MYCOTAXON

Volume LXXX, pp. 297-306 October-December 2001

STUDIES IN LEPIOTA IV.
Lepiota cristata and L. castaneidisca

Else C. Vellinga

University of California at Berkeley, 111 Koshland Hall # 3102, Berkeley CA 94720-3102, U.S.A. / National Herbarium, P.O. Box 9514, 2300 RA Leiden, the Netherlands
Email vellinga@uclink4.berkeley.edu; vellinga@nhn.leidenuniv.nl

Lepiota castaneidisca, though generally considered a synonym of *L. cristata*, is rediscovered as a separate species. It is macroscopically, molecularly, and ecologically distinct from *L. cristata*, and within the species two groups can be distinguished on molecular grounds. A third molecularly different variant, also with a hymeniform pileus covering and truncate to spurred spores, was discovered, but this variant is not morphologically distinguishable from *L. cristata*.

Keywords – Cryptic species, ITS-sequences, IGS1-sequences, California mycota

INTRODUCTION

Lepiota cristata (Bolton: Fr.) Kumm. was sampled from Europe, North America and China to study the variation in ITS-sequences as part of a research project assessing the phylogenetic relationships within and between the tribus Lepioteae Fayod and Leucocoprineae Singer within the family Agaricaceae (Fr.) Chevall. as recognized and defined by Singer (1986). Several distinct ITS-types were discovered, accompanied by phenotypical differentiation in one case.

MATERIAL AND METHODS

Material – Collections of *L. cristata* from a wide distribution area were sampled, as well as multiple collections from the same locality. A list of collections studied is given in the Appendix.
Morphology – Colour annotations in the macroscopical descriptions are from Munsell Soil Color Charts (1975). The notation [57,4,3] indicates that measurements were made on 57 spores in 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 – DNA was extracted from fresh and herbarium material; the internal transcribed spacer (ITS) and part of the intergenic spacer (IGS1) of the nuclear ribosomal repeat were amplified by the p[illegible]

and CNL12 and 5SA (Henrion et al., 1992) for the IGS1-region. Sequencing of both strands was performed with an ABI model 377 sequencer (Applied Biosystems, Foster City, California, U.S.A.) using a Thermo Sequenase™ Dye terminator Cycle Sequencing Pre-Mix Kit (Amersham Pharmacia Biotech, Piscataway, New Jersey, U.S.A.); the primers ITS1 or ITS5, ITS2, ITS3, and ITS4 were used for the ITS-region, and CNL12 and 5SA for the IGS1-region. The raw data were processed with the use of DNA Sequencing Analysis v.2.1.2 and Sequence Navigator v.1.0.1 (also of Applied Biosystems). The sequences thus obtained were aligned in ClustalW (online http://www.ch.embnet.org/software/ClustalW.html), with the following settings: opening gap penalty 10, end gap penalty 10, extending gap and separation gap penalty both 0.05. Sequences were analysed using PAUP 4.0b3a (Swofford, 2001). Gaps in the alignment of the IGS1-region were separately coded, following the 'simple indel coding method' as described by Simmons & Ochoterena (2000). Sequences have been deposited in Genbank, and the accession numbers are given in the Appendix.

LEPIOTA CRISTATA VERSUS L. CASTANEIDISCA

Lepiota cristata, a species with a hymeniform pileus covering and truncate to spurred spores, varies in pileus colour from very pale to dark brown, with the typical colour a kind of orange-brown; the annulus is attached to the stipe, but may be absent. Several varieties have been described from Europe (for an overview see Bon (1993)), and one from North America, viz. *L. cristata* var. *viridispora* Kyde & Peterson (1986). *Lepiota cristata* has a wide distribution area and is known from localities all around the Northern Hemisphere. It is also reported from the Southern Hemisphere, but these records may refer to different species (Horak, 1981). It grows especially in ruderal, locally nutrient-rich places, on woodchips, in city-parks, on roadsides etc., and is not commonly found in natural habitats.

The collections chosen for this study, all represent the more typical variant of the species, with orange-brown coloration. Despite this relative uniformity, the ITS-region exhibits considerable variation (Fig. 1). Collections from one area may have different ITS-sequences, whereas the ITS from widely dispersed collections may be identical. No correlation between morphology, geography, and ITS-sequences was found. This may be caused by introduction of the species in suitable, man-made, habitats. *Lepiota magnispora* Murrill, also a widespread but ecologically different species, has three, morphologically and geographically different, ITS-types (Vellinga, 2001b), but this kind of pattern is not present in *L. cristata*.

