Psathyrella codinae, a new species from Spain

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Résumé: Les caractères morphologiques ainsi que les données moléculaires et phylogénétiques suggèrent que *Psathyrella codinae* est une espèce nouvelle. La description est illustrée par des photographies du basidiome réalisées in situ ainsi que par des photographies de ses caractères microscopiques.

Abstract: Traditional morphology, sequence data, and phylogenetic analyses suggest that *Psathyrella codinae,* so far only known from Spain, is a new species to science. The description is supported by photographs of the basidiocarps and the microscopic features.

Keywords: Agaricales, Psathyrellaceae, *Psathyrella*, Catalonia, Mycobiota of Spain.

Introduction

At the end of May 2017, during a mycological trip, three basidiocarps of a *Psathyrella* species, growing in a knot of the trunk of a living *Quercus robur* L., were collected in Spain by the third author, and his friends Joaquim Carbó, Santi Gibert and Àngel Torrent, in the urban park “Park Nou” located near the city Olot (altitude about 480m - volcanic area Garrotxa – Girona).
None of the authors were able to identify without any doubt this species that has very exclusive morphological characteristics, so that further studies were required.

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Psathyrella *codinae* in situ (Photo: S. Gibert)

Material and methods

A photograph of the basidiomata was taken in situ by Santi Gibert and is reproduced with his permission.

The macromorphological characters where observed in fresh mature specimens.

The microscopic analyses were made by light microscope from section of fresh material or exsiccata. Forty mature spores from the upper stipe section of two basidiomata were measured in ammonia solution (NH_4OH 10%). The spore color was assessed additional in water and potassium hydroxide solution (KOH 5%). Cystidia and other microscopic structures were studied in pure ammonia solution or after staining by SDS Congo red. The colour codes are based on KÜPPERS (2007).

The diagram for the dispersion of the spore dimension was made with the program Piximètre (HENRIOT & CHEYPE 2016).

The sequencing was done by Pablo Alvarado (ALVALAB, Oviedo, Spain).

Total DNA was extracted from dry specimens blending a portion of them with the aid of a micropestle in 600 µl CTAB buffer (CTAB 2%, NaCl 1.4M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65º C. A similar volume of chloroform:isoamilalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifugated for 10 min at 13.000g, and the DNA in the supernatant was precipitated with a volume of isopropanol. After a new centrifugation of 15 min at the same speed, the pellet was washed in cold ethanol 70 %, centrifugated again for 2 min and dried. It was finally resuspended in 200 µl of double-distilled water. PCR amplification was performed with the primers ITS1F and ITS4 (GARDES&BRUNS 1993, WHITE et al. 1990) for ITS, and LR0R and LR5 (VILGALYS& HESTER 1990, CUBETAet al. 1991) for the 28S rDNAregion.

PCR reactions were performed under a program consisting of a hot start at 95º C for 5 min, followed by 35 cycles at 94º C, 54º C and 72º C (45, 30 and 45 s respectively) and a final 72º C step 10 min. PCR products were checked in 1 % agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked searching for putative reading errors, and these were corrected.

The raw sequence data were analyzed by Dieter Wächter (Thiersheim, Germany). Base calling and chromatogram creation were performed with FinchTV 1.4.0 (Geospiza, INC.). Bases were checked and corrected for errors manually. For tree inference we used the ITS1 (Internal Transcribed Spacer 1), 5.8S (5.8S rRNA gene), ITS2 (Internal Transcribed Spacer 2), LSU (Large Subunit 28S rRNA gene), β-Tub (β-Tubulin gene), ef-1α (Translation Elongation Factor 1-α gene) and ITS1-, 5.8S-, ITS2- and LSU-indel matrices coded with the simple indel coding method (SIC) (SIMMONS & OCHOTERENA 2000) using FastGap 1.2(BORCHSENIUS 2009).

The alignment of the individual partitions was performed with Prank Version 140603 (VEIDENBERG et al. 2016), refined by an iterative guide tree method. The best fitting partition scheme and optimum evolution models for the Bayesian analysis were calculated with Partitionfinder (LANFEAR et al. 2016), while the Bayesian information criterion (BIC) was used for the scoring. The topology of the 50% majority rule consensus phylogram, branch lengths and the a posteriori probabilities (PP values) were calculated using MrBayes v3.2.6 (RONQUIST & al. 2012). The ML bootstrap values were calculated using RAxML (STAMATAKIS 2006). The resulting phylogenetic tree (Fig. 2) is a section with relevant areas, created as part of another work, and was provided by Dieter Wächter. Since the phylogram is a small part of a large one, the marked outgroup consists of closest match Psathyrellaceae taxa, these are *Coprinellus* from section *Setulosi*, aswell as many other taxa, going down to the root of the Psathyrellaceae family. The upper outgroup marked with “Psathyrella cont.” on top of the phylogram are the following clades of the genus *Psathyrella*

Phylogenetic results

The position between the clades /supernula and /prona is surprising. Neither *Psathyrella multipedata* (Peck) A. H. Sm. and *Psathyrella supernula* (Britzelm.) Örstadius & Enderle on the one hand and *Psathyrella prona* (Fr.) Gillet and relatives on the other have substantial similarities. Even with *Psathyrella codinae* there is no significant morphological overlap. For this reason and the isolated phylogenetic position, the description of a new species is justified.

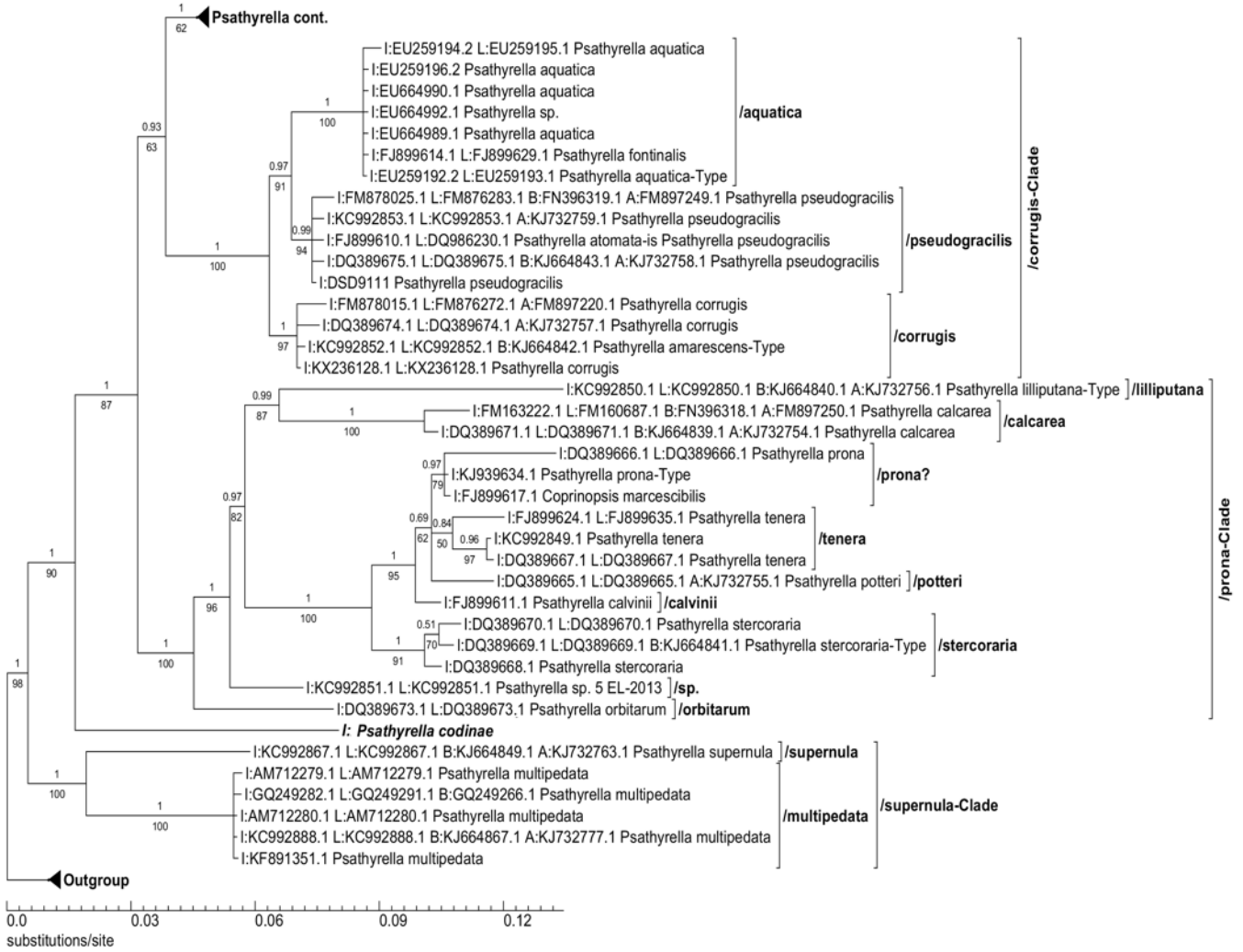


Fig. 2 - 50% majority rule consensus phylogram.

