

## *Mycocalicium llimonae*, a new species from the Iberian Peninsula

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**Abstract:** The new species, *Mycocalicium llimonae*, is described based on specimens collected from cones of *Pinus halepensis* in the west of the Iberian Peninsula. The new taxon is compared with the other species of the genus *Mycocalicium* in the Iberian Peninsula, particularly *Mycocalicium subtile*, and also with other genera in the *Mycocaliciaceae*.

**Key words:** calicioid fungi, Catalonia, *Mycocaliciaceae*, Spain

### Introduction

The genus *Mycocalicium* Ach. Vain (1890) is made up of *c.* 10 species worldwide (Kirk 2001) and, taxonomically, is one of the less well-known genera included in the *Mycocaliciaceae*. Their very small apothecia and the lack of good distinguishing characters, has often led to most species being either overlooked or misidentified as *Mycocalicium subtile* (Pers.) Szat.

Three *Mycocalicium* species are hitherto known from the Iberian Peninsula (Llimona & Hladun 2001): *M. minutellum*, *M. subtile* and *M. victoriae*. *Mycocalicium minutellum* is a problematic species which has often been referred to as *Mycocalicium subtile* var. *minutellum* Szat. by several authors (Schmidt 1970). *Mycocalicium minutellum* differs from *M. subtile* only in the size of the ascomata and is currently recognised as a synonym of *M. subtile* (Tibell 1987*b*; Vinuesa *et al.* 2001).

Most of the Iberian collections, previously identified as *M. minutellum*, do not correspond with the broadly accepted concept of that taxon. After careful examination we consider these specimens to be sufficiently

distinct in terms of morphology and ecology to merit description as a new species.

### Material and Methods

The study was based on material of *Mycocalicium minutellum* s. lat. collected by the authors from several localities in the north-east of the Iberian Peninsula. Voucher specimens from this study have been deposited in BCN. Additional material from AAU, MACB, MAF, MBH, MU, TU and VAB has also been examined. Light microscope observations of apothecia were made on thin sections (*c.* 10 µm) and ultra-thin sections (*c.* 4 µm) stained with lactic blue and heated gently. Ultra-thin preparations were prepared by SCT (Serveis científicotècnics, Parc Científic, Universitat de Barcelona). Ascus apex and ascospores were studied in squash preparations stained with lactic blue. Chemical tests of apothecia were made on squash preparations in water with either a 10% aqueous solution of potassium hydroxide (K) or 10% aqueous solution of nitric acid (N). SEM examinations were made on samples fixed on a glue surface and coated with gold.

Measurements of apothecium height, capitulum width, stalk width, ascus length and width, length and width of spores are given as in Titov (2000, 2001): (absolute minimum) *a* – *b* (absolute maximum), where *a* represents arithmetic mean minus the standard deviation and *b* arithmetic mean plus the standard deviation. Extreme values, arithmetic mean ( $\chi$ ), standard deviation ( $\sigma$ ) and number of measurements (*n*) are given within parentheses. Asci and spores were measured with an oil immersion objective ( $\times 100$ ) in squash preparations of apothecia mounted in water.

*Selected additional comparative material examined.*

*Mycocalicium albonigrum* (Nyl.) Tibell. **Greece:** Corfu: *c.* 8 km N of Corfu City (Kérkira), just W of Gouviá, dry meadow with scattered *Olea*-trees, on

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limestone ground, on wood of *Olea europaea*, in hollow tree, 23 v 1983, S. N. Christensen (MBH 1340).