Collections from forests of *Sequoia sempervirens* (D. Don) Endl. (Redwood), stands of *Cupressus macrocarpa* Gordon (Monterey Cypress) and other natural habitats in northern California that at first were thought to represent *L. cristata*, were examined molecularly. Surprisingly, on the basis of ITS-data, these formed two separate clades which are clearly distinct from *L. cristata* (Fig. 1). The IGS1-data support this (Fig. 2), though the dataset is much smaller. All the collections in these two clades have a hymeniform pileus covering, spores with a truncate to spurred base, and (narrowly) clavate cheilocystidia.

This situation may be compared with a totally unrelated group of fungi, viz. the genus *Sarcodon*, in which differences in ITS-sequences supported chemical and subtle macromorphological differences between two closely related species, which had not been recognized as separate species for over 50 years (*S. squamosus* (Schaeff.) Quél. and *S. imbricatus* (L.: Fr.) P. Karst.) (Johannesson et al., 1999). Just as in our case, the two *Sarcodon* species have different ecological requirements, viz. different host tree species.

A sister group of *L. cristata* was found in *Sequoia sempervirens* forests, and it is macroscopically and microscopically indistinguishable from that species; it is called *Lepiota* sp. in Figs 1, 2, and 4. This provides an example of genotypic differentiation with phenotypic similarity. A description of this taxon is given below. For a comparison with *L. cristata*, the reader is referred to descriptions of *L. cristata* from Europe by e.g. Bon (1993), Candusso & Lanzoni (1990) or Vellinga (2001a).

The second clade, comprising two terminal taxa, is macroscopically distinct from *L. cristata*. It differs in shape and colours of the pileus, viz. convex, lacking a distinct umbo, and reddish or pinkish brown (in the range of 5 YR, whereas the colours in *L. cristata* are in the range of 7.5 YR). These specimens are not recognized as *L. cristata* by European mycologists (e.g. H.A. Huijser, pers. comm.), though they are known under this name in the western U.S.A. (for a picture see Arora, 1986). However, these macroscopical characters are as those given by Murrill (1912) for his *L. castaneidisca* described from a collection found near Searsville Lake, San Mateo Co., California, under *Sequoia sempervirens*. Murrill's description is as follows: "Pileus fleshy, regular, convex, umbonate, gregarious, 1.5-4 cm. broad; surface dry, white, with small, imbricate, avellaneous to light-chestnut scales, the umbo chestnut with unbroken cuticle; lamellae free, white, broad, ventricose, rather close; spores ellipsoid, smooth, hyaline, 5-6 x 3μ; stipe cylindric, equal, hollow, glabrous, brownish-tinted, 4-7.5 cm. long, 3-10 mm. thick; annulus white, superior, delicate, inconspicuous."

Two years later, Murrill (1914) reconsidered his new species, and decided that it was synonymous with *L. cristata*, which he called *L. conspurcata* (Willd.) Morgan; this opinion was shared by Sundberg (1989), who like Smith (1966), studied the type collection. Indeed, the microscopical characters of the type collection are identical to those of *L. cristata*.

My conclusion is that Murrill's collection belongs to the same species as what is called *L. castaneidisca* 1 and 2 in Figs 1 and 2, and that the name *L. castaneidisca* should be used for this species. The ecology supports the use of this name. Murrill's collection was found growing under Redwood, and not in a man-made habitat, as would be the typical habitat for *L. cristata*. However, discriminating morphological characters between the two terminal taxa of the *L. castaneidisca* clade were not found, despite the consistent and significant differences in ITS and IGS1 (Figs 1 and 2). Collections which can be referred to either branch grow together in the same area. A full description of *L. castaneidisca* is given below. One collection, viz. 'ecv2411', is anomalous. It was found on a roadside pile of dirt, and macroscopically represents *L. cristata* in all aspects, although the molecular data suggest that this collection belongs to *L. castaneidisca*. Its characters are not included in the description of *L. castaneidisca* as given here.

Besides those spurred-spored species, very similarly looking species with ellipsoid spores occur in the same localities: *Lepiota thiersii* Sundb. without cheilocystidia, and *Lepiota neophana* Morgan sensu Sundberg (1989) with cheilocystidia have both been reported from Monterey Cypress groves (Sundberg 1989).