Description

Psathyrella codinae Deschuyteneer, A. Melzer& Pérez-De-Gregorio, sp. nov.

Mycobank no.: MB 824530
GenBank accession no.: MG696611 (ITS) MG674714 (LSU)

Etymology: Named in memory of the 150th anniversary of the birth of Joaquim Codina, the father of mycology of Catalonia.

Diagnosis
Pileus 8.5-15 mm latus, parabolicus, humidus paulo striatus, rubro-brunneus, in sicco pallescens. Velum fibrillosum, album,cellulae veli subcylindracea. Lamellae subdistantes, cinereo-brunneae, acie albida. Stipes 17-24 x 1.8-2.8 mm, cylindraceus, albus vel fuscotinctus, fibrillosus. Odor Raphani similis, sapor indistinctus.
Sporae 8-8.88-10 (-10.5) x (4.5-) 5.5-5.20-6 (-6.5) µm, ellipsoideae, ovoideae, sub microscopium rubro-brunneae, poro germinativo distincto et truncato instructae. Basidia 4-sporigera. Pleurocystidia 28-48 x 11-15 µm, numerosa, spathulata, interdum leviter crassi tunicata et brunnea. Cheilocystidia 30-55 x 8-12 µm, multa, lageniformia, obtusata vel capitata, raro furcata. Cellulae sphaeropedunculatae et clavatae rariorae. Fibulae adsunt.
Ad truncum vivum Querci robori.
Holotypus: Spain, Catalonia, Olot, 06. 05. 17, leg. Miquel À. Pérez-De-Gregorio, in herbario Senckenbergianum Görlitz (GLM-F112430) depositus.

Cap: 8.5-15 mm broad, paraboloid, surface smooth, faintly striate up to 1/3 from margin, young pale brown (ca. $Y_{50}M_{60}C_{30}$) to yellowish brown (ca. $Y_{80}M_{40}C_{10}$) towards margin, at maturity warm chestnut brown (ca. $Y_{50}M_{90}C_{60}$), center darker (ca. $Y_{50}M_{99}C_{80}$) hygrophanous, fading to pale flesh-coloured brown, without pinkish tones.
Lamellae: Subventricose, broadly adnate, subdistant, light greyish brown with a minutely white fimbriate edge.
Veil: Sparse, quickly volatile, forming in mature specimens numerous but fugacious white filaments on the cap and fascicles of fibrils appendiculate at the margin and on lower 2/3 of the stem.
Stipe: 17-24 x 1.8-2.8 mm, cylindrical, hollow, whitish or isabelline, then discolouring to dirty brown from the base, apex pruinose, densely covered by fibrillose veil remnants on lower 2/3, not radicant.
Trama: Smell slightly raphanoid, taste inconspicuous.
Spore: 8-10.6 (-11.3) x (4.5-) 5-6.3 (-8) µm, Ø = 8.9-10.3 x 5.2-5.8 µm, Q=(1.10-) 1.50-1.90 (-2.00), Ø = 1.7, smooth, ellipsoid to slightly ovoid in front view, laterally somewhat flattened and weakly amygdaliform, with a distinct central and truncate germ pore (1.5-1.8 µm wide) and a well visible apiculus. In water and ammonia solution dark reddish brown, in potassium hydroxide solution black brown with a reddish hue, nearly opaque.

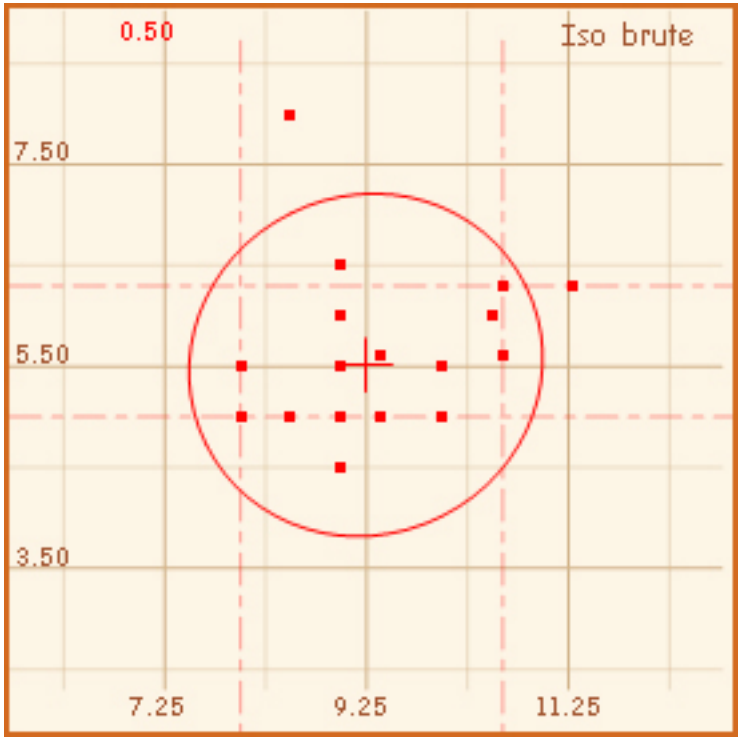


Fig. 3 –Sporogram.

