Psathyrella owyheensis A. H. Sm.
Seconde récolte connue de cette espèce réalisée en Espagne

Daniel Deschuyteneer, Joaquim Carbó, Dieter Wächter

Introduction

Psathyrella owyheensis a été décrite par Smith dans «Sands Basin, Owyhee County (d'où son nom), Idaho, United States », une région au climat Méditerranéen montagnard, dans une prairie pâturée par des bovins (Smith, 1972).

Dans une récente publication, Voto et al. (2015), après examen de deux exemplaires de l'holotype, ont établi une synonymie entre cette espèce et *Psathyrella carminei,* sur base de caractères biomoléculaires (correspondance ITS = 99% & TEF-1alpha = 98%) et micro-morphologiques.

Par comparaison, l'examen de nos spécimens nous permet également de conforter cette synonymie avec *Psathyrella carminei* Örstadius & E. Larss. (Örstadius et al., 2015) qui avait été découverte en Calabre (Italie), à proximité de la commune de Longobucco, en Novembre 2008, par Carmine Lavorato (d'où son nom), au sol, dans une forêt en présence de *Pinus nigra subsp. laricio* Maire.

Une nouvelle récolte de *Psathyrella owyheensis*, réalisée également sous climat méditerranéen, a été observée le 09/06/2018 par Joaquim Carbó, Carles Roqué et Àngel Torrent dans le « Parc Natural de les Capçaleres del Ter i del Freser », à proximité de la commune de Setcases (altitude 2125 m), région du Ripollès, dans la province de Gérone (Espagne).

L'étude abondamment illustrée de cette récolte, nous permet de préciser ses caractères écologiques, morphologiques et biomoléculaires.

Quinze à vingt exemplaires au port grégaire, apparaissaient tout comme la récolte de Smith dans une prairie pâturée par des bovins, à côté d'une légumineuse buissonnante, appelée localement "bàlec" *Genista balansae* (Boiss) Rouy**.** De loin, nos spécimens ressemblaient à *Marasmius oreades*, puis après les avoir ramassés, nous avons pensé qu'ils pouvaient correspondre à de grands *Panaeolina foeniscescii*, une hypothèse que nous avons rapidement éliminée après examen des caractères micro.

Psathyrella owyheensis was described by Smith in "Sands Basin, Owyhee County (hence its name), Idaho, United States", an area with a Mediterranean mountain climate, in a meadow grazed by cattle (Smith, 1972).

In a recent publication Voto et al. (2015), after examination of two samples of the holotype, established a synonymy between this species and *Psathyrella carminei* on the basis of biomolecular (ITS correspondence = 99% & TEF-1alpha = 98%) and micro-morphological characters.

By comparison, examination of our specimens, also allows us to support this synonymy with *Psathyrella carminei* Örstadius & E. Larss. (Örstadius et al., 2015), which had been discovered in Calabria (Italy), near the municipality of Longobucco, in November 2008, by Carmine Lavorato (hence its name), on the ground, in a forest in the presence of *Pinus nigra* subsp. *laricio* Maire.

A new collection of *Psathyrella owyheensis*, also in a Mediterranean climate, was observed on 09/06/2018 by Joaquim Carbó, Carles Roqué and Àngel Torrent in the "Parc Natural de les Capçaleres del Ter i del Freser", near the municipality of Setcases (altitude 2125 m), Ripollès region, in the province of Girona (Spain).

The extensively illustrated study of this collection, enables us to precise its ecological, morphological and biomolecular characteristics.

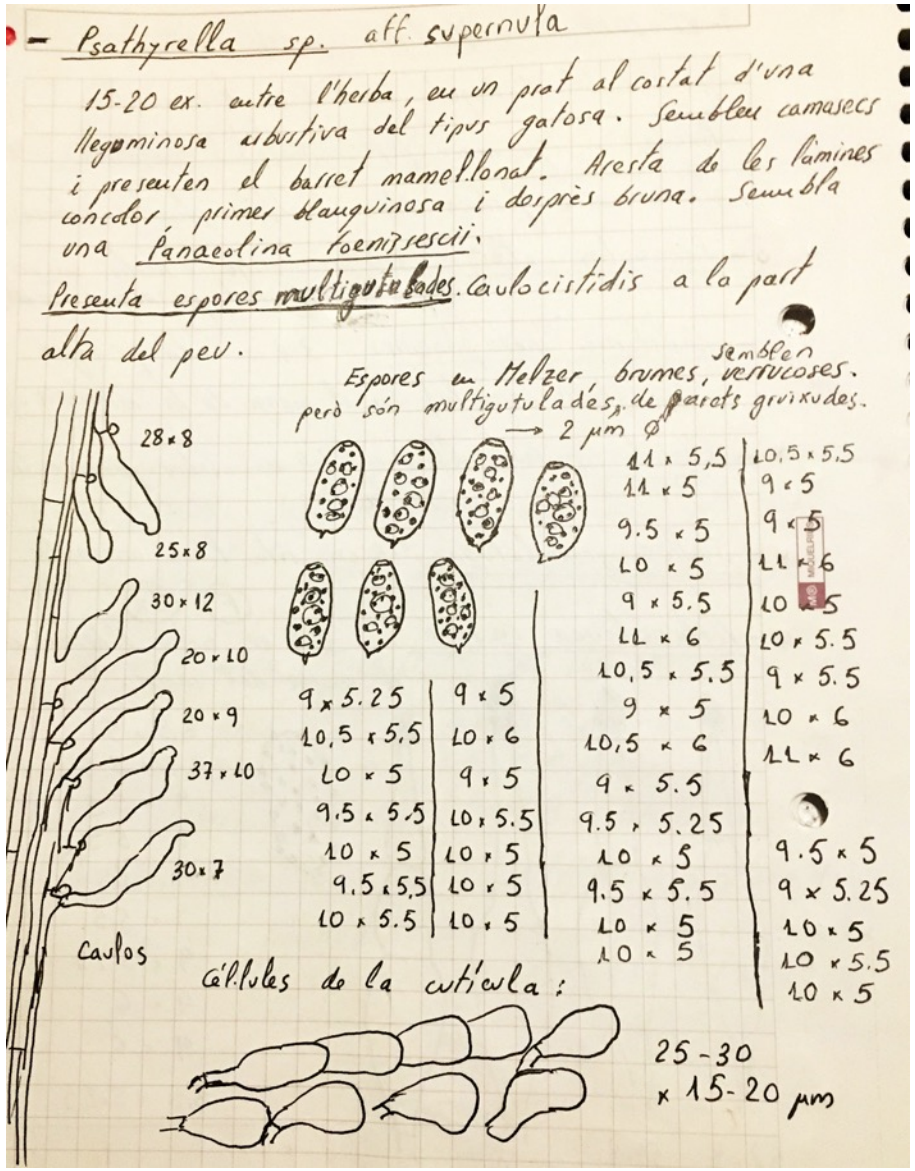
Fifteen to twenty specimens growing together, appeared just like the Smith collection in a meadow grazed by cattle, next to a bushy leguminous plant, locally called "bàlec" *Genista balansae* (Boiss) Rouy. From a distance, our specimens resembled *Marasmius oreades*, then after collecting them, we thought they might correspond to large *Panaeolina foeniscescii*, a hypothesis that we quickly eliminated after examining the microcharacteristics.

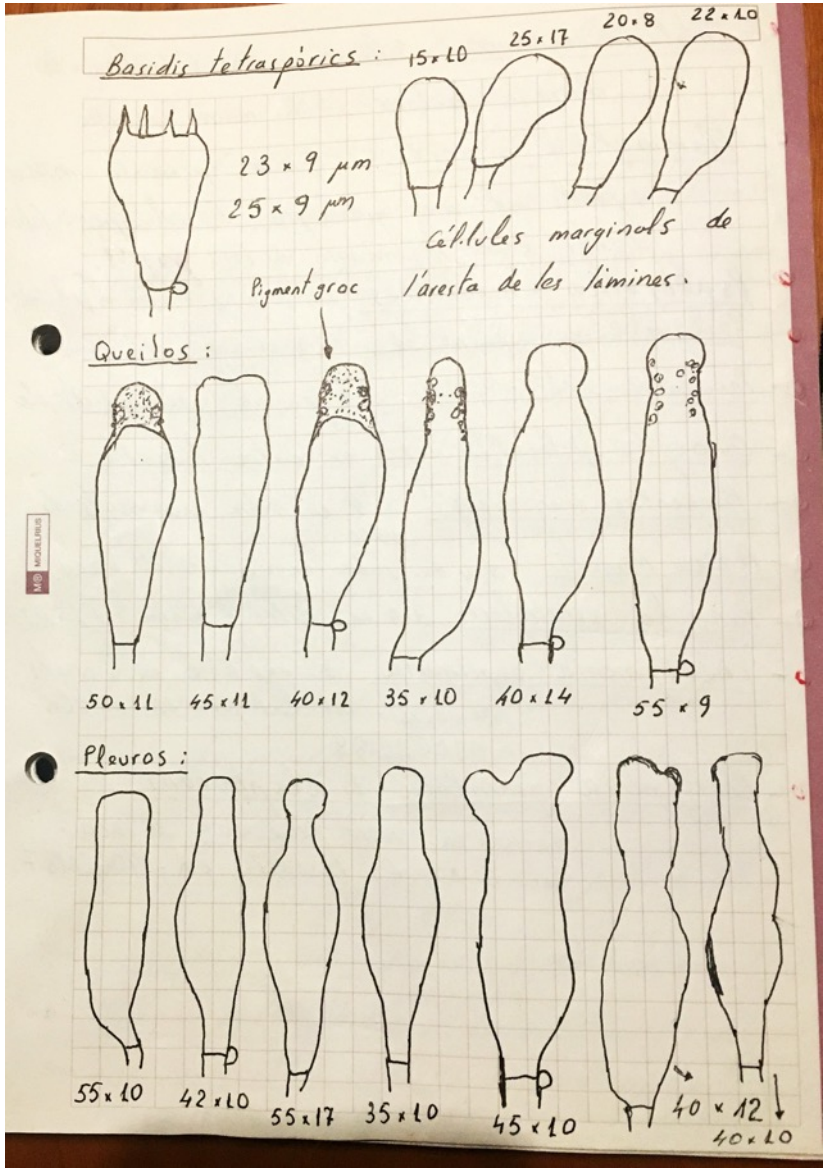
Description macroscopique partielle sur base des notes et photos réalisées in situ

Chapeau mesurant de 20 à 35 mm de diamètre, non strié, initialement conico-convexe, devenant plan-convexe avec apparition d'un large umbon discal obtus (la marge ayant tendance à s'éverser), initialement d'un beau brun foncé ; hygrophane, il décolore en beige grisâtre, finalement crème alutacé.
Voile fibrilleux, blanchâtre, rapidement volatile, présent sous forme de fibrilles au niveau de la marge des jeunes exemplaires.
Stipe : 35-70 x 3-4 mm, cylindrique, creux, blanchâtre à très faiblement ocracé, fibrilleux dans son tiers inférieur.
Lames adnées, ventrues, moyennement serrées, alternant avec lamelles & lamellules, beige grisâtre ; arête blanchâtre à concolore, mais sur base de la micro-scopie, il est fort possible que l'arête puisse apparaître surlignée.
Odeur non précisée.

