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# Experimental taxonomic studies in *Psilocybe* sect. *Psilocybe*

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The species of *Psilocybe* sect. *Psilocybe*, formerly classified in the genus *Deconica*, were investigated using morphology, mating behaviour and RAPD analysis. *Psilocybe inquilinus* and *P. crobula* do not seem to be closely related. Based on the morphology, two varieties could be accommodated in *P. subviscida*, namely as vars. *subviscida* and *velata*. The mating group of *P. montana* is characterized by rather thick-walled, dark spores with a fairly large germ-pore. Putative representatives of *P. muscorum* and *P. physaloides* freely interbreed with typical *P. montana*, and, consequently, these taxa are considered to represent one variable species. The ex-type strain of *P. chionophila* did not mate with isolates of *P. montana*. One collection of *P. chionophila* from a lowland habitat, morphologically resembling *P. montana*, was found to be interfertile with the ex-type strain of *P. chionophila*, but not with *P. montana*. We identified several collections as *P. magica*, which is morphologically similar to *P. schoeneti*. Mating studies showed that these specimen belong to the same biological species, but failed to mate with the ex-type of *P. schoeneti*.

## INTRODUCTION

*Psilocybe* belongs to the agaric family *Strophariaceae*. The core of this family consists of two large groups: *Psilocybe s. lat.* and *Pholiota s. lat.* (Noordeloos 1995, 1999a). However, different opinions exist on the generic concepts in these groups. *Psilocybe s. lat.* is often considered as a group of genera (*Psilocybe s. str.*, *Hypholoma*, *Stropharia*, and *Melanotus*), whereas some authors split *Pholiota s. lat.* in several genera such as *Pholiota s. str.*, *Hemipholiota*, *Flammula*, and others (e.g. Bon 1977, Watling & Gregory 1987). Continued studies, particularly with molecular methods, will certainly elucidate this conflicting situation.

Within *Psilocybe s. str.*, as conceived by Guzmán (1983, 1995), Orton (1960), Watling & Gregory (1987), and Stamets (1996), the species of sections *Psilocybe*, *Pratensae* and *Coprophilae* form a rather natural group, which formerly has been placed in a separate genus *Deconica*. Major problems, however, remain in the delimitation of the species in this complex. Characters used to distinguish species appear to be more variable than generally thought, and different taxonomic weight has been attributed to them by the various authors. Moreover, different interpretations of currently used names contribute to the confusion. For this reason we performed a critical study on the taxonomy of the group. This was achieved by morphological analysis, mating experiments to establish biological species concepts, and RAPD analysis of the genomic DNA.

So far the mating type system is only known for six species of *Psilocybe*. For five, *P. atrobrunnea*, *P. inquilinus*, *P. montana*, *P. semilanceata* and *P. velifera*, it is bifactorial (tetrapolar) heterothallic (Lamoure 1989), but *P. merdaria* is reported to be unifactorial (bipolar) heterothallic (Quintanilha, Quintanilha & Vasermanis 1941). In bifactorial species, each specimen meiotically produces two different alleles of the A and B factor, resulting in four mating types. Within a species many more alleles of these factors can occur, and it is therefore not unlikely that a haploid mycelium can mate with all four mating types of another specimen of that species. For some species, hundreds of alleles from both factors are known (Murphy & Miller 1997). In the present study the mating behaviour of a number of *Psilocybe* species has been studied.

RAPD fingerprinting has been applied to investigate isolates belonging to most of the biological species

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**Table 1.** Origin of isolates of species of *Psilocybe* studied, including RAPD type and mating group (nd, not done; nmo, no mating observed).

| Species | Isolate | Geographical origin | RAPD group | Mating group |
|---|---|---|---|---|
| *P.* aff. *castanella* | v 026 3 | Germany | F | nmo |
| *P. castanella* | v 240 1 | | nd | CASTANELLA |
| *P. castanella* | v 110 9 | Scotland | H | CASTANELLA |
| *P. castanella* | v 113 4 | Scotland | H | CASTANELLA |
| *P. castanella* | v 140 2 | Scotland | H | CASTANELLA |
| *P. castanella* | v 131 12 | Scotland | H | CASTANELLA |
| *P.* aff. *castanella* | v 022 10 | Germany | C | SPECIES 1 |
| *P. chionophila* | CBS 786.73 (as *P. montana*) | France | nd | CHIONOPHILA |
| *P. chionophila* | CBS 657.87 ex-type strain | France | B | CHIONOPHILA |
| *P. chionophila* | v 105 1 | The Netherlands | C | CHIONOPHILA |
| *P. crobula* | v 248 1 | Scotland | nd | CROBULA |
| *P. crobula* | v 124 1 | Scotland | C | CROBULA |
| *P. crobula* | v 078 2 | The Netherlands | D | CROBULA |
| *P. crobula* | v 150 3 | Austria | D | CROBULA |
| *P. crobula* | v 174 1 | The Netherlands | D | CROBULA |
| *P. crobula* | v 155 1 | Scotland | D | CROBULA |
| *P. crobula* | v 016 1 | Italy | D | CROBULA |
| *P. crobula* | v 017 17 | Italy | D | CROBULA |
| *P. crobula* | v 004 1 | Germany | D | nmo |
| *P. crobula* | v 020 5 | Germany | D | nd |
| *P. crobula* | v 130 3 | Scotland | D* | CROBULA |
| *P. inquilinus* | v 194 1 | The Netherlands | G | INQUILINUS |
| *P. inquilinus* | v 196 3 | The Netherlands | G | INQUILINUS |
| *P. inquilinus* | v 188 1 | The Netherlands | G | INQUILINUS |
| *P. inquilinus* | v 179 3 | The Netherlands | G | INQUILINUS |
| *P. magica* | v 116 1 | Scotland | I | MAGICA |
| *P. magica* | v 118 1 | Scotland | I | MAGICA |
| *P. magica* | v 152 1 | The Netherlands | I | MAGICA |
| *P. magica* | v 123 1 | Scotland | I | MAGICA |
| *P. montana* var. *macrospora* | v 212 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | CBS 597.87 | France | nd | MONTANA |
| *P. montana* var. *montana* | v 014 3 | Finland | nd | MONTANA |
| *P. montana* var. *montana* | v 176 1 | The Netherlands | nd | MONTANA |
| *P. montana* var. *montana* | v 215 3 | The Netherlands | nd | MONTANA |
| *P. montana* var. *montana* | v 195 2 | The Netherlands | nd | MONTANA |
| *P. montana* var. *montana* | v 061 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 094 1 | Finland | A | MONTANA |
| *P. montana* var. *montana* | v 096 1 | Finland | A | MONTANA |
| *P. montana* var. *montana* | v 129 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 151 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 154 21 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 171a 3 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 173 3 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 181 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 182 2 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 186 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 197 4 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 198 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 010 2 | Finland | A | MONTANA |
| *P. montana* var. *montana* | v 015 7 | Finland | A | MONTANA |
| *P. montana* var. *montana* | v 202 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 128 1 | The Netherlands | A | MONTANA |
| *P. montana* var. *montana* | v 206 1 | The Netherlands | A | nd |
| *P. montana* var. *montana* | v 039 2 | The Netherlands | A* | MONTANA |
| *P. montana* var. *montana* | v 069 4 | The Netherlands | A* | MONTANA |
| *P. montana* var. *montana* | v 011 4 | Norway | A* | MONTANA |
| *P. montana* var. *montana* | v 103 1 | Austria | N | MONTANA |
| *P. pratensis* | v 189 1 | The Netherlands | L | nmo |
| *P. schoeneti* | v 200 6 T | Germany | J | SUBVISCIDA |
| *P. subviscida* (received as *P. inquilinus*) | v 201 dk | Germany | E | nd |
| *P. subviscida* (received as *P. inquilinus*) | v 201 1 | Germany | E | nd |
| *P. subviscida* (received as *P. montana* f. *plana*) | h 3664 | The Netherlands | E | nd |

