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Re-examination of *Psilocybe subaeruginosa* and related species with comparative morphology, isozymes and mating compatibility studies

YU SHYUN CHANG AND ALAN K. MILLS

Department of Plant Science, University of Tasmania, GPO Box 252C, Hobart, Tasmania 7001, Australia

Comparative morphology, isozyme analysis and mating compatibility approaches were used to investigate the relationships between *Psilocybe subaeruginosa* and three closely related taxa, *P. australiana*, *P. eucalypta* and *P. tasmaniana*. The four names were found to represent one species, and the use of microscopic features such as coloured pleurocystidia or neck length of cheilocystidia were shown to be inappropriate taxonomic discriminators in the separation of the four taxa. Zymograms of extracellular enzymes of laccase, peroxidase, acid phosphatase, pectinesterase and polygalacturonase were useful tools for delineation of taxa within the genus *Psilocybe*. The name *P. subaeruginosa* is retained and *P. australiana*, *P. eucalypta* and *P. tasmaniana* are reduced to synonyms. A lectotype is designated.

Investigation of isozyme bands previously reported to be pectinlyase activities showed that they were non-enzymic in nature, and interpretation of these pectinlyase activities should be approached with caution.

Psilocybe subaeruginosa Clel. is a widespread fungus in south-eastern Australia (Cleland, 1927, 1934). *Psilocybe australiana* Guzman & Watling, *P. eucalypta* Guzman & Watling and *P. tasmaniana* Guzman & Watling are closely related to *P. subaeruginosa* (Guzman & Watling, 1978; Guzman, 1983), with the former two species occupying similar niches to *P. subaeruginosa*.

The four species exhibit a considerable overlap in both macroscopic and microscopic features. Guzman & Watling (1978) separated the species using single discriminating characters. The feature which distinguished *P. subaeruginosa* (and also Section *Subaeruginosae* Guzman) was the presence of coloured pleurocystidia, while the remaining three species have hyaline pleurocystidia (Guzman & Watling, 1978; Guzman, 1983). Similarly, *P. tasmaniana* was separated from the rest by its relatively long-necked cheilocystidia (> 5 μm) and coprophilous habitat, and *P. australiana* from *P. eucalypta* on the basis of spore size (Guzman & Watling, 1978; Guzman, 1983). In our experience, identification in the field is quite impossible.

Mating compatibility studies (Farr, Miller & Farr, 1977; Anderson, Korhonen & Ullrich, 1980; Fries, 1985; Boidin, 1986; Kile & Watling, 1988; Flynn & Miller, 1990) and isozyme analyses (Clare, Flentje & Atkinson, 1968; Franke, 1973; Garber, 1973; Blaich, 1977; Kerrigan & Ross, 1988) are techniques which have been used to clarify taxonomic problems in studies based on morphological and ecological data. Extracellular enzymes such as laccase have been shown to be useful biochemical markers in the systematics of *Agaricus* (Kerrigan & Ross, 1988); pectinesterase and polygalacturonase have proved to be effective in species delineation in *Sclerotinia*, *Penicillium* and *Rhizoctonia* (Cruickshank, 1983; Cruickshank & Pitt, 1987; Cruickshank, 1990).

Examination of recently collected and syntype specimens has indicated that the discrete separations proposed may not be valid. Hence, a re-examination of these four taxa seemed necessary, supplementing morphological examination of fresh and dried collections with mating compatibility studies and electrophoresis of extracellular enzymes. This study, part of a study of the Strophariaceae, focuses on three categories of investigation: (1) morphological examination of both fresh and dried collections; (2) electrophoresis of extracellular enzymes; and (3) mating compatibility studies.

MATERIALS AND METHODS

Specimens were collected mainly from southeast Tasmania, with a few from northwest Tasmania near Smithton and from type localities whenever possible. Comparisons were made with reliably identified or type material (see under taxonomic conclusion for citation of type specimens). Dried specimens are lodged at the Tasmanian Herbarium (HO), the Royal Botanic Garden, Edinburgh (E) and the Biology Branch Herbarium of New South Wales Department of Agriculture and Fisheries in Rydalmere, NSW (DAR). All cultures utilized in the study are lodged at DAR. Abbreviations of herbaria follow Index Herbariorum (Holmgren, Keuken & Schofield, 1981). Table 1 lists all the collections with information on localities, isolates, habitat and date of collection.

Pure cultures were obtained from fresh spore deposits whenever possible. The non-quantitative dilution method used to obtain monosporous isolates was similar to that used

Table 1. Sources of specimens and isolates used in the morphological, electrophoretic and mating compatibility studies. Numbers in parentheses refer to the number of isolates used

Collection	Locality	Isolate used	Habitat	Date
Psilocybe australiana				
CYS 95*	Garden, Hobart, Tasmania	NS	On woody litter	14 vii 88
CYS 112*	Fern Glades, near Mt Wellington, Tasmania	Monokaryons (4)	On ground litter	18 iv 89
CYS 132*	Myrtle Forest, Collinsvale, Tasmania	Monokaryons (4)	On ground litter	27 iv 89
CYS 135*	Mt Field National Park, Tasmania	Monokaryons (4)	On ground litter (2)	2 v 89
CYS 139*	Mt Field National Park, Tasmania	Monokaryons (4)	On leafy litter (2)	2 v 89
CYS 158*	Lake Dobson Road, Mt Field National Park	Monokaryons (4)	On ground litter	2 v 89
CYS 161*	Lady Barron Falls Track, Mt Field National Park	Monokaryons† (13)	On ground	2 v 89
CYS 170*	Myrtle Forest, Collinsvale, Tasmania	Monokaryons (4)	On rotten log	11 v 89
CYS 217 (= DAR 66084)	Tasman Peninsula, Tasmania	Monokaryons† (4)	On ground litter	25 v 89
CYS 233	Liffey Falls, on NW coast of Tasmania	Monokaryons (4)	On ground litter	14 vi 89
CYS 236 (= DAR 66085)	Liffey Falls, on NW coast of Tasmania	Monokaryons† (4)	On ground	14 vi 89
CYS 279	Univ. of Tasmania campus, Hobart, Tasmania	Monokaryons (2)	On ground	29 vi 89
CYS 280	Garden, Taroona, Tasmania	Monokaryons (4)	On mown lawn	1 vii 89
CYS 290	Lady Barron Falls Track, Mt Field National Park	NS	On leafy litter	3 vii 89
CYS 293	Lady Barron Falls Track, Mt Field National Park	Monokaryons	On ground litter	3 vii 89
CYS 369	Sandspit River Forest Reserve, Tasmania	NS	On mossy ground	15 v 90
Watling 10617 (holo)	Cotter dam, Blue Range, near Canberra, A.C.T.	—	On ground debris	iv 74
P. eucalypta				
CYS 362 (= DAR 63053)	Tidbinbilla Nature Reserve, near Canberra, A.C.T.	Monokaryons† (6)	On leafy litter (in *Eucalyptus* forest)	5 v 90
DAR 60750	Near Robertson, Old Kangaloon Rd, N.S.W.	—	On ground	1988
Watling 10656 (holo)	Tidbinbilla Nature Reserve, near Canberra, A.C.T.	—	On trackside	v 74
P. tasmaniana				
AKM 955	Colebrook, Tasmania	NS	On pasture land	vii 90
PDD 46240	Taranaki, New Zealand	—	On soil	1984
Watling 10393	NE Hobart, Nugent, Buckland, Tasmania	—	On dung	v 74
P. subaeruginosa				
CYS 515 (= DAR 66086)	Jumping Creek Reserve, Warandyte, Victoria	Monokaryons† (4)	On ground litter	vii 90
AD 5597	Mt Wilson, N.S.W.	—	At base of stump	5 vi 15
AD 5599 (syn)	Fitzroy Falls, N.S.W.	—	On ground litter	8 vi 19
AD 5600 (syn)	Craigie, Victoria	—	Along creek near decaying leaves	vi 17
AD 5602 (syn)	National Park, South Australia	—	On ground	6 viii 21
AD 5603 (syn)	National Park, South Australia	—	On woody debris	19 v 25
AD 5604 (syn)	Mt Lofty, South Australia	—	On ground	vi 23
P. semilanceata				
CYS 451 (= DAR 66087)	Neika, Tasmania	Monokaryons† (3)	On rich pasture	vii 90