Photo in situ. Joaquim Carbó - Parc Natural de les Capçaleres del Ter i del Freser" - 09/06/2018

Microscopie - Dessins de Joaquim Carbó

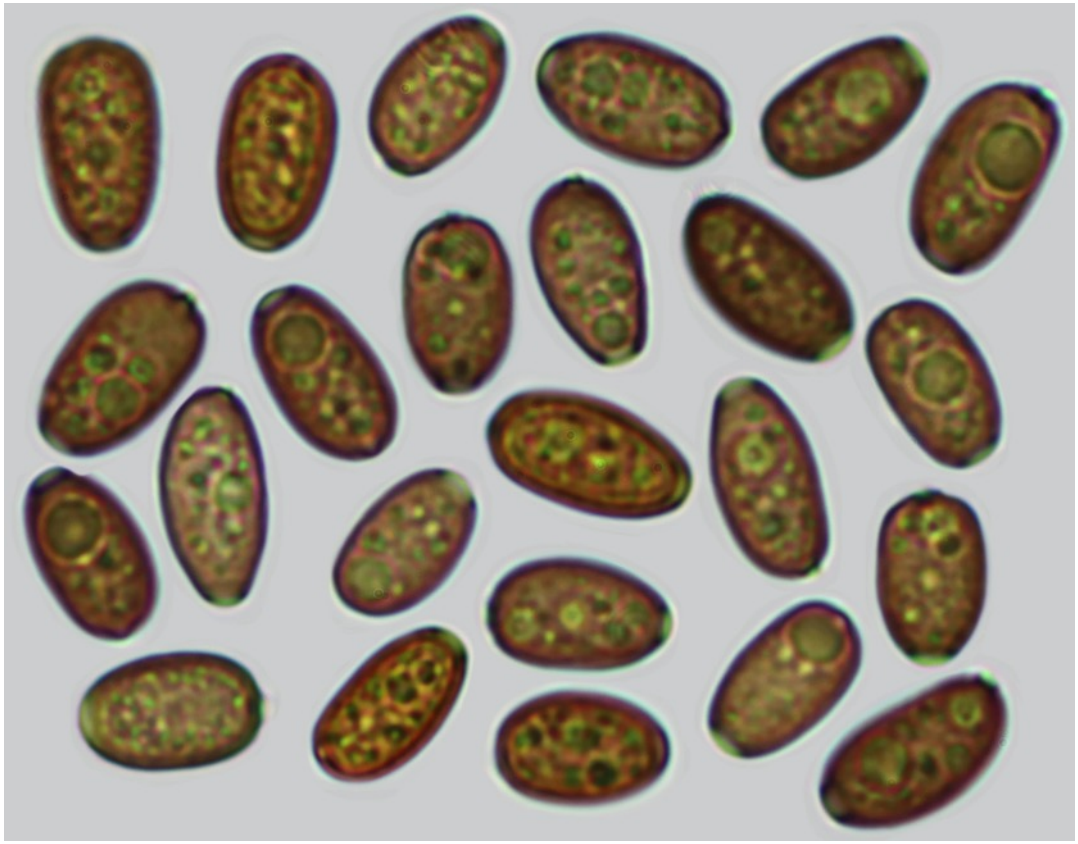




Microscopie réalisée sur trois exsiccata, dont un exemplaire jeune et deux exemplaires matures. Photos D. Deschuyteneer.

Basides tétrasporiques, et quelques-unes bisporiques.

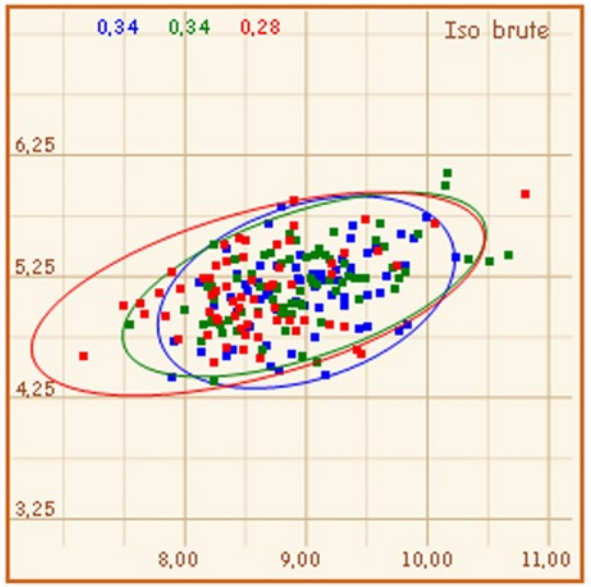
Spores polymorphes, lisses, brunes, non opaques, avec de nombreuses petites guttules, oblongues, ellipsoïdes et ovoïdes de face, asymétriques et le plus souvent légèrement amygdaliformes de profil ; les plus courtes à base parfois tronquée, leur donnant un aspect ovo-triangulaire ; pore germinatif central, large, convexe, à parfois tronqué**.** **Boucles** présentes.



Örstadius : 9-11 x 5-5,5 µm ; Me = 10x 5,2 µm Qe 1,9

Voto. : (7,5-)8,8-11,1(-12,5) x (3,8-)4,5-5,7(-6,5) µm ; Me = 9,4 x 5,2 µm ; Qe : 1,8-1,9

Diagramme comparatif des sporées des 3 exsiccata.



(N= 60)

(7,2)7,9-9,4(10,8) × (4,5)4,7-5,5(5,9) µm

Me = 8,6 × 5,1 µm ; Q = (1,5)1,52-1,8(2) ; Qe = 1,7

(N = 75)

(7,5)8,2-9,7(10,7) × (4,4)4,8-5,5(6,1) µm

Me = 9 × 5,2 µm ; Q = (1,5)1,6-1,9(2) ; Qe = 1,7

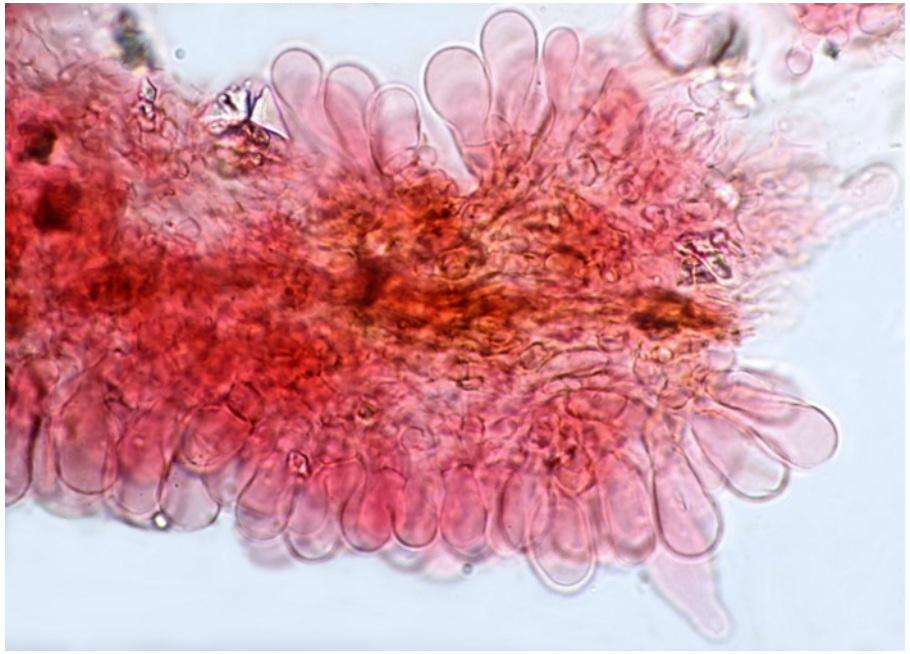
(N = 69)

(7,9)8,4-9,7(10,2) × (4,4)4,6-5,5(5,8) µm

Me = 9 × 5,1 µm ; Q = (1,5)1,6-1,9(2,1) ; Qe = 1,8

Arête entièrement occupée par des cellules marginales clavées et sphéropédonculées **(paracystides)** dont la paroi est généra-lement épaissie et colorée de brun jaunâtre, avec de rares cheilocystides éparses, signant l'appartenance de cette espèce au groupe « *spadiceo-grisea* ». Toute l'arête est infiltrée de fibres brun jaunâtre, surlignant les paracystides.