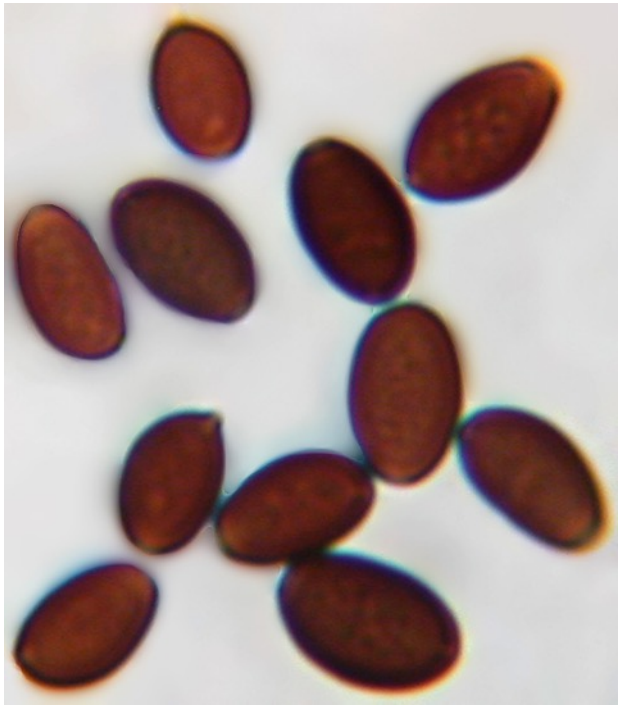
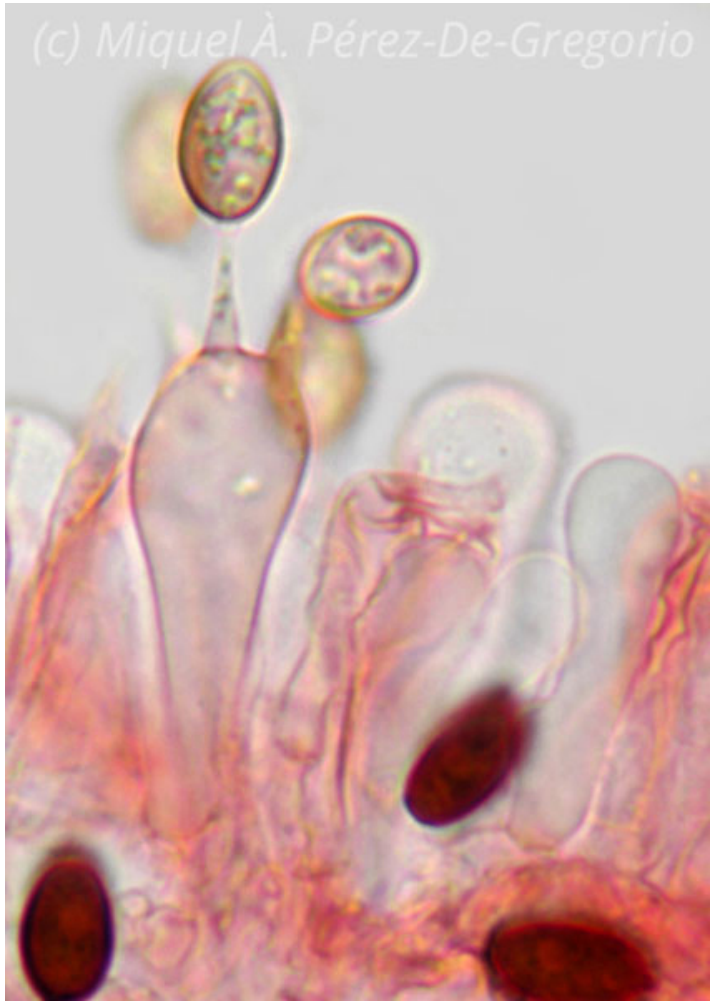
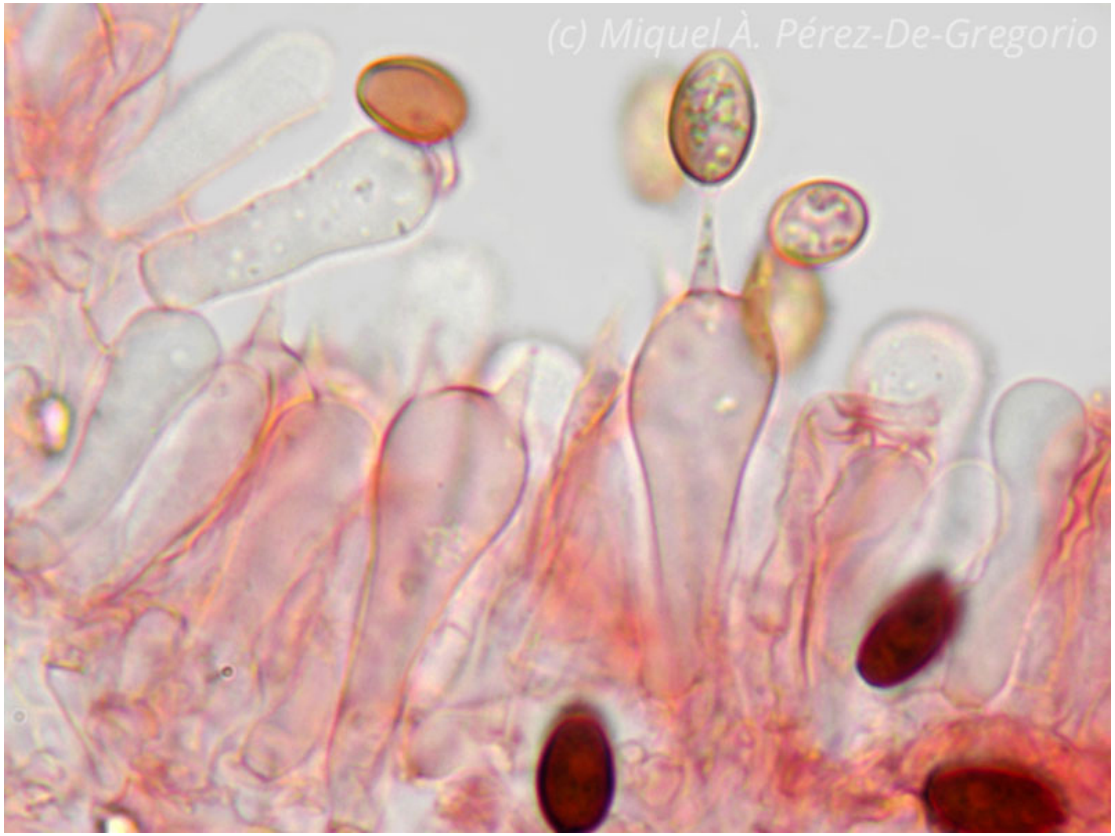


Fig. 4 – Spores in NH_4OH 10% (Photo: D. Deschuyteneer).

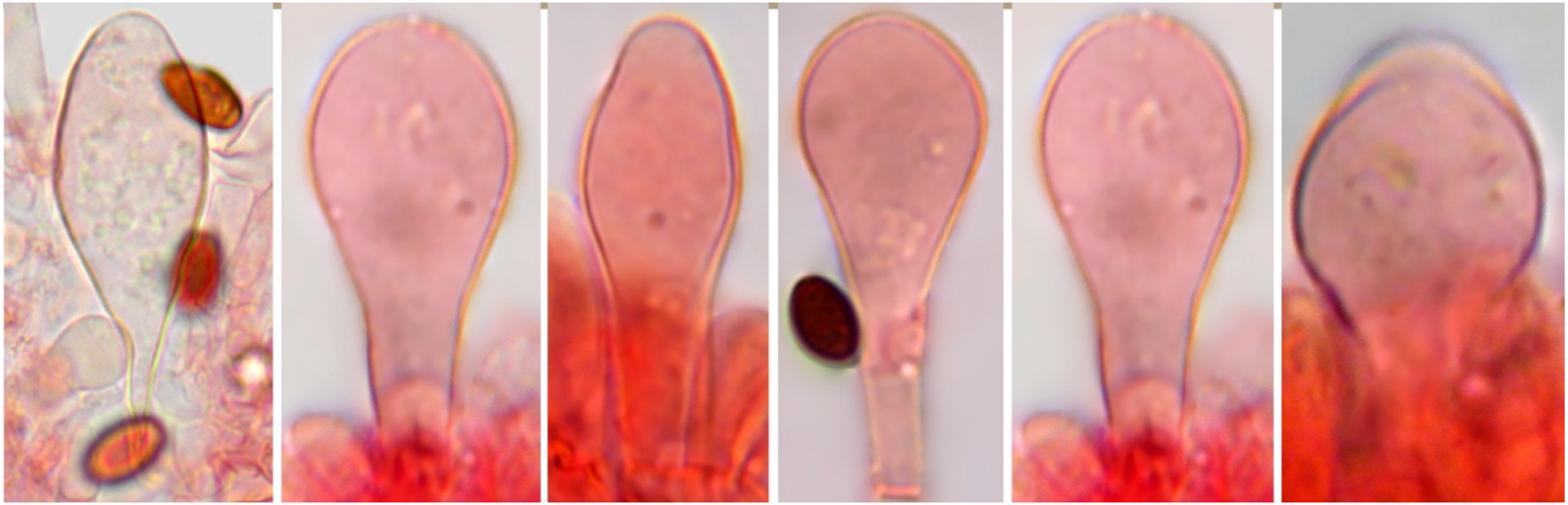
Basidia: 19-27 x 9.5-11 µm, clavate, 4-spored, rarely 2-spored.