* Part of collection lodged at Royal Botanic Garden, Edinburgh (E).
† Isolates and part of collection lodged at Biology Branch Herbarium, Rydalmere, New South Wales (DAR).
NS, No isolate, but specimens used in morphological study; holo, holotype; syn, syntype.

by Farr *et al.* (1977). All isolates used in the study were checked microscopically for the absence of clamps. All stock cultures were maintained on 2% malt extract agar (MA) incubated at 20 °C and then stored at 4°.

Morphological studies

Standard procedures (Guzman, 1983) were followed for the examination of macroscopic and microscopic characters of both fresh and dried material. A single basidiome from each collection was used in the measurements of microscopic characters. Mean values were based on measurements of 25 for spores and 10 for pleurocystidia. A diagrammatic representation of the microscopic characters measured is shown in Fig. 1. The colour codes and description were according to Methuen (Kornerup & Wanscher, 1978).

Fresh collections were sorted into four groups A, B, C and D on the basis of macroscopic and microscopic morphology, in particular spore size, colour of pleurocystidia and neck length of cheilocystidia. These four groups corresponded as nearly as possible to the putative species *P. australiana*, *P. eucalypta*, *P. tasmaniana* and *P. subaeruginosa*, respectively,

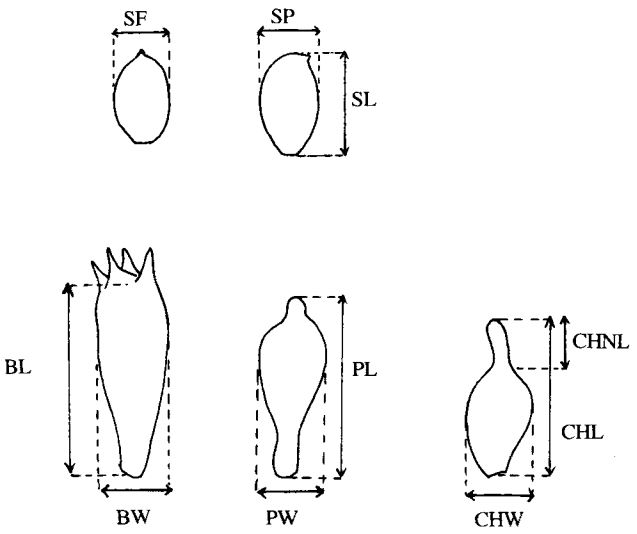


Fig. 1. Diagrammatic representation of microscopic characters measured for the morphological studies. SL, spore length; SF, spore width in face view; SP, spore width in profile; BL, basidia length; BW, basidia width; PL, pleurocystidia length; PW, pleurocystidia width; CHL, cheilocystidia length; CHW, cheilocystidia width; and CHNL, neck length of cheilocystidia.

although considerable difficulty was experienced in assigning some collections to a single group. Hyaline pleurocystidia were noted in most Tasmanian collections of fresh material, consequently they were grouped together as *P. australiana*. CYS 95 was noted to have cheilocystidia of longer neck length than the other Tasmanian collections; however, it was not from a coprophilous habitat, consequently it was grouped tentatively with the rest of the Tasmanian collections. AKM 955 was not collected by us but was known to have been collected from pasture land associated with animal grazing. The neck length of cheilocystidia was noted to be $\geqslant 5$ µm, while the likely association with dung placed this collection closer to *P. tasmaniana* than any other Tasmanian collections. Unfortunately the specimens were not viable when we obtained them. The two collections from mainland Australia were initially identified as *P. eucalypta* (CYS 362) and *P. subaeruginosa* (CYS 515). CYS 362 was collected from the type locality of *P. eucalypta* and the habitat (*Eucalyptus* forest) corresponded to the proposed habitat of *P. eucalypta*. CYS 515 contained some palely coloured pleurocystidia and was therefore tentatively placed in *P. subaeruginosa*.

Canonical discriminant function analysis was performed using the variables spore length, spore width (both face and side view), pleurocystidia length, pleurocystidia width and neck length of cheilocystidia. Normality was tested for each variable and appropriate transformation was applied before the variables were used in the analysis. The mean canonical variates generated were used in the scatter plots. These were also used in an Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) (Sneath & Sokal, 1973) and cluster analysis using the CLUSTER subprogram of SAS (SAS Institute Inc, 1988) to produce a dendrogram.

Electrophoretic studies

Five extracellular enzyme systems were selected based on results from preliminary trials, which also provided basic information on the incubation period for the production of various enzymes, ensuring as far as possible that the cultures were of equivalent physiological state. The enzymes used in electrophoresis were laccase, peroxidase, acid phosphatase, pectinesterase and polygalacturonase.

