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### Chlorophyllum and Macrolepiota (Agaricaceae) in Australia

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Abstract. A checklist of and a key to seven species of Chlorophyllum Massee and Macrolepiota Singer in Australia are given. Two species are described as new: C. nothorachodes Vellinga & Lepp from Australian Capital Territory and M. eucharis Vellinga & Halling from Queensland. Chlorophyllum hortense (Murrill) Vellinga is adopted as name for Leucoagaricus fimetarius (Cooke & Massee  $\rightarrow$  Sacc.) Aberdeen. Chlorophyllum brunneum (Farl. & Burt) Vellinga is the correct name for the species often referred to as M. rachodes in Australia. Macrolepiota clelandii Grgur. is variable in colour and especially in the number of spores per basidium and the shape of the cheilocystidia and encompasses all Australian collections under the names M. konradii, M. gracilenta, M. mastoidea and M. procera.

### Introduction

Representatives of the genera *Chlorophyllum* Massee and *Macrolepiota* Singer are easily recognised, by their big fleshy, often squamose basidiocarps with a prominent annulus, big spores (lengths in general >10  $\mu$ m), which usually are provided with a germ pore and presence of conspicuous cheilocystidia. Clamp connections are present in both genera, but in a very few species they are rare and hard to find or completely absent.

Vellinga et al. (2003) showed that Macrolepiota is polyphyletic, based on analyses of nrITS and nrLSU data from a wide range of species in the Agaricaceae. One clade, closely related to Agaricus L : Fr., is made up of members of Macrolepiota sect. Laevistipedes (Pázmány) Bon, C. molybdites (G.Mey. : Fr.) Massee, Endoptychum agaricoides Czern. and Leucoagaricus hortensis (Murrill) Pegler. The second clade, closely related to Leucoagaricus Singer and Leucocoprinus Pat., consists of the two other Macrolepiota sections, viz. section Macrolepiota and Macrosporae (Singer) Bon and several taxa that had not been formally assigned to any of these. Morphological characters support the recognition of the two clades as two genera.

For the first clade, the genus name *Chlorophyllum* has been proposed (Vellinga and De Kok 2002). The main characteristics of *Chlorophyllum* in its amended sense are the hymenodermal nature of the pileus covering, the smooth stipe and the germ pore, which is caused by a depression in the episporium; in one species the germ pore is absent.

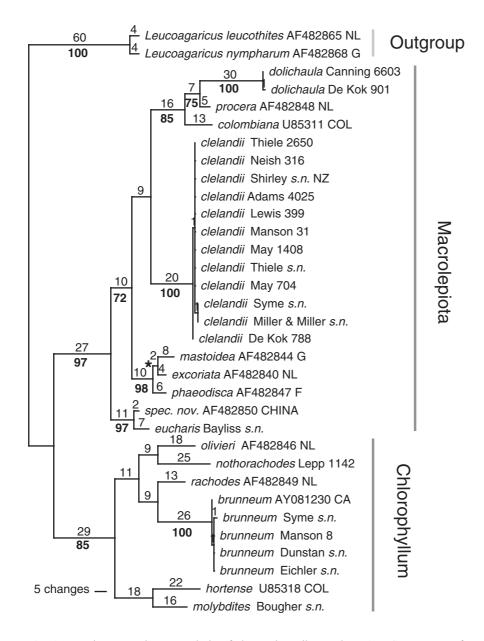
The genus *Macrolepiota* s.str. is characterised by a trichodermal pileus covering, made up of long, often thick-walled elements, the presence of a stipe covering, often visible as coloured bands in the full-grown specimens and a

germ pore caused by an interruption of the episporium covered by a hyalinous cap.

May and Wood (1997) provided references to the literature of seven Australian *Macrolepiota* species, one *Chlorophyllum* species and *Leucoagaricus fimetarius* (Cooke & Massee  $\rightarrow$  Sacc.) Aberdeen. At first there was much confusion over the applicability of the names 'konradii', 'mastoidea', 'gracilenta' and 'procera' to Australian collections. All these names were originally given to European species (Aberdeen 1962, 1992; Grgurinovic 1997). The confusion was partially resolved when Grgurinovic (1997) showed that Cleland's interpretation of *M. procera* (Vittad.) Singer represented a new species, *M. clelandii* Grgur., characterised by 2-spored basidia and long, cylindrical cheilocystidia. The present paper completes the process by showing that also 4-spored and pale-coloured specimens should be assigned to *M. clelandii*.

Modern material from the herbaria of CANB, MEL and the CSIRO Forestry and Forest Products, Wembley, forms the basis for the present overview. Morphological characters were examined and sequences of the internal transcribed spacer (ITS) of the nuclear ribosomal DNA were obtained. A key to the Australian species is provided to facilitate recognition. The overview of the species is in checklist format; the nomenclature is given in full, followed by selected descriptions and illustrations, lists of examined specimens and either a complete description (for the newly described taxa), or characteristics and notes on delimitation and variability.

A phylogenetic hypothesis for the relationships between the Australian species and their European relatives is given in Fig. 1, based on nrITS sequences. Representatives of all clades within *Chlorophyllum* and *Macrolepiota* are included.



