

La première partie de cette fiche (pages 1 à 20), rédigée en anglais par les auteurs, correspond à la description originale de l'holotype, publiée dans le bulletin de l'AMFB 10/2017. La seconde partie rédigée en français apporte une description et des informations complémentaires, sur base d'une récolte effectuée en France par Micheline Broussal.

## ***Psathyrella hellebosensis*, a new species from Belgium**

Daniel Deschuyteneer et Andreas Melzer

**Résumé :** *Psathyrella hellebosensis* est proposée comme espèce nouvelle. La description est illustrée par des photographies de l'espèce in situ ainsi que par des photos de ses caractères microscopiques. Ses caractères morphologiques ainsi que les données moléculaires, suggèrent qu'il s'agit d'une espèce inconnue à ce jour. Les différences avec *Psathyrella thujina* A. H. Sm., une espèce apparentée, sont discutées.

**Abstract :** *Psathyrella hellebosensis* is presented as a new species. The description is supported by photographs of the basidiocarps and the microscopic features. Morphological and molecular data suggest it is a species new to science. The differences with the related *Psathyrella thujina* A. H. Sm. are discussed.

**Key words :** *Psathyrellaceae*, *Psathyrella*, Mycobiota of Belgium

### **INTRODUCTION**

Between september and december 2015 and again, during the same period in 2016, the first author found numerous specimens of a *Psathyrella* species near Steenokkerzeel, Belgium. Habit and a certain quota of heart-shaped spores first suggested *Psathyrella panaeoloides* (Maire) Svrček ex Arnolds. But the construction of the lamellae edge, made of predominantly clavate cells forbade this suspicion. The second author was also unable to identify it, with any doubt. For this reason, a sequencing was commissioned ; the result indicated *Psathyrella thujina* A. H. Sm. However, taking into account the ecological and morphological differences with this species the agreement was not entirely satisfactory, so that further studies were required.

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**Fig. 1 – Habitat, the ruderal place where this species is growing (photo : D. Deschuyteneer).**

SEM photos were made by Myriam De Haan (Botanic Garden Meise). A portion of each sample was placed in a convolute of a filter paper (medium filtration rate ; particle retention >5 µm ; VWR) which was placed in a sample holder (stainless steel tube with meshed top and bottom) for critical point drying. The holders were submerged respectively for 30 min in 25 % ammonia, 2x20 min in 70 % ethanol, 2x30 min in dimethoxy-methane and left overnight, then 4x15 min in acetone, thereafter the samples were dried in a critical point dryer (Leica EP CDP 300).

The samples were placed in a High Resolution Fine Sputter Coater for FE-SEM (JFC-2300HR Coating Unit, JEOL) and coated with a layer of approximately 1.5 nm Pt/Pd (using Argon-gas, under 0.05 mbar). The scanning electron microscopy was carried out with a JEOL JSM-7100FLV Field Emission SEM. Look at the bottom of each photo to know the tension in kV and the working distance in mm for each SEM photo.

Two samples (AM1816, AM1849) and a German collection of *Psathyrella thujina* (AM1656) have been sequenced by the laboratory 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 1.4 M, EDTA pH 8.0 20 mM, Tris-HCl pH 8.0 100 mM). The resulting mixture was incubated for 15 min at 65° C. A similar volume of chloroform : isoamylalcohol (24:1) was added and carefully mixed with the samples until their emulsion. It was then centrifugated for 10 min at 13.000 g, 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 & al. 1990) for ITS, and LR0R and LR5 (VILGALYS & HESTER 1990, CUBETA & al. 1991) for the 28S rDNA region. PCR reactions were performed under a program consisting of a hot start at 95° C for 5 min, followed by 35 cycles at 94° C, 54° C and 72° C (45, 30 and 45 s respectively) and a final 72° C step 10 min. PCR products were checked in 1 % agarose gels, and positive reactions were sequenced with primer ITS4 and LR0R. Chromatograms were checked searching for putative reading errors, and these were corrected. BLAST was used to select the most closely related sequences from INSD public databases. Combined ITS-28S rDNA sequences were aligned in MEGA 5.0 (TAMURA & al. 2011) software with its Clustal W application and then corrected manually. The aligned loci were loaded in PAUP\* 4.0b10 (SWOFFORD 2001) and partitions subjected to MrModeltest 2.3 (NYLANDER 2004). Model GTR+Γ+I was selected and implemented in MrBayes 3.1 (RONQUIST & HUELSENBECK 2003), where a Bayesian analysis was performed (ITS and 28S rDNA data partitioned, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 190 000 generations, standard deviation having fell below 0.01. Finally a full search for the best-scoring maximum likelihood tree was performed in RaxML (STAMATAKIS 2006) using the standard search algorithm (2000 bootstrap replications). Significance threshold was set above 0.9 for posterior probability (PP). For comparative purposes, the sequences given in Table 1 were taken from the GenBank. *Lacrymaria velutina* (Pers.) Konrad & Maubl. was used as root.



Species	Voucher	Accession n°
<i>P. agraria</i> Enderle (inval.)	Enderle-KR0030008 (isotype)	KU307507
<i>P. ammophila</i> (Durieu & Lév.) P. D. Orton	LÖ160-00	KC992871
<i>P. ammophila</i>	LÖ359-11	KC992872
<i>P. carminei</i> Örstadius & E. Larss.	LÖ5-09 (type as <i>P. spec.</i> 11)	KC992880
<i>P. casca</i> (Fr.) Konrad & Maubl.	SZMC-NL-0440 (as <i>P. spadiceogrisea</i> )	FM878024 FM876282
<i>P. casca</i>	LÖ92-01 (as <i>P. spadiceogrisea</i> )	DQ389682
<i>P. clivensis</i> (Berk. & Broome) P. D. Orton	LÖ182-03	DQ389683
<i>P. dunensis</i> Kits van Waveren	WU 5995	AM712275
<i>P. fatua</i> (Fr.) P. Kumm.	LÖ132-97 (type)	DQ389681
<i>P. fatua</i>	LÖ231-08	KC992879
<i>P. jacobssonii</i> Örstadius	LÖ256-92	KC992855
<i>P. obtusata</i> (Pers.) A. H. Sm.	LÖ88-01	DQ389711
<i>P. phegophila</i> Romagn.	SZMC-NL-3527	FN396129 FN396198
<i>P. hellebosensis</i> Deschuyteneer & A. Melzer	LÖ379-06 (as <i>P. almerensis</i> )	KC992873
<i>P. thujina</i>	Smith66720 (type)	KC992876
<i>P. thujina</i>	LÖ31-04 (as <i>P. almerensis</i> )	KC992874
<i>P. spadiceogrisea</i> (Schaeff.) Maire	BRNM 705637	AM712276
<i>P. spec.</i>	LN0631	KC992877
<i>P. sublatispora</i> Örstadius, S. Å. Hanson & E. Larss.	LÖ190-97 (as <i>P. spec.</i> 6)	KC992854

Table 1 - Sequences taken from the GenBank.

