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## ***Hemimycena conidiogena,* a new cistophilous basidiomycete**

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**Abstract** - An ecological study of *Cistus* vegetation in Catalonia (Spain) revealed the existence of a previously undescribed species of *Hemimycena* section *Lactae* characterized by a yellowish tinge on mature basidiomata and the production of blastoconidia on cap and stipe. This species, close to *H. lactea*, is described as *Hemimycena conidiogena*. Ecological distribution, taxonomical affinities, systematic position and nature of blastoconidia are discussed.

**Résumé** – Une étude écologique des cistaies en Catalogne (Espagne) a révélé l'existence d'une espèce inédite du genre *Hemimycena*, section *Lactae*, caractérisée par la teinte jaunâtre des basidiomes mûrs et par la production de blastoconidies sur le chapeau et le pied. Cette espèce voisine de *H. lactea* est décrite ici sous le nom de *Hemimycena conidiogena*. La répartition écologique, les affinités taxinomiques, la position systématique et la nature des blastoconidies sont discutées.

**Key words** - Basidiomycetes, *Tricholomataceae*, *Hemimycena lactea*

## Introduction

Since 1997, two of us (J. V. and X. Ll.) have been surveying the fungal community of the rockrose bush (*Cistus* spp.) associations in Catalonia. Based initially on several plots located in the Cap de Creus i Serra de Verdera Natural Park (NE of Catalonia), the fieldwork was extended to similar sites including Collserola Park (Barcelona) and Serra de Prades (SW Catalonia). The fungal community of the first area has been described by Vila & Llimona (1998, 1999).

In both reports, one *Hemimycena* species was reported under the name *H. candida* (Bres.) Singer. During the C.E.M.M. Congress (Girona, 2001), two of the authors (P.-A. M. and M. À. P.-De-G.) rejected this identification; further studies revealed a previously undescribed species with interesting and unusual microscopic features, described here as *Hemimycena conidiogena*. Dr M. Noordeloos was kind enough to check our

observations and confirm the specificity of our taxon (cf Antonin & Noordeloos, 2004).

## Methods

The first collections were made during the establishment of fungal records, following Darimont's (1975) methodology simplified as set forth in Vila & Llimona (1999). The collections were photographed, described, and dried. Tissues were rehydrated in 5% KOH and stained with Congo red; additional observations were made in Melzer's and Cotton blue. Cytological observations (nuclei staining) were made in acetic carmine from exsiccates.

## Material studied

The examined collections are kept in the following herbaria: BCN-SCM and BCN-Myc (Centre de Documentació de Biodiversitat Vegetal, CERBIV, Parc Científic de Barcelona, Universitat de Barcelona), JVG (personal herbarium of Jordi Vila), PG (personal herbarium of Miquel À. Pérez-De-Gregorio), and LIP (Laboratoire de Botanique, Faculté des Sciences Pharmaceutiques et Biologiques, Université Lille II).

