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***Clitocybula canariensis* (Tricholomataceae), a new brown-rot fungus from the Canary Islands (Spain)**

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The new brown-rot fungus *Clitocybula canariensis* is described, based on morphological features and habitat. It was found fruiting on dead wood of *Pinus radiata* and is characterized by greyish-brown basidiomata with a grey fibrillose to scaly pileus, furfuraceous stipe at the apex, greyish decurrent lamellae, the presence of pileo-, caulo- and cheilocystidia and amyloid spores. *Clitocybula canariensis* is similar to the North American species *C. atrialba* in spore width and to *C. abundans* and *C. oculus* by the presence of cheilocystidia. The inclusion of this taxon in *Clitocybula* is discussed and differences with other cheilocystidiate species fruiting on coniferous wood are emphasized. *Clitocybula oculata* is placed into synonymy with *C. atrialba*. The presence of white-rot and brown-rot species within *Clitocybula* and the ability to degrade organopollutants are also discussed.

**Key words:** brown-rot, *Clitocybula*, macrofungi, Spain, taxonomy, Tricholomatales

## **Introduction**

In the course of a revision of “omphalinoid” fungi collected by one of the authors (RMD) in La Palma (Canary Islands, Spain), a new wood-rotting fungus of the genus *Clitocybula* (Singer) Métrod, was found and is here described, based on morphological and ecological features. The name proposed for the new taxon is *C. canariensis*, dedicated to the islands where the fungus was found.

The mycobiota of the Canary Islands is incompletely known and, probably, a high number of new species of agarics are still undescribed. Accordingly, seven new taxa of *Entoloma* (Fr.) P. Kumm. (Wölfel and Noordeloos, 2001) and a new species of *Hygrocybe* (Fr.) P. Kumm. (Bañares and Arnolds, 2002) have been recently described from the islands La Palma and La Gomera. The description of new species of the genus *Clitocybula* from

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poorly explored areas of the world is rather interesting, since some wood-rotting fungi have been reported to be implicated in bioremediation treatments for organopollutants (Pointing, 2001) and to decolourize synthetic dyes with structural similarities to lignin (Jarosz-Wilkolazka *et al.*, 2002). The white-rot fungus *Clitocybula duseinii* (Bres.) Singer [= *Hydropus duseinii* (Bresadola) Singer, Singer (1969, 1982)] isolated in North Patagonia (Argentina), has been reported to decolourize dye-containing effluent from a textile plant (Wesenberg *et al.*, 2002).

The genus *Clitocybula* includes about 10 species (Singer, 1978; Kirk *et al.*, 2001), mainly growing on wood, that were originally placed in *Collybia* (Fr.) Staude *nom. cons.*, *Clitocybe* (Fr.) Staude, *Fayodia* Kühner and *Omphalina* Qué. *nom. cons.* It was elevated to generic rank by Métrod (1952) and recent molecular phylogenetic analysis recognized it as a natural group within *hydropoid* clade (clade 27), together with *Gerronema* Singer *pro parte*, *Megacollybia* Kotl. & Pouzar and *Hydropus* Kühner *sensu stricto* (Moncalvo *et al.*, 2002). The reduced “cyphelloid” fungus *Porotheleum fimbriatum* (Pers.) Fr. is derived from *Hydropus* species, and is also included in this clade.

The main diagnostic characters of *Clitocybula* are the following: i) radially fibrillose to squamulose pileus surface; ii) absence of pleurocystidia and cellular hypoderm; iii) presence of cheilo-, pileo- and caulocystidia; iv) amyloid smooth spores; and v) lignicolous habitat. Molecular phylogenetic analyses have demonstrated that the genus *Hydropus sensu* Singaer (1986) is polyphyletic, and *Hydropus sensu stricto*, based on the type species *H. fuliginarius* (Fr.) Kühner ex Singer, is closely related to *Clitocybula*. However, *Hydropus* can be differentiated from *Clitocybula* by the absence of radially fibrillose pilei and the presence of laticifers (Kühner, 1938; Horak, 1968; Moncalvo *et al.*, 2002).

