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Psathyrella sect. *Cystidiosae* and first record of *Psathyrella cloverae* in the Dominican Republic

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type studies**Abstract:** The American species of *Psathyrella* sect. *Cystidiosae* are studied using morphological and molecular (nrITS, *tef1- α* , LSU) data. This study includes the first report of *P. cloverae* from the Dominican Republic herein described with images of the basidiomes in habitat and the main microcharacters. *P. varzeae* and *P. hesleri* are demonstrated to be later synonyms of *P. cloverae*. *P. smithii* is also treated as a later synonym though based only on morphological data due to the unsuccessful molecular analysis of its isotype. The distribution of *P. cloverae* covers a wide range of climate and vegetation types, and ranges from South America (Brazil) through Central America (Caribbean, Costa Rica, Mexico) up to North America. Based on non-type molecular studies, *P. cystidiosa* is phylogenetically closer to the Central American species *P. pivae* and is therefore considered to be a different species from *P. cloverae*.**INTRODUCTION**

The family Psathyrellaceae Vilgalys, Moncalvo & Redhead (synonyms: Zerovaemycetaceae Gorovij, *nomen anamorphosis*, restricted priority; Coprinaceae Overeem & Weese pro maxima parte, excluding the type name) includes, with phylogenetic support, several genera, including *Coprinellus* P. Karst., *Coprinopsis* P. Karst., *Homophron* (Britzelm.) Örstadius & E. Larss., *Lacrymaria* Pat., *Parasola* Redhead, Vilgalys & Hopple, *Psathyrella* Fr. ex Quél. and others. In the past the name used instead of Psathyrellaceae was Coprinaceae Overeem & Weese (inclusive of only two genera, *Coprinus* Pers. and *Psathyrella* Fr. ex Quél.), until Redhead *et al.* (2001) demonstrated, based on phylogenetic data, that the Coprinaceae also included a small number of taxa belonging to the different family Agaricaceae Chevall. Since these taxa also included *Coprinus comatus* (O.F. Müll.) Pers., the type name of *Coprinus*, in turn the type name of the family Coprinaceae, it was necessary to assign a new name, Psathyrellaceae, replacing Coprinaceae, to include the rest of the species.

For this family it is customary to informally distinguish species with a 'coprinoid' habit and species with a 'psathyrelloid' habit. The former group includes taxa usually characterized, among other characters, by the deliquescence of the basidiomes, while the members of the latter group do not usually deliquesce. Among the psathyrelloid taxa, there are two groups of species well characterized morphologically by possessing pleurocystidia more or less strongly thick-walled and usually incrustated.

In the past, Smith (1972), not yet possessing phylogenetic evidence, treated the members of these two groups in *Psathyrella* subgenus *Homophron* Britzelm. This subgenus was further split into the sections *Homophron* Britzelm., characterized by pale spores, and *Cystidiosae* A.H. Sm. 1972, characterized by well pigmented spores. Current phylogenetic knowledge (see Örstadius, Ryberg & Larsson 2015) demonstrates that in this group the presence/absence of veil, and not the spore colour, is the most relevant morphological character for separating the species. Consequently, we can morphologically distinguish the species with a veil, however scanty, and with well-pigmented spores, from those completely devoid of any trace of veil and with more or less pale spores. This distinction, although sometimes difficult to evaluate regarding the veil which is affected by the degree of freshness of the specimens, is necessary because the taxa of the first group belong to

the genus *Psathyrella* Fr. ex Quél., while the taxa of the second group constitute the genus *Homophron* (Britzelm.) Örstadius & E. Larss., phylogenetically very distant (Örstadius, Ryberg & Larsson 2015).

Basing on phylogenetic evidence, the Smith's taxon *Psathyrella* sect. *Cystidiosae* 1972, type name *P. cystidiosa* (Peck) A.H. Sm., is therefore not taxonomically based on the most important character. However, it is a nomenclaturally valid taxon and we propose an emendation to its definition (as see below).

Romagnesi (1982) had proposed *Psathyrella* sect. *Pygmaeae* Romagn., type name *P. pygmaea* (Bull.) Singer. However, since the type names of both sections belong to the same phylogenetically supported clade, the Romagnesi's section must be treated as a later synonym of *Psathyrella* sect. *Cystidiosae* (I.C.N. Art. 11.3).

Due to the presence of a veil our Dominican collection belongs to *Psathyrella* sect. *Cystidiosae* and corresponds phylogenetically to the holotype of *P. cloverae* (Crous *et al.* 2019). We therefore attempted to sequence holotypes, isotypes, and other representative collections related to American taxa to ascertain any presumed cases of synonymy. As we expected on a morphological basis, for *P. hesleri* and *P. varzeae* the ITS sequences obtained support their synonymy with *P. cloverae*. For *P. smithii* we propose its synonymy only on a morphological basis since repeated molecular analyses of the isotype have failed.

Regarding *P. cystidiosa*, we only obtained one ITS sequence out of five non-types collections from the MIN and TEN herbaria. That sequence does not nest in the '*cloverae* subclade' but beside *P. pivae* ('*pivae* subclade'). An LSU sequence, in GenBank, obtained from another non-type collection of *P. cystidiosa* (Crous *et al.* 2019) also nests beside *P. pivae*. Therefore, there is reliable molecular support to believe that this species is excluded from synonymy with *P. cloverae* notwithstanding a close morphological similarity.

Finally, we have evaluated which name, among *P. cloverae*, *P. hesleri*, *P. smithii* and *P. varzeae*, was to be chosen to have priority. *P. cloverae* and *P. hesleri* were described in the same work (Smith 1972), while *P. varzeae* and *P. smithii* were later. The name *P. hesleri* has a somewhat contorted history: Smith (1972) described it from a collection identified as *P. cystidiosa* that he considered to be misidentified. Our analysis confirms that he was correct in noting the misidentification, but mistaken in not recognizing it as his new species *P. cloverae*, thus creating a superfluous double. Therefore we propose to assign priority to the epithet *P. cloverae*.

MATERIALS AND METHODS

Morphology

The basidiomes of the Dominican collection were photographed fresh in the habitat by C.A. using a digital camera Nikon Coolpix 8400 and subsequently dried. Microscopic characters were studied and described by P.V. on dried material revived and observed in 5% KOH or 30% NH₄OH; in some cases Congo red was also added for enhanced clarity in images. The same procedure was used by P.V. for the micro revision of the isotype of *P. smithii*.

Special terms used in this paper are: 'indented' (referred to the spores): meaning that the base presents a lateral protrusion like a tooth (as it happens in other *Psathyrella* species, e.g. *P. albescens* Hesler & A.H. Sm.); 'lepto-' (referred to the cystidia): a prefix meaning thinned-wall; 'metuloid' (referred to the cystidia): meaning thick-walled; 'muricate' (referred to the cystidia): meaning the presence of incrustations on the cell walls; 'paracystidia': sterile cells of the gill edge with a clavate to ellipsoid shape, distinctly different from the pleurocystidia.

For the description of the vegetation present in the Dominican collecting site of *P. cloverae*, see Parra *et al.* (2018).

The herbaria cited in this paper are: Instituto Politécnico Nacional, Mexico city (Mexico), code ENCB; Field Museum of Natural History, Chicago (USA), code F; Jardín Botánico Nacional Dr. Rafael M. Moscoso, Santo Domingo (Dominican Republic), code JBSD; University of Michigan, Ann Arbor (USA), code MICH; University of Minnesota, St. Paul (USA), code MIN; Università degli Studi di Padova, Padua (Italy), code PAD; University of Tennessee, Knoxville (USA), code TENN; Royal Ontario Museum, Toronto (Canada), code TRTC. Their codes are taken from the online Index Herbariorum (<https://sweetgum.nybg.org/science/ih/>).

The authors of the images in the figures are shown in square brackets.

