Psathyrella senex, une espèce qui en cache une autre ? Psathyrella senex, a species which hides another one ?

Author's: D. Deschuyteneer and Dieter Wächter for the molecular part.

Psathyrella senex (Peck) A. H. Sm. 1972
in Memoirs of the New York Botanical Garden 24: 230 (1972)

MycoBank: 321366

Basionym: *Psilocybe senex* Peck 1888 in Rep. New York State Mus. 41: 70

Synonymy according to Index fungorum

Psilocybe senex Peck, *Rep. (Annual) Trustees State Mus. Nat. Hist., New York* 41: 70 (1888)

Psathyra senex (Peck) Morgan, *J. Mycol.* 13(4): 151 (1907)

Psathyra fibrillosa sensu Lange; fide Checklist of Basidiomycota of Great Britain and Ireland (2005)

Psathyrella fibrillosa sensu Maire; fide Checklist of Basidiomycota of Great Britain and Ireland (2005)

Drosophila ocellata Romagn., *Bull. mens. Soc. linn. Soc. Bot. Lyon* 21: 154 (1952)

Psathyrella ocellata (Romagn.) M.M. Moser, in Gams, *Kl. Krypt.-Fl.*, Edn 3 (Stuttgart) 2b/2: 222 (1967)

Psathyrella friesii Kits van Wav., *Persoonia* 9(2): 282 (1977)

Psathyrella trivialis Arnolds, *Biblthca Mycol.* 90: 437 (1982)

Psathyrella fulvescens var. brevicystis Kits van Wav., *Persoonia*, Suppl. 2: 281 (1985)

Psathyrella ploddensis Kits van Wav., *Persoonia* 13(3): 357 (1987)

Psathyrella senex group A
Author's: D. Deschuyteneer, Dieter Wächter.
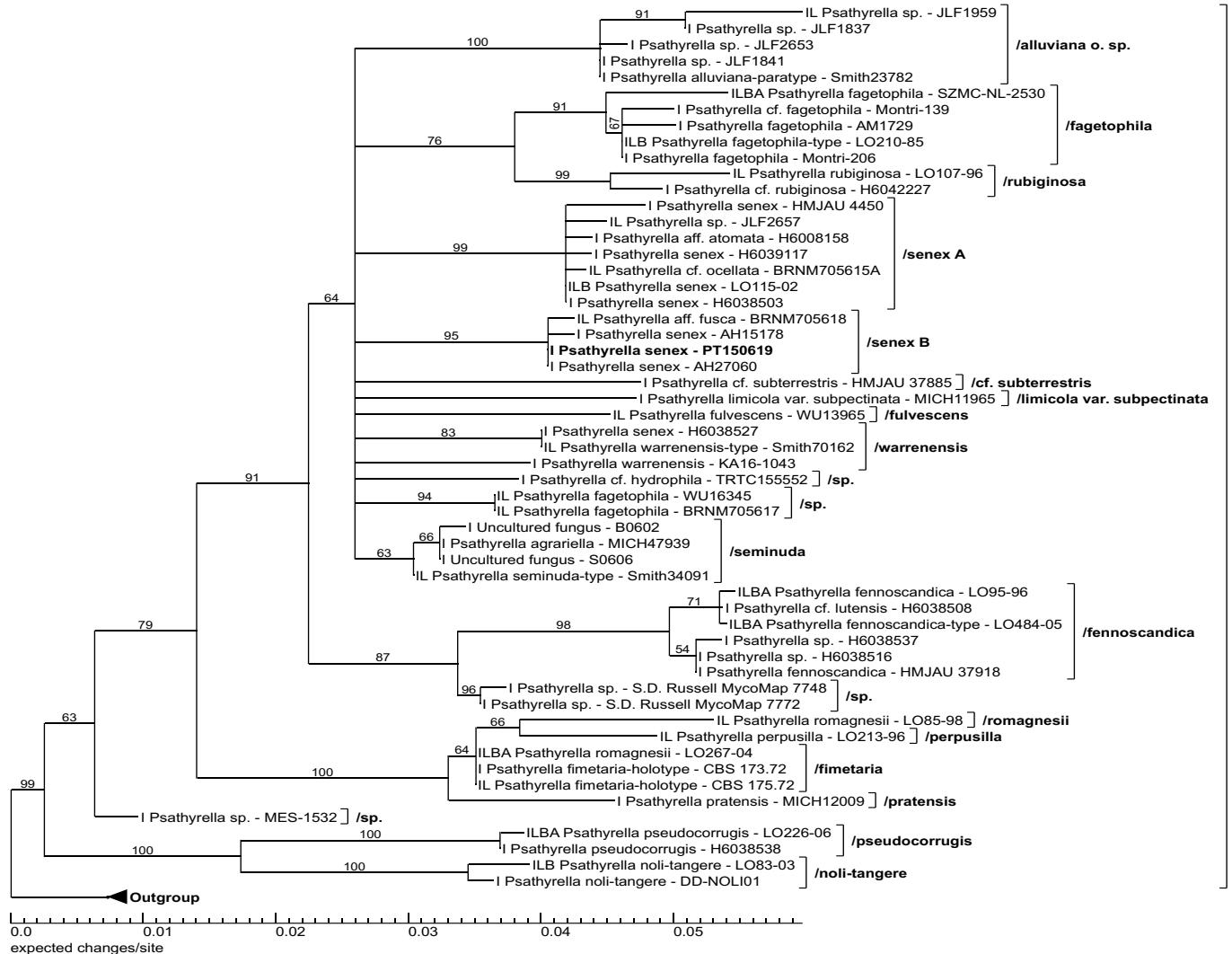
Psathyrella senex group A
Leg Leandro sanchez

7,80 - 9,80 / 4,05 - 5,10
ME= 8,55 x 4,45

Q= 1,75 - 2,15
QM= 1,90

queilos

pleuros

suprapellis

Photo in situ & micro Leandro Sanchez – Spain - **Genbank : MZ031395**

Sur restes de bois de *Acacia dealbata*, jusqu'à 22 mm diamètre.

On remnants of Acacia dealbata wood.

Basides clavées tétrasporiques. Basidia clavate, 4-spored ; 22-25 x 7-9 µm.

Cheilocystides abondantes. Cheilocystidia numerous ; 32-40 x 9-15 µm.

Pleurocystides nombreuses. Pleurocytidia numerous ; 37-48 x 11-13 µm.

