Découverte et description de *Psathyrella cladii-marisci* (Sicoli, NG Passal, De Giuseppe, Palermo & Pellegrino) dans la réserve naturelle de Torfbroek

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Introduction

Cette rare et nouvelle espèce a été décrite pour la première fois en mai 2019, par Sicoli & all lors de l'investigation de la mycoflore du Jardin Botanique de l'université de Calabre, Cosenza, Italy. Elle se développait autour et à la base d'un plant de *Cladium mariscus* taillé (d'où son nom), aux alentour du 10 Avril 2018.
Ce plant, avec la vase adhérente à ses racines, provenait du marais de Lago dell'Aquila (Laureana di Borrello, Calabria, southern Italy)

En septembre 2018, le premier auteur avait réalisé plusieurs récoltes de nombreux exemplaires de cette espèce, sans pouvoir la déterminer, dans la réserve naturelle de Torfbroek, une rare tourbière alcaline calcaire. Cette récolte a été séquencée et est presque identique à celle Sicoli ne différant que par une position, une base T présente dans l'holotype de Sicoli, absente dans ma récolte.

L'espèce se développait au sol, dans la tourbe, à la base de plantes que je ne pouvais identifier, car cette partie de la roselière avait été récemment fauchée. Une nouvelle croissance, a permis d'établir que cette espèce se développait très probablement à la base de *Cladium mariscus,* qui était présent en abondance à cet endroit, en compagnie entre autre de *Schoenus nigricans.*
Après la fauche, il est impossible d'établir de manière absolue que l'espèce soit spécifiquement liée à *Cladium mariscus ;* ceci est cependant fort probable, compte tenu du fait que toutes nos récoltes ont été réalisées uniquement dans la partie de la roselière où cette plante était présente.

Compte tenu de l'écologie très particulière, *Psathyrella typhae,* et en particulier *Psathyrella halophila* et *Psathyrella sulcatotuberculosa* étaient les hypothèses que nous avions envisagées, sans pouvoir aboutir à une détermination correcte, le séquençage ADN (ITS) ne permettant pas dans une première analyse, de séparer cette espèce de *Psathyrella candolleana,* une espèce multiple, dont l'écologie et l'aspect macroscopique est bien différent.

Une publication précisant les caractères morphologiques, écologiques et génétiques de cette espèce que nous ne parvenions pas à déterminer était en cours et est reprise ci-après.
Elle se veut être un complément largement illustré de la publication originale des auteurs : A new species of *psathyrella (Psathyrellaceae, Agaricales*) from Italy, Mycokeys 2019 ; 52: 89-102 ainsi que des corrections qu'ils souhaitaient apporter à leur publication originale. Ces dernières ont déjà été publiés dans une court correctif dans la même revue : micokeys.pensoft.net>article>38856.

This rare and new species was first described in May 2019 by Sicoli & all during the investigation of the mycoflora of the Botanical Garden of the University of Calabria, Cosenza, Italy. It developed all around and at the base of C*ladium mariscus* (hence its name) cut culms around the 10th of April 2018. This plant had been removed , together with the whole clump of mud attached to is roots from a natural marsh named Lago dell'Aquila (Laureana di Borrello, Calabria, southern Italy).

In September 2018, the first author had made several harvests of many specimens of this species without successfully identifying it, in the Torfbroek Natural Reserve, a rare alkaline calcareous marsh. This collection has been sequenced and is almost identical to that of Sicoli differing only by one position, a T base present in the sequence the holotype, absent in my specimen.

The species was growing on the ground, in the moss and at the base of a plant that he could not identify because this part of the reed bed had recently been mowed down. A new growth, made it possible to establish that this species most probably developed at the base of *Cladium mariscus* which was present in abundance at this place in the company of *Schoenus nigricans* among others. After mowing, it is impossible to establish in an unambiguous way that the species is specifically associated with *Cladium mariscus*, but this is very likely given that all our harvestings were made only in the part of the reed bed where this plant was present.
Considering the very specific ecology, *Psathyrella thujina* (formerly *P. almerensis*), *Psathyrella typhae, and in particular, Psathyrella halophila* and *Psathyrella sulcatotuberculosa* were the hypothesis we had considered without being able to make a correct determination, DNA sequencing (ITS and TEF alpha) not allowing in a first analysis to separate this species from *Psathyrella candolleana,* a multiple species whose ecology and morphology is very different.
A publication specifying the morphological, ecological and genetic characteristics of this species, which had not yet been determined, was in progress and is presented below. It is intended to be a richly illustrated complement to the authors' original publication: A new species of psathyrella (Psathyrellaceae, Agaricales) from Italy, Mycokeys 2019; 52: 89-102 as well as the corrections they wish to make to their original publication. These last ones were already published in a short corrigendum published in the same journal micokeys.pensoft.net>article>38856.

Cladium mariscus

Toutes les photos in situ ont été réalisées par D. Deschuyteneer, en septembre 2018, dans la réserve naturelle de Torfbroek, à la base ou sur les racines enfouies de *Cladium mariscus.* Ce marais alcalin abrite de nombreuses espèces rares comme *Parnassia palustris, Gymnadenia conopsea* (la grande orchidée moustique), *Carex lepidocarpa*, *Epipactis palustris*, et bien d'autres.

All the in situ photos were taken by D. Deschuyteneer - September 2018 in the Torfbroek Natural Reserve at the base or on the buried roots of *Cladium mariscus.* This alkaline marsh is host to many rare species such as *Parnassia palustris, Gymnadenia conopsea* (the great mosquito orchid), *Carex lepidocarpa*, *Epipactis palustris*, and many others.

Description macroscopique

Chapeau mesurant de 20 à 40 mm de diamètre, initialement campanulé ou conico-convexe, devenant rapidement plan convexe, avec souvent un large umbon obtus. Les jeunes exemplaires sont d'un beau brun noisette. Hygrophane, il devient assez rapidement beige-grisâtre en décolorant jusqu'à mi-rayon à partir de la marge, celle-ci ayant tendance a nettement s'éverser et se fissurer, étant donné les fortes chaleurs enregistrées en cette saison.
Lames larges de 2-3 mm, serrées, droites ou à peine subventrues, alternant avec des lamelles et lamellules, largement adnées, pâles au début avec un léger reflet rosâtre devenant rouille grisâtre ; arête fimbriée blanche.
Voile fibrilleux, blanchâtre, abondant sur les primordia, dont il relie la marge au stipe. Au cours de la croissance, il persiste sous forme de fibrilles éparses, disséminées sur le chapeau, et reste appendiculé au niveau de la marge, formant une fine guirlande de lambeaux triangulaires. Finalement il se colore de brunâtre sous l'effet de la sporée.
Stipe mesurant 15-35 x 2-3 mm, blanchâtre, court, creux, cylindrique, pruineux au sommet, fibrilleux ou glabre dans sa moitié inférieure, dont la base légèrement dilatée est non radicante.

Macroscopic description

Cap measuring 20-40 mm, initially campanulate, becoming quickly convex with often a large obtuse umbon. Beautiful hazelnut brown becoming beige-greyish from the margin which clearly tends to flip, and to fissurate due to the high heat recorded this season. .
Gills 2 - 3 mm broad, tight, largely adnate, straight or very only slightly ventricose, at first pale with a slight pinkish hue becoming rusty-greyish, white fimbriate edge.
Veil whitish fibrillous, abundant on the primordia connecting the margin to the stipe. During growth it persists as scattered fibrils on the cap, and remains appendiculate at the margin as triangular flaps and finally turns brownish.
Stipe measuring 30-35 x 2-3 mm, short, cylindrical, pruinous at the top, with a slightly dilated, not-rooting base.

A noter la tonalité rose des lames au stade précoce, et leur coloration rouille grisâtre à maturité, ce dernier aspect étant lié au caractère très pâle des spores.

Note the slightly pink tonality of the gills at the early stage and their greyish rust colouring at maturity, the latter aspect being linked to the very pale appearance of the spores.

Description microscopique

Basides clavées, tétrasporiques.

Spores à paroi un peu épaisse, lisses, très peu colorées, jaune pâle à beige jaunâtre dans l'ammoniaque à 10 %, légèrement grisâtre dans KOH à 5 % ; spores immatures presque hyalines, oblongues, ellipsoïdes à ovoïdes de face, asymétriques et légèrement phaséoliformes ou amygdaliformes de profil, parfois avec une légère dépression supra-hilaire ; pore germinatif central, discret, limité à un callus, conique. Rares spores de basides bisporiques non visualisées.

La sporée épaisse est de couleur brun grisâtre, avec une nette tonalité de rouille (une couleur inhabituelle pour ce genre).

Cheilocystides mesurant (22)30-60 x 10-15(20) µm, moyenne 41 x 13 (N=40) très denses, hyalines, à paroi fine, très polymorphes, essentiellement clavées, ven-trues, lagéno-ventrues, pas ou seulement légèrement pédicellées, rarement fourchues, souvent légèrement étranglées au sommet, mais également très souvent plus étroites et longuement cylindriques, à sommet largement obtus, parfois subcapité, les plus longues parfois septées et bouclées à ce niveau.

Les **cellules marginales « clavées et sphéropédonculées = paracystides** » sont peu fréquentes et présentent parfois une paroi épaissie.

Pleurocystides absentes. **Mediostrate** légèrement pigmentée. **Boucles** présentes.

Voile initialement constitué d'hyphes hyalines à extrémités dilatées, présentant de nombreuses boucles de connexion devenant brunâtres, et incrustées au cours de la croissance.

Pileipellis : un hyménoderme constitué d'une seule assise de cellules globuleuses et pyriformes. La trame piléique est constituée d' hyphes cylindriques peu pigmen-tées, non incrustées.