## Taxonomy

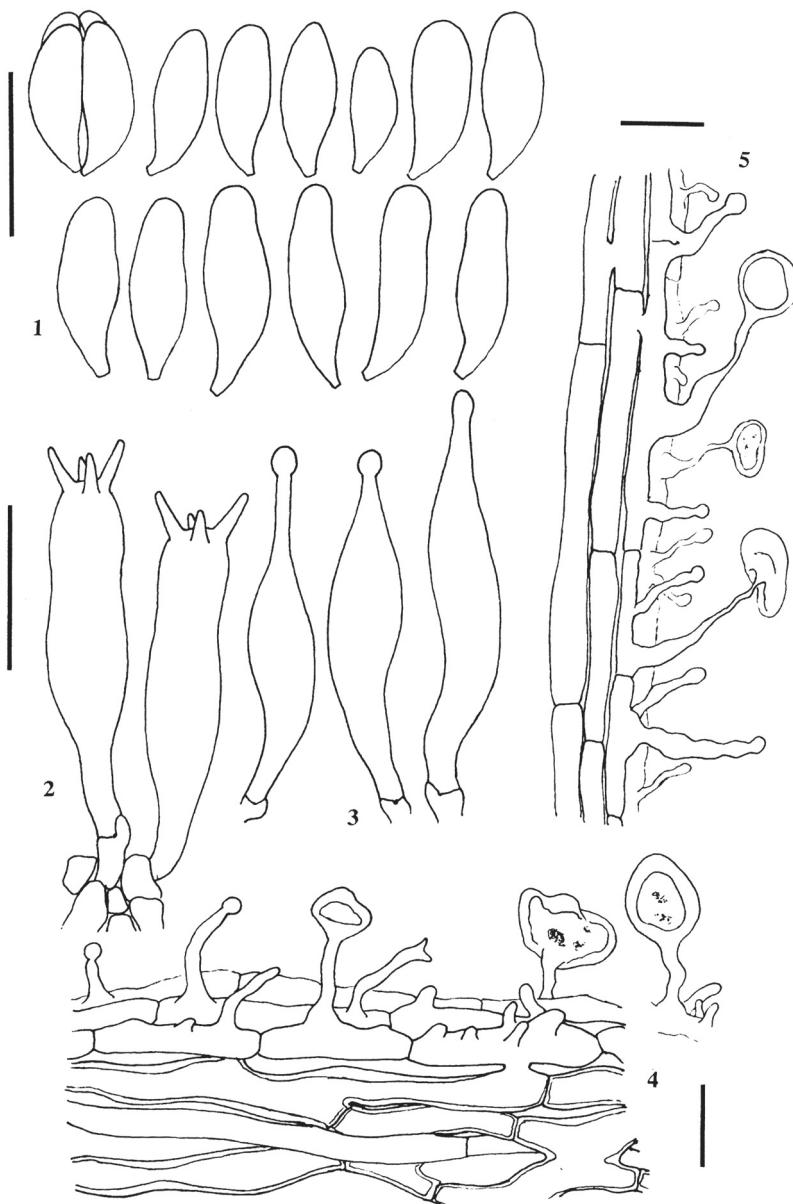
***Hemimycena conidiogena*** Vila, P.-A. Moreau & Pérez-De-Gregorio, sp. nov.

Fig. 1-11.

*Pileus 5-15 mm in diam., hygrophanus, laevis, a transparentia striata pure candida deinde dilute ochraceo-lutescente. Laminae adnatae, paucе densae, relative crassae, candidae deinde lutescentes. Stipes teres, 15-25 × 1-2 mm, albescens, leviter lutescente, subtiliter pruinosus, basaliter pilosus. Caro pertenuis, albescens; odor et sapor inconspicui. Sporae (9)-10-12,5-(13) × 3,5-4,5-(5) µm, hyalinae, inamiloideae, non cyanophilae, anguste obovatae vel flexuosaе, apice rotundatae, saepe conspicuam supraapicularem depressionem instructae. Basidia 22-30 × 6,5-8,5 µm, tetrasporata, Lamellae acie fertilia, cum rara vel interdum frequentes cheilocystidia, 18-30 × 5,5-7 µm, fusiformes, apice manifeste capitulati, × 2,5-3,5 µm. Pleurocystidia desunt. Evaginations ex breviter diverticulis aut pilis capitulatis usque ad 15-(20) µm longis; blastoconidia libera rara, 6,5-11 × 3,5-6 µm. Stipitipellis ex pileipelle homologam, cum pilos capitulatos longiores, ad 25-(35) µm, et cum capitulos latores, ad 8-9 µm, saepe in blastoconidiis transformatis. Fibulae in hymenio abundantes, rarae aut inconspicuae in alis partibus basidiomatis. Holotypus prope vicum Roses dictum, in NE Catalonia (Hispania), a X. Llimona et J. Vila lectus, 23-1-2001, 95 m altitudinis, sub Cisto monspeliense et C. albide, in herb. BCN-SCM B 4063 conservatus.*

*Etymology:* *conidium* = light dust, accessory spore, from the Greek κονίς = dust; and *gena* = that produces, as feminine adjective.

