Psathyrella confundens (Psathyrellaceae, Agaricales) — a moist growing new species from Europe

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NØKKELORD
Drosophila albidula, molekylær systematikk, ny art, taksonomi

SAMMENDRAG
Basert på fylogenetiske analyser og morfologi er den nye arten *Psathyrella confundens* beskrevet. Det er så langt kjent fra Frankrike, Tyskland og Sverige. Den tvetydige bruken av navnet *Drosophila albidula* blir diskutert. Arten gjenkjennes av små basidiomata, sparsomt slør, spisse cystidier, store sporer og et fuktig habitat. Karakterer som skiller den fra nært beslektede arter er gitt.

ABSTRACT
Based on phylogenetic analyses and morphology the new species *Psathyrella confundens* is described. It is so far known from France, Germany and Sweden. The ambiguous use of the name *Drosophila albidula* is discussed. The species is recognised by small basidiomata, scanty veil, acute cystidia, large spores, and a moist habitat. Separating characters of closely related species are given.

INTRODUCTION

Romagnesi (1952) gave the diagnosis of *Drosophila albidula* Romagn. without description: '*Drosophila albidula* nom. nov. (= *Ps. subatomata* Lange nec Karsten)' (Table 1). Kühner and Romagnesi (1953) provided a short description of *Drosophila albidula* (= *Psathyrella potteri* A.H. Sm.) with 13–16 × 7–8 µm large spores and a habitat 'on wilted grasses'. The cap was 5–20 mm broad and 'at first dull ochre brown or pale brownish'. Nothing was said about striation. According to Lange (1940) his *Psathyra subatomata* J.E. Lange (= *Psathyrella potteri*) was characterized by small basidiomata, pale ochre clay cap without striations. The gills were broadly adnate with white edge and the spores were given to 14–15.5 × 7.5 µm. The species grew on naked ground in stack-yard among rotten straw. Kühner and Romagnesi (1953) recognised *Drosophila atomata* ss. Bres. and *D. albidula* as two separate species, both in our view conspecific with *Psathyrella potteri*.

On our request Romagnesi sent on loan what he called the type of *Drosophila albidula*, collected in 1940 'on muddy soil of a shady path', herbarium number 233'. The material

was in a bad condition. The gill edge was collapsed and it was only possible to discern the shape and size of the cystidia. The spores measured 12–13.2 × 5.8–7.2 µm, compared with 13–16 × 7–8 µm given by Kühner and Romagnesi (1953) for their *D. albidula*, thus an indication of two different taxa. Romagnesi (1975) gave a complete description of *D. albidula* with 'barely chocolate brown' cap when young, 11.5–14.7 × 6.7–7.5 µm large spores, and a white gill edge. It is likely to believe that *Drosophila albidula* sensu Romagnesi 1975 represents *D. albidula* sensu Romagnesi 1952 (= coll. 233 above) while *D. albidula* sensu Kühner and Romagnesi (1953) most certainly is identical with *Psathyrella atomata* sensu Romagnesi 1975 (= *Psathyrella potteri*). Romagnesi (1975: 215) admits that collections of *Drosophila albidula*, not the ones based on the description from 1953, incorrectly were determined to *D. atomata* sensu Bres.

The validly published name *Psathyra subatomata* (Lange 1940) is not a later homonym of *Psathyrella subatomata* P. Karst. (Karsten, 1885). Romagnesi (1952) created the superfluous but legitimate name *Drosophila albidula* to replace *Psathyra subatomata*. He should have combined the epithet *subatomata* in *Drosophila*. *Psathyrella albidula* (Romagn.) n. c. made by Moser (1967) should not be considered as a new combination but as the new name *Psathyrella albidula* M.M. Moser nom. nov. The type goes back via *Drosophila albidula* Romagn. to Lange's material of *Psathyra subatomata*. The names *Drosophila albidula* Romagn., 1952, *Psathyrella albidula* M.M. Moser, 1967 and *Psathyrella prona* f. *albidula* (M.M. Moser) Kits van Wav., 1972, are all nomenclatural synonyms to *Psathyra subatomata* J.E. Lange, 1940. From a taxonomical point of view *Psathyrella albidula* sensu Moser is a misapplication lacking Latin diagnosis and type. The greater part of the text above is written in collaboration with professor Nils Lundqvist who thought that the epithet *confundens* would be a suitable name for a new species.