Caulocystides très longues, cylindro-lagéniformes, lagéno-ventrues, ou clavées, ressemblant à de long poils cylindriques, pouvant mesurer jusque 90-100 µm de long, parfois septés, et de temps en temps bouclés à ce niveau.

Microscopic description

Basidia: clavate, 4 spored

Spores slightly thick-walled, smooth, very slightly coloured, pale yellow to yellowish beige in NH4AOH 10%, slightly greyish in KOH 5%, immature spores almost translucent, oblong, ellipsoid to ovoid in face view, asymmetric and slightly phaseoliform or amygdaliform in side view, sometimes with a slight supra-hilair depression, very discreet central germ pore restricted to a callus, conical. Rare spores from unseen bisporic basidia.

The thick spore print is greyish brown in colour with a clear tonality of rust, a colour unusual for this genus.

Cheilocystidia measuring (22) 30 - 60 x 10-15 (20) µm, average 41 x 13 (N=40) densely packed, hyaline, with a thin wall, very polymorphic, essentially clavate, ventricose, lageno ventricose, not or only slightly pedicellate, rarely forked, often slightly constricted at the top, but also very often narrow and long cylindrical with wide obtuse top, sometimes subcapitate, the longest ones being sometimes septate.

Marginal cells "clavate and spheropedunculate = paracystidia" are not frequent and sometimes have a thickened wall.

Pleurocystidia absent. Gill trama slightly pigmented. **Clamp** connections present.

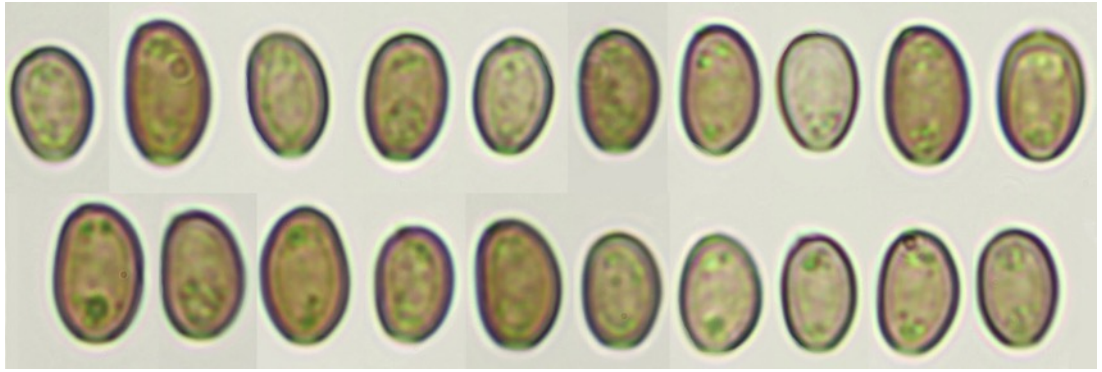
Veil initially composed of hyaline hyphae with dilated ends and lots of clamps, becoming brownish and encrusted during growth.

Pileipellis: a hymenoderm composed of a single layer of globular and pyriform cells. The pileitrama is made up of cylindrical hyphae slightly pigmented and not encrusted.

Caulocystidia numerous, cylindro-lageniform, lageno-ventricose or clavate, or looking like long cylindrical hairs up to 90-100 µm long, sometimes septate and from time to time showing clamps at this level.

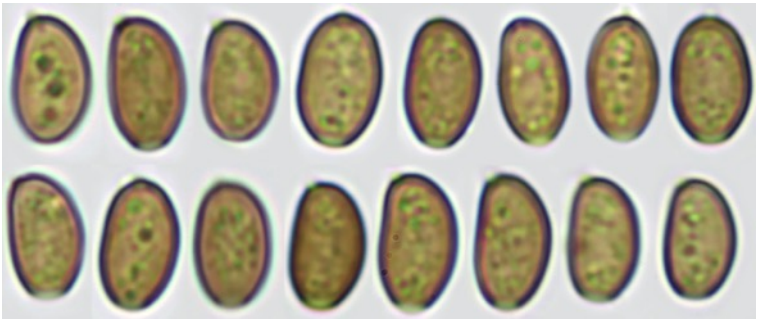
Spores mesurées avec piximètre
Mix de 5 sporées – (N= 150)
(5,4)6,2-7,5(8,3) × (3,8)4,1-4,9(5,6) µm
Me = 6,7 × 4,5 µm ;
Q = (1,2)1,4-1,6(1,7) Qe = 1,5

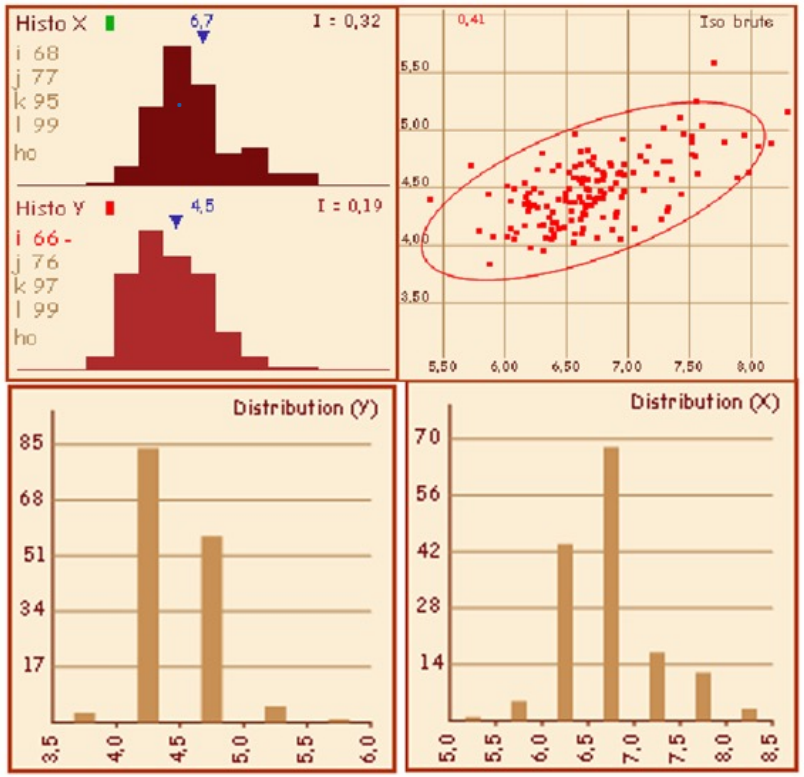
Spores de Torfbroeck très pâles dans NH_4OH à 10 %.
Very pale-coloured spores from Torfbroeck in NH_4OH 10 %.



Par comparaison les spores mesurées après examen de deux paratypes de Sicoli étaient un peu plus grandes :
In comparison, the spores measured on two investigated Sicoli paratypes were slightly bigger:
Paratype 1 : (N = 95)
(7,1)7,3-8,4(8,8) × (4,3)4,5-5(5,5) µm ; **Me = 7,9 × 4,8 µm**
Q = (1,5)1,6-1,75(1,8) ; Qe = 1,7
Paratype 2 : (N = 101)
(6,7)7,2-8,1(8,9) × (4,1)4,4-5(5,1) µm ; **Me = 7,7 × 4,7 µm**
Q = (1,4)1,5-1,7(1,9) ; Qe = 1,6