**Macroscopic description - Pileus** 5-15 mm in diam., initially conic or conico-convex, at maturity more extended-flattened, usually not umbonate, hygrophanous, slightly depressed in some old basidiomata; margin straight; surface smooth, striate, initially pure white, later gradually more yellowish to ochraceous. **Gills** not crowded, 16-20 reaching the stipe, 1 (2) series of lamellules, convex, adnate, comparatively thick,



**Fig. 1-5:** *Hemimycena conidiogena* (PAM01012301). 1: spores. 2: basidia. 3: cheilocystidia. 4: pileipellis (radial cut) and detail of a blastosporal structure (right). 5: stipitipellis (radial cut).

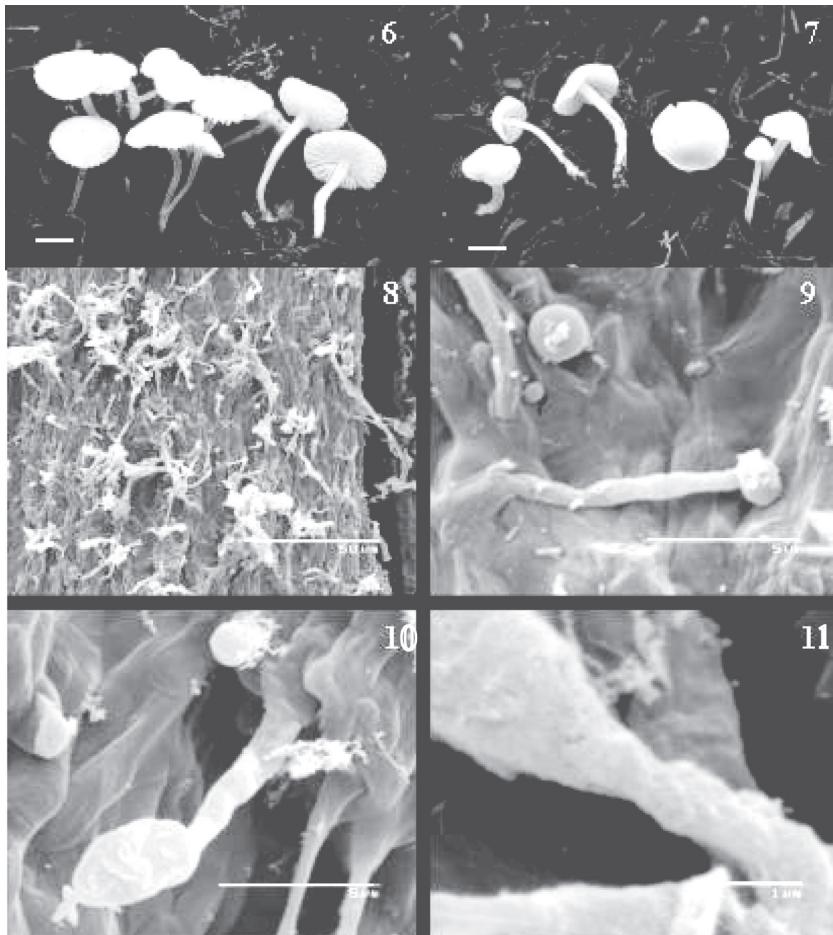
white, later yellowish or faint ochraceous; lamellulae present. **Stipe** 15-25 × 1-2 mm, cylindrical, whitish, later faintly yellowish, slightly translucent, at maturity with a thin pruina all over and basal hairs. **Flesh** very thin, whitish. Smell and taste not distinct.