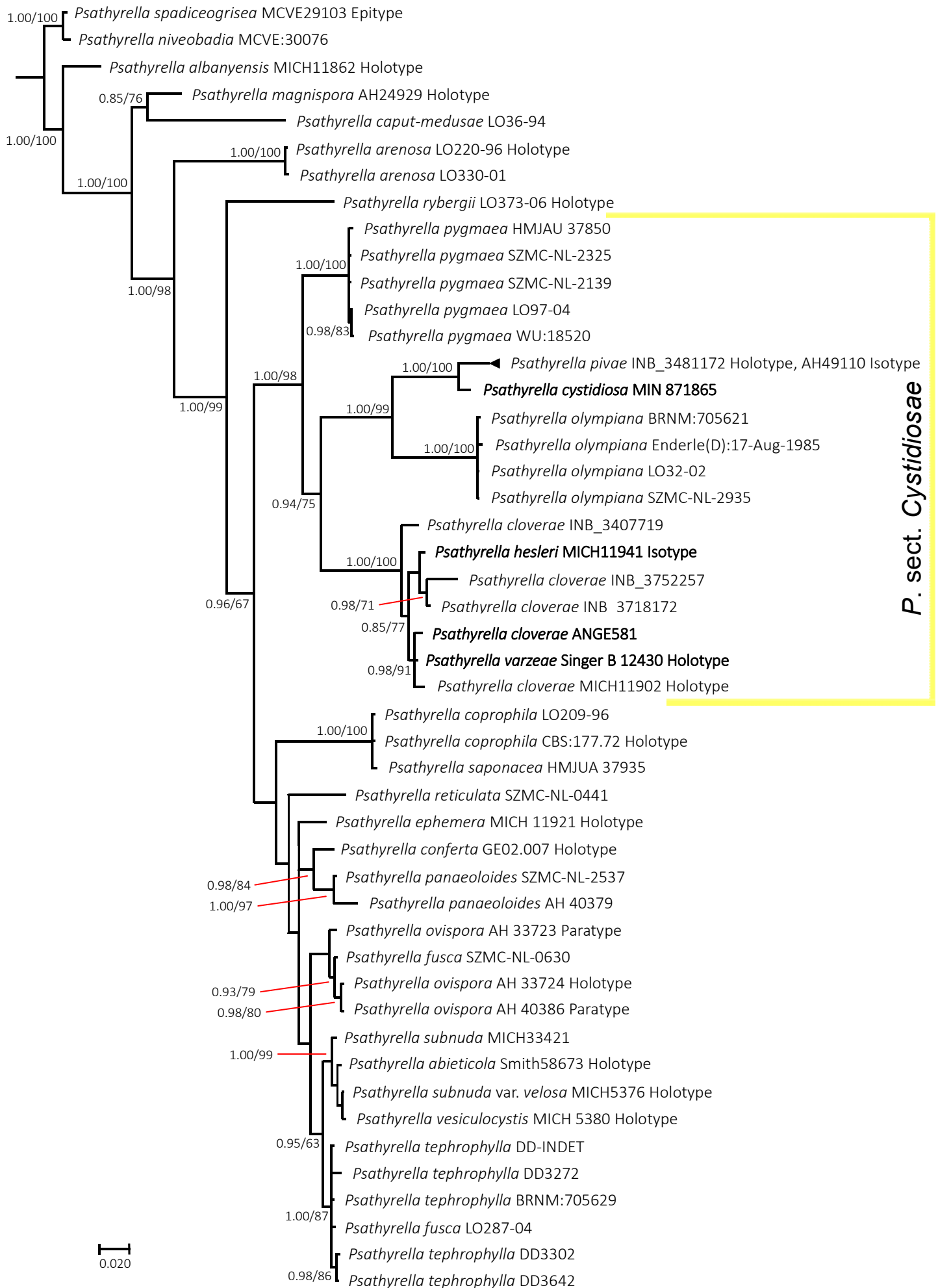


Figure 1 (above): A 50% majority rule ITS rDNA-28S rDNA- *tef1- α* consensus phylogram of the /cystidiosa clade of genus *Psathyrella* (with *P. spadiceogrisea* and *P. niveobadia* of /spadiceogrisea clade as outgroups) obtained using MrBayes from 2700 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or $\geq 70\%$ maximum likelihood bootstrap proportions (right). Nonsignificant support values are exceptionally represented inside parentheses. New sequences generated in this study are in bold

Table 1 (below). Fungal taxa, voucher numbers and GenBank accession numbers of the sequences used in the phylogenetic analyses; in red sequences generated in this work. Names in parentheses indicate misidentifications ('as') or prior synonyms (' \rightarrow ')

Taxa names of <i>Psathyrella</i>	Collection ID	ITS	<i>tef1-α</i>	LSU
<i>P. abieticola</i> Holotype	Smith58673/USA	KC992891	–	KC992891
<i>P. albanyensis</i> Holotype	MICH11862/USA	MF325952	MF521823	–
<i>P. arenosa</i> Holotype	LO220-96/Sweden	KC992895	KJ732784	–
<i>P. arenosa</i>	LO330-01/Sweden	KC992896	KJ732785	KC992896
<i>P. caput-medusae</i>	LO36-94/Sweden	KC992927	KJ732814	KC992927
<i>P. cloverae</i> Holotype	MICH11902/USA	MF966417	–	–
<i>P. cloverae</i>	INB_3407719/Costa Rica	MF966508	–	–
<i>P. cloverae</i>	INB_3752257/ Costa Rica	MF966509	–	–
<i>P. cloverae</i>	INB_3718172/ Costa Rica	MF966510	–	–
<i>P. cloverae</i>	ANGE581/Dominican Rep.	MZ856314	OK546186	–
<i>P. cloverae</i> (duplicate)	ANGE581/Dominican Rep.	PP476873	PP493201	–
<i>P. conferta</i> Holotype	GE02.007 (PC)/France	KC992890	–	KC992890
<i>P. coprophila</i> Holotype (\rightarrow <i>P. saponacea</i>)	CBS:177.72/UK	MH860433	–	MH872163
<i>P. coprophila</i> (\rightarrow <i>P. saponacea</i>)	LO209-96/Sweden	DQ389717	–	DQ389717
<i>P. cystidiosa</i>	MIN 871865/USA	PP469638	–	–
<i>P. ephemera</i> Holotype	MICH 11921/USA	NR_161023	–	–
<i>P. fusca</i>	LO287-04/Sweden	KC992892	KJ732779	KC992892
<i>P. fusca</i> (\rightarrow <i>P. tehrophylla</i>)	DD-INDET/Belgium	MK583508	–	–
<i>P. fusca</i> (\rightarrow <i>P. tehrophylla</i>)	DD3272/Belgium	MK577903	–	–
<i>P. fusca</i> (\rightarrow <i>P. tehrophylla</i>)	DD3302/Belgium	MK577904	–	–
<i>P. fusca</i> (\rightarrow <i>P. tehrophylla</i>)	DD3642/Belgium	MK577902	–	MK57791
<i>P. fusca</i> (\rightarrow <i>P. tehrophylla</i>)	BRNM:705629/Czech Rep.	AM712270	–	AM712270
<i>P. hesleri</i> Isotype (\rightarrow <i>P. cloverae</i>)	MICH11941/USA	PP476871	–	–
<i>P. magnispora</i> Holotype	AH24929/Spain	KC992863	–	–
<i>P. niveobadia</i>	MCVE:30076/Italy	MK400421	MK408675	–
<i>P. olympiana</i>	LO32-02/Sweden	DQ389722	KJ732817	DQ389722
<i>P. olympiana</i>	SZMC-NL-2935/Hungary	FN396103	FN396211	FN396156
<i>P. olympiana</i>	Enderle(D):17-Aug-1985 /Germany	AM712267	–	AM712267
<i>P. olympiana</i>	BRNM:705621/Czech Rep.	AM712268	–	AM712268
<i>P. ovispora</i> Holotype	AH 33724/Spain	MF966497	–	MN190260
<i>P. ovispora</i> Paratype	AH 33723/Spain	MF966496	–	MN190261
<i>P. ovispora</i> Paratype	AH 40386/Spain	MN190257	–	–
<i>P. ovispora</i> (as <i>P. fusca</i>)	SZMC-NL-0630/Hungary	FM878030	FM897256	FM876288
<i>P. panaeoloides</i>	SZMC-NL-2537/Hungary	FM878022	FM897255	FM876279

Taxa names of <i>Psathyrella</i>	Collection ID	ITS	<i>tef1-α</i>	LSU
<i>P. panaeoloides</i>	AH 40379/Spain	MF966501	–	–
<i>P. pivae</i> Holotype	INB_3481172/Costa Rica	MF966507	–	–
<i>P. pivae</i> Isotype	AH49110/Costa Rica	–	–	MN161533
<i>P. pygmaea</i>	HMJAU 37850/China	MG734744	MH161170	MH155959
<i>P. pygmaea</i>	WU:18520/Germany	AM712269		AM712269
<i>P. pygmaea</i>	SZMC-NL-2139/Hungary	FM878010	FM897258	FM876266
<i>P. pygmaea</i>	SZMC-NL-2325/Hungary	FM878011	FM897224	FM876267
<i>P. pygmaea</i>	LO97-04/Sweden	DQ389718	KJ732811	DQ389718
<i>P. reticulata</i>	SZMC-NL-0441/Hungary	–	FM897227	FN396193
<i>P. rybergii</i> Holotype	LO373-06/Sweden	KC992893	KJ732781	KC992893
<i>P. saponacea</i>	HMJUA 37935/China	MH155965	–	MH155960
<i>P. spadiceogrisea</i> Epitype	MCVE29103/France	MF325997	MF521779	–
<i>P. subnuda</i> (\rightarrow <i>P. abieticola</i>)	MICH33421/USA	MF326000	MF521778	–
<i>P. subnuda</i> var. <i>velosa</i> Holotype (\rightarrow <i>P. abieticola</i>)	MICH5376/USA	MF326005	MF521773	–
<i>P. varzeae</i> Holotype (\rightarrow <i>P. cloverae</i>)	Singer B 12430/Brazil	PP476872	–	–
<i>P. vesiculocystis</i> Holotype (\rightarrow <i>P. abieticola</i>)	MICH 5380/USA	MF326007	MF521772	–

Phylogenetic analyses

The phylogenetic analyses were commissioned to the Alvalab laboratory (Spain) which also generated all new sequences proposed in this paper except that of the isotype of *Psathyrella hesleri*, generated by Ida Broman Nielsen and Tobias Frøslev (Denmark).