In the large molecular phylogenetic study of psathyrelloid species (Örstadius et al. 2015) a collection of the new species was included, named *Psathyrella* sp LÖ312-92. In the phylogenetic analyses it came out as a distinct species together with *P. orbitarum* (Romagn.) M.M. Moser in the /prona clade. Due to the taxonomical confusion around the species name it was not further dealt with in that study.

MATERIALS AND METHODS

Colour names follow the Munsell soil colour charts (Munsell 1975), cited as Mu. in the text.

Micromorphological characters were observed using a Nikon Eclipse E200 light microscope equipped with phase contrast. Digital images were recorded with a Nikon Infinity 2 camera. For each collection, 10 to 20 mature spores were measured in water at ×1.000 magnification. Abnormally large or small spores were not considered. Other microscopic characters were studied in a 10 % NH_4OH solution and measured to nearest micron. To observe the hymenial cystidia, a complete lamella was cut off with a razor blade and soaked for a while. The gill edge was removed in order to check the cheilocystidia. The middle portion of the gill was cut out, crush-mounted, and pleurocystidia, basidia, subhymenium, and hymenophoral trama studied. The layers of the pileus were observed halfway from the margin by cutting tangential to the pileus, called a 'scalp'. The presence of clamps were checked. As for the shape of spores and cystidia the terminology of Vellinga (1988) was followed. Spores were mounted in a solution of ammonia before capturing digital images. Scale bars in figures of spores and cystidia represent 10 µm while scale bar in figure of basidioma represents 10 mm.