**Microscopic description** (based mainly on exsiccates: *PAM01012301*, isotype, and *PAM96012101*) - **Spores** (9)-10-12,5-(13) × 3,5-4,5-(5) µm, hyaline, not amyloid, not cyanophilic, with homogenous content (dry material), cylindrical with prominent apiculus to flexuous, with rounded apex, often wider in middle part and with often marked supraapical depression. **Basidia** 22-30 × 6,5-8,5 µm, all 4-spored, clamped, cylindrical, often tapering forwards toward the apex; sterigmata straight, conical, 3,5-4,5 µm long. **Gill edge** fertile, with scarce to locally frequent cheilocystidia (leptocystidia), 18-30 × 5,5-7 µm, hardly protruding, fusiform with distinct capitulate apex × 2,5-3,5 µm on a more or less differentiated, slender neck. **Pleurocystidia** none. **Subhymenium** 10-15 µm thick, densely pseudoparenchymatous, with small elements × 2-5 µm, somewhat shortly rameose near hymenium. **Gill trama** almost regular, with slender hyphae × 3-4-(5) µm, somewhat branched. **Suprapellis** a cutis of 1-(2) layers of short, prostrate articles, 25-60 × 3-4 µm, scarcely to frequently budding with short filiform diverticules and capitulate hairs up to 15-(20) µm long, with rounded, widely cylindrical to somewhat globulous-misshapen head, some collapsing, with depressed apex. Free blastoconidia scarce, looking like aborted spores, thin- or not distinctly thick-walled, with small granulations inside, 6,5-11 × 3,5-6 µm. **Subpellis** 15-20 µm thick, poorly differentiated from the flesh, with parallel hyphae × 3,5-6 µm, with thin to irregularly thickened, yellowish wall. **Stipitipellis** homologous to pileipellis, with longer capitulate hairs up to 25-(35) µm, and heads wider up to 8-9 µm, often becoming misshapen, more or less lobate or collapsed blastoconidia up to 12 µm long. **Clamp connections** abundant in hymenium and subhymenium, rare or inconspicuous in other tissues. No part of basidioma dextrinoid or metachromatic in Cresyl blue. No gloeoperlerous hyphae observed.

**Cytology** - The nuclear behaviour of *H. conidiogena* in basidia and spores is identical as that described by Valla (1967: 71). Spores contain usually 1 nucleus (sometimes 2); basidia contain 2-4 residual nuclei after spore ejection. Hyphae in subhymenium and basidioles are dicaryotic. Blastocnidia (attached) did not show distinct nuclei but a diffuse carminophilic content.

**Ecological characterisation** - *H. conidiogena* has been found only on litter of *Cistus monspeliensis*, in the bush association *Cisto-Sarrothamnetum catalaunici* O. de Bolòs 1956 sub association *cistetosum monspeliensis* Lapraz 1976. It belongs to the group of fungal species characteristic of the coenomycia *Russulo monspeliensi-Rectipilecium cistophili*, typified in Vila & Llimona (1999). Other species found sharing the same ecology are: *Agaricus porphyrizon*, *Clitocybe cistophila*, *Clitopilus scyphoides f. omphaliformis*, *Collybia catalaunica*, *C. dryophila*, *C. graveolens*, *C. inusitata*, *Crinipellis stipitaria*, *Flammulaster carpophilus*, *Lepiota clypeolaria*, *Leucoagaricus melanotrichus*, *Rectipilus cistophilus*, *Thelephora caryophyllea*.

Recent unpublished records have given a more precise idea of its ecology, as one of the saprobe species that colonize *Cistus monspeliensis* litter in winter, when a short



**Figs. 6-11.** *Hemimycena conidiogena*. **Figs. 6-7:** Sporophores *in situ*. Photo by J. Vila (bar = 1 cm). **Fig. 8-11:** Modified hairs producing blastoconidia (stipitipellis). SEM photos by J. Llistosella.

series of days free of desiccating wind enable the abundant dead leaves to conserve the humidity of December or January rainfall and dew.

