

A molecularly supported concept of *Marasmius epiphyllus* (*Basidiomycetes, Physalacriaceae*)

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Abstract – *Marasmius epiphyllus* and *M. tenuiparietalis* (*Basidiomycetes, Physalacriaceae*) are considered conspecific taxa. This result is supported by anatomic-morphological and molecular studies of specimens from central, western and southern Europe, including *Marasmius kablikianus* (*ad schedam*) and a recent collection of *M. epiphyllus* v. *plantaginis*.

***Marasmius epiphyllus* / *M. tenuiparietalis* / taxonomy / molecular studies**

Résumé – *Marasmius epiphyllus* et *M. tenuiparietalis* (*Basidiomycetes, Physalacriaceae*) sont considérés conspécifiques. Ce résultat est basé sur des études anatomiques-morphologiques et moléculaires sur des échantillons provenant d'Europe centrale, occidentale et méridionale, parmi eux *Marasmius kablikianus* (*ad schedam*) et une collection récente de *M. epiphyllus* v. *plantaginis*. Le placement de *M. favrei* dans la section *Epiphylli* est confirmé.

***Marasmius epiphyllus* / *M. tenuiparietalis* / taxonomie / études moléculaires**

INTRODUCTION

The small section *Epiphylli* was traditionally included in the genus *Marasmius* (e.g. Antonín & Noordeloos, 1993; Kühner, 1933, 1936; Singer, 1986). However, according to recent molecular studies, it is excluded from that genus and represents a not yet exactly settled group within family *Physalacriaceae* (e.g. Douanla-Meli & Langer, 2008; Owings & Desjardin, 1997; our studies). It contains

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species with small, marasmiod basidiocarps with a maximally 10 mm broad, white or whitish, membranaceous pileus, with a sometimes yellowish, ochraceous or orange-yellow centre. Their gills are well-developed, vein-like or sometimes absent. The stipe is insititious, filiform, pruinose, slightly pubescent or hairy. Under the microscope, they are characterized by cylindrical, ellipsoid, fusoid or obovate, non-dextrinoid basidiospores, well-developed cystidia on lamellae and stipe and a hymeniform pileipellis mostly composed of smooth or rough cells, rarely with apical projections (broom cells) and sometimes with well-developed pileocystidia. The type and most common species is *Marasmius epiphyllus* (Pers.: Fr.) Fr.

In the *M. epiphyllus* group, which was considered very variable, Singer (1969) described a new species, *Marasmius tenuiparietalis* Singer, which differed from *M. epiphyllus* by the better developed lamellae mostly reaching the pileus margin and especially by the predominantly thin-walled elements of the pileipellis. It has never been collected on remnants of herbaceous plants so far (on fallen leaves from deciduous trees only), suggesting a potential ecological difference between both taxa. Singer's species was also accepted by Antonín & Noordeloos (1993). However, results of recent anatomical and morphological studies showed that the wall thickness of pileipellis cells is very variable and it is very difficult to use this character for the delimitation of both taxa. In extreme cases, it is possible to distinguish specimens having thin- to slightly thick-walled cells (*M. tenuiparietalis*) from those having slightly to distinctly thick-walled ones (*M. epiphyllus*). The same problem arose when delimitation was based on the degree of the gill development. These problems led us to study specimens using molecular methods.