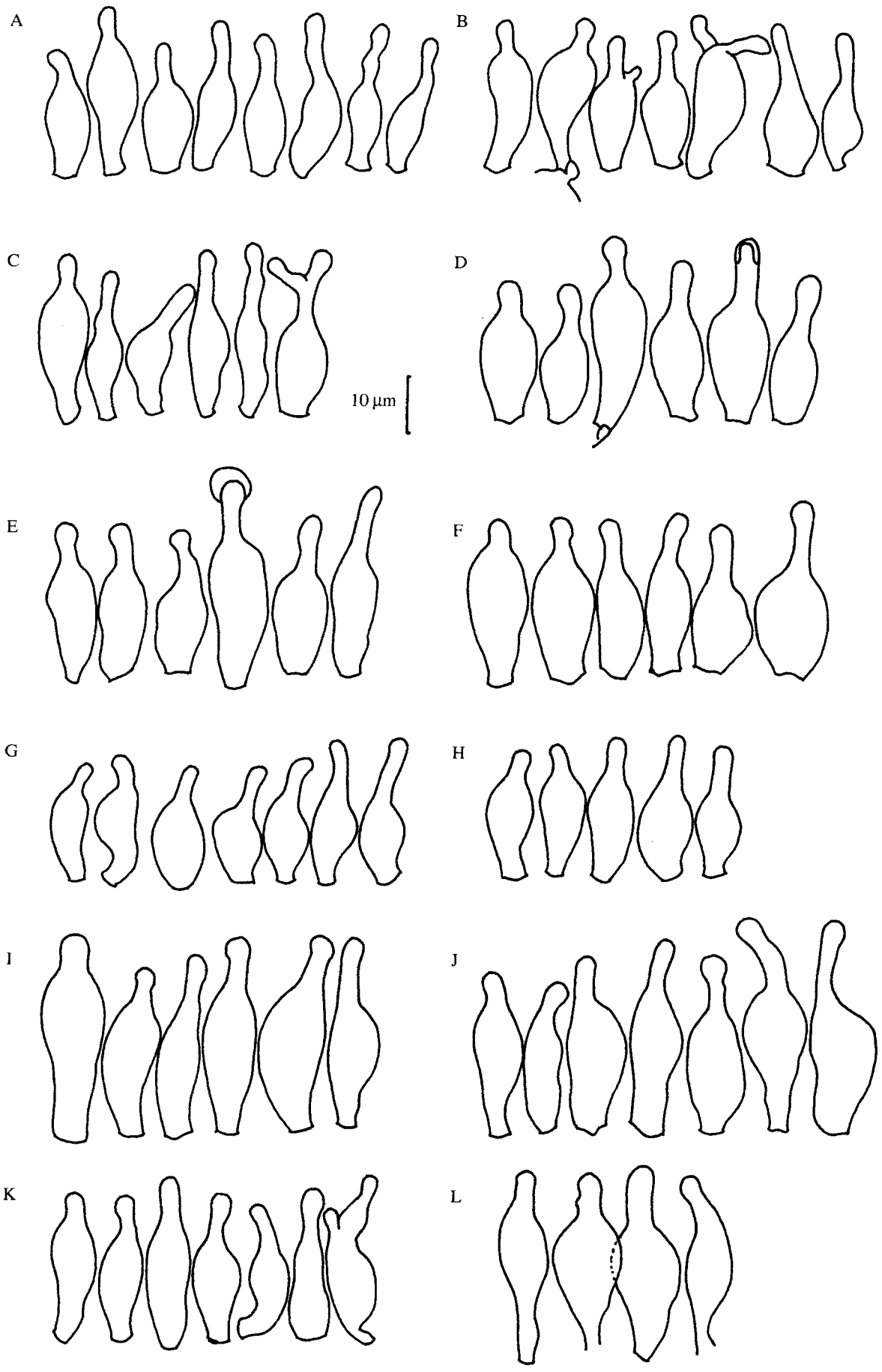
For the production of enzymes, cultures were grown in loosely capped 5 ml Bijou bottles, each containing 2 ml of growth medium autoclaved at 121° for 15 min and incubated at 20° stationary in the dark. To accomplish this, isolates from stock cultures were transferred on to fresh MA plates and incubated at 20° in the dark for 5–7 d, or longer in the case of slow-growing strains. Discs of 8 mm in diameter were cut from the actively growing edge of the colony and transferred to the selected growth medium.

A gel containing 13 monosporous isolates from one collection, CYS 161 (in group A), was run for each enzyme system to establish a preliminary estimate of the range of internal variations. Whenever possible, thereafter, at least two to four isolates from other collections in the same or different group(s) were used when making comparisons, and this

Table 2. The mean measurements and standard deviations (S.D.) of microscopic characters for *Psilocybe subaeruginosa*, *P. australiana*, *P. eucalypta* and *P. tasmaniana*

	P. subaeruginosa ($n = 8$)	*P. australiana* ($n = 17$)	*P. eucalypta* ($n = 3$)	*P. tasmaniana* ($n = 3$)
Spores				
Length (µm)	12·56 ± 0·38	13·05 ± 0·35	12·72 ± 0·62	12·62 ± 0·65
Width (SF, µm)	7·66 ± 0·28	7·87 ± 0·27	7·49 ± 0·16	7·44 ± 0·49
(SP, µm)	7·13 ± 0·43	7·66 ± 0·27	7·47 ± 0·12	7·40 ± 0·39
Pleurocystidia	Hyaline, rarely coloured	Hyaline, rarely coloured	Hyaline	Hyaline
Length (µm)	30·80 ± 1·95	29·99 ± 2·24	27·12 ± 2·23	29·15 ± 1·82
Width (µm)	9·76 ± 0·74	9·47 ± 0·76	8·45 ± 0·15	8·30 ± 1·91
Cheilocystidia	Hyaline, simple or bifurcate	Hyaline, simple or bifurcate	Hyaline, simple	Hyaline, simple or bifurcate
Neck length (µm)	6·39 ± 0·58	7·17 ± 0·89	6·68 ± 0·19	7·69 ± 0·68
Length (µm)	28·34 ± 1·13	27·45 ± 2·21	26·29 ± 6·30	27·83 ± 6·41
Width (µm)	7·89 ± 0·58	7·95 ± 0·78	8·27 ± 1·14	7·58 ± 0·44

SF, width of spore in face view; SP, width of spore in profile.


A
B
C
10 µm
D
E
F
G
H
I
J
K
L

included the isolates of a distinct outgroup species, *P. semilanceata* (Fr. ex Secr.) Kummer.

For laccase production, the growth medium consisted of 0·05% gallic acid in malate buffer (pH 4·0) (Cruickshank, personal communication). Cultures were incubated for 3 d at 20° stationary in the dark. Because of the short time required, laccase zymograms were used for the initial separation of species in addition to morphological characters.

For pectic enzymes, the growth medium was based on that used by Cruickshank & Pitt (1987), but modified by replacing $NH_4H_2PO_4$ with KH_2PO_4 and NH_4NO_3. Cultures were incubated at 20° in the dark for 14 d.

For peroxidase and acid phosphatase production, the medium was a 20% potato decoction (20 g chopped potato 100 ml^{-1} deionized water, simmered for 1·5 h then sieved through two layers of muslin). The cultures were incubated for 10 d at 20° in the dark. Polyacrylamide gel electrophoresis followed the system of Cruickshank & Pitt (1987).

Pectic enzymes were examined by the method of Cruickshank & Wade (1980). Laccase and peroxidase were examined by the method of Mills & Crowden (1968). In these two oxidase enzyme systems, to increase the resolution of the bands, gels were stained for 30 min at room temperature and then overnight at 4° in the staining solution. They were retained in water until photographic records were made. Acid phosphatase was examined by the method of Ho & Trappe (1987).

Photographic records of gels were prepared by contact printing under water on to high-contrast (No. 5) Ilfoprint paper.

All the isozyme data were analysed as phenetic characters. UPGMA (Sneath & Sokal, 1973) cluster analysis (using a SAS CLUSTER subprogram) was performed, based on the band frequency data to obtain a dendrogram. Very faint bands were excluded.

Mating compatibility studies

For Group A, 12 isolates from one collection (CYS 161) were paired in all possible combinations to determine the mating types. At least one isolate from each mating type was then crossed with monokaryons from other collections in the same group. Crosses were set up between the mating types of CYS 161 and the monokaryotic isolates of CYS 362 (Group B) and CYS 515 (Group D). All crosses were mon–mon pairings. Monokaryotic isolates of CYS 451 (*P. semilanceata*), a clearly separate species, were included in the crosses for comparison. The methodology described by Macrae (1967) was followed in the mating compatibility studies.

RESULTS

Morphological studies

The morphological comparisons between the four putative species were based on spores, pleurocystidia and cheilocystidia. Habitats were also included in the comparison. Table 2 shows the mean measurements of the microscopic characters used in the comparisons of the four groups.

On examination of fresh material designated as *P. subaeruginosa* (because of the presence of some coloured pleurocystidia), the majority of the pleurocystidia were found to be hyaline, with a few basidiomes having pallid yellow pleurocystidia. Herbarium material of *P. subaeruginosa* (including all the syntype collections) had hyaline, pallid yellow to occasional pale brown pleurocystidia. In all cases, when yellow coloration of cystidia was observed basidia and hyphae in the subhymenium and trama were similarly coloured. The majority of pleurocystidia in the fresh collections designated as *P. australiana* were hyaline, with some weakly coloured pleurocystidia noted in one collection.