Most species of *Clitocybula* are known from North America (Bigelow, 1973; Singer, 1978), but a few species have been reported also from Europe (Bon, 1997), Asia and tropical areas (Singer, 1978). They grow mainly on dead wood or logs of both, hardwoods and conifers, and very rarely on earth (Bigelow, 1973; Singer, 1978). Four species and two varieties have been reported from Europe (cf. Bon, 1997) and only three species and one variety have been found previously in Spain: *C. lenta* (Maire) Malençon & Bertault (cf. Maire, 1933; Calonge and Tellería, 1980; Esteve-Raventós and Moreno, 1985; Ortega, 1992; Constantino and Siquier, 1996; Mendaza, 1999 as *C. lenta* var. *odorata* Bon), *C. lacerata* (Lasch) Singer (cf. Tabarés and Pascual, 1997; Llistosella *et al.*, 1998) and *C. taniae* Vila (Vila, 2002). *Clitocybula lenta* has also been reported from Morocco fruiting on hardwoods (Malençon and Bertault, 1975).

The new species here proposed, *C. canariensis*, was found in La Palma (Canary Islands), fruiting on dead wood of *Pinus radiata* D. Don (type material: AH 35146) and on unidentified dead wood. It is described below on the basis of morphological and ecological features, and is compared with *C. atrialba* (Murrill) Singer because of similar spore width, and compared with *C. abundans* (Peck.) Singer and *C. oculus* (Peck.) Singer, because of the presence of cheilocystidia.

### Materials and methods

The material studied of *C. canariensis* is deposited in AH and the private herbarium of R.M. Dähncke (Dä). Type material and additional collections of *C. atrialba* and *C. oculata* were borrowed from NY and compared with the new species.

A total of ten samples of *P. radiata* wood degraded by *C. canariensis* (AH 36146 holotype) were collected by one of the authors (RMD), and examined by JMB to determine type of rot. Type of rot can be determined by the colour of wood and relating the proximity of the decay to an identifiable fungal fruit body (Rayner and Boddy, 1988).

Drawings were made with a camera lucida device coupled to an Olympus BX 50 microscope. Mycological terminology is in agreement to Kirk *et al.* (2001). Colour of basidiomata was determined according to Munsell (1994). Thirty spore measurements from at least one basidiome of each studied collection were obtained to determine spore size following the Q method of Heinemann and Rameloo (1985).

### Key to species of the genus *Clitocybula* in North America and Europe

1. Pileocystidia acuminate, with thick walls (-3 µm); strong nitrous smell; in Mediterranean areas.....*Leucoinocybe lenta* (Maire) Singer [= *C. lenta* (Maire) Malençon & Bertault] 2
1. Pileocystidia not so; without such smell.....2
2. Cheilocystidia present.....3
2. Cheilocystidia absent.....6
3. Cheilocystidia irregularly cylindrical to subclavate, showing greyish-brown vacuolar content; Canary Islands, on *Pinus* debris. *C. canariensis* Barrasa, Esteve-Rav. & Dähncke 4
3. Cheilocystidia hyaline .....4
4. Cheilocystidia fusiform-acuminate to rostrate, thin-walled; Catalonia (Spain), gregarious on grassy areas in littoral dunes.....*C. taniae* Vila 5
4. Cheilocystidia clavate, subclavate or subcylindrical; often growing caespitose.....5