MATERIAL AND METHODS

The macroscopic description is based on our own observations. The type specimens available have been studied. Microscopic studies are usually based on dried material using light microscopes Olympus BX-50 with a magnifications up to 1000 x. Observations were made on mounts in Congo-red, 10% Ammonia, 10% KOH, and the Melzer's reagent. The following abbreviations have been used: E = quotient of length and width of the spores, and Q = the mean value of E in all collections studied, L = number of entire lamellae, l = number of lamellulae between each pair of entire lamellae. A list of studied specimens is included in Tab. 1. *Marasmius* sp. (PRM 870457) represents a slightly, especially macroscopically aberrant collection growing on *Petasites kablikianus* named as *Marasmius kablikianus* on herbarium specimen label by Z. Pouzar. Genomic DNA extraction, polymerase chain amplification (PCR) and sequencing were performed as described by Vašutová *et al.* (2008). DNA fragments of about 1.5 kilobases (kb) spanning the nuclear ribosomal (nr) ITS regions and the D1/D2 domains of the nrLSU were amplified using standard PCR protocols typically employing a short (10 sec) annealing step at 54°C and 35 cycles. For certain herbarium specimens the DNA was apparently to degraded to allow amplification of the 1.5 kb fragment, in these cases we sequenced only a fragment of the nrITS regions. The following primer combinations were used: the primer pairs ITS1F/TW13 or ITS1F/TW14 served as standard PCR and sequencing primers. The primers LROR and ITS4 (<http://plantbio.berkeley.edu/~bruns/tour/primers.html>) were used as additional PCR and sequencing primers. DNA was sequenced using the Big Dye terminator Cycle

Table 1. Origins and accessions of the investigated specimens

<i>Herbarium</i>	<i>EMBL number</i>	<i>Identified as</i>	<i>Locality</i>	<i>Ecology</i>
BRNM 695779	FN293007	<i>M. epiphyllus</i>	Italy, Borgo val di Taro, Brunelli, 21 Oct. 2005 leg. V. Antonín 05.239	On petiols of fallen leaves of <i>Castanea sativa</i> and <i>Quercus pubescens</i> .
BRNM 695733	FN293008	<i>M. epiphyllus</i>	Czech Republic, Komárov, Soběslavská blata, Komárovský chobot, 28 Sept. 2005 leg. V. Antonín 05.178	On petiols of fallen leaves of <i>Populus tremula</i> .
PRM 870457	FN293009	<i>Marasmius</i> sp.	Slovakia, Čingov, Lesnica valley, 26 Sept. 1984 leg. Z. Pouzar	On petiols and laminae of <i>Petasites kablikianus</i> .
PRM 902346	FN293010	<i>M. tenuiparietalis</i>	Czech Republic, Praha-Suchdol, Tiché údolí valley, 31 Oct. 2004 leg. Z. Pouzar	On leaves – <i>Quercus robur</i> , <i>Q. rubra</i> , <i>Fraxinus excelsior</i> , <i>Crataegus</i> , <i>Carpinus betulus</i> and <i>Acer pseudoplatanus</i> .
BRNM 523367	FN293011	<i>M. tenuiparietalis</i>	Slovakia, Zlatá Baňa, Chabzdová, 26 Sept. 1990 leg. V. Antonín 90.97	On leaves of <i>Populus tremula</i> .
PRM 894159	FN293012	<i>M. tenuiparietalis</i>	Czech Republic, Borkovice, 22 Sept. 2000 leg. F. Kotlaba	On nervae of folia of <i>Populus tremula</i> .
K 40466	FN293013	<i>M. tenuiparietalis</i>	UK, England, Oxfordshire, Bix, the Warburg Reserve, 2 Oct. 1996 leg. N.W. Legon	On petioles of <i>Fraxinus excelsior</i> and <i>Fagus sylvatica</i> .
BRNM 714560	FN293015	<i>M. epiphyllus</i> var. <i>plantaginis</i>	Spain, Girona, Sant Pere Pescador (L'Alt Emporda), 24 Dec. 2007 leg. N. Maceu	On remnants of <i>Plantago crassifolia</i> .
LIP	FN293014	<i>M. tenuiparietalis</i>	France, Savoie, St. Jean de Chevelu, 25 Oct. 1998 leg. P.-A. Moreau 98102509	On petiols of <i>Fraxinus excelsior</i> .
BRNM 523372	FN293016	<i>M. tenuiparietalis</i>	Slovakia, Červenica, Dubnícke rašeliníšte, 24 Sept. 1990 leg. V. Antonín 90.81	On leaves of <i>Populus tremula</i> , rarely <i>Fagus sylvatica</i> .
BRNM 695419	FN293017	<i>M. favrei</i>	Czech Republic, Horní Rozsčicka, 19 Oct. 2000 leg. J. Čáp	On petiols of <i>Sorbus aucuparia</i> .