1



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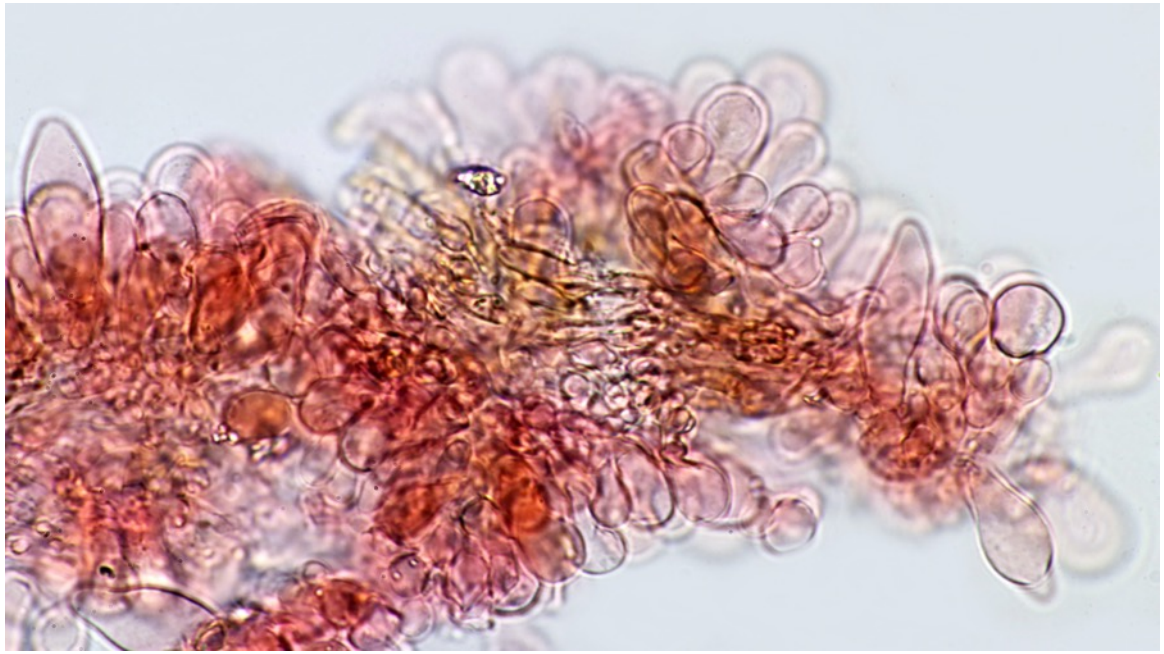
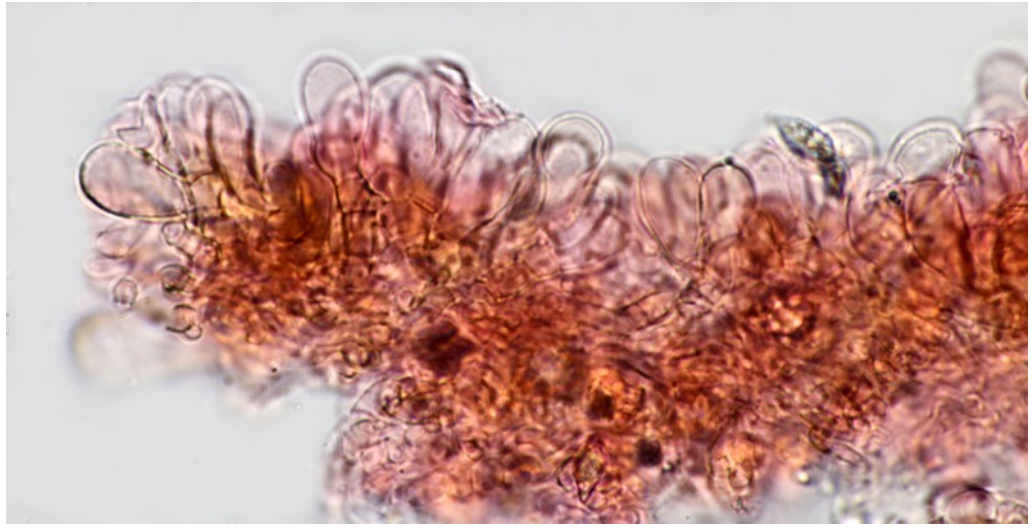
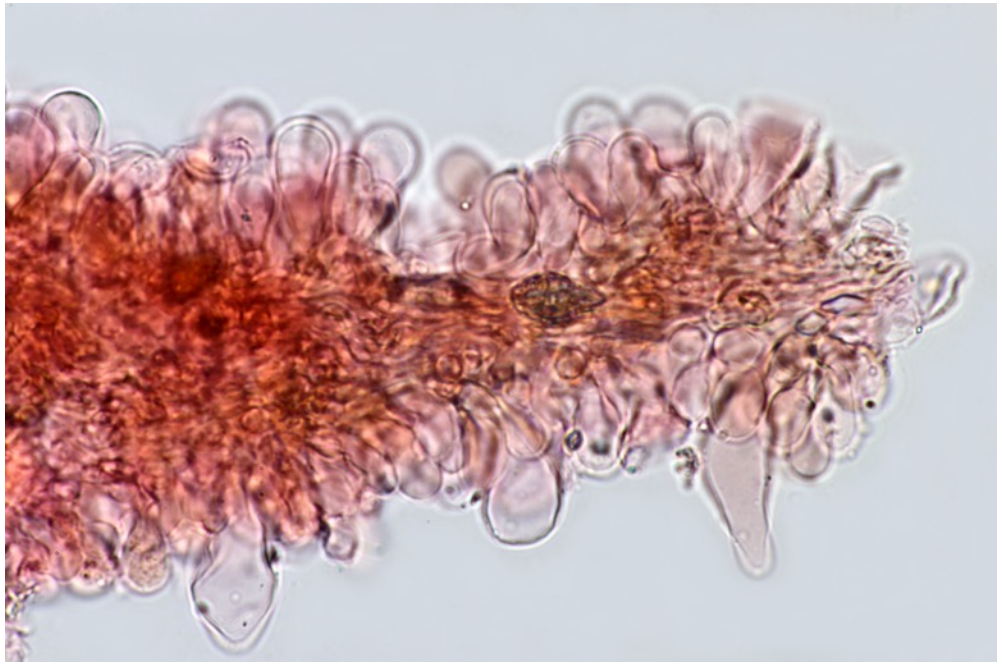
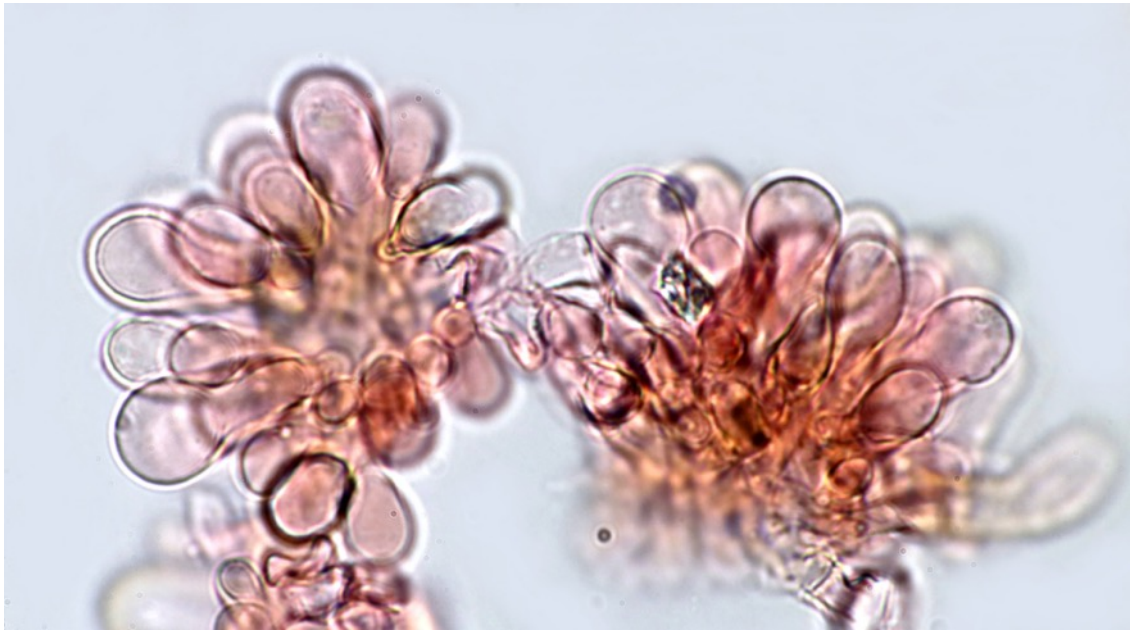
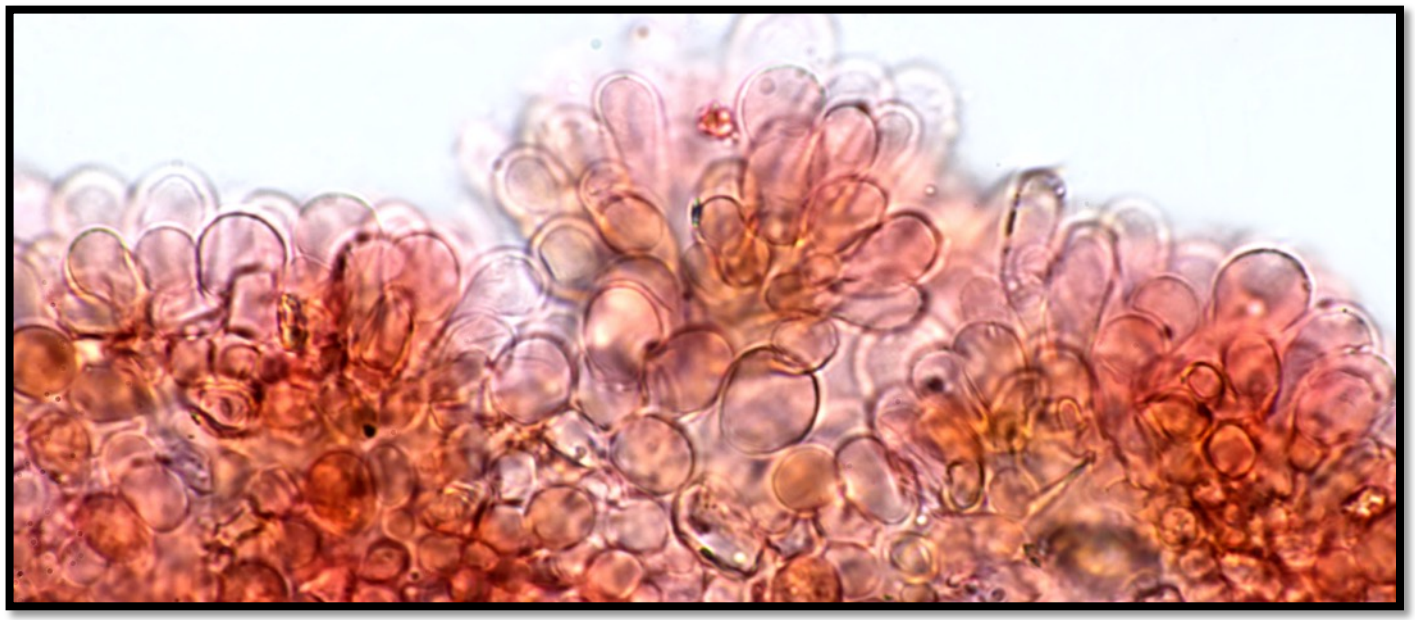


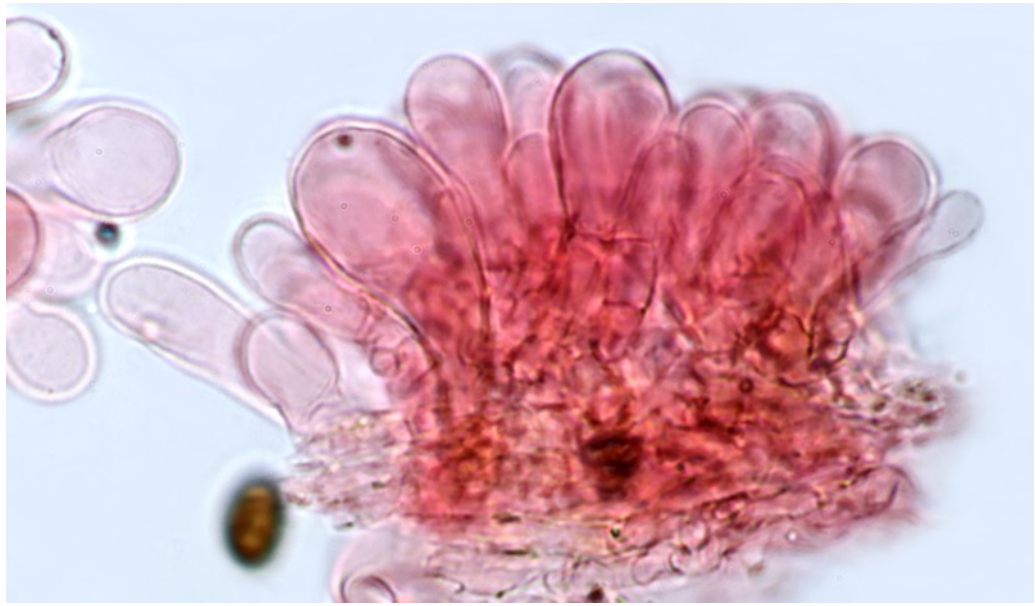


Paracystides clavées de l’arête, à paroi épaissie brun jaunâtre, surlignées de fibres brun jaunâtre.