**Table 1.** (*cont.*)

| Species | Isolate | Geographical origin | RAPD group | Mating group |
|---|---|---|---|---|
| *P. subviscida* (received as *P. montana* f. *plana*) | h 4039 1 | The Netherlands | E | nd |
| *P. subviscida* (received as *P. inquilinus*) | CBS 197.39 | Unknown | E | nd |
| *P. subviscida* var. *subviscida* | h 232 | The Netherlands | nd | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 216 | The Netherlands | nd | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 218 | The Netherlands | nd | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 219 | The Netherlands | nd | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 246 | The Netherlands | nd | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | h 231 | Austria | nd | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 227 | The Netherlands | nd | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 245 | The Netherlands | nd | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 065 5 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 097 30 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 100 1 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 101 1 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 127 1 | Scotland | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 142 1 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 102 1 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 143 2 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 013 4 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 013 6 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 107 1 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 106 1 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *subviscida* | v 003 dk | Switzerland | E | nd |
| *P. subviscida* var. *velata* | v 136 1 | The Netherlands | E | SUBVISCIDA |
| *P. subviscida* var. *velata* | v 098 9 | Austria | E | SUBVISCIDA |
| *P. subviscida* var. *velata* | v 153 1 | The Netherlands | K | nd |
| *P. subviscida* var. *subviscida* | CBS 330.39 | Unknown | nd | SUBVISCIDA |

observed, in order to identify them with these biological species where mating experiments were not performed, and, secondly, to further provide an independent molecular measure to test the developed biological and morphological species concepts.

The results of these studies are presented in the present paper. Keys, full descriptions of the accepted species, type-studies and nomenclatoral discussions have been published elsewhere (Noordeloos 1999a, 2001).

## MATERIALS AND METHODS

### *Species examined and morphological analyses*

The species, specimens and isolates studied are listed in Table 1. Our research concentrated on the species of the '*Deconica*' complex which includes *Psilocybe apelliculosa*, *P. crobula*, *P. castanella*, *P. chionophila*, *P. flocculosa*, *P. inquilinus*, *P. magica*, *P. montana*, *P. micropora*, *P. phyllogena*, *P. pratensis*, *P. subviscida* and *P. xeroderma*. Material was collected in northern, western and central Europe, as well as received from a number of collaborators, listed in Acknowledgements; all isolates used are deposited in CBS and voucher specimens are in L. About 200 collections were studied in this research. The macromorphological characteristics were scored on the fresh material and from notes attached to herbarium specimens. The microscopical data have been obtained from the dried specimens using standard procedures as described in Kuyper (1988).

### *Isolation of mono- and dikaryons, and mating experiments*

Haploid monokaryotic strains were obtained from spore prints. Suspensions of various concentrations were made in a 0.02% Tween 80 solution and smeared over 2% malt extract agar (MEA) plates. Germinating spores were isolated and later tested for the absence of clamps. Inocula of haploid strains were mated on MEA 2%, about 10 mm apart, and the Petri dishes were sealed with elastic tape. Samples were prepared from the contact zone 2–3 wk after contact and examined for the presence of clamp connections. In case of negative results they were re-examined after 5–6 wk.

### *Isolation of DNA and RAPD analysis*

For DNA isolation, cells were harvested from 3–6 wk-old cultures grown on cellophane sheets covering 2% malt extract agar and lyophilized after harvest. Genomic DNA was isolated according to the CTAB method (O'Donnell *et al.* 1997). For RAPD analysis 20 decamer primers (Operon Technologies, Alameda, CA) were investigated. The following primers gave the most reliable results: OPA01, OPA02, OPA03, OPA04, OPA05, OPA07, OPA08, OPA10 and OPA12. Polymerase chain reaction using these primers was performed in 200 µl reaction tubes containing 5 pmol primer, 0.1 mM dNTP, 15 mM $MgCl_2$ and 0.125 U Super/Therm (ITK diagnostics, Uithoorn, The Netherlands) in 25 µl reaction