sequencing Kit v 3.1 (Applied Biosystems, Foster City, CA) and 3130xL Genetic Analyzer (Applied Biosystems). Forward and reverse sequences were assembled, aligned and edited with the SeqScapeV2.5 software (Applied Biosystems). Sequences were deposited in the EMBL Nucleotide Sequence Database (accession no. FN293007-FN293018). *Xerula pudens* (AF321490, sequence obtained from public DNA database) was selected as outgroup based on a preliminary nrLSU-based phylogeny of *Physalacriaceae* (data not shown). The dataset was restricted to the ITS1, 5.8S and ITS2 regions plus a few basepairs from the adjacent nrSSU and nrLSU regions, for which a complete dataset without missing data was available. The sequences were aligned with MAFFT 6.240 (Katoh *et al.*, 2005) using the linsi settings, resulting in an alignment of 657 basepairs. No data were excluded from the alignment. Parsimony analysis was calculated with TNT (Goloboff *et al.*, 2008) using the implicit enumeration option, which allows an exact search of small datasets. Standard bootstrap with replacement and implicit enumeration was run for 100 replicates. Bayesian phylogenetic analysis of the sequence data was performed with MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) using the GTR+I+G model and default heating parameters. The analysis was run for 1,000,000 generations, sampling frequency was 100 and burnin was set to 1000.

RESULTS

According to the phylogenetic analysis all investigated species of the section *Epiphylli* form a monophyletic group with high posterior probability and bootstrap support (Fig. 1). All specimens of the *M. epiphyllus* group except sample BRNM 523372 (from Slovak Republic) are part of a homogenous group without internal phylogenetic resolution. This result is confirmed by direct inspection of the alignment (Tab. 2). Sequences obtained from specimens classified as *M. epiphyllus*, *M. epiphyllus* var. *plantaginis*, *M. tenuiparietalis*, and *Marasmius* sp. (see Material and Methods) were nearly identical. In position 149 of the ITS1 region two variants (A, G) and mixed bases (R = A+G) occurred. In all other potentially polymorphic sites, either the consensus base or mixed bases including the consensus base were found. Mixed bases might be interpreted as resulting from 1) incomplete homogenisation (concerted evolution) of the nrDNA repeats, 2) presence of two different haplotypes in heterokaryotic mycelia, or 3) sequencing errors or PCR errors. Even if all minor and possibly erroneous variants (Y or K instead of T) are counted as polymorphisms, the variation in the commonly highly variable ITS1 region is lower than 1.6%, a value far below the 3% threshold which is conventionally used for species discrimination (e.g. Ryberg *et al.*, 2008). While the sequences from all but one specimen from the *M. epiphyllus* group from different geographic locations, substrates and habitats (Tab. 1) were almost indistinguishable, one specimen from Slovak Republic (BRNM 523372) classified as *M. tenuiparietalis* was markedly different in the investigated nrDNA region (Fig. 1). This sample seems to belong to a different species. However, morphologically the specimen fits well the concept of *M. epiphyllus* as outlined below. Unfortunately, this cryptic species is known from one specimen only. For all other specimens it can be concluded that they are conspecific, given the low variation in the ITS1 region, the absence of phylogenetic structure (Fig. 1) and the lack of correlation of morphological characters and DNA polymorphisms.