## RESULTS

### PHYLOGENETIC RESULTS

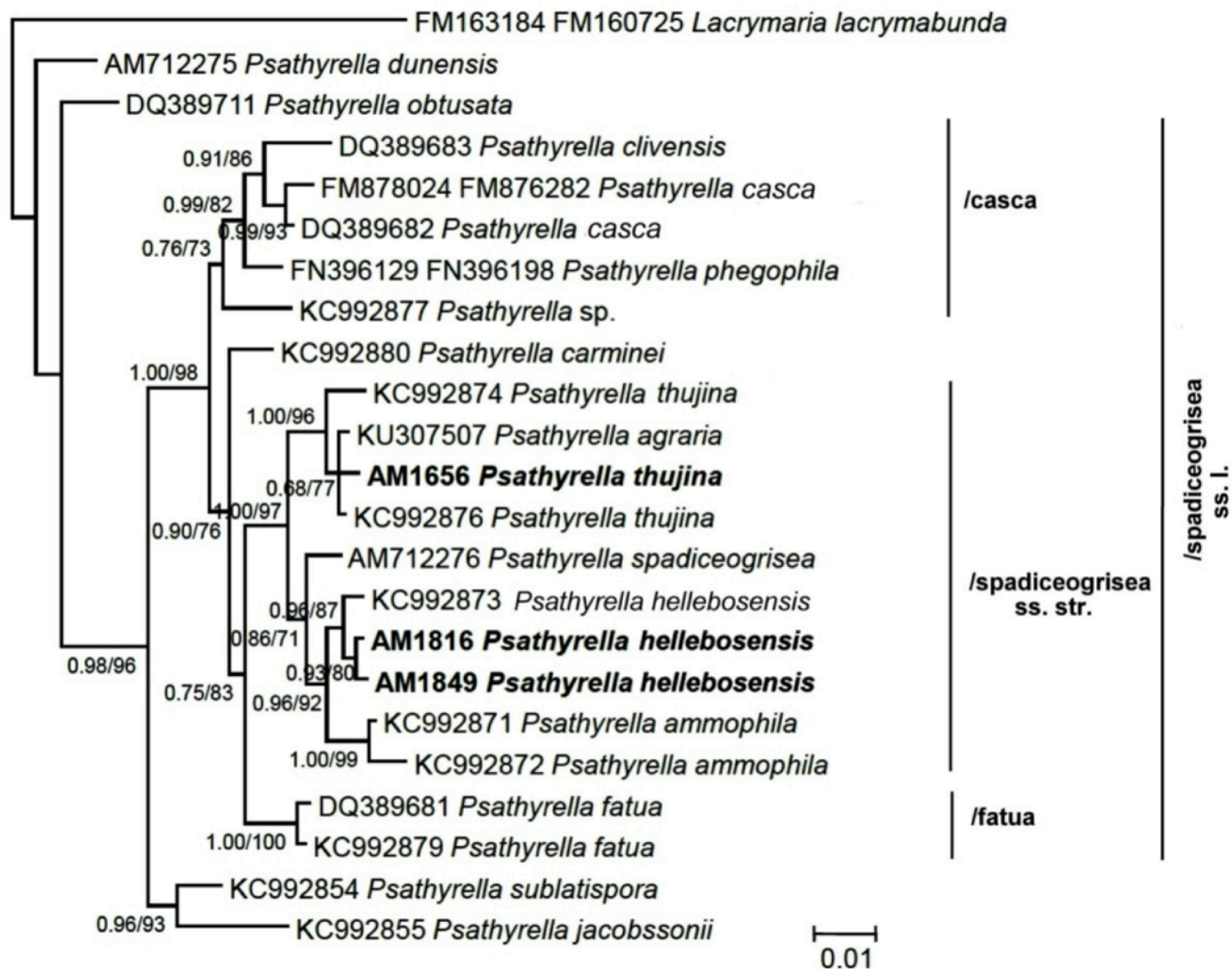


Fig. 2 - Maximum likelihood tree showing the placement of *Psathyrella hellebosensis* within the *spadiceogrisea*-group.

## **DESCRIPTION**

***Psathyrella hellebosensis*** D. Deschuyteneer & A. Melzer, spec. nov.

MycoBank no. : MB 820034

GenBank accession numbers : KY680789 (ITS), KY680790 (LSU)

Etymology : named after a small forest (in Flemisch : Hellebos) next to the place where this species has been found.

## **DIAGNOSIS**

Pileus 20-35 mm latus, parabolicus deinde convexus, humidus striatus, hygrophanus, rubro-brunneus vel brunneus, in sicco pallescens. Velum fibrillosum, album. Lamellae subdistantes, cinereo-brunneae vel brunneae, acie concolorae vel albidae. Stipes 40 x 2-4(5) mm, cylindraceus, albus vel fuscotinctus, fibrilloso-flocculosus. Odor et sapor indistincti.

Basidia 4-sporigera. Sporae 6,9-8,8(-10) x 4,4-5 µm, ovoideae, ellipsoideae, sub microscopium rubro-brunneae, poro germinativo distinctae. Pleurocystidia 32-60 x 9,5-17 µm, utriformia, obtusa vel capitata, aliquando furcata. Cheilocystidia 22-45(-55) x 9-15 µm, pleurocystidiis similia ; cellulis sphaeropedunculatis et clavatis multum immixtae, quod non raro crassitunicatis et brunneissunt. Cellulae veli subcylindraceae, hyalinae. Fibulae adsunt. Ad terram cum putridis plantis.

Holotypus : Belgium, Brabant, Steenokkerzeel, 10.11.2016, leg. D. Deschuyteneer (Ca. 15), in herbario Universitatis Lipsiae (LZ P-7615) depositus.

## **Fig. 3 - *Psathyrella hellebosensis* in situ.**

**a) young specimen,**

**b) view of the bottom of the cap,**

**c) a group of specimen,**

**d) old specimen (photos : D. Deschuyteneer)**

**Fig. 3 a - *Psathyrella hellebosensis* in situ, young specimen**





**Fig. 3 b - *Psathyrella hellebosensis* in situ. View of the bottom of the cap.**





**Fig. 3 c - *Psathyrella hellebosensis* in situ. A group of specimen.**





**Fig. 3 - *Psathyrella hellebosensis* in situ. Older specimen.**



**Habitat** : gregarious or subcaespitose, on soil among decaying grass and plant remnants and some wood chips.

**Cap** : 20-35 mm broad, at first paraboloid, dark reddish brown (ca.  $Y_{50}M_{80}C_{50}$ ), faintly striate when moist, later convex, in final stage flattening but often with a large umbo and a brighter festooned margin (ca.  $Y_{50}M_{40}C_{10}$ ), hygrophanous, drying out to pale greyish brown (ca.  $S_{40}Y_{10}M_{00}$ ) or greyish yellow brown (ca.  $Y_{30}M_{10}C_{00}$ ), usually slightly rugulose. Veil well developed on young fruitbodies as white fibrils or flocci reaching up to 1/3 from margin, but rapidly disappearing, on stem present in early stages, forming scattered patches or adpressed remnants.

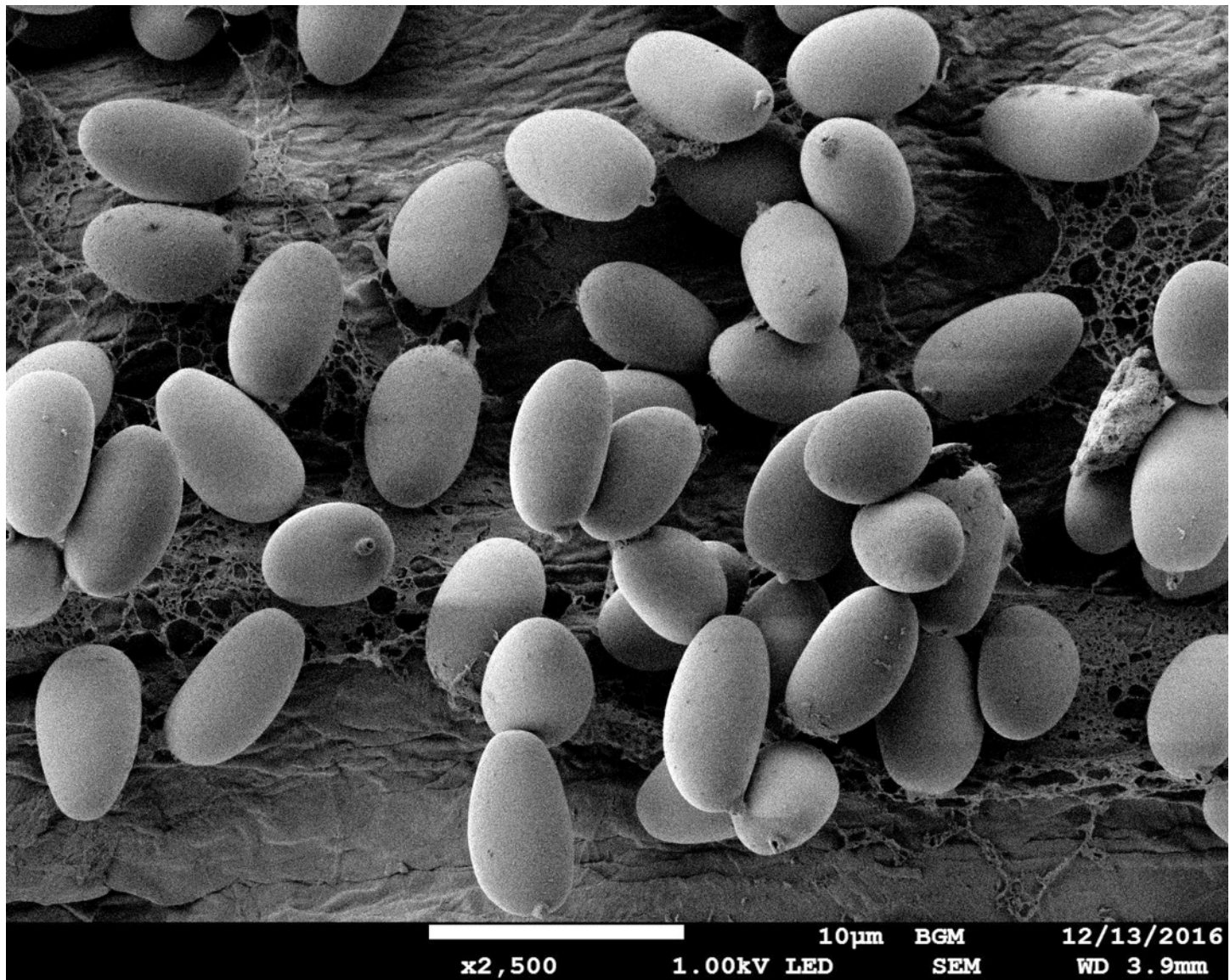
**Lamellae** : Subdistant, straight or slightly ventricose, 2-4 mm wide, broadly adnate, greyish brown to dark brown, edge concolorous, paler or white.