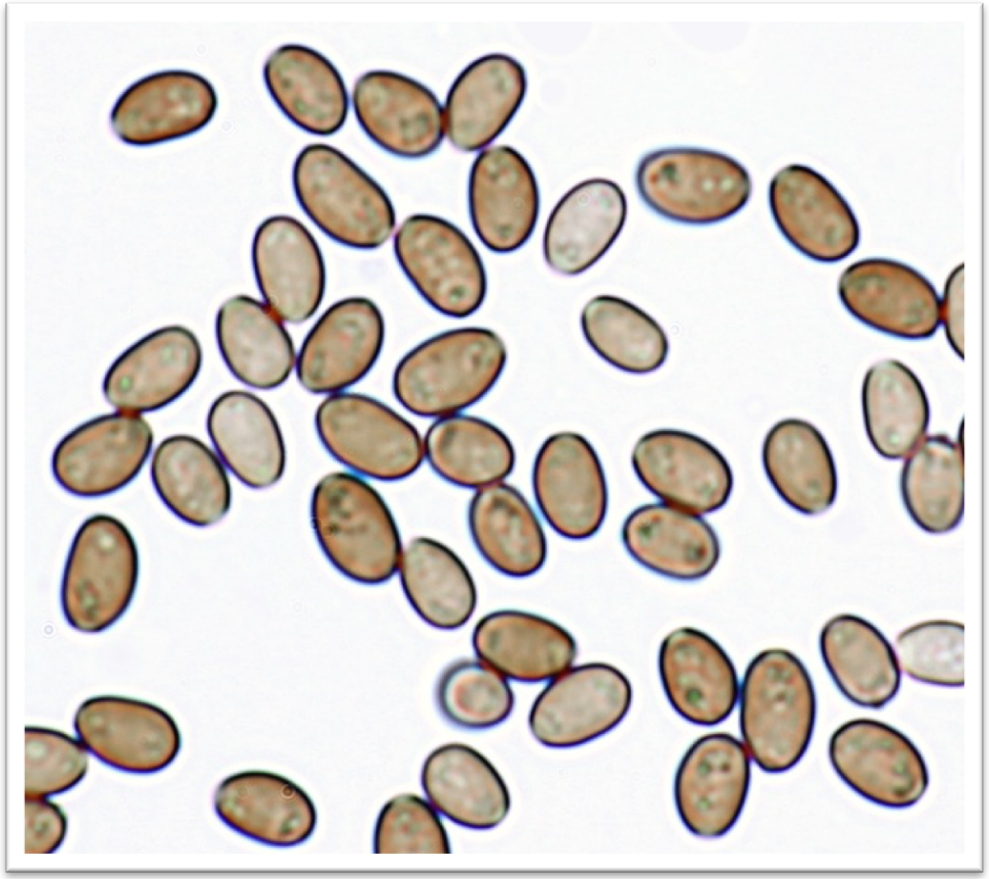
Spores from Sicoli paratype



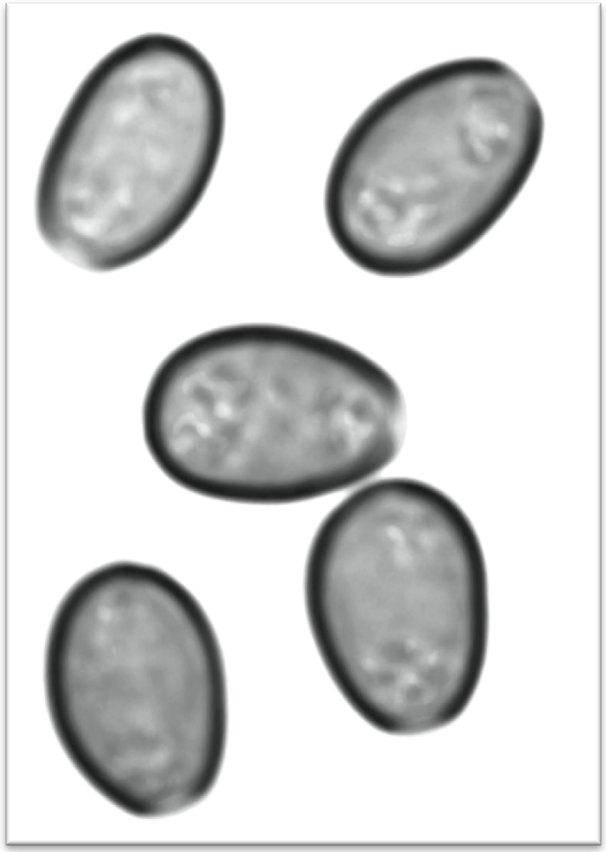


Spores immatures presque hyalines.

Immature spores almost hyaline.



slightly thick-walled spores



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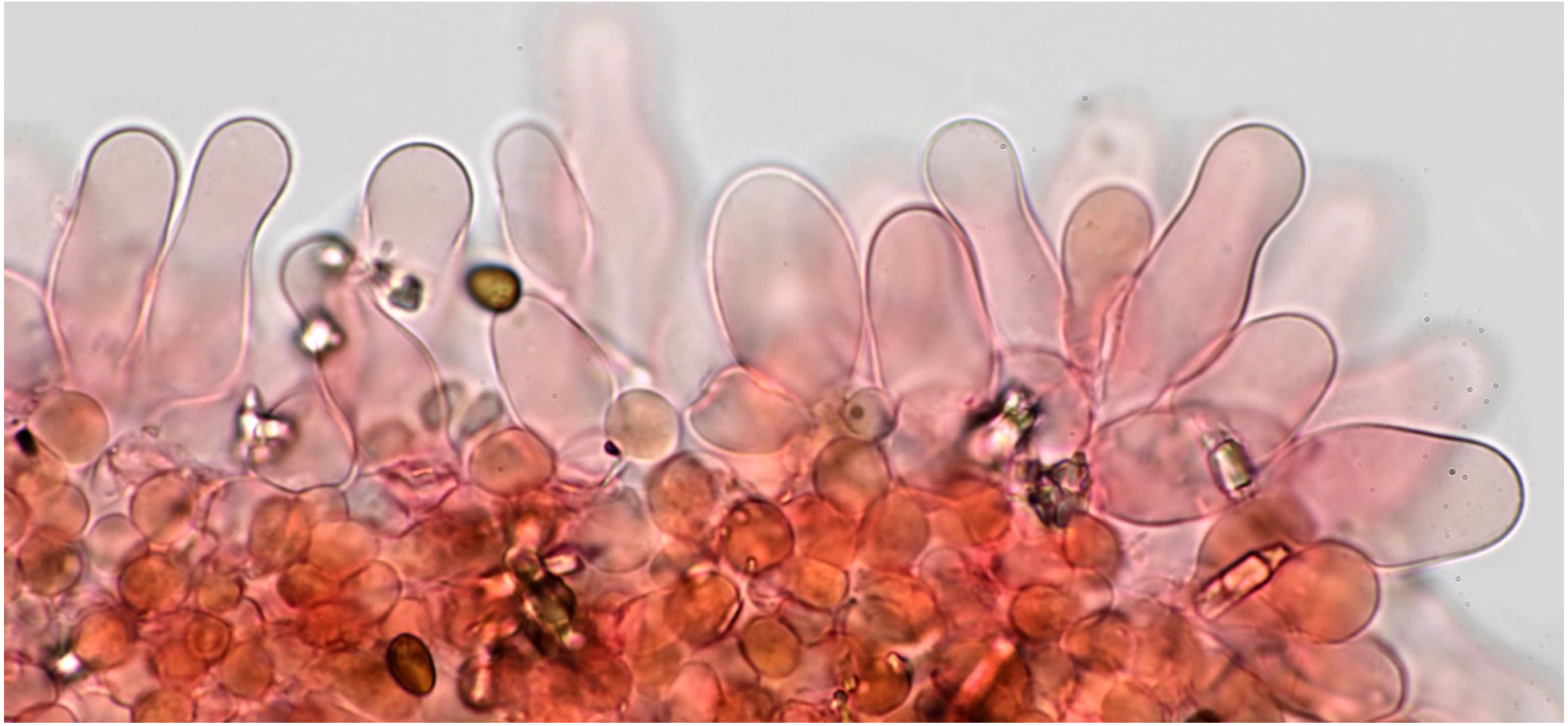
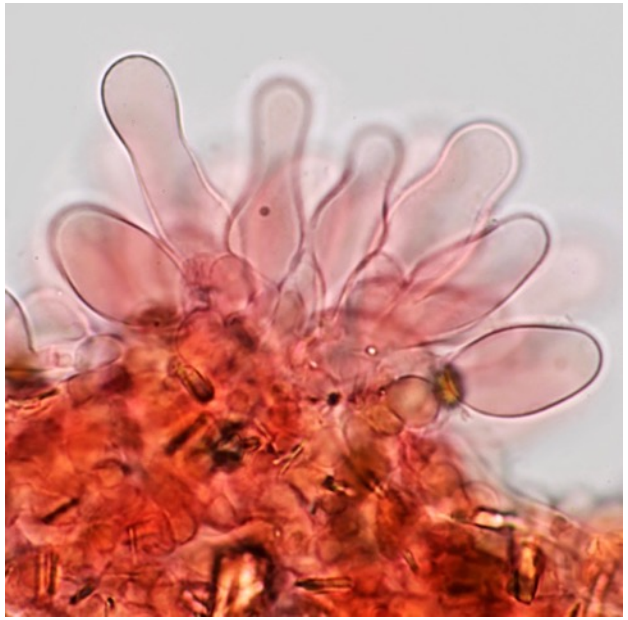
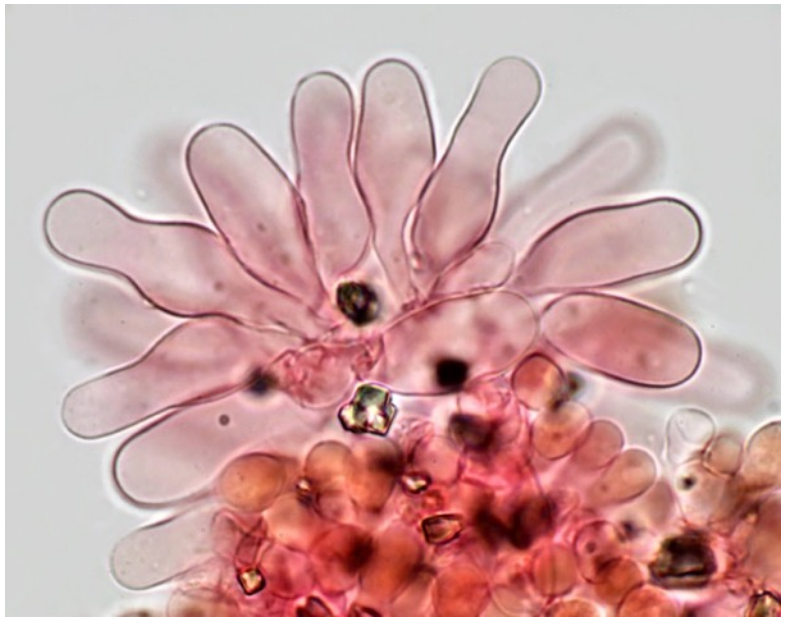
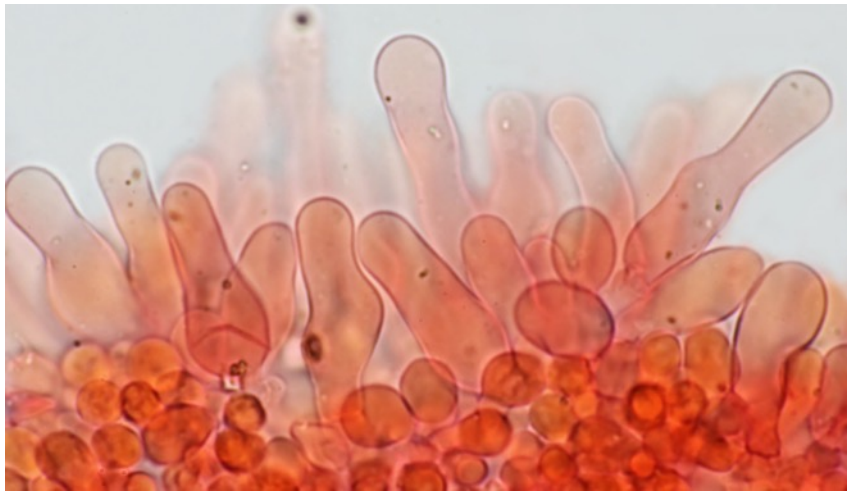
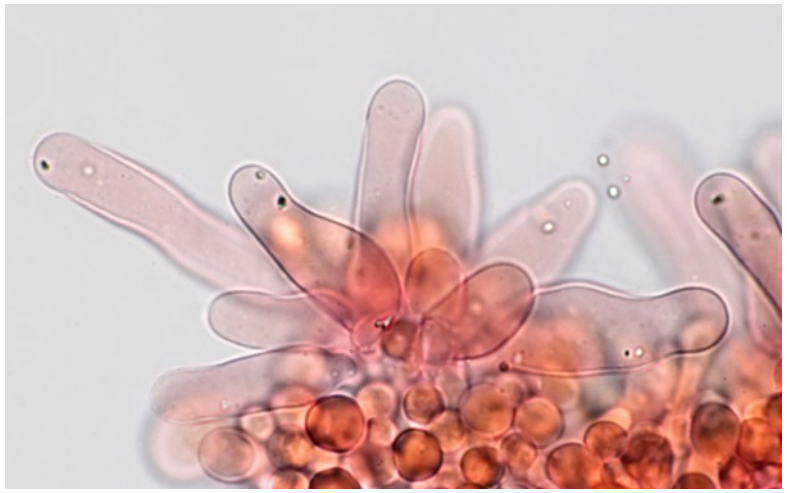
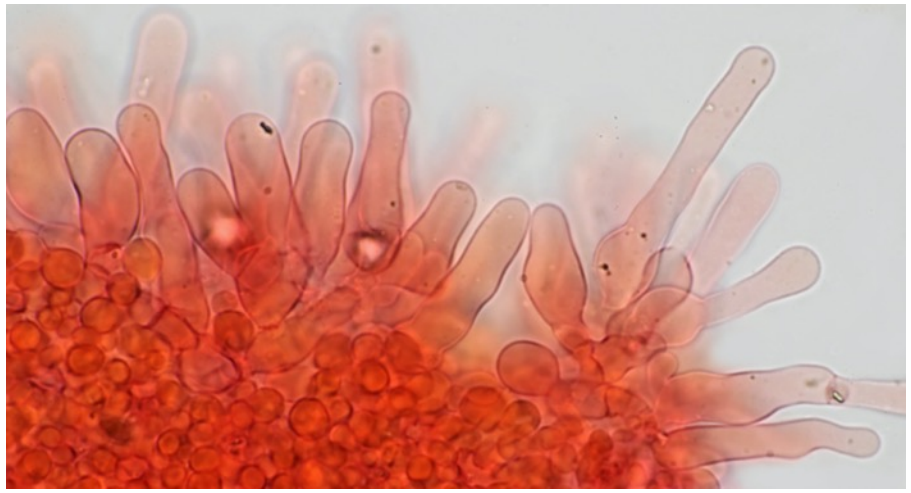