BLASTn (Altschul *et al.* 1990) was used to select the most closely related ITS rDNA sequences from the International Nucleotide Sequence Database Collaboration public database (INSDC, Arita *et al.* 2021), as well as LSU and *tef1- α* sequences of the same collections. Sequences first were aligned in MEGA 5.0 (Tamura *et al.* 2011) with its Clustal W application and then realigned manually as needed to establish positional homology. Aligned loci were loaded in MrBayes 3.2.6 (Ronquist *et al.* 2012), where a Bayesian analysis was performed (three partitions: ITS, LSU, *tef1- α* ; model GTR+G+I, two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until the average split frequencies between the simultaneous runs fell below 0.01 after 0.36 M generations. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis 2014) using the standard search algorithm (same partitions, GTRGAMMAI model, 2000 bootstrap replications). The significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

RESULTS

Emendation

Psathyrella sect. *Cystidiosae* A.H. Sm. emend. Voto
Memoirs of the New York Botanical Garden **24**:246 (1972)

Species of *Psathyrella* having pleurocystidia with crystalliferous to incrustated apex, and with walls in most cases yellowish and slightly to distinctly thickened.

Synonym: *Psathyrella* sect. *Pygmaea* Romagn., *Bull. trimest. Soc. mycol. Fr.* **98**(1):10 (1982)

Notes

This emendation is proposed basing on the characters of all species so far known to phylogenetically belong to this section. As shown by the phylogram, *P. rybergii* Örstadius & E. Larss. is a sister node to the “*cystidiosa* clade”; its pleurocystidia share a pale yellow pigment but are neither incrustated nor thick-walled therefore it is ruled out of the section.

This section comprises only two species in Europe: *P. pygmaea* (Bull.) Singer and *P. olympiana* A.H. Sm., both also present in North America. More species, and more names, are present in the American continent, beside the autonomous species, which are discussed below.

Taxonomy

P. cloverae A.H. Sm.

Memoirs of the New York Botanical Garden **24**:248. 1972

Typus: E. Clover 1229 (MICH11902), USA, Texas, Hidalgo, Canal South Mission, 2.VI.1933

Molecular heterotypical synonyms proposed here:

= *Psathyrella hesleri* A.H. Sm., *Mem. N. Y. Bot. Gard.* **24**: 249. 1972;

= *Psathyrella varzeae* Singer, *Fieldiana Botany* **21**: 102. 1989.

Morphological heterotypical synonyms proposed here:

= *Psathyrella smithii* Guzmán, *Bol. Soc. Mex. Mic.* **8**:94. 1974.

Description of the Dominican collection (Fig. 2-8).



Fig. 2. *Psathyrella cloverae*. ANGE581. Bar: 10mm [C. Angelini]

Macroscopic characters

Pileus (young and fresh specimens not observed) 18 – 32 mm; convex, sometimes undulate; margin radially ribbed-striated up to two thirds of the radius, with inconspicuous whitish residues of veil; surface smooth, hygrophanous, grey-brownish at first, often with an olivaceous shade while discoloring, finally beige to whitish with a (pale) greyish violaceous necropigment from the margin.

Lamellae approx. 30, intermixed with 3 – 7 lamellulae; adnate; not or slightly ventricose; greyish purplish brown; edge pale.

Stipe 15 – 36 × 3.0 – 6.0 mm, cylindrical or slightly tapering upwards, straight to somewhat flexuous, base equal or somewhat swollen; surface white, flocculose at apex, minutely flocculose elsewhere.

Context white in stipe.

Macroscopic characters

Spores (n = 20) (6.82) 7.20 – 8.00 (8.60) × (4.39) 4.55 – 5.20 (5.80) μm, on average 7.51 × 4.76 μm, Q = (1.35) 1.48 – 1.69 (1.73), on average 1.58; in face view oval to oval-pentagonal or elliptic, base broadly rounded to truncate, in side view adaxially flattened, subamygdaliform, subphaseoliform, sometimes weakly indented above the base; in water and in ammonia orange- to reddish- brown, in KOH grey-brown (cocoa-brown); germ pore distinct, 0.8 – 1.5 μm broad, broadly rounded to truncate; ; apiculus very small.

Basidia 4-spored, very rarely 2-spored, 16.0 – 19.5 × 7.5 – 9.0 μm, clavate.

Pleurocystidia (33.0) 40.0 – 50.0 × (12.5) 13.0 – 18.0 (22.0) μm; (ventricose) fusoid, ventricose-lageniform, utriform, mucronate-clavate; metuloid with walls 0.7 – 1.4 (2.0) μm thick at extreme base and 3.0 – 5.2 μm thick in neck, often completely occluding the space in extreme apex, hyaline to yellowish in ammonia; apex subacute to obtuse, sometimes rounded, muricate with incrustations; pedicel often elongate; abundant near the edge.

Cheilocystidia of three kinds, a) similar to pleurocystidia, scarce; b) paracystidia 15.0 – 26.0 × 8.8 – 13.0 (17.0) μm, clavate to ellipsoid, thin-walled, abundant; c) intermediate with paracystidioid shape and thickened walls or with more or less utriform shape and thin walls, scattered.

Pileipellis composed of a paraderm of cellular elements.

Caulocystidia more or less similar to pleurocystidia or smaller in size, occasionally thin-walled; scattered to abundant. *Clamp connections* present.

Habitat and collection examined: gregarious, growing on a fallen degraded trunk in a humid rain forest with deciduous trees; Dominican Republic, P.to Plata, Sosúa, Puerto Chiquito, (19°44'50.1"N 70°31'34.2"W), 4 January 2016, *legit* C. Angelini ANGE581, exsiccatum in JBSD124849, duplicate in PAD H0062119; GenBank: MZ856314 and PP476873- ITS, OK546186 and PP493201- *tef1-α* (same collection sequenced twice).