**Stem** : 40 x 2-4(5) mm, cylindrical, white and pruinose in the upper part; whitish, isabelline to sometimes pale brown in the lower third, with some veil remnants, hollow, not rooting.

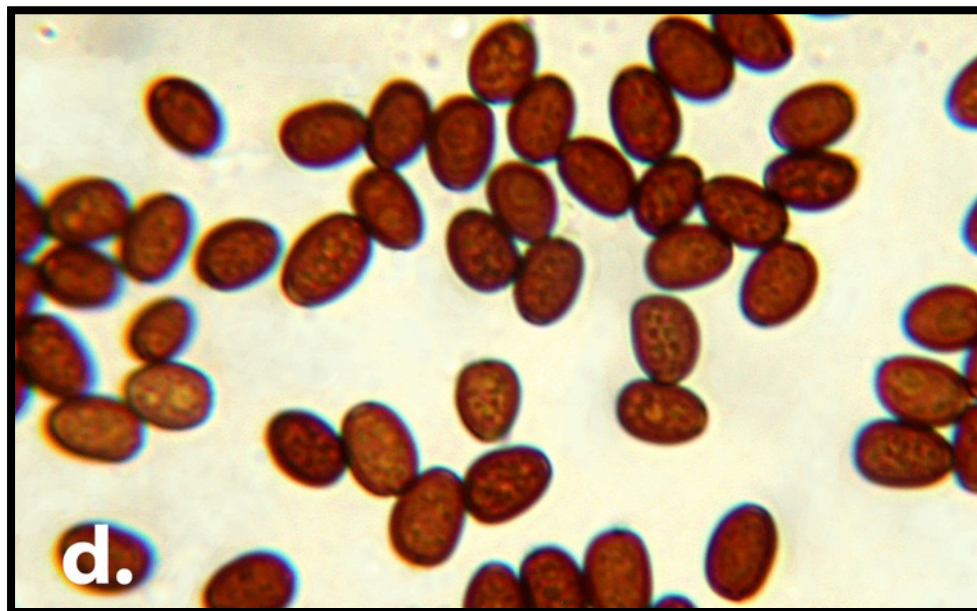
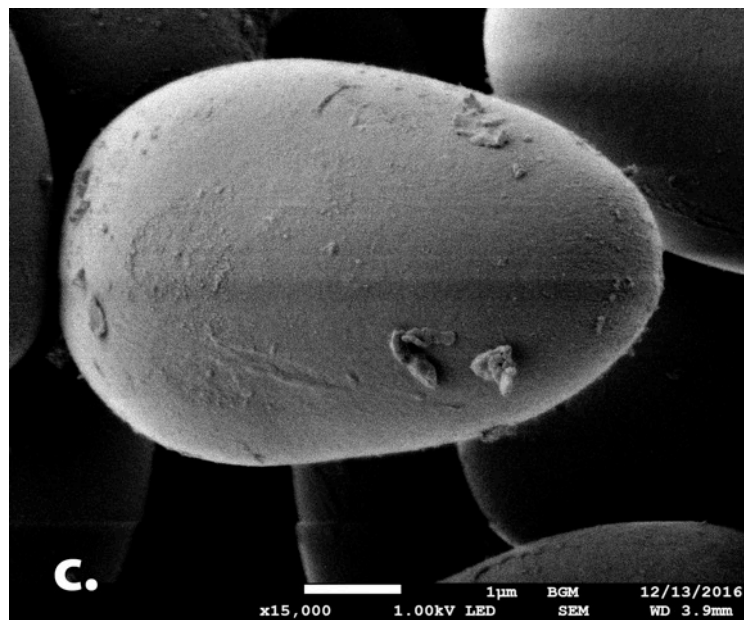
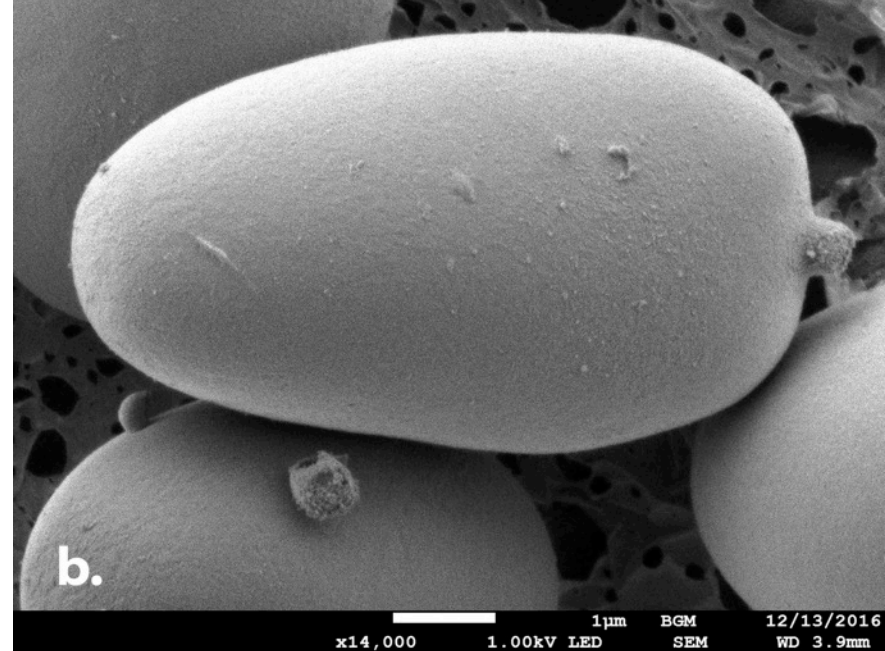
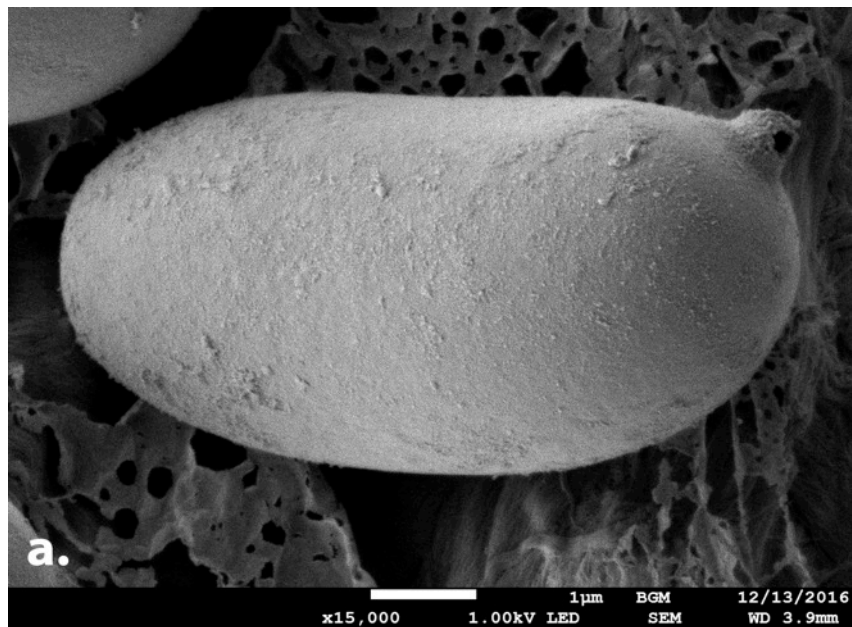
**Flesh** : In the cap up to 2-3 mm thick, light brown. Smell and taste not distinctive.

**Spores** : 6,9-8,8 (-10) x 4,4-5  $\mu$ m, av. 7,7-8,9 x 4,7-4,9  $\mu$ m, av.  $Q=1,60-1,89$ , in front view slightly to strikingly ovoid to very distinctly ovoid, the smallest ones nearly heart-shaped ; in side view only rarely and weakly phaseoliform, adaxially flattened, germ pore distinct (1,5-2  $\mu$ m wide) and central. In water and ammonia solution (10 %) reddish brown, in KOH (5 %) grey brown, not opaque.