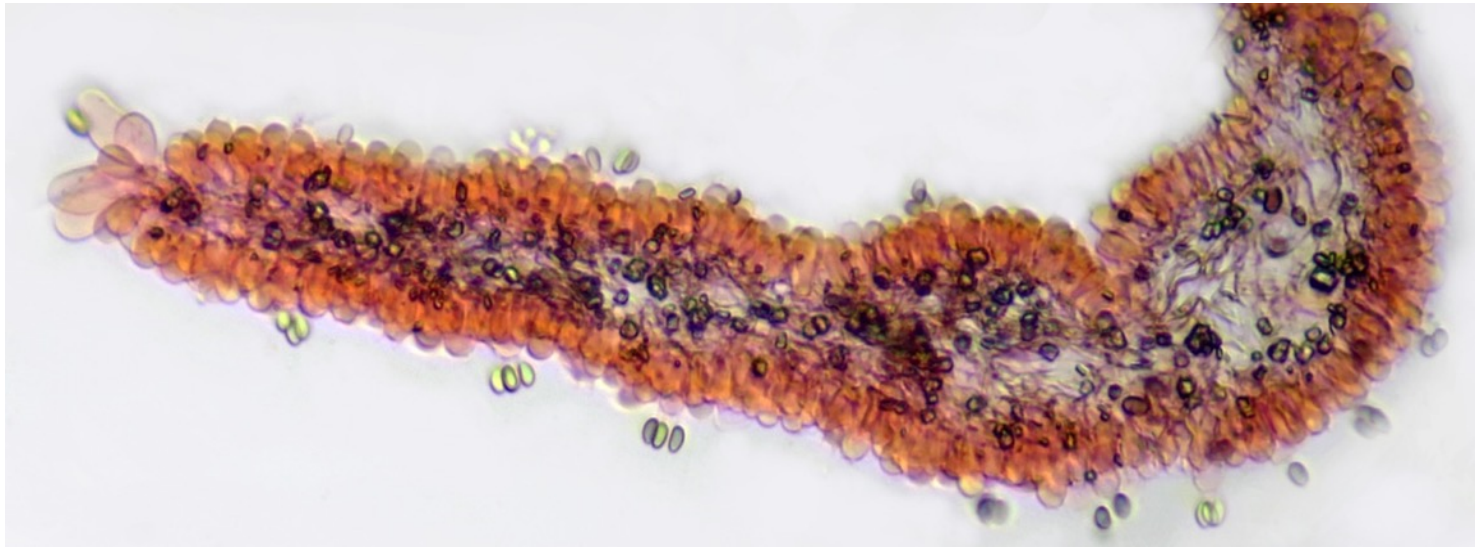
Cheilocystidia of the **Torfbroeck** specimens (N=40) : (22) 30 - 60 x 10-15 (20) µm ; Me = 41 x 13 µm.

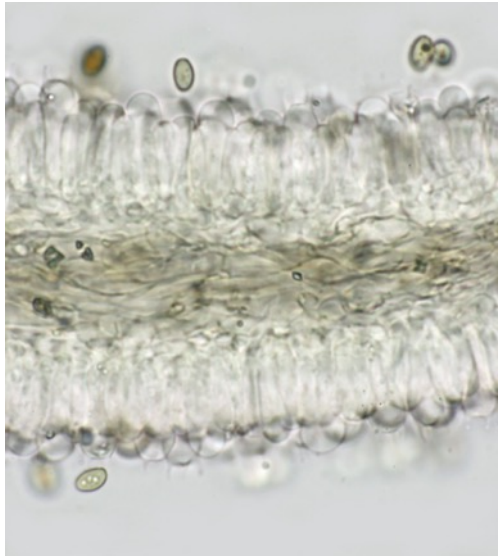




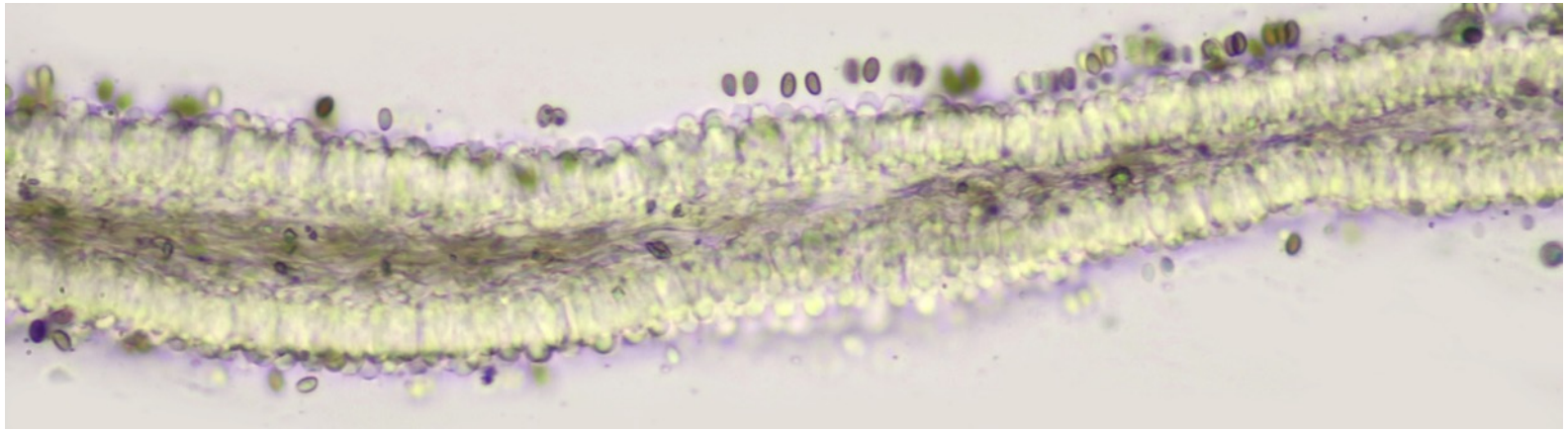
Cheilocystidia of **Sicoli paratypes** (N = 100): (25) 30,8 - 44,6 (54,5) × (6,1) 8,8 - 13,5 (16,1) µm ; Me = 37,5 × 11,2 µm.

Pleurocystidia absent, gill trama slightly pigmented, basidia clavate 4-spored







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Caulocystides très longues, cylindro-lagéniformes, lagéno-ventrues, ou clavées, ressemblant à de long poils cylindriques, pouvant mesurer jusque 90-100 µm de long, parfois septés, et de temps en temps bouclés à ce niveau.