**Fig. 1.** Maximum parsimony analysis of the nuclear ribosomal DNA ITS-sequences of *Macrolepiota* and *Chlorophyllum* taxa. Three most parsimonious trees were retrieved and the one with the lowest lnL is presented. Bootstrap supports >70% are indicated under the branches. The analysis is based on 197 parsimony-informative characters; tree length = 456, CI = 0.6425, RC = 0.5402. An asterisk indicates the branch collapsing in the strict consensus tree. Collections are identified with collection numbers or GenBank accession numbers; country of origin is indicated for non-Australian collections (CA = California, USA, CHINA = China, COL = Colombia, F = France, G = Germany, NL = the Netherlands, NZ = New Zealand).

#### Materials and methods

### Morphology

Herbarium material was used for morphological examination. Colour annotations are according to Kornerup and Wanscher (1967). The notation [57,4,3] in descriptions indicates that measurements were made on a total of 57 spores from four samples in three collections. The following abbreviations are used: avl for average length, avw for

average width, Q for quotient of length and width and avQ for average quotient.

#### Molecular identification

Methods for DNA extraction, PCR reactions, sequencing, analysing, aligning and phylogenetic analysis of the data were as in Vellinga (2001*b*). The GenBank accession numbers for ITS sequences are given with the lists of examined collections.

#### Key to the Australian Chlorophyllum and Macrolepiota species

| 1. | Sp | ore              | print green   |
|----|----|------------------|---|
| 1. | Sp | pore print white |   |
|    | 2. | Ba               | usidiocarps overall white   |
|    |    | 3.               | Pileus with whitish squamules and yellowish umbo; stipe wine-red when damaged; basidia 2-spored, rarely 4-spored; spores                |
|    |    |                  | 8.0–12.5 × (5.5–)6.0–7.0 µm, without germ pore  |
|    |    | 3.               | Pileus with some dark small squamules at the pileus centre; stipe not changing colour when damaged; basidia 4-spored; spores            |
|    |    |                  | $11.5-16.5 \times 8.0-11.5 \mu$ m, with germ pore covered by hyaline cap  |
|    | 2. | Ba               | usidiocarps with distinct dark brown patches and squamules  |
|    |    | 4.               | Pileus covering hymenodermal, with closely packed narrowly clavate to cylindrical terminal elements; spores with truncate apex and germ |
|    |    |                  | pore without hyalinous covering   |
|    |    |                  | 5. Clamp connections absent at the base of basidia and cheilocystidia   |
|    |    |                  | 5. Clamp connections present, at least at the base of the basidia and cheilocystidia  |
|    |    | 4.               | Pileus covering trichodermal, made up of cylindrical, long, often septate elements; spores with a germ pore, covered by a hyalinous cap |

### 1. Chlorophyllum molybdites (G.Mey. : Fr.) Massee, Kew Bull. 1898: 136, 1898

Agaricus molybdites G.Mey., Pr. Fl. Essequ.: 300, 1818; Agaricus molybdites G.Mey. : Fr., Syst. Mycol. 1: 308, 1821; Mastocephalus molybdites (G.Mey. : Fr.) O.Kuntze, Rev. Gen. Pl. 2: 860, 1891; Leucocoprinus molybdites (G.Mey. : Fr.) Pat., Bull. Soc. Mycol. Fr. 29: 215, 1913; Macrolepiota molybdites (G.Mey. : Fr.) Moreno, Bañares & Heykoop in Mycotaxon 55: 467, 1995. Agaricus morganii Peck, Bot. Gaz. 4: 137, 1879; Lepiota morganii (Peck) Sacc., Syll. Fung. 5: 30, 1887; Mastocephalus morganii (Peck) O.Kuntze, Rev. Gen. Pl. 2: 860, 1891; Chlorophyllum morganii (Peck) Massee, Kew Bull. 1898: 136, 1898. Agaricus glaziovii Berk., Vidensk., Meddel. 1879–1880: 32, 1880; Pholiota glaziovii (Berk.) Sacc., Syll. Fung. 5: 751, 1887. Lepiota ochrospora Cooke & Massee, Grevillea 21: 73, 1893. Chlorophyllum esculentum Massee, Kew Bull. 1898: 136, 1898; Lepiota esculenta (Massee) Sacc. & Syd., Syll. Fung. 16: 2, 1901.

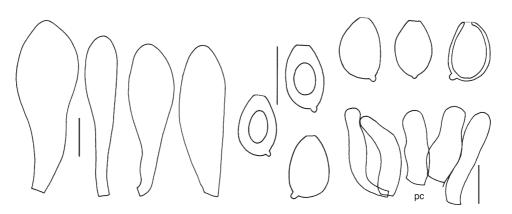
### Selected description and illustrations: Grgurinovic, Larger Fungi South Australia: 439–440, fig. 289, 1997.

*Chlorophyllum molybdites* is characterised by a white pileus with brown squamules, a double-crowned annulus, broad-truncate green spores with wide germ pore, clavate cheilocystidia and very rare clamp connections. *Chlorophyllum molybdites* is widespread in irrigated lawns, where it occurs during the hotter months. It is easily recognised by the greenish, truncate spores. It causes gastro-intestinal problems, especially when eaten raw, but is also considered a good edible mushroom. Reid and Eicker (1991) gave a comprehensive overview of the literature on this species, including a more extensive list of synonyms than the one given here.