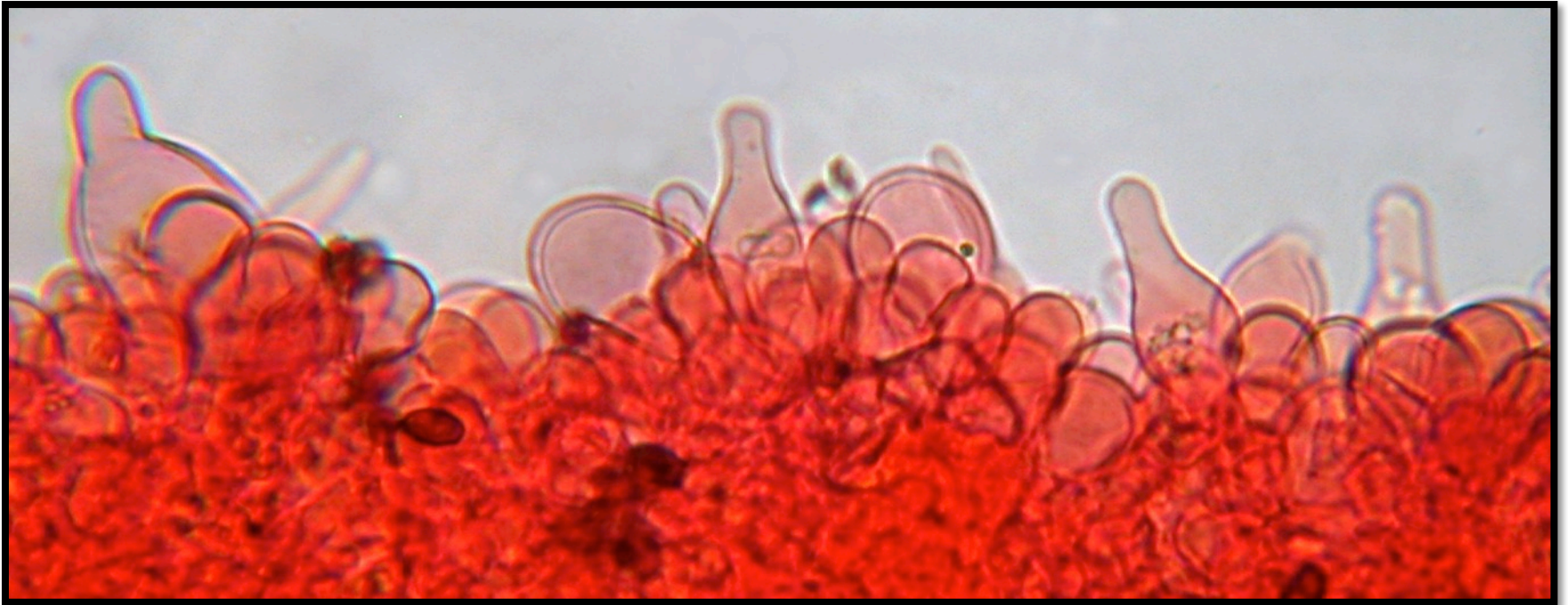
**Fig. 4 – Spores : SEM-photo, overview.**



**Fig. 5 – Spores :** a) SEM-photo, side view, b) SEM-photo, front view. c) SEM-photo of the smallest heart-shaped spores. d) Spores in water (photo : D. Deschuyteneer).



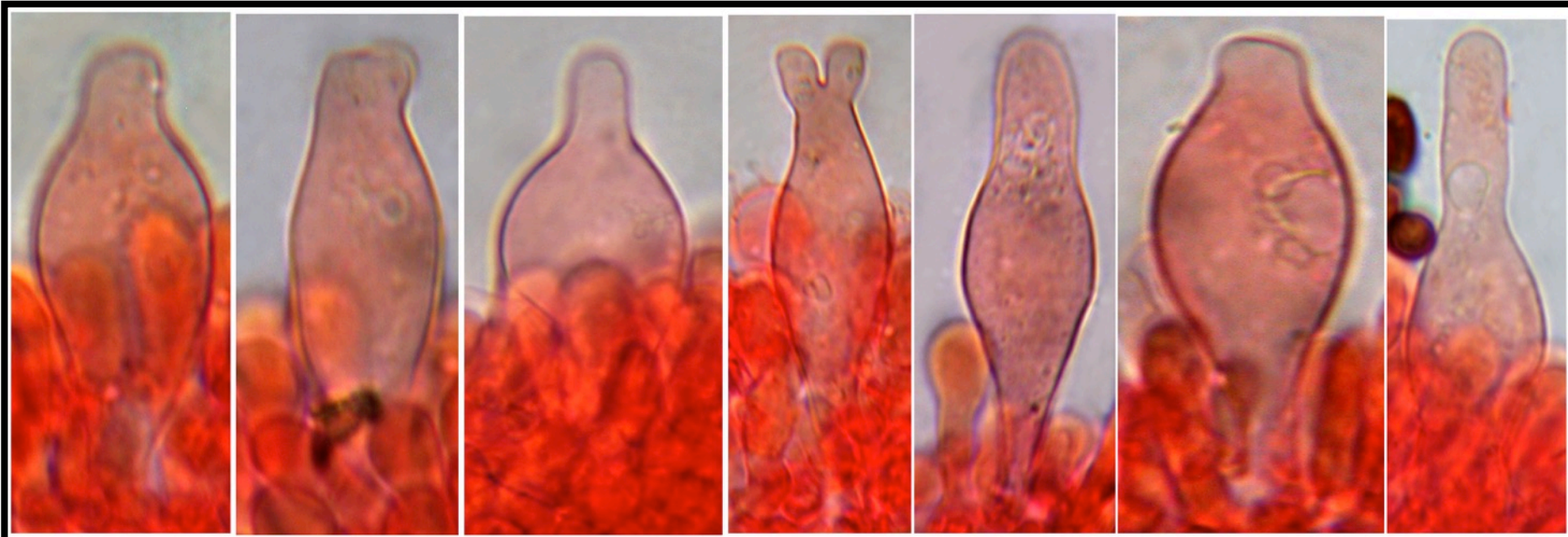
**Cheilocystidia** : 22-45(-55) x 9-15  $\mu\text{m}$ , mostly utriform to subutriform, rarely lageniform, often with a short and broad neck and an obtuse, bifurcated or irregularly branched top, thin-walled and colourless, very rare to scattered, heaped only on some spots, totally missing near the cap margin. Lamellae edge dominated by densely packed sphaeropedunculate and clavate cells, 13,7-46,5 x 8-22  $\mu\text{m}$ , the largest ones often with slightly thickened and pale brownish pigmented wall.



**Fig. 6 – Lamellae edge** (photo : D. Deschuyteneer).



**Pleurocystidia** : 32-60 x 9,5-17  $\mu\text{m}$ , predominantly similar to the cheilocystidia, rarely fusiform, thin-walled and colourless, scattered to numerous.



**Fig. 7 – Pleurocystidia** (photo : D. Deschuyteneer).

**Basidia** : 17.7-30 x 8-9.5 µm, 4-spored, clavate, sphaeropedunculate.

**Lamellae trama** : distinctly brown in ammonia solution (10 %) from membranal pigment.

**Veil** : made of subcylindrical, branched, hyaline cells.

**Clamps** : present.

**Caulocystidia** : numerous, very long sometimes more than 100 µm long, similar to the cheilocystidia or fusiform to digitiform, sometimes forked or septated.

**Material examined** : Belgium, Steenokkerzeel, 30.12.2015 (AM1816, now LZ P-7615, holotype) and 10.11.2016 (AM1849, AM1850, AM1851), leg. D. Deschuyteneer.

Additional examined material (*Psathyrella thujina*) : Germany, Schleswig-Holstein, Pampau, 15.06.2012, leg. Torsten Richter (AM1549) ; - Germany, Mecklenburg-Vorpommern, Ahrenshoop, 31.10.2013, leg. Torsten Richter (AM1656).

## DISCUSSION

In the literature, there are some descriptions of *Psathyrella thujina* or their synonyms : ARONSEN (1993), DE MEULDER (1999), ESTEVE-RAVENTÓS & VILLARREAL (2002), KITS VAN WAVEREN (1985), LUDWIG (2007), MUÑOZ & CABALLERO (2012), ÖRSTADIUS & KNUDSEN (2008), SMITH (1972). Most of the collections are not genetically tested, but a correct determination is to assume.

The delineation of *Psathyrella thujina* against *Psathyrella hellebosensis* without the help of molecular biology tools is extremely difficult, since there appears to be overlap in the features. All the subtle characteristics are important in their entirety.

The habit of most basidiomata of *Psathyrella hellebosensis* seems relatively stocky ; the stem is quite short and thick in relation to the cap diameter. Also, there is a distinct tendency to caespitose growth. Due to the high dependence on the environmental conditions (sunlight, humidity), the cap color and the veil are of little significance.

No definitive statements can be made regarding the ecological requirements, but the Belgian collections grew on not very dry, but also not wet soil, to be called as ruderal. Accompanying plants are *Ranunculus repens* L., *Ranunculus ficaria* L., *Symphytum officinale* L., *Urtica dioica* L. and *Rumex obtusifolius* L. These plants indicate nitrate-rich and not too dry loamy soil. The collection LÖ379-06 was found “on a moist place” (Leif Örstadius, pers. comm.).