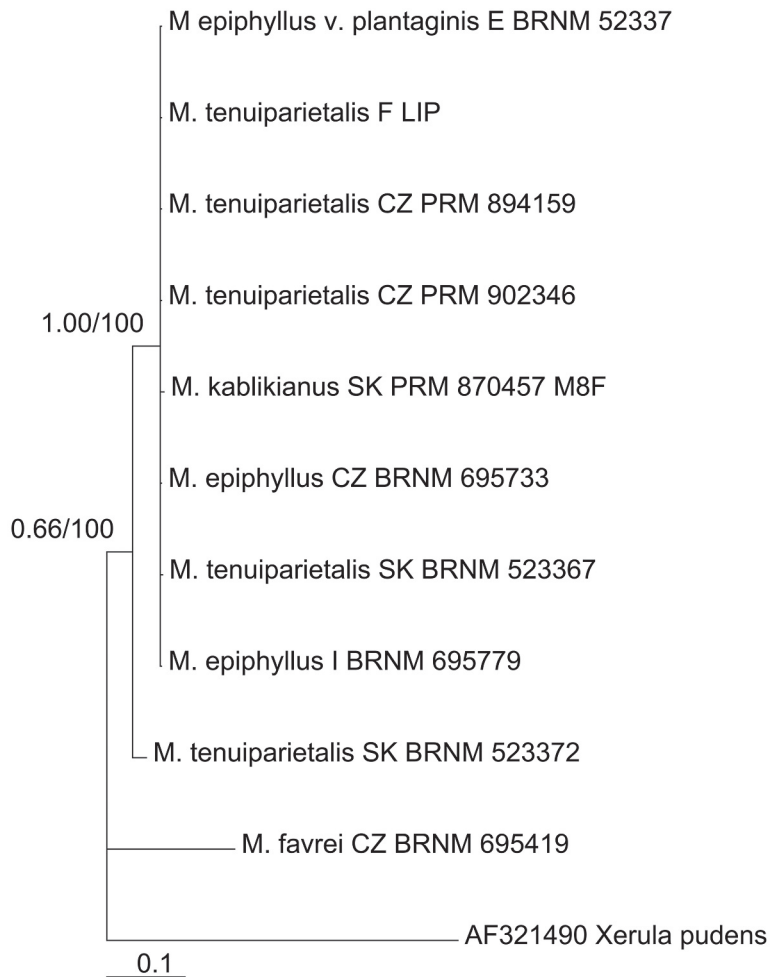


Fig. 1. Bayesian majority rule consensus phylogram of the *Epiphylli* rooted with *Xerula pudens*. The topology is identical with the strict consensus parsimony bootstrap tree. Internal edge labels indicate Bayesian posterior probabilities respectively parsimony bootstrap values. Herbarium accession numbers are noted along with the taxon labels.

Description of *Marasmius epiphyllus*

Marasmius epiphyllus (Pers.: Fr.) Fr., Epicr.: 386. 1838.

≡ *Agaricus epiphyllus* Pers., Syn. Meth. Fung.: 468. 1801; *Agaricus epiphyllus* Pers.: Fr., Syst. Mycol. 1: 139. 1821; *Androsaceus epiphyllus* (Pers.: Fr.) Pat., Hymen. Eur.: 105. 1887.

= *Agaricus squamula* Batsch, Elench. Fung. Cont. prima: 91. 1786; *Marasmius squamula* (Batsch) J. Schröt. in Cohn, Kryptog.-Fl. Schles. 3(1): 556. 1889.

= *Marasmius subvenosus* Peck, Ann. Rep. N. Y. State Mus. Nat. Hist. 23 (1869): 125. 1872.

Table 2. Polymorphic positions in the ITS1 region of an alignment of *M. epiphyllus* sequences (excluding BRNM 523372, strongly deviant, and Moreau 98102509, too short). Numbers indicate base position in the ITS1 (total length 313 bp).