It was shown earlier that other lepiotaceous species described from Europe, e.g. *Melanophyllum haematospermum* (Bull.: Fr.) Kreisel, and *Cystolepiota pulverulenta* (Huijsman) Vellinga, are represented by a distinct ITS-type in California habitats (Vellinga, 2001b), though the morphology does not show variation. Further research on different genes is needed to show whether these types represent separate species.

The number of lepiotaceous fungi in Redwood forests and especially in Monterey Cypress stands is high, and these fungi obviously play an important role in the decomposition process. Clearly, when comparing or assessing species diversity it is essential to take into account the morphologically hidden genetic differences.

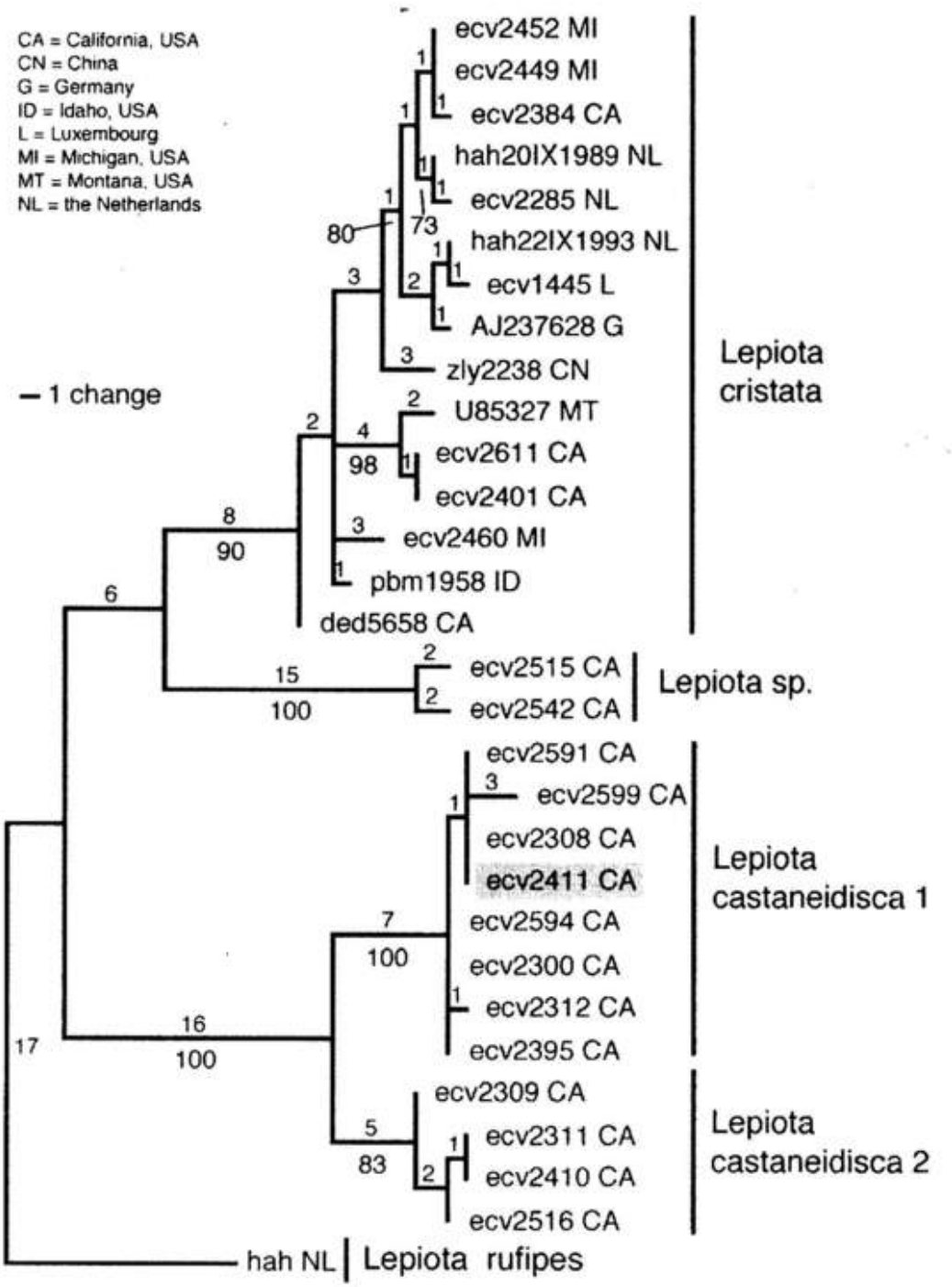

Fig. 1. Relationship between *L. cristata*, *L. castaneidisca*, and *L.* spec., with *L. rufipes* Morgan sensu European authors as an outgroup, based on parsimony analysis of the nuclear ribosomal DNA ITS-sequences. Collections are indicated with the collector's initials and collection number or date, or with a Genbank accession number, and a country or state code. Collection 'ecv2411' (shaded) is morphologically identical to *L. cristata*. One of 300 most parsimonious trees is shown. Bootstrap supports > 70 % are indicated under the branches, based on 500 replicates. The analysis is based on 72 parsimony-informative characters; tree length = 116, CI = 0.7414, RC = 0.6958.