**SPECIMENS EXAMINED - SPAIN - BARCELONA:** CAN FERRER, SERRA DE COLLSEROLA, BARCELONA (BARCELONÈS), UTM 31T 4294588, alt. 240 m under *Cistus monspeliensis*, 23-10-2000, leg. X. Llimona and J. Vila, BCN-Myc.- *Ibid.*, 25-1-2001, leg. X. Llimona and J. Vila, JVG 1010125-1. **GIRONA:** Near SANT PERE DE RODES, EL PORT DE LA SELVA (ALT EMPORDÀ), UTM 31T 5144686, alt. 470 m, under *C. monspeliensis* and *C. albidus*, 4-12-1999, leg. O. Llimona and X. Llimona, BCN-Myc.- *Ibid.*, 22-4-2000, leg. O. Llimona and X. Llimona, BCN-Myc.- COL·LECCió DE LA PERAFAITA, ROSES (ALT EMPORDÀ), UTM 31T 5194682, alt. 250 m, under *C. monspeliensis*, 9-12-2000, leg. X. Llimona and O.

Llimona, *BCN-Myc.*- CAN MARÉS, ROSES (ALT EMPORDÀ), UTM 31T 5174677, alt. 130 m, under *C. monspeliensis*, 28-11-2000, leg. X. Llimona, J. Vila, L. Muñoz, L. Peiró and M. Conejero, *JVG 1001128-30*.- COLLET SOBRE LA FALCONERA, ROSES (ALT EMPORDÀ), UTM 31T 5174676, alt. 95 m, under *C. monspeliensis* and *C. albidus*, 23-1-2001, leg. X. Llimona and J. Vila, *BCN-SCM B 4063 (HOLOTYPE)*, *JVG 1010123-20 (ISOTYPE)*, and *PAM 01012301 (ISOTYPE, LIP)*.- *IBID.*, 22-2-2001, leg. X. Llimona, J. Vila, L. Muñoz, C. Gutiérrez and M. Conejero, *BCN-Myc.*- Along the way to SANT QUIRZE DE COLERA, RABÓS (Alt Empordà), UTM 31T 50469, alt. 160 m, under *C. monspeliensis*, 21-1-1996, leg. M.À. Pérez-De-Gregorio, *PG960121*, and PAM n° 96012101, *LIP*.

## Discussion

**Systematic position** - White species of *Mycena* (Pers.) Roussel or *Hemimycena* Singer with non-dextrinoid flesh have not been covered in monographs since the revisions of Kühner (1938), Smith (1947), and Valla (1969). Although Kühner (*op. cit.*: 538), Smith (*op. cit.*: 149) and again Kühner (1980: 729) pointed out the close affinities between their sections *Adonidae* (Fr.) Quél. and *Lactae* in a wide sense of *Mycena*, most contemporary authors follow Singer's (1986) classification, placing the second section in the genus *Hemimycena*. Maas Geesteranus (1990) also points out close affinities between *Mycena* and *Hemimycena* and the transient position of *Mycena flavoalba*. Because we believe that a generic separation between both groups of species is possible, we have chosen to follow Singer's classification. Antonín & Noordeloos (2004) agree with this position.

Within this group of white species, *H. conidiogena* is characterized by the yellowish tinge of the mature basidiomata (an exceptional feature in *Hemimycena*), by its elongated spores, and also by its preferentially cisticolous habitat, strongly selective for *Cistus monspeliensis*. But the most unusual characteristic is the presence of "hairs" generating blastoconidia on pileus and stipe (*inde nomen*), showing analogies with those of *H. lactea*.

Presence of hairs on surfaces excludes the possibility of a species allied to *Hemimycena* sect. *Candidae*: *H. candida* (Bres.) Singer (Kühner 1938) and *H. ochrogaleata* (J. Favre) Singer (Favre 1955; Lamoure 1973-1974; Breitenbach & Kränzlin 1991), both with deeply decurrent gills. In keys including *Mycena* and allied genera (Kühner 1938; Smith 1947; Kühner & Romagnesi 1953; Moser 1978; Maas Geesteranus 1990), *H. conidiogena* may key out both as *Mycena* sect. *Adonidae* or *Hemimycena* sect. *Lactae*, according to the importance given to colour of basidiomata. Moreover, *H. conidiogena* reminds strongly of *Mycena flavoalba* in fresh as well as in dry condition. The classification of such a coloured species within *Hemimycena* is justified by a comparative analysis of unamyloid-spored taxa in both genera (**Tab. 1**).