*Collection examined*: AUSTRALIA: Queensland, Townsville, Davies Rd, CSIRO lab., *N.L. Bougher*, 5.v.1988; GenBank AY083209 (CSIRO Forestry and Forest Products, Wembley E4022).

#### Chlorophyllum nothorachodes Vellinga & Lepp, sp. nov. (Fig. 2)

Chlorophylli brunneo in habitu similis; sporae  $9.0-12.0 \times 6.0-8.0 \mu m$ ; cheilocystidia  $22-44 \times 6.5-17 \mu m$ , tenuiter clavata fusiformiaque; cellulae pileum tegentes parvae praecipue cylindraceae; sine fibulis; praeterea in spatii interne transcripti sequentia ('ITS') differt.



**Fig. 2.** *Chlorophyllum nothorachodes*—spores, cheilocystidia and elements of pileus covering (pc). All from holotype. Scale bars =  $10 \mu m$ .

*Holotypus hic designatus*: 'Australia, Australian Capital Territory, Stirling, 8 km SW of Capital Hill, Canberra, 19.i.1995, H. Lepp 1142 (CANB 601128)'.

*Etymology*: nothorachodes is derived from the Greek word 'vo $\theta o \zeta$ ' meaning 'false' and the epithet 'rachodes', as this species closely resembles *C. rachodes* (Vittad.) Singer.

Pileus up to 280 mm, eventually more or less applanate with a wavy margin, with vague low umbo, dry, with central star-shaped patch and concentrically arranged small patches, brown, tan-coloured (6E5-6E6), slightly darker at centre, on a cream to pale brown radially fibrillose background; margin fringed and exceeding lamellae. Lamellae free, crowded, ventricose, cream with brown eroded lamella edge. Stipe up to 250 mm long and 25-40(-60) mm wide, with bulbous base,  $\pm$  oval in cross-section, hollow, dirty white above annulus, dirty white to brownish below annulus (with minute pale brown punctae in youngest specimens), with some short, longitudinal dark brown fibrils (near 7F8) near the base, becoming dull brownish-orange on bruising. Annulus loose, well-developed, about 3/4 of the way up the stem, pale brown below, white above, with a floccose edge. Context off-white and dull in pileus and off-white in stipe; some parts turn orange on being cut, but not uniformly; the colour change (to reddish-orange, 7A8-8A8) happens where the flesh is injured or where the blade drags when the flesh is being cut-not everywhere where the flesh is exposed to the air; the layer at the interface between lamellae and flesh turns orange red on being cut. Smell and taste not recorded.

Spores [64,2,1] 9.0–12.0(–13.0) × 6.0–8.0(–9.0)  $\mu$ m, avl × avw = 9.8 × 6.7  $\mu$ m, Q = 1.25–1.7, avQ = 1.45, amygdaloid-ellipsoidal to amygdaloid-oblong in side view, with truncate or rounded base with broad germ pore, ellipsoidal to oblong in frontal view, thick-walled, congophilous, metachromatic in Cresyl Blue and dextrinoid. Basidia 29–36 × 9–11  $\mu$ m, 4-spored, without clamp connection at the base. Lamella edge sterile. Cheilocystidia 22–44 × 6.5–17  $\mu$ m, narrowly clavate to fusiform, with brown intracellular pigment, without clamp connection at the base. Pleurocystidia absent. Pileus covering a tight-knit

hymeniderm, made up of  $12-28 \times 4.0-8.0$  µm, cylindrical, narrowly clavate or narrowly lageniform elements, thick-walled, with brown intracellular pigment, covered by a waxy layer. Clamp connections absent (not observed at the base of basidia and cheilocystidia, nor in the pileitrama).

*Habitat and distribution*: gregarious, in garden on rich soil; known from a suburb of Canberra (Australian Capital Territory).

*Chlorophyllum nothorachodes* resembles *C. brunneum* in the field, but differs in the non-marginate bulb, the more fusiform cheilocystidia, the small and narrow pileipellis elements and especially in the absence of clamp connections.

Another clamp-less species is *M. venenata* Bon ([not validly described, as Bon in Bon *et al.* (1979) failed to designate a collection of one date as the type]. The pileus squamules in this species are radially arranged and not concentrically as in the other *Chlorophyllum* species. It resembles *C. nothorachodes* in the shape of the cheilocystidia.

On the basis of ITS sequence analyses, *C. nothorachodes* is a sister taxon to *C. olivieri* (Barla) Vellinga, a species known from Europe and north-western USA (Vellinga *et al.* 2003). In *C. olivieri* the scales on the pileus are almost the same colour as the background, often an olivaceous drab. Both *C. olivieri* and *C. rachodes* are characterised by spheropedunculate cheilocystidia and a double complicated annulus.

*Collection examined*: AUSTRALIA: Australian Capital Territory: Stirling, 8 km SW of Capital Hill, Canberra (35°21'30"S, 149°02'30"E, 600 m alt.), *H. Lepp 1142*, 19.i.1995; GenBank AF482855 (Holotype, CANB 601128).