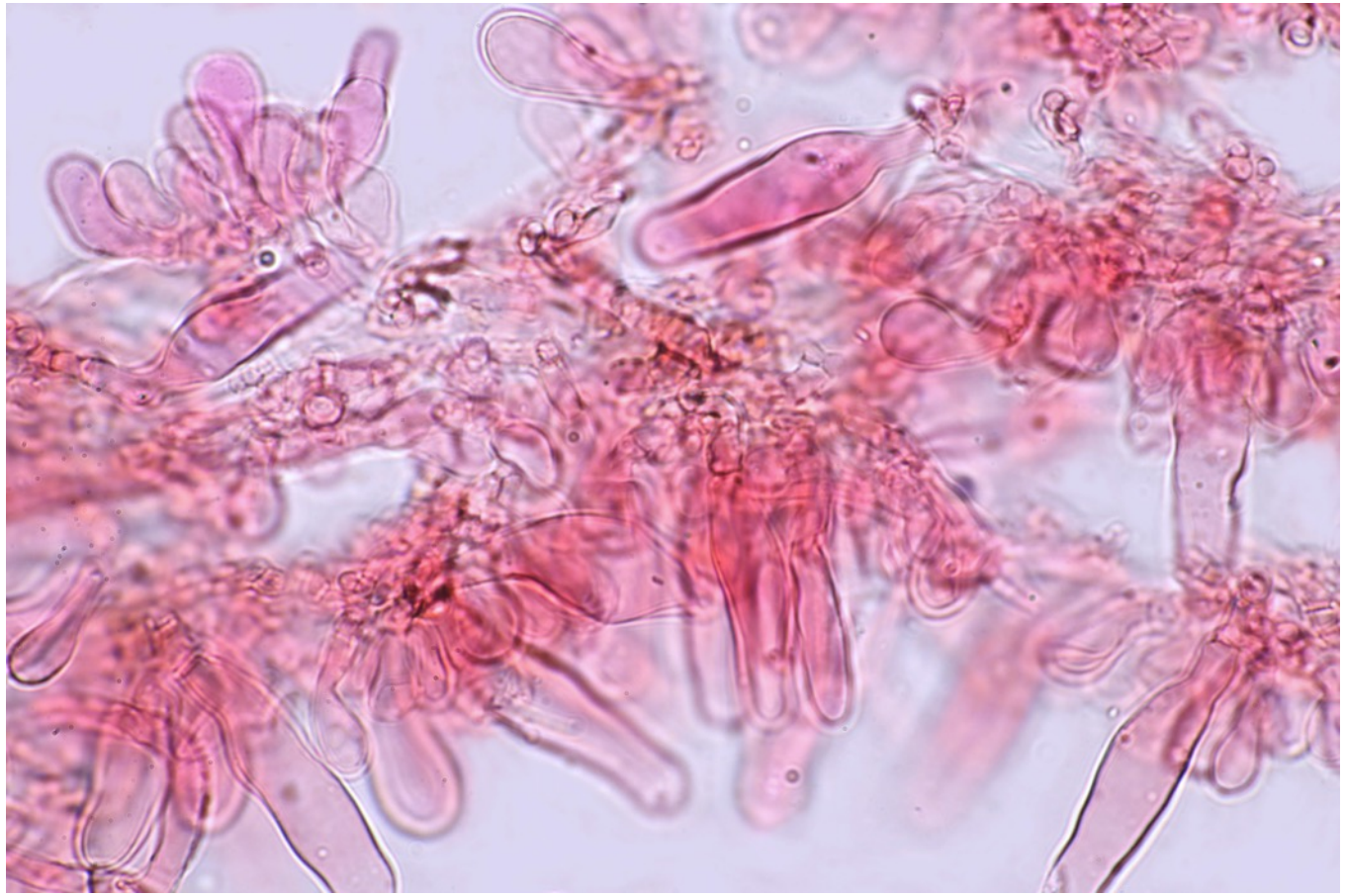








Cheilocystides hyalines, à paroi fine (parfois légèrement épaissie), polymorphes, essentiellement cylindro-lagéniformes, peu nombreuses sur l'exemplaire jeune mais devenant nombreuses et souvent à sommet tronqué (plus rarement fourchu) sur les exemplaires plus développés, et toujours mêlées à de nombreuses cellules marginales clavées (paracystides).



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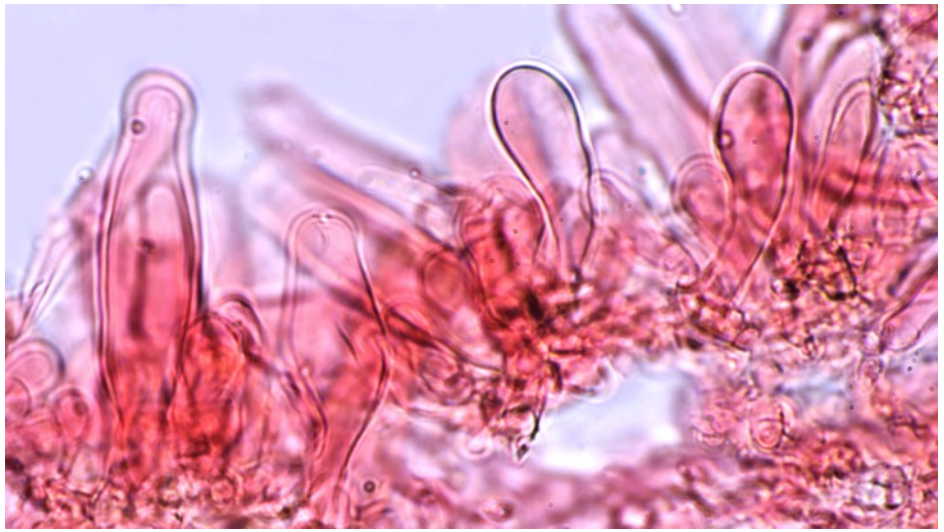
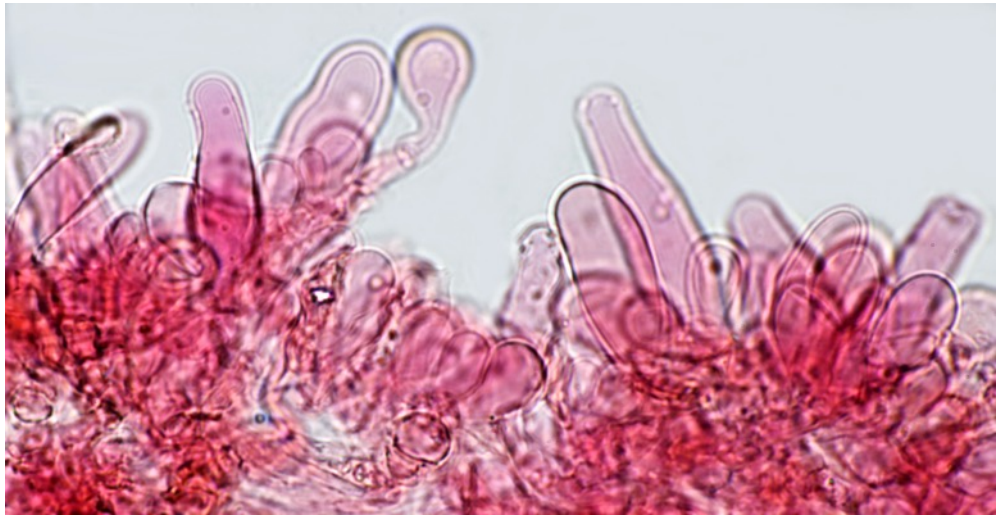
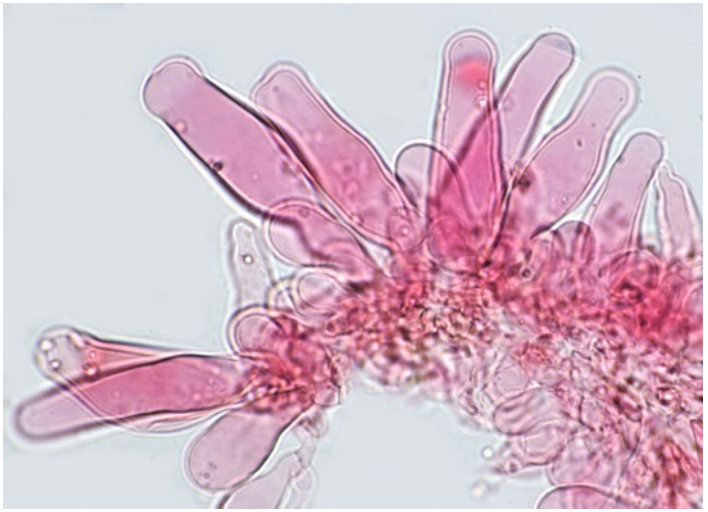
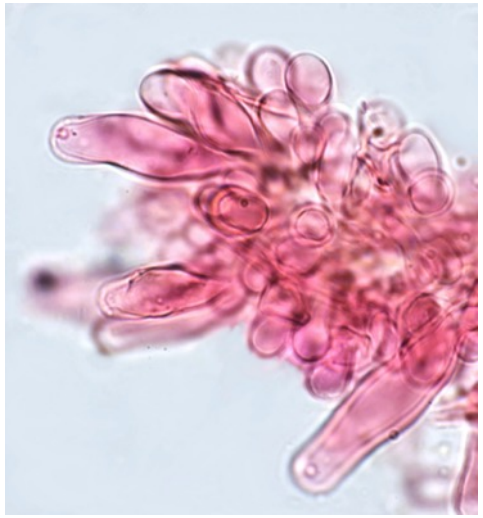
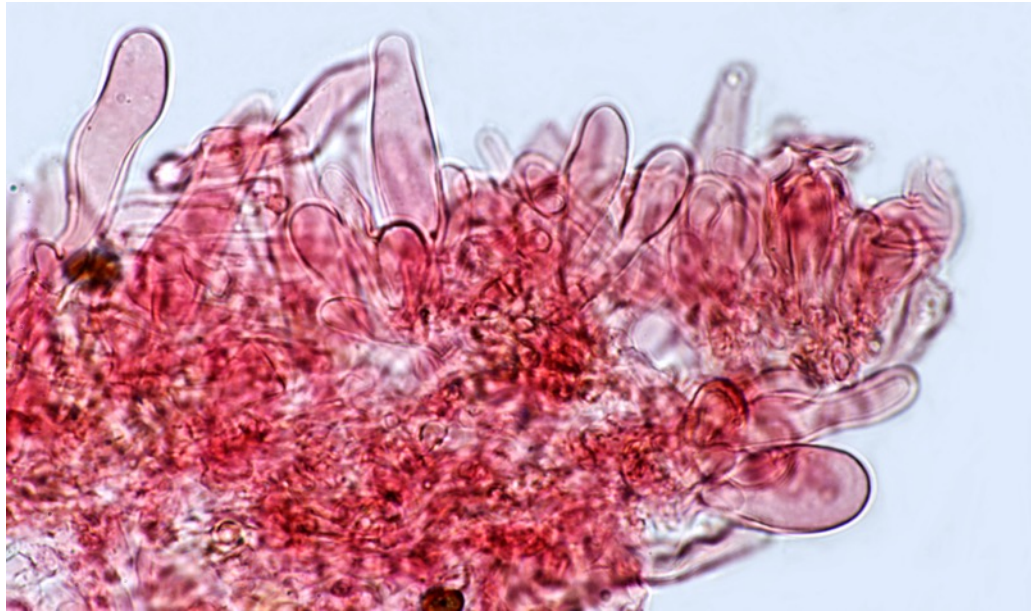


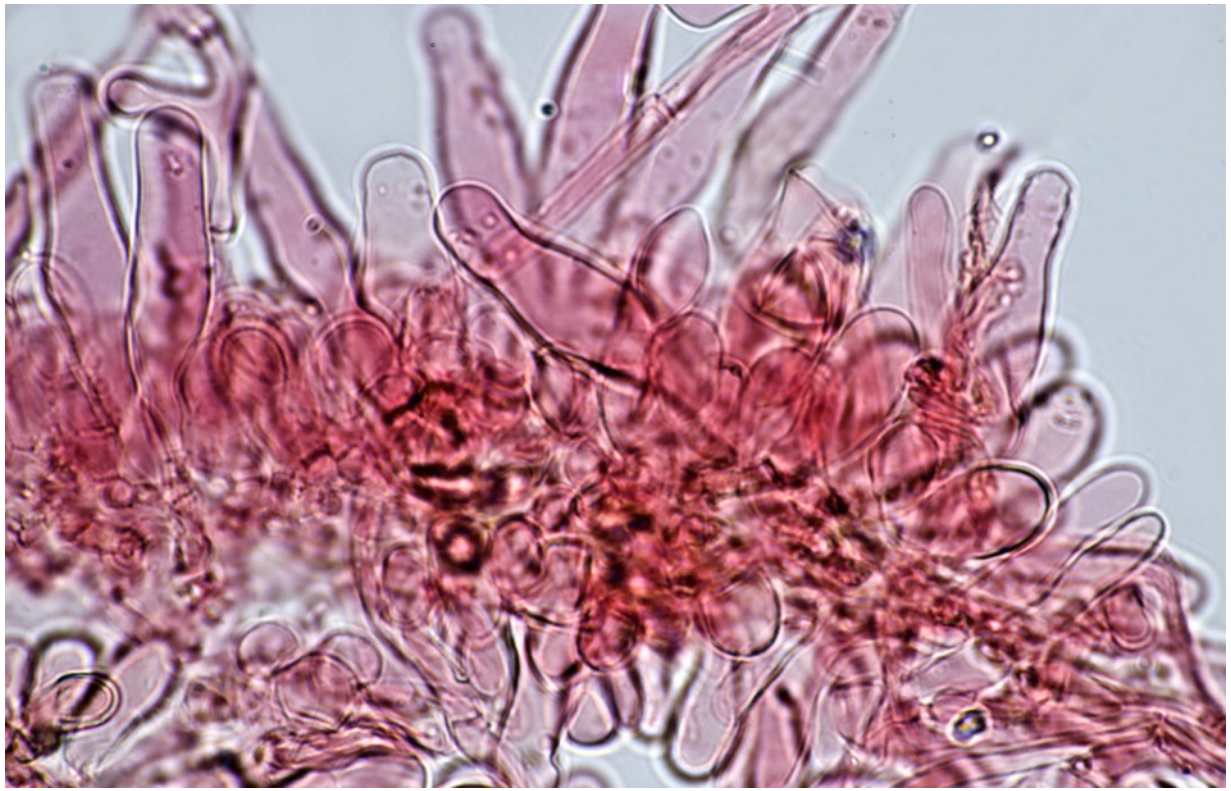
Cheilocystides à paroi fine (rarement légèrement épaissie) essentiellement cylindro-lagéniformes, à sommet largement obtus, souvent tronqué, parfois fourchu, mêlées à de nombreuses paracystides.