*Mycocalicium subtile* (Pers.) Svat. **Spain:** Albacete: Alcaraz, Sierra del Relumbrar, Cañada del Conejo, 832 m, on wood of *Juniperus oxycedrus*, 27 v 1978, J. M. Egea (MU 778); Riopar, Sierra del Calar del Mundo, Cañada de los Mojones, on bark of *Pinus nigra*, 17 ix 1984, J. M. Egea & P. P. Moreno (MU 6530); Lago de las Truchas, on wood of *Pinus pinaster*, 26 v 1984, J. M. Egea & P. P. Moreno (MU 6649, 6650). **Castellón:** Azuebar, Sierra del Espaldán, Barranco de la Mosquera, 30SYK2517, 625–750 m, on bark of *Quercus suber*, 6 iii 1992, S. Fos (VAB-LICH 4721). **Ciudad Real:** Fuencaliente, Sierra de la Quintana, umbría Burcio del Pino, 30SUH9351, 1010 m, on wood of *Quercus pyrenaica*, 13 iii 1998, G. Sarrión (MACB 76753); río Valmayor, 30SUH9454, slope near river side, in shadows, on branch of *Quercus ilex*, 22 i 1998, G. Sarrión (MACB 76754). **Jaen:** Orquera, Sierra de Segura, Cortijo de Rocanales, on bark of *Pinus nigra*, 7 viii 1985, P. P. Moreno (MU 6531); Bajada al vivero Montesinos, on bark of *Pinus nigra*, 7 viii 1985, P. P. Moreno (MU 6529). **Lleida:** Espot, Parque Nacional de Aigüestortes i Estany de Sant Maurici, Plaça dels Arbres, 31TCH41, 1400–2000 m, on wood of *Pinus uncinata*, 21 i 1997, X. Llimona (BCN 14505). **Madrid:** Montejo de la Sierra, 30TVL5851, on wood of dead *Fagus sylvatica*, in a sunny place, 21 vii 2003, G. Amo & A. R. Gutiérrez (MACB). **Teruel:** Puebla de Vallverde, Sierra de Jalambre, Cerro Verde, 30TXK6848, 1500 m, on bark of *Pinus nigra*, S. Fos (VAB-LICH 11065).

*Mycocalicium subtile* (Pers.) Svat. [*sub Mycocalicium minutellum* (Ach.) Nádov.]: **Denmark:** Livø: at Skovején W of Majdal, on wood of dead *Abies*, covering large part of the trunk, 31 x 1988, S. N. Christensen (MBH 5365, 5366).—**Poland:** *Western Carpaty:* Tatra National Park, Filipczanski Wierch., 1200 m, on stump of *Pinus cembra*, 23 ix 1988, V. Alstrup & M. Olech (MBH 638).—**Sweden:** Västerbotten Län: Storsandsberget, wood of *Pinus/Picea*, 29 vi 1981, V. Alstrup (MBU 81170).

*Mycocalicium victoriae* (C. Knight ex F. Wilson) Tibell. **Spain:** **Barcelona:** Rubí, Oliverar, 31TDF19, on wood of old *Olea europaea*, 21 v 1994, X. Llimona (BCN 14504). **Castellón:** Eslda, 30TYK2918, 450 m, on bark of *Quercus suber*, 9 x 1987, Muñoz (VAB-LICH 9210); Ahín, Sierra de Espaldán, Barranco de la Mosquera, 30SYK2518, 700 m, on bark of *Quercus suber*, 28 ix 1996, Muñoz (VAB-LICH 0463). **Ciudad Real:** Fuencaliente, puertas de la finca Valmayor, 30SUH9255, 770 m, on wood of *Quercus pyrenaica*, 22 i 1998, G. Sarrión (MACB 76755); Robledo de las Hoyas, 30SUH8156, 270 m, north face of trunk of *Quercus pyrenaica*, 25 v 1996, G. Sarrión (MACB 76757). **Granada:** Polopos, Haza de Lino, Sierra de la Contraviesa, 30SUF8156, 1300 m, on bark of *Quercus suber*, 3 iv 1991, S. Fos (VAB-LICH 9210). **Mallorca:** Puerto de Soller, on naked wood of the cleaved trunk of an old carob tree in the vicinity of the town, 26 iii 1970, M. S. Christiansen (MBH 6216). **Tarragona:** Vimbodí, entre Poblet y la Pena, 31TCF3779, 738 m, on bark of *Castanea* sp., 15 vii 2003, N. L. Hladun & G. Figueras (BCN 14066).

