

Description d'une récolte Française de *Psathyrella obscuotristis* (Enderle & M.Wilhelm ex Enderle & M. Wilhelm 2008).
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Daniel Deschuyteneer & Dieter Wächter

Synonymes : aucun connu.

Synonyms: none known.

Introduction

Cette espèce assez charnue a été récoltée par Annie Duvert, en septembre 2018, sur un tronc moussu pourrissant, dans la forêt domaniale de Laigue, située au nord-est de l'Oise, dans la région Hauts-de-France par 4,9°27'32" nord, 2°56'30" est. Cette forêt, dont l'altitude est située entre 35 et 129 m, est essentiellement peuplée de futaies de chênes, de charmes et de hêtres.

This fairly fleshy species was collected by Annie Duvert in September 2018 from a rotting mushy trunk, in the "Forêt de Laigue" located, in the « Hauts-de-France » region at 49°27' 32 » north, 2°56'+30"east.

This forest, whose altitude is between 35 and 129 m, is mainly populated by oaks, hornbeams and beech trees.

Résumé: cette espèce de la section *Hydrophylae* est caractérisée par son habitat lignicole sur feuillus, la forte pigmentation de l'ensemble de ses structures, ses très petites spores ellipsoïdes à ovoïdes, à parois épaisses dépourvues de pore germinatif et ses cheilocystides et pleurocystides clavées et sphéropédonculées rostrées.

Summary: This species of the *Hydrophylae* section is characterized by its hardwood lignicole habitat, the strong pigmentation of all its structures, its very small thickwalled ellipsoid to ovoid spores without germ pore, its clavate and spheropedonculate cheilocystidia and its pleurocystidia which are often rostrate.

Description macroscopique - Macroscopic description.

Espèce charnue apparaissant sur bois mort de feuillus, de manière isolée ou en petits groupes, dont les stipes sont parfois connés par leur base.

A fleshy species that appears on dead hardwood, either alone or in small groups, whose stipes are sometimes connected by their base.

Chapeau mesurant de 10 à 50 mm de diamètre, brun sombre, initialement convexe à marge enroulée, devenant plan-convexe, non strié, non hygrophane.

Cap measuring 10 to 50 mm in diameter, dark brown, initially convex with a curled margin, becoming flat-convex, not striated, not hygrophanous.

Lames alternant avec lamelles et lamellules, brun-noisette devenant brun-marron, larges, ventrues, serrées, adnées, dont l'arête fimbriée est blanche ou plus pâle, presque concolore.

Lamellae close, alternating with lamellulae, hazelnut-brown becoming chesnut-brown, broad, ventricose and adnate, with a white or paler fimbriated edge.

Stipe mesurant 20-40 x 36 mm, habituellement cylindrique, nettement fibrilleux, blanchâtre, se tachant de rouille par la base.

Stipe measuring 20-40 x 36 mm, usually cylindrical, strongly fibrillous, whitish, stained with rust from the base.

Voile fibrilleux abondant, reliant la marge au stipe chez les sujets jeunes, formant parfois à ce niveau une zone sub-annulaire, et persistant généralement au cours de la croissance sous forme d'un réseau dense de fibrilles et lambeaux blanchâtres adhérents à la marge du chapeau.

Veil white abundant fibrillous, connecting the margin of the cap to the stipe in young specimens, sometimes forming a sub-annular zone at this level, and generally persistent during growth as a dense network of fibrils and whitish flaps adherent to the margin of the cap.

Chair épaisse, brune ; saveur non précisée, mais odeur douce ou terreuse avec une pointe subtile d'amande amère selon les données de la littérature.

Flesh thick, brown, odour and taste not specified, but sweet or earthy with a subtle bitter almond odour according to the literature.



Photos in situ : Annie Duvert



Photos in situ : Annie Duvert

L'examen et les photos des caractères microscopiques ont été réalisés par le premier auteur sur exsiccatum.

Basides 12-25 x 4-6 μm , clavées et cylindriques, tétrasporiques.

Spores mesurant (4,5-)4,6-**4,9**-5,2(-5,4) \times (3,2-)3,6-**3,8**-4(-4,2) μm , à parois épaisses, lisses, brunes dans l'ammoniaque, grises dans la potasse 5%, non opaques, ellipsoïdes à ovoïdes de face, asymétriques de profil et généralement amygdaliformes, parfois guttulées ; pore germinatif absent ; apicule très petit.

Pleurocystides mesurant 22-35(-40) x 9-13 μm , (N=25), peu nombreuses, clavées, sphéropédonculées, rostrées, parfois à parois épaisses et colorées de jaune-brun.

Cheilocystides mesurant 18-40(-45) x 8-15 μm , clavées, sphéropédonculées, rostrées, identiques aux pleurocystides nombreuses, éparses ou groupées, parfois à parois épaisses et colorées de jaune-brun, mélangées à de nombreuses basides et basidioles. Les **rostres**, souvent fort développés ont une longueur de 3 à 15 μm .

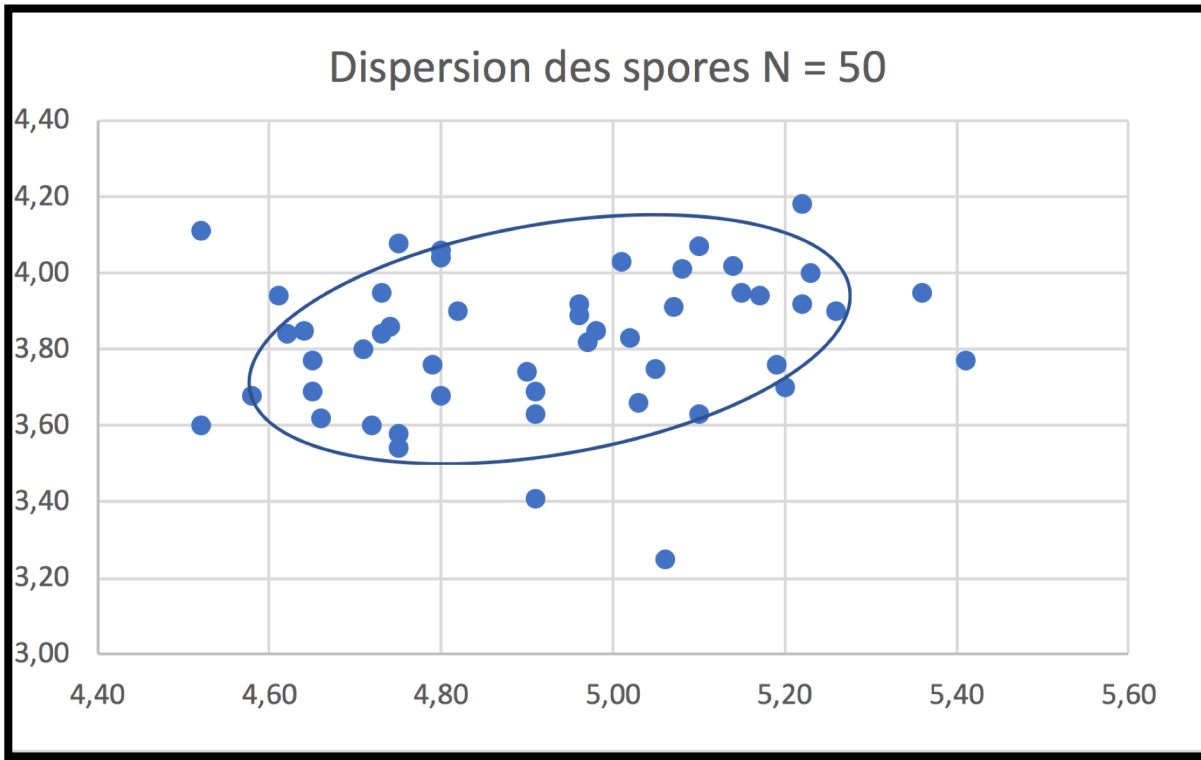
Méδιοstrate fortement pigmentée de brun, dont les hyphes sont partiellement incrustées.