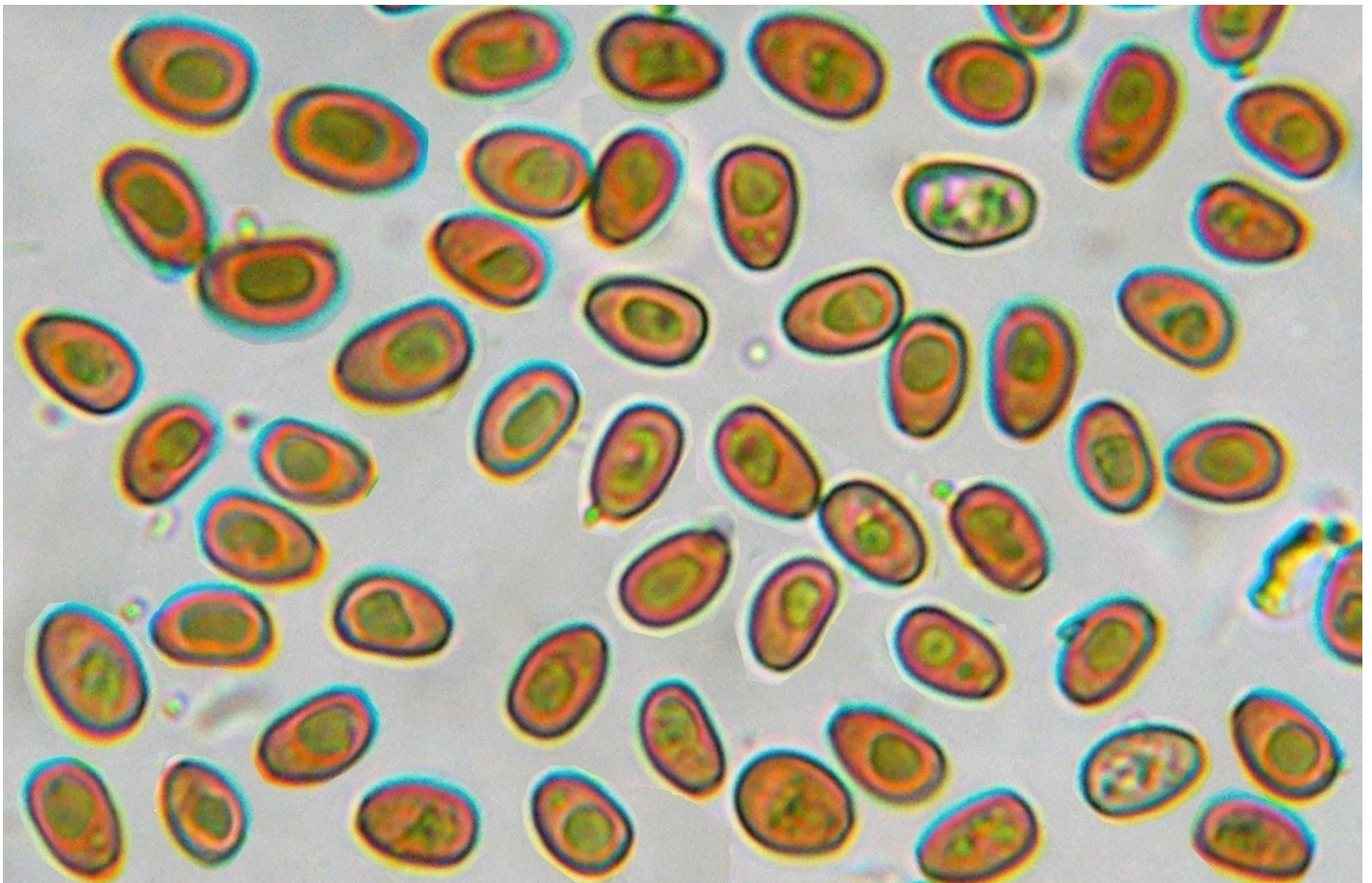


Fig. 3: *P. cloverae*, ANGE581. Spores in water [P. Voto]

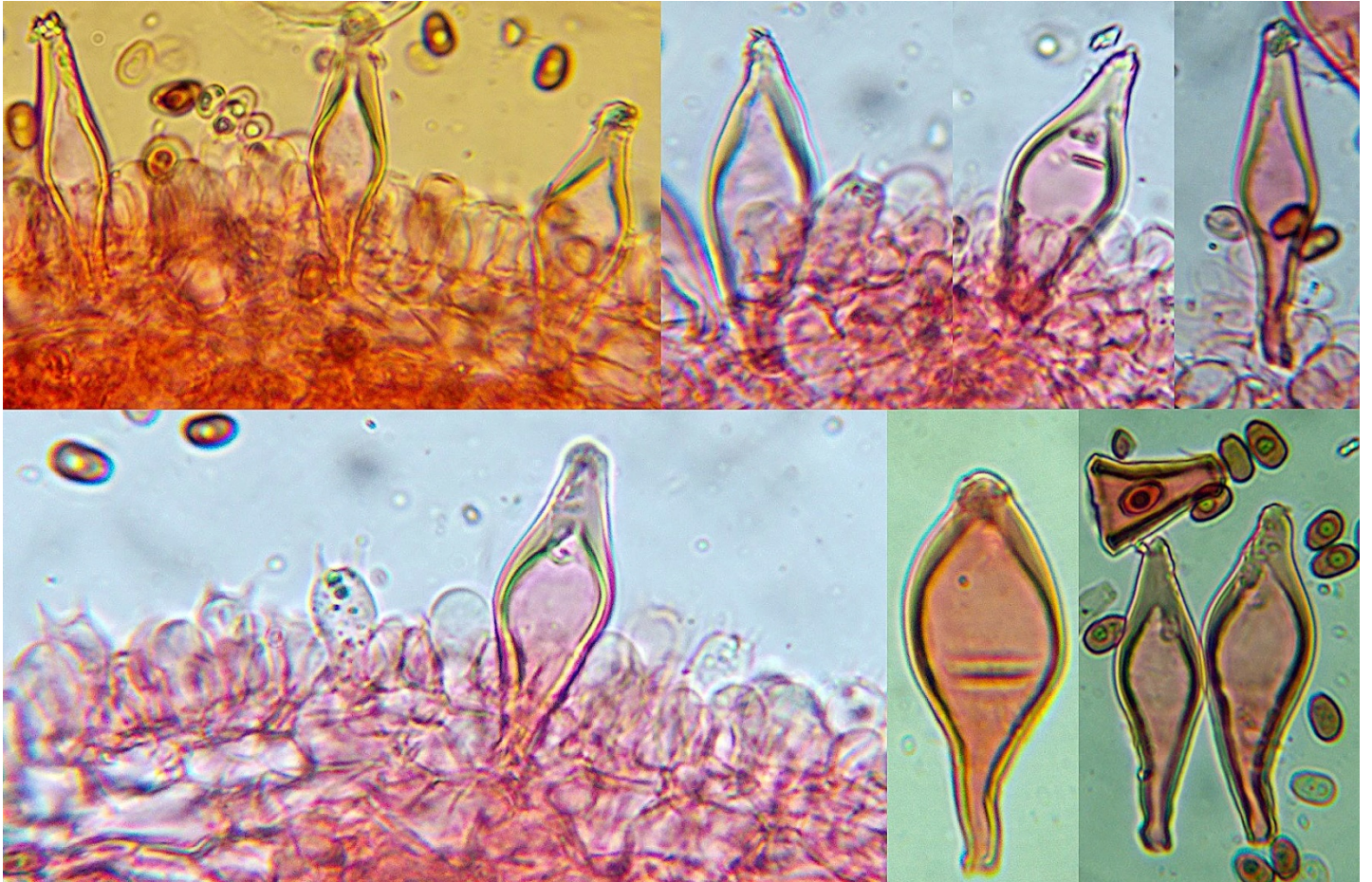


Fig. 4: *P. cloverae*, ANGE581. Pleurocystidia in Congo red [P. Voto]

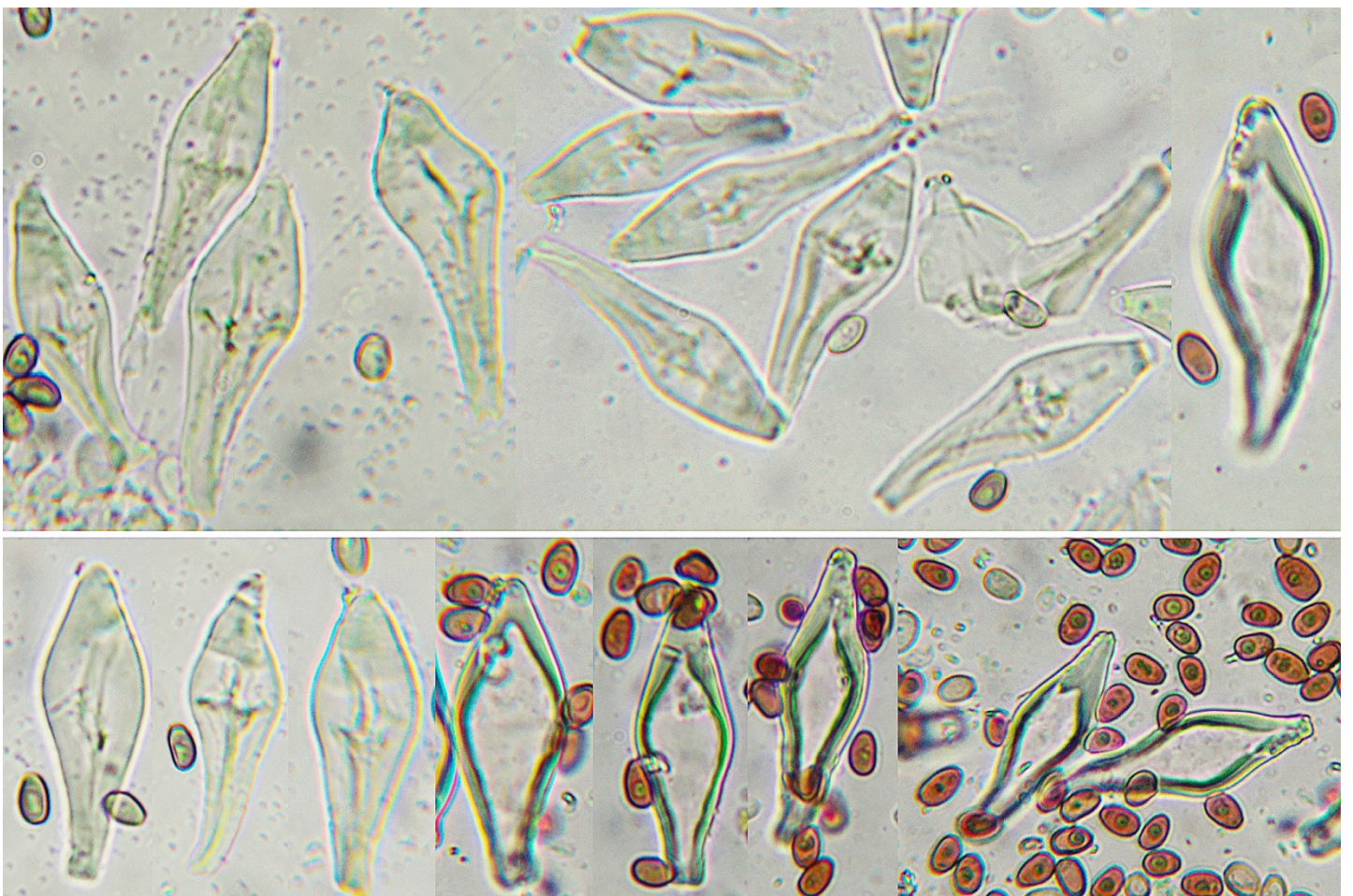


Fig. 5: *P. cloverae*, ANGE581. Pleurocystidia in 5% KOH (top), in ammonia (bottom) [P. Voto]