**Table 2.** Diagnostic characters of the accepted morphospecies in *Psilocybe* sect. *Psilocybe*.

| | *inquilinus* | *crobula* | *castanella* | *subviscida* var. *subviscida* | *subviscida* var. *velata* | *montana* var. *montana* | *montana* var. *macrospora* |
|---|---|---|---|---|---|---|---|
| Pileus striate | Yes | Poor | Poor/not | Yes | Poor/not | Yes | Yes |
| Peeling pellicle | Yes | Yes | No | No/poor | No/poor | No/poor | No/poor |
| Veil on pileus | Poor | Abundant | Present | Absent | Present | Absent | Absent |
| Lamella colour | Pale brown | Pale brown | Pale brown | Pale brown | Pale brown | Dark brown | Dark brown |
| Veil on stipe | Absent | Abundant | Present | Absent | Present | Absent | Absent |
| Spore length | 7–10 | 5.5–8 | 6.5–8 | 6–8.5 | 6.5–8 | 7–9 | 8.5–11 |
| Spore width | 4–6 | 3.5–6 | 4–5.5 | 4–5 | 4–5 | 4–6 | 5–7 |
| Spore breadth | 5–7 | 4–6 | 4–5.5 | 4–5.5 | 4–5.5 | 4.5–6 | 6.5–8.5 |
| Spore F/I index | $<50\%$ | $>50\%$ | $<25\%$ | $<50\%$ | $<50\%$ | 50–60 % | 50–60 % |
| Spore wall | Thin | Thin | Thin | Thin | Thin/thick | Thick | Thick |
| Spore wall colour | Pale brown | Pale brown | Pale brown | Pale brown | (Pale) brown | Dark brown | Dark brown |
| Germ-pore | Small to medium | Medium to small | Small | Large | Large | Large | Large |
| Pleurocystidia | Absent | Absent | Absent | Absent | Absent | Absent | Absent |

volumes. PCR used 40 cycles with the following parameters: denaturation at 94 °C for 60 s, annealing at 34 ° for 60 s and extension at 72 ° for 120 s. In addition, the (ATG)5 primer was used using the following PCR conditions: denaturation at 94 ° for 60 s, annealing at 48 ° for 60 s and extension at 72 ° for 120 s. PCR products were visualized by conventional electrophoresis using 1.5 % agarose in $1.0 \times$ TBE. Ethidium bromide-stained gels were photographed on a transilluminator ($\lambda = 300$ nm).

### *Computer-assisted analysis of RAPD genomic fingerprints*

The large number and high complexity of the fingerprint patterns obviated the use of computer-assisted pattern analysis. Polaroid pictures of ethidium bromide stained agarose gels were digitized using a Hewlet Packard Scanjet II cx and DeskScan II v.2.1a software and stored as TIFF files. The images were converted, background was subtracted and the patterns were normalized according to the molecular size markers, and analyzed with Bionumerics software (version 2.0; Applied Maths, Kortrijk, Belgium). Similarity matrices of the gel tracks were calculated using the pairwise Pearson's product-moment correlation coefficient ($r$ value), an approach that compares the whole densitometric curves of the fingerprints (Häne *et al*. 1993, Rademaker & de Bruijn 1997, Rademaker *et al*. 1999). Only informative parts of the lanes were included in the analysis. The genomic fingerprints patterns were compared ranging from 240 bp to 3.0 kb for ATG(5), OPA02, OPA07, and OPA12, 240 bp to 4.0 kb for OPA01, 125 bp to 2.5 kb for OPA03, OPA08, OPA10, 175 bp to 2.5 kb for OPA04, and 125 bp to 3.0 kb for OPA05 profiles. Using the similarity coefficients from each experiment, a consensus dendrogram was obtained from the composite data set with the unweighted pair group method using arithmetic averages (UPGMA; Sokal & Michener 1958). The different RAPD profiles were also compared by clustering of the product–moment correlation coefficients ($r$-values) and the Kendall's *tau* correlation coefficients.

## RESULTS AND DISCUSSION

### *Morphology*

The mating studies revealed at least 12 biological species. Most of them were represented with several to many isolates, namely *P. inquilinus*, *P. crobula*, *P. subviscida* with var. *subviscida* and var. *velata*, *P. castanella*, *P. montana* var. *montana* and var. *macrospora*, *P. chionophila*, and *P. magica*. Some other species have been isolated only once (*P. phyllogena*, *P. micropora*, *P. xeroderma*, *P. pratense*, and *P. schoeneti*). It was possible to distinguish these biological species, with only a few exceptions, by morphological criteria. The diagnostic characters for each of the recognized species are presented in Table 2. The main characters used to distinguish species were the following:

(1) *Pileal surface*. The surface of the pileus, whether dry or viscid, has always been considered an important criterion. However, our study revealed that this must be considered with care. A gelatinous cuticle, that can be entirely lifted off with a needle (separable pellicle) proved to be of good diagnostic value, and is used to distinguish *P. inquilinus* and *P. crobula* from related taxa. However, a slightly viscid pellicle which is only partly separable proved to be of minor value. Within *P. montana*, for example, collections with a dry pileus and those with a slightly viscid pileus were interfertile. For this reason we see no grounds to differentiate *P. montana s. str*. with a dry pileus from *P. muscorum* with a viscid pileus.

(2) *Presence or absence of veil*. The veil has always been considered a valuable character to distinguish species. In most cases this proved to be true, with the exception of *P. subviscida*, where the veil did not prove to be a discriminating character (see below).