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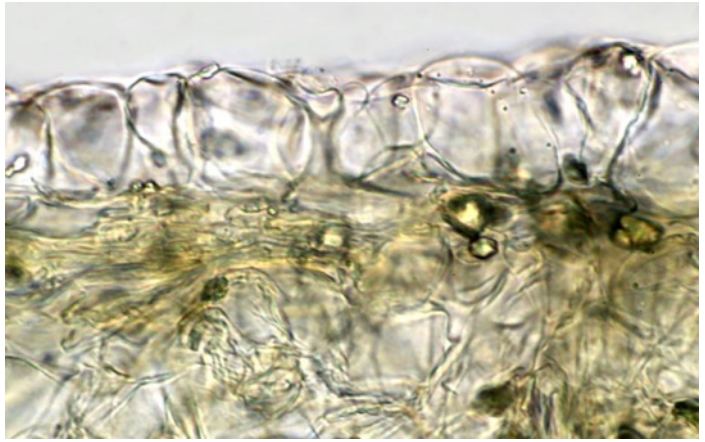
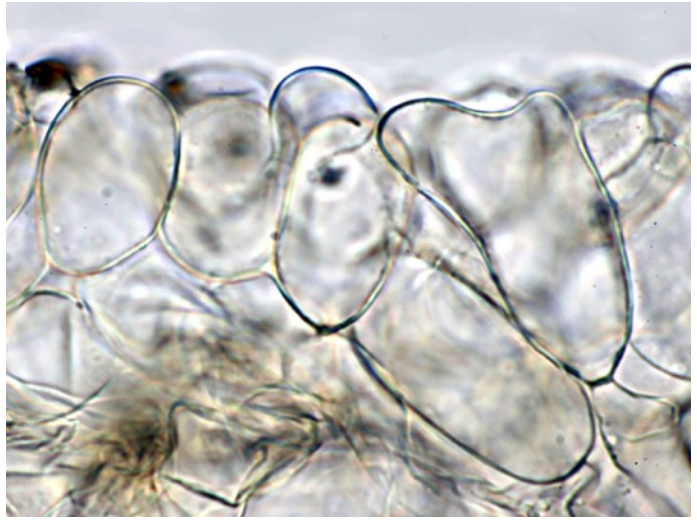
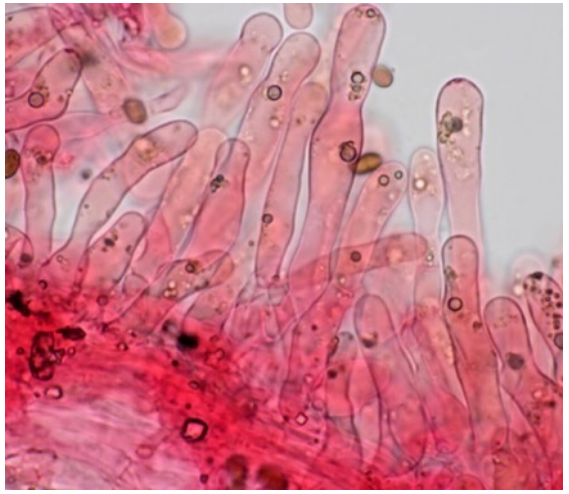
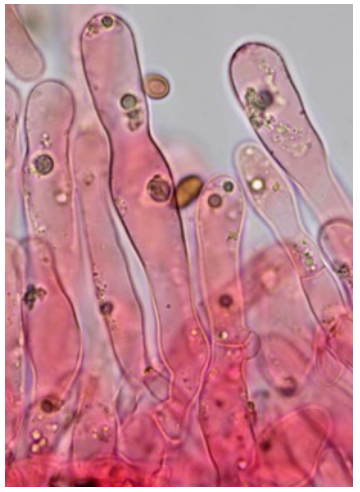
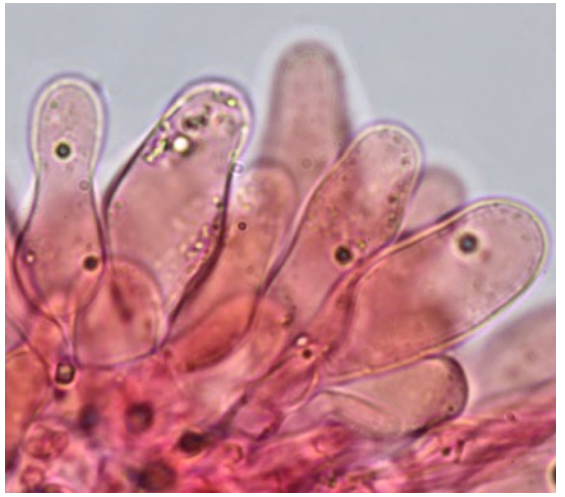




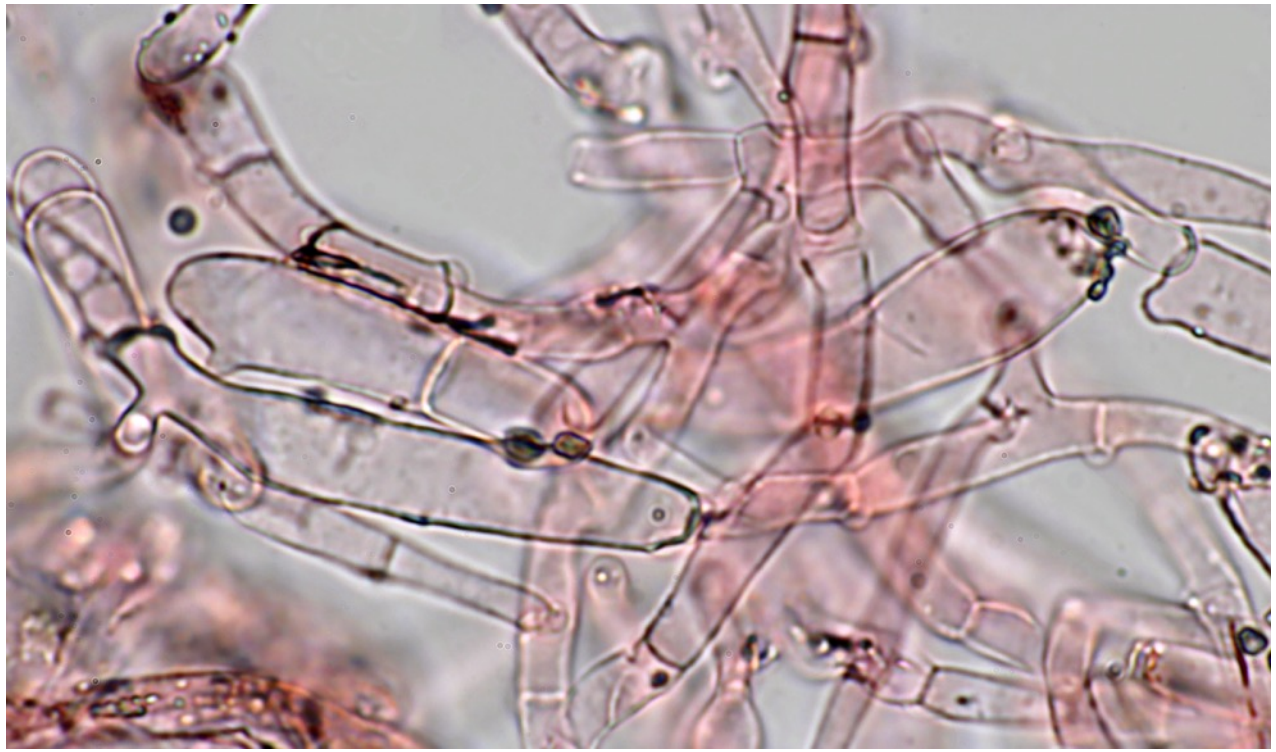






Voile initialement constitué d'hyphes hyalines à extrémités dilatées, présentant de nombreuses boucles de connexion devenant brunâtres, et incrustées au cours de la croissance.

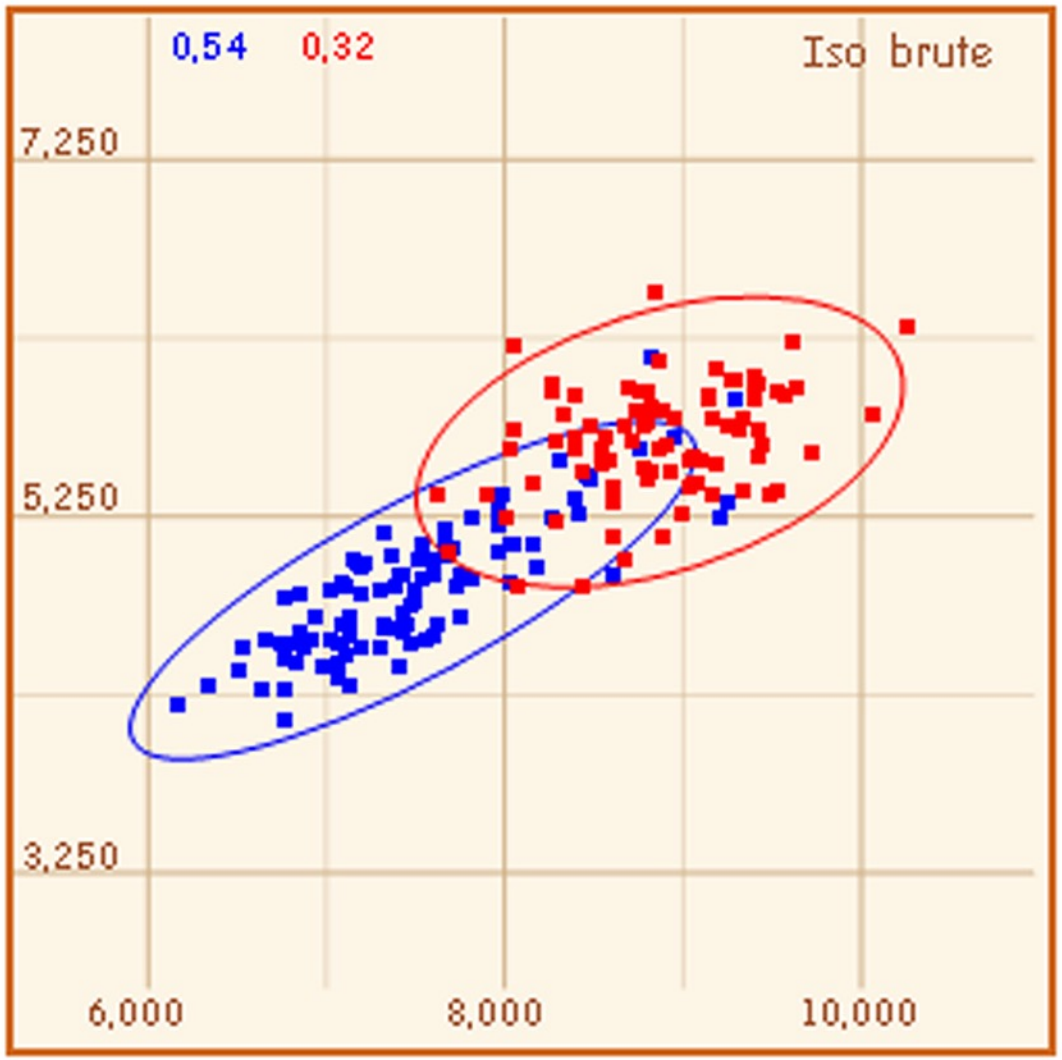
Veil initially composed of hyaline hyphae with dilated ends and lots of clamps, becoming brownish and encrusted during growth.