<i>EMBL number</i>	<i>Herbarium</i>	36	86	120	149	258	271
FN293007	BRNM 695779	T	.	T	R	T	T
FN293008	BRNM 695733
FN293009	PRM 870457	.	.	Y	A	Y	.
FN293010	PRM 902346	.	.	.	A	.	.
FN293011	BRNM 523367	.	.	.	G	.	.
FN293012	PRM 894159	Y
FN293013	K 40466	.	.	.	G	.	.
FN293014	LIP	.	K
FN293015	BRNM 714560	.	.	.	A	.	K

= *Marasmius tremulae* Velen., Novit. Mycol. Novis.: 17. 1947; non *Marasmius tremulae* sensu Kühner (1947); J. Favre (1951) (= *Marasmius favrei*)

= *Marasmius tenuiparietalis* Singer, Beih. Nova Hedwigia 29: 99. 1969.

Pileus 1-5 (10) mm broad, membranaceous, hemispherical to conico-convex or convex, then appanate to slightly depressed, rarely with a broad, obtuse papilla, with slightly involute or deflexed margin when young, later with straight or even reflexed or dentate margin, with regular then undulating marginal zone, white or very pale brown or ochraceous when young then brilliantly white, becoming slightly yellowish on drying when old, slightly pubescent under lens, radially rugulose. Lamellae distant, L = 6-11, l = 0-1 (2), poorly developed as irregular ridges or veins, reaching or not reaching margin of pileus, sometimes almost venose, anastomosing, often forked, more rarely well-developed and reaching the margin of the pileus, broadly adnate or shortly decurrent, sometimes loosening from stipe in a false collarium, white to cream with entire, concolorous edge. Stipe 5-35 × 0.2-0.6 mm, usually central, rarely eccentric, cylindrical, filiform, white when young then turning brown, dark brown or grey-brown from base upward, when mature only with narrow white zone at apex, downwards dark brown, grey-brown or blackish brown at base, entirely finely pubescent to furfuraceous, with slight broadening at base but without a distinct disc. Smell and taste indistinct. Telepods sometimes present.

Spores (7.5) 8.5-12.5 (14) × 3.0-4.5 (5.0) µm, E = (1.9) 2.1-2.9 (3.2), Q = 2.5-2.6, slenderly ellipsoid to slightly lacrimoid, non-dextrinoid. Basidia (23) 26-40 × (6.5) 8.0-10 µm, clavate, 4-, rarely 2-spored, clamped. Basidioles (14) 19-26 (30) × (2.0) 4.0-9.0 µm, clavate, cylindrical or fusiform, clamped. Hymenial cystidia 27.5-58 × 5.0-9.0 µm, slenderly fusiform to slightly lageniform, rarely utriform, mostly with a long neck, sometimes with a small slimy cap, thin-walled, clamped. Subhymenium made up of thin-walled, hyaline, cylindrical, 2.0-3.0 (5.5) µm wide hyphae. Pilei- and hymenophoral trama irregular, made up of hyaline, cylindrical, branched, interwoven, thin-walled, 2.0-9.0 µm wide, non-

dextrinoid, clamped hyphae. Pileipellis hymeniform, made up of clavate or broadly fusiform, sometimes lageniform, thin- to slightly or distinctly thick-walled cells, (8.0) 12-27 (34) × (5.0) 8.0-11 (22) μm, sometimes with a gelatinous cap, hyaline, clamped. Pileocystidia (13) 20-41 × (4.0) 6.0-9.5 μm, lageniform or fusiform, sometimes with a slimy cap, clamped. Stipitipellis a cutis of narrow, thick-walled, hyaline (apex) or brown (basal part), clamped, 4.0-15 μm wide hyphae. Caulocystidia present in two types: 1) non-differentiated, cylindrical, 4.0-10 μm wide, patent or more or less appressed terminal endings of surface hyphae, and 2) true cystidia, (5.0) 13.5-34 (47) × (3.0) 4.5-8.5 (10) μm, fusiform, lageniform or almost cylindrical, thin-walled or slightly thick-walled, hyaline or sometimes yellowish at base, sometimes with a slimy cap.