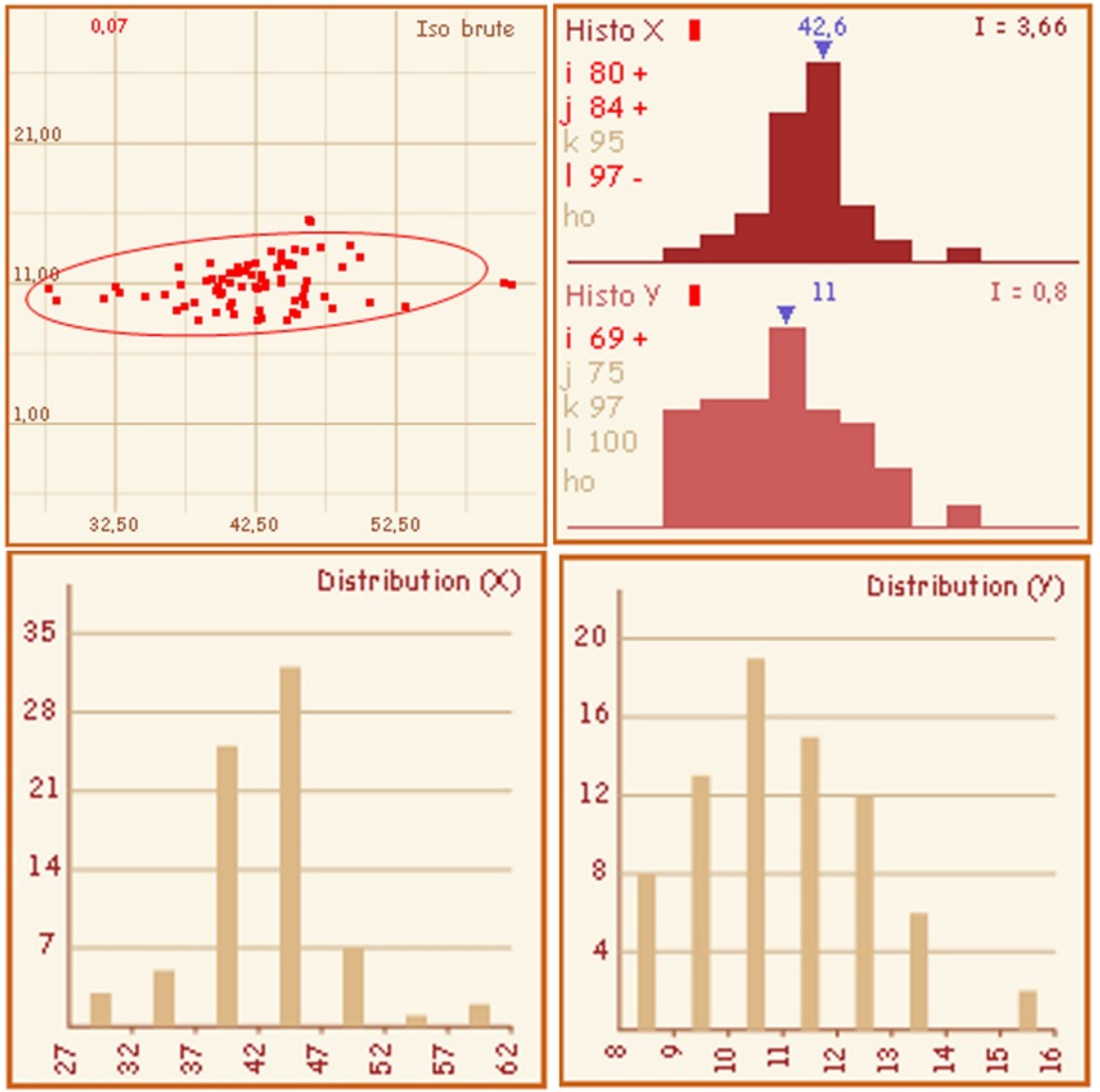








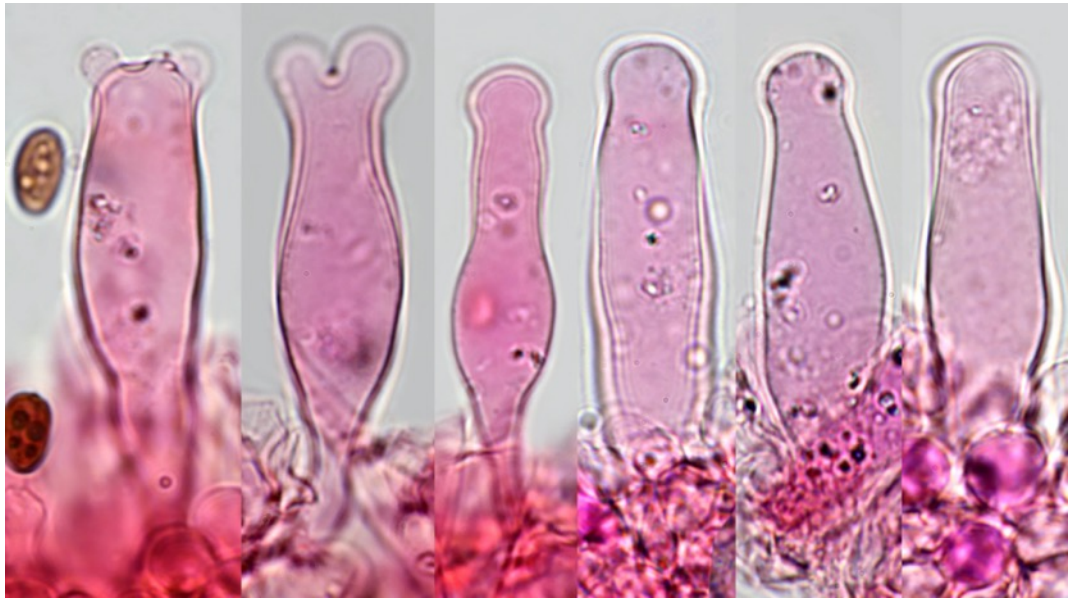


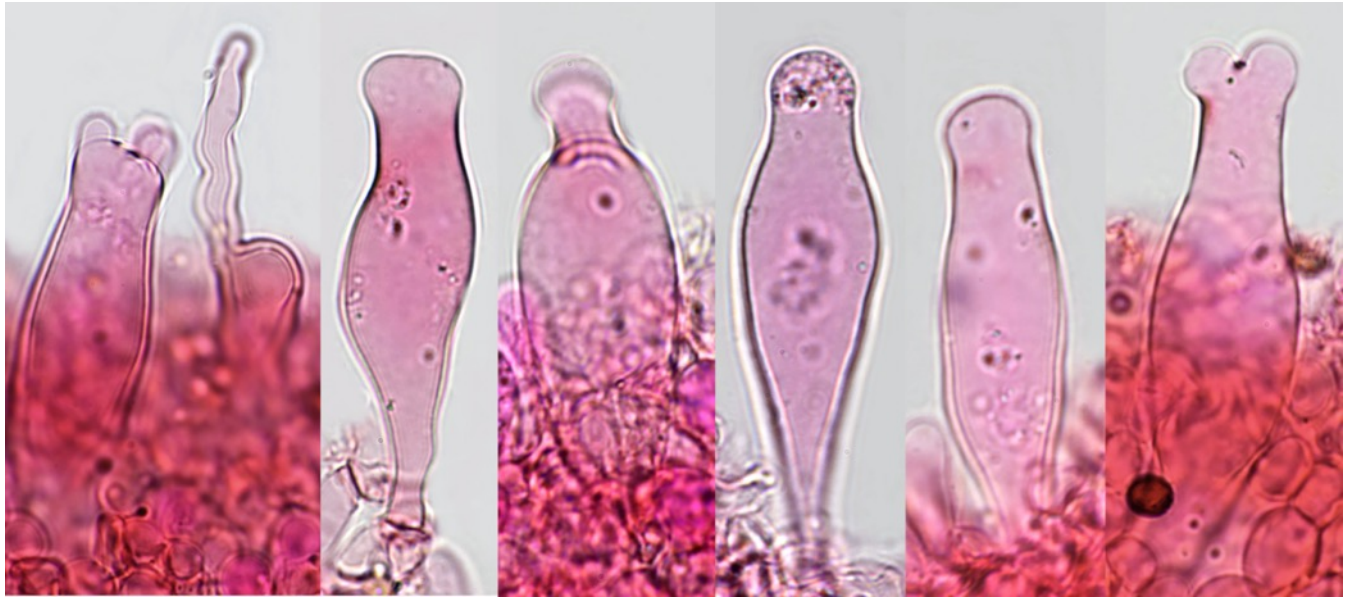


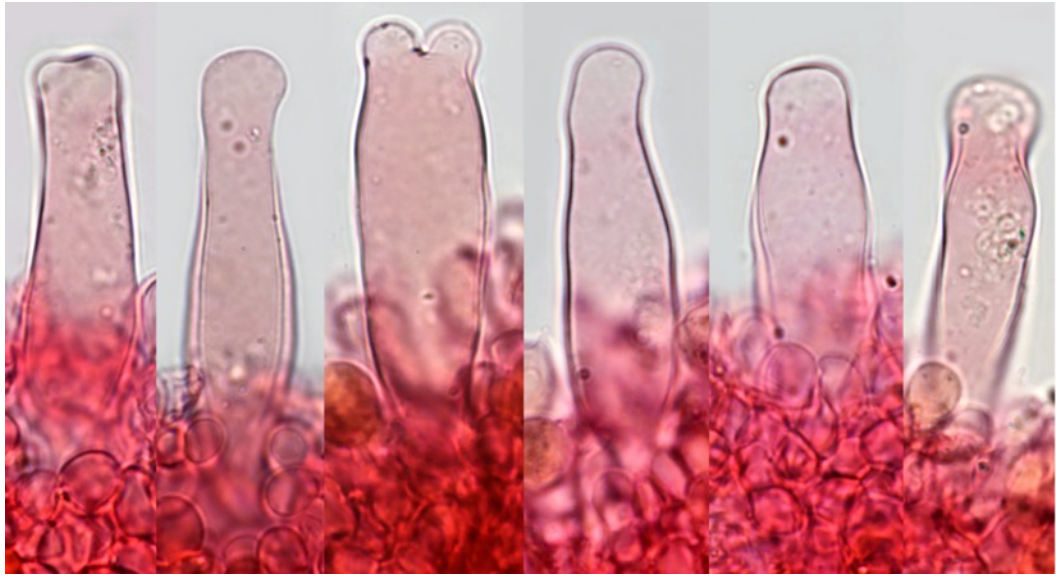
Mesures des cheilocystides avec Piximètre : (N=75)