(3) *Spores*. Size of spores sometimes varies considerably within a species, a feature particularly striking

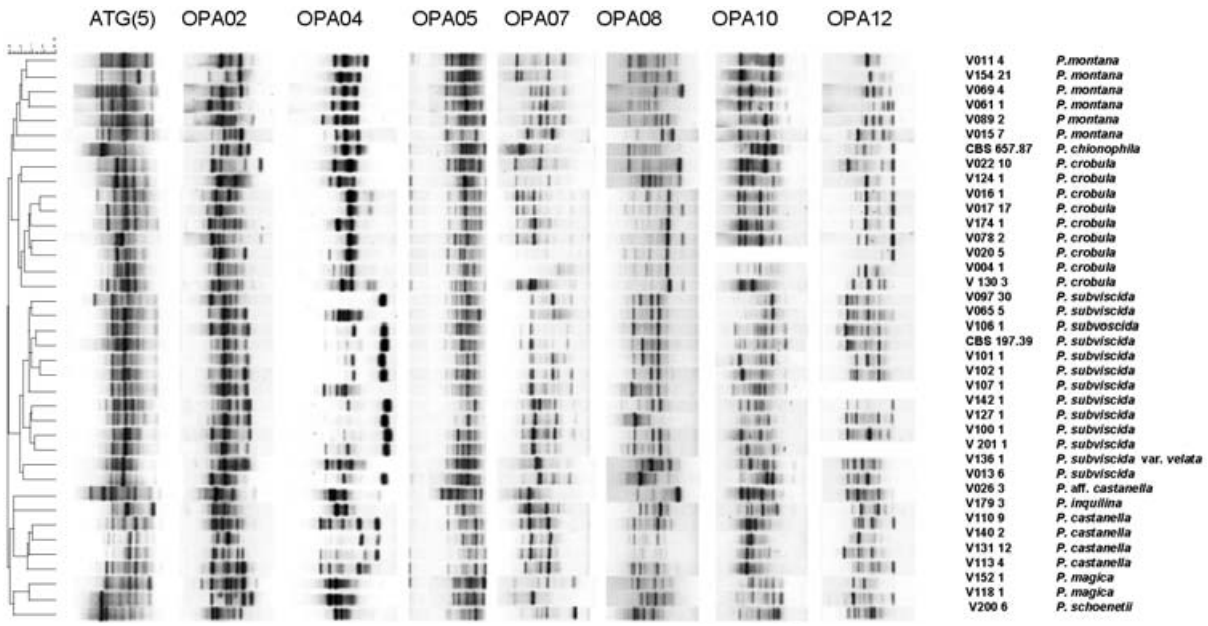


**Fig. 1.** UPGMA/product-moment cluster analysis of eight linearly combined fingerprint profiles of representatives of *Psilocybe* species.

in *P. montana*. In other cases, however, small differences in size are decisive and prove to be good tools to define the species. See comments on *P. inquilinus* versus *P. crobulus*, below.

(4) *Cystidia*. Guzmán (1983, 1995) attributed much weight to the size of the cheilocystidia as a character to distinguish species in sects. *Psilocybe* and *Pratensae*. Our morphological studies show that these differences are not applicable. Size and, to a lesser extent shape, of the cheilocystidia may vary a great deal within one biological species. For details see Noordeloos (2001).

It was not possible to identify the species by the morphological characters displayed in pure culture. However, a number of strains produced straight to more or less spirally arranged chains of arthroconidia as seen in *Pseudohelicomyces*, the anamorph *Psilocybe* (Valenzuela & Garnica 2000). These strains belong to *P. montana* var. *macrospora* and *P. subviscida*. The anamorph is lacking in a number of strains of these species, either monokaryotic or dikaryotic. After subjection to preservation by freezing at ultra-low temperatures, the number of strains forming arthroconidia increased. This strongly suggests the involvement of a stress factor in the production of the anamorph, which may be comparable to that reported by Petersen (1995) for *Oudemansiella canarii*.

### *RAPD analysis*

RAPD analysis from *Psilocybe* revealed an overall large diversity of profiles using ten different random primers. Cluster analysis of the individual primer profiles yielded clusters that showed limited correlation with the accepted classification of the species. Species-specific clusters were observed in dendrograms obtained from the composite data set of 4 to 8 randomly primed fingerprint profiles at a dendrogram correlation between 30 and 60 (Fig. 1). *Psilocybe montana*, *P. subviscida* and *P. magica* showed single species clusters. *P. chionophila* was found within the *P. montana* cluster. *P. crobula* isolates constituted a cluster together with one *P. castanella* isolate that actually has an ITS sequence identical to *P. crobula* (S.J.W.V. & T.B., unpubl.). The other *P. castanella* isolates grouped together and were flanked by a *P.* aff. *castanella* and a *P. inquilinus* strain. *P. schoeneti* was found separated from the other groups. Within the species-specific clusters, a large number of polymorphisms were observed. These discriminatory bands allowed the assessment of diversity of these groups down to the specimen or population level. Analyzing the *Psilocybe* collections using six linearly combined randomly primed fingerprint profiles showed similar results. However, when using four linearly combined randomly primed fingerprint profiles some more exceptions to the species differentiation were found (data not shown).

Cluster analysis of eight linearly combined fingerprint profiles of descendants of *Psilocybe* isolates v 013 (*P. subviscida*) and v 069 (*P. montana*) are given in Fig. 2. A high overall similarity, larger than approximately 0.8, can be observed between the descendants. Moreover, divergence is reflected in polymorphisms among the descendants of each isolate.

Ten different randomly primed fingerprints were applied. To analyze the congruence between these experiments, their similarity matrices were compared. The resulting *r*-values were calculated and presented in a similarity matrix and derived dendrogram (Fig. 3). The observed concordance between any of the fingerprint methods was low, with an average *r*-value of $0.256 \pm 0.116$ standard deviation. The highest correlations,

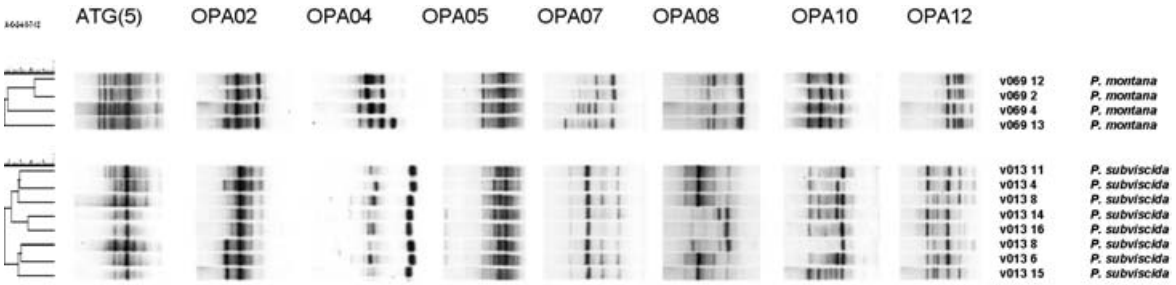


**Fig. 2.** UPGMA/product-moment cluster analysis of eight linearly combined fingerprint profiles of descendants of *Psilocybe subviscida* isolate v 013 (top) and *P. montana* isolate v 069 (bottom) respectively.