Neck length of cheilocystidia was found to range from 3 to 12 μm in all putative species. The mean neck length of cheilocystidia was measured as > 5 μm in all four putative species (Table 2). Figure 2 illustrates the range of neck length noted in the cheilocystidia of the four groups.

Figure 3 illustrates the scatter plot using the mean canonical variates generated from the spore characters. There were no distinct clusters resolved along either of the axes. Collections with relatively broader spores tended to become the outliers as a result of variation along the second canonical axis resulted from contrast between spore length and spore width (side view).

Figure 4 shows the scatter plot of the mean canonical variates generated from the cystidia variables. Again no distinct clusters were resolved from the scatter plot using the first two mean canonical variates. Neck length of cheilocystidia, which was earlier proposed to be a diagnostic character for *P. tasmaniana* (Guzman & Watling, 1978), contributed only slightly to variation along the first canonical axis.

The dendrogram generated from the cluster analysis is shown in Fig. 5. All four putative groups, A–D, were intermixed in their apparent relationships, i.e. none of the putative groups was separable on the selected parameters.

The range of habitat appeared quite varied for all the four putative species. For the group referred to as *P. australiana* alone, the habitat ranged from on ground (soil or mossy ground), ground litter (leafy litter of mixed foliage of *Eucalyptus, Nothofagus* and manfern [*Dicksonia antarctica* Labill.] fronds or woody litter such as fallen twigs or branches), on rotten logs, dead stumps and manfern trunks to mown lawn. Both *P. eucalypta* and *P. subaeruginosa* shared similar habitats (see Table 1).

Electrophoretic studies

Cruickshank & Wade (1980) have associated yellow-stained bands with pectinlyase activities. Karlsson & Stenlid (1991) also found these yellow bands of 'pectinlyase', but for reasons

Fig. 2. The range of neck length of cheilocystidia noted in both fresh and herbarium material of *Psilocybe australiana*: A CYS 161, B CYS 217, C CYS 95, D CYS 280, E CYS 290 and F CYS 369; *P. tasmaniana*: G Watling 10393 (holotype) and H AKM 955; *P. eucalypta*: I CYS 362; and *P. subaeruginosa*: J AD 5603 (lectotype), K CYS 515 and L AD 5600 (syntype).

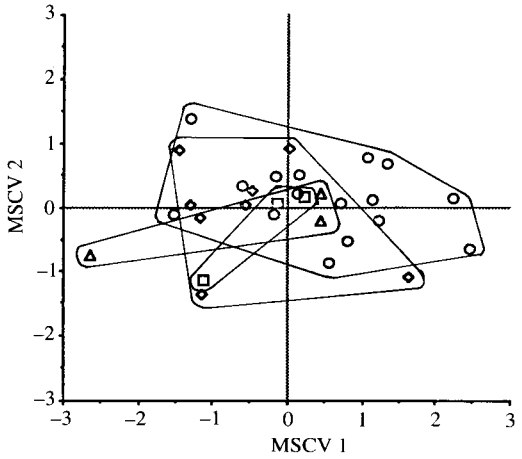


Fig. 3. Plot of the mean canonical variates from canonical discriminant function analysis of spore variables (SL, SF and SP) of the four putative groups A (○), B (□), C (△) and D (◇).

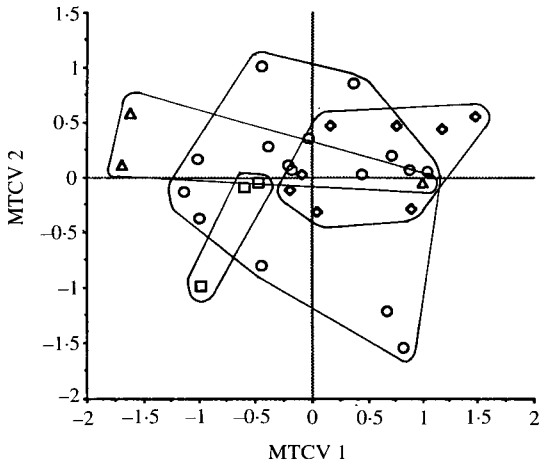


Fig. 4. Plot of the first two canonical variates from the canonical discriminant function analysis of the cystidia variables (PL, PW and CHNL) of the four putative groups A–D (for notation see Fig. 3).

of variable behaviour excluded them from their final analysis. These yellow bands were noted in the course of this electrophoretic study. Further investigations into the enzymic nature of these bands revealed that they were non-enzymic and could be mimicked to a large extent by organic acids. Thus, extreme care should be exercised when interpreting these 'pectinlyase' activities. Cruickshank (personal communication) has been consulted during the course of the investigation and agrees with this caution.

In each enzyme system, allelic designations were not assigned but observations regarding recognizable loci were noted. Each band was scored as an independent phenetic character and numbered from the cathodic end.

Laccase (Lac). Seven bands were scored consistently (Fig. 6i). Of these, Bands 1 and 4 were present exclusively in the isolates of CYS 451 (*P. semilanceata*). Allelic designations were not assigned, though five loci were recognizable in the laccase activities. The first four bands corresponded to four monomorphic loci and Bands 5–7 appeared to be alleles of a polymorphic locus. Of the isolates in the three putative groups, Bands 2 and 3 were dominant (shared by 84·44% and 71·11% of isolates respectively).

Peroxidase (Per). As a result of the influence of peroxide on some laccases (Blaich & Esser, 1975), only bands that appeared after the addition of peroxide were included for comparison. Consequently two bands (R_F = 0·35 and 0·38) were excluded (Fig. 6ii). The remaining seven bands were scored. These bands appeared to correspond to six loci. Bands 1 and 2 were alleles of a polymorphic locus and the remaining bands corresponded to five monomorphic loci. Band 4 was dominant (80%) in the isolates of the three putative groups. Band 7 was detected in only two isolates; as a result of its infrequent occurrence it was excluded from the cluster analysis. Bands 3, 5 and 6 occurred exclusively in the isolates of CYS 451.

Acid phosphatase (AcP). No extracellular AcP activity was detected in the isolates of CYS 451. Of the remaining isolates, ten bands (Fig. 6iii) were consistently scored; they appeared to correspond to seven loci. Three polymorphic loci (R_F = 0·11, 0·13; R_F = 0·24, 0·26; R_F = 0·30, 0·33) were noted while the rest were monomorphic. Band 5 was dominant throughout the isolates of the three putative groups (77·8%).