5. Stipe greyish to dark grey, squamulose; spores subglobose to broadly elliptic, 5-6.5 × 4-5.5 μm; gregarious to subcaespitose on hardwoods..... *C. oculus* (Peck) Singer
5. Stipe white, only pruinose at apex; spores 4.5-6(-7.5) × 3.5-5.5(-6) μm; densely caespitose, either on hardwoods or conifers ..... *C. abundans* (Peck) Singer
6. Spores 6-9 μm broad; stipe furfuraceous to squamulose over entire length, covered with brown to blackish squamules.....  
..... *C. atrialba* (Murrill) Singer [= *C. oculata* (Murrill) H.E. Bigelow]
6. Spores 3-5(-6) μm broad; stipe not squamulose .....7
7. Spores globose, 3-4(-5) μm broad ..... *C. familia* (Peck) Singer<sup>1</sup>
7. Spores ellipsoid to broadly ellipsoid, 5-8 × 4.5-6 μm ..... *C. lacerata* (Lasch) Singer<sup>2</sup>

<sup>1</sup>*C. familia* var. *compressa* (Romagn.) H.E. Bigelow: pileus darker, brown-ochraceous, lamellae adnate to subdecurrent, taste bitterish; Europe.

<sup>2</sup>*C. lacerata* var. *odorata* Bon: strong smell of orange blossom or tangerine; Europe.

## Results

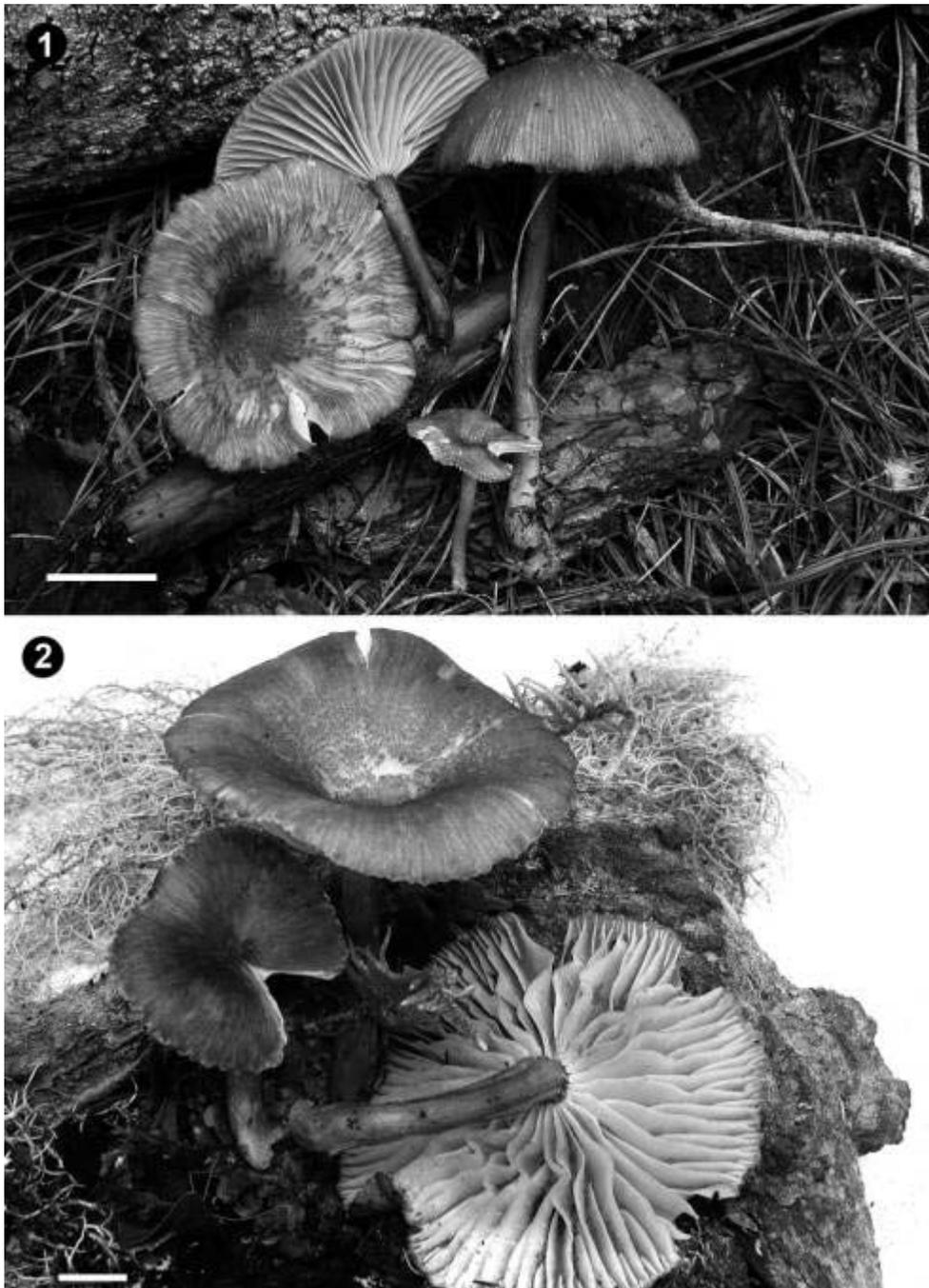
### *Clitocybula canariensis* Barrasa, Esteve-Rav. & Dähncke, **sp. nov.** (Figs. 1, 2)