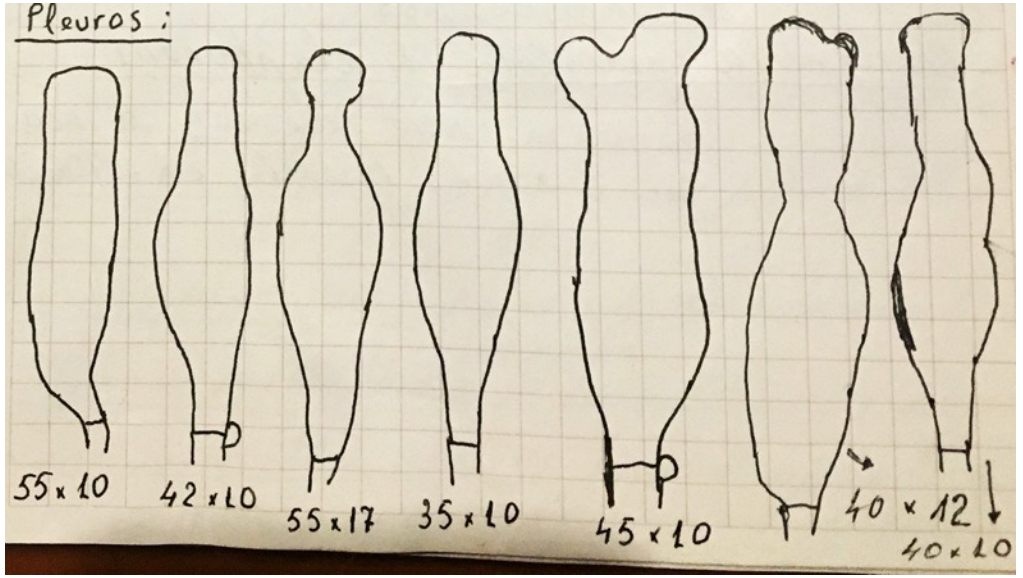
(27,7)37-47,4(60,7) × (8,3)9,1-12,9(15,5) µm
Me = 42,6 × 11 µm

Pleurocystides nombreuses, polymorphes, hyalines à paroi fine, et essentiellement lagéniformes sur l'exemplaire jeune, dont le sommet sur les exemplaires matures (adultes), est souvent tronqué ou fourchu, et dont la paroi est parfois modérément épaissie.





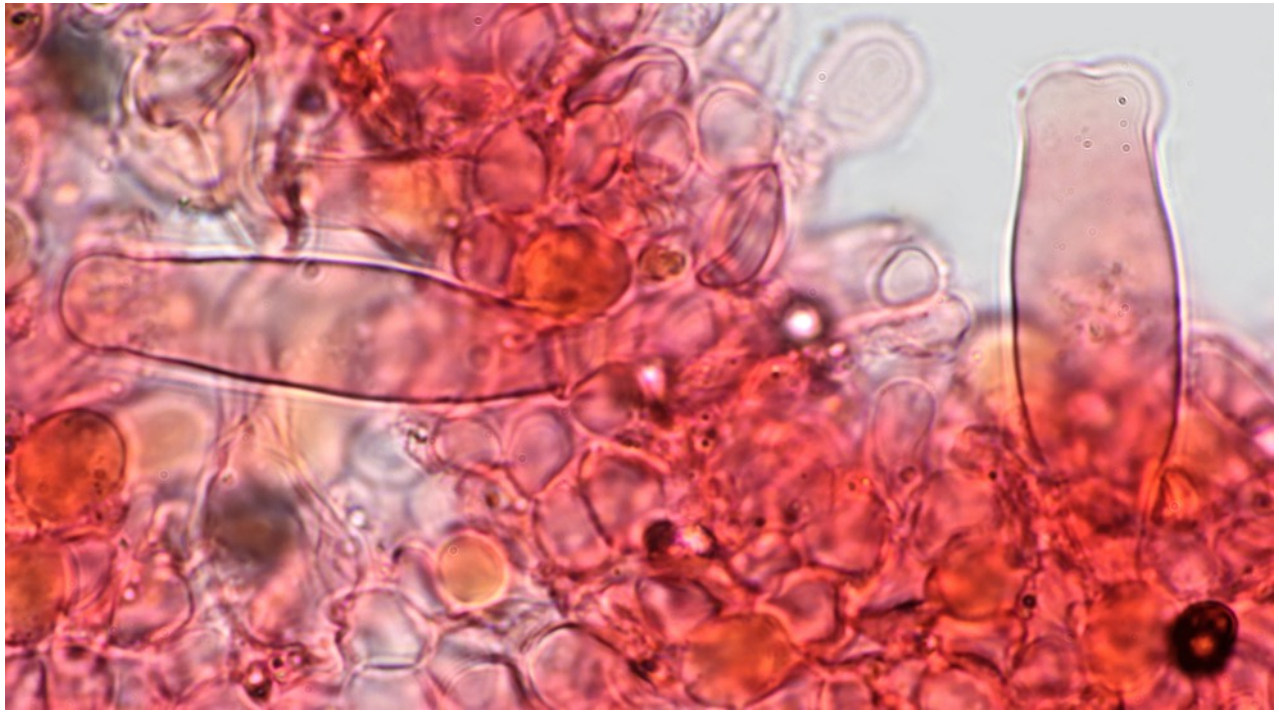


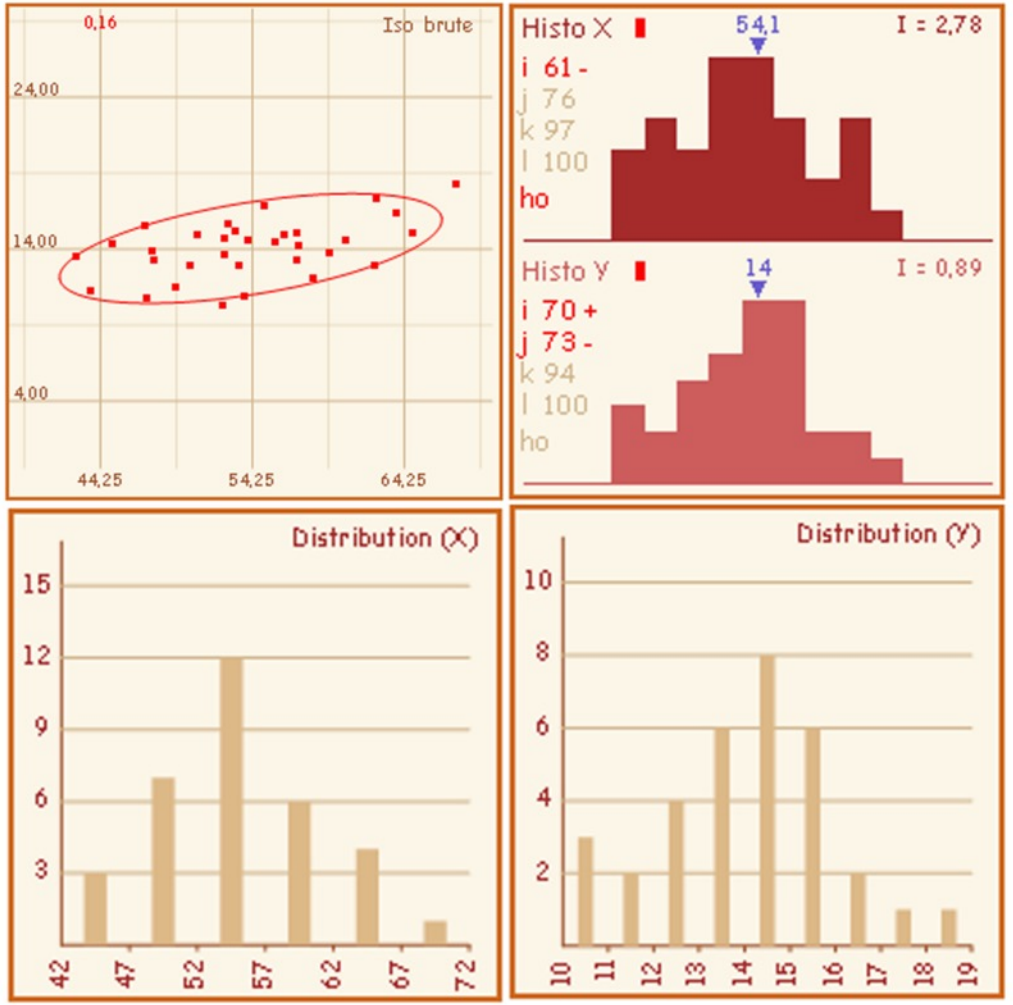


Mesure des pleurocystides (mix des 3 exsiccata) : (N = 33)

(42,6)47,2-62,4(67,6) × (10,3)11,2-16,4(18,3) µm

Me = 54,1 × 14 µm





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 for AM1656, AM1816/LZP-7615, AM1849, AM1913, DD0222, DD0223, DD2111, DD2112, DD2113, DD2115, DD2116, DD2117, DD2602, DD3019, DD8849, DD-NIV01, DSD9141 and JC20180609.5 was performed with the ITS4 [1] primer and for DD2602 additionally with the ITS1F [2] primer. For DD2601 only the ITS1F [2] primer was used. For the LSU region for JC20180609.5 the LR5 [3] primer was used and AM1816/LZP-7615, DD-NIV01 and DSD9141 the LR0R [4] primer. For the ef-1α region for DD2111, DD2112, DD2113, DD2115, DD2116, DD2117 and JC20180609.5 the EF1-1567R [5] primer was used. Sequences of AM1816/LZP-7615, AM1849 and AM1656 were edited by Alvalab (Oviedo, Spain). The other 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 sobsection Spadiceogriseae by divergence matrix and corrected if 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 [7] and Unite [8] (essential ones of the /saponacea s.l. clade see Table 1). Region boundaries for the ITS- and LSU-region were carried out with ITSx [9] and HMMER [10] including the databases. As outgroup, the sequence sets of the most closely related clade of the ingroup were used, i.e. the /jacobssonii s.l and the /microrhiza s.l. clades. 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 [11] 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 [12] using the SIC = "Simple Indel coding" [13] method. After each alignment step, an ML analysis with RAxML [14] (model: GTRCAT, refining under GTR+G for DNA, GTR2+G with acquisition bias correction according to Lewis [15] 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 [16], 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 [17]. For the final partitioning, the guide tree of the last iteration step was used. As information criterion the Bayesian Information Criterion (BIC) [18] used was after comparison with the Corrected Akaike Information Criterion (AICc) [19] 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 [14]. For all DNA partitions, the GTR substitution matrix [20] under the CAT model [14] was used. The final optimization took place under gamma distribution [14]. For the binary partitions, the "Two State Time-Reversible Model" with acquisition bias correction [15] 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%, except at the critical nodes, shown in red ML supprot values on the branches. The phylogram in Fig. 1 was edited with Treegraph [21]. The Outgroup has been collapsed for a better view.