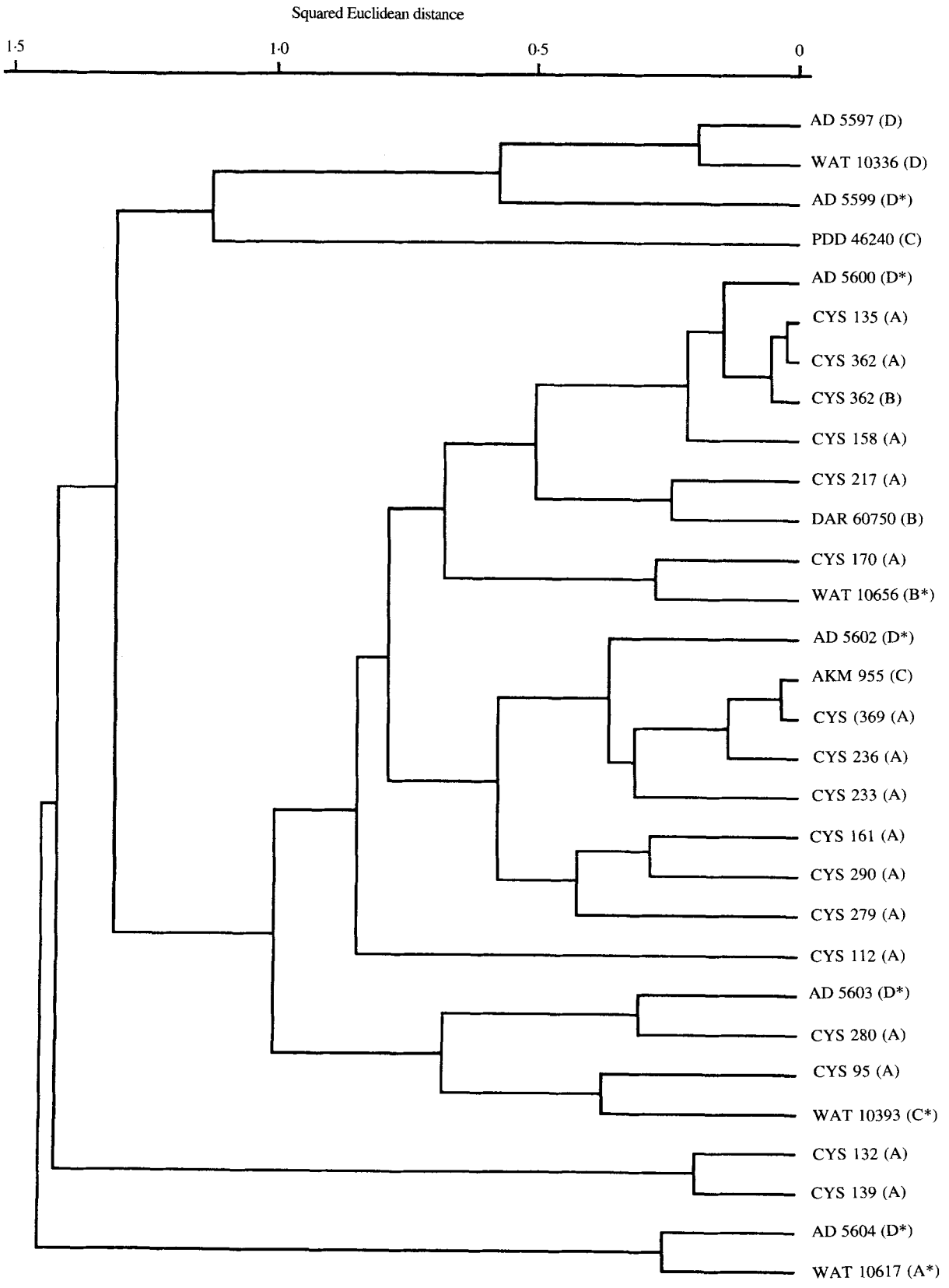
Pectinesterase (PE) and polygalacturonase (PG). Variations were noted in the PE activities across the isolates of the three putative groups. A total of 16 bands was consistently scored and numbered from 1 to 14 from the cathodic end (Fig. 6iv) and 1′ and 2′ for the two backrunners (of negative R_F values). Bands 1′ and 2′ were present exclusively in the isolates of CYS 451; again, all the bands detected in the isolates of CYS 451 were not present in the remaining isolates. Of the isolates of the three putative groups, Band 3 was shared by 95% of the isolates, followed by Bands 8 and 9 of 64·4% and 84·4% respectively.

No PG activity was detected in the isolates of CYS 451. Five prominent bands were consistently noted in the remaining isolates (Fig. 6v). Two bands of R_F 0·14 and 0·18 were noted in a single isolate, and one of them may be alternating with the band of R_F 0·24. However, the present results could not confirm this. Seven bands were scored consistently. Band 3 was shared by 91·11% of the isolates in the three putative groups.

Figures 7–10 show representation of the zymograms of isolates of CYS 161, 158 and 279 (group A, *P. australiana*), CYS 362 (group B, *P. eucalypta*), CYS 515 (group D, *P. subaeruginosa*) and CYS 451 (*P. semilanceata*, an outgroup species) of the five enzyme systems. All the zymograms of isolates of *P. semilanceata* were very different.

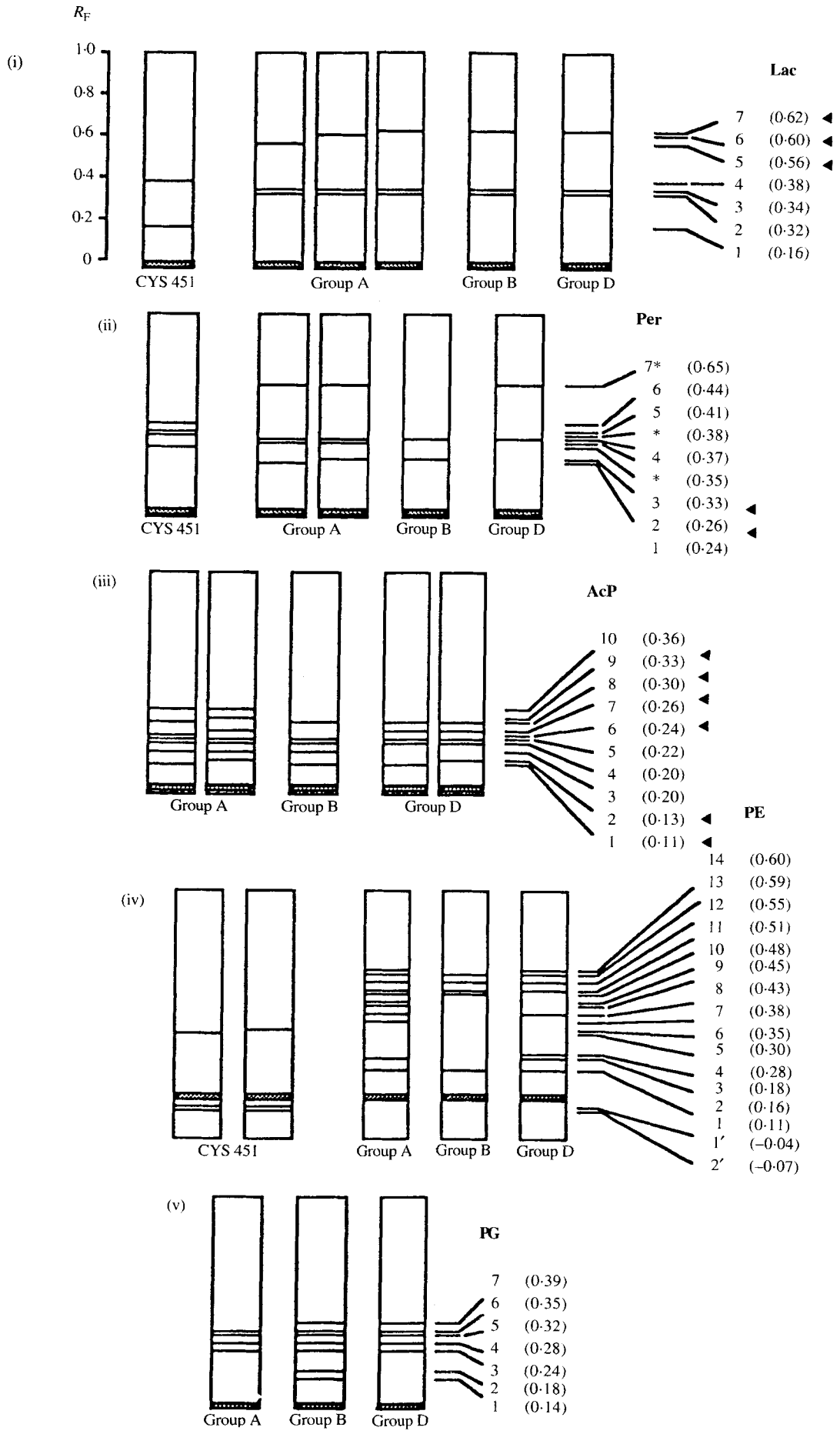
Unfortunately, no isolate of *P. tasmaniana* was available for electrophoretic study.