Except for the general colours of basidiomata, all characters of *H. conidiogena* match *Hemimycena* definition. As mentioned in our description, the yellow pigment is localized in hyphal wall. Consequently, the colour appears here as a derived character, not homologous to the vacuolar pigmentation of *Adonidae* (especially *M. flavoalba*; Kühner 1938). Pigment localization of another critical species with ochraceous colours, *H. ochrogaleata*, is still unclear (Lamoure 1973-1974: 57) but might also be parietal.

Because of this combination of features, *H. conidiogena* belongs unambiguously to *Hemimycena* sect. *Lactae*, and the comparative analysis with *H. lactea* suggests a close relationship between both species.

**Table 1.** Determinant characters between *Mycena* sect. *Adonidae* and *Hemimycena* sect. *Lactae* (data from Kühner 1938; Smith 1947; Maas Geesteranus 1990).

	<i>Mycena</i> sect. <i>Adonidae</i>	<i>Hemimycena</i> sect. <i>Lactae</i>	<i>H. conidiogena</i>
Common characters		Spores not amyloid Subpellis not pseudoparenchymatous Cheilocystidia numerous, often bearing mucilage Hair-like caulocystidia present	
Colour of basidiomata	Pale yellow, orange to bright pink (vacuolar pigment)	Pure white	Yellowish cream
Dextrinoidity (Melzer's reagent)	Negative to positive (Kühner 1938)	Negative	Negative
Metachromatic coloration (Cresyl blue)	Positive	Negative	Negative
Pileal structure	Brush-like, sometimes with hairs	Hairs frequent, brush-like structures variable	Hairs abundant, brush-like structure poorly developed.

**Comparison with other species of *Hemimycena*** - Although many species of *Hemimycena* have been described during the XX<sup>th</sup> century (especially Kühner 1938, Smith 1947, Orton 1960, Kühner & Valla 1972, Malençon & Bertault 1975, Redhead 1982, Singer 1986, Romagnesi 1992, etc.), *Hemimycena* sect. *Lactae* has remained a paucispecific section, including several cosmopolitan and common species (*H. cucullata*, *H. lactea*, *H. pseudolactea*) and several infrequent or less known taxa (*H. delicatella*, *H. rickenii*, *H. gypsella*). Macroscopically, *H. conidiogena* does not resemble any of these species, which are all pure white or, at the most, whitish.

The peculiar spore shape observed in *H. conidiogena* is the same as described and illustrated by Kühner (*loc. cit.*) for “*Mycena lactea*” (fig. 233) and “*M. pseudolactea*” (fig. 226) and by Smith for “*M. gypsea*” (*loc. cit.*, fig. 13 (3), “*M. pseudolactea*” (fig. 15 (2)) and “*M. delicatella*” (fig. 15 (3)). The typical slender-capitulate, almost lecithiform shape of cheilocystidia is also present in *H. lactea* (Kühner, *loc. cit.*, as irregularly distributed) and in *H. delicatella* (Smith 1947: 163, as abundant - there are also pleurocystidia).

*H. delicatella* (Peck) Singer has been verified only from North America (Smith 1947: 161) and Japan (Hongo 1954), with clavate to cylindrical cheilocystidia and small spores (7,5-9,5 × 2,5-3,5 µm); Smith (1947: 163) and Maas Geesteranus (1980: 409) make it a synonym of *H. lactea* ss. Kühner.

*H. lactea* (Pers.: Fr.) Singer, in its four-spored form (Kühner 1938; Valla 1969; Kühner & Valla 1972 ; Malençon & Bertault 1975), shows fusiform cheilocystidia with slender neck and capitulate apex and hair-like elements in pileipellis. Because of these last morphological features, *H. lactea* is obviously the closest relative of *H. conidiogena*.