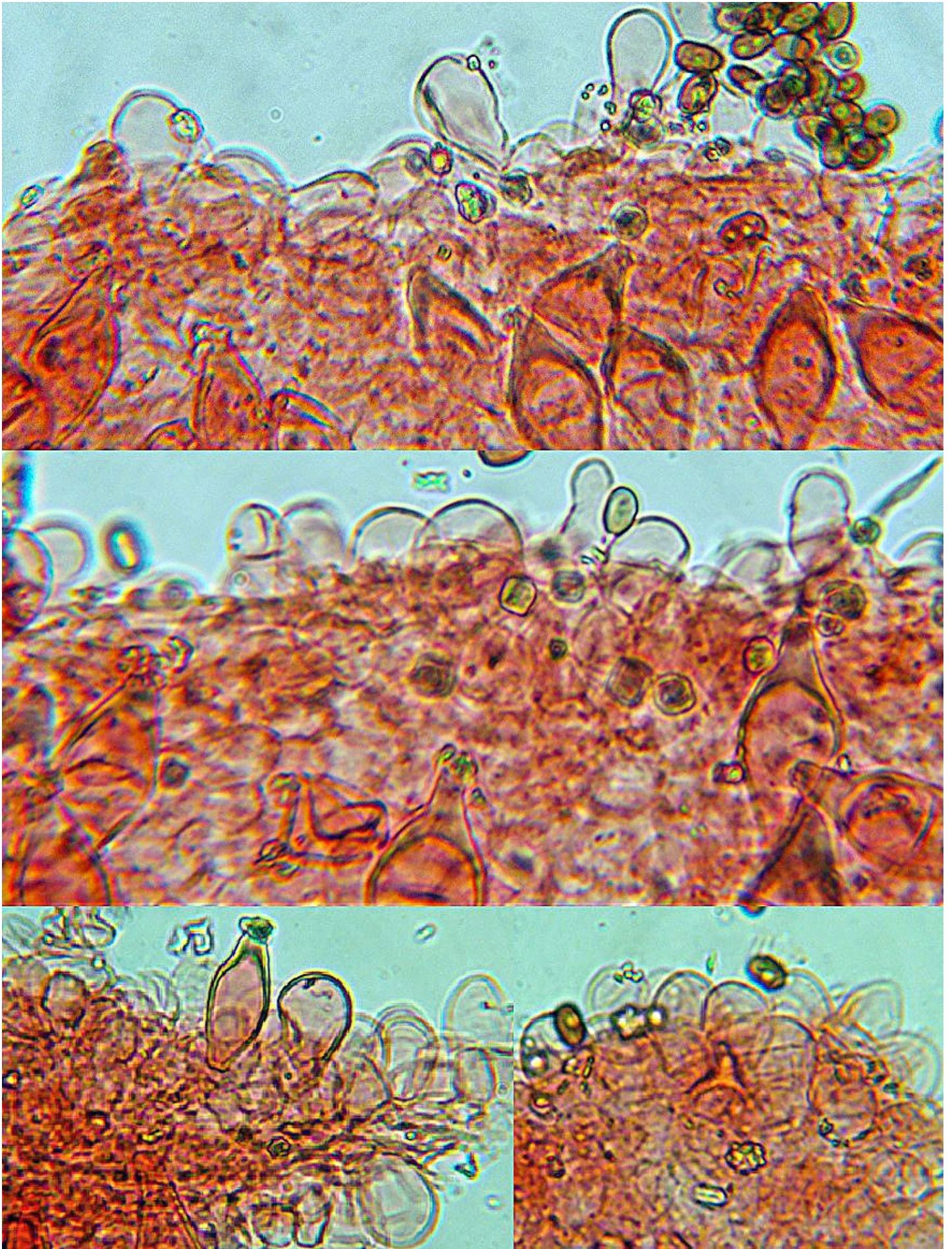


Fig. 6: *P. cloverae*, ANGE581. Gill edge in Congo red [P. Voto]

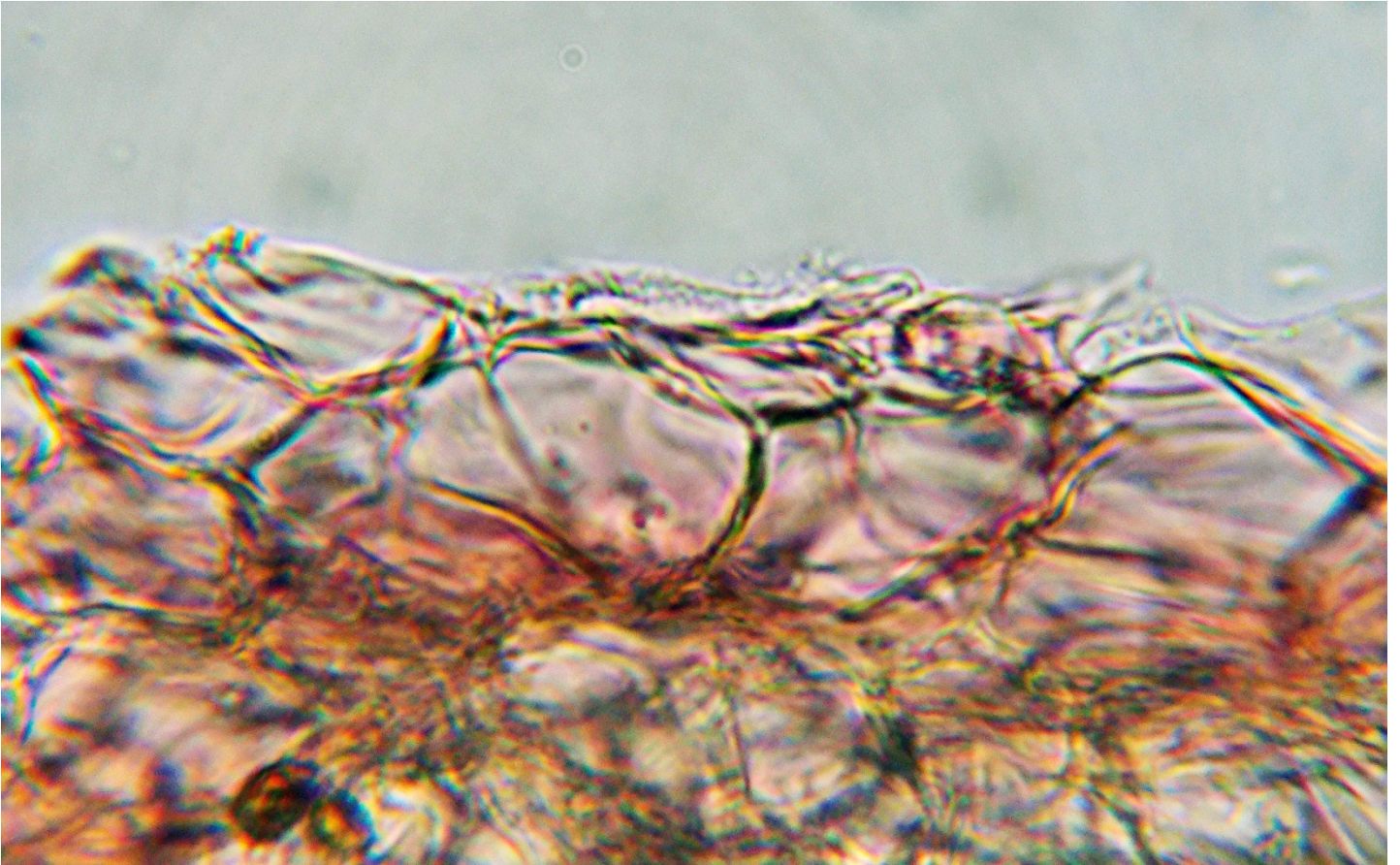


Fig. 7: *P. cloverae*, ANGE581. Pileipellis in Congo red [P. Voto]