DISCUSSION

The description above corresponds to the concept presented in Antonín & Noordeloos (2010). In 2007, a very similar taxon from this group growing on *Plantago* remnants, *Marasmius epiphyllus* var. *plantaginae* Heim, was re-collected after more than 70 years in Spain (Macau, 2008; Moreau & Macau, 2008). A formal new combination *Marasmius epiphyllus* var. *plantaginis* (Heim) P.-A. Moreau & Macau [Basionym: *Androsaceus epiphyllus* var. *plantaginae* Heim 1934 = *Marasmius plantaginis* (Heim) Singer 1986] was also proposed by Moreau & Macau (2008). Macroscopically, it differs by less numerous (L = 0-4) and more venose lamellae and an apparently glabrous pileus and stipe. Microscopically and molecularly, however, it fits to the variability of *M. epiphyllus*. Therefore we keep this taxon as a variety of *M. epiphyllus*.

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REFERENCES

- ANTONÍN V. & NOORDELOOS M.E., 1993 — A monograph of *Marasmius*, *Collybia* and related genera in Europe. Part 1: *Marasmius*, *Setulipes*, and *Marasmiellus*. *Libri Botanici* 8: 1-229. Eching.
- ANTONÍN V. & NOORDELOOS M.E., 2010 — A monograph of marasmioid and collybioid fungi in Europe. — IHW-Verlag, Eching, 480 p.
- DOUANLA-MELI C. & LANGER E., 2008 — Phylogenetic relationship of *Marasmius mbalmayoensis* sp. nov. to the tropical African *Marasmius bekolacongoli* complex based on nuc-LSU rDNA sequences. *Mycologia* 100(3): 445-454.
- GOLOBOFF P., FARRIS J. & NIXON K., 2008 — TNT, a free program for phylogenetic analysis. *Cladistics* 24:774-786.
- KATOH K., KUMA K., TOH H. & MIYATA T., 2005 — MAFFT version 5: improvement in accuracy of multiple sequence alignment. describes [ancestral versions of] the G-INS-i, L-INS-i and E-INS-i strategies. *Nucleic Acids Research* 33: 511-518.
- KÜHNER R., 1933 — Études sur le genre *Marasmius*. *Botaniste* 25: 57-116.
- KÜHNER R., 1936 — Nouvelles recherches sur le genre *Marasmius*. *Annales Société Linnéenne Lyon* 79 (1935): 99-120.
- MACAU N., 2008 — *Marasmius epiphyllus* var. *plantaginae*. *Bolets de Catalunya* 27: pl. 1331.
- MOREAU P.-A. & MACAU N., 2008 — Un taxon poco conocido del litoral mediterráneo: *Marasmius epiphyllus* var. *plantaginis*. *Revista Catalana de Micologia* 30: 65-70.

- OWING P. & DESJARDIN D.E., 1997 — A molecular phylogeny of *Marasmius* and selected segregate genera. *Inoculum* 48 (3): 29.
- RONQUIST F. & HUELSENBECK J.P., 2003 — MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.
- RYBERG M., NILSSON R.H., KRISTIANSSON E., TÖPEL M., JACOBSSON S. & LARSSON E., 2008 — Mining metadata from unidentified ITS sequences in GenBank: A case study in *Inocybe* (Basidiomycota). *BMC Evolution Biology* 8: 50. doi: 10.1186/1471-2148-8-50
- SINGER R., 1969 — Mycoflora australis. *Nova Hedwigia, Beiheft* 29: 1-405.
- SINGER R., 1986 — The Agaricales in modern taxonomy. 4th ed. Koenigstein, 981 p., 88 pl.
- VAŠUTOVÁ M., ANTONÍN V. & URBAN A., 2008 — Phylogenetic studies in *Psathyrella* focusing on sections *Pennatae* and *Spadiceae* – new evidence for the paraphyly of the genus. *Mycological Research* 112 (10): 1153-1164.