Mycobank number: MB500699.

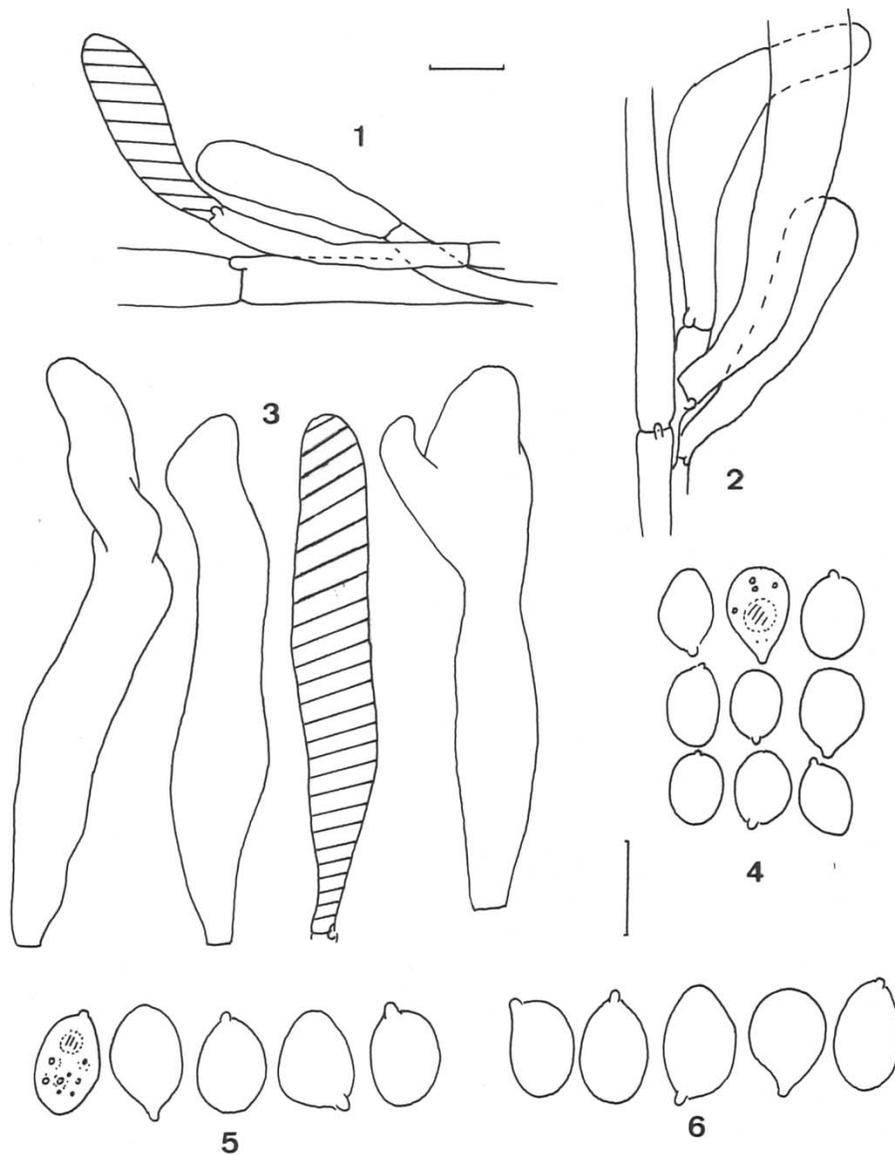
*Etymology*: From the Canary Islands, referring to the type locality.