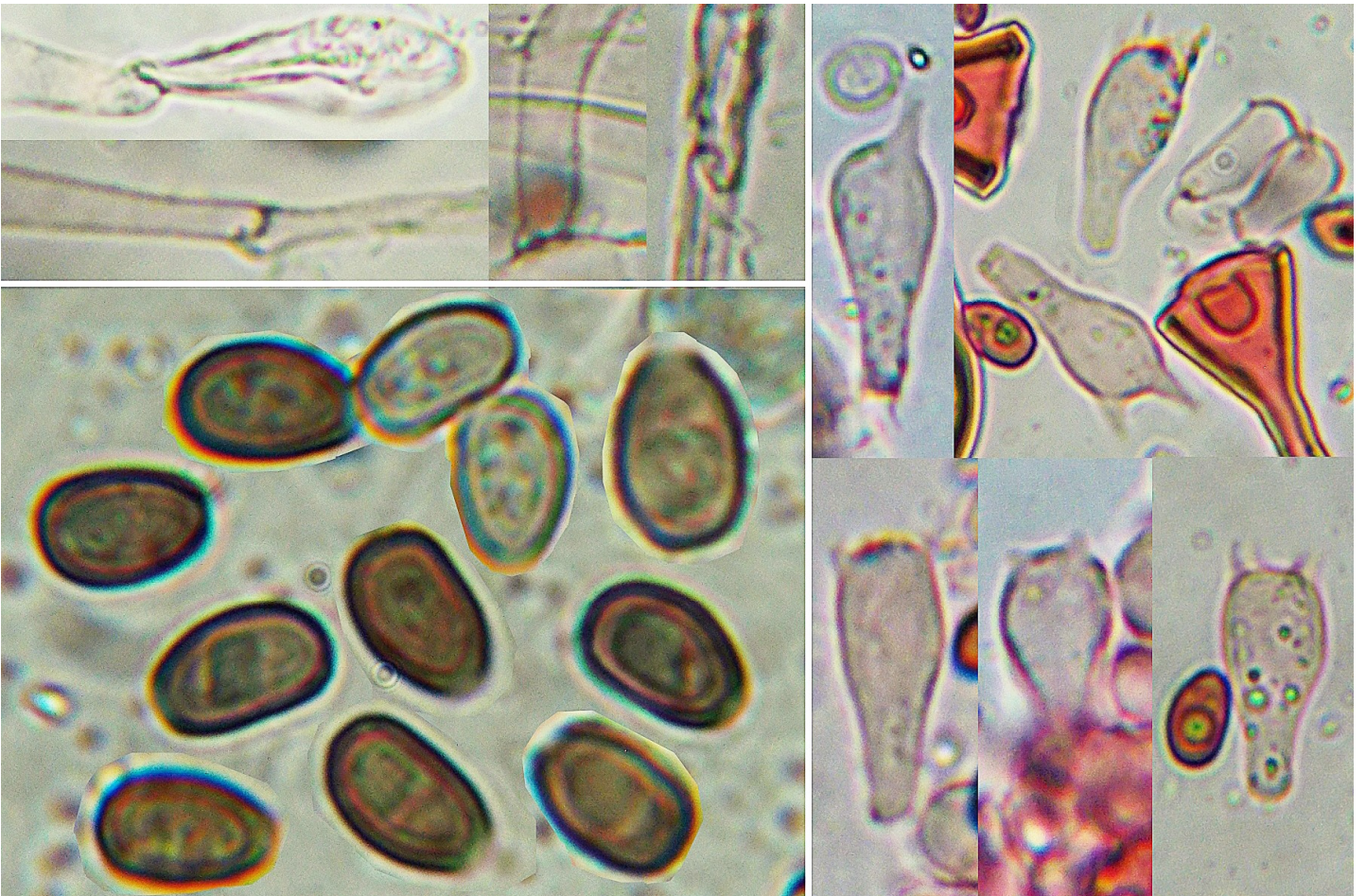


Fig. 8: *P. cloverae*, ANGE581. Clamps in pileipellis in Congo red (top left), spore germ pore in 5% KOH (bottom left), basidia in Congo red (right) [P. Voto]

General description of *Psathyrella cloverae*

(Based on ANGE581 and the original descriptions of all species synonymized)

Macroscopic characters

Pileus 15– 40 (80) mm broad; obtusely conic then through conical-convex to convex, margin sometimes radially lobed when expanded; usually translucently striate up to two thirds of radius; dull cinnamon brown to strong brown, hygrophanous, discoloring from centre through pale brown, dull greyish brown, dull dingy cinnamon brown to honey yellow, yellowish brown, ochraceous-yellow, olivaceous-yellow, finally beige to whitish often with greyish violaceous necropigment from margin; surface lubricous; veil made of a coating of delicate white fibrillose flecks, initially dense, later fugacious.

Lamellae approx. 30– 40 intermixed with 3 – 7 lamellulae, adnate, narrow, possibly intervenose, whitish then dark purplish brown, edge fimbriate, pale.

Stipe (15) 20– 90 (108) × (1.0) 2.0– 6.0 mm, equal or slightly broadening downward (up to 8.0 mm broad), base often abruptly swollen or with a narrow marginate bulb; hollow; at the top flocculose, below at most minutely flocculose-pruinose but glabrescent and silky; white, discoloring pallid above and brownish below.

Context pale brownish, very thin and fragile in pileus, white in stipe; smell indistinctive, taste indistinctive to bitter. Spore print dark (greyish, purplish) brown.

Microscopic characters

Spores (6.5) 7.0 – 9.5 (10.5) × 4.0 – 5.5 (6.0) μm , in face view oblong, elliptic, (broadly) oval, oval-pentagonal, base broadly rounded to truncate, in profile adaxially flattened, subamygdaliform, subphaseoliform to phaseoliform or weakly indented above the base, smooth, in water and in ammonia orange- to reddish- brown, in KOH grey brown (cocoa-colour) or chocolate-brown, in Melzer's reagent tawny to reddish tan; germ pore distinct, 0.8 – 1.5 μm broad, mostly broadly rounded to truncate; apiculus very small.

Basidia 11– 30 × 7– 9 μm , 4-spored, very rarely 2-spored, (narrowly) clavate, hyaline in KOH.

Pleurocystidia metuloid, 33– 90 × 8– 22 (26) μm ; narrowly to ventricose fusoid, also ventricose-lageniform, utriform, mucronate-clavate, pedicel short to elongate; walls 0.7 – 1.4 (2.0) μm thick at extreme base and 3.0 – 5.2 μm thick in neck, often completely occluding the space in extreme apex, hyaline to yellowish in ammonia; apex obtuse to acute, sometimes rounded, mostly with (sub) hyaline incrustations in fresh material; cell body (sub) hyaline in KOH, content not distinctive; very abundant.

Cheilocystidia metuloid like the pleurocystidia but smaller, sometimes simply clavate, not abundant; paracystidia thin-walled, 15– 30 × 7– 13 (17) μm , clavate to ellipsoid or sometimes subutriform, hyaline in KOH, numerous.

Caulocystidia similar to pleurocystidia but generally smaller, somewhat more versiform, possibly thin-walled, and with apex obtuse to subacute; scattered to abundant.

Pileipellis a paraderm composed of a layer of vesiculose to sometimes clavate or pedicellate cells 1 – 4 deep with (sub) hyaline, sometimes subgelatinous wall in KOH.

Clamp connections numerous.

Habitat and collection examined: scattered to gregarious or caespitose, saprotroph on the ground or on rotten deciduous wood in or out of forest, March through September; in tropical to temperate climate, in boreal and austral hemisphere. Brazil: Amazonas, Lago Januári (*P. varzeae*, holotype). Dominican Republic: (ANGE581). Mexico: Chichotla, Mitzalhoc falls (*P. smithii*, holotype). USA: Tennessee, Knox Co., Knoxville (*P. hesleri*, holotype); Texas, Hildalgo Co., Mission (*P. cloverae*, holotype).

NOTES

By this study it emerges that *P. cloverae* is a taxon embracing a series of synonyms described throughout a great portion of the entire American continent, from Northern Brazil to the northern states of the USA, apparently independent of the type of climate and vegetation.

Its distinctive characteristics are its small to medium size; a brown pileus discoloring from the centre into various shades of yellowish and olivaceous shades, conical then convex, deeply striate when fresh; a dense but

fugacious veil of fibrillose flocks on the pileus but scarcely present on the stipe; hyaline to yellowish, metuloid and muricate pleurocystidia; leptoparacystidia and leptocheilocystidia present on the gill edge; spores generally $7.0 - 9.5 \times 4.0 - 5.5 \mu\text{m}$, usually in part oval-pentagonal in front view and in part (sub) phaseoliform in side view, of a medium brown colour and with a large germ pore; a detriticolous habitat.