Boucles présentes. Clamps present.
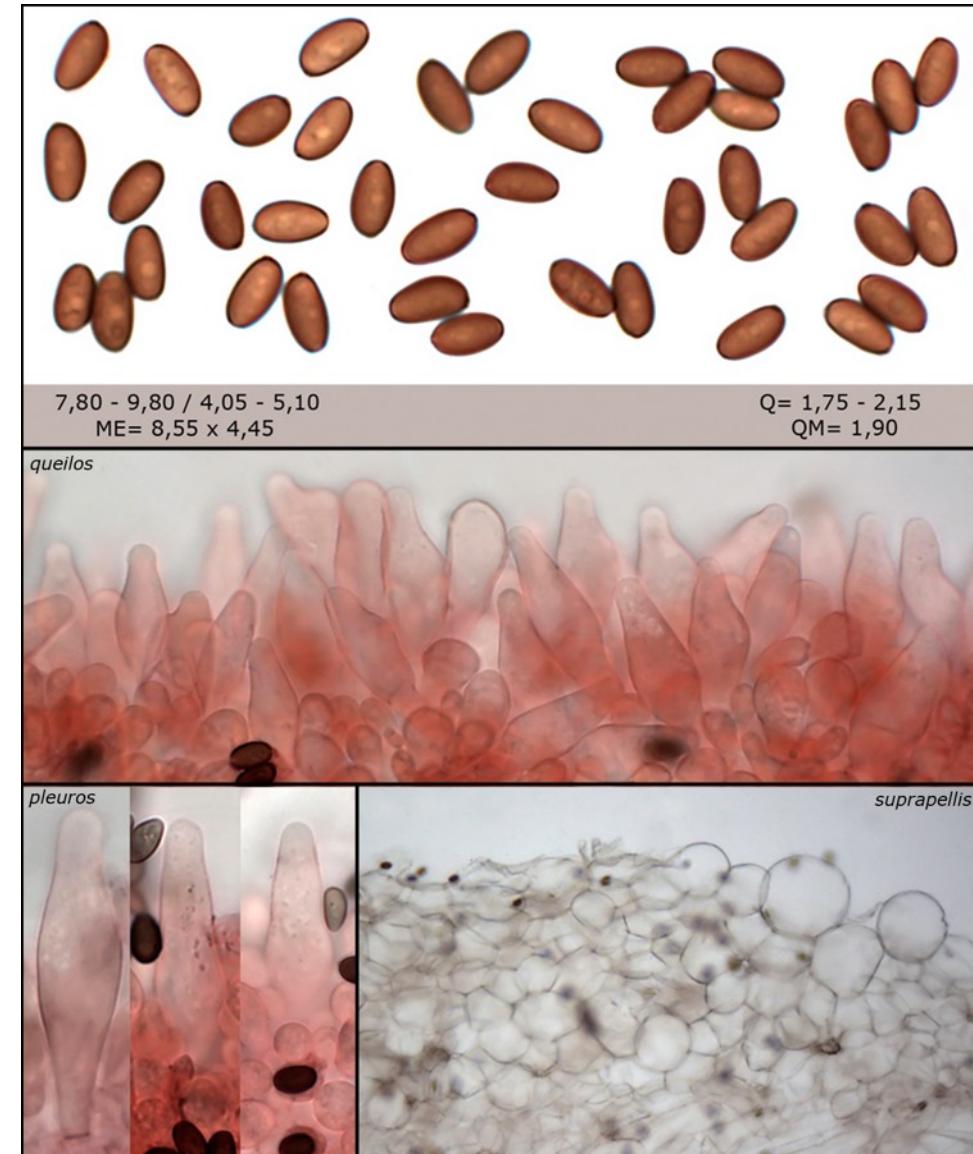
Spores lisses, brun jaunâtre moyen, ellipsoïdes de face, asymétriques et légèrement amygdaliformes de profil, pore germinatif central distinct, large, convexe.
Boucles présentes.
Spores smooth, medium yellowish brown, ellipsoid in front view, asymmetrical and slightly amygdaliform in profile, with a distinct, broad, convex central germ pore.
Clamps present.
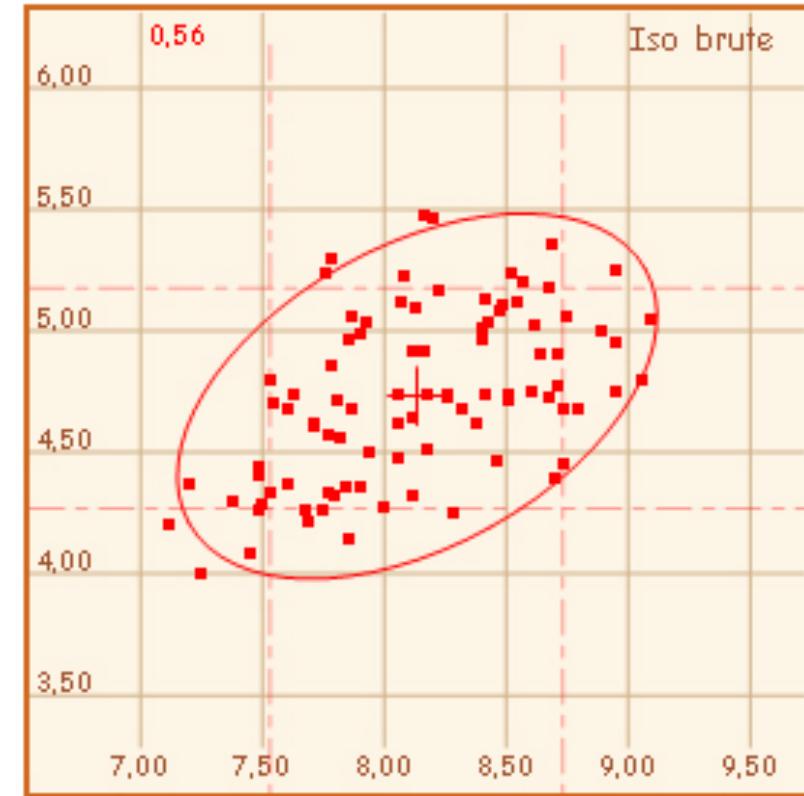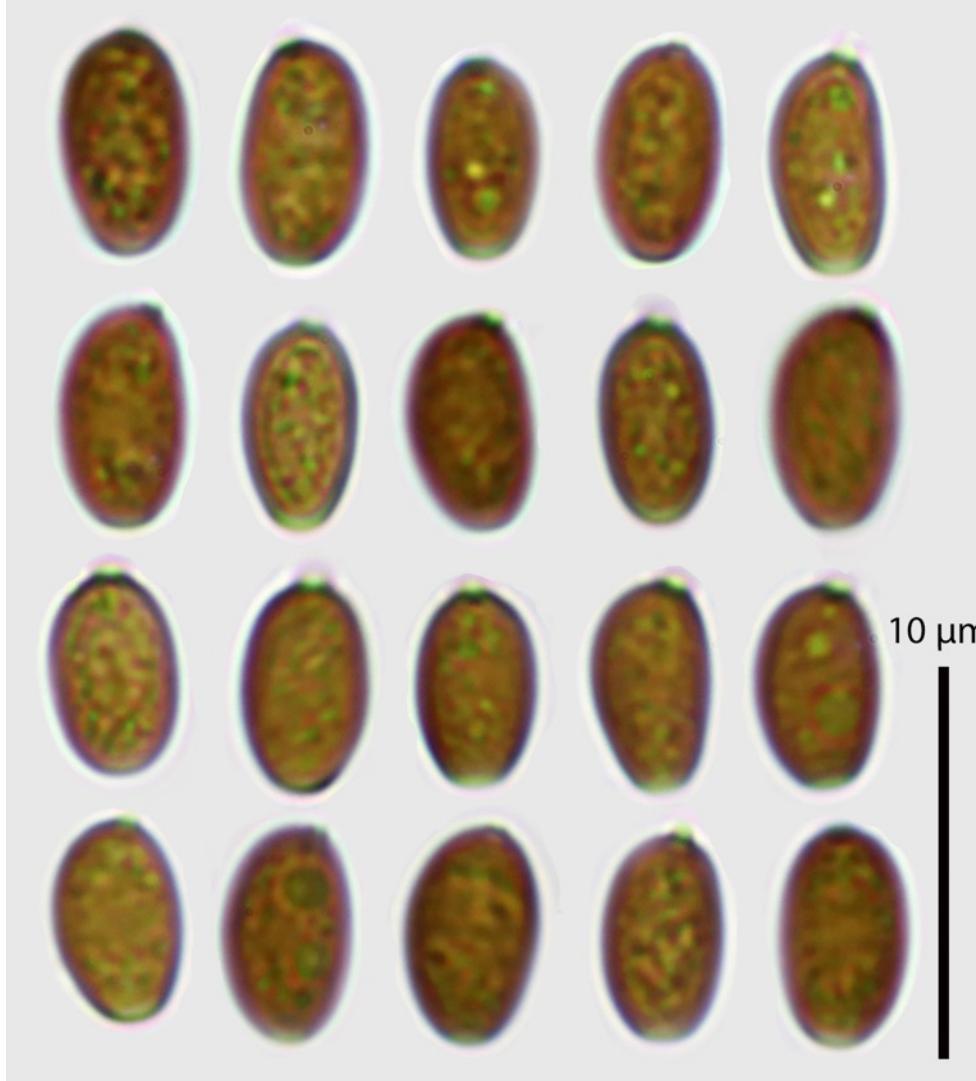
N = 90
(7,1) 7,5 - 8,7 (9,1) × (4) 4,3 - 5,2 (5,5) µm ;
Me = 8,1 × 4,7 µm ;
Q = (1,5) 1,6 - 1,9 (2) ; Qe = 1,7

Cheilocystides abondantes, hyalines, à paroi fine, lagéniformes et sub-utriformes, à sommet obtus.
Paracystides ou basidioles clavés, bouclés, nombreux, de petites dimensions, à paroi fine
Cheilocystidia abundant, hyaline, thin-walled, lageniform and sub-utriform, with obtuse apex.
**Paracystidia or basidioles** clavate, clamped, numerous, small, thin-walled

N = 75
(24,1) 27,4 - 35,9 (40,2) × (7,3) 8,2 - 10,7 (13,4) µm
Me = 31,8 × 9,4 µm
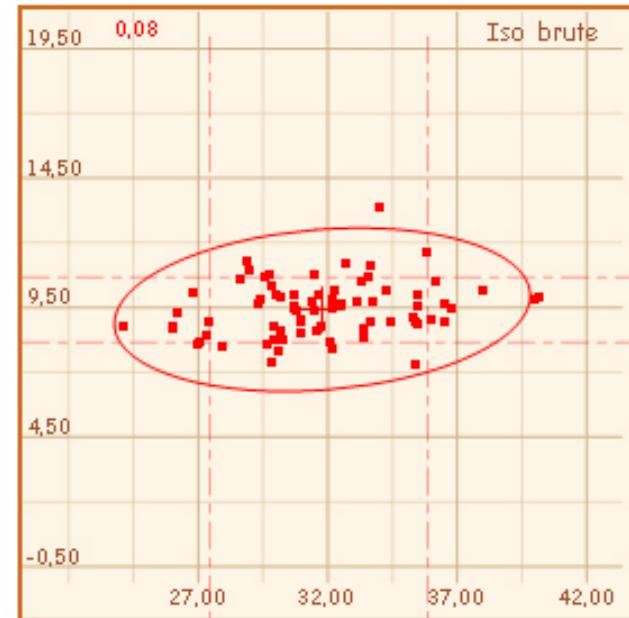
Pleurocystides très nombreuses, hyalines, à paroi fine, analogues aux cheilocystides.
Pleurocystidia very numerous, hyaline, thin-walled, similar to cheilocystidia

N = 60
(28,1) 32,9 - 48,3 (51,3) × (8,6) 10 - 13,3 (14,9) µm
Me = 40,6 × 11,6 µm.
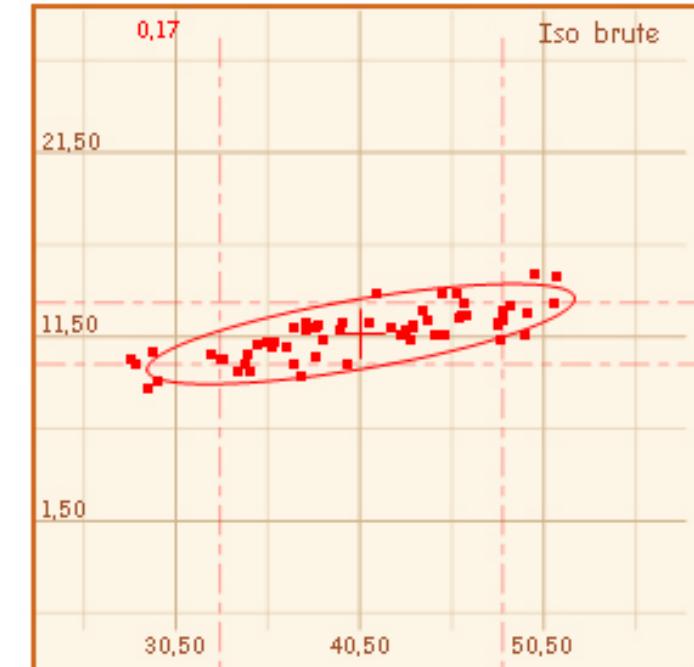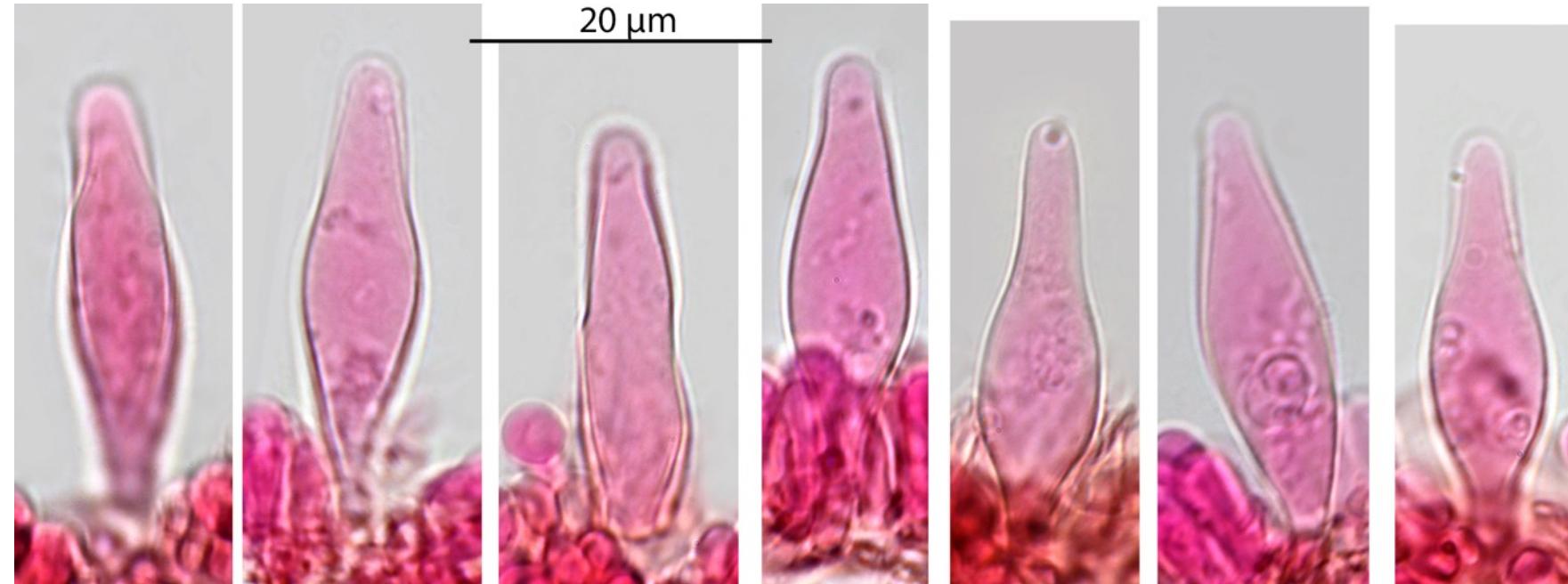
Pileipellis, un hymenoderme constitué de une couche de cellules globuleuses.
Pileitrame constitué de fibres cylindriques, nettement pigmentées de brun jaunâtre.
Pileipellis, an hymeniderm consisting of one layer of globular cells.
Pileitrama consisting of cylindrical fibers, brightly pigmented with yellowish brown.