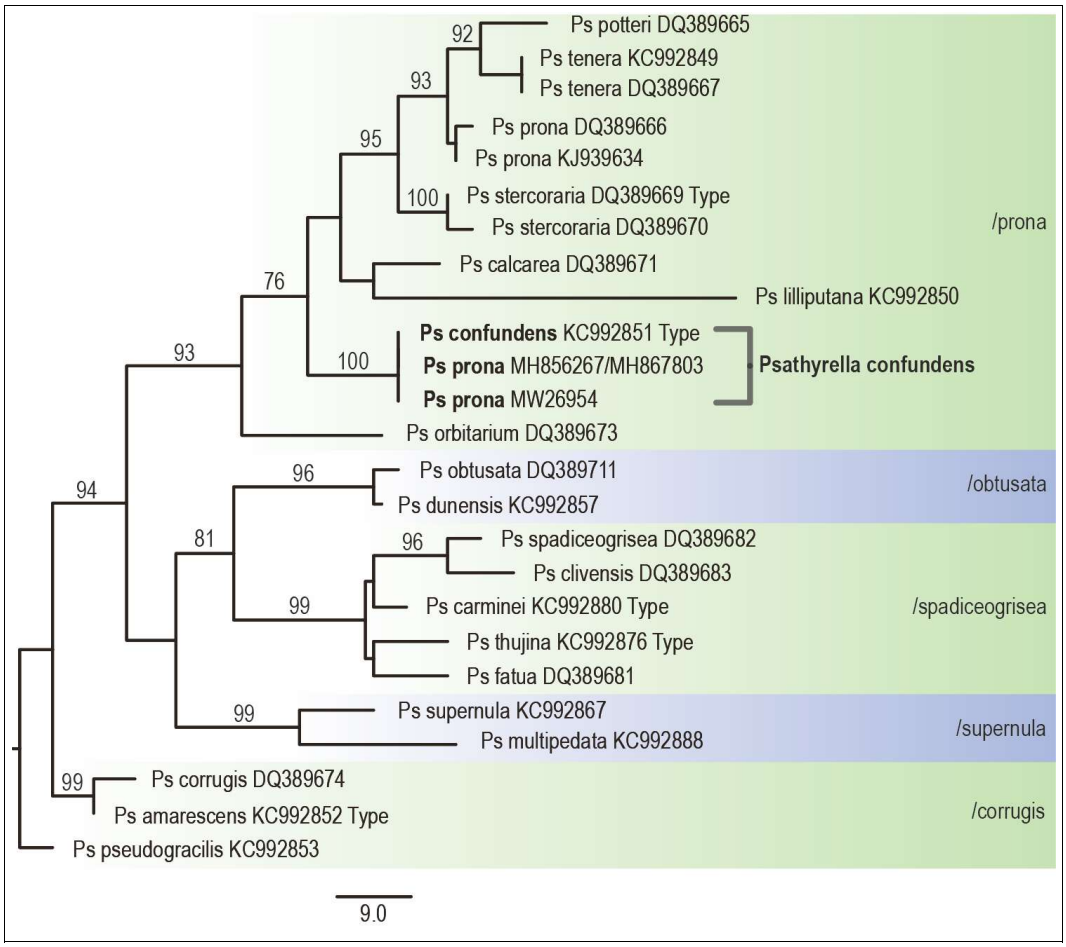

Figure 1. Phylogram showing the position of *Psathyrella confundens* in the /prona clade based on ITS and LSU sequence data. Bootstrap values are indicated on branches, the major supported clades are marked with names. Sequences originating from type specimens are indicated.

A nuc rDNA ITS and LSU data set to present *P. confundens* in a phylogenetic context was compiled based on the results from the multi gene analysis of Psathyrellaceae in Örstadius et al (2015). Representatives from the /prona, /spadiceogrisea, /obtusata and /supernula clades were selected. Rooting of trees are made with representatives from the /corrugis clade. In addition, the ITS sequence of *P. confundens* was blasted in GenBank (Clark et al. 2016) to seek additional available sequence data of similar and closely related taxa. Two sequences deposited as *Ps. prona* (Fr.) Gillet, one originating from Belgium (MH867803) and one from Portugal (MW269541) were found and added to the data set.

Alignment of the data set was performed using the L-INS-i strategy implemented in MAFFT 7.017 (Katoh and Standley 2013). The alignment was adjusted using ALIVIEW 1.17.1 (Larsson 2014). For inference of phylogenetic relationships of the dataset, heuristic searches for the most parsimonious trees were performed using PAUP* under the maximum parsimony (MP) criterion (Swofford 2003).

Table 1. An overview of names, spore sizes, etc.

Year	Name and author	L. Örstadius and E. Larsson	Spores
1940	*Psathyra subatomata* J.E. Lange	*Psathyrella potteri*	14-15.5 x 7.5 µm
1952	*Drosophila albidula* Romagnesi nom. nov.	*P. potteri* and/or *P. confundens*	The author refers to Lange 1940
1953	*Drosophila albidula* ss. Kühner and Romagnesi	*P. potteri*	13-16 x 7-8 µm
1953	*D. atomata* ss. Kühner and Romagnesi who refer to Bresadola	*P. potteri*	12-16 x 6.5-8 µm
1967	*Psathyrella albidula* (Romagn.) M.M. Moser n. c.	*P. potteri*	14-15.5 x 7.5 µm
1972, 1985	*Psathyrella prona* f. *albidula* Kits van Waveren	*P. potteri* and/or *P. confundens*	13-16 x 7-8 µm
1975	*Drosophila albidula* Romagnesi	*P. confundens*	11.5-14.7 x 6.7-7.5 µm
1975	*Drosophila atomata* ss. Romagnesi	*P. potteri*	14-16-(17) x 7-8.2 µm
-	Coll. 233 from 1940, type of *D. albidula* examined by Örstadius	*P. confundens*	12-13.2 x 5.8-7.2 µm

All transformations were considered unordered and equally weighted and gaps were treated as missing data. Heuristic searches with 1000 random-addition sequence replicates and TBR branch swapping were performed. Relative robustness of clades was assessed by the bootstrap (BT) method using 1000 heuristic search replicates with 10 random taxon addition sequence replicates and TBR branch swapping, saving 100 trees in each replicate.