Voile constitué d'hyphes cylindriques partiellement bouclées teintées de jaune.

Pileipellis : un hyménoderme constitué de une à deux couches de cellules globuleuses et sphéropédonculées teintées de jaune.

Pilleitrame : hyphes cylindriques très pigmentées de jaune et partiellement incrustées.

Boucles présentes à tous les niveaux.



Mesures des spores effectuées avec Piximètre.

(4,5-) 4,6-5,2 (-5,4) × (3,2-) 3,6-4 (-4,2) μm

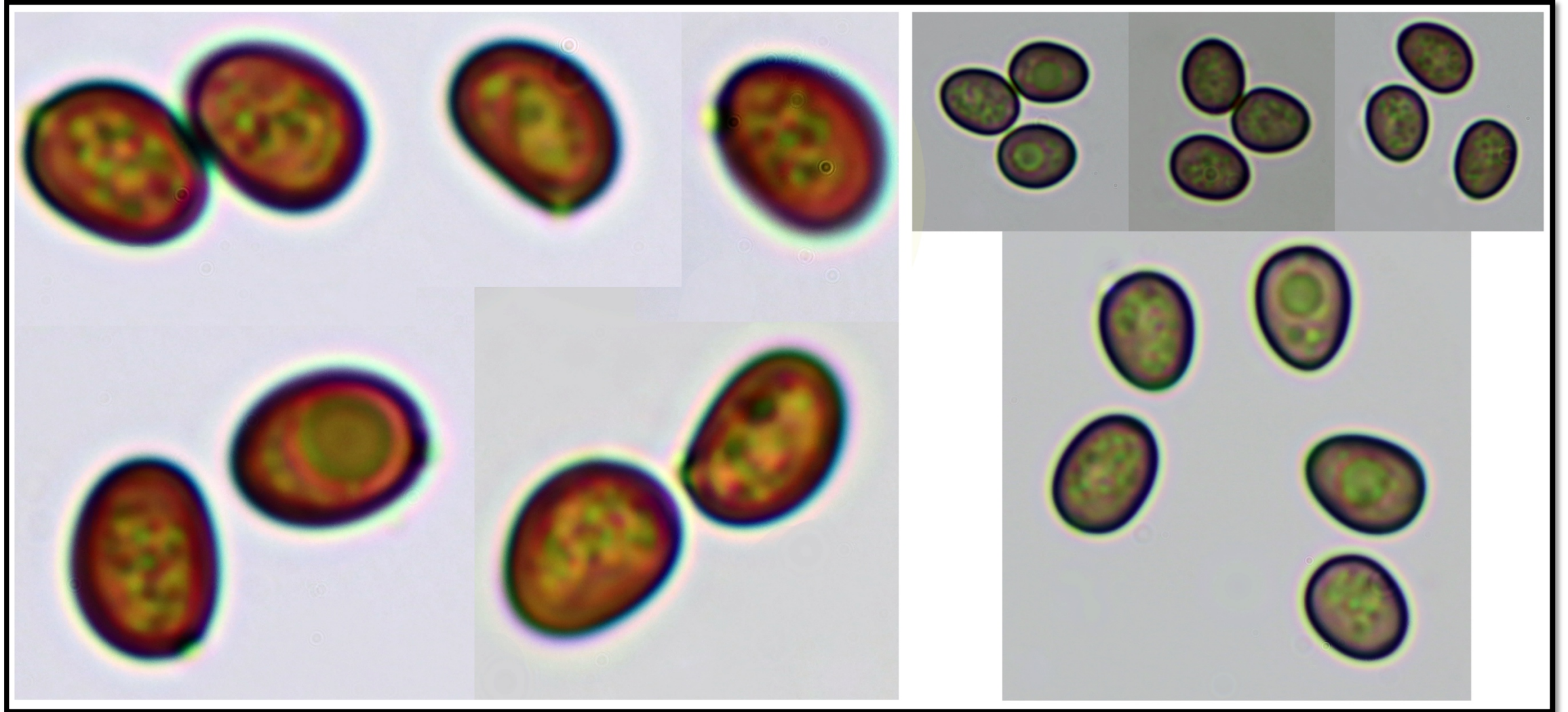
Q = (1,1-) 1,2- 1,4 (-1,6) ; N=50

Me= 4,9 × 3,8 μm ; Qe =1,3

Cheilocystidia inclusive mucron length	Mucron length	Pleurocystidia inclusive mucron length	Mucron length
18	12	0	20
20	13	0	21
22	10	0	22
24	12	6	22
25	11	5	24
26	10	8	25
26	11	0	25
28	10	7	25
28	10	5	27
28	12	0	27
28	14	5	28
30	11	0	28
30	11	0	29
30	11	5	30
30	10	6	30
30	11	8	31
32	9	11	31
32	10	5	31
32	12	4	31
33	9	6	32
34	10	5	34
34	11	6	35
34	13	9	35
36	10	4	39
38	8	17	40
38	10	8	
38	11	8	
40	10	15	
41	13	16	
45	15	5	