The dendrogram produced from the cluster analysis is shown in Fig. 11. CYS 451, being a distinct outgroup species, was clearly separated from all the collections of the three



* Type material.

Fig. 5. Dendrogram from a cluster analysis based on all the mean canonical variates generated from the canonical discriminant function analysis.


R_F
(i)
1·0
0·8
0·6
0·4
0·2
0
CYS 451
Group A
Group B
Group D
Lac
7 (0·62)
6 (0·60)
5 (0·56)
4 (0·38)
3 (0·34)
2 (0·32)
1 (0·16)
(ii)
Per
7* (0·65)
6 (0·44)
5 (0·41)
* (0·38)
4 (0·37)
* (0·35)
3 (0·33)
2 (0·26)
1 (0·24)
(iii)
AcP
10 (0·36)
9 (0·33)
8 (0·30)
7 (0·26)
6 (0·24)
5 (0·22)
4 (0·20)
3 (0·20)
2 (0·13)
1 (0·11)
PE
14 (0·60)
13 (0·59)
12 (0·55)
11 (0·51)
10 (0·48)
9 (0·45)
8 (0·43)
7 (0·38)
6 (0·35)
5 (0·30)
4 (0·28)
3 (0·18)
2 (0·16)
1 (0·11)
1′ (–0·04)
2′ (–0·07)
(iv)
(v)
PG
7 (0·39)
6 (0·35)
5 (0·32)
4 (0·28)
3 (0·24)
2 (0·18)
1 (0·14)

putative groups. A higher degree of affinity was expected between the Tasmanian collections, and this was evident in the dendrogram. The close link of the two mainland collections (CYS 362 and 515) may indicate the effect of geographical distance on gene flow; however, they were not distinctly different from the Tasmanian collections.

Mating compatibility studies

The results indicated a tetrapolar incompatibility system for CYS 161 (group A, putative *P. australiana*), and four mating types (A_1B_1: 1, 3, 4, 10, 12 and 16; A_1B_2: 8 and 17; A_2B_1: 5 and A_2B_2: 2 and 11) were recovered from the polarity matrix. The four mating types were intercompatible with the monokaryotic isolates of all other collections in group A (Table 3) and indicated the involvement of a multiple allelic system. The results showed that isolates of both CYS 362 (group B) and CYS 515 (group D) were intercompatible with CYS 161, 236 and 217 (all group A) as well as between themselves (Table 3). The isolates of collections from groups A, B and D were all interincompatible with *P. semilanceata* (Table 3).

DISCUSSION

Only one morphological species was identified, and this corresponded to a single biological species from the results of morphological, electrophoretic and mating compatibility studies.

This re-examination has shown that the proposed use of coloured pleurocystidia as a taxonomic criterion is not valid when considering *P. subaeruginosa*. Results from this study show that pleurocystidia are hyaline in almost all the material examined, and only occasionally is pale yellow coloration noted in pleurocystidia of some material, but then the rest of the gill tissue is also similarly coloured. None of the material examined has chocolate brown pleurocystidia as suggested by Guzman (1983). Since this criterion, i.e. coloured (brown) pleurocystidia, also characterizes Section *Subaeruginosae* Guzman, the position of *P. subaeruginosa* in Section *Subaeruginosae* is not tenable, and the name should instead be transferred to Section *Cynaescens* Guzman, which is characterized by the presence of hyaline pleurocystidia.

Neck length of cheilocystidia of 5 μm or more has been used by Guzman & Watling (1978) to separate *P. tasmaniana* from *P. australiana* and *P. eucalypta*. In the same paper it is noted that there was inconsistency between Table 1 (p. 208) and drawings (Fig. 1D and G, p. 205) of the cheilocystidia of *P. australiana* and *P. eucalypta*. The drawings show the neck length of cheilocystidia of both these species to be greater than 5 μm, and this contradicts the measurements given in their table 1. Our study of the holotype and syntype specimens of these taxa supports the information contained in the drawings, as neck length of cheilocystidia was found to be generally 5 μm or more. Since all the four proposed species share similar neck length, the distinction based on neck length of cheilocystidia between *P. tasmaniana* and the remaining three species becomes untenable. Thus, neck length of cheilocystidia has little taxonomic value here. Results from the canonical discriminant function analysis enhanced the finding that these four putative groups were not separable on the proposed morphological criteria.

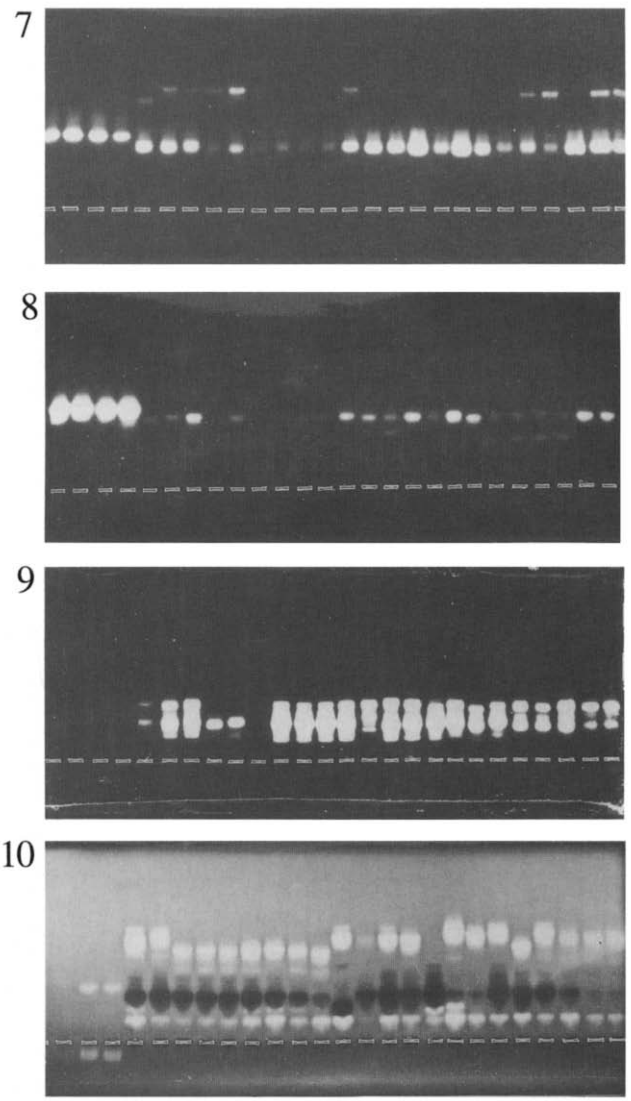
The coprophilous habitat has been used by Guzman & Watling (1978) in addition to neck length of cheilocystidia to separate *P. tasmaniana* from *P. australiana* and *P. eucalypta*. Guzman & Watling (1978) allude to a wider habitat preference, but list 'dung' as the habitat for *P. tasmaniana* in their table 1 (p. 207). However, *P. subaeruginosa* has also been reported on dung (Cleland, 1927, 1934) and the New Zealand material of *P. tasmaniana* was not collected from dung (see Table 1). It appears that the habitat of *P. tasmaniana* is more varied than initially envisaged, so habitat is also not a valid criterion for the separation of species.