The three taxa we list among the synonymies represent species which had been separated only by weakly sustainable morphological differences, as commented below.

a) Notes on *Psathyrella hesleri*

In mycoportal.com there are two collections reported with the same date and place of collection (21 July 1948, United States, Tennessee, Knox, Knoxville, Highland Ave., in lawn, 35.96083333 -83.93222222) but with two different names and voucher numbers: *P. cystidiosa*, TENN-F-018562, and *P. hesleri*, TENN-F-017725. P. B. Matheny contributed to resolving this ambiguity by hypothesizing that TENN-F-018562 was considered Peck's true "*P. cystidiosa*" until Smith (1972), examining that collection, described it as a new species, *P. hesleri*, and housed the portion of exsiccata received for study in TENN-F-017725 (holotype) and MICH 11941 (isotype). Then, TENN-F-018562 was never updated with the name change and Smith did not refer to TENN-F-018562 under *P. hesleri* in his monograph.

Smith (1972) only compares *P. hesleri* with *P. subagraria* (G.F. Atk.) A.H. Sm., easily differentiated by thin-walled cystidia, and with *P. spadicea* P. Kumm. [currently *Homophron spadiceum* (P. Kumm) Örstadius & E. Larss.], strongly distinguishable by the absolute absence of veil and pale spores. No comparison is made with the truly closest species, such as *P. cloverae* and *P. cystidiosa*, although he places these species very close to each other in his key.

As no sequences of *P. hesleri* were available in the databases, we tried to sequence the holotype, TENN-F-017725, a non-type collection, MIN 920377, and the isotype, MICH 11941; we succeeded to generate an ITS sequence only from the latter (GB PP476871) and the phylogenetic evidence supports its synonymy with *P. cloverae*.

b) Notes on *Psathyrella smithii*

Guzmán & Johnson (1974) describe this species from a veil-less Mexican collection on the ground among grass, with a striate, fissurated pileus with greyish yellowish brown, darker in the centre, colours, and spores $(6.6) 7.2 - 8.4 \times (4.2) 4.8 - 6 \mu\text{m}$, elliptic-globose in front view, subphaseoliform in side view. They comment that *P. cystidiosa* only differs by the spore size and the absence of incrustations on pleurocystidia (for this assumed absence of incrustations see below, comments on *P. cystidiosa*).

Our revision of the isotype Johnson 1973-051132 (TRTC 154105) follows (Fig. 9-11):

Spores (n = 123) $(6.29) 6.65 - 7.74 (8.46) \times (3.85) 4.41 - 5.16 (5.56)$, on average $7.14 \times 4.76 \mu\text{m}$; Q = $(1.24) 1.36 - 1.64 (1.82)$, on average 1.51; inconspicuously tridimensional, in front view elliptic, ovoid, subpentagonal, base broadly rounded to truncate or sometimes weakly oblique to indented (like in *P. albescens*), in side view adaxially flattened to subphaseoliform or sometimes phaseoliform to subamygdaliform; in water orangish brown, in ammonia more brownish; germ pore distinct but small, $0.8 - 1.5 \mu\text{m}$ broad.

Pleurocystidia $35.0 - 72.0 \times 10.0 - 34.5 \mu\text{m}$; fusiform to claviform, sometimes utriform, apex in fusiform and utriform cells obtuse to sometimes subacute or mucronate, peduncle short to elongate and narrow to stocky; walls up to $2.5 \mu\text{m}$ thickened towards the apex, greenish to weakly yellowish in ammonia; apical crystals mostly scarce to absent.

Clamp connections numerous.

The spore shape of *Psathyrella smithii*, as described in the protolog and as observed in our revision, is precisely the same as the shape found in the Dominican material (compare figure 40 in Guzmán & Johnson 1974 and our figure 3). Pleurocystidia walls are hyaline to pale coloured as in *P. cloverae*. We find no differences between *P. smithii* and *P. cloverae*.

Unfortunately, we have made multiple unsuccessful attempts to sequence this isotype Johnson 1973-051132 (we housed the remaining material at PAD H0062120 as isoparatype). We could not obtain the holotype at ENCB (Mexico).



Fig. 9: *P. smithii* isotype Johnson 1973-051132 (TRTC 154105). Exsiccata received [P. Voto]

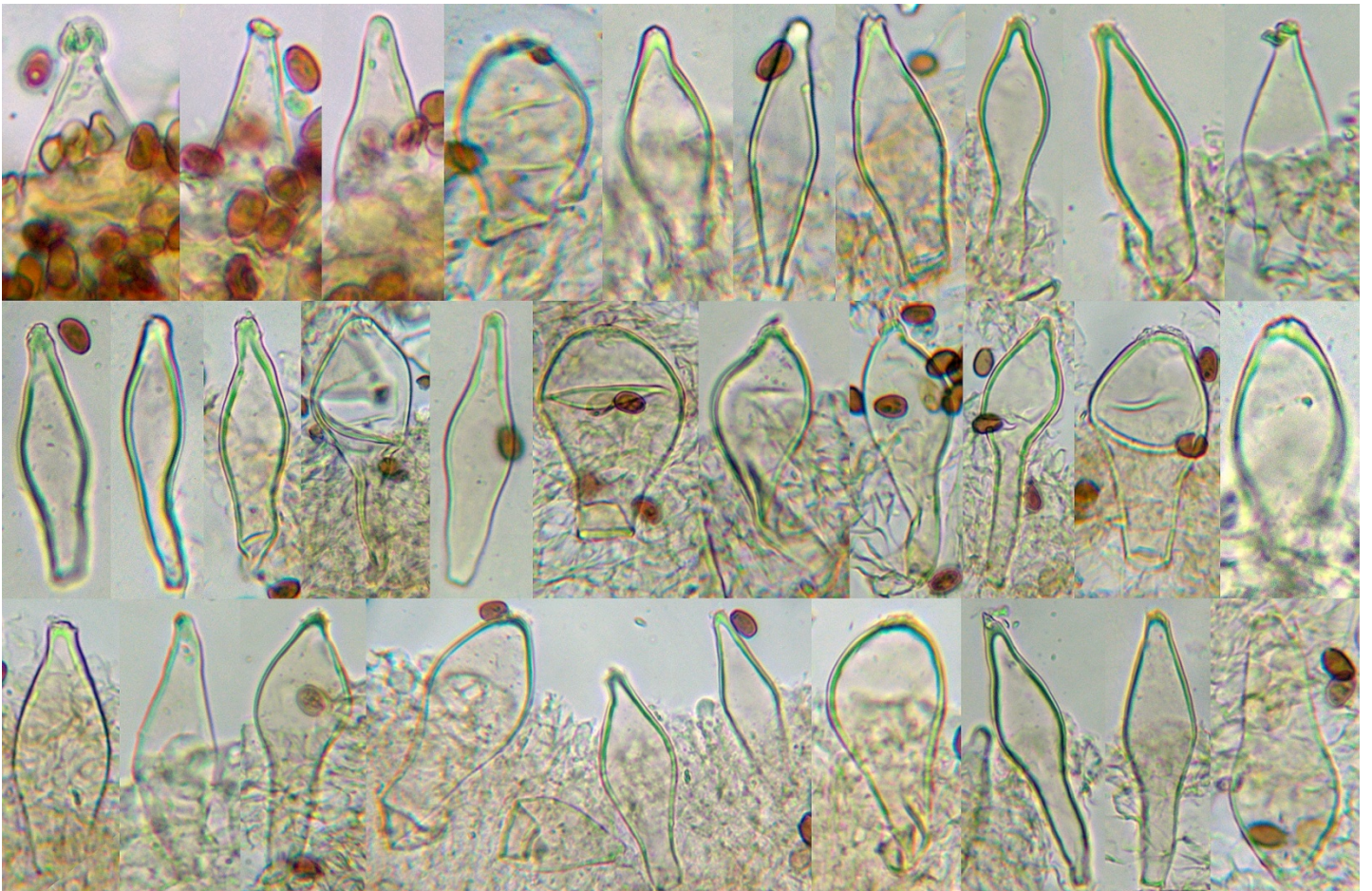


Fig. 10: *P. smithii*, isotype Johnson 1973-051132. Hymenial metuloid cystidia in Congo red [P. Voto]