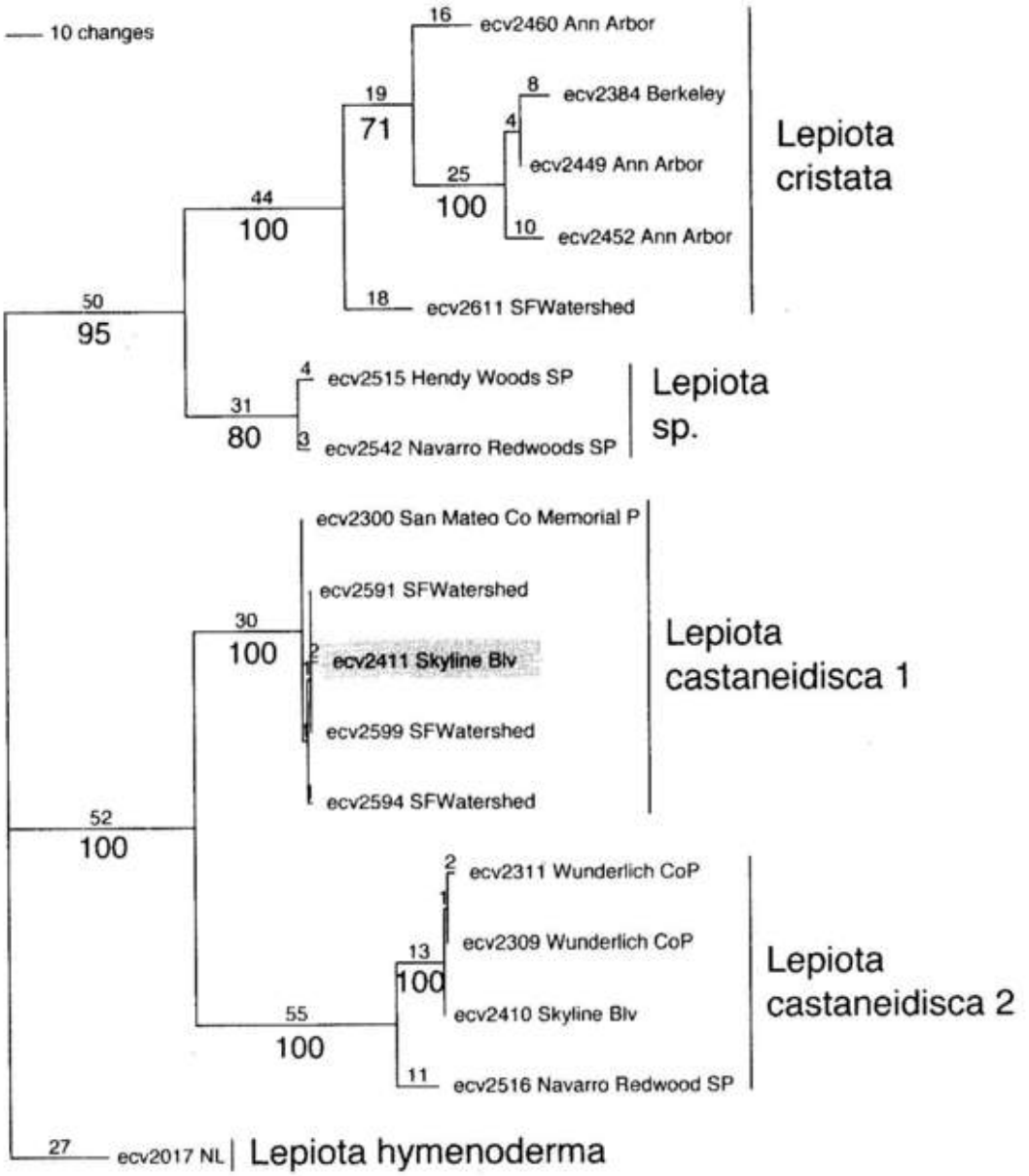

Fig. 2. Relationships between *L. cristata*, *L. castaneidisca*, and *L.* spec., with *L. hymenoderma* D.A. Reid as an outgroup, based on parsimony analysis of the nuclear ribosomal DNA IGS1-sequences. Collections are indicated with the collector's initials and collection number, and the place of origin. Collection 'ecv2411' (shaded) is morphologically identical to *L. cristata*. One of 18 most parsimonious trees is shown. Bootstrap supports > 70 % are indicated under the