# 3. *Chlorophyllum brunneum* (Farl. & Burt) Vellinga in *Mycotaxon* 83: 416, 2002 (Fig. 3)

Lepiota brunnea Farl. & Burt, Icones farlowianae: 8, pl. 6, 1929; Macrolepiota rachodes var. brunnea (Farl. & Burt) Candusso in Candusso & Lanzoni, Lepiota s.l., Fungi Eur. 4: 535, 1990; Macrolepiota brunnea (Farl. & Burt) Wasser, tribes Cystodermateae Singer and Leucocoprineae Singer of the CIS and Baltic States (Libri Botanici 9): 82, 1993. Lepiota bohemica Wichansky in Mykol. Sb. 38: 103, 1961 (not valid); Macrolepiota bohemica (Wichansky) Krieglst. &

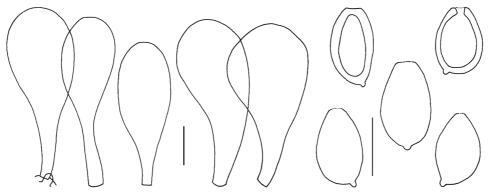


Fig. 3. *Chlorophyllum brunneum*—spores and cheilocystidia. From Syme. Scale bars =  $10 \,\mu m$ .

Pázmány in Pázmány in Z. Mykol. 51: 52, 1985 (not valid); Macrolepiota rachodes var. bohemica (Wichansky) Bellù & Lanzoni in Beitr: Kenntn. Pilze Mitteleur. 3: 191, 1987 (not valid); Lepiota rachodes var. hortensis Pilát, Klíčkurcování našich hub hřibovity ch a bedlovity ch. Agaricales: 422, 1951 (not valid, no Latin description); Macrolepiota rhacodes var. hortensis (Pilát) Wasser, Flora Grib. Ukr. Agar. Grib.: 298, 1980 (not valid).

Misapplied names: Macrolepiota rachodes sensu Australian authors; M. rachodes var. hortensis sensu Arora, Mushrooms Demystified, Edn 2: 297, fig. p. 297, 1986; sensu Phillips, Mushrooms Other Fungi Great Britain: 25, 1981.

Selected description and illustrations: Bougher and Syme, Fungi Southern Australia: 188–189, 1998; Grgurinovic, Larger Fungi South Australia: 441–443, fig. 290, 1997 (both as Macrolepiota rachodes).

*Chlorophyllum brunneum* is characterised by medium-sized basidiocarps, a simple annulus, an abruptly, often marginate, bulbous stipe base, often truncate spores, predominantly clavate (not spheropedunculate) cheilocystidia and clamp connections at the base of cystidia and basidia.

*Chlorophyllum rachodes*, with which it is often confused, is characterised by big basidiocarps, a double annulus, a bulbous, but not marginate, stipe base and predominantly spheropedunculate to broadly clavate cheilocystidia. Grgurinovic (1997) noted the difference in the size and shape of the cheilocystidia between the Australian specimens and those described by Pegler from Kenya (1977), but stopped short of concluding that the Australian specimens represent a different species. *Chlorophyllum brunneum* is a sister taxon to *C. rachodes* in the ITS sequence analysis (Fig. 1).

De Kok and Vellinga (1998) did not recognise the present species as a separate taxon, but merged it with *C. rachodes*, probably because the species is very rare in the Netherlands and it might have a more southern distribution. The main characters to distinguish *C. brunneum* from *C. rachodes* have always been the presence of a big bulb, big squames on the pileus and the truncate spore apex, characters that are variable and not restricted to *C. brunneum*.

The name *Macrolepiota rachodes* var. *hortensis* Pilát has been widely used for this species (see misapplied names), but was invalidly published, as no Latin diagnosis was provided (Pilát 1951; Bellù and Lanzoni 1987; De Kok and Vellinga 1998). Pilát (1951) described this variety as having big basidiocarps, but all other characters fit within the concept of *C. brunneum*.

*Chlorophyllum brunneum* was also known as *M. bohemica* Wichansky, but Wichansky (1961) did not designate a type specimen of one particular date. An extensive overview of the taxa in this complex is given by Vellinga (2003).

*Chlorophyllum brunneum* occurs in various man-made habitats in Europe and North America. It is very common in California and it may have been introduced into Australia where it is found under imported trees (*Cupressus macrocarpa*, *Pinus radiata*) and on compost heaps. It has been found in Australia at least since the late 19th century (Cooke 1892; Grgrurinovic 1997, studied a collection from 1913). Other *Chlorophyllum* species, like *C. hortense* and *C. molybdites*, also occur in man-made habitats and are widely distributed.

*Collections examined*: AUSTRALIA: Victoria, Midlands, Avenel, home block (36°54'S, 145°14'E), *H. Manson 8, 1992*; GenBank AY083206 (MEL 2030630); Victoria, Gippsland Plain, Melbourne, suburb of South Yarra, Royal Botanic Gardens, W side of Nursery (37°50'S, 144°59'E), *R. Dunstan*, 22.iii.1996; GenBank AY083207 (MEL 2032908); Victoria, Gippsland Plain, Melbourne, bayside suburb of Beaumaris, Beaumaris coastal reserve (37°59'30"S, 145°02'30"E), *J. Eichler*, 6.iv.1995; GenBank AY083208 (MEL 2030616); Western Australia, Denmark area, Lot 406 (57°34'S, 117°21'E) *K. Syme*, 22.v.1996; GenBank AY083205 (CSIRO Forestry and Forest Products Wembley E5628).