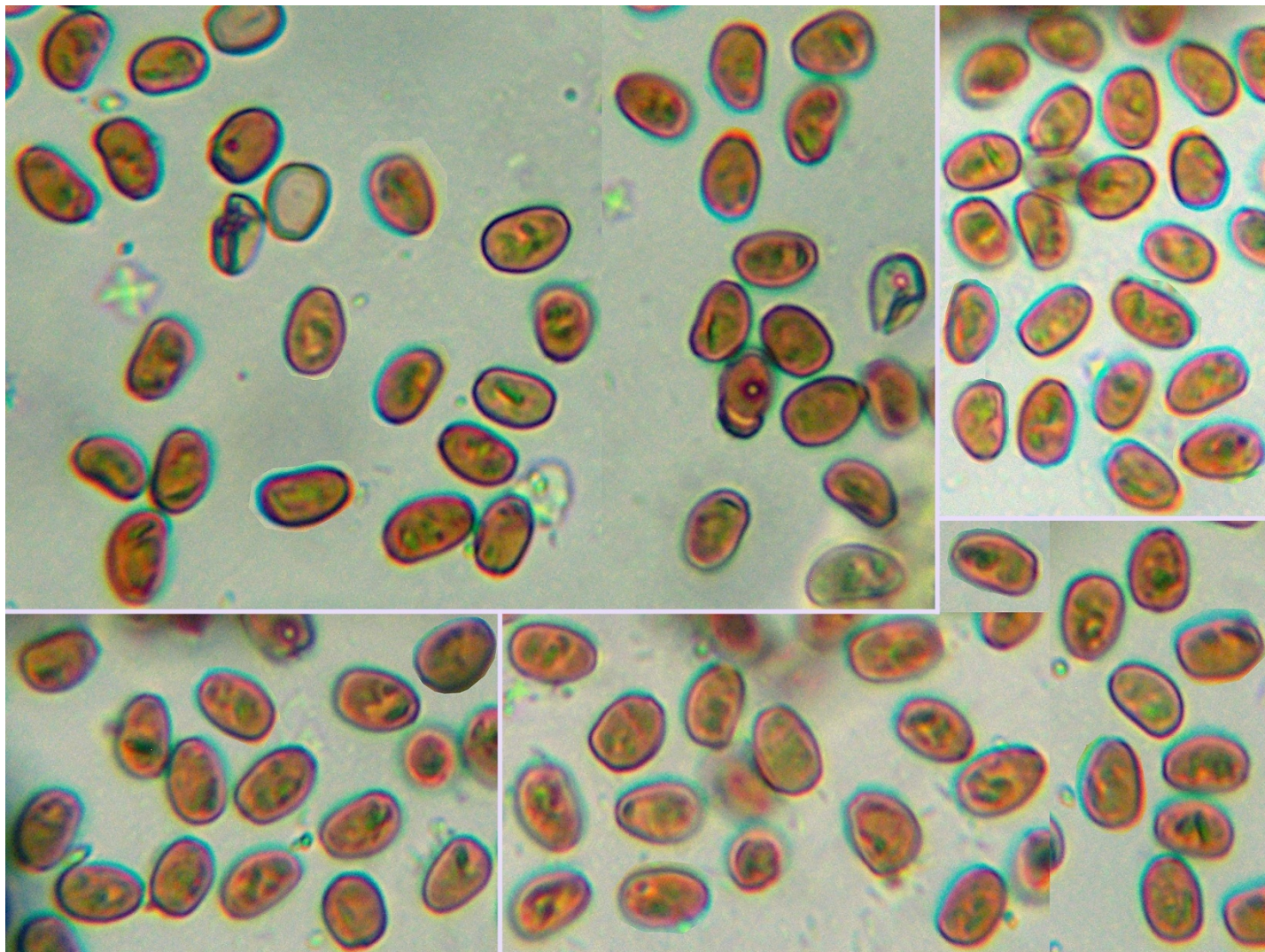


Fig. 11: *P. smithii*, isotype Johnson 1973-051132. Spores in water [P. Voto]

c) Notes on *P. varzeae*

Singer (1989) only compared this species with *P. cystidiosa*, ignoring *P. hesleri*, *P. cloverae* and *P. smithii*. He differentiated it from *P. cystidiosa* by a truncate germ pore, a darker colour of the pileus, somewhat shorter spores and metuloid cystidia, a longer stipe, and the habitat in varzea vegetation of the Amazon forest. All these morphological characters are usually subject to an intraspecific range of variability in *Psathyrella* species. We succeeded generating an ITS sequence (GB PP476872) from the holotype, F 1053727, voucher Singer B 12430, and the phylogenetic evidence supports the synonymy with *P. cloverae*.

***P. cystidiosa* (Peck) A.H. Sm., Mem. N. Y. Bot. Gard. 24: 250. 1972.**

Basionym *Psilocybe cystidiosa* Peck, Bull. N.Y. St. Mus. 167: 46. 1913 ('1912')

Smith (1972), in his observations, only distinguished *P. cloverae* from *P. cystidiosa* by the copious universal veil and long narrow basidia of the former but he himself specified that the veil of *P. cloverae* is delicate, therefore much liable to be often found already reduced or absent as occurred, for example, in the holotype collection of the synonymous *P. varzeae*.

Also, Smith (1972) did not mention any incrustations on the pleurocystidia of *P. cystidiosa* but they are reported as present in the description by Padamsee (2001).

In addition to the Smith's (1972) description, we gathered some additional descriptive information as follows.

Padamsee (2001) (cumulative description of four collections examined) reports the pileus deep yellowish brown at margin and medium yellowish brown at centre when young, light greyish yellowish brown at margin

and light yellowish brown at centre when mature, mostly glabrous with scattered micaceous particles, slightly ridged, possibly rugulose; spores (7.5) 7.9 – 9.5 × (4.1) 4.3 – 5.1 (5.3) μm, on average 8.3 – 8.9 × 4.7 – 4.9 μm, obovate in front view, reniform in side view; pleurocystidia crystalliferous and with yellowish thickened-walls; habitat on ground or on fallen branches.

Kuo's website (see reference) reports a collection from Illinois identified as *P. cystidiosa* and described with the pileus brown and drying out to yellowish buff or brownish, the margin becoming finely lined, no veil seen on about 20 specimens collected, spores 7 – 9.5 × 4 – 5 μm, crystals present in the image of the pleurocystidia.

The Minnesota Department of Natural Resources (see reference) hosts a page with some data on *P. cystidiosa*: pileus up to 30 mm broad, with concentric zones of colour from light yellowish brown at the centre to deep yellowish brown towards the edge; stipe 16-50 × 1-3 mm, white; habitat on soil or on dead wood in shady places of deciduous forests; the species is considered rare and endemic to Minnesota (USA).

McLaughlin (1998, 1999) also offers some comments on Minnesota's collections.

Since no ITS sequences of *P. cystidiosa* were available in the databases, we attempted to sequence five non-type collections (MIN 859496, MIN 871863, MIN 871864, MIN 871865 and MIN 890991) the first four of which from Padamsee (2001) which we consider a reliable reference for this species. We obtained an ITS sequence only from the first (GenBank acc. no. PP469638). The LSU region of MIN 859496 had been previously sequenced (Padamsee *et al.* 2008). Because these two sequences are nested beside the holotype of *P. pivae* Heykoop, G. Moreno & M. Mata, outside the clade of *P. cloverae*, there is sufficient phylogenetic support to consider *P. cystidiosa* different from *P. cloverae*. Morphologically, *P. pivae* differs by larger spores.

From all morphological data that we have gathered on *P. cystidiosa*, it seems that a solid morphological difference between it and *P. cloverae* is difficult to delineate. Following Smith (1972) the scarcity of veil of *P. cystidiosa* from young stage seems to be a sufficiently appreciable difference (the pileus is glabrous in the basionym description by Peck 1913). This is so at least in case of collections with distinctly developed veil which would exclude *P. cystidiosa*.

Furthermore, all the descriptions consulted seem to indicate two other possible differences regarding the pileus.

A distinct striation is usually reported for *P. cloverae* while it is defined as absent to scarce for *P. cystidiosa* (the pileus is sometimes obscurely striate on the margin when dry in the basionym description by Peck 1913).

The pileus colour of *P. cystidiosa* is usually reported as pale brown to deep or medium yellowish brown discoloring to light (greyish) yellowish brown colour (it is defined as pale brown when moist, and yellowish drab with a brownish centre when dry in the basionym description by Peck 1913). In contrast, for *P. cloverae* the colour is saturated brown, cinnamon brown to strong brown or tawny discoloring to greyish brown, pale cinnamon, honey-yellow, often with an olivaceous shade. These two differences are evidently only partial as they are strongly conditioned by the degree of freshness and discoloration of the specimens due to hygrophanity.

Finally, Smith (1972), citing Peck (1913), reports that the pileus of *P. cystidiosa* sometimes becomes lacerate when expanded; this character was not reported by Padamsee (2001) but was detected in the *P. smithii*'s type collection by Guzmán & Johnson (1974).

Eventually, cases will occur where only the molecular analyses can differentiate the two species with certainty.

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