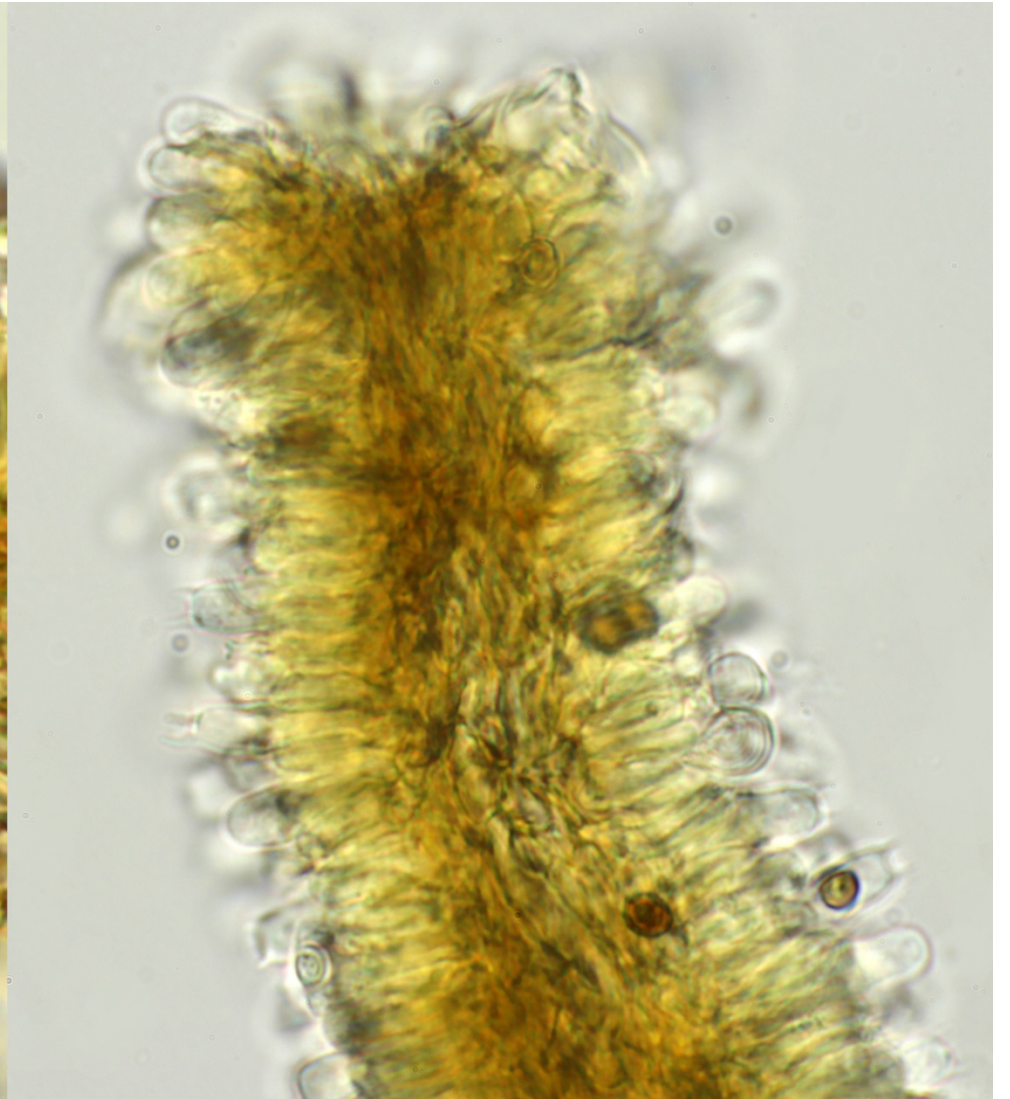
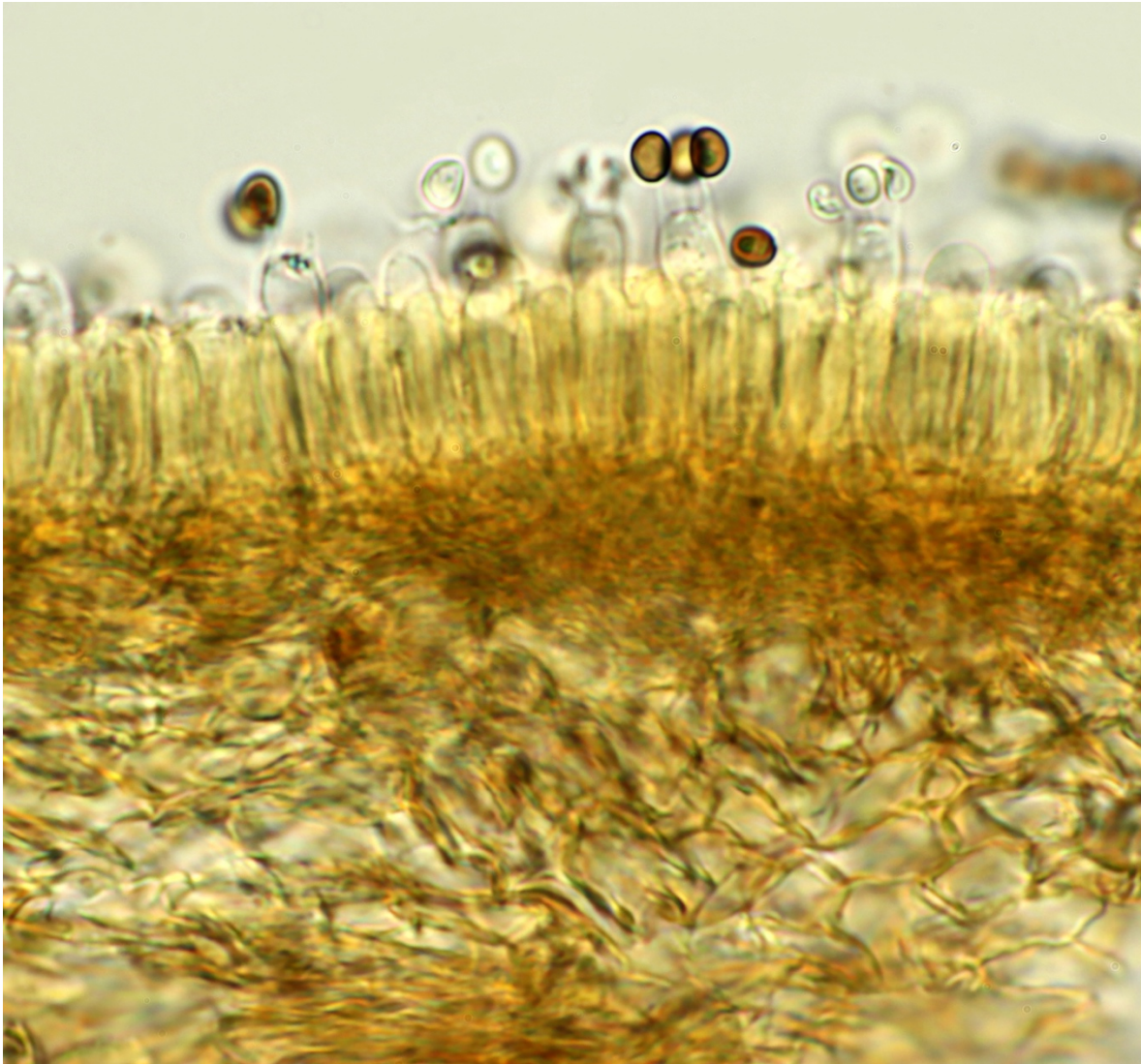
Enderle	4,6-5,6(6) x 3,6-4,1 μm
Melzer - récolte de Friebes - AM1823	4,4-5(-5,6) x 2,5-3,1 μm, i. M. 4,9 x 2,8 μm, mittl. Q=1,75
Walley & Volders	4,7-5,1-5,8 x 3,3-3,6-4 μm

Spores measuring, (4,5-) 4,6-4,9-5,2 (-5,4) × (3,2-) 3,6-3,8-4 (-4,2) μm; Q = (1,1-) 1,2- 1,4 (-1,6) ; N=50;
thick walled, smooth, brown in NH₄OH 10%, grey in KOH 5%, not opaque, in front view ellipsoid to ovoid, in side view asymmetric and generally amygdaliform, sometimes guttulated, germ pore absent, very small apicle.