## The Species

### *Mycocalicium llimonae* Hladun & Muñiz sp. nov.

Thallus non manifestus autem substratus argentatum. Apothecia pusilla, (179·2) 188·44–287·93 (332·8) µm alta; capitula nitida nigra, hemisphaerica, pulverulenta, ad (25·6) 45·64–136·33 (204·8) µm diametro. Stipia brunnei vel spadicei, ad (12·8) 22·85–43·19 (51·2) µm diametro. Excipulum parum effusum, brunneum, ex stratis externis stipi continuum. Hypothecium olivinum vel fuliginium. Hymenium aeruginosum. Asci (31·5) 36·4–44·9 (49·8) µm longi, (2·49) 2·5–3·5 (4·15) µm lati, cylindrici, apicibus incrassatis. Sporae non septatae, ellipsoideae, spadiceae, (4·98) 5·15–6·68 (9·13) × (1·66) 2·34–3·27 (4·15) µm, ornamento minuto rugosi.

Typus: Spain, Catalonia, Barcelona, Sant Cugat del Vallés, Tibidabo, Vista Rica. 31TDF28, 400 m, on pine cone of *Pinus halepensis*, 21 November 2003, N. L. Hladun, G. Figueras & D. Muñiz (BCC(Lich 14507)—holotypus).

(Fig. 1)

*Thallus* crustaceous immersed, inconspicuous, but substratum becoming silvery.

*Apothecia* small, (179·2) 188·44–287·93 (332·8) µm high ( $\chi=238·18$ ,  $\sigma=49·74$ ,  $n=30$ ). *Capitulum* shiny black, lenticular to subspherical, often irregular, with a granular surface resembling a thin mazaedium, (25·6) 45·64–136·33 (204·8) µm diam. ( $\chi=90·98$ ,  $\sigma=45·34$ ,  $n=30$ ). *Epithecium* thin, brown to pale brown, 4–7 µm wide, composed of amorphous material. *Hypothecium* dark green to olivaceous brown, 60–75 µm wide. *Excipulum* brown, poorly to well-developed, arranged as a continuation of the outer layers of the stalk, comprising three or four periclinally arranged rectangular hyphae, 5–6 µm wide, slightly darker than the hyphae from the stalk. *Stalk* brown to pale brown or white (12·8) 22·85–43·19 (51·2) µm diam. ( $\chi=33·02$ ,  $\sigma=10·17$ ,  $n=30$ ); consisting of pale brown to hyaline hyphae, 2–3 µm wide, irregularly interwoven, with a thick gelatinous coat. All parts of the apothecium K– or K+ becoming darker. Brownish parts N– or N+ deep reddish brown. *Asci* (31·5) 36·4–44·9 (49·8) × (2·49) 2·5–3·5 (4·15) µm (length:  $\chi=40·67$ ,  $\sigma=4·26$ ,  $n=30$ ; width:  $\chi=3·00$ ,  $\sigma=0·50$ ,  $n=30$ ), cylindrical, apex of ascus strongly thickened. *Spores* uniseriate and slightly obliquely