Psathyrella senex group B

Psathyrella PT150619 in the phylogram – Genbank : MN657231 - leg Patrice Tanchaud

Récolte de Patrice 03/2017 – Charente-Maritime – commune de Ste Gemme
Chapeau de 4 -15 mm de diamètre – écologie salbulicole.
Cap diameter : 4-15 mm on sandy soil. 2019-1420-ALV21789 PT150619 = ok, 100% Psathyrella senex (MF966488)
https://www.mycocharentes.fr/pdf1/2934.pdf

Spores lisses, à paroi fine, brun foncé dans l'eau et l'ammoniaque, grisâtre dans la potasse, non opaques, contenant parfois de multiples gouttes lipidiques, oblongues à ellipsoïdes de face, assymétriques de profil et alors simplement « aplaties » (je cherche un meilleur qualificatif)ou légèrement amygdaliformes. Sommet de la spore conique à convexe avec un large pore germinatif central parfois tronqué. Pas de spores phaséoliformes ou présentant une dépression suprahilaire.

Spores smooth, thin-walled, dark brown in water and ammonia, greyish in KOH, not opaque, sometimes containing multiple lipid drops, oblong to ellipsoidal in front view, asymmetric in profile and then simply flattened or slightly amygdaliform, apex conical to convex with a large central germ pore sometimes truncated. No phasedoliform spores or with suprahilar depression.
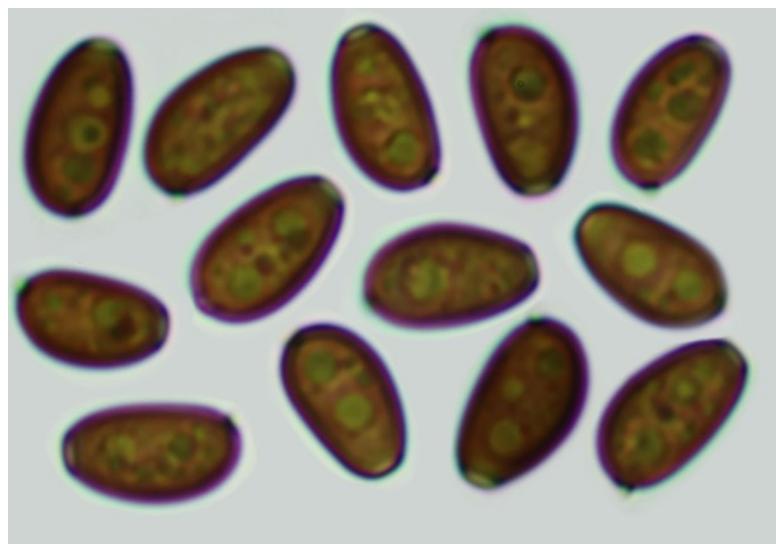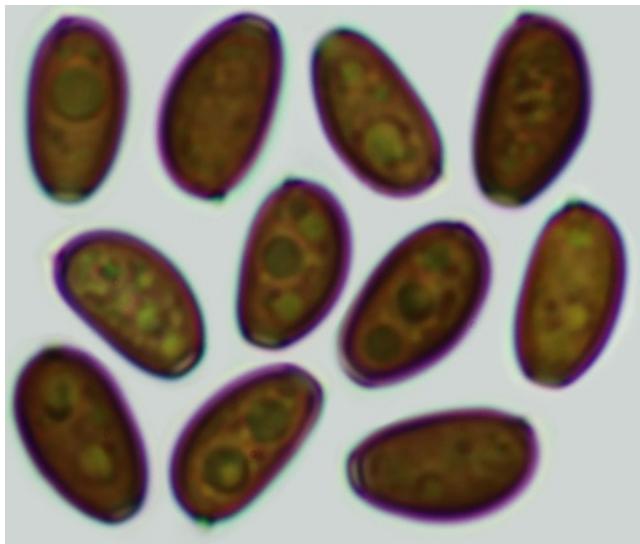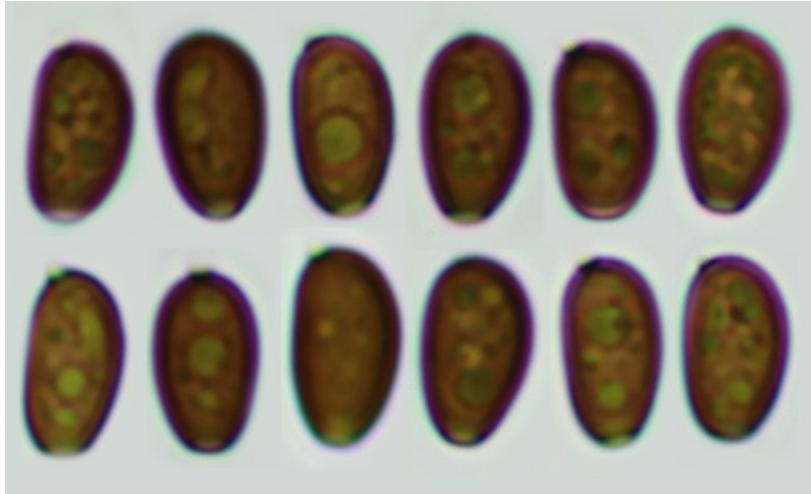

Spores mesurées avec Piximètre : (N = 100)
(6,9) 7,5 - 8,5 (8,8) × (4) 4,2 - 4,8 (5,1) µm
Me = 8 × 4,6 µm
Q = (1,5) 1,7 - 1,9 (2,1) ; **Qe = 1,8**
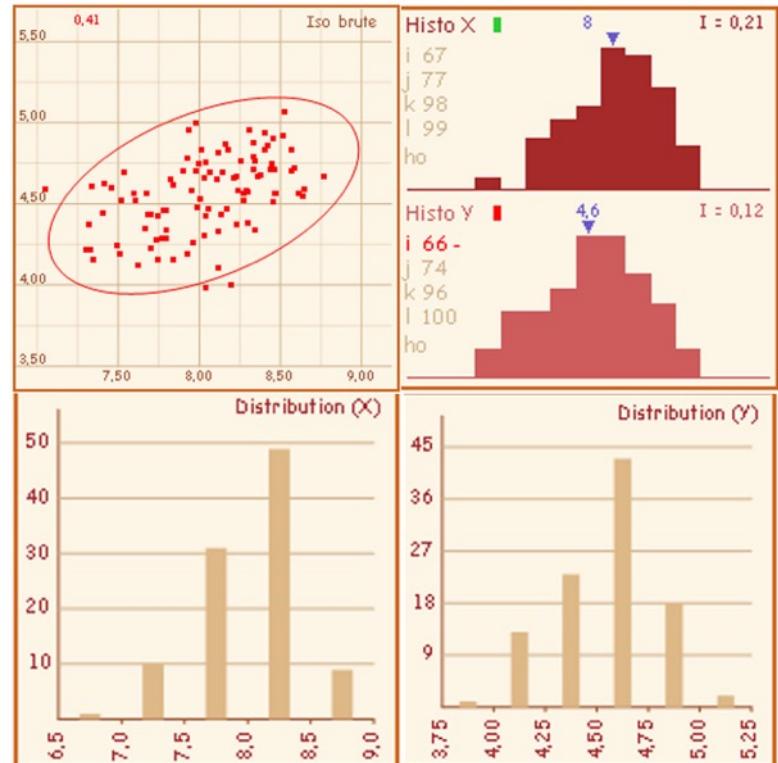
Cheilocystides hyalines, à paroi <u>fine,</u> généralement très denses et réparties <u>uniformément</u> tout au long de l'arête. Toutefois sur certaine lames elles sont davantage éparses, voire clairsemées. Elles sont essentiellement de type ventrues ou lagéno-ventrues, moins souvent lagéniformes ou cylindriques, parfois légèrement rétrécies au <u>sommet largement obtus</u> avec parfois une amorce de col, peu ou pas pédicellées.