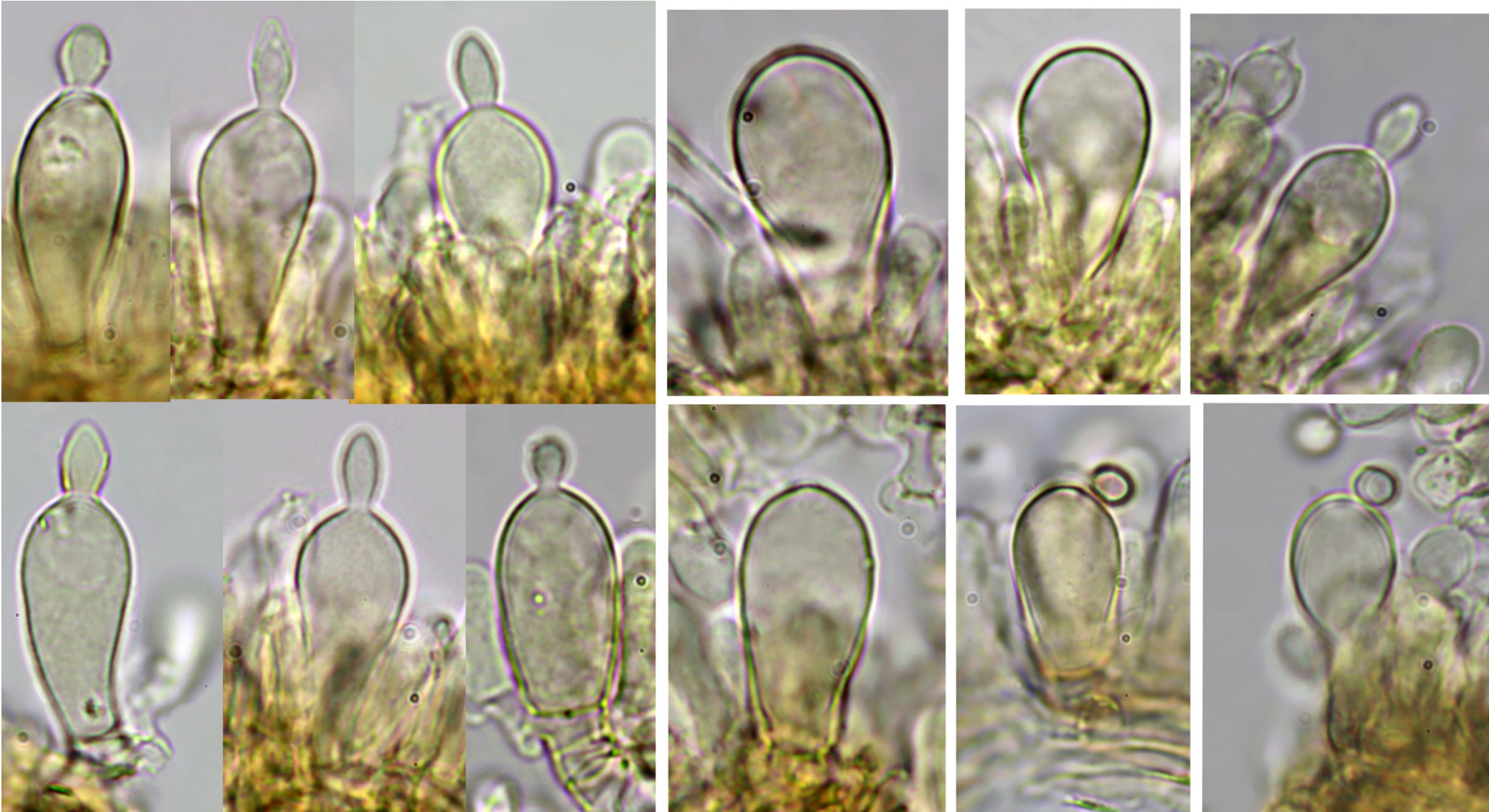


Basidia 12-25 x 4-6 μm , 4spored, clavate and cylindrical.