branches, based on 1000 replicates. The analysis is based on 251 parsimony-informative characters; tree length = 428, CI = 0.7874, RC = 0.7019.

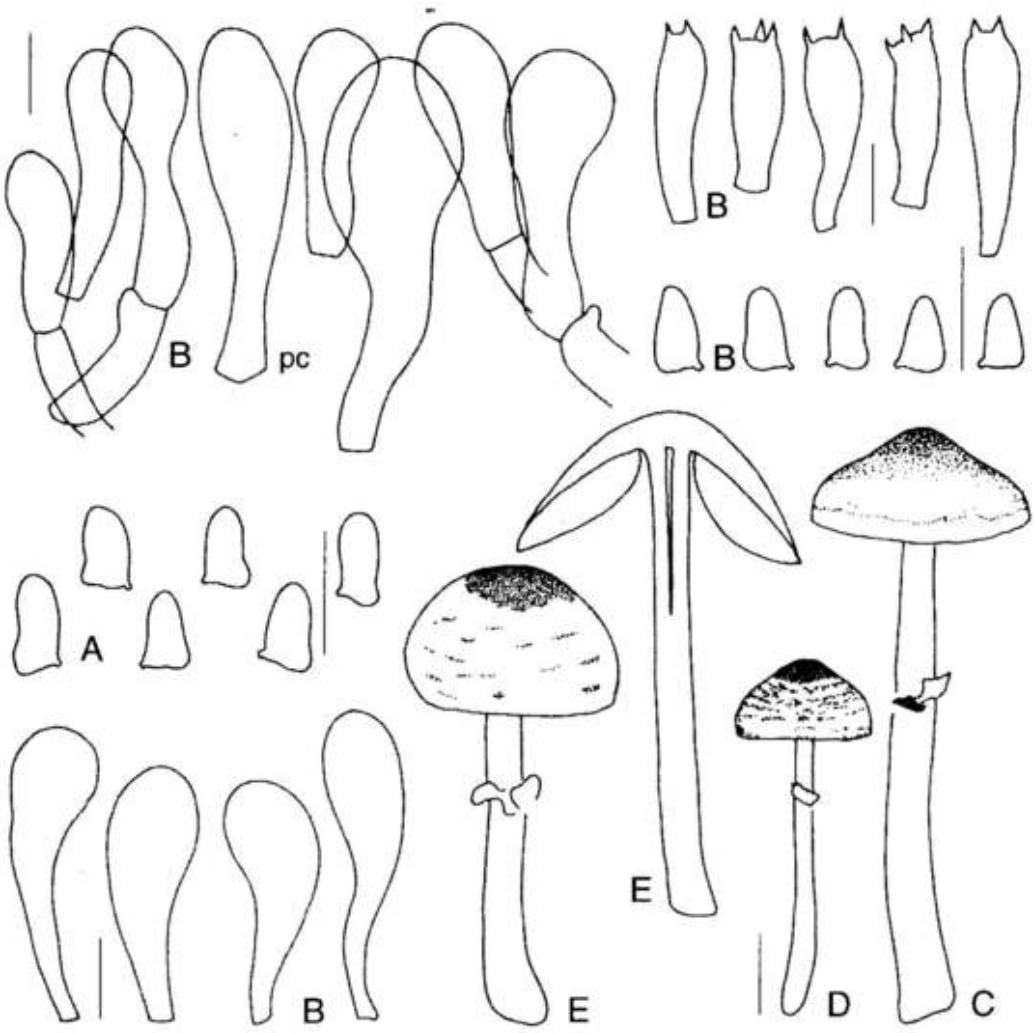

Fig. 3. *Lepiota castaneidisca* – Habitus, spores, basidia, cheilocystidia, and pileus covering (pc). A. from holotype; B. from coll. E.C. Vellinga 2516; C. from coll. E.C. Vellinga 2300; D. from coll. E.C. Vellinga 2395; D. from coll. E.C. Vellinga 2410. Scale bar is 1 cm for basidiocarps and 10 µm for microscopic structures.