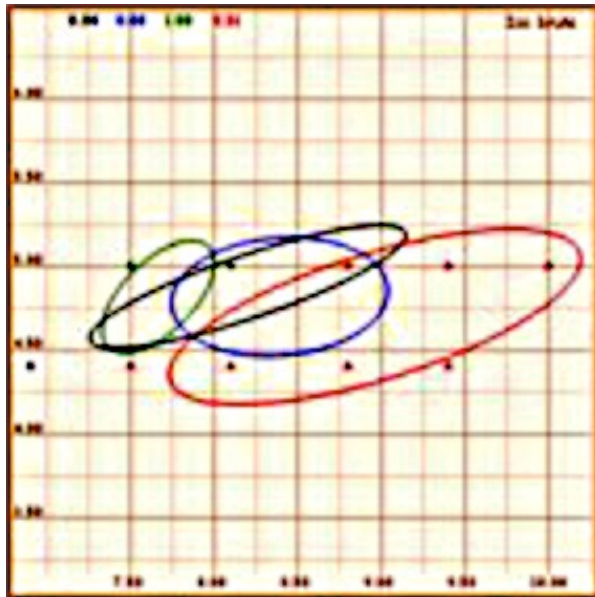
In contrast, *Psathyrella thujina* is almost always reported from the immediate vicinity of water bodies, according to SMITH (1972) “gregarious on black muck in a spring area” and LUDWIG (2007) “im Uferbereich unter *Salix* auf Laubresten”. ARONSEN (1993), DE MEULDER (1999), ESTEVE-RAVENTÓS & VILLARREAL (2002), KITS VAN WAVEREN (1985), KRISAI-GREILHUBER (1992), ÖRSTADIUS & KNUDSEN (2008) always mention a closeness of typical plants of the shore zone (*Cirsium*, *Epilobium*, *Juncus*, *Phragmites*, *Scirpus* and *Typha*). The German collections were found at *Phragmites* (AM1549) and *Bolboschoenus maritimus* (L.) Palla (AM1656). An exception is mentioned at MUÑOZ & CABALLERO (2012), the mushrooms were growing “con restos vegetales (paja seca y otras gramíneas) usados como abono, en una joven plantación de olivos” [at plant remnants (dry straw and other grasses) used as fertilizer, in a young olive grove]. Guillermo Muñoz (pers. comm.) designated the habitat as “very very moist soil”.

The molecular study suggests that even *Psathyrella agraria* Enderle (inval.) is identical with *P. thujina*. The mushrooms were found on “schwarzer Riederde” with possibly buried remains of maize. The place is located in the “Donausmoos”, an area with moor and swamp, is therefore very moist. ENDERLE (1996) expressed the suspicion, this ecology refers to *P. thujina* (literally : *P. almerensis*). According ENDERLE (1996) have the spore dimensions of 9.7-11.6(-12.5) x (5.2-)5.5-6(-6.5)  $\mu\text{m}$ , are thus significantly larger than those of *P. hellebosensis*.

Most of the most micromorphological features are inconstant and not very useful for the distinction between very closely related species ; e. g., the cheilo- and pleurocystidia are variable in size, shape and number and correspond as much with *P. hellebosensis* as with *P. thujina*.

The most important characteristics are spore size and shape. Of course, here a certain variability must be conceded, as usual in the genus.

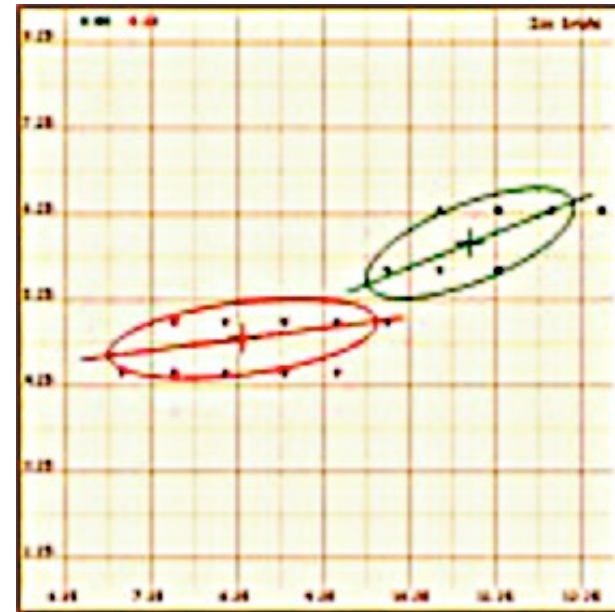
Diagram 1 illustrates the dispersion of spore dimensions of the four collections. In comparison to *Psathyrella thujina* (table 2), however, the spores are smaller and the overlap is low (diagram 2).



**Diagram 1 - Distribution of spore sizes within *P. hellebosensis*.**

Red : AM1849 ; green : AM1850.

Blue : AM1851 ; black : AM1816.



**Diagram 2 - Distribution of spore sizes.**

Red : *P. hellebosensis*.

Green : *P. thujina* (AM1549, AM1669).



Equally important is the spore shape. It ranges in different proportions from oblong up to broadly ovoid with a remarkably obtuse base, the shortest spores being often almost subtriangular. The spores of *Psathyrella thujina* are predominantly referred or drawn as ellipsoid with a conical base. In addition, the spores in lateral view appear less frequently and only slightly phaseoliform.

The collection LÖ379-06 has slightly larger spores than *P. hellebosensis*, on average 9.6 x 5.6 µm (Leif Örstadius, pers. comm.), but the shape agrees well. The base is mostly obtuse (judged after a photo, provided by Leif Örstadius) but they are never heart shaped.

source	spore length (µm)	spore width (µm)	av. spore size (µm)
ARONSEN (1993)	9-11.5 (-12.2)	5-6.8	
DE MEULDER (1999)	(8-) 9-10.4	4.7-5.7	
ESTEVE-RAVENTÓS & VILLARREAL (2002)	(9-) 9.15-11.5	5.5-6.42 (-6.5)	10.36 x 5.97
KITS VAN WAVEREN (1985)	9-11.5	4.5-6.5	9.3-10.8 x 5.2-6.1
LUDWIG (2007)	8-10.5	4.5-6	
MUÑOZ & CABALLERO (2012)	9-10.5	5-6	
ORSTADIUS & KNUDSEN (2008)	8.5-11	5-6	
SMITH (1972)	9-12	5-6	
AM1549	(10-) 10.5-12.5	5.5-6.2	11.4 x 6.1
AM1669	10-11.3	5.5-6.3	10.5 x 5.8

**Table 2 : spore size of *Psathyrella thujina*.**

## ACKNOWLEDGMENTS

We are grateful to Pablo Alvarado (Oviedo, Spain) for sequencing the specimens and creating the consensus tree, Myriam De Haan (Botanic Garden Meise, formerly National Botanic Garden of Belgium), for the SEM photos, Leif Örstadius (Kristianstad, Sweden) for informations about his collections of *Psathyrella thujina*, Guillermo Muñoz (Calahorra, Spain) for informations about the location of his record, and Bernard Clesse (Fagnolle, Belgium) for the determination of several accompanying plants in the habitat.

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**Seconde partie** (pour plus de détails merci de vous référer à la description originale en anglais dans la première partie de cet article).

Alors que Andreas Melzer et moi même étions occupés à rédiger la description originale de *P. hellebosensis*, Micheline Broussal, que je remercie vivement, nous a adressé des photos macroscopiques et microscopiques ainsi que des exsiccata d'une espèce qu'elle ne pouvait déterminer.

Par le plus curieux des hasards, elle venait de récolter également *Psathyrella hellebosensis*. Cette seconde récolte connue, me donne l'occasion d'apporter quelques précisions par rapport à la récolte de l'holotype.

L'holotype (photo de droite) a été récolté à proximité du Hellebos (ce qui lui a donné son nom, signifiant en français, bois du diable), en zone rudérale, sur un terrain vague où sont entreposés des branchages et des souches, de feuillus et de conifères, avant leur transformation en mulch. Le sol est également très riche en débris végétaux divers.