Discussion : dans l'état actuel de nos connaissances, et sur base des deux récoltes de *Psathyrella cladii marisci* observées, il semble bien que cette espèce soit un saprotrophe électif de *Cladium mariscus,* qui se développe sur les restes enfouis des plants coupés, et qui apparaît dans des marais et tourbières non salés, drainés par de l'eau douce.
D'autres psathyrelles, dépourvues de pleurocystides, qui affectionnent les tourbières et roselières marécageuses, sont reprises ci-après en précisant les critères qui permettent de les différentier.
Psathyrella typhae est une espèce beaucoup plus petite, gracile, se développant également en tourbière calcaire, sur tiges et feuilles mortes de diverses plantes aquatiques dont *Typha* spp., *Phragmytes* spp., *Carex* spp., *Acorus* spp. …, qui possède de plus grandes spores, mesurant 9-12,5(-13) x 5-8(-8,5) µm, très pâles et dépourvues de pore germinatif.
Psathyrella sulcatotuberculosa a de petites spores sans pore germinatif comme P. claddi marisci mais a une écologie plus large, d'autres cheilocystidies, et parfois un chapeau " sulcate-tuberculosa " ridé.
Psathyrella halophila est une espèce macroscopiquement proche, qui apparaît cependant en milieu halophile, ce qui correspond à une écologie déjà bien différente. Toutefois, cette espèce a retenu toute notre attention, car elle a également été décrite en présence de *Cladium mariscus* (Siquier & Carbo & Perez di Gregorio op cit.). Elle s'en distingue par des caractères génétiques mais également morphologiques illustrés ci-après.
Sur le plan microscopique, cette dernière espèce présente des spores nettement plus grandes, plus colorées, avec un pore germinatif beaucoup plus net ; sur le plan génétique, les séquences comparées montrent de multiples différences.

In the present state of our knowledge and on the basis of the two collections of *Psathyrella cladii marisci* observed, it seems that this species is an elective saprotroph of *Cladium mariscus*, which grows on the buried remains of cut plants and appears in unsalted marshes and peatlands drained by soft water.
Other Psathyrellas without pleurocystidia that prefer peat bogs and swampy reed beds are listed below, specifying the criteria that allow them to be distinguished.
Psathyrella typhae is a much smaller species, also developing in limestone bogs, on stems and dead leaves of various aquatic plants including *Typha spp., Phragmytes spp., Carex spp., Acorus spp. ...* which has larger very pale spores measuring 9-12.5 (-13) x 5-8 (-8.5) µm, whithout germ pore.
Psathyrella sulcatotuberculosa has small spores whithout a germ pore like P. claddi marisci but has a wider ecology, other cheilocystidia, and sometimes a wrinkled « sulcate-tuberculose » cap.
Psathyrella halophila is a macroscopically close species that nevertheless appears in a halophilic environment, which corresponds to an ecology that is already very different. However, this species has caught our attention because it has also been described in the presence of *Cladium mariscusi (Siquier & Carbo & Perez de Gregorio op cit.)*. It differs from it by genetic but also morphological features, as illustrated below.
Microscopically, this last species has much larger spores, more colourful with a much clearer germ pore and genetically the compared sequences show multiple differences.

Comparison between the size of the spores of
Psathyrella cladii marisci from Torfbroeck (blue ellipse)
&
Psathyrella litoralis (holotype of Corriol) = *P. halophila* (red ellipse)



Bien qu'il existe un chevauchement des dimensions autour des 7,5-8,5 x 5-5,5 µm, les spores de *P. cladii mariscii* apparaissent nettement plus courtes et plus étroites, ce qui est nettement évident, lorsqu'on photographie un mix de ces sporées (voir plus bas).

Although there is an overlap in dimensions around 7,5-8,5 x 5 -5,5 µm, the spores of *P. cladii marisci* appear much shorter and more narrow, which is evident when photographing a mix of these spores (see below).

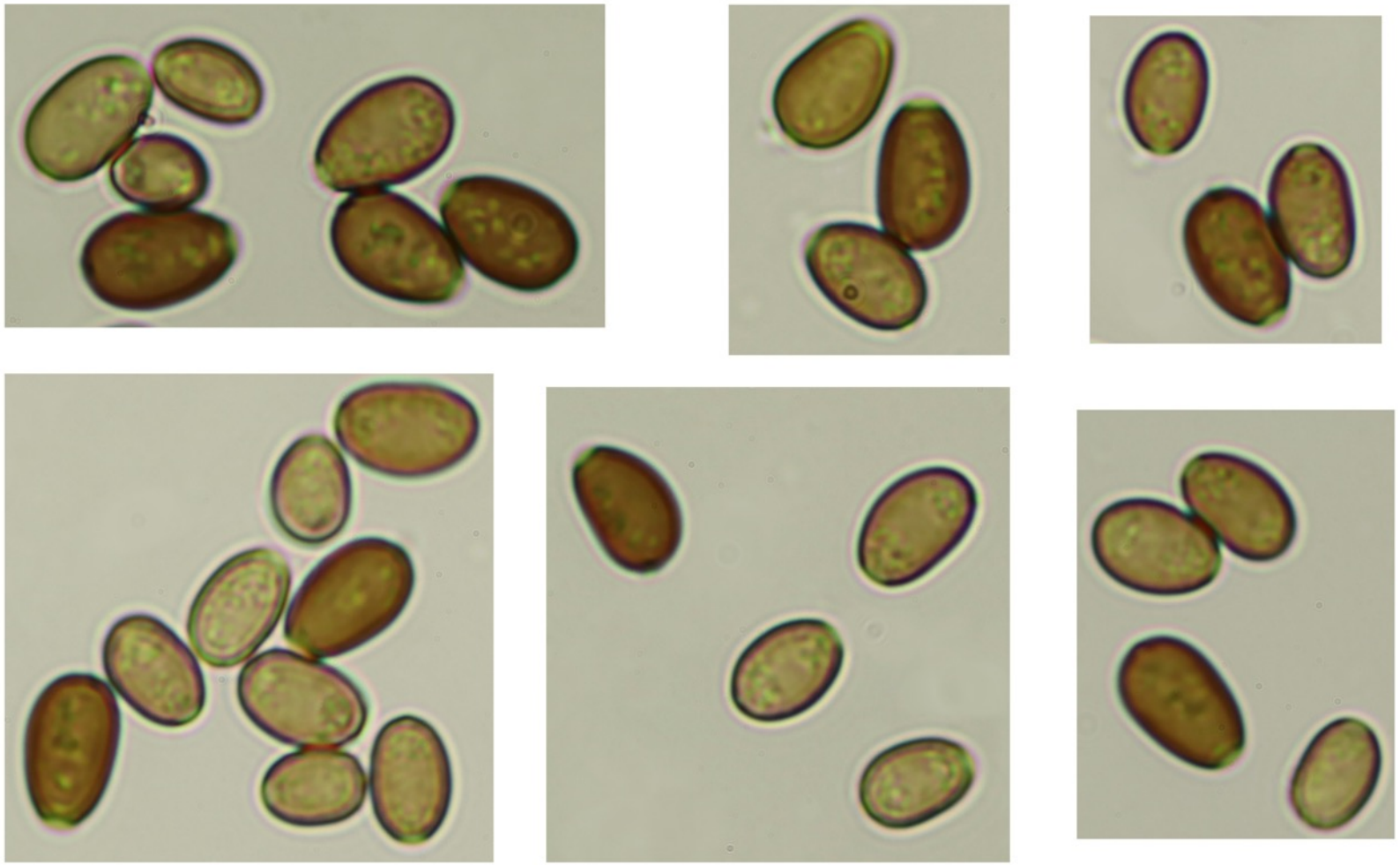
New spores measurements of specimens collected 06/09/2019
Psathyrella cladii marisci from Torfbroeck
N = 102
6,2)6,8-8,4(9,3) × (4,1)4,4-5,3(6,1) µm
Me = 7,5 × 4,8 µm
Q = (1,4)1,5-1,6(1,8) ; Qe = 1,5

Spores measurements of
Psathyrella litoralis holotype = *Psathyrella halophila*
N = 90
7,6)8,3-9,4(10,3) × (4,9)5,3-6(6,5) µm
Me = 8,9 × 5,7 µm
Q = (1,3)1,4-1,7(1,8) ; Qe = 1,6

Mix of *Psathyrella halophila* (specimens from Carbó J. & Perez de Gregorio M.A.) & *Psathyrella cladii-marisci* from Torfbroeck

Les spores de *P. cladii-marisci* apparaissent plus petites et beaucoup plus pâles, avec un pore germinatif central très discret, limité à un callus.