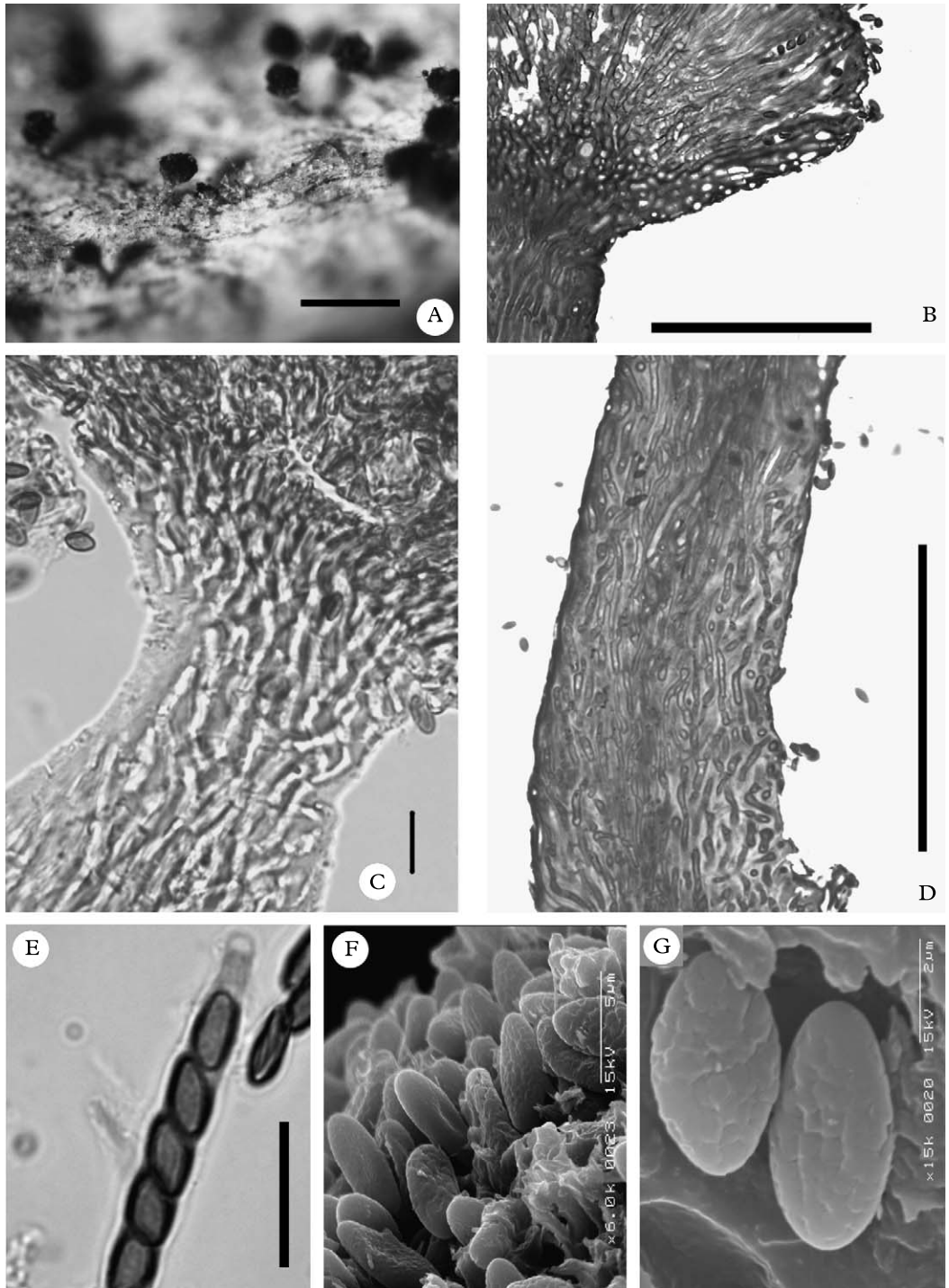


FIG. 1. *Mycocalicium llimonae* (holotype). A, habitus; B, vertical section of apothecium showing the exciple in lactic blue; C, detail of structure of the stalk; D, vertical section of stalk in lactic blue; E, ascus in lactic blue; F & G, ascospores (SEM). Scales: A=0.5 mm; B, D=50 µm; C & E=10 µm; F=5 µm; G=2 µm.



orientated; non-septate, ellipsoidal, pale brown, slightly greenish in fresh samples, (4·98) 5·15–6·68 (9·13) × (1·66) 2·34–3·27 (4·15)  $\mu\text{m}$ , (length:  $\chi=5\cdot99$ ,  $\sigma=0\cdot80$ ,  $n=61$ ; width:  $\chi=2\cdot83$ ,  $\sigma=0\cdot49$ ,  $n=61$ ); surface smooth or with very minute verrucose ornamentation visible in SEM.

*Pycnidia* not observed.

**Etymology.** This species is named in honour of Prof. X. Llimona (Barcelona) with gratitude for his teaching.

**Ecology and habitat.** This taxon grows saprophytically on pine cones on dry branches of *Pinus halepensis*, but can also occur on *P. sylvestris*, *P. piniae*, *P. pinaster*, generally growing in open environments under the influence of the sea.

**Distribution.** *Mycocalicium llimonae* ranges from the thermo- to the mesomediterranean belts in the east of the Iberian Peninsula where it is widely distributed from sea level up to 900 m. It is known only from the Iberian Peninsula and seems to be a common species in *Pinus halepensis* forest.