Les cellules marginales clavées **(paracystides ou basidioles**) de petites dimension sont <u>peu fréquentes</u> même à proximité de la marge du chapeau, parfois à paroi épaisse brunâtre. L'arête est dans ce cas soulignée de fibres brunâtres (Voir page 9)
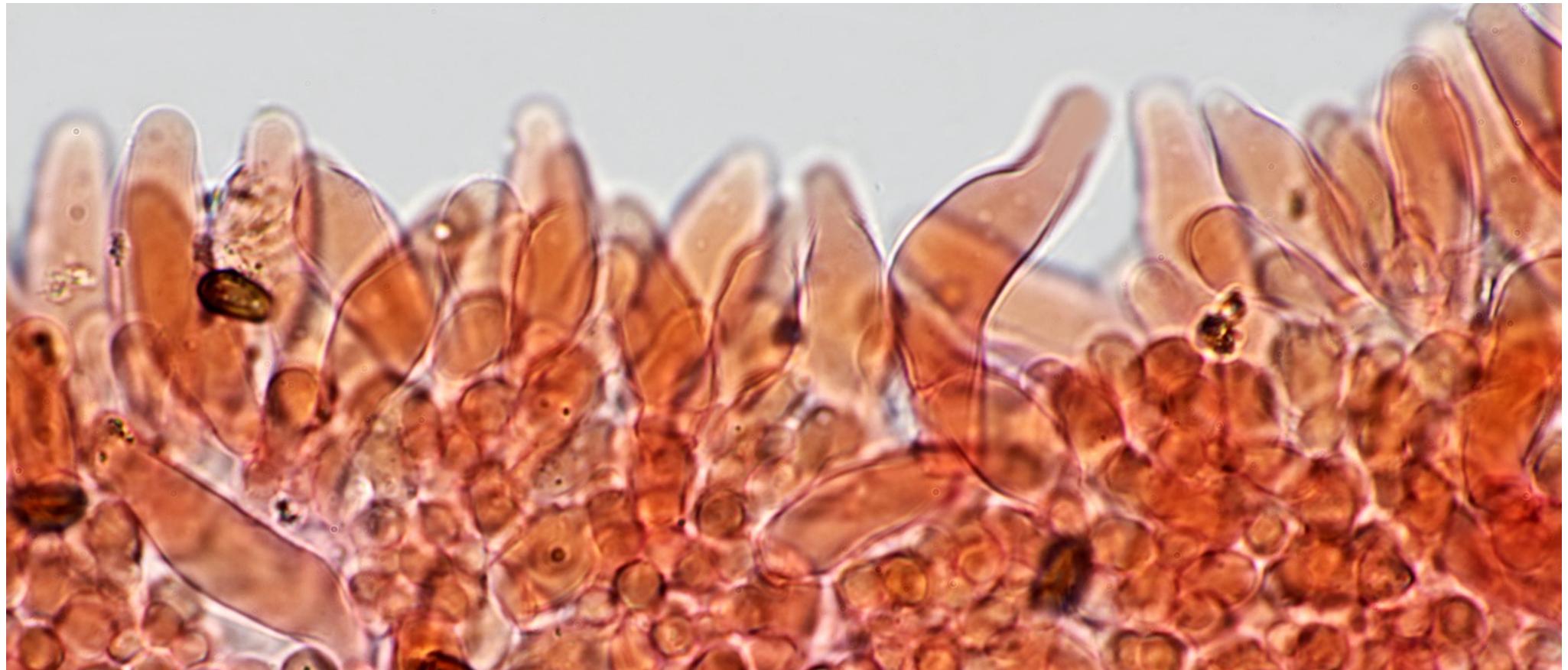
Mesures des cheilocystides : (N=81) ; (20,4) 25,3 - 35,6 (40) × (7,9) 8,5 - 10,8 (11,6) µm ; Me = 29,7 × 9,5 µm.

Cheilocystidia hyaline, thin-walled, generally very dense and uniformly spread along the edge. However, on some gills they are more scattered, even sparse. They are essentially of the utriform or lageno-utriform type, less often lageniform or cylindrical, sometimes slightly narrowed at the top with a broad short neck and an obtuse apex, not or only slightly pedicellate. Smaller, clavate marginal cells (paracystidia or basidioles) are sparse even near the margin of the cap, sometimes with a thick brownish wall. In this case, the edge is underlined with brownish fibrils (See page 9).

Measures of the cheilocystidia : (N=81) ; (20,4) 25,3 - 35,6 (40) × (7,9) 8,5 - 10,8 (11,6) µm ; Me = 29,7 × 9,5 µm.

Comme indiqué dans la description de l'espèce par Melzer, sur une des lames examinées et à proximité de la marge du chapeau, les cheilocystides sont soulignées de fibres brunâtres, et les paracystides sont teintées de brun et à paroi épaisse brunâtre.

As indicated in Melzer's description of the species, on one of the gill examined and near the margin of the cap, the cheilocystidia are underlined with brownish fibres, and the paracystidia are brownish with a thick brownish wall.

Pleurocystides à paroi fines, très nombreuses, lagéniformes,, à long col s'atténuant progressivement jusqu'au sommet obtus ou subobtus, le plus souvent nettement pédicellées. Quelque pleurocystides peu fréquentes à sommet fourchu et à paroi modérément épaissie. Quelque pleurocystides peu fréquentes à sommet fourchu et à paroi modérément épaissie.

Pleurocystidia thin-walled, very numerous, lageniform, with a long neck that gradually attenuates, apex obtuse or subobtuse, most often pedicellate. Some rare pleurocystidia with forked apex and moderately thickened walls.
Some rare pleurocystidia with forked apex and moderately thickened walls.
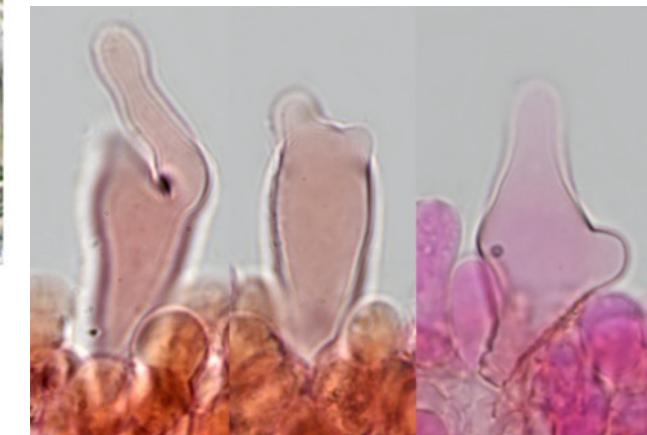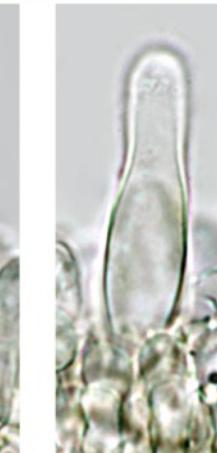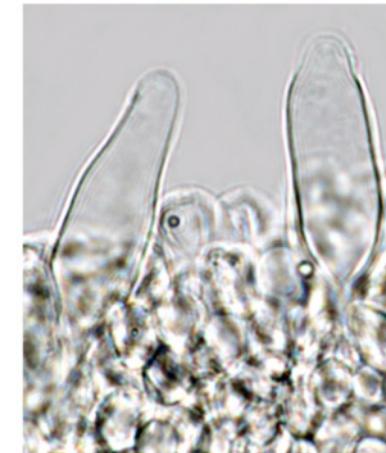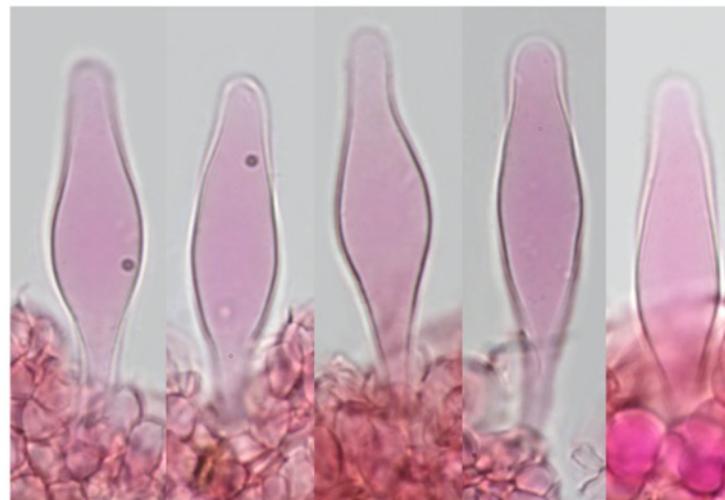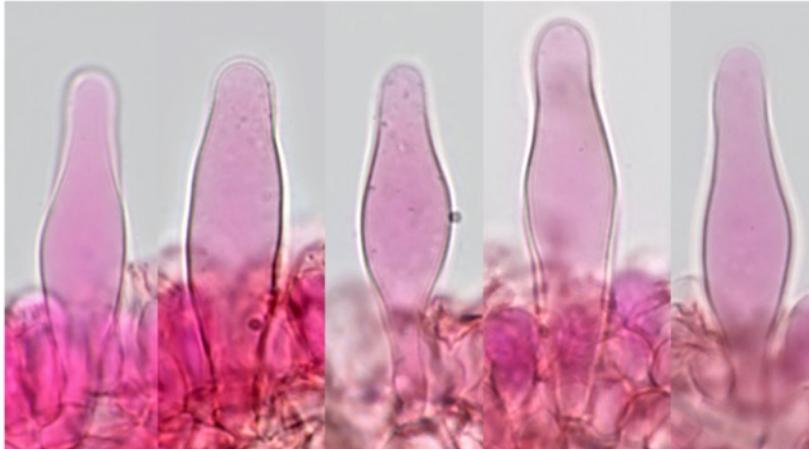
Mesures des pleurocystides : (N = 50)
(29,5) 34,2 - 43,9 (50,4) × (8,7) 9,3 - 11,1 (11,8) µm
Me = 39,6 × 10,2 µm

Psathyrella senex group B

Psathyrella AH 15178 – leg Gabriel Moreno
Identified by G. Moreno and Michel Heykoop as Psathyrella senex (Peck) A. H. Smith
One complete very fragile exsiccatum in low condition - Removal of 2 gills for microscopic study.

Psathyrella senex (Peck) A.H. Sm.
GUADALAJARA, Tamajón, entre restos leñosos y acículas de *Pinus nigra*
subsp. salzmannii en suelo arenoso, 25–X–1992, *leg.* M. Heykoop, **AH**
15178, secuencia de ITS en GenBank MF966484.