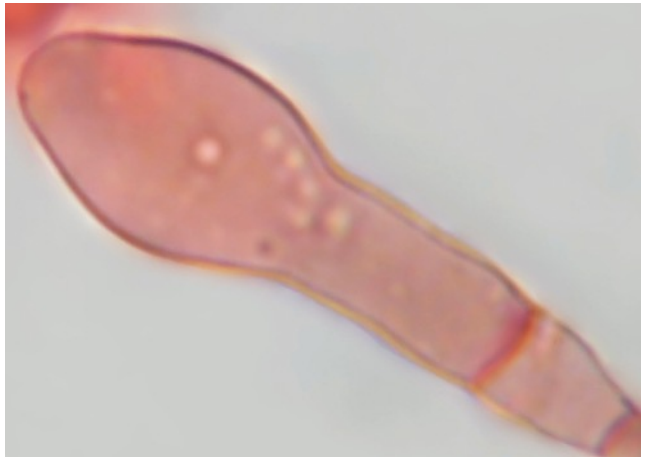


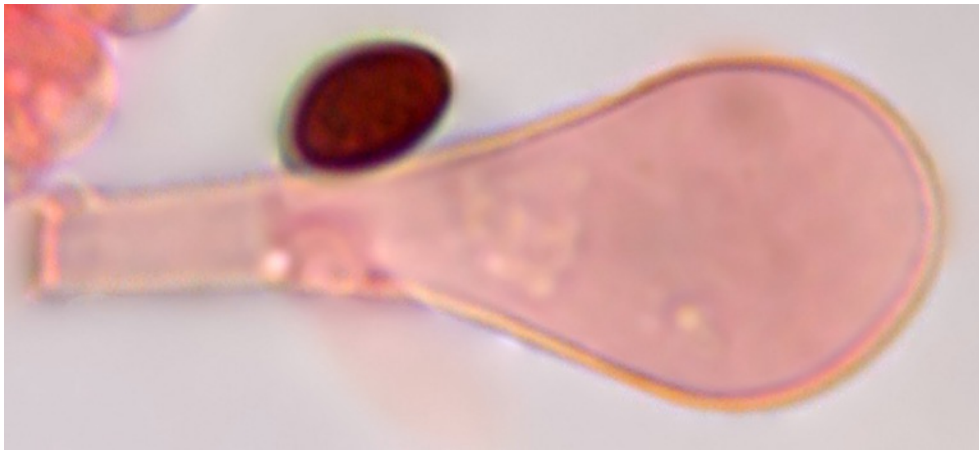


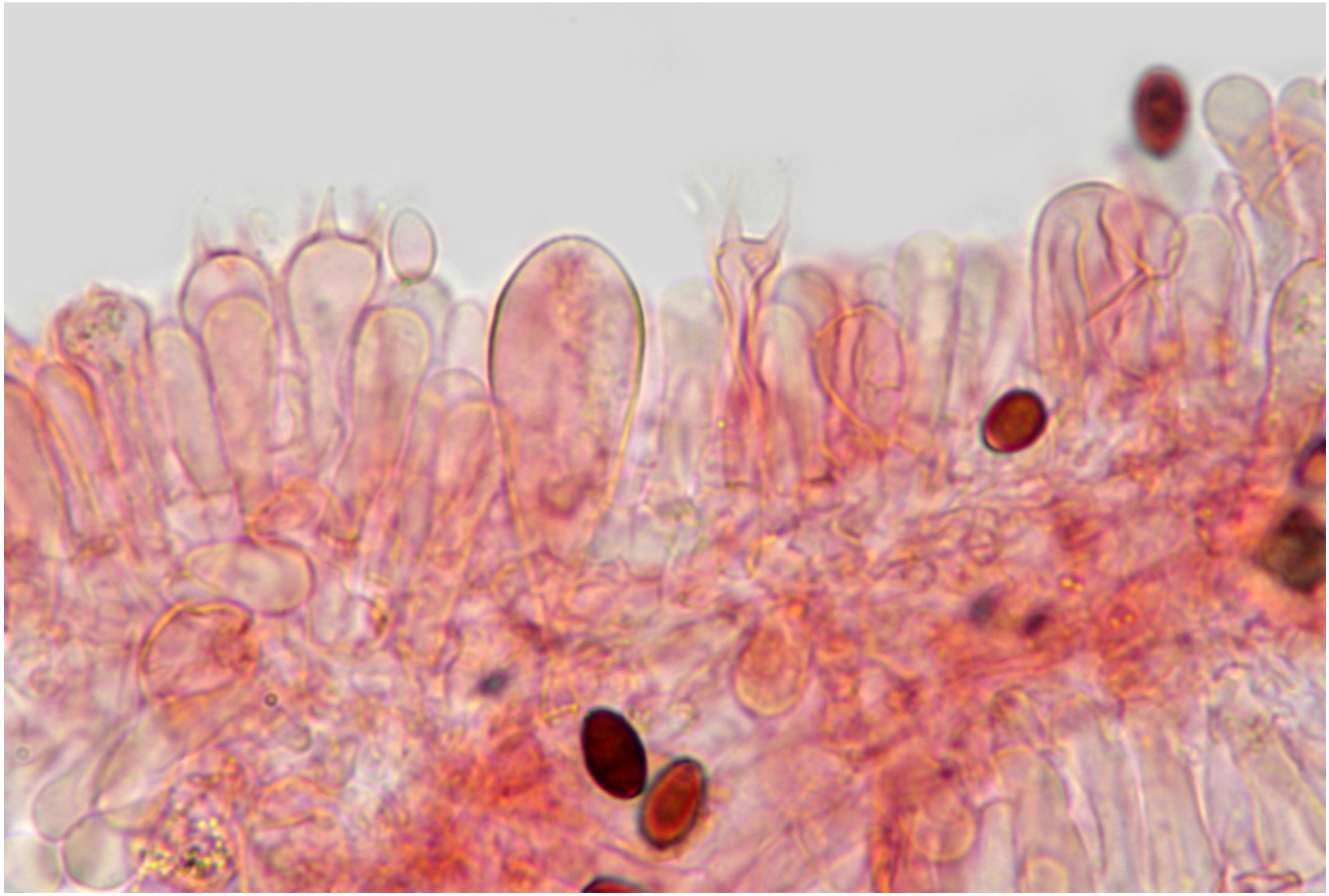
Photos : Miquel À. Pérez-De-Gregorio

Pleurocystidia: 28-48 x 11-17.7 µm, very numerous, mostly spatulated and strongly pedunculated, also but less frequently clavate or ventricose with a short neck and an obtuse or subcapitate apex, often with a faintly thickened (0.5 µm) brown coloured wall.