**Selected specimens examined.** **Spain:** **Barcelona:** La Beguda Alta, 31TDF09, 300 m, on cones of *Pinus halepensis*, 30 v 2004, D. Muñiz (BCN); Serra de Collserola, Castellfortí, 31TDF28, 350 m, on cones of *Pinus halepensis*, 30 iv 2004, A. Gómez-Bolea, N. L. Hladun & D. Muñiz (BCN); Montserrat, el Bruc, 31TCF90, 540 m, on cones of *Pinus halepensis*, 12 xii 2003, G. Figueras (BCN); Sant Cugat del Vallés, Tibidabo, 31TDF28, 400 m, on cones of *Pinus halepensis*, 21 xi 2003, N. L. Hladun, G. Figueras & D. Muñiz (BCN); Sant Martí de Tous, 31TCG70, 460 m, on cones of *Pinus halepensis*, 12 x 1995, X. Llimona (BCN); Sant Sadurní d'Anoia, Can Catasús, 31TCF98, 200 m, on cones of *Pinus halepensis*, 15 x 2004, D. Muñiz (BCN); Vilanova i la Geltrú, Pineda de Sant Gervasi, 31TDG96, sea-level, on cones of *Pinus halepensis*, 26 xi 2002, X. Llimona (BCN). **Girona:** Cap de Creus, Roses, La Falconera, 31TEG28, 80 m, on cones of *Pinus pinea*, 22 ii 2001, X. Llimona (BCN), on cones of *Pinus halepensis* on sunny soil, 18 iii 2001, X. Llimona (BCN); Llançà, Cap Ras, 31TEG19, 30 m, on cones of *Pinus halepensis*, 08 xii 2000, 02 i 2001, X. Llimona (BCN); Port de la Selva, near the church, 31TEG18, 50 m, on cones of *Pinus halepensis*, 18 iv 2005, X. Llimona (BCN); Tossa de Mar, Pineda de Cala Bona, 31TDG29, 0–5 m, on cones of *Pinus halepensis*, 30 xii 1997, X. Llimona & P. Hoyo (BCN), 10 ii 1998, X. Llimona (BCN). **Lleida:** Castellidans, 31TCF19, 200 m,

on cones of *Pinus halepensis*, 21 xi 1994, 02 v 1996, X. Llimona (BCN); Km 5–6, road from Maials to Almatret, 31TBF87, 340 m, on cones of *Pinus halepensis*, 10 x 1995, X. Llimona (BCN); Mas de Melons, 31TCF09, 300 m, on cones of *Pinus halepensis*, 02 v 1996, X. Llimona (BCN). **Tarragona:** Ametlla de Mar, Torrent del Pi, 31TCF13, 0–5 m, on cones of *Pinus halepensis*, 22 i 1996, X. Llimona (BCN); Alcanar, 31TBE99, 320 m, on cones of *Pinus halepensis*, 07 x 1997, X. Llimona (BCN); Hospitalet de l'Infant, Cala Bea, 31TCF23, 0–5 m, on cones of *Pinus halepensis*, 30 i 1996, 18 xii 1987, X. Llimona (BCN); Serra de l'Espelta, Masriudoms, 31TCF24, 500 m, on cones of *Pinus halepensis*, 13 i 1998, X. Llimona (BCN); near el Perelló, old road, 31TCF02, 120 m, on cones of *Pinus sylvestris*, 16 iv 2002, X. Llimona (BCN); Prades, la Baltasana, 31TCF37, 1050–1100 m, on cones of *Pinus pinaster*, 15 vii 2005, D. Muñiz (BCN); El Mèdol, 31TCF65, 20 m, on cones of *Pinus halepensis*, 22 i 1996, X. Llimona (BCN).

## Discussion

*Mycocalicium llimonae* could be confused with species of *Chaenothecopsis* or *Phaeocalicium* with non-septate ascospores and short apothecia, such as *Chaenothecopsis resinicola* Tibell & Titov, but it differs by the presence of pruina, the structure of the stalk and ecology. *Chaenothecopsis* and *Phaeocalicium* species occur mainly in shaded and wet situations whereas *M. llimonae* grows in open and sunny exposed sites. The thickened apex of the ascus, size of asci and spores, place this new taxon in *Mycocalicium* according to Schmidt's classification (Schmidt 1970).