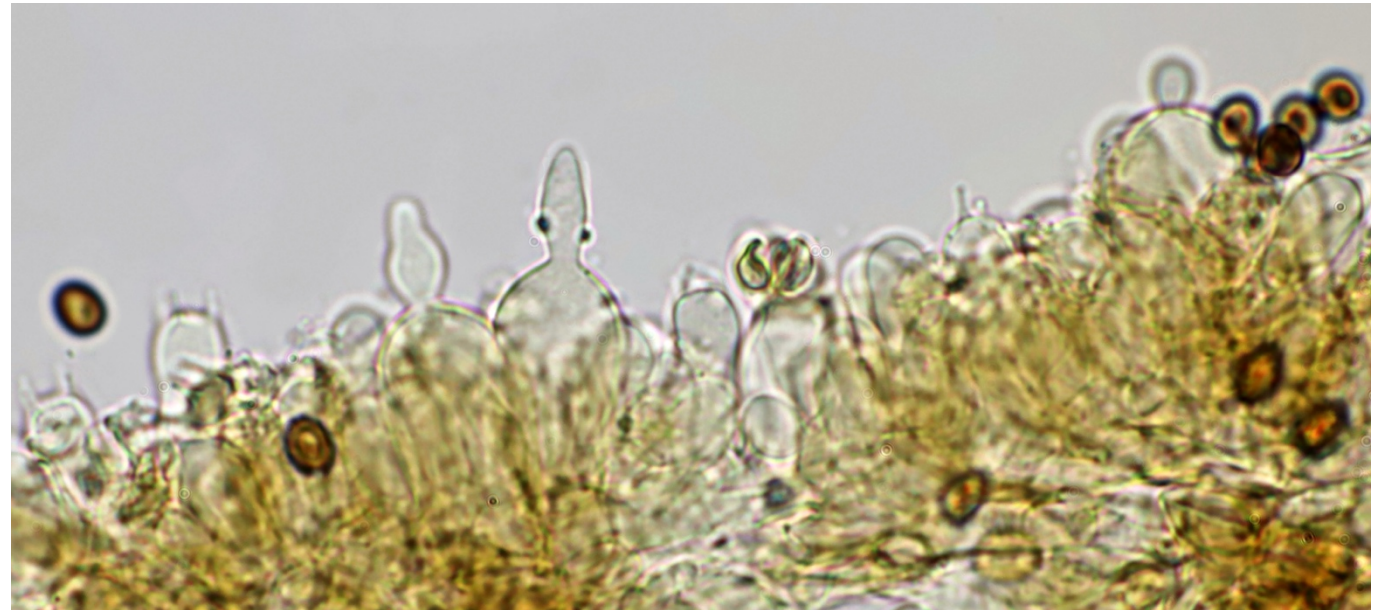
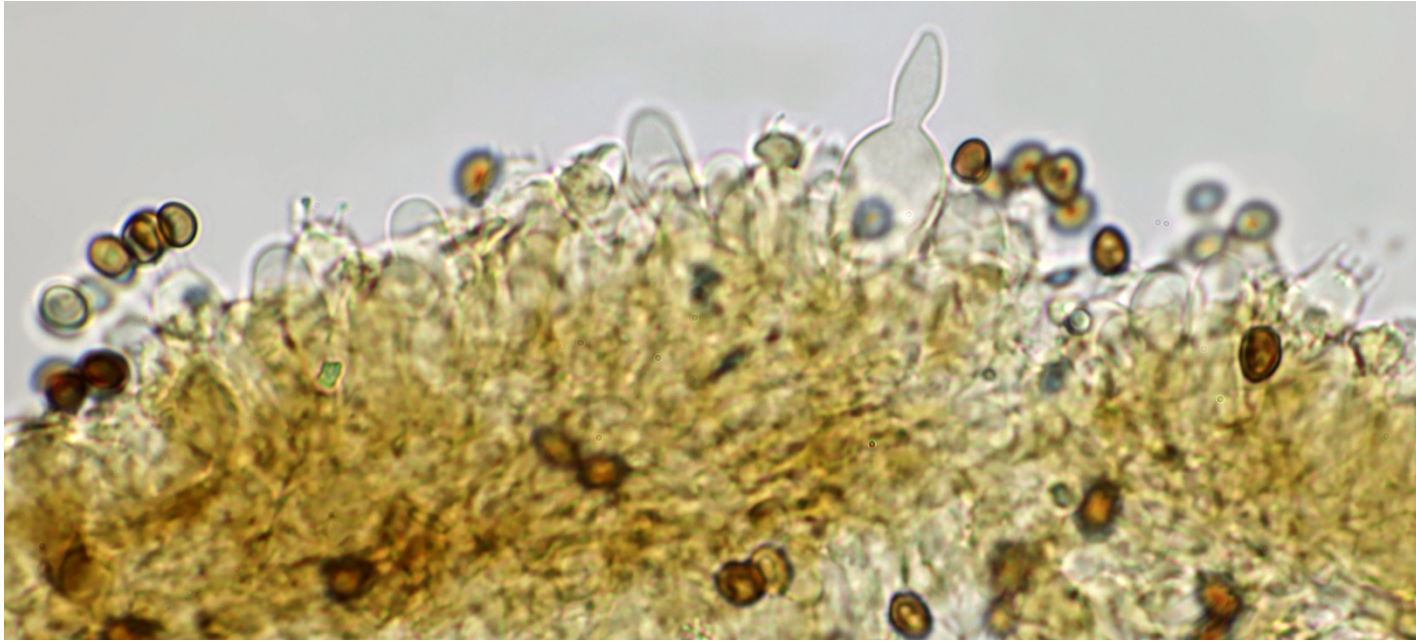
Lamellae trama, strongly brown pigmented, whose hyphae are partially encrusted.



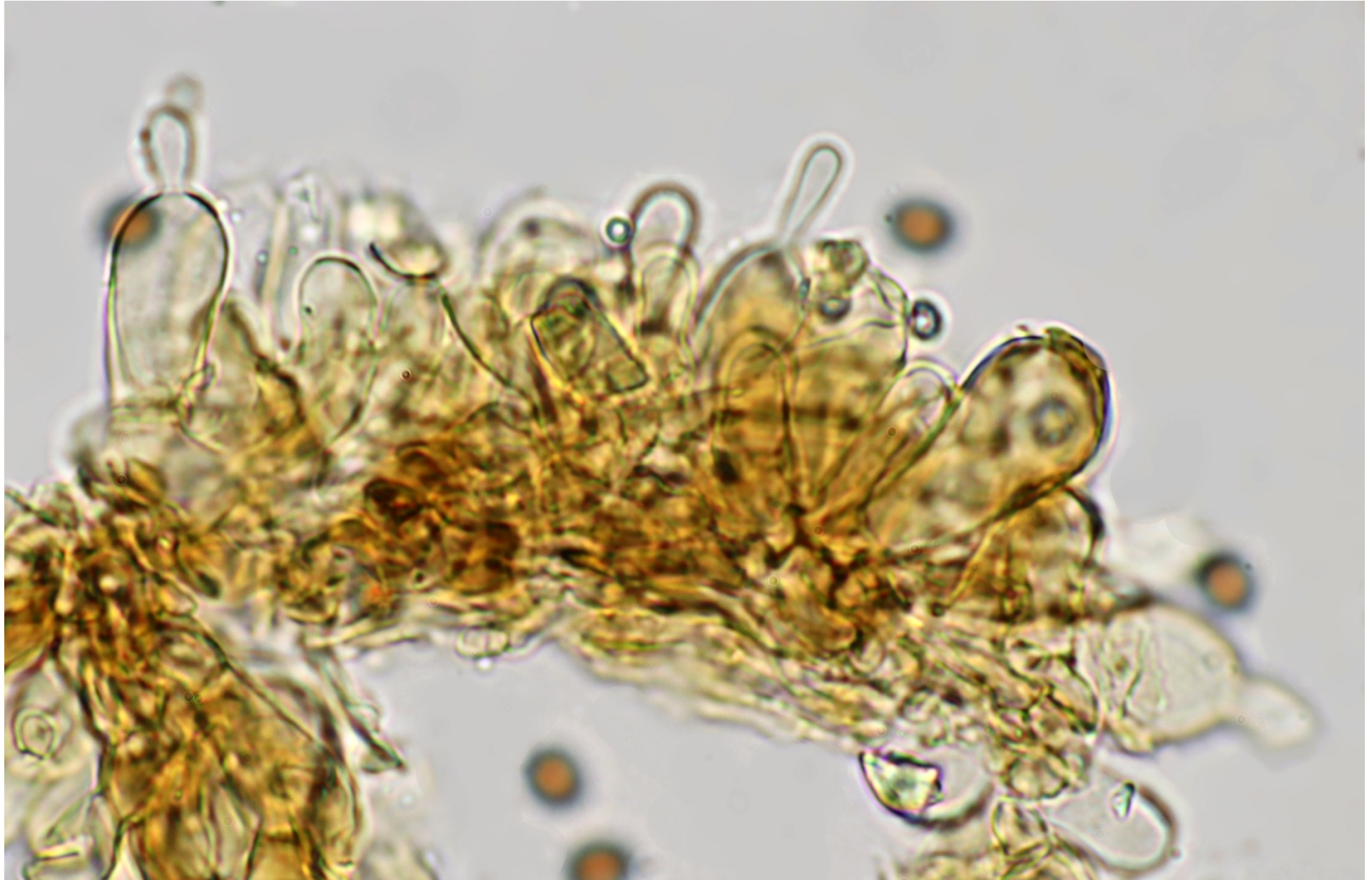
Pleurocystidia measuring 22-35(-40) x 9-13 μm (N=25); few in number, clavate, spheropedonculate, rostrate, sometimes thick-walled and yellow-brown coloured.