The spores of *H. conidiogena* are longer ( $10\text{--}12,5 \times 3,5\text{--}4,5 \mu\text{m}$ ) than in those mentioned species. The main differences between *H. lactea* (four-spored form) and *H. conidiogena* are summarized in **Tab. 2**.

**Table 2.** Comparison between *Hemimycena lactea* (4-spored forma) and *H. conidiogena*

	<i>Hemimycena lactea</i>	<i>Hemimycena conidiogena</i>
Cap	1-1,5 (2) cm, pure white, remaining so, sulcate.	0,5-1,5 cm, pure white, then yellowish-ochraceous, sulcate.
Stem	0,5-1 mm diam., hyaline white, pruinose.	1-2 mm diam., whitish, then faintly yellowish, finely pruinose.
Spores	(6,5) 7-10 (11,5) $\times$ (2) 2,5-3 (3,5) $\mu\text{m}$ .	(9) 10-12,5 (13) $\times$ 3,5-4,5 (5) $\mu\text{m}$ .
Basidia	$23\text{--}27 \times 5,5\text{--}6 \mu\text{m}$ , 4-spored.	$22\text{--}30 \times 6,5\text{--}8,5 \mu\text{m}$ , 4-spored.
Suprapellis	A cutis with abundant, thin, erect, non-capitate hairs.	A cutis with scarce to locally numerous short capitate hairs ("conidiophores").
Stipitipellis	A cutis with numerous, irregular, filiform to capitulate hairs.	A cutis with rather numerous capitate hairs (some modified in "conidiophores").
Flesh	Hyphae with hyaline, thin walls ( $<0,5 \mu\text{m}$ ).	Hyphae with yellowish thickened walls, up to 1 (1,5) $\mu\text{m}$ thick.
Ecology	Coniferous litter	<i>Cistus</i> spp. litter.

Valla (1969) compares these formations to those observed on mycelium of *Clitocybe senilis* (Fr.) Gillet, and to the "acremoniform branches" described from *Psilocybe* cultures. Recently Norvell (1998) also reported similar structures as "tibiiform diverticula" on the mycelium of *Phaeocollybia* spp., and suggested an excretive (protective) function for them, as shown on a few dark-spored Agarics (Hutchinson et al., 1996).

In fact, since De Bary (1859) described chlamydospores in *Asterophora lycoperdoides* Sow.: Fr., mitospores have rarely been described from basidiomata of Agarics; most of them have been identified from mycelium cultures (Vandendries, 1937; Oddoux 1957; Watling 1979; Stalpers 1987).

Clémençon (1997: 286) distinguishes three kinds of mitospores in Hymenomycetes: arthroconidia (from hyphal fragmentation), chlamydospores (differentiated with thick walls) and blastic mitospores (differentiated with thin walls). Following this classification, mitospores of *Hemimycena* should be classified as blastic mitospores, more precisely as blastoconidia (introduced by Stalpers, *op. cit.*, for *Galzinia*). Clémençon (*op. cit.*: 334) reports analogue formations as sympoduloconidia.

Up to now, blastoconidia are only reported in one agaric: *Baeospora myosura* (Fr.) Singer, from mycelial cultures (Kendrick & Watling 1979; Clémençon, *op. cit.*: 287). All mitosporic formations compared by Valla (1969) to those of *H. lactea* can be considered as arthroconidia according to these recent classifications. In Watling's conspectus (1979), these formations would be called "blastic conidia", and compared to mycelial conidia illustrated from *Hohenbuehelia* (*loc. cit.*: 457, fig. 19.1 O). Because of the analogies with *Baeospora* conidia, we have chosen to report them as "blastoconidia". Cultures and more efficient research of nuclear material in these conidia will be necessary to interpret thoroughly these structures.

### Acknowledgments

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