*Pileo* griseo-fusco, 30-70 mm lato, convexo vel umbilicato, sulcato, minute fibrilloso-furfuraceo vel squamato. *Lamellis* griseolis, decurrentibus, subdistantis, acie brunneolis ad maturitatis. *Stipite* concoloro, 40-100 × 4-8 mm, cylindrico, ad apicem atro-punctato. *Inodoro*. *Sporis* 7.2-9.5 × 5.8-7.2 μm, laevis, subglobosis vel ovoideis, debiliter amyloideis. *Cheilocystidiis* 55-80(-100) × 10-15 μm, cylindricis versus subclavatis, saepe leviter lobatis vel apices, pigmento vacuolari fusco repletis. *Dermatocystidiis* plerumque vesiculosi in pileo et stipite. Ad ligno putrido in *Pineto*.

*Basidiomata* gregarious or isolated. *Pileus* 30-70 mm diam., convex at first, then expanding to applanate with depressed disc, finally umbilicate to infundibuliform; margin slightly decurved at first, becoming straight in depressed pilei, entire or crenulate, not or hardly striate at extreme margin; surface matted-fibrillose to furfuraceous, especially at centre, soon radially streaked towards the margin on a whitish background, the disc remaining uniformly furfuraceous to scaly in a concentric pattern; surface slightly hygrophanous, colour sepia to greyish brown (Munsell 10YR 4/1-2, 3/1-2), slightly paler on drying. *Lamellae* arcuate-decurrent, ending abruptly adnate towards the stipe forming a pseudocollarium (*Pseudoclitocybe cyathiformis*-like), subdistant (L = 35-45; l = 1-3), occasionally intervenose; colour light grey (Munsell 2.5Y 7/1-2, 6/1); edges even to finely crenate, concolorous at first, browning in mature specimens. *Stipe* 40-100 × 4-8 mm, central, cylindrical to slightly enlarged at base, elastic; rhizomorphs not seen; surface scurfy to furfuraceous at the upper half and decorated with dark-greyish dots, smooth towards the base, concolorous to pileus and covered with whitish mycelium at extreme base, fistulose with age. *Context* whitish in pileus, pale



**Fig. 1.** *Clitocybula canariensis*. **1.** Basidiomata (from holotype, AH 35146). **2.** Basidiomata (from AH 35147). Bars = 10 mm.



**Fig. 2.** *Clitocybula canariensis* (from holotype AH 35146). 1. Pileocystidia. 2. Caulocystidia. 3. Cheilocystidia. 4. Spores. *Clitocybula atrialba* (from holotype NY 259). 5. Spores. *Clitocybula oculata* (from holotype NY 835). 6. Spores. Bars= 10  $\mu$ m (large bar for spores).