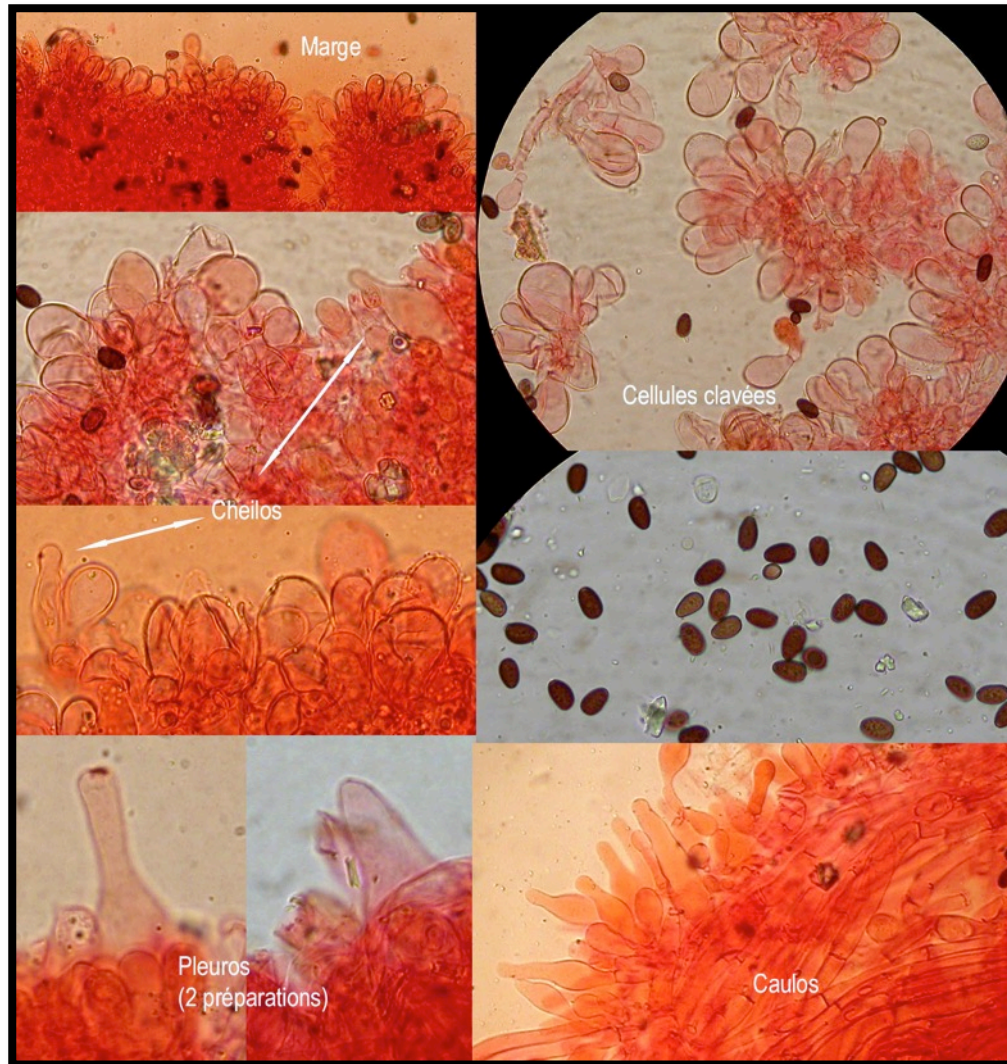
Compte tenu de la récolte de Micheline, l'habitat semble à priori plus diversifié, puisque cette seconde récolte a été effectuée en France en milieu sableux-limoneux, dans le bois du Boucanet (influence salée, embouchure du Vidourle), au pied de *Inula crithmoides*, sans qu'aucune relation avec des tiges desséchées de cette plante ait pu être établie. Il est probable que l'espèce se soit développée à proximité de ces plantes, car l'humidité qui régnait dans leur entourage immédiat devait être plus importante.

Macroscopiquement, la récolte de Micheline (photo de gauche) est strictement identique à l'holotype, bien que les exemplaires soient ici à un stade de déshydratation plus avancé et que la balance des couleurs ne soit pas optimale.



Contrairement à plusieurs récoltes de l'holotype effectuées en 2016 et 2017, qui laissait supposer une croissance grégaire à subcespiteuse, quatre à cinq exemplaires étant souvent connés par leur base, les exemplaires de Micheline croissaient de manière isolée et ont été rassemblés pour les besoins de la photo.

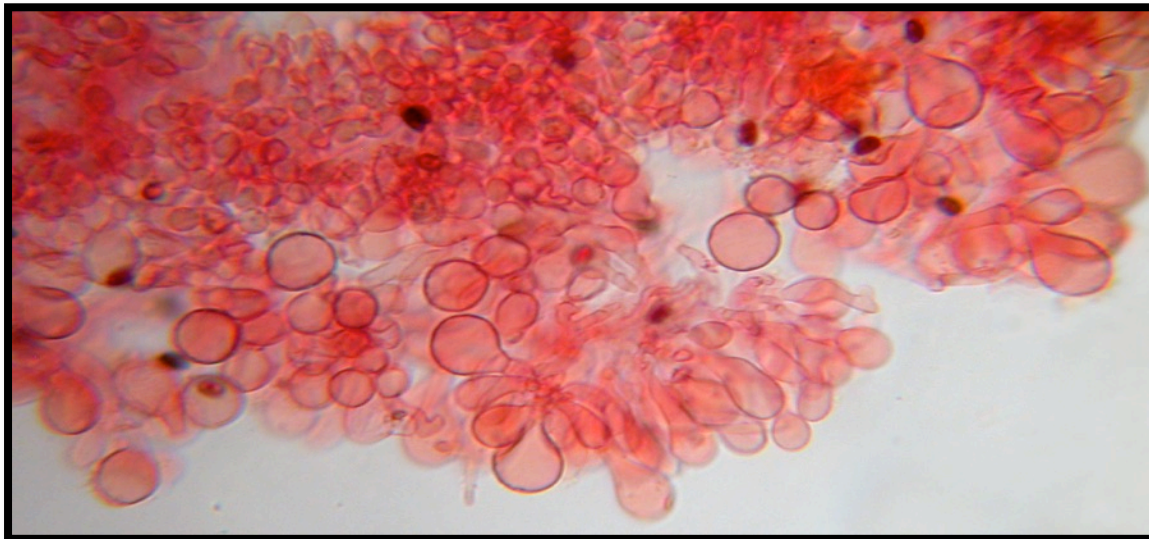
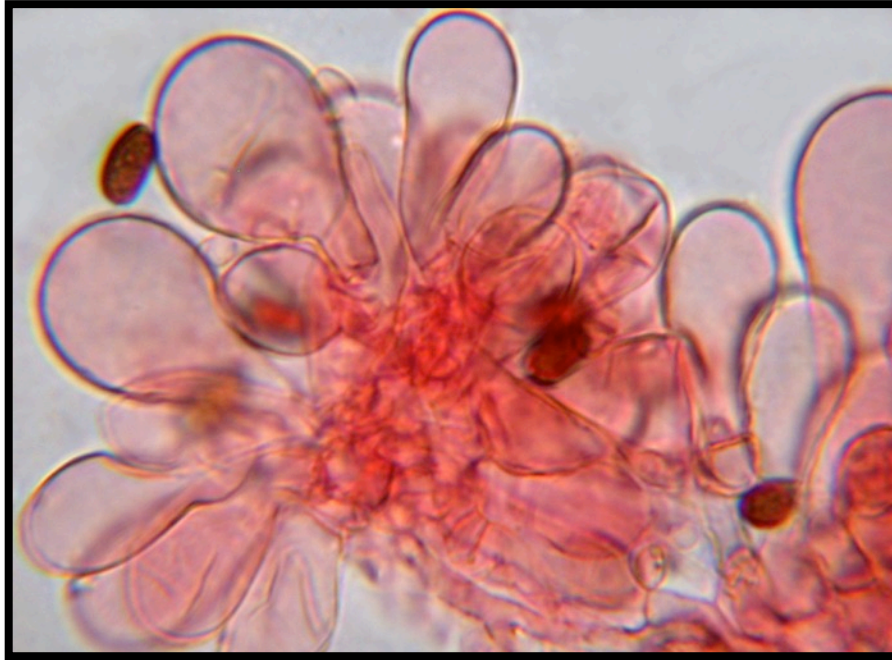
La microscopie ci-après fournie par Micheline était identique (avec une faible variabilité) à celle observée lors de l'observation de l'holotype.