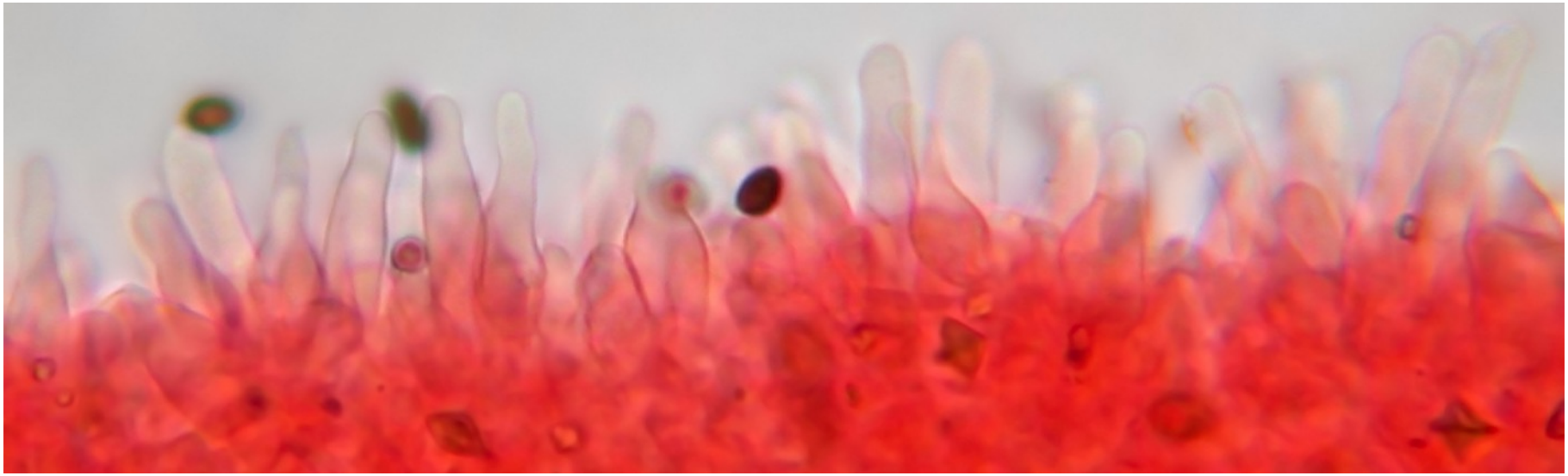


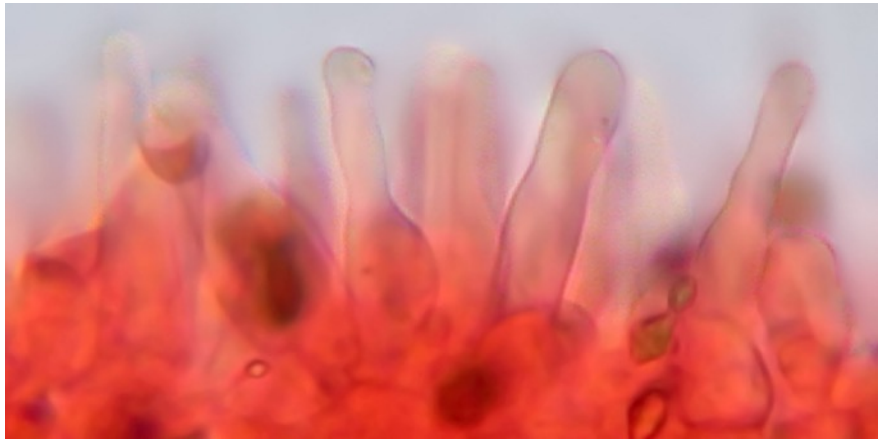




Basides et pleurocystides – photo Miquel À. Pérez-De-Gregorio

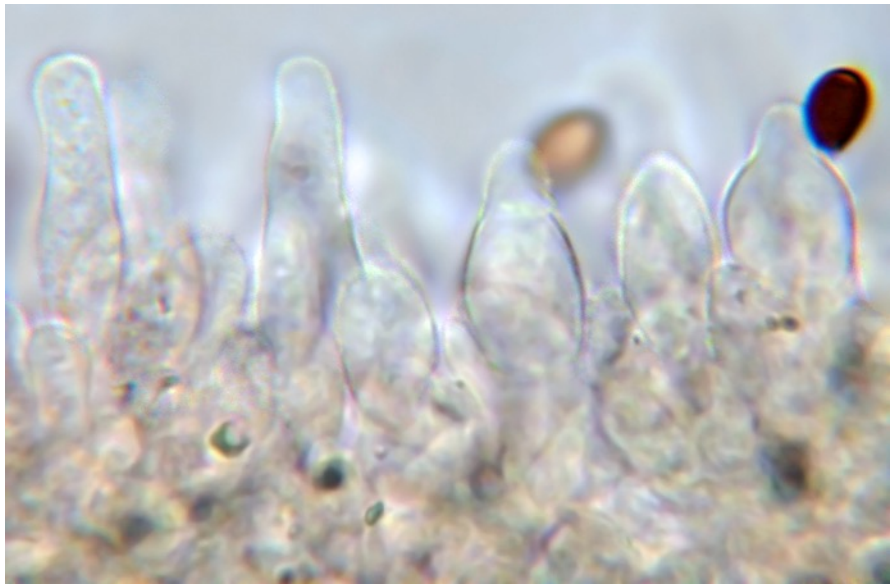
Cheilocystidia: Of two types,
A) predominantly lageniform and sublageniform, not distinctly pedunculated, with a long, large and sometimes flexuous neck, tapering to an obtuse and rarely bifurcated apex; also, but less usually ventricose with a subcapitate apex, 27-55 x 8-12 µm, colourless and thin walled, very numerous and densely packed all along the edgewithout gaps,
B) clavate and spheropedunculated, 16.5-22 x 11-13.7 µm, hidden by the other cheilocystidia, colourless, rare.