grey in the stipe. *Odour* not distinctive. *Taste* mild. *Spore deposit* pale cream. *Basidiospores* 7.2-9.5  $\times$  5.8-7.2  $\mu$ m ( $Q_m = 1.23$ -1.28-1.33), subglobose to ovoid, smooth, pluriguttulate, weakly amyloid, with prominent apiculus. *Basidia* 28-45  $\times$  7-9  $\mu$ m, clavate, four-spored, filled with hyaline to pale brown vacuolar pigment, sterigmata up to 3  $\mu$ m long. *Cheilocystidia* numerous, 55-80(-100)  $\times$  10-15  $\mu$ m, mostly cylindrical, also subclavate or narrowly utriform, with undulate to lobate outline towards the apex, mostly filled with pale brown

intracellular pigment. *Lamellar edge* heterogeneous. *Hymenophoral trama* of parallel to subparallel hyphae 4-10 µm in diam., mostly cylindric, filled with intracellular brown pigment. *Pileipellis* consisting of a cutis of interwoven hyphae, 4-20 µm in diam., with pileocystidia protruding or recumbent, similar in shape and somewhat smaller than cheilocystidia, all elements filled with intracellular brown pigment. *Oleiferous hyphae* not seen. *Caulocystidia* grouped in clusters on the upper stipe surface, similar to cheilocystidia, filled with brown pigment. *Stipe trama* sarcodimitic.

*Habitat*: gregarious on dead wood of *Pinus radiata*.

*Type of rot*: brown rot.

*Known distribution*: La Palma Island (Canary Islands).

*Material examined*: SPAIN, Canary Islands, La Palma, Pared Vieja, 1200 m, on dead wood of *Pinus radiata*, 18 September 2004, R.M. Dähncke (Dä 1965; AH 35146 **holotype**); *ibid.*, Pequeño Perú, 750 m, on dead wood, 24 November 2003, R.M. Dähncke (Dä 1788; AH 35147); *ibid.*, Refugio de la Pared Vieja, 1200 m, on dead wood, 24 October 2004, R.M. Dähncke (Dä 2005; AH 35148).

*Material examined for comparison*: *Clitocybula atrialba* (Murrill) Singer: **U.S.A.**: WASHINGTON, Seattle, on decayed buried wood in woods, October 20-November 1, 1911 (NY, W.A. Murrill 259-holotype + photo.); *ibid.* (NY W.A. Murrill, 249); *ibid.* (No. XII, paratype?); WASHINGTON, Lower Tahoma Creek, Mt. Rainier Nat. Park, 21 September 1954, A.H. Smith, H.E. Bigelow # 2103 part, card. (MASS donated to NY s.n.); WASHINGTON, Bremerton, on cones + among rotting sticks, 19 November 1930, J.B. Flett #43 (NY 87, two collections); CALIFORNIA, Lily Lake, Marin County, 12 December 1971, Victoria Thynne, H.E. Bigelow 17046 (MASS donated to NY s.n.). CALIFORNIA, La Honda, near Palo Alto, dense redwood forest with a few deciduous trees on west slopes of Santa Cruz mountains, below 1000 ft. elevation of cascade mountains and in river valley, 25 November 1911, W.A. Murrill and L.A. Abrams (NY 1263). *Clitocybula oculata* (Murrill) Bigelow: **U.S.A.**: OREGON, Mill City, virgin forest of conifers and a few hardwood trees on foothills of cascade mountains and in river valley, elevation 800-1200 ft., 9 November 1911 (NY, W.A. Murrill 835-holotype).

*Notes*: All the collections studied were collected on dead wood. All wood degraded samples examined showed brown colour, indicating that *C. canariensis* is a brown-rot fungus. Whilst type collection (AH 35146) was found on dead wood of *Pinus radiata*, two additional collections (AH 35147 and AH 35148), were found fruiting on a log of unidentified wood in a laurel forest mixed with *Pinus* sp.

## Discussion

The genus *Clitocybula* is distributed mainly in North America (Bigelow, 1973; Singer, 1978). The type material of *C. canariensis* was found on dead wood of Monterey pine (*Pinus radiata*), a North American conifer that has been introduced frequently in plantations in the Western Canary Islands. This might suggest that *C. canariensis* could have also been introduced in the islands with plantations of this pine. Although additional collections of *C.*

*canariensis* were found growing on unidentified dead wood in mixed forest of conifers and deciduous trees, the possibility that they were also fruiting on *Pinus radiata* wood can not be rejected.