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[8] **Unite:** Kõljalg U, Nilsson RH, Abarenkov K, Tedersoo L, Taylor AFS, Bahram M, Bates ST, Bruns TD, Bengtsson-Palme J, Callaghan TM, Douglas B, Drenkhan T, Eberhardt U, Dueñas M, Grebenc T, Griffith GW, Hartmann M, Kirk PM, Kohout P, Larsson E, Lindahl BD, Lücking R, Martín MP, Matheny PB, Nguyen NH, Niskanen T, Oja J, Peay KG, Peintner U, Peterson M, Põldmaa K, Saag L, Saar I, Schüßler A, Scott JA, Senés C, Smith ME, Suija A, Taylor DL, Telleria MT, Weiß M, Larsson K-H. 2013. Towards a unified paradigm for sequence-based identification of Fungi. Molecular Ecology, DOI: 10.1111/mec.12481

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

[11] **Mafft 7.372** (used over mafft.cbrc.jp)

- Nakamura, Yamada, Tomii, Katoh 2018 (Bioinformatics 34:2490–2492) - Parallelization of MAFFT for large-scale multiple sequence alignments.
- Katoh, Rozewicki, Yamada 2017 (Briefings in Bioinformatics, in press) - MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization.
- Yamada, Tomii, Katoh 2016 (Bioinformatics 32:3246-3251) additional information - Application of the MAFFT sequence alignment program to large data-reexamination of the usefulness of chained guide trees.
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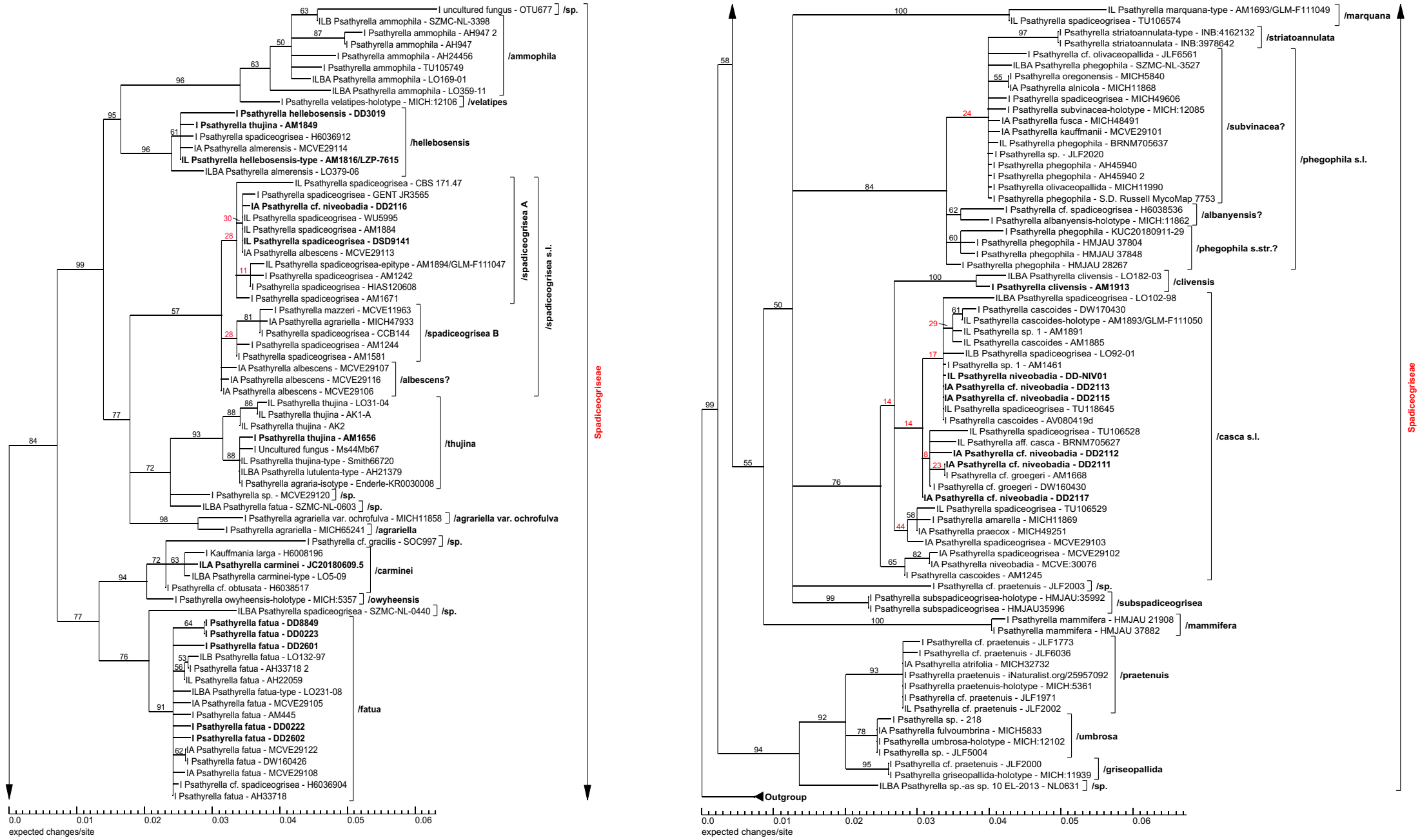

Fig. 1 50% collapsed maximum likelihood consensus phylogram. Red support values mean: no collapsing was done. The values on the branches are ML bootstrap values. Abbreviations: I: ITS region, L: LSU region, B: β-tubulin region, A: ef-1α region. **Psathyrella carminei - JC20180609.5** in the phylogram is our species described here as Psathyrella owyheensis.

Discussion

L'aspect de l'arête occupée par d'abondantes paracystides, en particulier sur les sujets jeunes, et les dimensions des spores orientent sans équivoque vers une espèce de la mouvance *« spadiceogrisea ».*
Il nous est apparu intéressant au travers de nos observations de préciser la variabilité infragénérique de cette espèce dont les pleurocystides en particulier sont nettement plus polymorphes que celles observées par Örstadius et indiquées dans sa description de *Psathyrella carminei.*
D'autre part, l'aspect macroscopique de notre récolte ainsi que ses caractères micro-morphologiques dont, en particulier l'aspect et les dimensions des spores, les pleurocystides à sommet souvent tronqué, parfois fourchu ou subcapité ainsi que l'écologie en prairie pâturée par des bovins, sont en parfaite concordance avec les observations Smith et de Voto *(op. cit).* Ces caractéristiques viennent conforter la synonymie établie par Voto *(op. cit.)* sur base biomoléculaire et micro-morphologique entre *Psathyrella owyheensis et Psathyrella carminei.*
Il sera intéressant de préciser dans de futures récoltes l'aspect macroscopique de l'arête, puisque sur base des observations microscopiques, il est probable qu'elle puisse être surlignée de rouge-brun dans certaines récoltes.
L'odeur de nos spécimens n'a pu être appréciée in situ, et nous espérons pouvoir la préciser lors de prochaines récoltes, l'holotype étant décrit comme ayant une odeur de poisson, un caractère probablement inconstant.

The edge appearance with, especially in young specimens, abundant paracystidia, and the spores dimensions indicate an clear trend for a species of the "spadiceogrisea" group.
It seemed interesting to us through our observations, to specify the infrageneric variability of this species, whose pleurocystidia in particular are clearly more polymorphic, than those observed by Örstadius and indicated in his description of *Psathyrella carminei.*
On the other hand, the macroscopic aspect of our collection as well as its micro-morphological characteristics, and in particular the appearance and dimensions of the spores, the pleurocystidia with often truncated, sometimes forked or subcapitate apex, and the ecology in grassland grazed by cattle, are in perfect agreement with the observations of Smith and Voto *(op. cit).*
These characteristics support the synonymy established by Voto *(op. cit.)* on a biomolecular and micro-morphological basis between *Psathyrella owyheensis* and *Psathyrella carminei*.
It will be interesting to specify in future observations the macroscopic aspect of the edge, since on the basis of microscopic observations, it is likely that it can be red-brown underlined in some specimens.
The odour of our samples could not be appreciated in situ, and we hope to be able to specify it in the future, as the holotype is described as having a fishy odour, a character that is probably not constant.

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Littérature :

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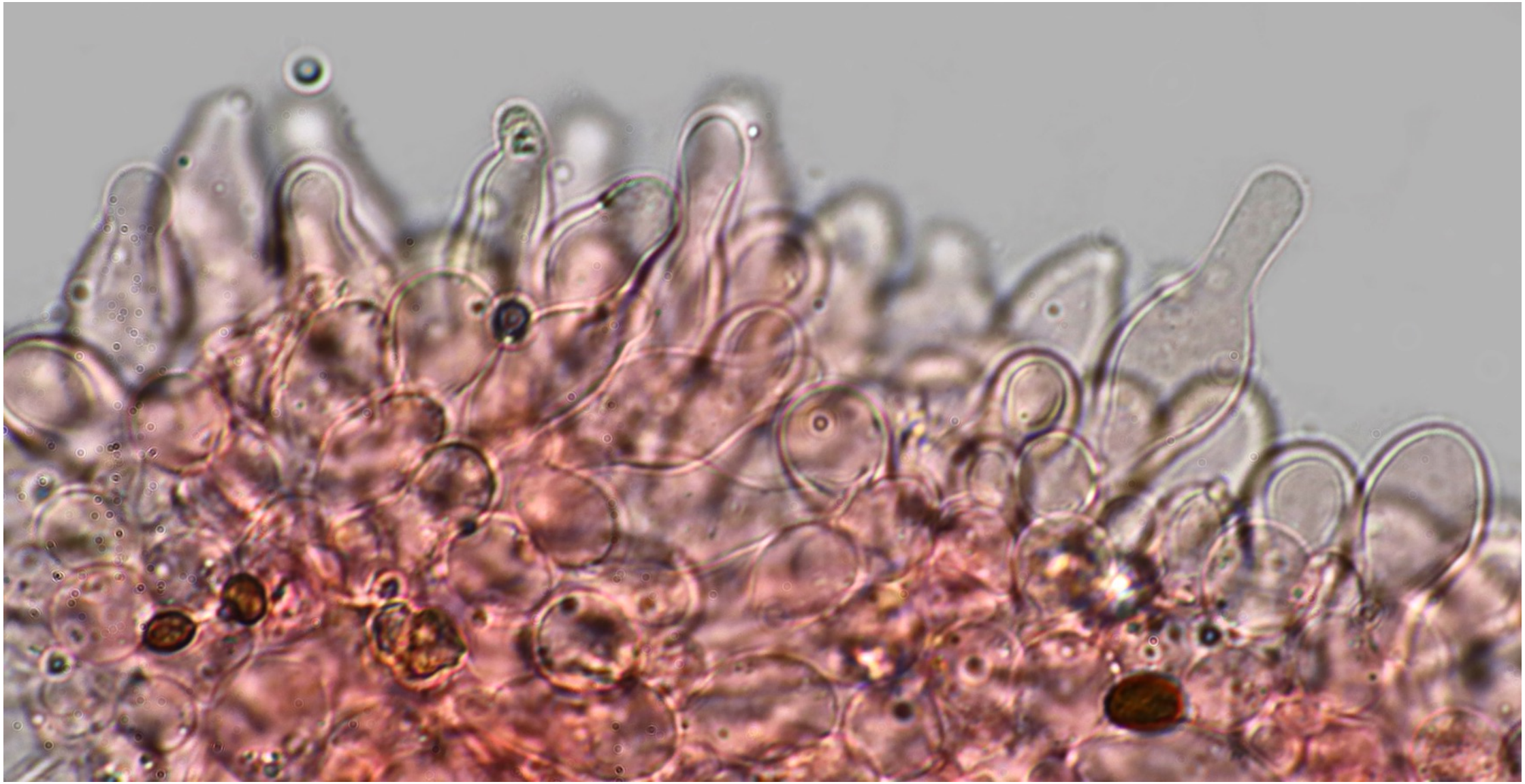
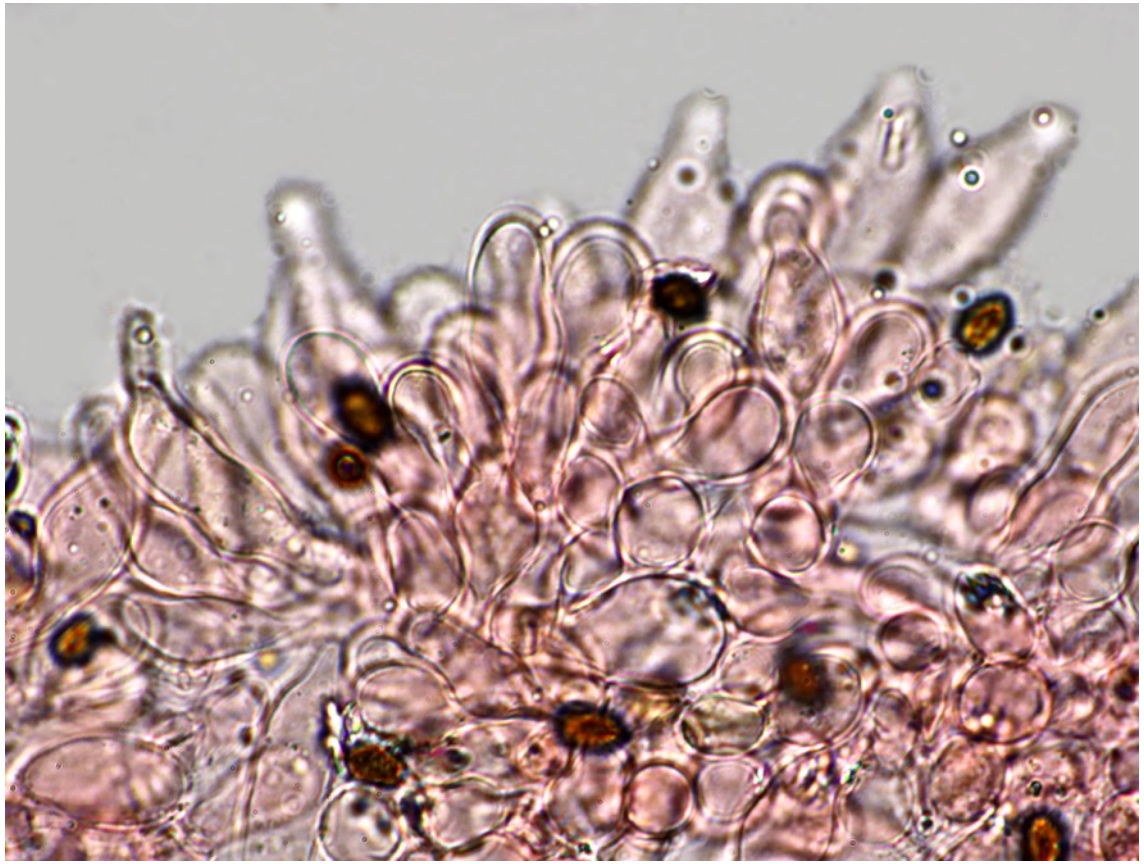
Récolte de Fouad Ouchène 2021