*Mycocalicium llimonae* can also be confused with the two species of *Mycocalicium* from the Iberian Peninsula, *M. subtile*, specifically with some specimens previously identified as *M. minutellum* (Ach.) Nád., and *M. victoriae*. These two species, however, differ in apothecium size, colour and structure of stalk, asci size and spore dimensions and colour (Table 1). The height of the apothecium of *M. subtile* varies from 0·7–2·0 mm (*sensu* Vainio 1927) or 0·4–0·8 mm (*sensu* Tibell 1998) and, in *M. subtile* var. *minutellum* s.l., it ranges between 0·6 and 0·8 mm (Schmidt 1970; Vainio 1927). In *M. llimonae* it is rather shorter, up to 0·4 mm. In addition, the stalk is brownish black to black and made up of dark brown

TABLE 1. Comparison of morphological characters of *Mycocalicium* species

	<i>Mycocalicium subtile</i>	<i>Mycocalicium victoriae</i>	<i>Mycocalicium albonigrum</i>	<i>Mycocalicium limonae</i>
Apothecium height	[0·4–2·0 mm]*	[0·8–1·8 mm]	[0·7–1·3 mm]	(179·2) 188·44–287·93 (332·8) µm
Stalk colour	Shining black	Shining black	Shining black	Brown to white
width	20–120 µ	80–100 µ	[0·07–0·12 mm]	(12·8) 22·85–43·19 (51·2) µm
section colour	Dark brown, olivaceous green	Wall reddish, hyaline within	Dark brown	Hyaline
structure	Periclinal parallel hyphae	Periclinal interwoven hyphae	Periclinal parallel hyphae	Irregular interwoven hyphae, gelatinous coat
Capitulum width	160–350 µm	100–400 µm	150–320 µm	(25·6) 45·64–136·33 (204·8) µm
Excipulum colour	Brown	Reddish brown	Brown	Brown
structure	3–4 layers of periclinal hyphae	Pseudoparenchymatous tissue of isodiametric cells	Large, almost isodiametric, cells with thin walls	3–4 layers of periclinal hyphae
Hypothecium colour	Dark brown, sometimes green	Greenish	Dark brown	Dark green to brown
Ascus length	(42·33) 45·14–54·73 (55·61) µm	(52·29) 52·78–59·52 (63·91) µm	[35–45 µm]	(31·5) 36·4–44·9 (49·8) µm
width	(2·49) 2·99–4·00 (4·98) µm	(3·32) 4·02–5·02 (4·98) µm	[3–4 µm]	(2·49) 2·5–3·5 (4·15) µm
Spore length	(5·81) 6·83–8·61 (11·62) µm	(7·47) 7·99–9·64 (10·73) µm	(7) 7·09–9·24 (12) µm	(4·98) 5·15–6·68 (9·13) µm
width	(2·49) 2·9–4·04 (4·98) µm	(3·32) 3·9–5·19 (5·81) µm	(2·5) 3·11–4·19 (4·5) µm	(1·66) 2·34–3·27 (4·15) µm
colour	Dark brown	Dark brown	Dark brown	Pale brown (greenish in fresh collections)

[ ]\*Denotes measurements from the literature.

to dark olivaceous-brown, periclinally arranged, hyphae in *M. subtile* whereas in *M. llimonae* it is made up of pale brown to hyaline, interwoven hyphae, covered by a gelatinous coat. Furthermore, the asci and spore sizes are larger in *M. subtile* than *M. llimonae*, and the spores of *M. subtile* appear darker.

*Mycocalicium victoriae* differs from *M. llimonae* in the ascomata size, ascus and spore size as well as in the structure of the stalk. However, this species also differs in the structure of the excipulum, which is formed by larger isodiametric cells constituting a pseudoparenchymatous tissue (Yoshimura and Shinada 1980). The stalk of *M. victoriae* is made up of a reddish brown outer part, 20–25 µm wide, consisting of interwoven hyphae with thickened walls and a central part consisting of hyaline periclinally arranged hyphae (Tibell 1987).

A further species, *Mycocalicium albonigrum*, not reported from the Iberian Peninsula, resembles *M. llimonae* in its ecology and some morphological features and could be confused with the new taxon. *Mycocalicium albonigrum*, however, has larger apothecia with stalks formed by dark olivaceous brown hyphae arranged periclinally and an excipulum made up of almost isodiametric cells. In addition, *M. albonigrum* shows a short apical canal in semi-mature asci.

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