(A)

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| OPA10 | 100 | | | | | | | | | |
| OPA12 | 30.8 | 100 | | | | | | | | |
| OPA07 | 33.1 | 19.0 | 100 | | | | | | | |
| OPA08 | 18.2 | 27.8 | 41.2 | 100 | | | | | | |
| ATG(5) | 20.2 | 19.8 | 24.2 | 24.6 | 100 | | | | | |
| OPA02 | 13.2 | 25.1 | 35.8 | 16.7 | **60.2** | 100 | | | | |
| OPA05 | 25.1 | 28.5 | 34.1 | 21.5 | **51.5** | **49.3** | 100 | | | |
| OPA04 | 15.8 | 26.4 | 22.1 | 16.8 | 24.4 | 27.8 | 38.0 | 100 | | |
| OPA03 | ND | 24.9 | 28.1 | ND | 11.6 | ND | ND | 28.3 | 100 | |
| OPA01 | ND | 16.7 | 16.0 | ND | 6.8 | ND | ND | 19.5 | 6.2 | 100 |

(B)

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| OPA10 | 100 | | | | | | | | |
| OPA12 | 16.3 | 100 | | | | | | | |
| ATG(5) | 9.9 | 10.6 | 100 | | | | | | |
| OPA02 | 4.3 | 12.0 | **34.0** | 100 | | | | | |
| OPA05 | 10.6 | 16.1 | **29.1** | **30.6** | 100 | | | | |
| OPA07 | 13.4 | 8.0 | 11.3 | 13.6 | 12.6 | 100 | | | |
| OPA08 | 5.8 | 11.6 | 9.8 | 7.1 | 7.2 | 20.1 | 100 | | |
| OPA03 | ND | 16.3 | 7.1 | ND | ND | 17.6 | ND | 100 | |
| OPA04 | 6.6 | 15.5 | 12.3 | 15.0 | 22.3 | 9.1 | 5.1 | 17.2 | 100 |
| OPA01 | ND | 9.2 | 4.4 | ND | ND | 8.4 | ND | 1.4 | 10.0 | 100 |

**Fig. 3.** Comparison of the different RAPD profiles. (A) Clustering of the product moment correlation coefficients (*r*-values) between the different RAPD fingerprinting methods. The matrix shows the product moment correlation (*r*-) values. The three highest *r*-values between these methods were found between ATG(5), OPA02 and OPA05 primed fingerprints profiles and are printed in bold, while the lowest *r*-value was found between OPA01 and OPA03 is underlined. (B) Clustering of the Kendall's *tau* correlation coefficients between the different RAPD fingerprinting methods. The three highest *tau* values between these methods were found between ATG(5), OPA02 and OPA05 primed fingerprints profiles and are printed in bold, while the lowest *tau* value, found between OPA01 and OPA03, is underlined.

with *r*-values of 0.602, 0.511 and 0.493, were observed among the (ATG)5, OPA02 and OPA05 primed genomic fingerprinting methods. The low genetic similarities of the separate OPA01 and OPA03, OPA04 and OPA07 and OPA02 and (ATG)5, primed fingerprints are illustrated by scatter plots including linear fit lines in Fig. 4. The corresponding *r*-values are 0.062, 0.221 and 0.602 respectively and a high scatter can be observed in each plot.

The RAPD fingerprinting method is most commonly used for the discrimination of populations. However, it has been found useful for the distinction of species of *Phytophthora* (Cooke *et al.* 1996). In our study, RAPD was shown to be functional for discriminating species in *Psilocybe* sect. *Psilocybe* using several profiles. However, care should be taken when only a limited amount of fingerprints are combined in one analysis since clustering is likely to yield several subspecific groups per species. The discriminatory power of the RAPD technique to distinguish between individual specimens was also illustrated by the observation of polymorphisms among descendants of a single isolate (see above).

### *Mating experiments*

All intercompatibility groups (ICGs) tested proved to be bifactorial heterothallic. This indicates that the mating system in *Psilocybe* consists of a homogenic

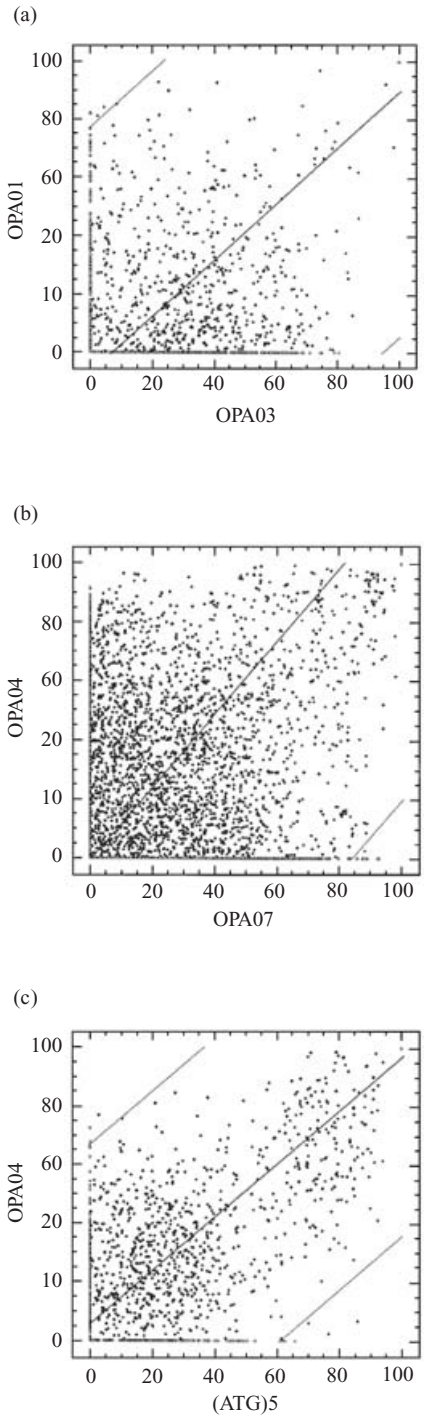


**Fig. 4.** Scatterplot of genetic similarities derived from different RAPD fingerprint analyses. The relationships between OPA01 and OPA03 (a), OPA04 and OPA07 (b), and OPA02 and ATG(5) (c) are indicated by first degree regression curves. Confidence intervals are as indicated.