MOLECULAR RESULTS

The aligned ITS and LSU dataset consisted of 25 sequences and 1628 characters. After exclusion of ambiguous data, mainly from the beginning and the end of the data set, 1552 characters remained for the analysis. Of these, 1370 were constant, 78 were variable but parsimony uninformative, and 104 were parsimony informative. The MP analysis yielded 6 equally most parsimonious trees (length = 317 steps, CI = 0.6593, and RI = 0.7818). One of these trees is presented in Fig. 1. Bootstrap analysis recovered four supported clades corresponding to /prona (93%), /obtusata (96%), */spadiceogrisea* (99%) and /supernula (99%). The three sequences of *C. confundens* form a terminal strongly supported clade (100%) within / prona, with *P. orbitarum* as a sister species.

TAXONOMY

Psathyrella confundens Örstadius & E. Larss. sp. nov. – Figs. 2-5.

MycoBank: MB838658

Etymology: The epithet refers to the ambiguous use of the name *Drosophila albidula* Romagn.

Holotype: Sweden: Skåne: Nosaby, Eknabben, on moist soil in a rich deciduous wood, 12. September 1992, leg. L. Örstadius, LÖ312-92 (Herb. GB-0131144, ITS and LSU sequence GenBank KC992851).

Basidiomata small, psathyrelloid. *Cap* 5–15 mm diam, at first conical, campanulate, conico-convex, then convex with a low umbo and a regular margin, when old sometimes with

conspicuously distant furrows, dark reddish brown (Mu. 5YR 3/3, Munsell 1975), dark brown, rusty brown, rather dull, ochraceous brown, drying to dirty pale, grey, cream, or pink tinges, slightly to distinctly hygrophanous, when moist striate at margin or further towards centre; *veil* fibrillose, covering almost entire primordium, when mature only scattered fibrils remaining, evanescent. *Gills* distant to medium spaced, L = 14–20, adnate, ventricose, when young ochraceous grey, becoming grey (Mu. 5YR 6/1), when old blackish brown; *edge* white pruinose, sometimes red underlined. *Stipe* 15–120 × 0.5–2 mm, cylindrical, often slightly flexuous, with a small bulb at base, pale brown, pulverulent at apex, lower part with fibrils from veil. *Smell* not distinctive; *taste* mild. *Spores* 11.5–13.5 × 6–7.5 µm (av. 12.2–12.8 × 6.5–6.9 µm, Qav. = 1.9–2), oblong, subcylindrical, subovoid, in profile sometimes with a slight suprahilar depression, hardly amygdaliform, in water red (Mu. 2.5YR 4/8); germ pore distinct. *Basidia* 4-spored, 20–36 × 10–12 µm. *Pleurocystidia* 35–80 × 9–16 µm, lageniform, sometimes flexuous, scattered to numerous, pale. *Cheilocystidia* of two types: A: 25–65 × 7–16 µm, similar in shape and frequency to pleurocystidia, B: 16–40 × 10–20 µm, clavate, numerous especially close to cap margin (Fig. 5). Scalp cap ½-way from margin: *pileipellis* made up of subglobose to ellipsoid 10–40 µm wide cells; *pileitrama* with strongly incrusted hyphae. *Hymenophoral trama* made up of rather strongly pigmented hyphae. *Veil cells* 30–60 × 4–12 µm. *Clamp connections* seen at stem base mycelium.

Figure 2. Basidioma from the type locality, Sweden: Skåne: Nosaby, Eknabben, LÖ294-01. Scale bar 10 mm.