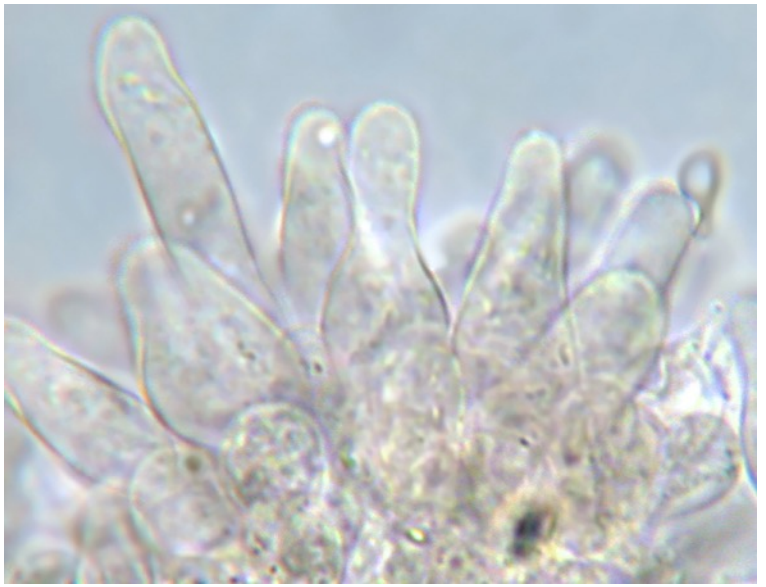


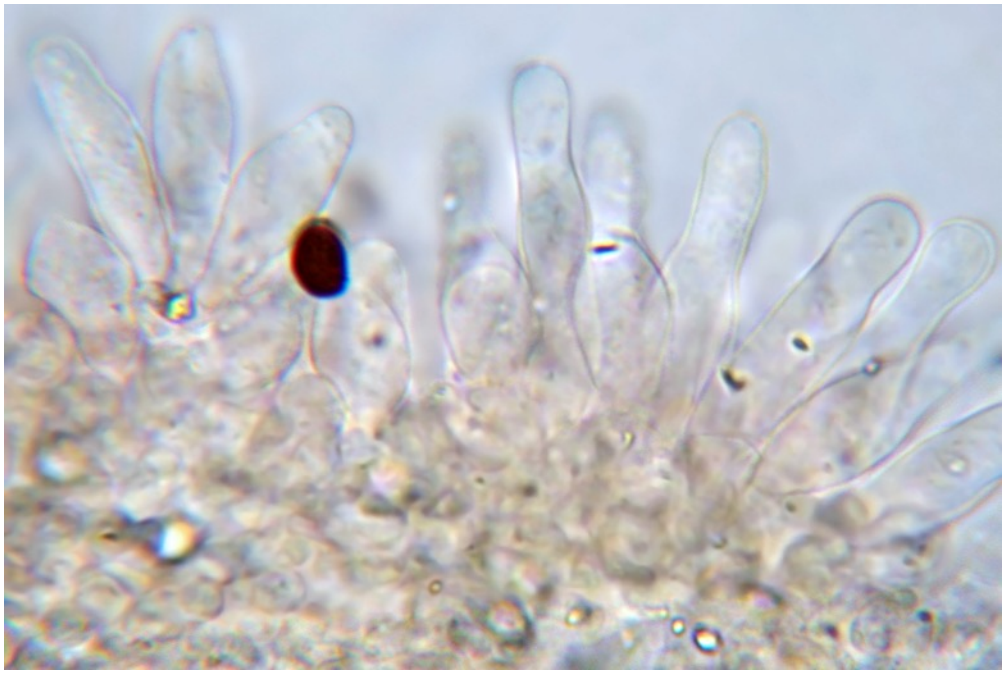


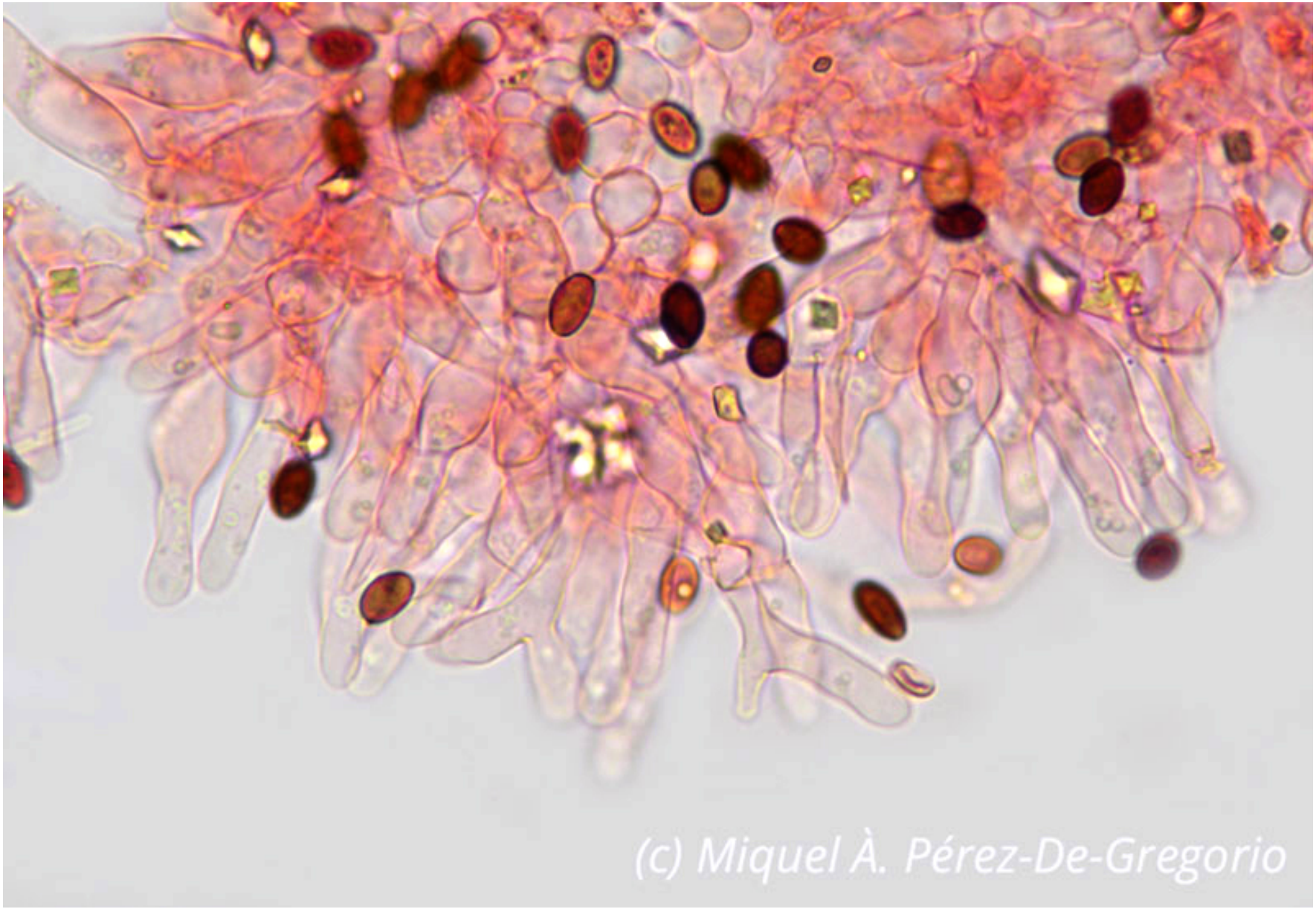
Edge x 400

Cheilocystidia in NH4OH 10%

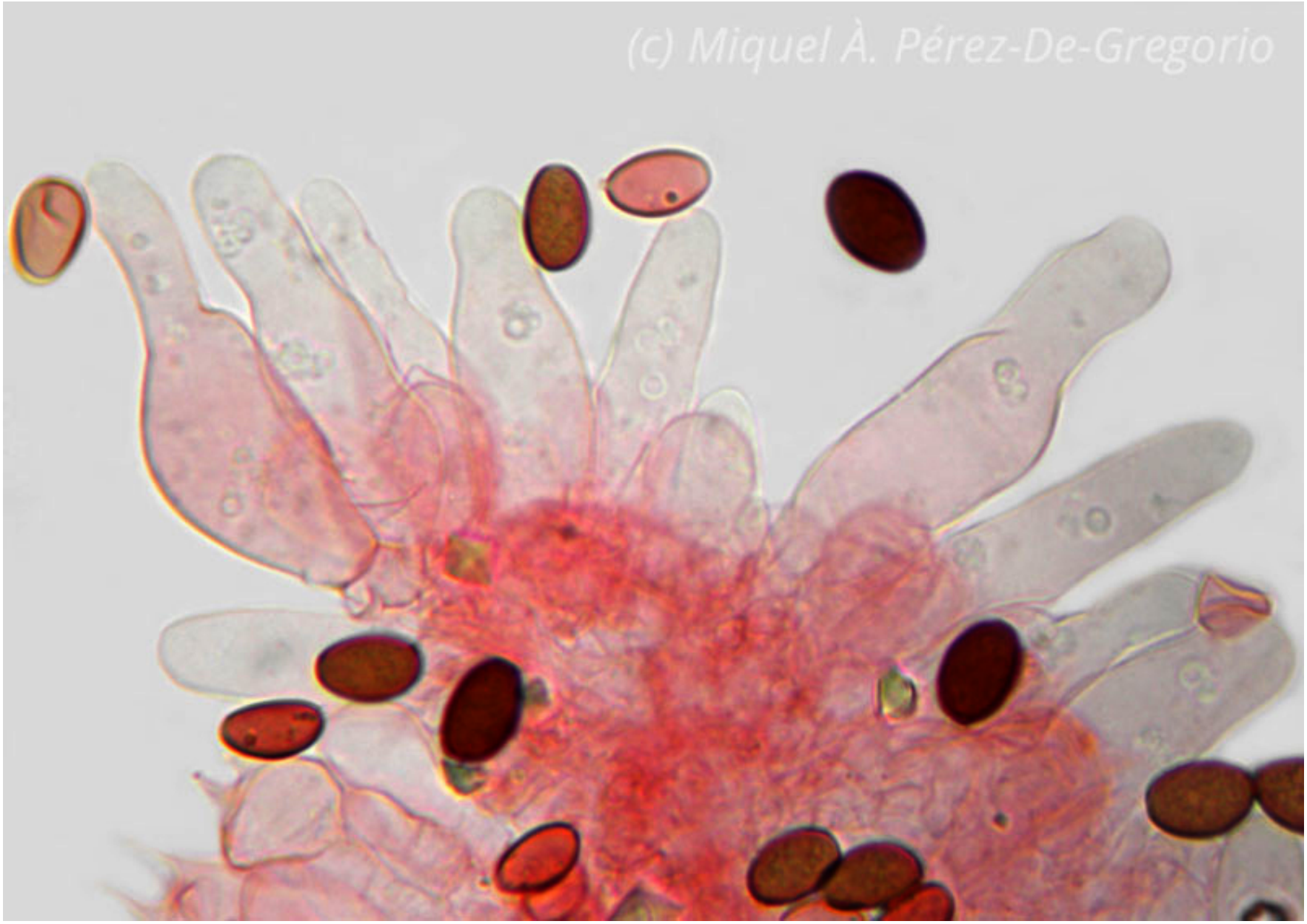








cheilocystidia



cheilocystidia

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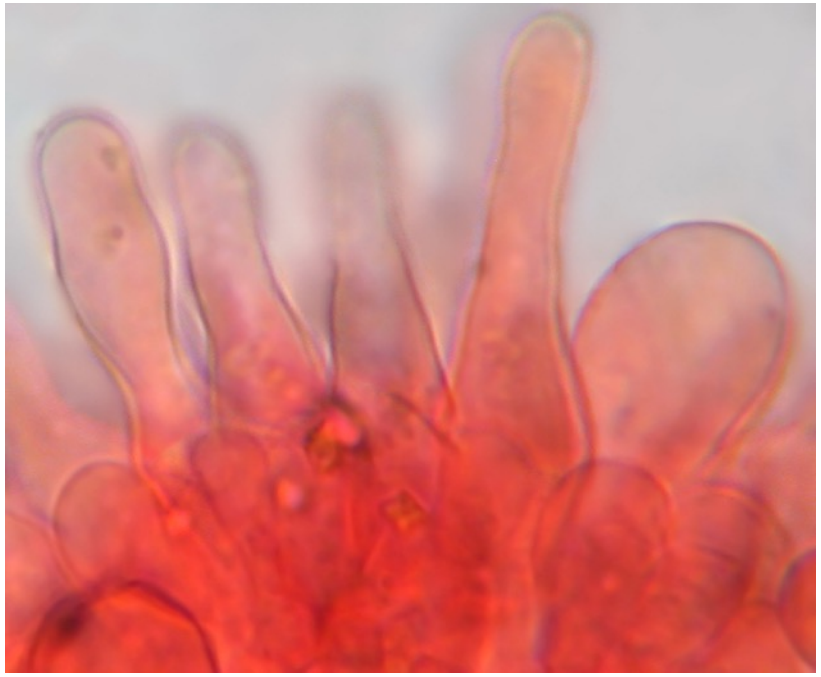
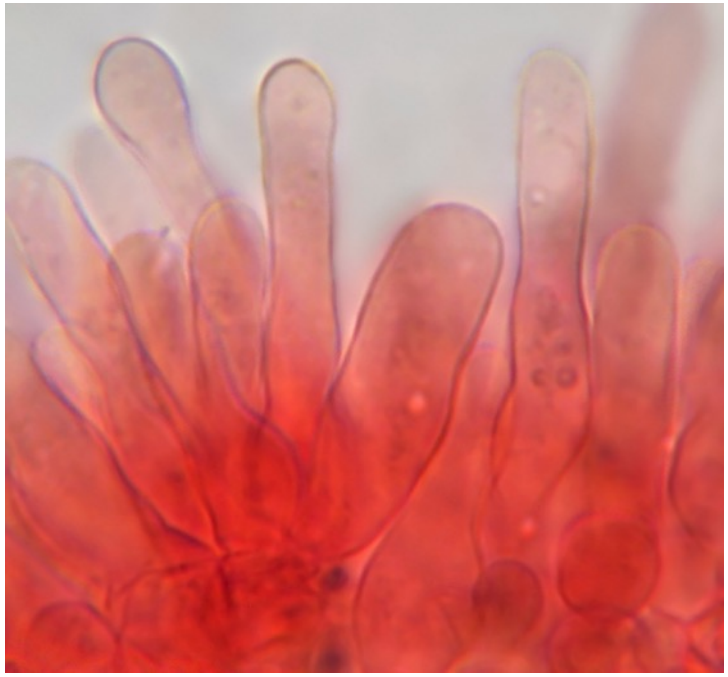
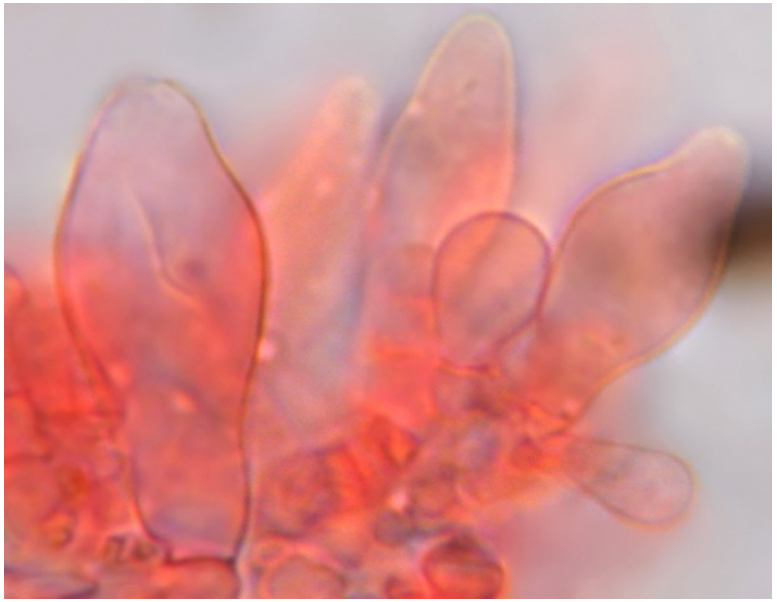
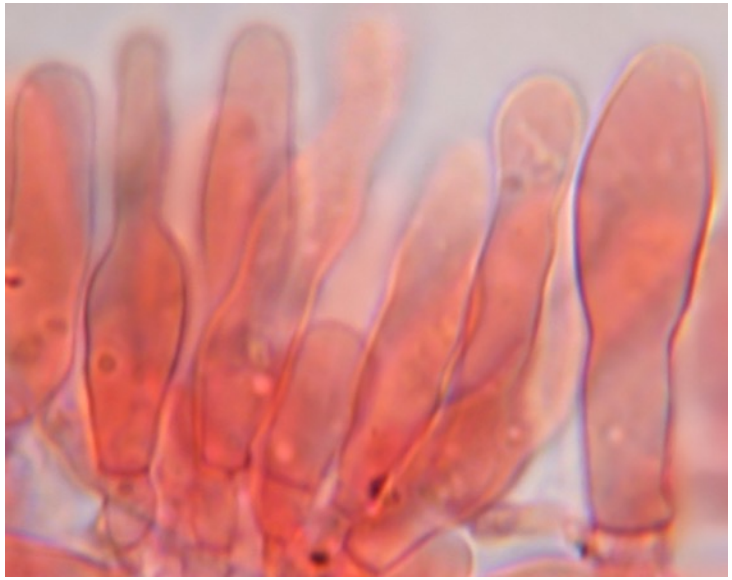
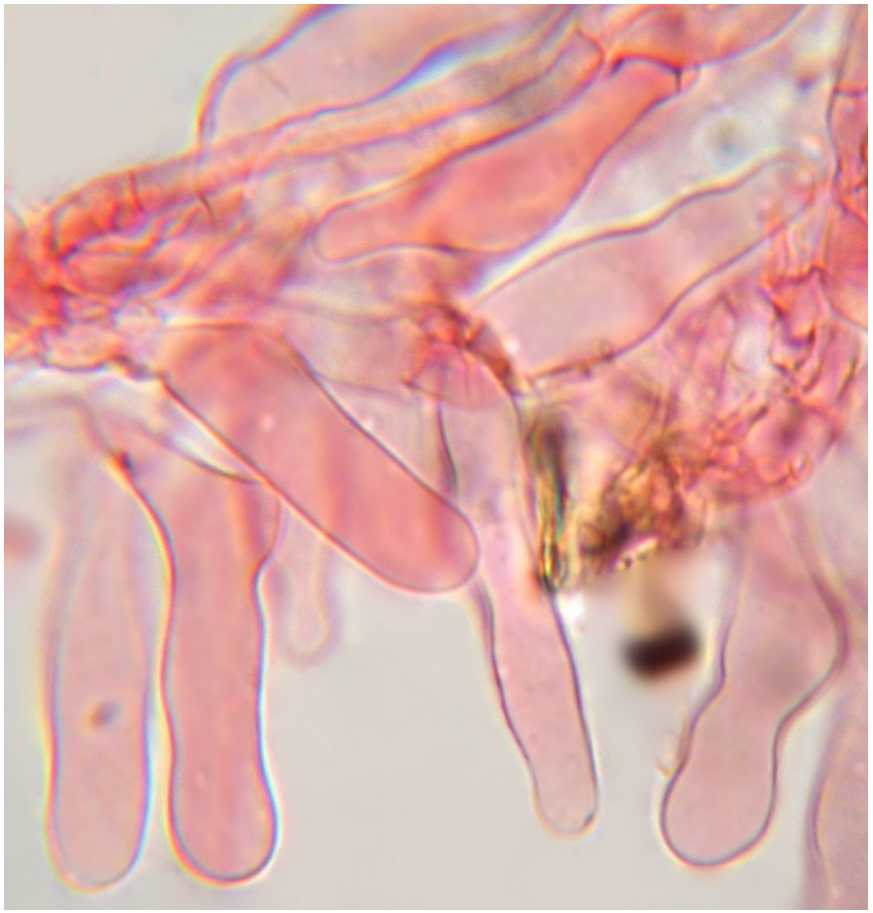








Caulocystidia: Similar to the cheilocystidia or versiform, sometimes with a slightly thickened wall, occasionally up to 90 µm long, numerous.
Gill trama: Yellowish brown; noteworthy is the presence of numerous greenish pyramidal crystals in the hymenium.
Cutis: Several layers of usually clavate and spheropedunculated cells.
Clamps: Present
Habitat: On a stump of living *Quercus robur* L. (Common oak).



caulocystidia

Discussion: English and French text

Psathyrella codinae is primarily characterized by medium-sized dark spores, spatulated pleurocystidia and their slightly thickened walls. No European species is even approximately similar.
In addition to the molecular biological study has been tested, whether it is a species already known but not included in GenBank. The review of the North American species in SMITH (1972) showed that none of the descriptions looked like *Psathyrella codinae*.