### 4. *Chlorophyllum hortense* (Murrill) Vellinga in *Mycotaxon* 83: 416, 2002

Lepiota hortensis Murrill, N. Amer. Fl. 10 (1): 59, 1914; Leucoagaricus hortensis (Murrill) Pegler, Kew Bull. Add. Ser. 9: 414, 1983. Lepiota humei Murrill, Lloydia 6: 220, 1943. Lepiota mammillata Murrill, Lloydia 6: 220, 1943. Lepiota subfulvidisca Murrill, Lloydia 6: 221, 1943. Lepiota alborubescens Hongo, Mem. Fac. Lib. Arts Educ. Shiga Univ., Nat. Sci. 12: 40, 1962; Macrolepiota alborubescens (Hongo) Hongo, Trans. Soc. Mycol. Japan 27: 107, 1986. Leucoagaricus bisporus Heinem., Bull. Jard. Bot. Nat. Belg. 43: 8, 1973.

Misapplied name: Leucoagaricus fimetarius sensu Aberdeen, Lepiotoid Genera (Agaricales) in South-eastern Queensland: 8–9, 1992; Lepiota fimetaria sensu Young, Common Australian Fungi: 88. 2000.

Selected description and illustrations: Aberdeen, Lepiotoid Genera (Agaricales) in South-eastern Queensland: 8–9, 1992 (as L. fimetarius); Akers & Sundberg in Mycotaxon 62: 401–419, 1997.

*Chlorophyllum hortense* is characterised by medium-sized, pale basidiocarps with whitish squames on the pileus and a yellowish umbo, a strong reddening reaction when the stipe is damaged, a simple annulus, spores without a germ pore, 2-spored basidia, cylindrical cheilocystidia and clamp connections.

Akers and Sundberg (1997) discussed the placement of the species and accommodated it in *Leucoagaricus*, on account of the absence of a germ pore, though they acknowledged that the presence of clamp connections suggested a close relationship to *Macrolepiota*. Molecular work by Vellinga *et al.* (2003) has now shown that the species belongs to the same clade as *Chlorophyllum molybdites* and *C. rachodes* and a position within *Leucoagaricus* was ruled out (see also Fig. 1). Aberdeen (1992) used the name *Leucoagaricus* fimetarius for this species, but Vellinga (2002) pointed out several problems with this name, the most serious being that the original description represents a totally different species than that of Aberdeen. Accordingly, the name *Lepiota* fimetaria (Cooke & Massee  $\rightarrow$ ) Sacc. is not used for the present species.

*Chlorophyllum hortense* occurs on dung, compost enriched garden soil etc., and has a wide (sub-)tropical distribution.

Collection examined: AUSTRALIA: New South Wales, Sydney, Quakers Hill, A.M. Young, 29.i.1982 (L).

### 5. *Macrolepiota dolichaula* (Berk.) Pegler & Rainer, *Kew Bull.* 23: 365, 1969

Agaricus dolichaulus B. & Br., Trans. Linn. Soc., Bot. 27: 150, 1870; Leucocoprinus dolichaulus (B. & Br.) Pat., Bull. Trimest. Soc. Mycol. Fr. 29: 215, 1913; Leucocoprinus dolichaulus (B. & Br.) Boedijn, Sydowia 5: 221, 1951 (superfl. comb.). Agaricus beckleri Berk., J. linn. Soc. 13: 156, 1872; Lepiota beckleri (Berk.). Sacc., Syll. Fung. 5: 56, 1887. Agaricus stenophyllus Cooke & Massee, Grevillea 15: 98, 1887; Lepiota stenophylla (Cooke & Massee) Sacc., Syll. Fung. 9: 4, 1891.

Selected descriptions and illustrations: Grgurinovic, Larger Fungi South Australia: 445–446, fig. 292, pl. 24, 33d, 1997; Pegler, Preliminary Agaric Flora East Africa: 308–309, fig. 67–2, 1977; Shepherd and Totterdell, Mushrooms Toadstools Australia: 74–75, 1988.

*Macrolepiota dolichaula* forms big, whitish basidiocarps, with a long stipe and an umbonate pileus with small uplifted

squamules on the pileus; average spore sizes are  $12.4-14.8 \times 8.6-9.4 \mu m$ , avQ = 1.45-1.6; cheilocystidia are broadly clavate to speheropedunculate.

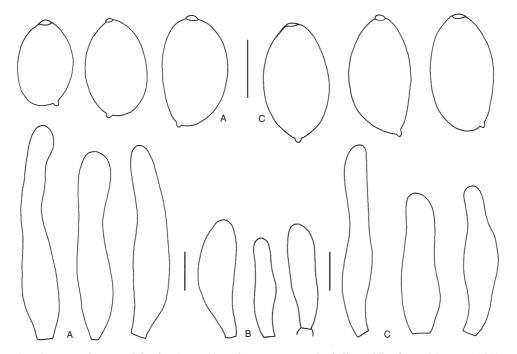
The species is widespread and also reported from East Africa and South-east Asia (Pegler 1977 and 1986).