Drawings and notes M. Heykoop

Cheilocystidia numerous, densely packed, hyaline, utriform, lageno-utriform, and clavate, underlined <u>in few places </u>with yellow-brown coloured fibers, mixed with lots of small clavate or globulous paracystidia which are infrequently thickwalled.
Measurements done with Piximètre : N = 100 ; (24,2) 28,9 - 36,9 (40,5) × (8,5) 9,9 - 14 (16,6) µm ; **Me = 32,6 × 12 µm.**
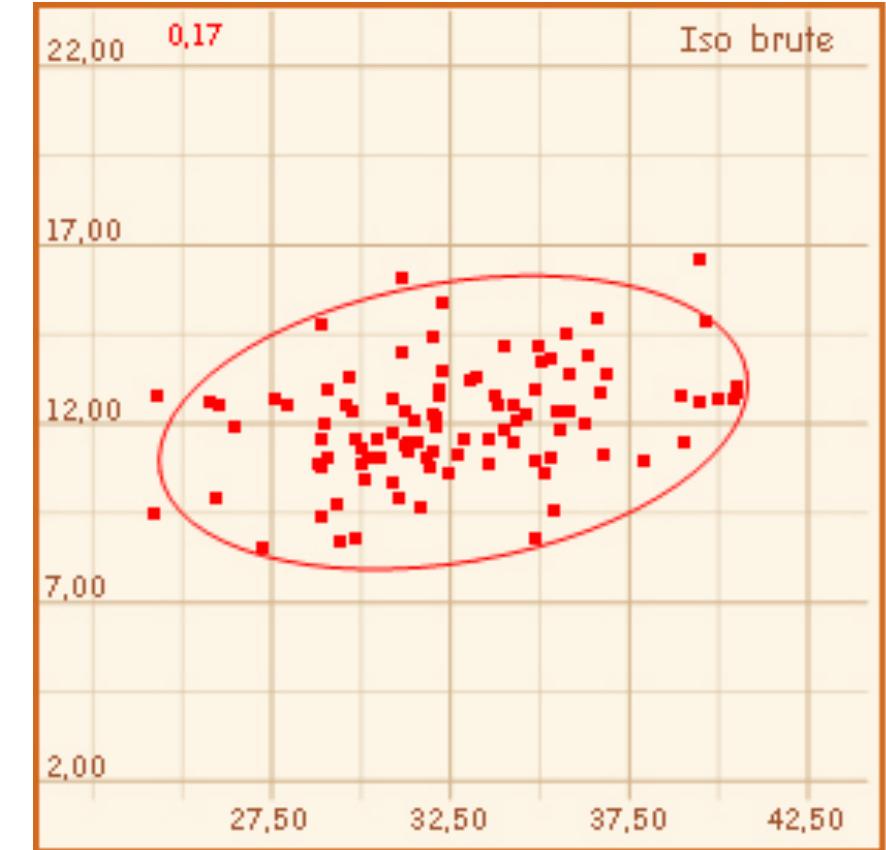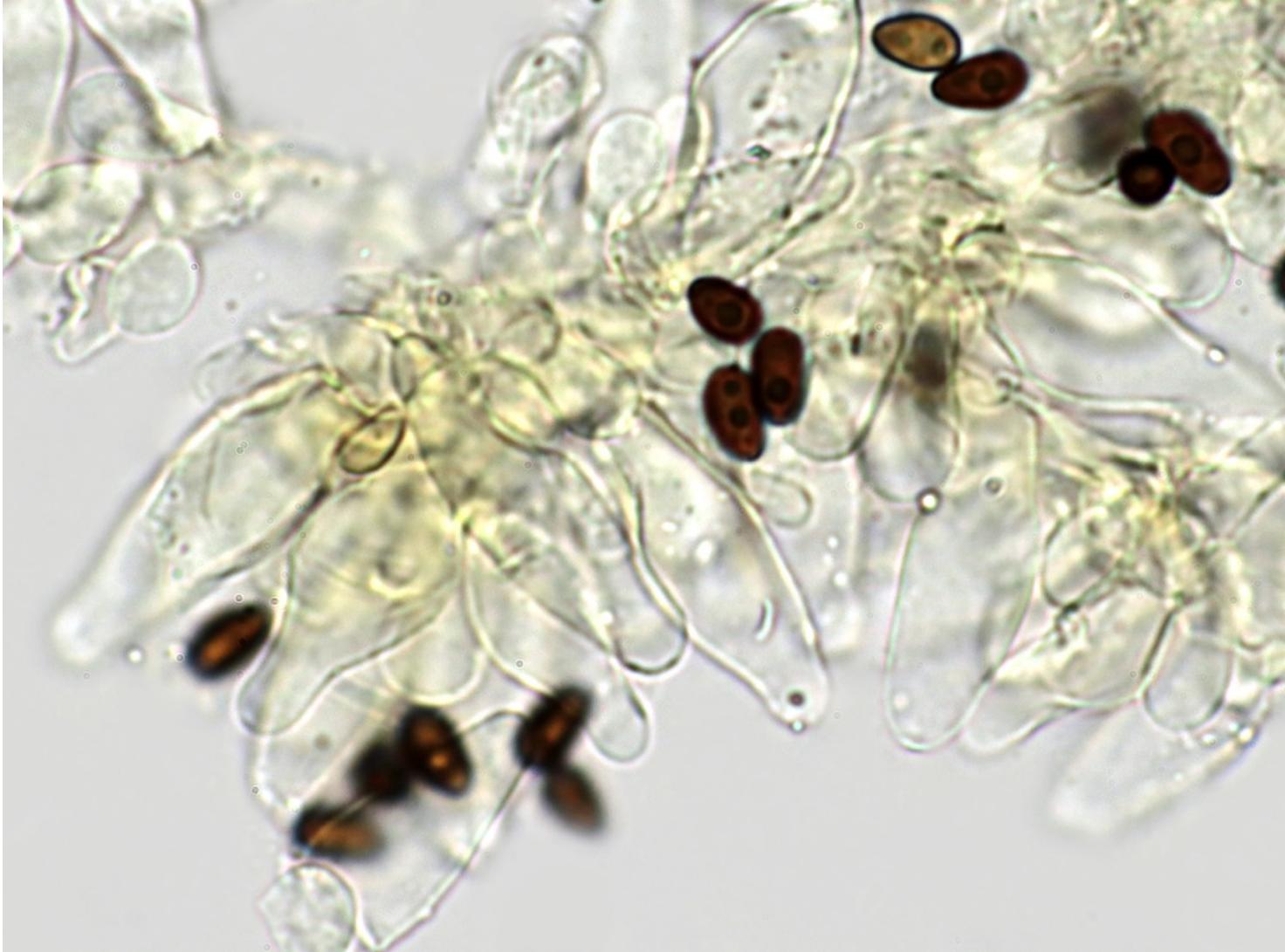
Cheilocystidia mixed with lots of paracystidia especially near the margin of the cap.

Few paracystidia are thickwalled
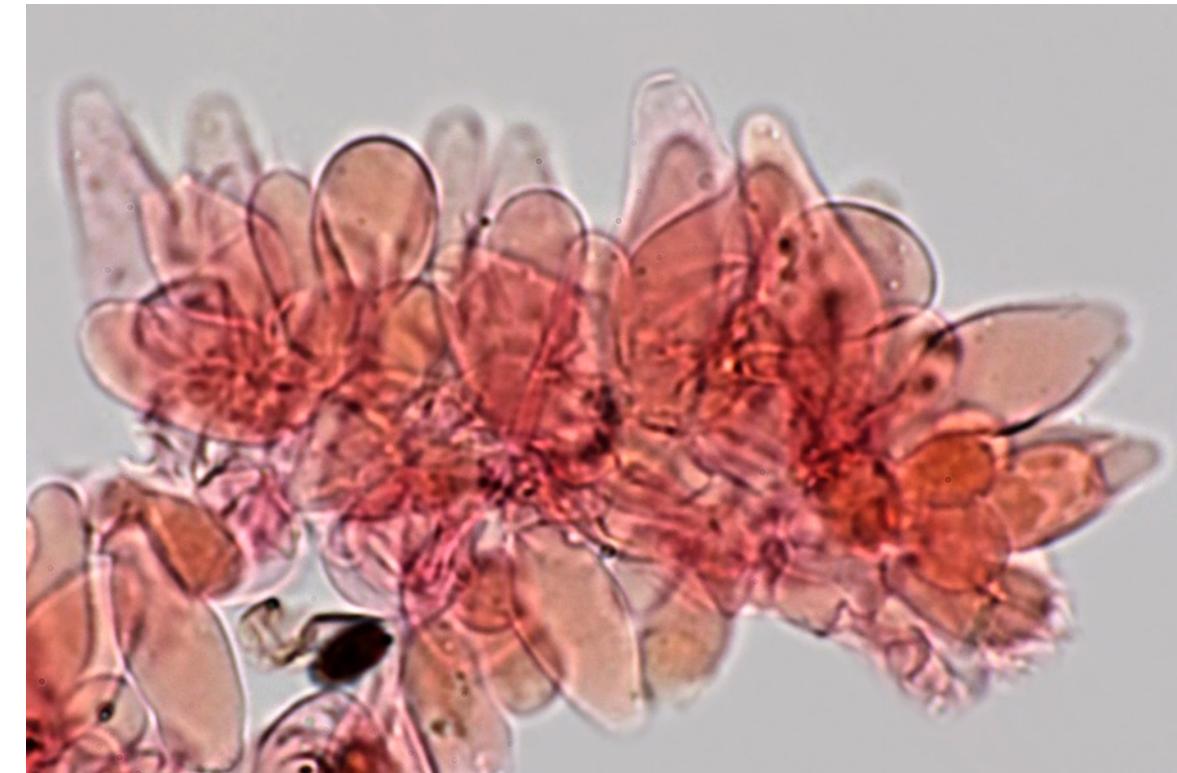
Pleurocystidia very numerous, hyaline, lageniform and sub-utriform , with obtuse apex
Measuring : N = 38 ; (36,5) 39,1 - 51,2 (54,2) × (9,5) 10,7 - 13,1 (15,6) µm ; **Me = 44,8 × 12,1 µm.**
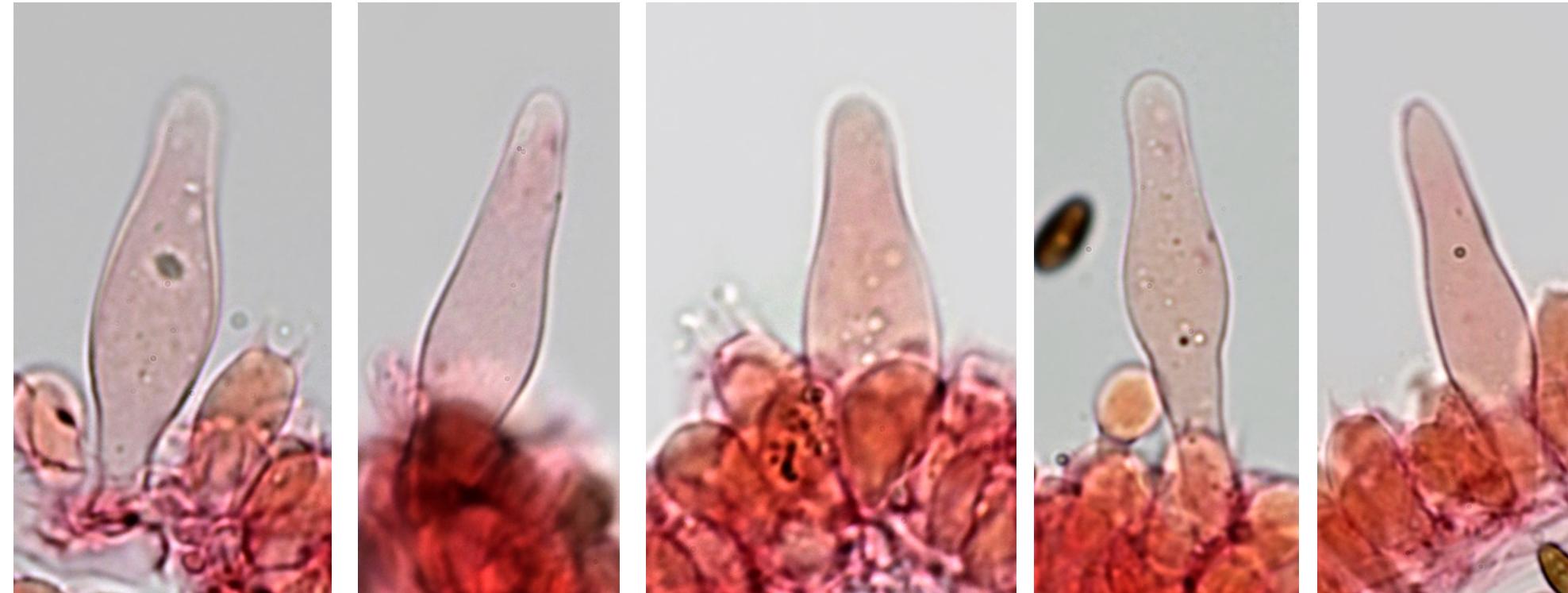
Basidia : 4-spored – Clamps : present
Spores : smooth , brown , not opaque , ellipsoid in face view, asymetric and flatened on one side in profile, germ pore large, central.