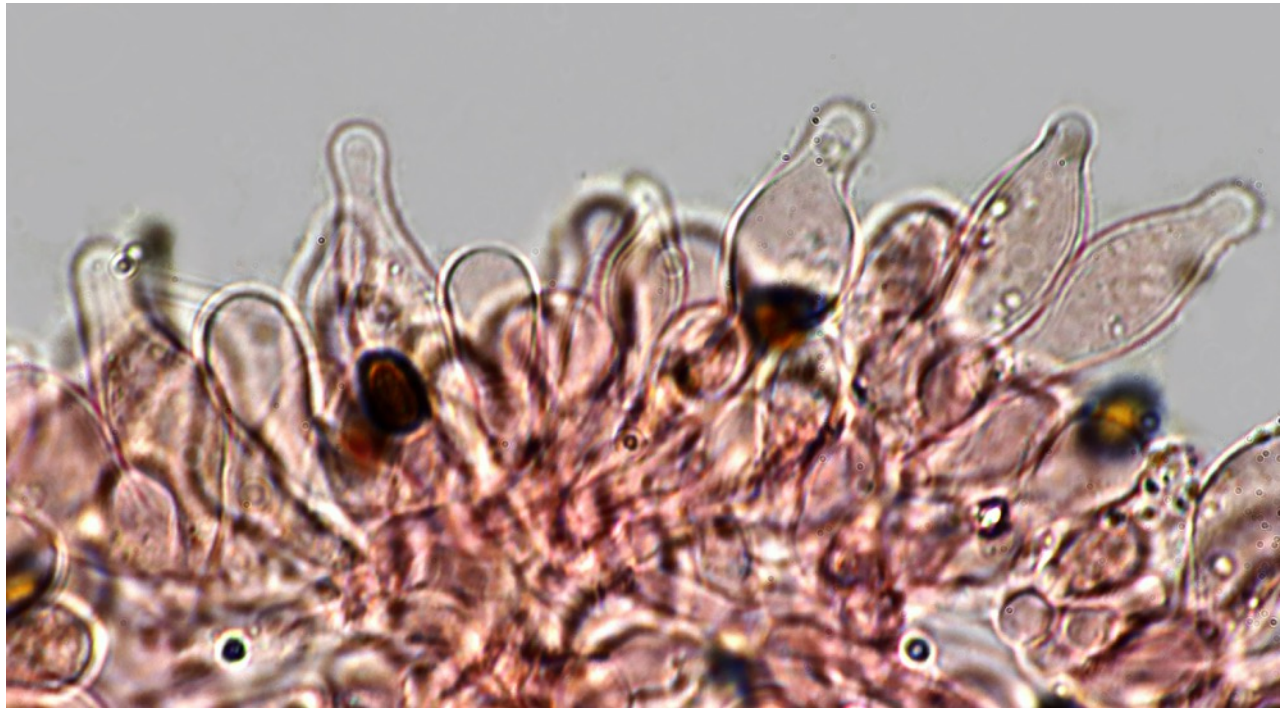
Dans l'herbe sous Platanus, Prunus, Salix et Pinus à 10-15 m. Strasbourg (bords du canal de la Bruche), 16 mai 2021.
Diameter 2 cm
Distinct fishy smell.

Gill edge covered with clavate, thin-walled hyaline paracystidia mixed with some thin-walled hyaline, lageniform and utriform cheilocystidia similar to pleurocystidia.



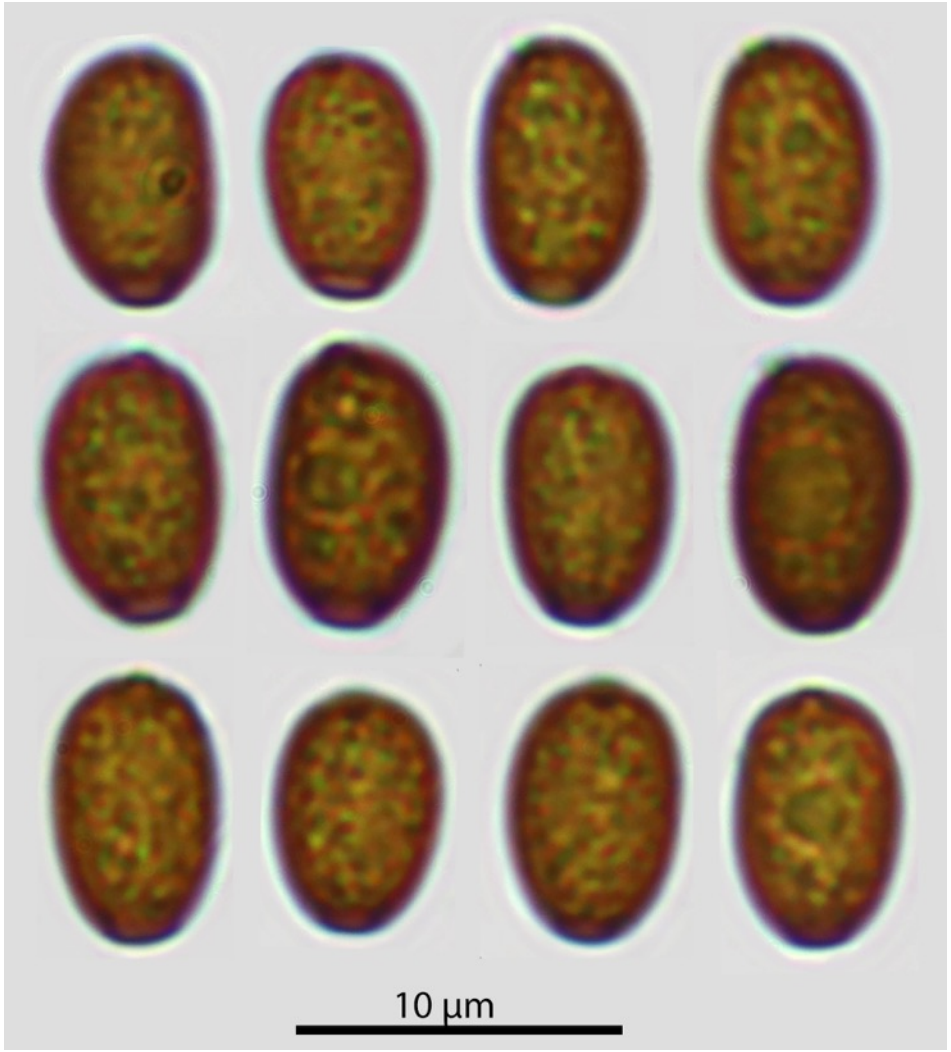
Gill edge





Basisia : 4-spored – Clamps : present

Spores : smooth, orange-brown, not opaque, ellipsoïd and ovoid, base sometimes truncated in face view, adaxially flattened or slightly amygdaliform in profile, germ pore like a callus, distinct, central.



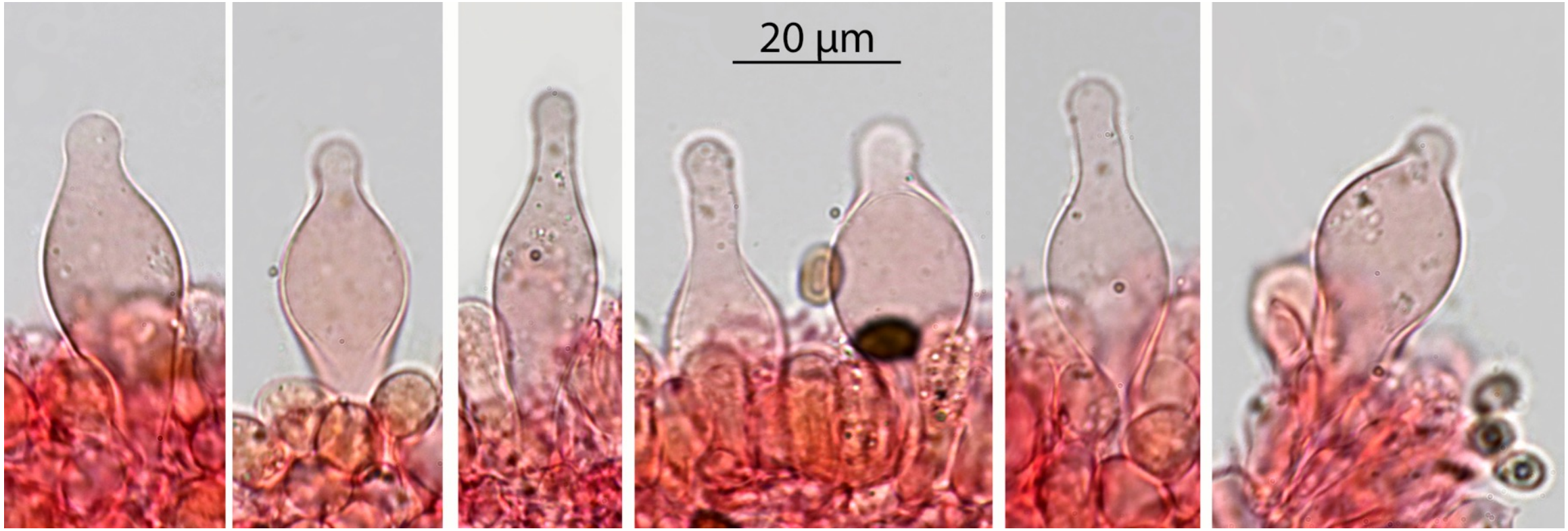
N = 32

(7,3) 7,5 - 8,5 (8,6) × (4,7) 4,8 - 5,4 (5,5) µm

Me = 8 × 5,1 µm ;

Q = (1,4) 1,5 - 1,7 ; Qe = 1,6

Pleurocysidia very numerous, hyaline, thin-walled, mainly utriform but also lageniform, with short, wide, sometimes mucronate neck and obtuse top.



(34,8) 35,6 - 43,8 (45,4) × (12,5) 13,5 - 16,9 (17,5) µm ; Me = 39,7 × 15,3 µm

Pleurocystidia