Also, a review of species with thickened cystidia walls outside the USA and Europe did not produce any results. Cystidia of *Psathyrella chiloensis* Singer are somewhat similar, but there is no veil present and the spore dimensions are only 5.5-6.5 x 2.8-3.7 µm (VALENZUELA & al. 1994).

For the latter reason *Psathyrella hesleriaffinis* Singer (SINGER 1978*)* should also be rejected.

Psathyrella phaeocystidiata Singer has large spores and cystidia with an approximately similar shape but the apex clearly wears crystals; in addition, this species has a ring on the stem (SINGER 1973).

Psathyrella amaura (Berk. & Broome) Pegler has very pale spores without a germ pore (KITS VAN WAVEREN 1995).

Psathyrella codinae est principalement caractérisée par ses spores sombres de taille moyenne ainsi que par ses pleurocystides spatulées dont la paroi est légèrement épaissie et teintée. Aucune espèce Européenne n'est similaire.
En sus de l'étude de biologie moléculaire nous nous sommes assurés qu'il ne s'agissait pas d'une espèce déjà connue mais non répertoriée dans GenBank.
L'examen des espèces nord-américaines décrites dans Smith (1972) a montré qu'aucune des descriptions ne correspondait à celle de *Psathyrella codinae* et une revue des espèces possédant des cystides à paroi épaisse hors Europe et Etats-Unis n'a pas donné davantage de résultats**.**

Psathyrella chiloensis Singer possède des cystides quelque peu similaires, mais l'espèce est dépourvue de voile et les spores, beaucoup plus petites**,** ne mesurent que 5,5-6,5 x 2,8-3,7 µm (Valenzuela et al., 1994).

Psathyrella hesleriaffinis Singer (Singer 1978) se différencie également facilement de notre espèce par la taille de ses spores.

Psathyrella phaeocystidiata Singer possède de grandes spores et des cystides d'aspect proche, mais leur apex est nettement coiffé de cristaux et de plus cette espèce est annelée (Singer 1973).

Psathyrella amaura (Berk & Broome) Pegler se distingue également de notre espèce par ses spores très pâles et (un espace en trop) dépourvues de pore germinatif (Kits van Waveren 1995).

Measurements

	Spores		Pleurocystides		cheilocystides	
1	8	5	28	12	30	10
2	8	5	30	11	30	12
3	8	5	30	13	32	11
4	8	5	32	14	32	11
5	8	5,5	33	13	33	10
6	8	5,5	33	13	34	11
7	8,5	5	35	15	35	9
8	8,5	5	35	15	35	11
9	8,5	5	36	12	35	11
10	8,5	5	37	13	36	11
11	8,5	5	38	12	39	12
12	9	4,5	38	12	40	7
13	9	5	40	13	40	9
14	9	5	40	14	40	10
15	9	5	40	14	40	10
16	9	5	45	13	40	14
17	9	5	48	14	42	8
18	9	5			42	9
19	9	5			44	9
20	9	5			45	8
21	9	5			45	8
22	9	5,5			45	8
23	9	6			45	9
24	9	6			48	10
25	9	6,5			55	9
26	9,5	5				
27	10	5				
28	10	5				
29	10	5,5				
30	10,5	6				
	8,88	5,20				

Acknowledgments

We are grateful to Dr André Fraiture who reviewed this article, to Pablo Alvarado Garcia for the sequencing and to Dieter Wächter for creating the consensus tree; to Santi Gibert, Joaquim Carbó and Àngel Torrent for the forwarding of the exsiccata, the photo taken at the growthplace and the dispersion diagram of the spores.
Carles Roqué suggested the species name. The fieldwork allowing the discovery of this fungal species has been funded by the city hall of Olot within the research project “Catàleg dels fongs en l’àmbit dels espais interurbans del ParcNou - Pla de Llacsi la Moixinad’Olot”.

Bibliography

Borchsenius F., 2009 - FastGap 1.2., Department of Biosciences, Aarhus University, Denmark. Published online at http://www.aubot.dk/FastGap_home.htm.
Cubeta M. A., Echandi E., Abernethy T. & Vilgalys R., 1991 - Characterization of anastomosis groups of binucleate *Rhizoctonia* spp. using restriction analysis of an amplified ribosomal RNA gene. Phytopathology 81: 1395-1400.
Gardes M. & Bruns T. D., 1993 - ITS primers with enhanced specificity for basidiomycetes-application to the indentification of mycorrhizas and rusts. Molecular Ecology 2: 113-118.
Geospiza Inc.: FinchTV 1.4.0. http://www.geospiza.com
Henriot A. & Cheype J.-L., 2016 - Piximètre. piximetre.fr
Kits van Waveren E., 1995 - The Berkeley & Broome species of *Psathyrella* in the Kew Herbarium. Kew Bulletin 50(2): 307-325.
Küppers H., 2007 - DuMont Farbenatlas (10. Auflage). Köln, 165 p.
Lanfear R., Frandsen P. B., Wright A. M., Senfeld T. & Calcott B., 2016 - PartitionFinder 2: new methods for selecting partitioned models of evolution formolecular and morphological phylogenetic analyses. Molecular Biology and Evolution 34(3): 772-773. DOI: dx.doi.org/10.1093/molbev/msw260
Ronquist F., Telenko M., van der Mark P., Ayres D. L., Darling A., Höhne S., Larget B., Liu L., Suchard M. A. & Huelsenbeck J.P., 2012 - MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. Systematic Biology 61(3): 539-542.
Simmons M. P. & Ochoterena H., 2000 - Gaps as characters in sequence-based phylogenetic analyses. Systematic Biology 49: 369-381.
Singer R., 1973 - Diagnoses fungorum novorum Agaricalium III. - Beihefte zur Sydowia 7: 1-106.
Singer R., 1978 - Interesting and new species of Basidiomycetes from Ecuador II. - Nova Hedwigia 29(1-2): 1-98.
Smith A. H., 1972 - The North American Species of *Psathyrella*. Memoirs of the New York Botanical Garden 24: 1-633.
Stamatakis A., 2006 - RAxML-VI-HPC: maximum likelihoodbased phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688-2690.

Valenzuela E., Moreno G., Garnica S. & Grinbergs L., 1994 - Agaricales senso lato de Chile. II. Boletín de la SociedadMicológica de Madrid 19: 281-304.
Veidenberg A., Medlar A. & Löytynoja A., 2016 - Wasabi: An Integrated Platform for Evolutionary Sequence Analysis and Data Visualization. *Molecular Biology and Evolution 33(4): 1126-1130. DOI:*https://doi.org/10.1093/molbev/msv333
Vilgalys R. & Hester M., 1990 - Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. Journal of Bacteriology 172(8): 4238-4246.
White T. J., Bruns T., Lee L. & Taylor J. W., 1990 - Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis M. A., Gelfand D. H., Sininski J. J.& White T. J. (eds) PCR protocols, a guide to methods and applications. Academic Press, New York, pp 315–322.