Figure 3. Type locality of *Psathyrella confundens*, Sweden: Skåne: Nosaby, Eknabben.

Habit and habitats: Solitary to gregarious, moist to wet, 'on very wet clay soil' (Romagnesi 1975, as *Drosophila albidula*), among leaves, on sticks or twigs, on moss, fruits in autumn September - October.
Distribution: The species is confirmed from Sweden, Portugal and Belgium, but rarely recorded from France, Germany and Sweden. Kits van Waveren (1972, 1985) reported *Psathyrella prona* f. *albidula* (Table 1) from the Netherlands and Switzerland. Exact distribution is unknown due to confusion with related species.

Specimens examined
France: Bourgogne-Franche-Comté: Yonne, Sens, 'on the muddy earth of a shady path', 18. Sept. 1940, H. Romagnesi 233 (PC, type of *Drosophila albidula*), (Fig 4).
Germany: Bayern: Leipheim, by Donau-Brücke, down by the poplars, 22. Oct. 1996, M. Enderle (KR).
Sweden: Skåne: Nosaby, Eknabben, on moist soil in a rich deciduous wood, 2. Oct. 1986, L. Örstadius, LÖ168-86 (GB) (Fig. 2); 12. Sept. 1992, L. Örstadius, LÖ312-92, HOLOTYPUS (GB), (Fig 2, 5); 16. Oct. 2001, L. Örstadius, LÖ294-01 (GB), (Figs 2, 3).

Figure 4. Spores from the type of *Drosophila albidula,* France: Bourgogne-Franche-Comté: Yonne, Sens, Romagnesi. 233. Scale bar 10 µm.

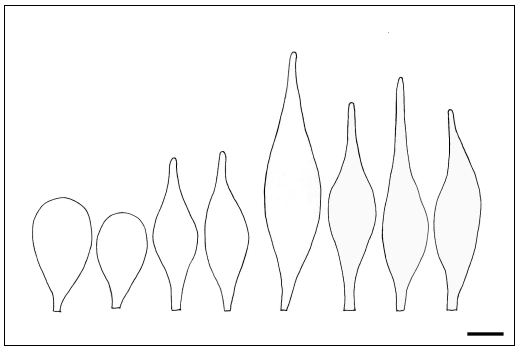

Figure 5. Hymenial cystidia from the holotype (cheilocystidia to the left, pleurocystidia to the right), Sweden: Skåne. Nosaby, Eknabben, LÖ312-92. Scale bar 10 µm.

Notes: The description is partly taken from Romagnesi (1975) as *Drosophila albidula* and our examination of material from France, Germany and Sweden. Romagnesi always found a white gill edge. The Swedish and German material varied from a pure white to a slightly or distinctly pigmented edge. *Psathyrella confundens* is recognised by small basidiomata, a fibrillose veil when young, acute ending cystidia, large spores, and a moist habitat. *Psathyrella prona* (Fr.) Gillet is separated by 2-spored basidia and slightly larger spores that often are limoniform with a more or less pronounced suprahilar depression. *Psathyrella potteri* differs in an early drying and hardly striate cap, a preference of growing on manured soil, and broader spores. The separating morphological features between *P. orbicularis* (Romagn.) Kits van Wav. and *P. confundens* are not convincing but the former can have a pseudorrhiza and it grows not only moist but also in dry forests or open grassland. Moreover, the spores are slightly narrower (av. 5.1–6.5 µm) and the cheilocystidia of type B are scattered close to cap margin opposite to *P. confundens* that often has a deep layer of cells. *Psathyrella orbitarum* (Romagn.) M.M. Moser differs in having smaller spores (av. 10.1–11.6 × 5.1–6 µm). Genetically *P. confundens* is closely related to *P. orbitarum* but the two differ by

15 substitutions in the ITS1 region, and 9 substitutions and one 2bp insertion/deletion event in the ITS2 region.

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