Collections examined: AUSTRALIA: Australian Capital Territory, Canberra, Black Mountain, CSIRO site, *R.P.J. de Kok 901*, 14.iv.2000; GenBank AY083193 (CANB); New South Wales, *c*. 2 km from Monteagle towards Frenfell, 470 m alt., *K.R. Thiele 2651*, 10.v.2000 (MEL); New South Wales: between Blakney Ck and Bevendale, just off road inside entrance to 'Kunama Cottage' (34°36'S, 149°07'E, 520 m alt.), *E.M. Canning 6603*, 4.iv.1989; GenBank AF482839 (CANB); Victoria, Midlands/Eastern Highlands, 3 km E of Alexandra, Ultima Thule Creek Rd, near entrance to Chittagong homestead. (37°12'S, 145°45'E, 260 m alt), *N.H. Sinnott 2590*, 28.iii.1993 (MEL 2030609).

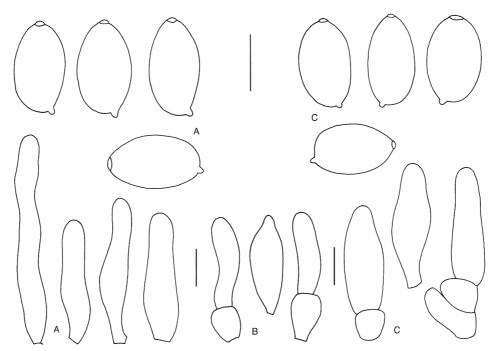
# 6. *Macrolepiota clelandii* Grgurinovic, *Larger Fungi South Australia*: 443, 1997 (Figs 4, 5)

Misapplied names: Macrolepiota procera sensu Cleland in several publications (fide Grgurinovic 1997); Macrolepiota gracilenta sensu Willis, Victorian Fungi, 1941 (fide Aberdeen 1962); Macrolepiota konradii sensu Bougher & Syme, Fungi Southern Australia: 186–187, 1998; sensu Shepherd & Totterdell, Mushrooms Toadstools Australia: 75, 1988; Macrolepiota mastoidea sensu Young, Common Australian Fungi, Edn 2: 91, 2000.

Selected descriptions and illustrations: Grgurinovic, Larger Fungi South Australia: 443–445, fig. 291, pl. 25a. 1997; Bougher & Syme, Fungi southern Australia: 186–187. 1998 (as *M. konradii*); Shepherd & Totterdell, *Mushrooms* 



**Fig. 4.** *Macrolepiota clelandii*, 2-spored variant—spores and cheilocystidia from (*A*) May 1408, (*B*) Thiele, (*C*) De Kok 788. Scale bars =  $10 \mu m$ .



**Fig. 5.** *Macrolepiota clelandii*, 4-spored and mixed-spored variants—spores and cheilocystidia from (*A*) Miller & Miller, (*B*) Neish 316 and (*B*) Syme. Scale bars =  $10 \mu m$ .

Toadstools Australia: 75. 1988 (as M. konradii); M. Taylor, Mushrooms Toadstools: fig. 9, p. 21. 1981 (as Lepiota sp.).

*Macrolepiota clelandii* is a slender, small- to medium-sized *Macrolepiota* species, characterised by the dark brown pileus centre and small dark brown squamules on the pileus. Microscopically it is easy to recognise because of the 2-spored basidia, the huge spores  $(14.0-28.5 \times 9.0-15.5 \ \mu m)$  and the cylindrical to narrowly lageniform cheilocystidia; the pileus covering is a trichoderm, made up of brown-walled cylindrical elements, with terminal elements  $22-50 \times 7-12 \ \mu m$ .

However, there are several variations on this pattern: pale variants do occur (e.g. collection May 704); length of cheilocystidia differs considerably (from 24-32 µm in collection Thiele s.n., to 33-58 µm long in May 1408) and the number of spores produced per basidium is not always two. Grgurinovic (1997) pointed out the occurrence of 4-spored basidia and it appears that the proportion of 2-spored, v. 3-spored or 4-spored basidia is quite variable. One of the collections studied is predominantly 4-spored and has consequently smaller spores than 2-spored basidiocarps, the cheilocystidia are short and relatively wide. This collection has been described and illustrated by Bougher and Syme (1998; as *M. konradii*). Other collections have predominantly 3-spored basidia, or 50% 2-spored basidia and cylindrical cheilocystidia (Neish 316, Miller & Miller s.n.). Microscopical details are given in Fig. 5 with typical 2-spored variants for comparison in Fig. 4. The ITS-sequences of those 4-spored and mixed collections fall within the scope of *M. clelandii* (Fig. 1).

Taxa with predominantly 2-spored basidia are rare within the Agaricaceae. *Agaricus bisporus* (J.E.Lange) Imbach, *Chl. hortense* and *Leucoagaricus sericifer* f. *sericatellus* (Malençon) Vellinga all have 2-spored basidia and 4-spored variants of these taxa are known as well, some of which have been officially described: *A. bisporus* var. *burnetti* Kerrigan & Callac and *L. sericifer* (Locq.) Vellinga f. *sericifer*. Spores of those 4-spored variants are smaller than those produced by 2-spored basidia.