DESCRIPTIVE PART

Lepiota castaneidisca Murrill in Mycologia 4: 232. 1912. – Fig. 3.

Pileus 8-32 mm, when young blunt-conical-paraboloid with inflexed margin, expanding via conico-convex, and campanulate-convex to convex with slightly inflexed to deflexed margin, orange reddish, warm (pinkish to reddish) brown to pale orange-brown (2.5 YR 4/4, between 5 YR 5/6 and 2.5 YR 5/8, 5 YR 5-4/6, 7.5 YR 7/8) at centre, in old specimens fading and losing the reddish tinges (c. 7.5 YR 6-5/4-6), smooth and closed, much paler around umbo (5 YR 7/5, 5 YR 7/3, 5 YR 8/4, 7.5 YR 8/5), and splitting up, especially in outer zone, into small pale, sometimes pale pink-cream, concentrical patches, on radially fibrillose whitish background; margin exceeding lamellae.

Lamellae, L = c. 40-45, l = 1-5, moderately crowded to moderately distant, free, slightly ventricose 2.5-5 mm wide, whitish when young, distinctly cream-coloured in older specimens, sometimes with pinkish sheen, with white irregular to fimbriate edge. Stipe 25-65 x 2-6 mm, cylindrical, slightly widened towards base, hollow, lengthwise creamy innately fibrillose, pinkish in lower part, discolouring reddish when bruised, especially in lower half, and in older specimens, with annulus, with white mycelial cords. Annulus ascending when young, in old specimens present as some remmants on stipe, completely brownish or with patches on underside as on pileus, with fringed upper rim, and cottony white inside. Context whitish, white-cream in pileus, firm and dull, in stipe shiny cream to red-brown in old specimens in lower half. Smell strong, sweet fruity-aromatic, of cut basidiocarps with rubber component of the *L. cristata* smell, sometimes with cod-liver oil component. Taste not recorded.

Spores [119,8,8] 5.0-9.0 x 3.0-4.0 µm, avl x avw = 6.4-8.1 x 3.1-3.7 µm, Q = 1.6-2.5, avQ = 1.95-2.2, in side-view triangular, clyindrical with spurred base, in frontal view oblong, hardly congophilous, dextrinoid, not colouring in Cresyl Blue. Basidia 18-30 x 5.0-8.0 µm, 4-spored; a few 2-spored basidia present. Lamella edge sterile; cheilocystidia 20-44 x 6.5-13.5 µm, narrowly clavate, clavate, more rarely cylindrical or spheropedunculate with short to long pedicel. Pleurocystidia not observed. Pileus covering a hymeniderm made up of elements of different length, 16-62 x 8.0-18 µm; most elements colourless, some with brown intracellular pigment, with slightly thickened walls; elements covered by a colourless layer. Stipitipellis a cutis of cylindrical non-coloured hyphae, c. 2.0-3.0 µm wide. Clamp-connections present in all tissues.

Habitat & distribution – Gregarious, terrestrial, and apparently saprotrophic, in *Sequoia sempervirens* groves, in stands of *Cupressus macrocarpa*, and in mixed *Quercus agrifolia* Nee forests. November to January. Widespread in coastal central and northern California.

Lepiota sp. (close to *L. cristata*) – Fig. 4.

Pileus 12-30 mm, campanulate when young, expanding to plano-convex and finally applanate, with low and/or very small umbo, at centre orange-brown, red-brown, or pinkish-orange-brown (c. 5 YR 5/6, 7.5 YR 4/4, 5/6, 6/6), closed and smooth at centre, around centre breaking up into concentrical rings of patches, more irregularly arranged near margin, on whitish background, paler than at centre. Lamellae, moderately crowded, free, slightly ventricose, cream or creamy, with white eroded edge. Stipe 35-60 x 1,5-3 mm, cylindrical, but slightly widened at utmost base, hollow, shiny white innately fibrillose on reddish background in lower half. Annulus in young specimens as a funnel around stipe, sometimes only clinging to pileus margin, later disappearing, white, with brown patches on underside. Smell strong, like *L. cristata*: like rubber and sweet.