Arête - Edge

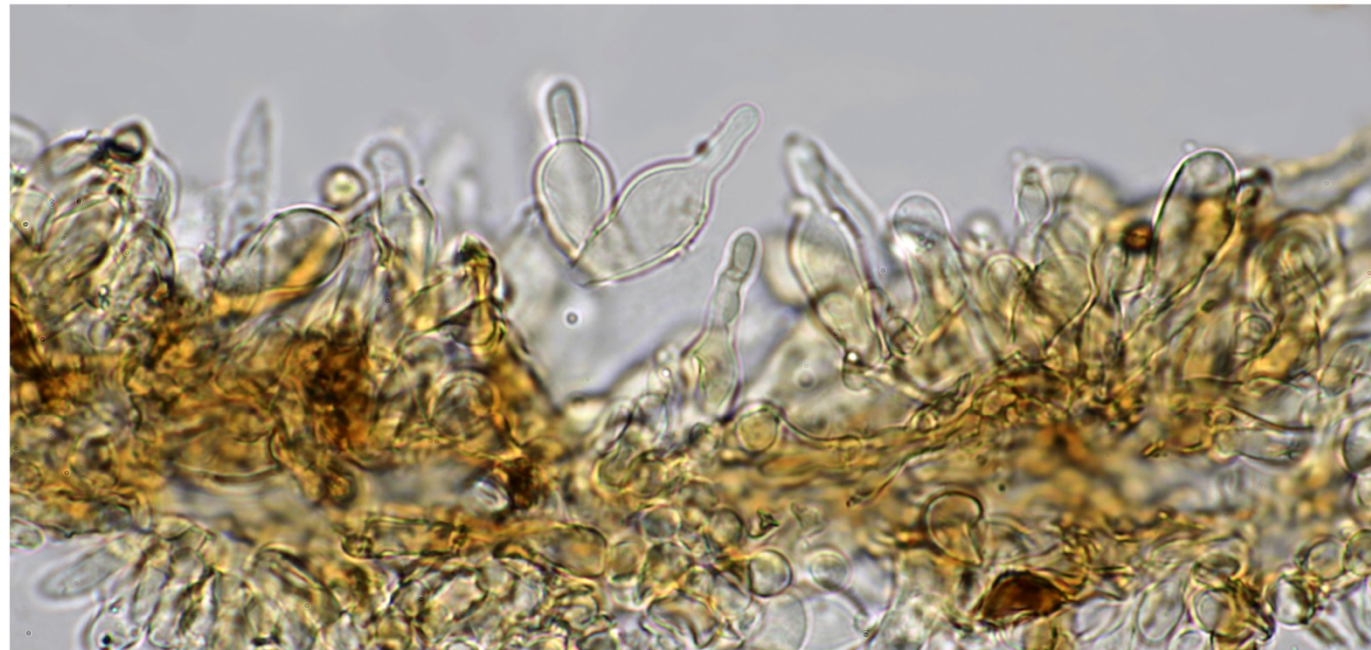
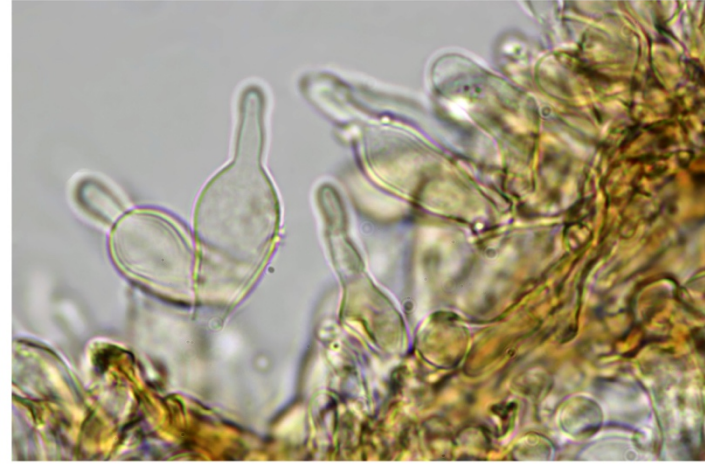
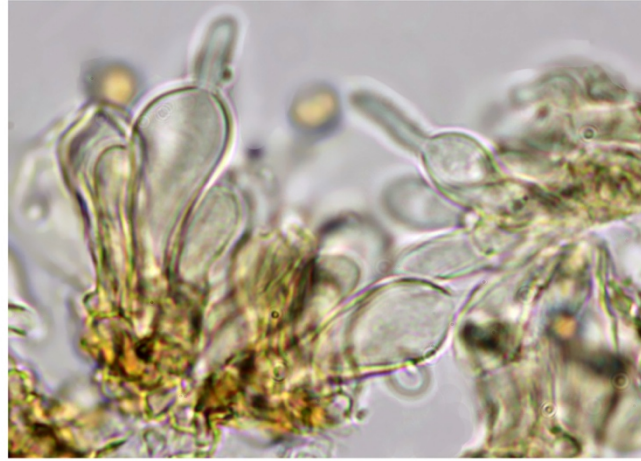