Morphological examination of material from New Zealand indicated from this study that *P. subaeruginosa* is not limited to Australia.

The electrophoretic data are particularly encouraging when considering wider studies in the Strophariaceae. Isolates obtained from fresh collections and identified as *P. australiana*, *P. eucalypta* and *P. subaeruginosa* on the basis of morphological criteria all produced zymograms with a high degree of uniformity in all the selected enzymes, and this was supported by the results of the UPGMA cluster analysis. This illustrates the potential of extracellular enzymes as biochemical markers in the genus *Psilocybe*. *P. semilanceata*, which is morphologically distinct from *P. subaeruginosa*, produced zymograms which were distinctively different from those of *P. subaeruginosa*. Thus, species delineation for *Psilocybe* in Tasmania could be achieved through direct comparison of the zymograms. The results are reproducible, and in agreement with Cruickshank's (1990) finding that 'intergel comparisons were meaningful and results could be presented as composites from several gels'.

The results of these isozyme analyses correlated closely with the results of morphological and mating studies. This electrophoretic technique may prove to be a very useful adjunct in taxonomic studies, especially where spore germination is an intractable problem but tissues from wild isolates are more easily available.

Fig. 6. Schematic representations of isozyme patterns. R_F scale the same from (i) to (v). Bands marked with arrows were alleles of polymorphic loci. (i) Laccase (Lac) isozyme patterns of CYS 451 (*P. semilanceata*), Group A (putative *P. australiana*), Group B (putative *P. eucalypta*) and Group D (*P. subaeruginosa*). Bands 1–7 numbered from the cathodic end. (ii) Peroxidase (Per) isozyme patterns of the same four groups as (i). Bands marked with an asterisk were excluded from cluster analysis. (iii) Acid phosphatase (AcP) isozyme patterns in the three putative groups A, B and D. (iv) Pectinesterase (PE) isozyme patterns of the same four groups as in (i). (v) Polygalacturonase (PG) isozyme patterns.



Figs 7–10. Representations of the zymograms of the five enzyme systems of the monokaryotic isolates of three of the four putative groups (A, B and D) and the outgroup species, *P. semilanceata*. In all figures from left: CYS 451 (01, 03 and 04) (*P. semilanceata*), CYS 158 (04), CYS 279 (03), CYS 217 (02, 03, 05 and 08), CYS 236 (01–03) (all group A), CYS 362 (01, 02, 04 and 05) (group B), CYS 515 (01, 02, 03 and 05) (group D) and CYS 161 (01, 02, 10 and 11) (group A). **Fig. 7.** Lac. **Fig. 8.** Per. **Fig. 9.** AcP. **Fig. 10.** PE and PG (× 0·8).

TAXONOMIC CONCLUSION

Morphological examination, selected enzyme analyses (including cluster analysis) and mating compatibility experiments all indicate that a single species has been represented by four names. For reasons of nomenclatural priority, the name *Psilocybe subaeruginosa* Cleland must be retained, with *P. australiana* Guzman & Watling, *P. eucalypta* Guzman & Watling and *P. tasmaniana* Guzman & Watling reduced to synonymy. An emended description modified from Guzman's is presented.

Psilocybe subaeruginosa Clel. *Trans. & Proc. Roy. Soc. South Australia* **51**: 305 (1927). Type citation: Cleland did not specify a type in his cited collections. Guzman (1983) assumed Cleland 13251 (AD) to be the type, and since this collection cannot be located, clearly lectotypification is required.

Lectotype (here chosen): South Australia, National Park, AD 5603! Isolectotype: South Australia, National Park, AD 5602! Allotypes: South Australia, Mount Lofty, AD 5604! New South Wales, Fitzroy Falls, AD 5599!; Victoria, Craigie, AD 5600!

Psilocybe australiana Guzman & Watling, *Notes from the Roy. Bot. Garden Edinburgh* **36**: 206 (1978). Holotype: New South Wales, near Canberra, Cotter Dam, Blue Range, Watling 10617 (E!).

Psilocybe eucalypta Guzman & Watling, *Notes from the Roy. Bot. Garden Edinburgh* **36**: 204 (1978). Holotype: A.C.T., near Canberra, Tidbinbilla Nature Reserve, Watling 10656 (E!).

Psilocybe tasmaniana Guzman & Watling, *Notes from the Roy. Bot. Garden Edinburgh* **36**: 207 (1978). Holotype: Tasmania, NE of Hobart, Nugent, Buckland, Watling 10393 (E!).

Selected illustrations: Cleland (1934, fig. 25, p. 141); Cole, Fuhrer & Holland (1978, pl. 5); Fuhrer (1985, p. 75 as *Psilocybe* sp.); Shepherd & Totterdell (1988, p. 93 as *Psilocybe* sp.).

Pileus 11–60 mm in diam., when young conic to convex, campanulate, then convex to plano-convex or expanded umbonate; greasy to tacky or more rarely subviscid when moist, surface glabrous, even, remnants of veil as white fibrils attached along the margin, margin slightly striate, hygrophanous, greyish yellow (4A5–C5), then pale brownish (5C5, 5D7) to dull brown (6E5) when older, often with bluish green tints, drying pallid brownish or straw colour. *Lamellae* adnate to adnexed. Pallid yellow (3A2) when veil breaks, becoming brownish-fuscous (5E5–6F7) with spores, edges pallid. *Stipe* 35–140 × 2–7·5 (10) mm, sometimes flexuous, equal, finely striate, mealy above, fine fibrils sometimes adherent below, base slightly swollen and passing sometimes into a broad mass of white mycelium, stuffed but sometimes hollow, cartilaginous. Surface whitish, streaked with dark greyish-brown, often blotched greenish-blue. *Veil* a whitish cobweb in young stages, occasionally leaving indefinite traces as somewhat superior annulus. *Context* white to pale yellow (3A2–3) in the pileus and stipe, but becoming brownish in the stipe. Turning blue when bruised or on drying.

Spore print violaceous black. *Spores* (9·6–) 10·8–15 (–15·8) × (6·4–) 6·6–8·8 × (5·6–) 6–7·5 (–8·7) µm, subellipsoid in face view, slightly inequilateral in profile, thick-walled, pale to dark yellowish-brown, with broad germ pore. *Basidia* (20·6–) 24–38·3 (–42·5) × 6·6–11·7 µm, 4-spored or rarely 2-spored, hyaline, or yellowish-brown, ventricose or subcylindric to subpyriform. *Cheilocystidia* (17–) 20–40·8 (–48) × (4–) 5·5–16·7 µm, similar to the pleurocystidia in form and colour, long-necked, 5 µm or more, abundant, usually forming a sterile band, frequently with a hyaline drop at the apex. *Pleurocystidia* (18·3–) 20–47·3 (–50) × (4·8–) 6–16·7 µm, fusoid-ventricose, subpyriform, mucronate or with a more or less elongated neck 2–4·5 µm broad, hyaline, some very pallid yellow, very rarely with brown contents or deeply coloured.