*Clitocybula canariensis* is similar to *C. atrialba* in pileo- and caulocutis, and in spore width. Within *Clitocybula*, two groups of species were established according to spore size (Bigelow, 1973). *Clitocybula canariensis*, together with *C. atrialba*, are included in the group having large spores (6-9  $\mu\text{m}$  wide). *Clitocybula canariensis* is differentiated from *C. atrialba* in the matted-fibrillose to furfureaceous or adpressed-scaly pileus, greyish sepia colors, decurrent lamellae which end abruptly adnate towards the stipe and in the presence of cheilocystidia. On the other hand, *C. canariensis* is similar to *C. oculus* and *C. abundans* (two species having spores less than 6  $\mu\text{m}$  wide), in the presence of cheilocystidia. The presence/absence of different types of cystidia on fungal tissues, has been emphasized and correlated with molecular phylogenetic analysis in several omphalinoid agarics. Thus, the genera *Contumyces* Redhead, Moncalvo, Vilgalys and Lutzoni, *Loreleia* Redhead, Moncalvo, Vilgalys and Lutzoni and *Sphagnomphalia* Redhead, Moncalvo, Vilgalys and Lutzoni, were established not only on the basis of molecular analysis, but also on morphology and types of cystidia (Redhead *et al.*, 2002). In agreement with this taxonomic criterion, *C. canariensis* would have to be considered phylogenetically related to cheilocystidiate species within the genus *Clitocybula*, but this assumption should be corroborated with molecular phylogenetic analyses.

After the examination of herbarium material (including types) of *C. atrialba* and *C. oculata*, we could not find significant morphological differences between both. They seem to be identical in caulo- and pileocystidia and spore size and shape. Dry specimens were also very similar in colour of pileus, lamellae and stipe. Both can also be found on mixed forests of coniferous and hardwoods (Bigelow, 1973). *Clitocybula oculata* was found only once by W.A. Murrill in 1911 and type material (NY 835) includes only a small broken basidioma. The only difference observed by Bigelow (1973), was related to the more pallid to avellaneous colour of pileus. Therefore, we consider this chromatic difference within the variation of a single species and consider them conspecific.

Like many *Clitocybula* species, *C. canariensis* is also a wood-rotting fungus and produces brown rot. Wood-rotting fungi are characterized by their ability to degrade lignin and cellulose and are the most important agents implicated in wood degradation. Among wood-rotting fungi, white-rot fungi are the most efficient in extensive lignin degradation. These fungi secrete three extra cellular enzymes (lignin peroxidase, manganese peroxidase and laccase)

that are essential to degrade the complex and recalcitrant polymer of lignin (Rayner and Boddy, 1988; Hatakka, 1994). Interestingly, these enzymes are non-specific of substrate and can also degrade other recalcitrant environmental compounds with similar chemical structure to lignin (Reddy, 1995). Moreover, ligninolytic enzymes have been reported to be very variable depending of fungal species (Wesenberg *et al.*, 2003) and more fungal screening of wood-rotting fungi to degrade organopollutants would be necessary. In this way, the South American white-rot fungus *C. duseinii* has been recently reported to decolourize raw mixed-dye wastewater from a textile dye-producing plant (Wesenberg *et al.*, 2002). Although no specific peroxidases were isolated from culture liquids (Freitag and Morrel, 1992), some brown-rot basidiomycetes [i.e. *Coprinus micaceus* (Bull.) Fr. and *Fomitopsis pinicola* (Sw.) P. Karst.], have been reported also to be effective decolourizers of polymeric dyes in solid-agar media (Jarosz-Wilkolazka *et al.*, 2002). This indicates different mechanisms in decolourization of dyes between white-rot and brown-rot fungi. Since many of the species included in the genus *Clitocybula* seem to be specialized in wood decay, the description of the new brown-rot fungi *C. canariensis* from poorly known mycological areas, such as Canary Islands, is interesting in order to know its ability to degrade polluting substances.

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