Arête - Edge



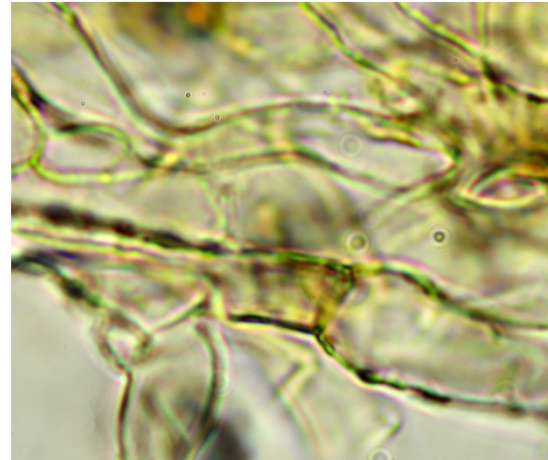
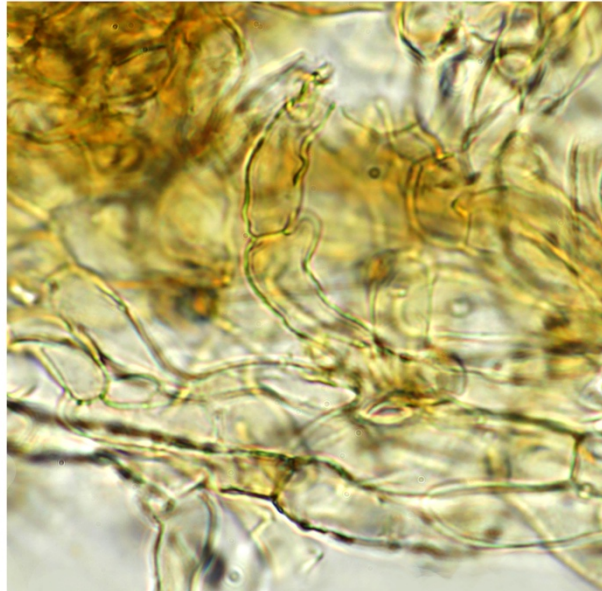
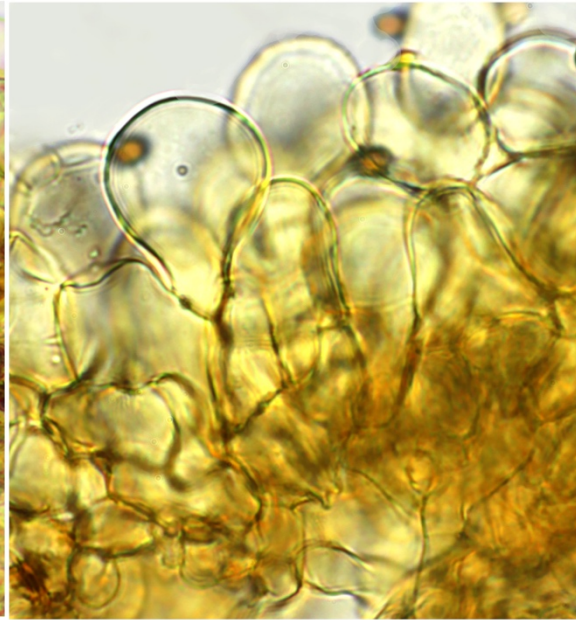
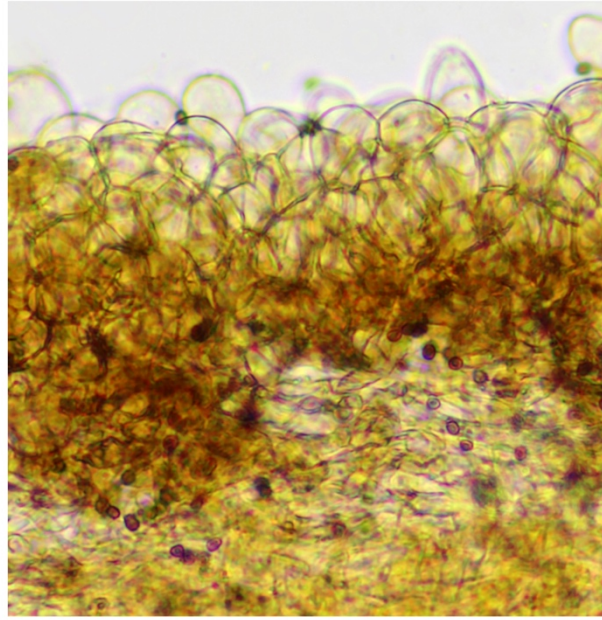
Cheilocystidia measuring 18-40 (-45) x 8-15 μm ; clavate, spheropedonculate, rostrate, similar to pleurocystidia, numerous, scattered or in clusters, sometimes thick-walled and yellow-brown coloured, mixed with many basidia and basidiols.

The rostrum, often highly developed, has a length of 3 to 15 μm .



Pileipellis: an hymeniderm of one or two layers of globular and spheropedonculate yellow-tinged cells.

Pilleitrama: cylindrical strongly yellow-tinged hyphae often encrusted.



Séquençage ADN (fraction-ITS) réalisé sur exsiccatum

Référence 2018-1094-ALV17438 DD-MELI1 = ITS ok, 93% *Psathyrella obscuotristis* (KC992931)

Genbank Acession number/Version: **MK129248.1**

Bien que la concordance du séquençage ADN de notre récolte avec KC992931 ne soit que de 93%, l'ensemble des caractères macroscopiques et microscopiques permettent une détermination certaine et le clade *obscuotristis* a été étendu de manière à intégrer ces nouvelles données.

Although the concordance of the DNA sequencing of our specimens with KC992931 is only 93%, all macroscopic and microscopic characteristics allow a definite determination and the *obscuotristis* clade has been extended to include these new data.

2018-1094-ALV17438 DD-MELI1 = ITS ok, 93% *Psathyrella obscuotristis* (KC992931)

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GTAAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGCGGAAGGATCATTAAATGAAATAATTATGGTGTTGGTTGTAGCTGGCTTTTAG
GAGCATATGTGCACACCCATCATTTTTTATCTTTCCACCTGTGCACTTATTGTAGGTCTGGATAATGCTCACTCTCACAATGAGTGGATAC
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Sequencing and phylogenetic analysis

DNA Extraction, Amplification and Sequencing of the fungus was performed by Alvalab (Oviedo, Spain). The phylogenetic analysis was done by Dieter Wächter (Thiersheim, Germany). The genomic DNA was extracted from dried fruiting bodies. Amplification of the ITS region was performed with the ITS4 primer [1]. The initial base calling was done with FinchTV [2]. The nucleotide sequence was checked manually for errors, as well as the base calling at unsafe regions (trails, low confidence scores, stutters and polymorphs) on the basis of existing sequences of the */Hydrophilae*-clade by divergence matrix and corrected if necessary. In the present case only a trimming of the trails was necessary. The following molecular phylogenetic markers were used for the phylogenetic analysis: ITS1 (Internal Transcribed Spacer 1), 5.8S (5.8S rRNA Gene), ITS2 (Internal Transcribed Spacer 2), LSU (Large Subunit 28S rRNA Gen), β -tub (exons of the β -tubulin gene), ef-1 α (exons of the ef-1 α gene). The nucleotide sequences for the tree inference were taken from NCBI [3] and Unite [4] (essential ones of the */Hydrophilae*-clade see Table 1). Region boundaries for the ITS- and LSU-region were carried out with ITSx [5] and HMMER [6] including the databases. As outgroup, the sequence sets of the most closely related clades of the Ingroup were used, i.e. the */pygmaea* down to the */gordonii* clade. Due to the rapidly evolving, indel-rich areas of the ITS region, it can only be aligned veridical by using an iterative multigene-guide tree. The initial alignment of the ITS region was performed with Mafft [7] using the FFT-NS-2 method. The initial alignment of the LSU gene was carried out using E-INS-i method. The indel matrices for the ITS and LSU regions were each coded with SeqState [8] using the SIC = "Simple Indel coding" [9] method. After each alignment step, an ML analysis with RAxML [10] (model: GTRCAT, refining under GTR+G for DNA, GTR2+G with acquisition bias correction according to Lewis [11] for indel partitions) was carried out and the resulting best tree was used as a guide tree for the refinement of the ITS1 and ITS2 MSA. The iterative alignments were done with Prank [12], whereby the switches -once and -uselogs were set. Tracing values were recorded, evaluated statistically and thus the end of the iteration loop of the alignment was determined. The partitioning of all alignments and the indel matrices as well as the model selection for the DNA alignments was done with Partitionfinder [13]. For the final partitioning, the guide tree of the last iteration step was used. As information criterion the Bayesian Information Criterion (BIC) [14] used was after comparison with the Corrected Akaike Information Criterion (AICc) [15] and evaluation with respect to over- or under-partitioning. The partitioning scheme of the final phylogeny was:

- DNA-partition 1: ITS1 + ITS2
- DNA-partition 2: LSU
- DNA-partition 3: 5.8S + β -tub-Codon 1 + β -tub-Codon 2
- DNA-partition 4: β -tub-Codon 3 + ef-1 α -Codon 3
- DNA-partition 5: ef-1 α -Codon 1
- DNA-partition 6: ef-1 α -Codon 2
- Binary partition (gap matrices): ITS1 + ITS2 + LSU

The final maximum likelihood analysis was done with RAxML 8.2.10 [10]. For all DNA partitions, the GTR substitution matrix [16] under the CAT model [10] was used. The final optimization took place under gamma distribution [10]. For the binary partitions, the "Two State Time-Reversible Model" with acquisition bias correction [11] was used. 1000 ML bootstrap inferences were calculated. Of these, 1000 trees were sampled and the best tree was labeled with the ML bootstrap support values and collapsed to the ML bootstrap value of 50%. The phylogram in Fig 1 was edited with Treegraph [17]. The upper Psathyrella clades (*/noli-tangere* to */fibrillosa*) has been collapsed for a better representation.