Subhymenium subcellular, hyaline to pale yellowish or brownish with diffused to irregularly incrusted pigment on the thick walls; sometimes diffused blue pigment is observed in KOH slides. *Trama* parallel, hyaline or brownish, with thick-

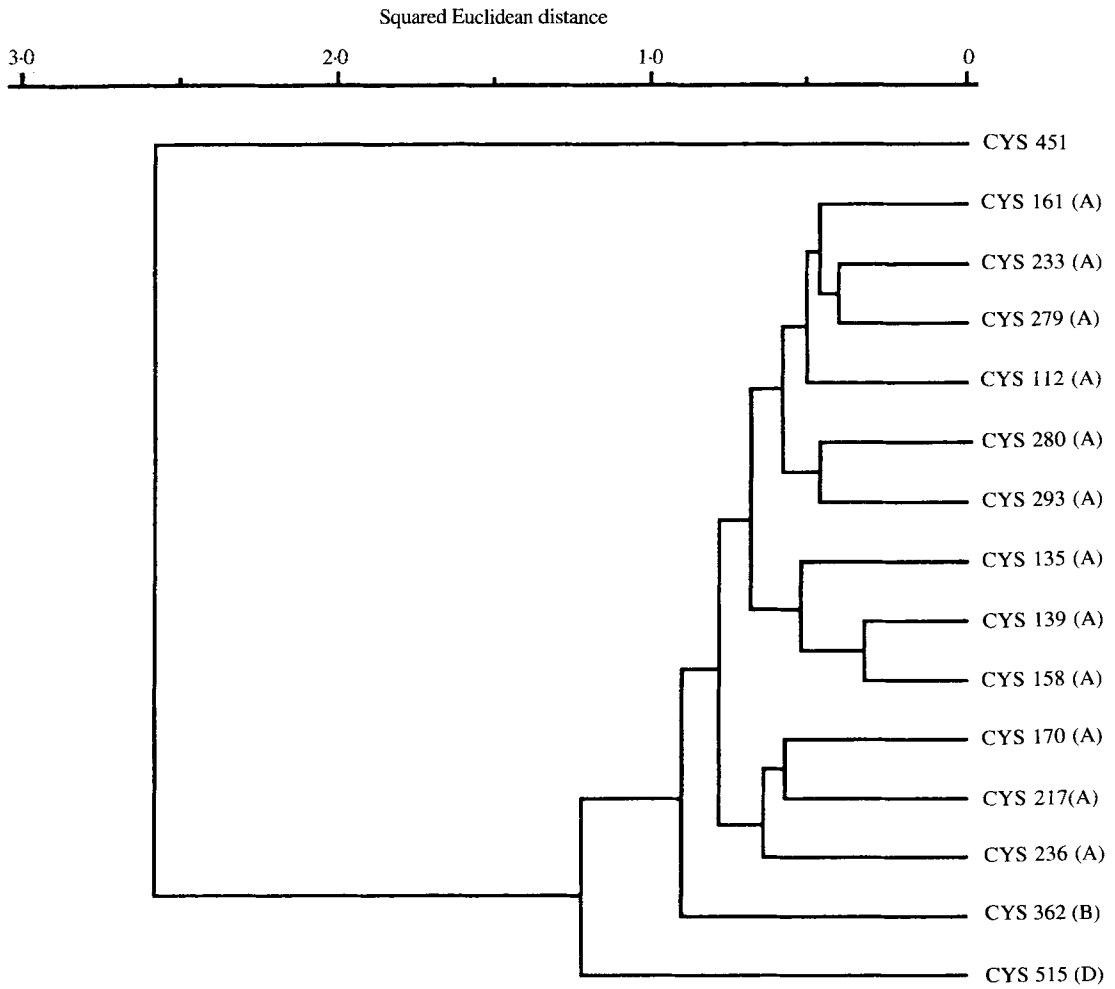


Fig. 11. Dendrogram from the UPGMA cluster analysis based on band frequencies. The number refers to collection (Table 1) and the putative group in parentheses.

walled hyphae (walls 1–1·5 μm thick). *Epicutis* formed by a thin layer of gelatinized, repent hyphae, more or less 5 μm diam., hyaline to brownish. *Hypodermium* hyaline to brownish, formed by subglobose to broad, elongate hyphae. Clamp connections very common and conspicuous.

Habitat and distribution. Solitary to gregarious, on rich soil among grass or mosses, or on dung, on leafy litter of mixed forest foliage of *Eucalyptus*, *Nothofagus* and manfern (*Dicksonia antarctica* Labill.) fronds, or woody litter such as fallen twigs, rotten logs, dead stumps and manfern trunks, mainly in deeply shaded places, occasionally in more exposed areas. Fruiting in April–August. Known only from Australasia.

AD 5603 was chosen as the lectotype, as it agrees most closely with Cleland's original description and has a range of microscopic features which are well within the range noted in the syntype and fresh collections.

Specimens examined: P. subaeruginosa Clel.

AUSTRALIA: South Australia, Morialta, AD 5606; New South Wales, National Park, AD 5598; Mt Wilson, AD 5597; near Robertson, Old Kangaloon Road, DAR 60750 (as *P. eucalypta*); Victoria, Dandenong Ranges, AD 5601; Jumping Creek, Warandyte, CYS 515 (HO, DAR); Tasmania, Mount Field National Park, pathside to Russell Falls, Watling 10336, 10387 (E); Colebrook, AKM 955 (as *P. tasmaniana*); see Table 1 for other Tasmanian collections (as *P. australiana*).

NEW ZEALAND: Waikato, PDD 45554, 54315; Bay of Plenty, PDD 54517; Auckland, PDD 55207 (all as *P. australiana*); New Plymouth, PDD 48120, PDD 48122 (all as *P. eucalypta*); Taranaki, PDD 46240 (as *P. tasmaniana*) and PDD 45296 and 45331 (as *Psilocybe* aff. *tasmaniana*).

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Table 3. Mating tests between the tester strains of CYS 161 and monokaryons of collections in putative groups A, B and D and CYS 451 (*P. semilanceata*), as well as between putative groups B and D

Species and isolate no.	No. of monokaryons	Species and isolate no.	No. of monokaryons	No. of pairings	Total no. positive pairings	Total no. negative pairings
P. australiana		*P. australiana*				
CYS 161	4	× CYS 112	4	16	16	0
(1, 2, 5, 8)		(1, 2, 3, 4)				
		× CYS 132	4	16	16	0
		(1, 2, 3, 4)				
		× CYS 158	4	16	16	0
		(1, 2, 3, 4)				
		× CYS 170	4	16	16	0
		(1, 2, 3, 4)				
		× CYS 217	4	16	12	4
		(1, 3, 5, 8)				
		× CYS 236	4	16	16	0
		(1, 2, 3, 4)				
		× CYS 279	4	16	16	0
		(1, 2, 3, 4)				
		× CYS 280	4	16	16	0
		(1, 2, 3, 4)				
P. eucalypta		*P. australiana*				
CYS 362	5	× CYS 161	4	20	14	6
(1, 2, 3, 4, 5)		(2, 5, 10, 17)				
		× CYS 217	2	10	5	5
		(3, 8)				
		× CYS 236	2	10	10	0
		(1, 2)				
P. subaeruginosa		*P. australiana*				
CYS 515	4	× CYS 161	4	16	16	0
(1, 2, 3, 5)		(2, 5, 10, 17)				
		× CYS 217	2	8	8	0
		(3, 8)				
		× CYS 236	2	8	8	0
		(1, 2)				
		P. eucalypta				
		× CYS 362	4	16	11	5
		(1, 2, 4, 5)				

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