Measuring with Piximètre : N = 99
(7,9) 8,3 - 9,3 (10) × (4,3) 4,6 - 5,1 (5,5) µm ; **Me = 8,8 × 4,9 µm ;**
Q = (1,6) 1,7 - 1,9 (2) ; Qe = 1,8
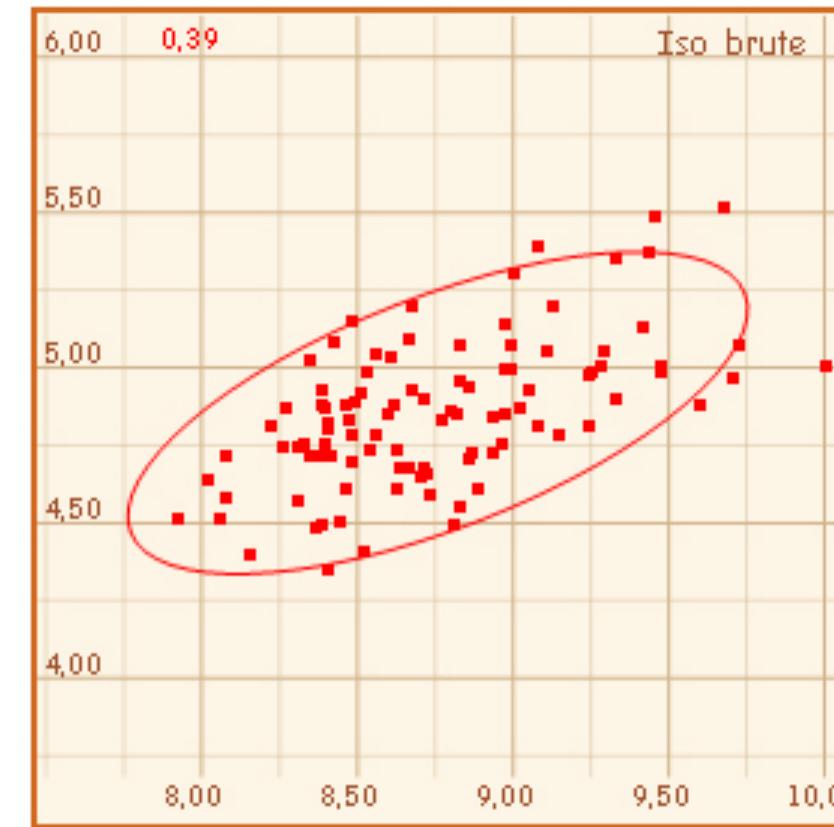
Psathyrella AH 27060 – leg Gabriel Moreno
Identified by G. Moreno and Michel Heykoop as Psathyrella senex (Peck) A. H. Smith
One complete exsiccatum and one third of a cap - Removal of 2 gills from each specimen for microscopic study.

Psathyrella senex (Peck) A.H. Sm.
CIUDAD REAL, Parque Nacional de Cabañeros, Valle del Brezoso,
Arroyo de Brezoso, en humus y restos leñosos de *Quercus pyrenaica*,
18–X–2001, *leg.* G. Moreno, E. Llarandi & M. Heykoop, **AH 27060**,
secuencias de ITS en GenBank MF966488.