The spores of *P. cladii-marisci are* significantly smaller, much paler with a very discreet central germ pore restricted to a callus.



Memo note

Giuseppe Pellegrino compared my sequence with that of Sicoli and found out they are almost identical, except for one position where in Sicoli's sequence there is a T base, which is missing in mine. (underlined in red)

>P. cladii-marisci TTTTACACACCCCATTTGAATGATTTAGAATGTAGTCAATGGGCTTTCATGCCTATAAAAAACTATACAA
>DDSUTUC_ITS_final TTTTACACACCCCATTTGAATGATTTAGAATGTAGTCAATGGGCTTTCATGCCTATAAAAAACTATACAA
>P. cladii-marisci CTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT
>DDSUTUC_ITS_final CTTTCAGCAACGGATCTCTTGGCTCTCGCATCGATGAAGAACGCAGCGAAATGCGATAAGTAATGTGAAT
>P. cladii-marisci TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
>DDSUTUC_ITS_final TGCAGAATTCAGTGAATCATCGAATCTTTGAACGCACCTTGCGCTCCTTGGTATTCCGAGGAGCATGCCT
>P. cladii-marisci GTTTGAGTGTCATTAAATTCTCAACCTCACCAGTTTTGTAACGAGACAGGTGAAGGCTTGGAT**T**GTGGGG
>DDSUTUC_ITS_final GTTTGAGTGTCATTAAATTCTCAACCTCACCAGTTTTGTAACGAGACAGGTGAAGGCTTGGAT**--**GTGGGG
>P. cladii-marisci GTTTTGCAGGCTGCCTCAGTGCTGGTCTGCTCCCCTGAAATGCATTAGCGAGCTCATATTGAGCTTCCGT
>DDSUTUC_ITS_final GTTTTGCAGGCTGCCTCAGTGCTGGTCTGCTCCCCTGAAATGCATTAGCGAGCTCATATTGAGCTTCCGT
>P. cladii-marisci CTATTGGTGTGATAATTATCTACGCCGTGGATTGGACTCATGCTTGCTTCTAACCGTCCGCAAGGACAAT
>DDSUTUC_ITS_final CTATTGGTGTGATAATTATCTACGCCGTGGATTGGACTCATGCTTGCTTCTAACCGTCCGCAAGGACAAT
>P. cladii-marisci TTACTTGACCAATTTGACCTCAAATCAGGTAGGACT
>DDSUTUC_ITS_final TTACTTGACCAATTTGACCTCAAATCAGGTAGGACT

Memo notes

Comparaisons entre les séquences de *P. cladii marisci* & les séquences de P. halophila (holotype) et P. littoralis

La séquence ITS de *P. cladii-marisci* est bien **différente** de celle de *P. halophila* & *P. littoralis* (surligné en rouge) : 24 différences