incompatibility system only. There are no indications of an additional heterogenic incompatibility system, as in *Heterobasidion annosum* (Chase & Ullrich 1990a, b). From the literature it appears that the nuclear behaviour is normal (Boidin 1964) with uninucleate spores forming an uninucleate mycelium from the start. The secondary mycelium is regularly binucleate. The ICGs support the accepted species concepts, contrary to for example the situation in *Hebeloma* (Aanen & Kuyper 1999).

Confrontations between haploid strains of different isolates belonging to the same ICG generally resulted in the production of clamp connections, indicating that the species are multi-allelic for both mating type factors. No attempts were made to estimate the number of alleles. Nearly all confrontations between haploid strains of different conspecific specimens were positive (Table 1, Figs 5–7). This concerns *P. montana* (Fig. 5), *P. subviscida* (Fig. 6), *P. crobula*, *P. inquilinus*, *P. castanella*, *P. chionophila*, and *P. magica* (Fig. 7). In some cases living material was lost during the research period, resulting in some missing mating data.

Interspecific matings sometimes resulted in the occasional occurrence of false clamps, which were ignored. A few positive cases of partial intercompatibility did occur. Four strains of *P. montana* (v 010, v 011, v 015 and v 215) regularly showed clamp connections with four strains of *P. subviscida* (v 013, v 098, v 102, v 143), but only in one of the possible combinations, except for collection 11, which displayed clamps in two combinations. Strain v 015 also showed rare clamps with *P. inquilinus* (v 179, v 188). Strain v 202 of *P. montana* produced clamp connections with strain v 124 of *P. crobula*. Strain v 219 of *P. subviscida* produced clamps with strain v 155 of *P. crobula*. Strain v 100 of *P. subviscida* produced occasional clamps with strain v 017 and v 174 of *P. crobula* and with strain v 196 of *P. inquilinus*. Strains v 110 and v 131 of *P. castanella* produced occasional clamps with strain v 127 respectively v 107 of *P. subviscida* and v 130 respectively v 124 of *P. crobula*.

There is no doubt that most strains involved in the interspecific matings belong to the species they have been assigned to, because the RAPD data support the morphological conclusions. This is the case for strains v 010, v 011, v 015 of *P. montana* (v 202 and v 215 were not studied by molecular means), for strains c 013, c 098, v 100, v 102, v 107, v 127, v 143 of *P. subviscida*, for strains v 179, v 188, v 196 of *P. inquilina*, for strains v 130 and v 155 of *P. crobula*, and for strains v 110, v 131 of *P. castanella*.

The literature contains several reports of strains that function as bridges between species; they show regular mating with more than two species. For example, Petersen & Ridley (1996) reported a New Zealand strain of *Pleurotus pulmonarius* which was universally compatible with four different species of *Pleurotus*. This may be comparable with the situation in *Rhizoctonia*, where bridging isolates may show abundant anastomoses with three or more anastomosis groups (Carling 1996).

### *Morphological* vs *biological species concepts*

From this study it is clear that some morphological species concepts, as used for example in Kuyper (1988) must be adjusted due to the results of our mating experiments.