Spores [40,2,2] 5.0-7.0 x 3.0-4.0 µm, avl x avw = 6.1 x 3.2 µm, Q = 1.65-2.1, avQ = 1.9, in side-view triangular, clyindrical with spurred base, in frontal view oblong, hardly congophilous, dextrinoid, not colouring in Cresyl Blue. Basidia 20-24 x 6.0-8.0 µm, 4-spored. Lamella edge sterile; cheilocystidia 19-35 x 7.0-11 µm, narrowly clavate, clavate, to spheropedunculate, slightly thick-walled, with refractive contents. Pleurocystidia not observed. Pileus covering a hymeniderm made up of elements of different length, 21-64 x 5.0-14 µm, most elements colourless, some with brown intracellular pigment, with slightly thickened walls; elements covered by a colourless layer. Stipitipellis a cutis of cylindrical, colourless hyphae,2.0-3.0 µm in

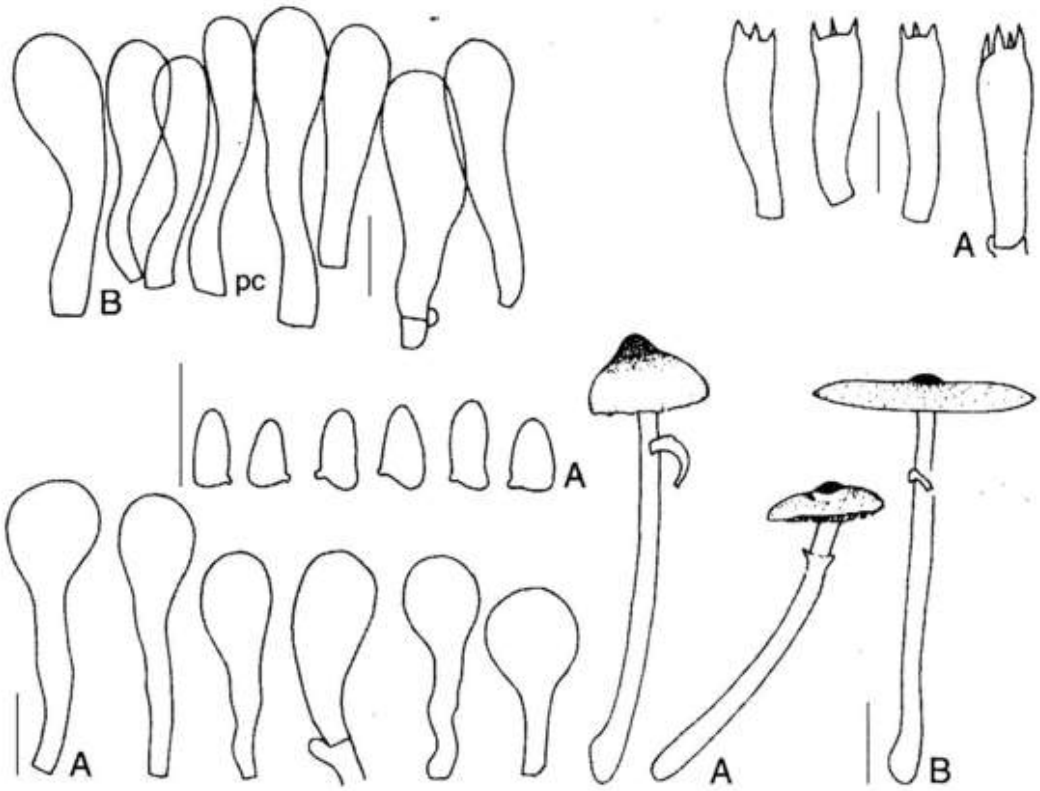

Fig. 4. *Lepiota* sp. – Habitus, spores, basidia, cheilocystidia, and pileus covering (pc). A. from coll. E.C. Vellinga 2542; B. from coll. E.C. Vellinga 2515. Scale bar is 1 cm for basidiocarps and 10 µm for microscopic structures.

diameter. Clamp-connections present in all tissues

Habitat & distribution – In groups, terrestrial, in woods of *Sequoia sempervirens*. November. Known from two localities in Mendocino Co. (California).

ACKNOWLEDGMENTS

Collections for this study were received from Dr Z.L. Yang (HKAS, Kunming, China), Henk A. Huijser (Nuenen, the Netherlands), P. Brandon Matheny (WTU, Seattle, WA), and the herbaria at SFSU (Dennis E. Desjardin) and NY. Research funding was provided by the Foundation 'Rijksherbariumfonds Dr. E. Kits van Waveren'. John Lennie's and Tom Bruns' comments on earlier drafts were of great value.

