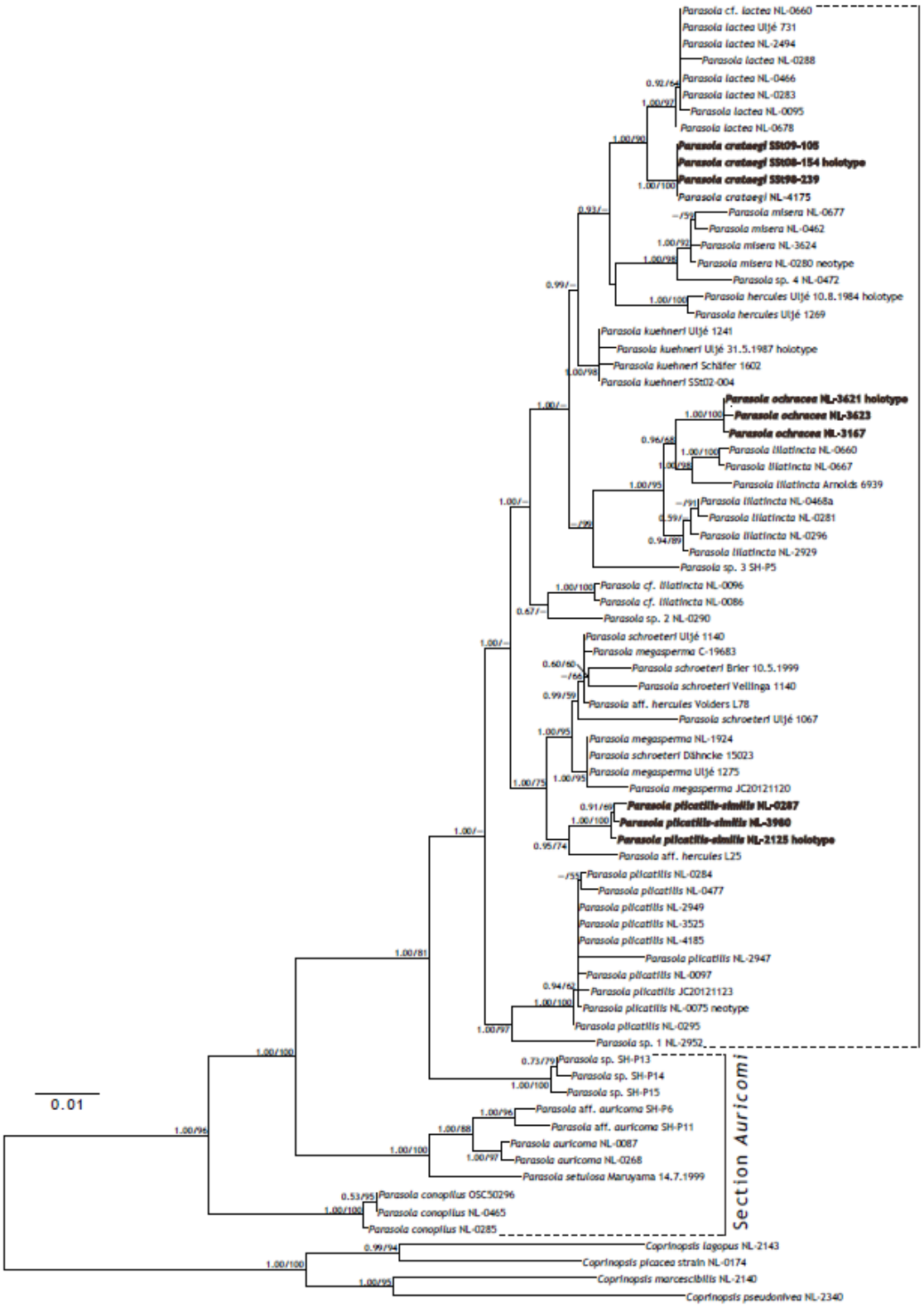

Figure 2. Macromorphology of *P. kuehneri* and the newly described *Parasola* species. A, B: *P. crataegi*, C, D: *P. kuehneri*, E, F: *P. ochracea*, G: *P. plicatilis-similis*. Photos: A–D: G. Schmidt-Stohn; E, G: L. Nagy. Bars = 1 cm.

Figure 3. Illustration of the diversity of basidiospore shapes and sizes in the newly described species. A. *Parasola crataegi*. B. *P. ochracea*. C. *P. plicatilis-similis*.

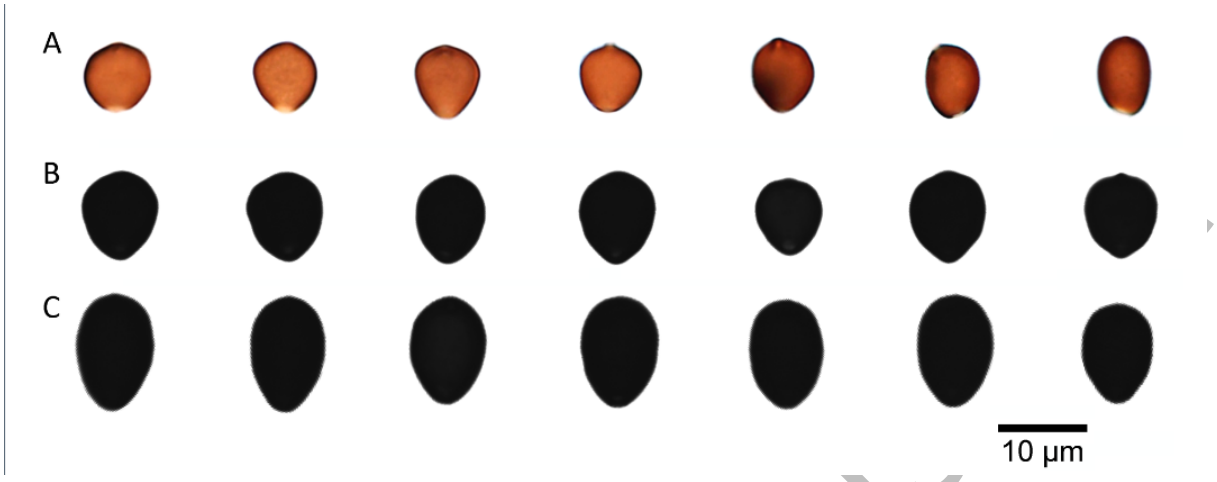

Figure 4. Cheilo- and pleurocystidial characters of *Parasola* species. A, B. *P. crataegi.* A. Cheilocystidia. B: Pleurocystidia. C. *P. ochracea* cheilocystidia. D, E. *P. plicatilis-similis.* D. Pleurocystidia. E: Cheilocystidia.