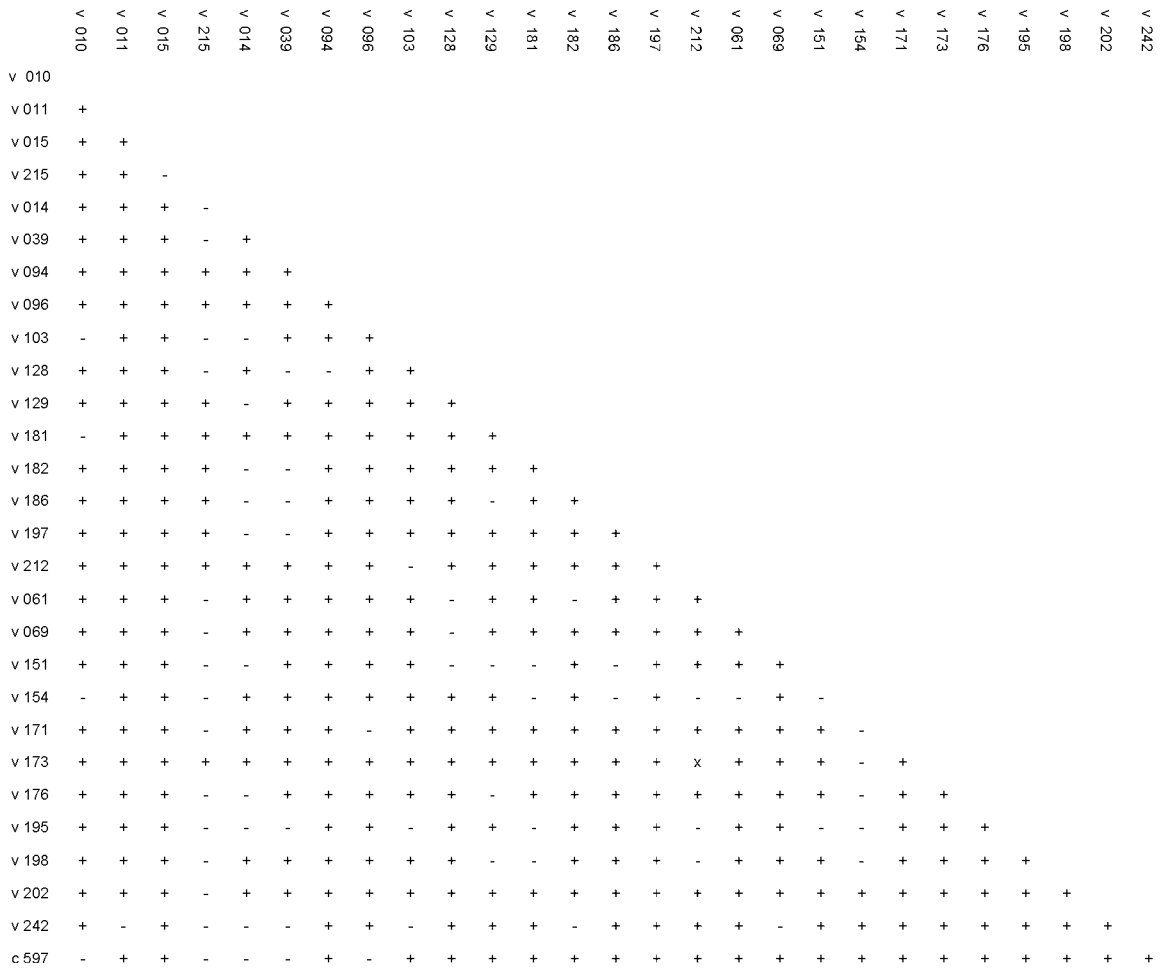


**Fig. 5.** Mating scheme of isolates of *Psilocybe montana* (+, positive mating; −, negative mating).

## Psilocybe inquilinus *vs* P. crobula

Both species share a strongly gelatinised pileipellis, which can be lifted entirely from the pileus with a needle as a transparent pellicle. Although both taxa sometimes have been considered as extremes of one variable taxon (e.g. Höiland 1978), the mating experiments prove that they must be considered separate biological species, which can be distinguished on account of substrate preference and small differences in spore-size and shape (Table 2). This is also supported by the RAPD patterns, which suggest that *P. inquilinus* and *P. crobula* are not very closely related.

## Psilocybe subviscida

During our studies, many collections have been derived from one mating pool, very likely representing a biological species, but showing considerable morphological variation. The whole group, which inhabits raw humus of grasses, straw and partly digested dung, can be characterized by a relatively firm, fibrillose stipe. The surface of the pileus varies from relatively dry to rather viscid. A veil may be present and even very distinct in the form of flecks adhering to the margin of the pileus and a fibrillose annuliform zone on the stipe, but is absent in many collections. The presence of a veil seems to be correlated with the thickness of the spore wall. The oldest name for this species appears to be *Psilocybe subviscida*. Application of the morphological species concept as used by Kuyper (1988) would have resulted in the distinction of two species: *P. subviscida* and another which could be named either *P. bullacea* or *P. graminicola*. However, on account of the mating results, we distinguish two varieties in *P. subviscida*. The variety with thin-walled spores and lacking a veil represents the var. *subviscida*, whereas that with a veil and thick-walled spores has been described as var. *velata* (Noordeloos 1999b). The varieties freely interbreed and the RAPD results also show that isolates from both are similar. Type studies show that *P. graminicola* is a synonym of the latter variety. The name *P. bullacea* has been frequently misapplied to var. *velata*. For further details see Noordeloos (2001).

## Psilocybe montana

The mating group of *Psilocybe montana* is characterized by rather thick-walled, dark spores with a fairly large germ-pore. Many authors (e.g. Watling & Gregory 1987) distinguish several species in this group, including *P. muscorum* and *P. physaloides*, mainly on characters

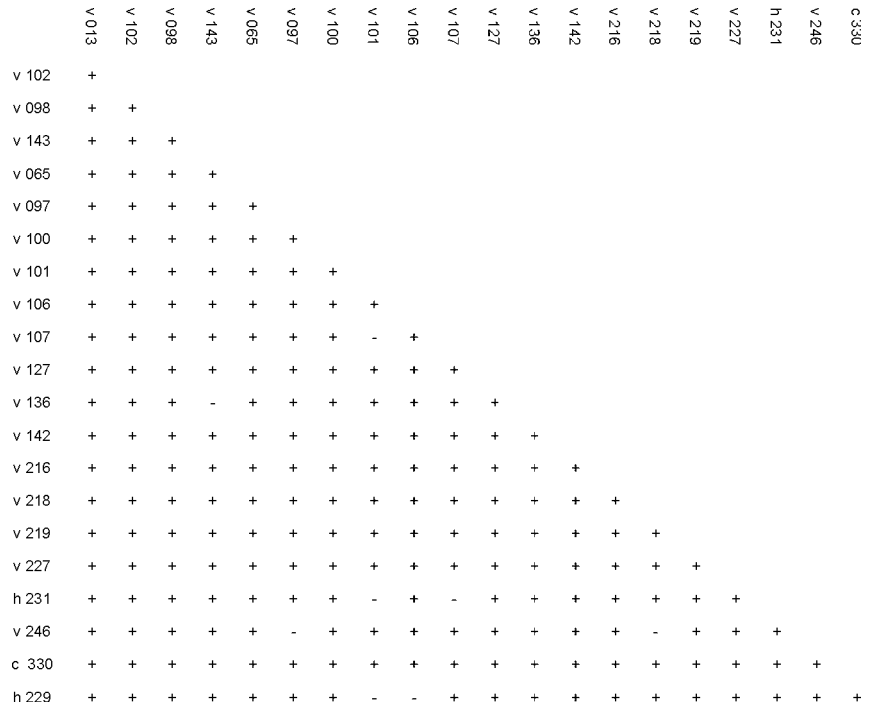


**Fig. 6.** Mating scheme of isolates of *Psilocybe subviscida* (+, positive mating; −, negative mating; a small + is an incidental positive interspecies mating).