The spores from 2-spored basidia in *M. clelandii* and *C. hortense* have 4 nuclei; *C. hortense* spores of 4-spored basidia have 2 nuclei (pers. obs.).

*Macrolepiota mastoidea* (Fr. : Fr.) Singer (including *M. konradii*; Vellinga 2001*a*) belongs to a different clade within *Macrolepiota* than *M. procera* and *M. clelandii* (Fig. 1; Vellinga *et al.* 2003, Fig. 1). Clamp connections are very rare and difficult to observe in *M. mastoidea*, although quite common at the base of basidia in *M. clelandii*. ITS sequences of *M. mastoidea* and allied species [*M. psammophila* Guinb., *M. subsquarrosa* (Locq.) Bon] hardly show any variation (unpublished results) and are close to *M. excoriata* (Schaeff. : Fr.) Wasser (Fig. 1).

*Macrolepiota procera* and allies differ in the structure of the pileus covering from *M. clelandii* and *M. mastoidea*; the terminal elements are thick-walled and situated on thin-walled upright cylindrical elements. Macroscopically the big patches of velar material on the pileus are striking.

*Macrolepiota clelandii* is quite common in southern Australia and in New Zealand.

Collections examined: AUSTRALIA: Australian Capital Territory, Cork Oak Plantation, W of Black Mountains Reserve (35°16'S, 149°04'E, 600 m alt.), L.G. Adams 4025, 15.v.1988; GenBank AY083197 (CANB); Australian Capital Territory, Upper Cotter River, along track from Murray Cap to ford in the Cotter River, R.P.J. de Kok 788, 3.iv.1999; GenBank AY083195 (CANB); New South Wales, between Blakney Ck and Bevendale, 'Kunama Cottage' (34°36'S, 149°07'E, 520 m alt.), E.M. Canning 6606, 9.iv.1989 (CANB); Victoria, Midlands, Avenel, home block (36°54'S, 145°14'E), H. Manson 31, 21.vi.1993; GenBank AY083199 (MEL 2030615); Victoria, Eastern Highlands, betweeen Toolangi and Black Range state forests, Murrindindi Scenic Reserve, Murrindindi River walking track, near Suspension Bridge camping area (37°24'S, 145°34'E), N.H. Sinnott 2949, 5.vi.1994 (MEL 2030628); Victoria, Eastern Highlands, Kinglake NP, Lyrebird Circuit Walk, near car park for walk to Mason Falls (37°29'54"S, 145°14'48"E), T.W. May 1408, 29.iii.1999; GenBank AY083200 (MEL 2059641); Victoria, Eastern Highlands, Kinglake NP, 'The Gums' camping ground, Eucalyptus Rd (37°28'19"S, 145°23'31"E), P.G. Neish 316, 13.vi.1997; GenBank AY083194 (MEL 2039178); Victoria, Gippsland Highlands, Morwell NP, side of track prior to crossing Fosters Gully (38°22'S, 146°23'E), S.H. Lewis 399, 30.iv.1998; GenBank AY083198; (MEL 2046436); Victoria, Wannon, Portland district, plantation near Kentbruck (38°07'S, 141°13'E), T.W. May 704, 9.vi.1990; GenBank AY083202 (MEL 227060); Victoria, East Gippsland, Martins Ck (c. 10 miles N of Orbost) (37°34'S, 148°27'E), 1990, K.R. Thiele; GenBank AY083201 (MEL 229171); Victoria, 52 km N of Orbost on the Bonang Rd, Martins Ck, alt. 320 m, K.R. Thiele 2650, 17.v.2000; GenBank AF482838 (MEL); Western Australia, along South Coast Hwy, Walpole-Nornalup NP (59°34'S, 116°40'E), K. Syme, 9.v.1995, GenBank AY083203 (CSIRO Forestry and Forest Products, Wembley E5627); Western Australia, Manjimup, Diamond Tree, O.K. Miller & H. Miller, 11.vi.1989; GenBank AY083204; (CSIRO Forestry and Forest Products, Wembley E0630). NEW ZEALAND: North Island, Auckland, Manurewa, Hill Rd, Oxford Park, C. Shirley, late spring 1999, GenBank AY0832196 (Herbarium Shirley).

## 7. *Macrolepiota eucharis* Vellinga & Halling, sp. nov. (Figs 6, 7)

Pileus areolatus squamis nigris, stipes basi volvatus, sporae 10.8–15.5  $\times$  7.1–9.1 µm, cheilocystidia 25–53  $\times$ 

 $5.0-12 \ \mu m$  cylindracea tenuiter clavata fusiformiaque, fibulae non-observatae.

*Typus hic designatus*: 'Australia, Queensland, 10 km west of Millaa Millaa on Palmerston Highway, 4.ii.1992, R.E. Halling (Holotype CSIRO Forestry and Forest Products, Wembley E4506; Isotype NY)'.

*Etymology*: eucharis is derived from the Greek word 'ευχαρις', meaning 'charming, lovely, attractive'.