Table 1 List of relevant sequences used in this publication

Species	Voucher	ITS	LSU	β -Tub	ef-1 α
<i>Conocybe appendiculata</i>	2226	JF908596.1			
Cf. <i>Psathyrella</i> sp.	AH1166	HM583851.1			
<i>Psathyrella piluliformis</i>	F19690	HM240540.1	HM240540.1		
<i>Psathyrella</i> sp.	F19734	HQ604761.1	HQ604761.1		
<i>Psathyrella piluliformis</i>	FPD	MF686489.1			
<i>Psathyrella piluliformis</i>	H6038507	UDB021187			
<i>Psathyrella mucrocystis</i>	H6038542	UDB021220			
<i>Psathyrella pertinax</i>	H6038548	UDB021225			
<i>Psathyrella piluliformis</i>	HMJAU 37922	MG734716.1			
<i>Psathyrella pertinax</i>	HMJAU 6830	MG734735.1			
<i>Psathyrella maculata</i>	KA13-0933	KR673660.1			
<i>Psathyrella maculata</i>	KA13-1206	KR673679.1			
<i>Psathyrella</i> sp.	MES-1490	KY462469.1			
Uncultured fungus	S63	FJ820551.1			
<i>Psathyrella piluliformis</i>	SCD17	MF755280.1			
<i>Psathyrella oboensis</i>	SFSU DED 8234	NR_148107.1			
<i>Psathyrella</i> sp.	TENN61733A	FJ596872.1			
<i>Psathyrella</i> sp.	TENN61767-H1	FJ596886.1			
<i>Psathyrella piluliformis</i>	WU20516	AM712272.1	AM712272.1		
<i>Psathyrella echinata</i>	ZT12073	KC992925.1	KC992925.1	KJ664900.1	
<i>Psathyrella pertinax</i>	SZMC-NL-2350	FM878012.1	FM876269.1	FN396262.1	FM897259.1
<i>Psathyrella piluliformis</i>	SZMC-NL-2936	FN396113.1	FN396164.1	FN396306.1	FN396219.1
<i>Psathyrella piluliformis</i>	SZMC-NL-3923	FN396136.1	FN396185.1	FN396316.1	FN396235.1
<i>Psathyrella maculata</i>	CBS206.33	GQ249281.1	GQ249290.1	GQ249265.1	GQ249273.1
<i>Psathyrella piluliformis</i>	LO162-02	DQ389699.1	DQ389699.1	KJ664899.1	KJ732808.1
<i>Psathyrella pertinax</i>	LO259-91	DQ389701.1	DQ389701.1		KJ732809.1
<i>Psathyrella mucrocystis</i>	LO103-98	DQ389700.1	DQ389700.1	KJ664901.1	KJ732810.1
<i>Psathyrella obscurotristis</i>	Wilhelm489	KC992931.1	KC992931.1		KJ732816.1
<i>Psathyrella maculata</i>	CBS 208.33	MH855417.1			
<i>Psathyrella maculata</i>	CBS 209.33	MH855418.1	MH866869.1		
<i>Psathyrella maculata</i>	ECO-TA-HO 7880	MF156265.1			

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FinchTV 1.4.0: Geospiza, Inc.; Seattle, WA, USA; <http://www.geospiza.com>

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HMMER 3.1b2 (February 2015): <http://hmmer.org/> - Copyright (C) 2015 Howard Hughes Medical Institute. Freely distributed under the GNU General Public License (GPLv3)

Mafft 7.372 (used over mafft.cbrc.jp)

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Discussion

Sur base de certains de ses caractères microscopiques, *Psathyrella obscuotristis* pourrait éventuellement être confondue avec :

Psathyrella laevissima, petite espèce, macroscopiquement très différente, peu charnue, au chapeau plan convexe le plus souvent nettement ridé, dont le voile est peu développé, rapidement labile, qui présente quelques pleurocystides rostrées et qui possède de petites spores mesurant en moyenne 5-6(-6,5) x (2,5-)3-4(-4,2) μm , avec un petit pore germinatif central distinct.

Psathyrella piluliformis, une espèce assez grande et charnue, à croissance sub-fasciculée, qui possède également de petites spores mesurant en moyenne 5-6,1 x 2,9-3,5 μm dont le pore germinatif est indistinct et dont les cystides ne sont pas rostrées.

Psathyrella umbrina une espèce au voile abondant, qui possède des cystides rostrées et des spores de dimensions nettement supérieures, mesurant en moyenne 6,7-7,5 x 4,5-4,8 μm , dont le pore germinatif est absent ou indistinct.

Psathyrella maculata de la section *Pseudostropharia*, qui possède des cystides mucronées (pas rostrées), de petites spores mesurant en moyenne 4,6-5,5 x 3-3,4 μm avec un pore germinatif indistinct ou absent, mais dont le chapeau est largement recouvert d'écailles brunâtres.

Based on some of its microscopic characters, *Psathyrella obscuotristis* may possibly be confused with:

Psathyrella laevissima, a small and macroscopically very dissimilar species, not very fleshy, with a flat-convex cap that is usually clearly wrinkled, with a scarce veil, quickly volatile, with some rostrate pleurocystidia and small spores measuring on average 5-6(-6,5) x (2,5-) 3-4(-4,2) μm , having a small but distinct central germ pore.

Psathyrella piluliformis, a medium size and fleshy species, most often fasciculate, which has also small spores averaging 5-6,1 x 2,9-3,5 μm with an indistinct germ pore without rostrate cystidia.

Psathyrella umbrina, a species with abundant veil, rostrate cystidia, and significantly larger spores averaging 6,7-7,5 x 4,5-4,8 μm without or with an indistinct germ pore.

Psathyrella maculata of the *Pseudostropharia* section, which has mucronate (not rostrate) cystidia, small spores averaging 4,6-5,5 x 3-3,4 μm with an indistinct or absent germ pore but whose cap is largely covered with brownish scales.

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Auteurs - Authors

Daniel Deschuyteneer, Spreeuwenhoek 12, 1820 Perk, Belgique. danieldeschuyteneer@gmail.com

Dieter Wächter, Burgstrasse 5, 95707 Thiersheim, Germany. info@nocrotec.com