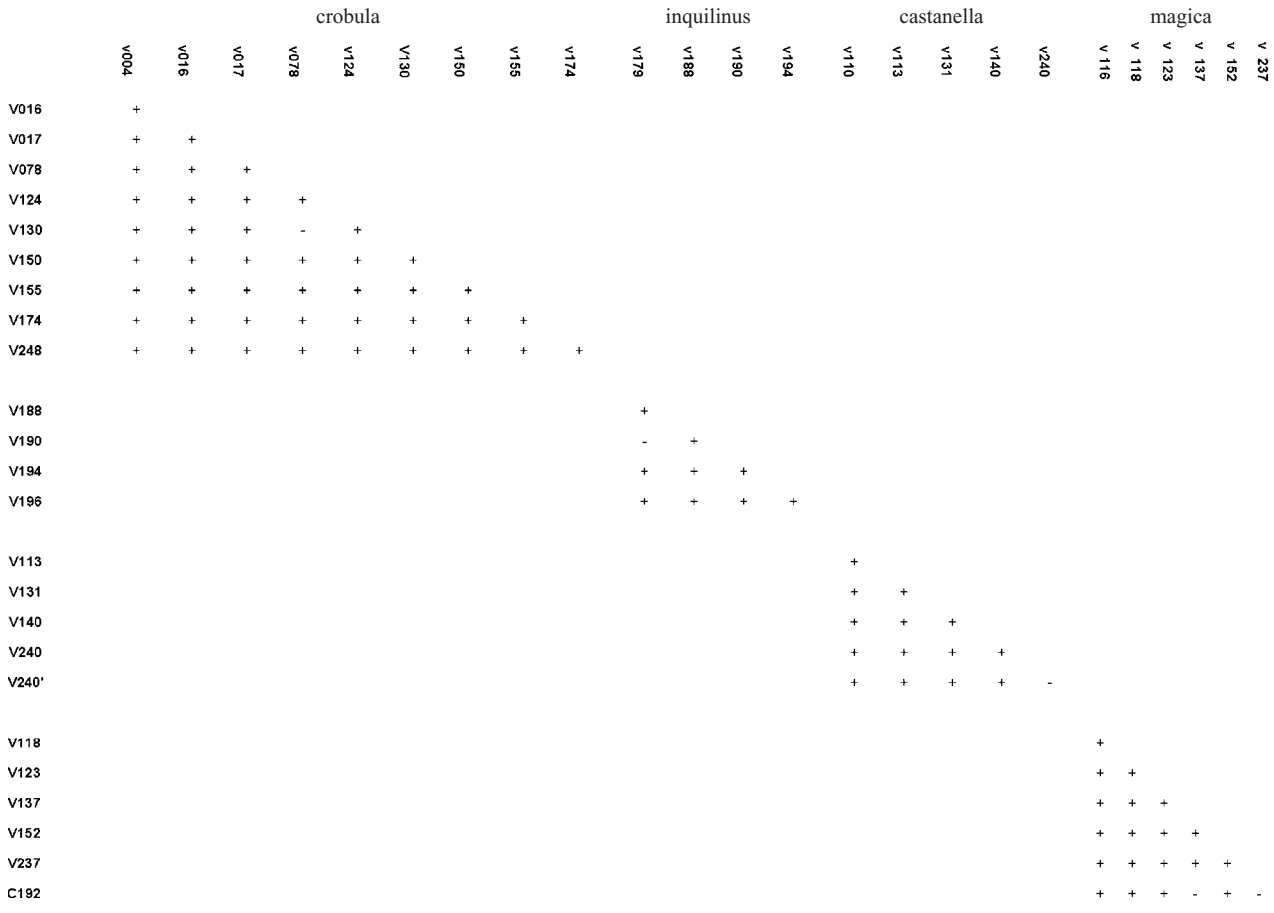


**Fig. 7.** Mating scheme of isolates of *Psilocybe crobula*, *P. inquilinus*, *P. castanella*, and *P. magica* (+, positive mating; −, negative mating).

such as transparency of the pileus, a dry *vs* viscid surface of the pileus, and small differences in spore size. Our studies show that collections with these characters freely interbreed, and therefore these taxa have been merged into one variable species. One collection with extremely large spores, far exceeding the range of all other collections, has been described as var. *macrospora* (Noordeloos 1999b).

### Psilocybe chionophila *vs* P. montana

Lamoure (1977) described *Psilocybe chionophila* as a species close to *P. montana*, from which it differed mainly by the habitat, snow-beds in the Alps and parasitic on the moss *Polytrichum norvegicum* (syn. *P. sexangulare*). Lamoure showed that isolates of *P. chionophila* did not mate with those of *P. montana*. In addition, small differences were noted in the size and shape of the spores. We were able to use an ex-type strain of *P. chionophila* in our studies, and this did not mate with isolates of *P. montana*. However, one collection from The Netherlands (V105) which resembled *P. montana* was interfertile with the ex-type strain of *P. chionophila*, and not with any isolates of *P. montana*. Morphologically *P. chionophila* could not be distinguished from *P. montana*, except for a slight difference in spore shape. The RAPD pattern shows both taxa are very close, and this is supported by sequence data (S.V. & T.B., unpubl.). It seems likely that the intersterility of both taxa is of recent origin. The observation of an interfertile strain from The Netherlands occurring on bare soil in a *Fagus* forest without any mosses growing nearby (v 105) suggests that the parasitic behaviour of *P. chionophila* on mosses is not obligatory.

### Psilocybe magica *vs* P. schoeneti

Bresinky (1976) described *Psilocybe schoeneti* as a new species from southern Germany growing in association with the moss *Campylium stellatum* in peat bogs. This species is characterized by a veil along the margin of the pileus, flattened spores, and cheilocystidia with a relatively broad neck, which is often more or less capitate. Svrček (1989) described a very similar species from the Czech Republic under the name *P. magica*. During our fieldwork several collections of a species fitting the concept of this latter species were made in Scotland and The Netherlands. Mating studies showed they all belong to the same biological species. However, they failed to mate with the ex-type strain of *P. schoeneti*. Despite the morphological resemblance of our collections with both the type of *P. schoeneti* and *P. magica*, on account of the intersterility with the ex-type strain, that are not able to synonymise both names, and so use the name *P. magica* for our collections.

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