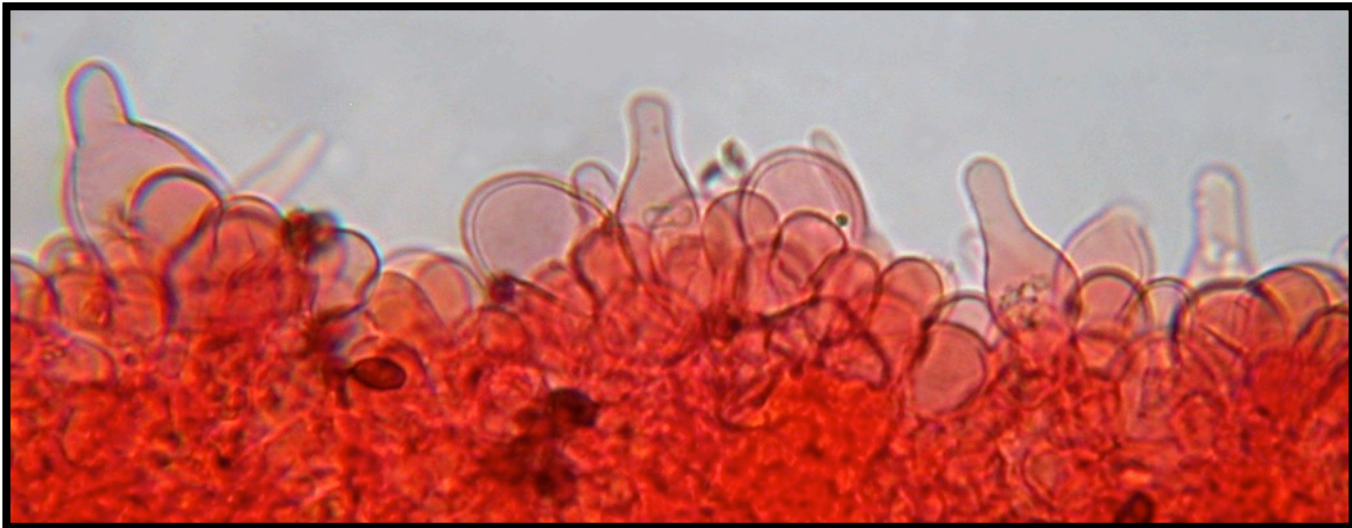
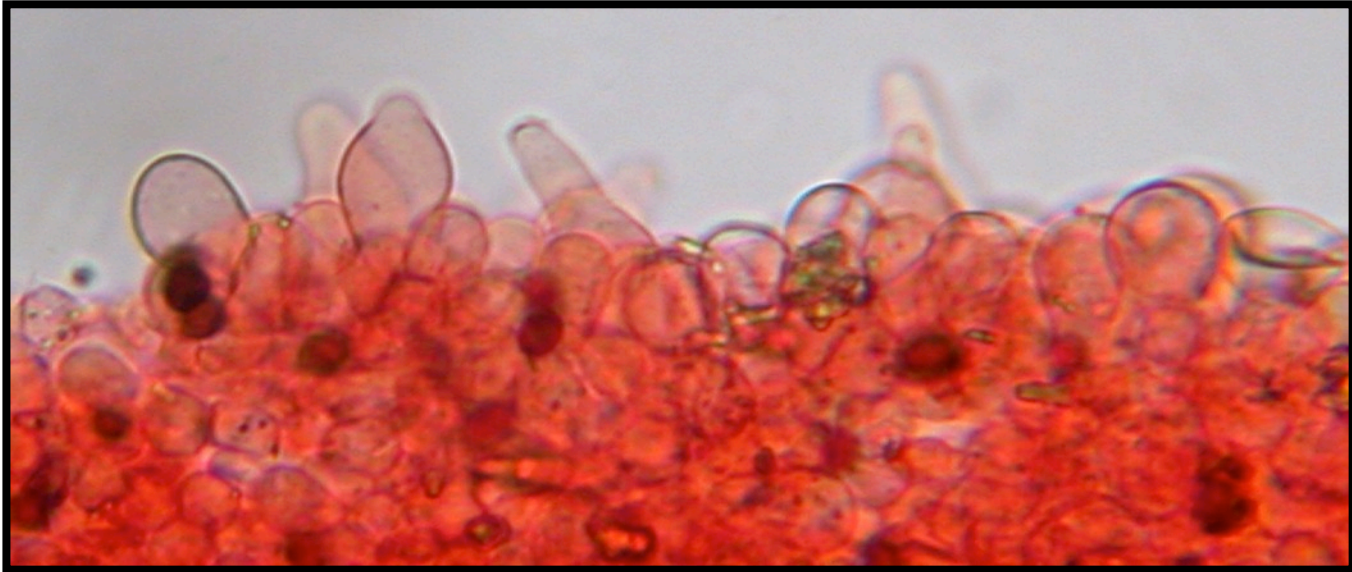
**Deux caractères microscopiques essentiels, qui frappent l'observateur sont la marge et les spores.**

1/ La marge est constituée essentiellement de « cellules marginales » clavées sphéropédunculées à paroi parfois légèrement épaissie.

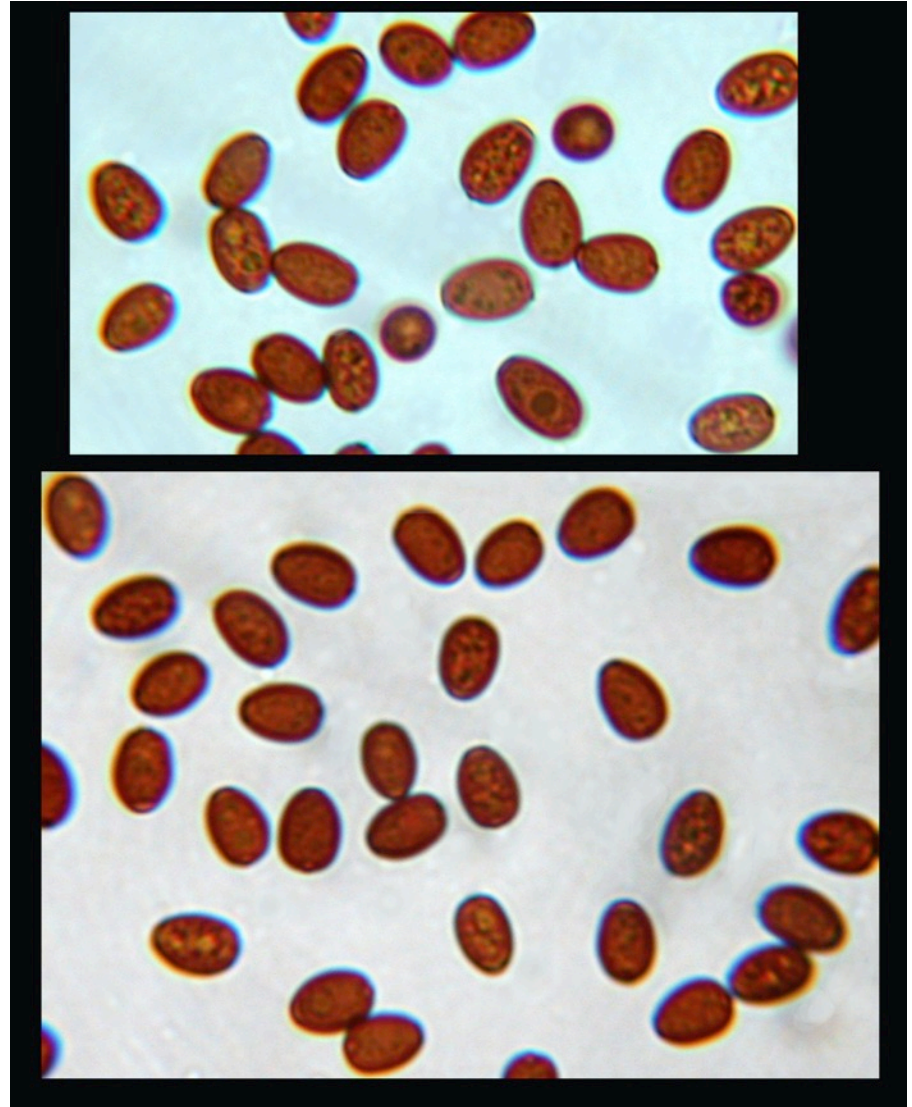
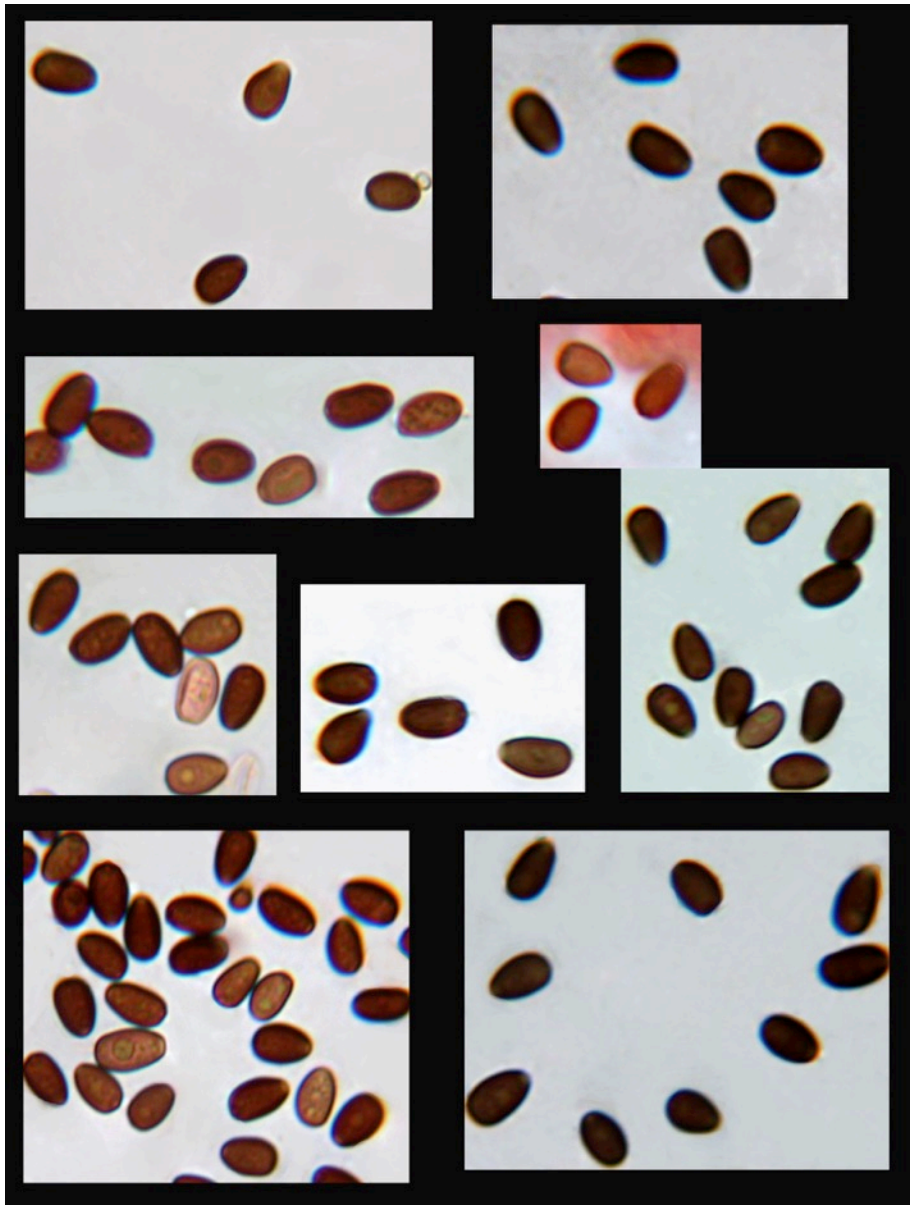