Waiting for photo in situ

Michel Heykoop drawings and notes

Cheilocystidia numerous, densely packed, hyaline, utriform and lageno-utriform, underlined by yellow-brown coloured fibers mixed with lots of small clavate or globulous paracystidia wich are thickwalled and yellow brown coloured in one of the two specimen.
Measuring done with Piximètre : N = 49 ; (22,6) 24,8 - 33,7 (43,2) × (8) 9,1 - 11,6 (13,8) µm ; Me = 29,6 × 10,3 µm.
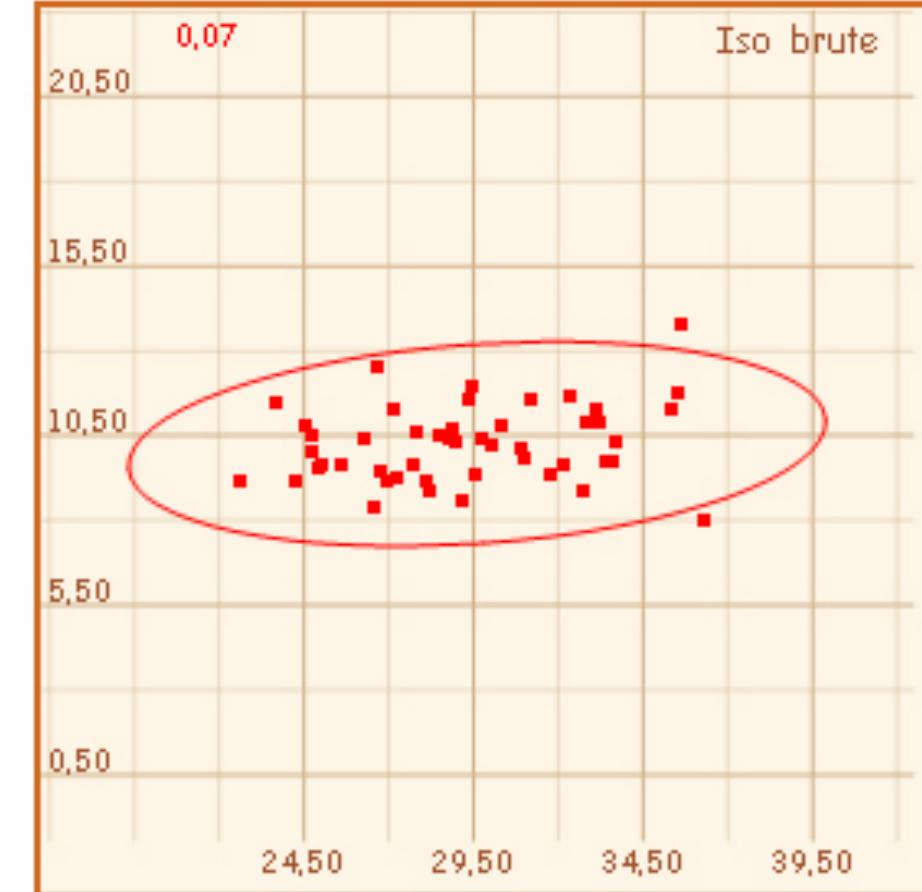
Cheilocystidia and paracystidia wich are thickwalled and yellow brown coloured in one of the two specimen.
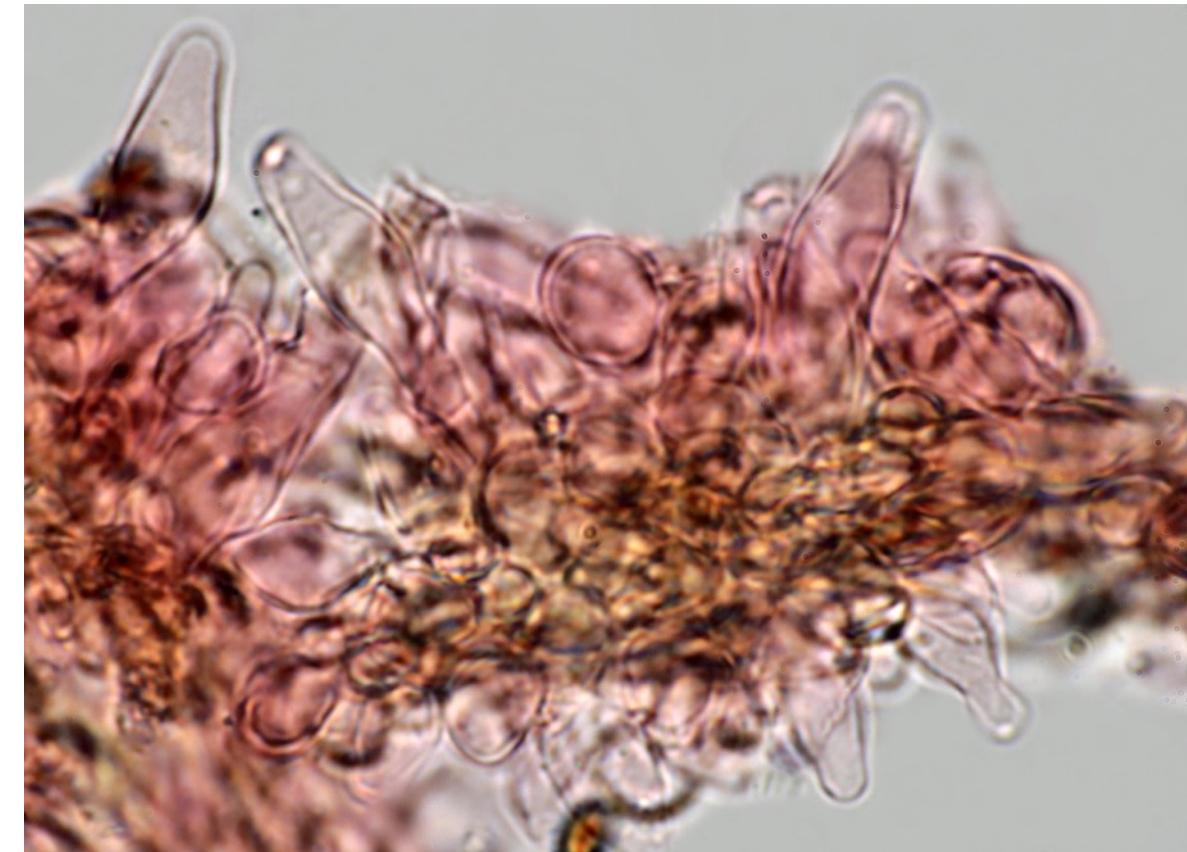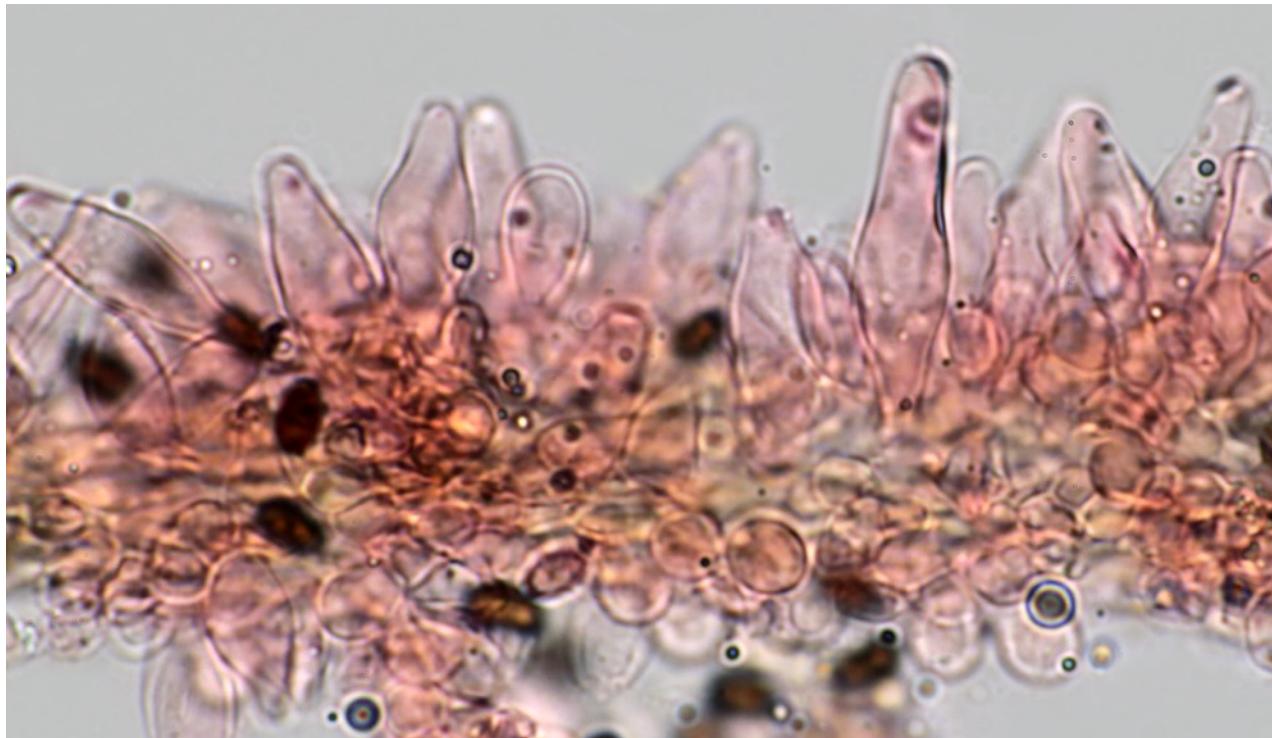
Pleurocystidia very numerous, hyaline, lageniform and sub-utriform, with obtuse apex.
Measuring : N = 50 ; (30,9) 32,5 - 44,5 (48,3) × (8,9) 9,3 - 11,2 (13,2) µm ; Me = 37,8 × 10,4 µm.
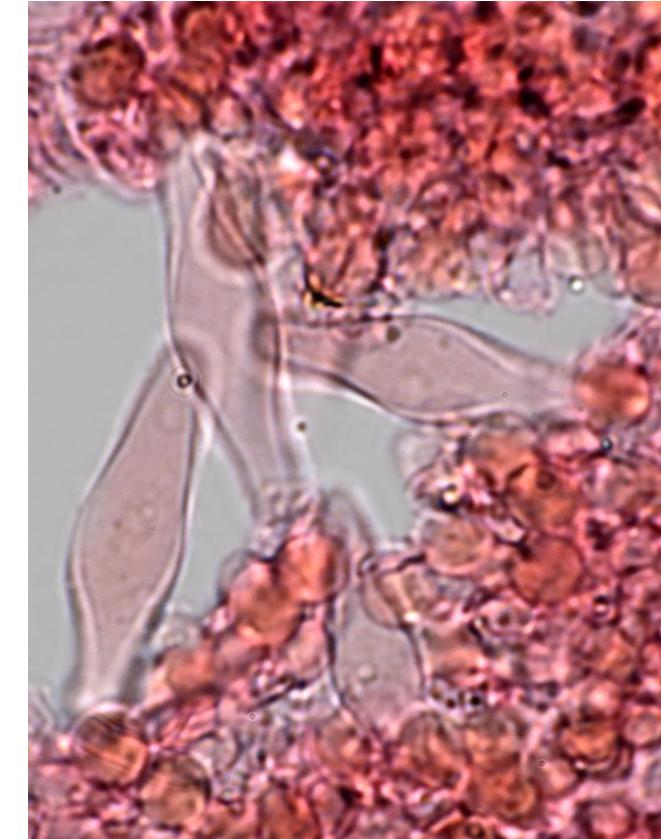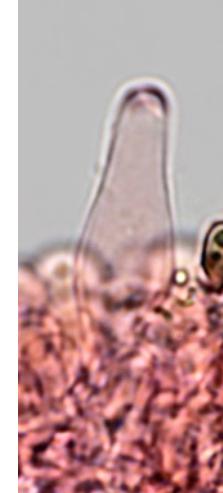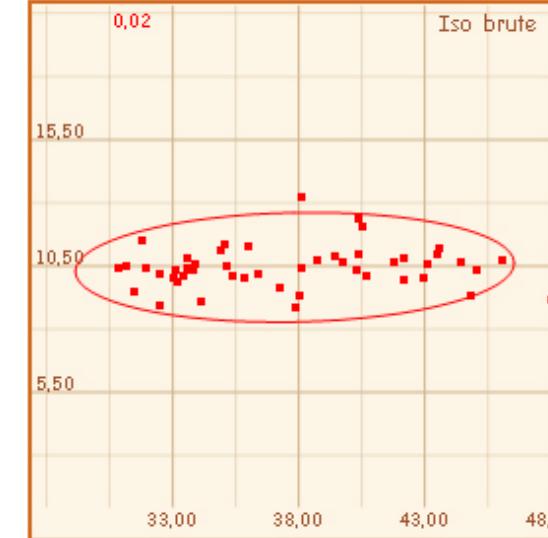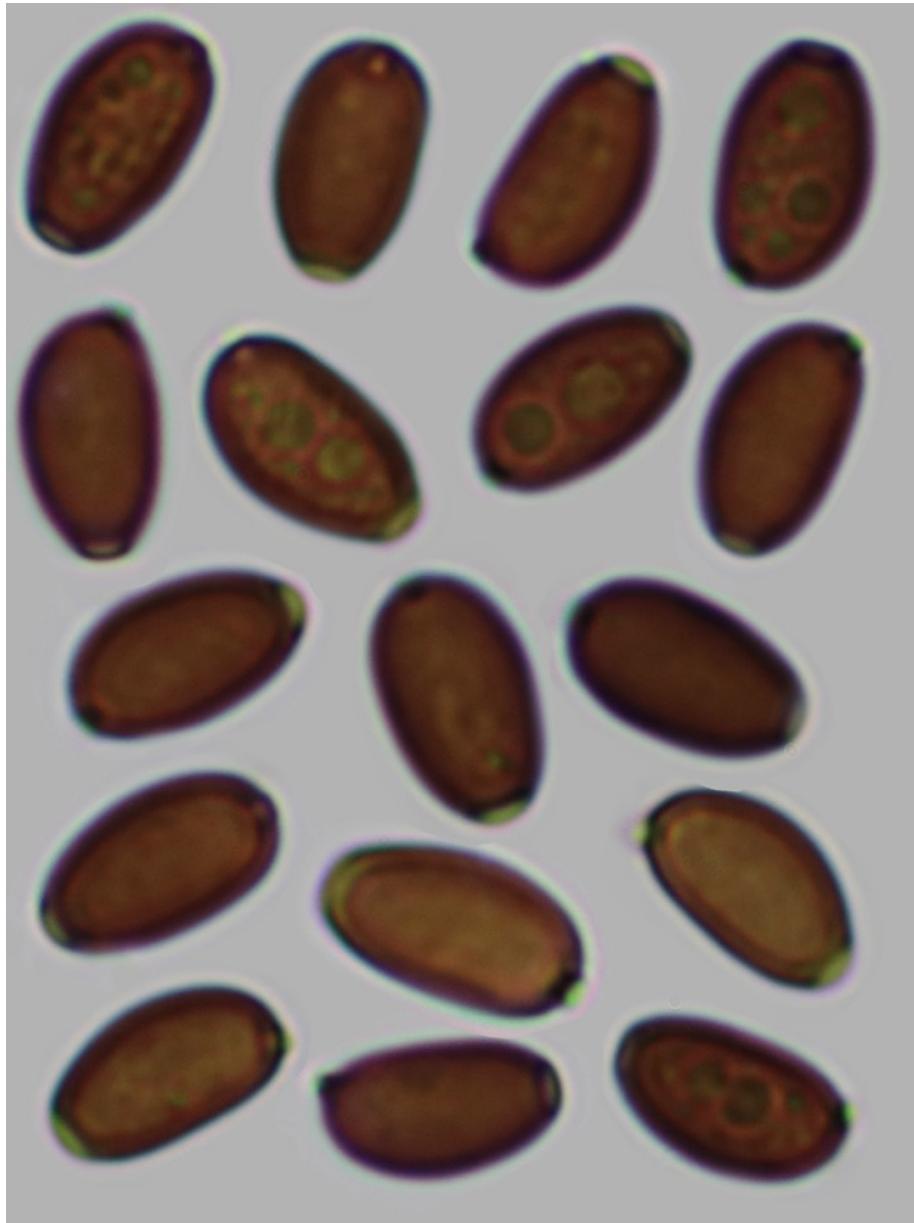
Basidia : 4-spored
Spores : smooth , brown , not opaque , ellipsoid in face view, assymetric and flatened on one side in profile, germ pore large, central.