P. halophila P. littoralis (synonymisée OK – avec seulement 6 bases de différence sur 730 surlignées en jaune)

```
                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                          10         20         30         40         50
P. cladii marisci MK080112        ---------- ---AAGTCGT AACAAGGTTT CCGTAGGTGA ACCTGCGGAA
P. halophila MG825900             ------GAAG TAAAAGTCGT AACAAGGTTT CCGTAGGTGA ACCTGCGGAA
P. littoralis MG825901            TTAGAGGAAG TAAAAGTCGT AACAAGGTTT CCGTAGGTGA ACCTGCGGAA

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                          60         70         80         90        100
P. cladii marisci MK080112        GGATCATTAA CGAATATCTA TGGCGTTGGT TGTAGCTGGC TCCTAGGAGC
P. halophila MG825900             GGATCATTAA CGAATATCTA TGGCGTTGGT TGTAGCTGGC TCCTAGGAGC
P. littoralis MG825901            GGATCATTAA CGAATATCTA TGGCGTTGGT TGTAGCTGGC TCCTAGGAGC

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         110        120        130        140        150
P. cladii marisci MK080112        ATT-GTGCAC GCCCGTCATT CATATCATCT TTCCACCTGT GAACCATGTG
P. halophila MG825900             ATTTGTGCAC GCCCGTCATT CATATCATCT TTCCACCTGT GAACTATGTG
P. littoralis MG825901            ATTTGTGCAC GCCCGTCATT CATATCATCT TTCCACCTGT GAACTATGTG

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         160        170        180        190        200
P. cladii marisci MK080112        TAGGCCTGGA TACCCCTCGC TTTGGCAACA AAGCGGATGC GAGGATTGCT
P. halophila MG825900             TAGGTCTGGA TACCCCTCAC TTTGGCAACA AAGTGGATGC AAGGATTGCT
P. littoralis MG825901            TAGGYCTGGA TACCCCTCAC TTTGGCAACA AAGTGGATGC AAGGATTGCT

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         210        220        230        240        250
P. cladii marisci MK080112        GCGTCGACAA GG-CCGGCTC TCTTTGAATT TCCAGGTTCT ATGTCTTTTT
P. halophila MG825900             GTGTCGACAA GGGCCGGCTC TCTTTGAATT TCCAGGTCCT ATGTCTTTTT
P. littoralis MG825901            GTGTCGACAA GG-CCGGCTC TCTTTGAATT TCCAGGTCCT ATGTCTTTTT

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         260        270        280        290        300
P. cladii marisci MK080112        ACACACCCCA TTTGAATGAT TTAGAATGTA GTCAATGGGC TTTCATGCCT
P. halophila MG825900             ACACACCCCA TTCGAATGAT GTAGAATGTA GTCAATGGGC TTTCAAGCCT
P. littoralis MG825901            ACACACCCCA TTCGAATGAT GTAGAATGTA GTCAATGGGC TTTCAAGCCT

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         310        320        330        340        350
P. cladii marisci MK080112        ATAAAAAACT ATACAACTTT CAGCAACGGA TCTCTTGGCT CTCGCATCGA
P. halophila MG825900             ATAAAACACT ATACAACTTT CAGCAACGGA TCTCTTGGCT CTCGCATCGA
P. littoralis MG825901            ATAAAACACT ATACAACTTT CAGCAACGGA TCTCTTGGCT CTCGCATCGA
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                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         360        370        380        390        400
P. cladii marisci MK080112        TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
P. halophila MG825900             TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG
P. littoralis MG825901            TGAAGAACGC AGCGAAATGC GATAAGTAAT GTGAATTGCA GAATTCAGTG

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         410        420        430        440        450
P. cladii marisci MK080112        AATCATCGAA TCTTTGAACG CACCTTGCGC TCCTTGGTAT TCCGAGGAGC
P. halophila MG825900             AATCATCGAA TCTTTGAACG CACCTTGCGC TCCTTGGTAT TCCGAGGAGC
P. littoralis MG825901            AATCATCGAA TCTTTGAACG CACCTTGCGC TCCTTGGTAT TCCGAGGAGC

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         460        470        480        490        500
P. cladii marisci MK080112        ATGCCTGTTT GAGTGTCATT AAATTCTCAA CCTC-ACCAG TTTTGTAACG
P. halophila MG825900             ATGCCTGTTT GAGTGTCATT AAATTCTCAA CCTCCACTCG TTTTGTAACA
P. littoralis MG825901            ATGCCTGTTT GAGTGTCATT AAATTCTCAA CCTCCACTCG TTTTGTAACR

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         510        520        530        540        550
P. cladii marisci MK080112        AGACAGGTGA AGGCTTGGAT -TGTGGGGGT TTTGCAGGCT GCCTCAGTGC
P. halophila MG825900             AGACGGGTGA AGGCTTGGAT GTGGGGG-TC TTTGCAGGCT GCCATAGTGC
P. littoralis MG825901            AGACGGGTGA AGGCTTGGAT GTGGGGGGTC TTTGCAGGCT GCCATAGTGC

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         560        570        580        590        600
P. cladii marisci MK080112        TGGTCTGCTC CCCTGAAATG CATTAGCGAG CTCATATTGA GCTTCCGTCT
P. halophila MG825900             TGGTCTGCTC CCCTGAAATG CATTAGCGAG CTCATATCGA GCTTCCGTCT
P. littoralis MG825901            TGGTCTGCTC CCCTGAAATG CATTAGCGAG CTCATATCGA GCTTCCGTCT

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         610        620        630        640        650
P. cladii marisci MK080112        ATTGGTGTGA TAATTATCTA CGCCGTGGAT TGG-ACTCAT GCTTGCTTCT
P. halophila MG825900             ATTGGTGTGA TAATTATCTA CGCCGTGGAT TGGGACTCGT GCTTGCTTCT
P. littoralis MG825901            ATTGGTGTGA TAATTATCTA CGCCGTGGAT TGGGACTYGT GCTTGCTTCT

                                  ....|....| ....|....| ....|....| ....|....| ....|....|
                                         660        670        680        690        700
P. cladii marisci MK080112        AACCGTCCGC AAGGACAATT TACTTGACCA ATTTGACCTC AAATCAGGTA
P. halophila MG825900             AACTGTCCGC AAGGACAATC TACTTGACCA ATTTGACCTC AAATCAGGTA
P. littoralis MG825901            AACYGTCCGC AAGGACAATC TACTTGACCA ATTTGACCTC AAATCAGGTA

                                  ....|....| ....|....| ....|....| ..
                                         710        720        730
P. cladii marisci MK080112        GGACTACCCG CTGAACTTAA ---------- --
P. halophila MG825900             GGACTACCCG CTGAACTTAA GCATATCAA- --
P. littoralis MG825901            GGACTACCCG CTGAACTTAA GCATATCAAT AA
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Biomolecular study : Dieter Wächter

Sequencing and phylogenetic analysis

DNA extraction, amplification and sequencing of the fungi were 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], for the LSU region the LR5 [2] and for the ef-1α region the EF1-1567R primer [3] was used. The initial base calling was done with FinchTV [4]. 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 /candolleana s.l. clade 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 [5] and Unite [6] (essential ones of the clade shown in Fig. 1 see Table 1). Region boundaries for the ITS- and LSU-region were carried out with ITSx [7] and HMMER [8] including the databases. As outgroup, the sequence sets of the most closely related clade of the ingroup were used, i.e. the /vinosofulva 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 [9] 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 [10] using the SIC = “Simple Indel coding” [11] method. After each alignment step, an ML analysis with RAxML [12] (model: GTRCAT, refining under GTR+G for DNA, GTR2+G with acquisition bias correction according to Lewis [13] 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 [14], 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 [15]. For the final partitioning, the guide tree of the last iteration step was used. As information criterion the Bayesian Information Criterion (BIC) [16] used was after comparison with the Corrected Akaike Information Criterion (AICc) [17] 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 [12]. For all DNA partitions, the GTR substitution matrix [18] under the CAT model [12] was used. The final optimization took place under gamma distribution [12]. For the binary partitions, the “Two State Time-Reversible Model” with acquisition bias correction [13] 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 [19]. The outgroup has been collapsed for a better view.

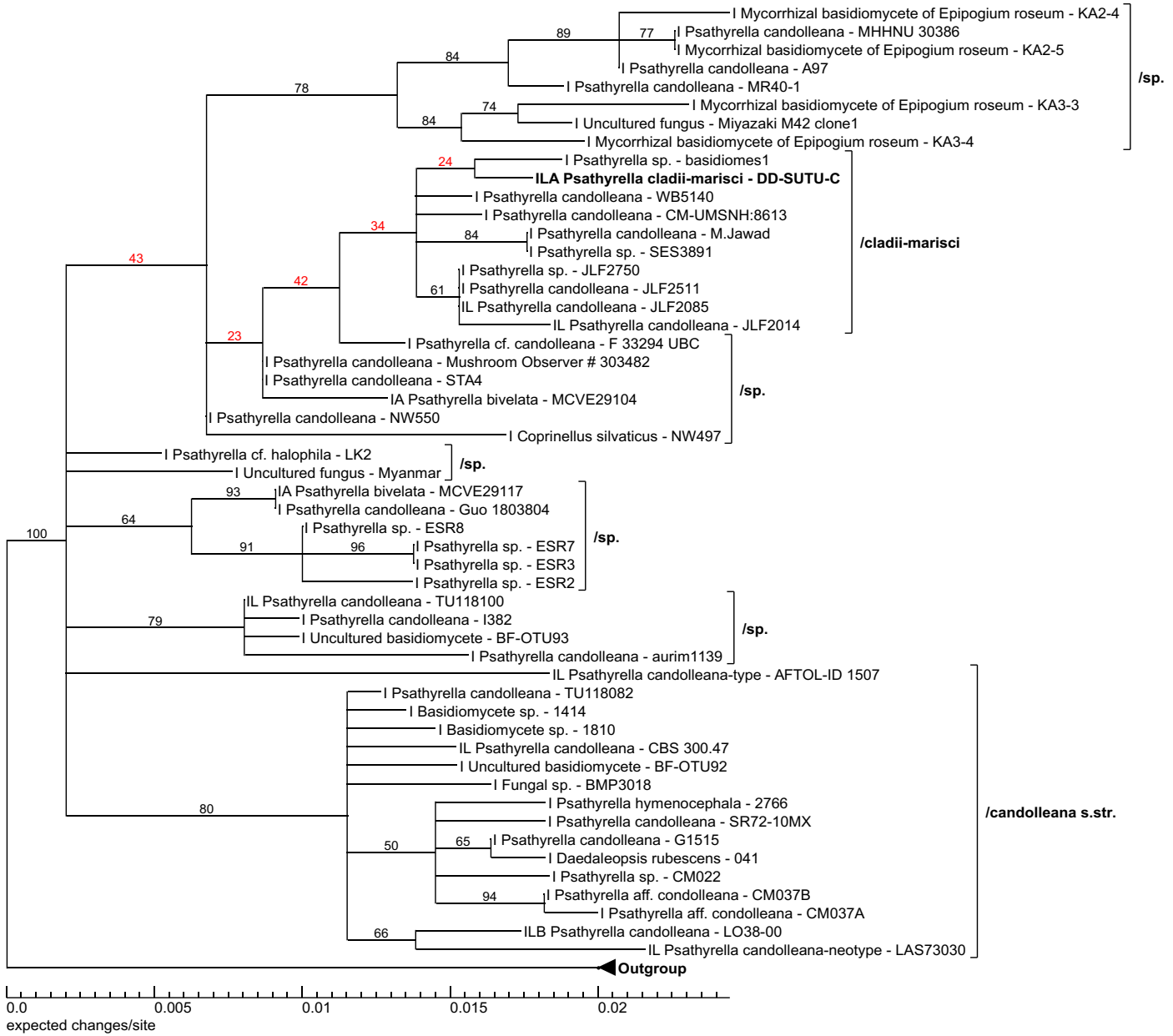

Fig. 1 50% collapsed maximum likelihood consensus phylogram. The values on the branches are ML bootstrap values. Red support values mean: no collapsing was done. Abbreviations: I: ITS region, L: LSU region, B: β-tubulin region, A: ef-1α region.

Table 1 List of relevant sequences used in this publication

Species	Ref.-ID	ITS	LSU	β-Tub	ef-1α
Basidiomycete sp.	1810	AM930993.1			
Basidiomycete sp.	1414	AM930990.1			
Coprinellus silvaticus	NW497	EU520144.1			
Daedaleopsis rubescens	041	EU661889.1			
Fungal sp.	BMP3018	HQ833000.1			
Mycorrhizal basidiomycete of Epipogium roseum	KA2-4	AB176574.1			
Mycorrhizal basidiomycete of Epipogium roseum	KA2-5	AB176575.1			
Mycorrhizal basidiomycete of Epipogium roseum	KA3-3	AB176578.1			
Mycorrhizal basidiomycete of Epipogium roseum	KA3-4	AB176579.1			
Psathyrella aff. condolleana	CM037A	KP826737.1			
Psathyrella aff. condolleana	CM037B	KP826738.1			
Psathyrella bivelata	MCVE29104	MF325961.1			MF521812.1
Psathyrella bivelata	MCVE29117	MF325962.1			MF521811.1
Psathyrella candolleana	MR40-1	KU324797.1			
Psathyrella candolleana	A97	MK247759.1			
Psathyrella candolleana	MHHNU 30386	MK214387.1			
Psathyrella candolleana	WB5140	KY940508.1			
Psathyrella candolleana	CM-UMSNH:8613	MH347300.1			
Psathyrella candolleana	M.Jawad	LC481953.1			
Psathyrella candolleana	JLF2085	MK996304.1	MN031146.1		
Psathyrella candolleana	JLF2511	MK996307.1			
Psathyrella candolleana	JLF2014	MK996303.1	MN031145.1		
Psathyrella candolleana	STA4	LC458687.1			
Psathyrella candolleana	NW550	EU520251.1			
Psathyrella candolleana	AFTOL-ID_1507	DQ494689.1	DQ110874.1		
Psathyrella candolleana	SR72-10MX	KT697973.1			
Psathyrella candolleana	LAS73030	KM030175.1	KM030175.1		
Psathyrella candolleana	LO38-00	DQ389720.1	DQ389720.1	KJ664864.1	
Psathyrella candolleana	TU118082	UDB015850			
Psathyrella candolleana	G1515	MK247863.1			
Psathyrella candolleana	CBS 300.47	MH856260.1	MH867795.1		
Psathyrella candolleana	Guo_1803804	KX394805.1			
Psathyrella candolleana	I382	GU062309.1			
Psathyrella candolleana	TU118100	UDB015469	UDB015469		
Psathyrella candolleana	aurim1139	DQ093650.1			
Psathyrella cf. candolleana	F_33294_UBC	MH752459.1			
Psathyrella cf. halophila	LK2	LK2_ITS			
Psathyrella cladii-marisci	DD-SUTU-C	follows	follows		follows
Psathyrella hymenocephala	2766	FJ168608.1			
Psathyrella sp.	SES3891	follows			
Psathyrella sp.	basidiomes1	MK080112.1			
Psathyrella sp.	JLF2750	MN017262.1			
Psathyrella sp.	CM022	KP826731.1			
Psathyrella sp.	ESR8	MK226170.1			
Psathyrella sp.	ESR2	MK226164.1			
Psathyrella sp.	ESR7	MK226169.1			
Psathyrella sp.	ESR3	MK226165.1			
Psathyrella tuberculata	Mushroom Observer # 315413	MH497604.1			
Uncultured basidiomycete	BF-OTU92	AM902043.1			
Uncultured basidiomycete	BF-OTU93	AM901757.1			
Uncultured fungus	Miyazaki M42 clone1	AB306300.1			
Uncultured fungus	Myanmar	AB306305.1			

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[2] Rehner SA, Samuels GJ (1994) Taxonomy and phylogeny of Gliocladium analysed from nuclear large subunit ribosomal DNA sequences. Mycological Research 98: 625-634

[3] **FinchTV 1.4.0**: Geospiza, Inc.; Seattle, WA, USA; http://www.geospiza.com

[4] **NCBI**: National Center for Biotechnology Information, U.S. National Library of Medicine 8600 Rockville Pike, Bethesda MD, 20894 USA - https://www.ncbi.nlm.nih.gov/

[5] **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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[7] **HMMER 3.1b2** (February 2015): http://hmmer.org/ - Copyright (C) 2015 Howard Hughes Medical Institute. Freely distributed under the GNU General Public License (GPLv3)

[8] **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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