REFERENCES

Arora, D., 1986. Mushrooms demystified. A comprehensive guide to the fleshy fungi. Ed. 2. Ten Speed Press, Berkeley. 959 pp, 217 pl.

Bon, M., 1993. Flore mycologique d'Europe 3. Les Lépiotes. Doc. mycol. Mémoirs hors série no. 3. Lepiotaceae Roze.

Candusso, M. & G. Lanzoni, 1990. Lepiota s.l. Fungi europaei 4. Giovanna Biella,

Saronno. 743 pp, 80 pl.
Gardes, M. & Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. Mol. Ecol. 2: 113-118.
Henrion, B., F. Le Tacon & F. Martin, 1992. Rapid identification of genetic variation of ectomycorrhizal fungi by amplification of ribosomal RNA genes. New Phytologist 122: 289-298.
Holmgren, P.K., N.H. Holmgren & L.C. Barnett, 1990. Index Herbariorum Part 1. The herbaria of the world. Ed. 8. Regnum vegetabile 120: xi + 1-693.
Horak, E., ('1980') 1981. On Australasian species of Lepiota S.F. Gray (Agaricales) with spurred spores. Sydowia 33: 111-144.
Johannesson, H., S. Ryman, H. Lundmark & E. Danell, 1999. Sarcodon imbricatus and S. squamosus – two confused species. Mycol. Res. 103: 1447-1452.
Kyde, M.M. & J.L. Peterson, 1986. A variant of Lepiota cristata. Mycologia 78: 851-853.
Munsell soil color charts, 1975. Baltimore, Maryland.
Murrill, W.A., 1912. The Agaricaceae of the Pacific Coast II. Mycologia 4: 231-262.
Murrill, W.A., 1914. Agaricaceae. North American Flora 10 (1): 41-65.
Simmons, M.P. & H. Ochoterrena, 2000. Gaps as charcters in sequence-based phylogenetic analyses. Syst. Biol. 49: 369-381.
Singer, R., 1986. The Agaricales in modern taxonomy. Ed. 4. Koeltz Scientific Books, Koenigstein. 981 pp, 88 pl.
Smith, H.V., 1966. Contributions toward a monograph on the genus Lepiota, I. Type studies in the genus Lepiota. Mycopath. & Mycol. appl. 29: 97-117.
Sundberg, W.J., 1989. Lepiota sensu lato in California III. Species with a hymeniform pileipellis. Mycotaxon 34: 239-248.
Swofford, D. L. 2001. PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4. Sinauer Associates, Sunderland, Massachusetts.
Vellinga, E.C., 2001a. Lepiota. In M.E. Noordeloos, Th.W. Kuyper & E.C. Vellinga (eds). Flora agaricina neerlandica 5 (in the press).
Vellinga, E.C., 2001b. Studies in Lepiota III. Some species from California, U.S.A. Mycotaxon 80: 285-295.

APPENDIX

List of collections examined for this study, and Genbank accession numbers for the ITS- and IGS1-sequences resp. Herbarium abbreviations are according to Holmgren et al. (1990).

Lepiota cristata
China, Sichuan Prov., Xiangcheng Co., in Maxingou near Riying (alt. 3600 m.), 9-VII-1998, Z.L. Yang 2238, AF391044 (HKAS).
Luxembourg, Hollenfels, 28-IX-1988, E.C. Vellinga 1445, AF391027 (L).
The Netherlands, prov. Zuid-Holland, Leiden, 11-X-1998, C. Bas (coll. E.C. Vellinga 2285), AF391048 (L); prov. Noord-Brabant, Eindhoven, Philips de Jong park, 20-IX-1989, H.A. Huijser, AF391043 (L); prov. Limburg, Wijlre, Stokhem, 22-IX-1993, H.A. Huijser, AF391042 (herb. Huijser).
U.S.A., California, Alameda Co., Berkeley, 9-XII-1999, E.C. Vellinga 2384, AF391049; AF391069 (UC); San Mateo Co., San Francisco Watershed, near Pulgar Water Temple, 10-XII-1999, E.C. Vellinga 2401, AF391046 (UC); ibidem, 8-XII-2000, E.C. Vellinga 2611 AF391045, AF391068 (UC); Santa Barbara Co., Los Padres National Forest, Figuarora Campground, 30-I-1993, D.E. Desjardin 5658, AF391050 (SFSU); Idaho, Bonner Co., Priest Lake, 24-IX-2000, S. Clark (coll. P.B. Matheny 1958), AF391051 (WTU); Michigan,