Measuring with Piximètre : N = 70
(6,8) 7,6 - 8,5 (8,7) × (4,2) 4,5 - 4,9 (5,1) µm ; Me = 8 × 4,7 µm ;
Q = (1,5) 1,6 - 1,8 (1,9) ; Qe = 1,7
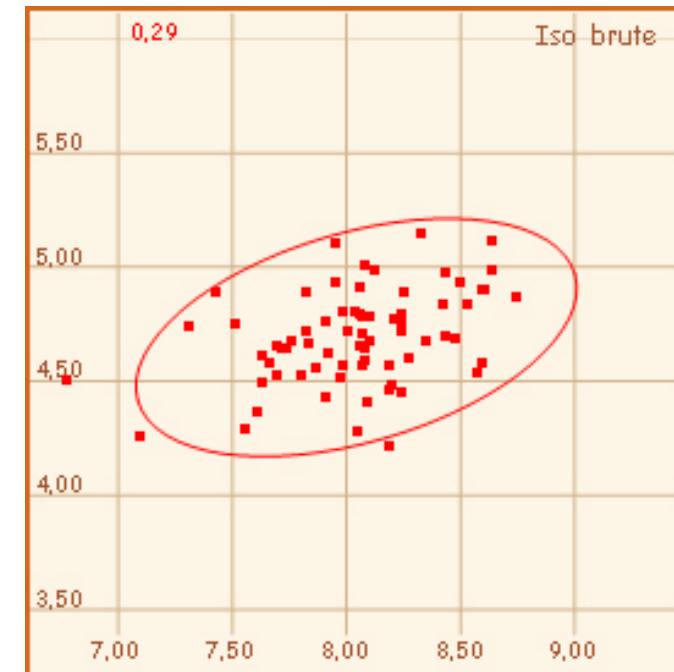
Sequencing and phylogenetic analysis
DNA extraction, amplification and sequencing of the fungus was performed by Alvalab (Oviedo, Spain). The phylogenetic analysis was done by Dieter Wächter (Thiersheim, Germany). The genomic DNA was extracted from dried fruiting bodies. Amplification of the ITS region was performed with the ITS4 primer [1]. The initial base calling was done with FinchTV [2]. The nucleotide sequences were 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 /noli-tangere 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-1α (exons of the ef-1α gene). The nucleotide sequences for the tree inference were taken from NCBI [3] and Unite [4] (essential ones of the /noli-tangere-clade s.l. 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 clade of the ingroup were used, i.e. the the Cystopsathyra section clade and the /piluliformis s.l. 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 alignments of the LSU-, β-tub and ef-1α genes 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 (ns) 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: 5.8S
•DNA-partition 3: LSU + β-tub-Codon 1
•DNA-partition 4: β-tub Codon 2 + ef-1α Codon 2
•DNA-partition 5: β-tub Codon 3 + ef-1α Codon 3
•DNA-partition 6: ef-1α Codon 1
•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 Outgroup has been collapsed for a better view.
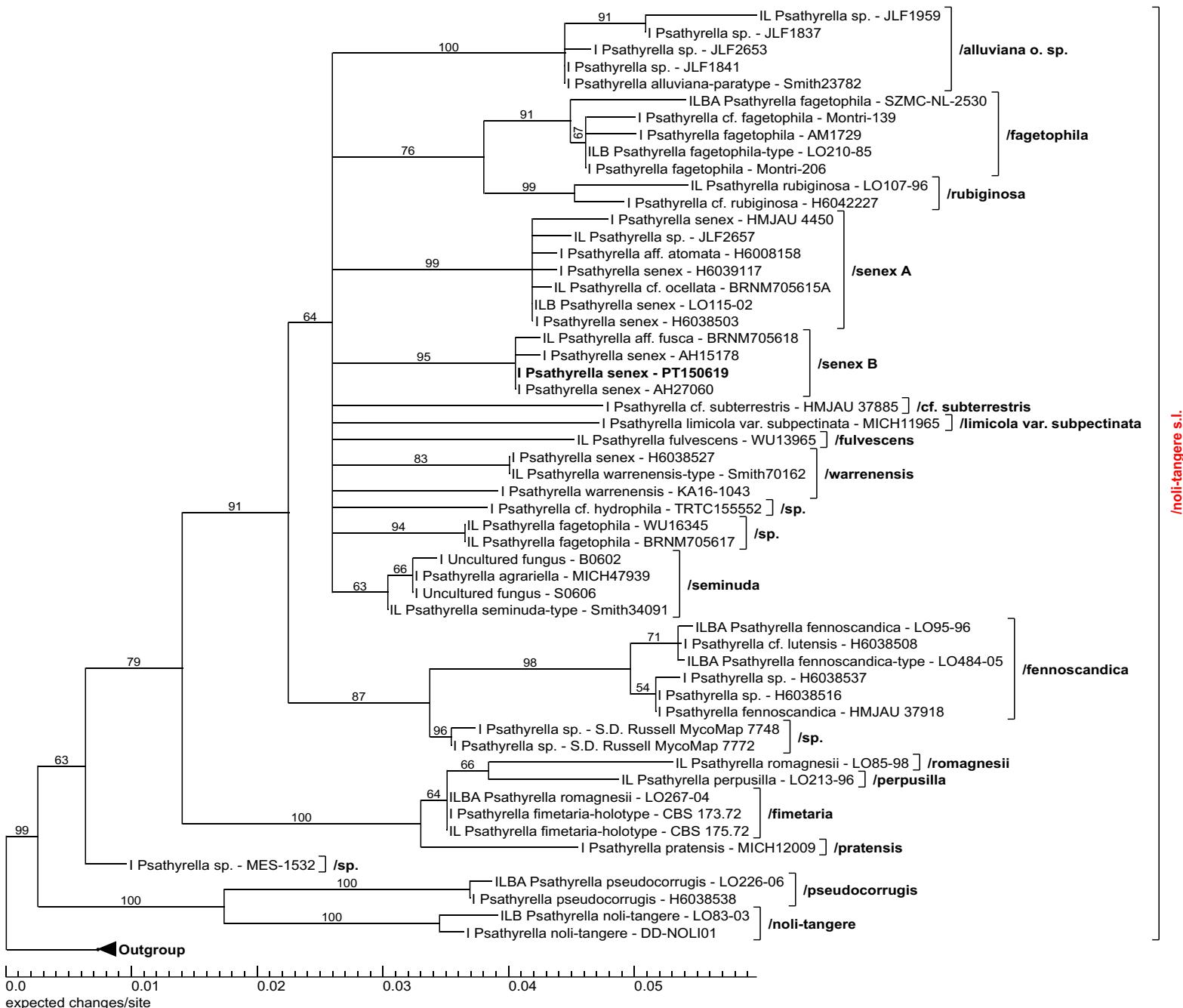
Fig 1 50% collapsed maximum likelihood consensus phylogram. The values on the branches are ML bootstrap values. Abbreviations: I: ITS region, L: LSU region, B: β-tubulin region, A: ef-1α region.

Table 1 List of relevant sequences used in this publication

Species	Ref.-ID	ITS	LSU	β-Tub	ef-1α
Psathyrella sp.	JLF1841	MN017246.1			
Psathyrella sp.	JLF2653	MN017258.1			
Psathyrella sp.	JLF1837	MN017245.1			
Psathyrella sp.	JLF1959	MN017249.1	MN031140.1		
Psathyrella alluviana o. sp.	Smith23782	follows			
Psathyrella fagetophila	AM1729	MK045662.1			
Psathyrella fagetophila	LO210-85	KC992902.1	KC992902.1	KJ664879.1	
Psathyrella fagetophila	SZMC-NL-2530	FM878003.1	FM876259.1	FN396322.1	FM897222.1
Psathyrella fagetophila	Montri-206	MK028505.1			
Psathyrella cf. fagetophila	Montri-139	MK028506.1			
Psathyrella cf. rubiginosa	H6042227	UDB021234			
Psathyrella rubiginosa	LO107-96	KC992905.1	KC992905.1		
Psathyrella aff. atomata	H6008158	UDB021144			
Psathyrella senex	HMJAU 4450	MG734732.1			
Psathyrella senex	LO115-02	DQ389712.1	DQ389712.1	KJ664880.1	
Psathyrella senex	H6039117	UDB021231			
Psathyrella cf. ocellata	BRNM705615A	AM712266.1	AM712266.1		
Psathyrella senex	H6038503	UDB021183			
Psathyrella sp.	JLF2657	MN017259.1	MN031153.1		
Psathyrella senex	AH15178	MF966484.1			
Psathyrella senex	AH27060	MF966488.1			
Psathyrella senex	PT150619	follows			
Psathyrella aff. fusca	BRNM705618	AM712264.1	AM712264.1		
Psathyrella cf. subterrestris	HMJAU 37885	MG734747.1			
Psathyrella limicola var. subpectinata	MICH11965	MF325980.1			
Psathyrella fulvescens	WU13965	AM712265.1	AM712265.1		
Psathyrella senex	H6038527	UDB021206			
Psathyrella warrenensis	Smith70162	KC992906.1	KC992906.1		
Psathyrella warrenensis	KA16-1043	MK351682.1			
Psathyrella cf. hydrophila	TRTC155552	JN021089.1			
Psathyrella fagetophila	WU16345	AM712262.1	AM712262.1		
Psathyrella fagetophila	BRNM705617	AM712263.1	AM712263.1		
Uncultured fungus	S0606	FM999571.1			
Uncultured fungus	B0602	FM999574.1			
Psathyrella seminuda	Smith34091	KC992907.1	KC992907.1		
Psathyrella agrariella	MICH47939	MF325950.1			
Psathyrella sp.	H6038537	UDB021215			
Psathyrella fennoscandica	HMJAU 37918	MG734723.1			
Psathyrella sp.	H6038516	UDB021196			
Psathyrella cf. lutensis	H6038508	UDB021188			
Psathyrella fennoscandica	LO484-05	KC992903.1	KC992903.1	KJ664881.1	KJ732790.1
Psathyrella fennoscandica	LO95-96	KC992904.1	KC992904.1	KJ664882.1	KJ732791.1
Psathyrella sp.	S.D. Russell MycoMap 7748	MK532816.1			
Psathyrella sp.	S.D. Russell MycoMap 7772	MK532817.1			
Psathyrella romagnesii	LO85-98	DQ389716.1	DQ389716.1		
Psathyrella perpusilla	LO213-96	DQ389714.1	DQ389714.1		
Psathyrella romagnesii	LO267-04	DQ389715.1	DQ389715.1	KJ664889.1	KJ732802.1
Psathyrella fimetaria	CBS 175.72	MH860432.1	MH872162.1		
Psathyrella fimetaria	CBS 173.72	MH860430.1			
Psathyrella pratensis	MICH12009	MF325994.1			
Psathyrella sp.	MES-1532	KY462474.1			
Psathyrella pseudocorrugis	LO226-06	KC992917.1	KC992917.1	KJ664888.1	KJ732801.1
Psathyrella pseudocorrugis	H6038538	UDB021216			
Psathyrella noli-tangere	LO83-03	DQ389713.1	DQ389713.1	KJ664890.1	
Psathyrella noli-tangere	DD-NOLI01	follows			

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