Les cheilocystides peu nombreuses sont majoritairement lagéniformes à ventrues, et il est assez fréquent dans la récolte de Micheline, tout comme dans les récoltes de l'holotype, d'observer de cheilocystides plus fusiformes prolongées par un long bec, parfois flexueux, à sommet obtus, ou aplati, représentant dans ce cas des formes transitionnelles vers des cystides à sommet fourchu, qui sont assez fréquentes.

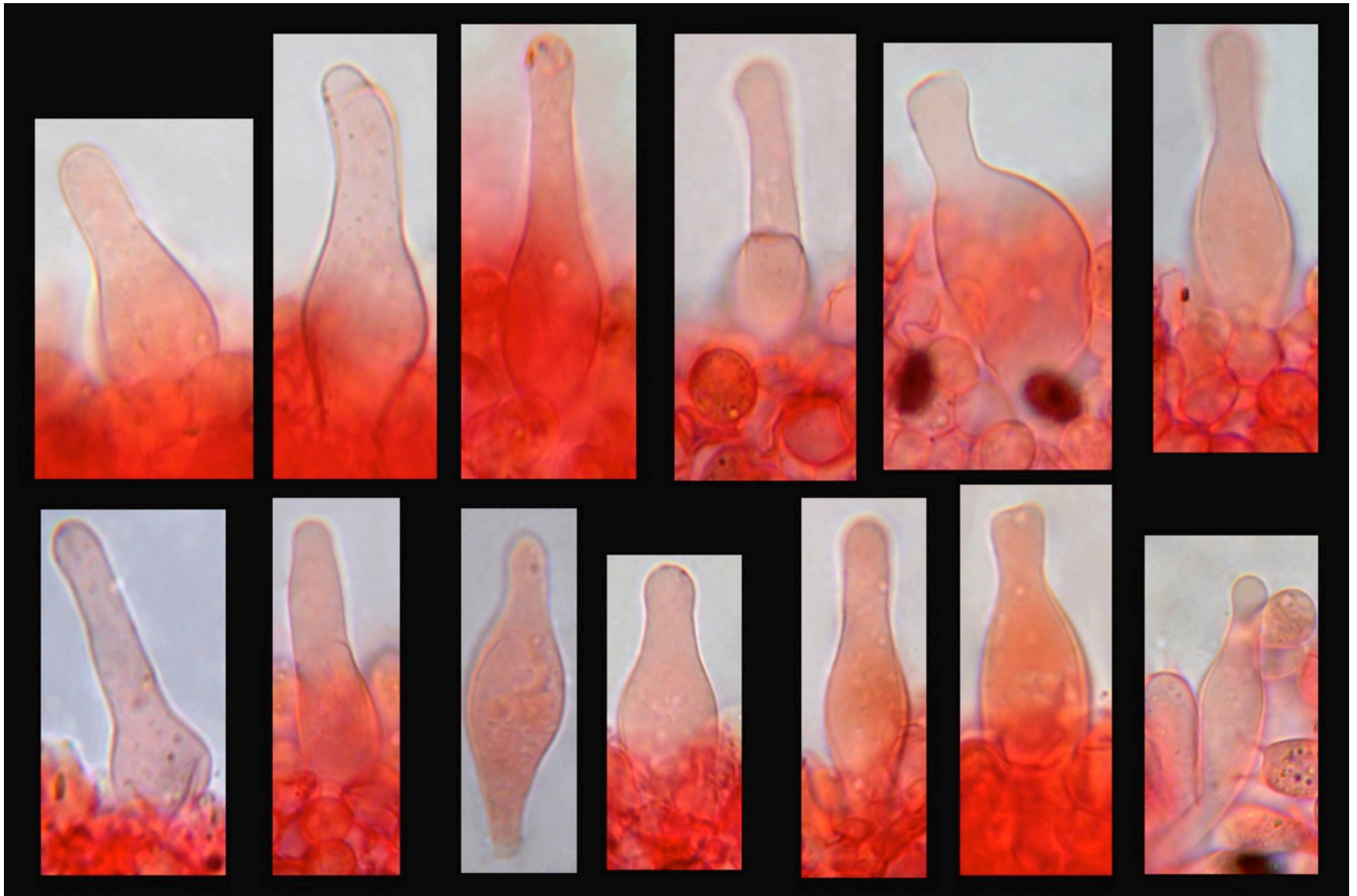


2/ Le deuxième caractère essentiel est l'abondance de petites spores subtriangulaires mélangées à de nombreuses spores de plus grande taille, oblongues. Du fait de ce caractère, je suis persuadé que cette espèce a par la passé souvent été confondue avec *P. panaeoloides*.



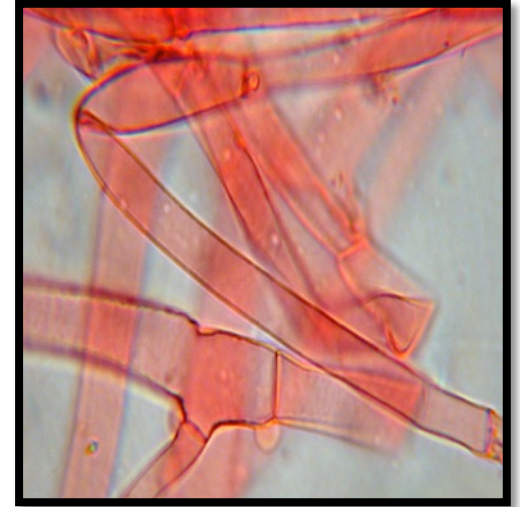
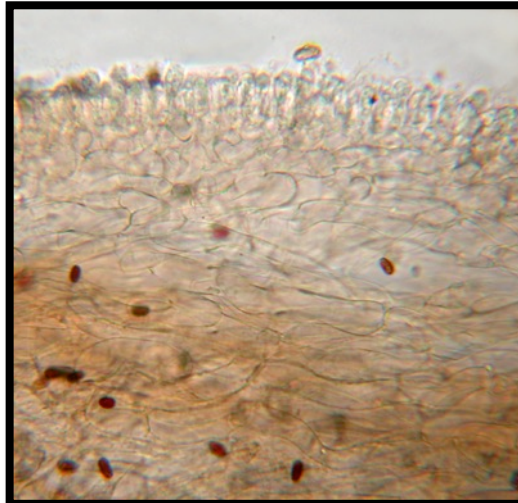
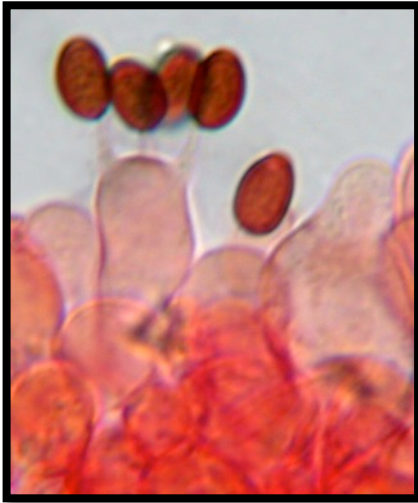


Les nombreuses pleurocystides sont analogues aux cheilocystides.





Basides clavées tétrasporiques ; Médiostrate nettement pigmentée ; voile formé de fibrilles ramifiées.



Caulocystides abondantes