Pileus up to 6 cm broad when expanded, parabolic at first then convex, dry, surface breaking up into areolate black scales, more densely packed toward disc, scattered and detersile toward margin, overlying a felty, radially oriented subsquamulose-fibrillose layer; scales black. on greyish-brown (6E5-6E4) background. Lamellae remote from stipe, crowded, white with fimbriate, concolourous edge, up to 8 mm broad. Stipe 11-14 cm long, 6-7 mm broad near apex, equal with a bulbous base, terete, dry, uniformly fibrillose, dark brown (7F4); stipe base volvate to marginately bulbous, paler to concolourous with stipe surface, with free limb, with some dark minute scales, as on pileus surface. Annulus ascending, simple, white above and below, with dark minute patches on underside near margin, with fringed margin. Context in pileus white, not changing colour, 7 mm thick in pileus. Smell and taste mild.

Spores [31,2,2]  $10.8-15.5 \times 7.1-9.1 \mu m$ ,  $avl \times avw = 12.0-12.4 \times 7.9 \mu m$ , Q = 1.4-1.8, avQ = 1.53-1.58, ellipsoidal to oblong and amygdaloid in side view, with apical and central germ pore, thick-walled, congophilous, dextrinoid, metachromatic in Cresyl Blue and cyanophilous. Basidia 27-40  $\times$  9.5-13  $\mu m$ , 4-spored, some 2-spored. Lamella edge sterile; cheilocystidia 25-53  $\times$  5.0-12  $\mu m$ , cylindrical, narrowly clavate, or slightly fusiform, slightly thick-walled, colourless. Pleurocystidia absent. Pileus covering (squamules) a trichoderm, made up of

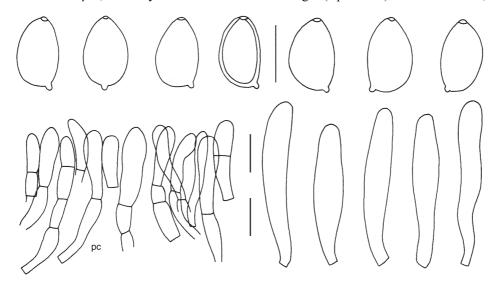


Fig. 6. *Macrolepiota eucharis*—spores, cheilocystidia and elements of pileus covering from holotype. Scale bars =  $10 \mu m$ .

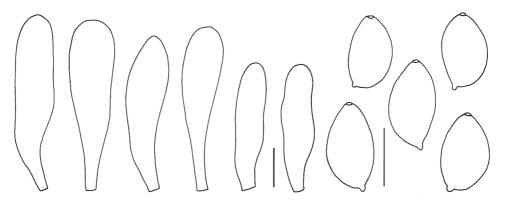


Fig. 7. Macrolepiota eucharis—spores and cheilocystidia from Bayliss. Scale bars = 10 µm.

brown-walled elements; terminal elements  $20-80 \times 6.0-14 \mu m$ , cylindrical with rounded apex. Pileus covering (background) made up of radially arranged adnate hyphae with incrusted brown pigments. Stipitipellis a cutis covered by irregularly wavy dark brown hyphae. Clamp connections not observed at the base of basidia and cheilocystidia.

Habitat and distribution: solitary or in small groups, in rain forests, under *Eucalyptus grandis* and *Allocasuarina littoralis*. Known from two places in Queensland.

*Macrolepiota eucharis* is a small, elegant species, characterised by the dark grey to black pileus, a volvate base to the stipe, small spores and narrowly clavate and cylindrical cheilocystidia.

*Volvolepiota brunnea* (Rick) Singer comes close but differs in the pileus covering which is made up of clavate elements with emerging hyphae; in young specimens these clavate elements are very prominent, in older specimens, the hyphae form the prominent layer. These hyphae are colourless at the apex and the brown pigment is situated in the cell walls of the lower parts of the hyphae and in the clavate elements. Coarsely incrusted short-celled cylindrical hyphae are present as well. Heinemann and De Meijer (1996) gave an elaborate description of this taxon.

*Macrolepiota eucharis* is a sister species to another new species from China, with even smaller spores (8–10  $\mu$ m), which also has a slightly volvate stipe base (Fig. 1). These two species appear to be basal to the other clades within the genus *Macrolepiota*, indicating that the ancestral species were volvate, had small spores and lacked clamp connections at the base of basidia and cheilocystidia.

*Collections examined*: AUSTRALIA: Queensland, Mareeba, Davies Creek Rd, *B. Bayliss*, 3.ii.1992, GenBank AF482854 (CSIRO Forestry and Forest Products, Wembley E4505); Queensland, 10 km W of Millaa Millaa on Palmerston Hwy, *R.E. Halling*, 4.ii.1992 (Holotype CSIRO Forestry and Forest Products, Wembley E4506).

#### Acknowledgments

The curators of CANB, MEL and CSIRO Forestry and Forest Products, Wembley were so kind as to send material on loan.

Rogier P. J. de Kok, Kevin R. Thiele and Clive Shirley lent me collections as well. Jan Frits Veldkamp helped with the Latin diagnoses and John Lennie, Tom D. Bruns and Machiel E. Noordeloos made useful comments on an earlier version of the manuscript.

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Manuscript received 2 May 2002, accepted 11 December 2002