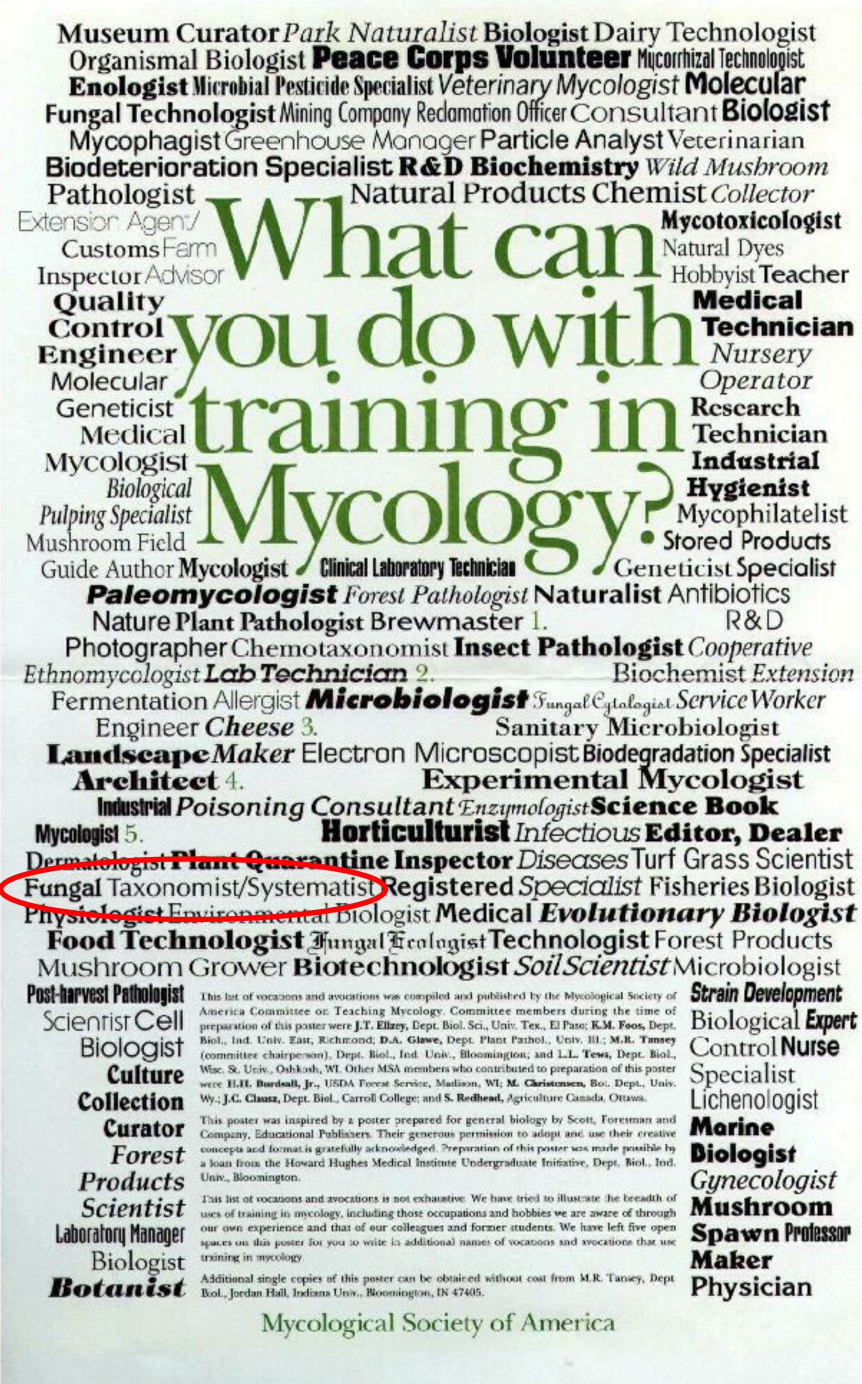
Morphology in the age of molecular techniques

Slavomír Adamčík, Miroslav Caboň, Dušan Senko, Brian P. Looney



Warning
to taxonomists?

making new species
is only a gate to science !

Taxonomic tools

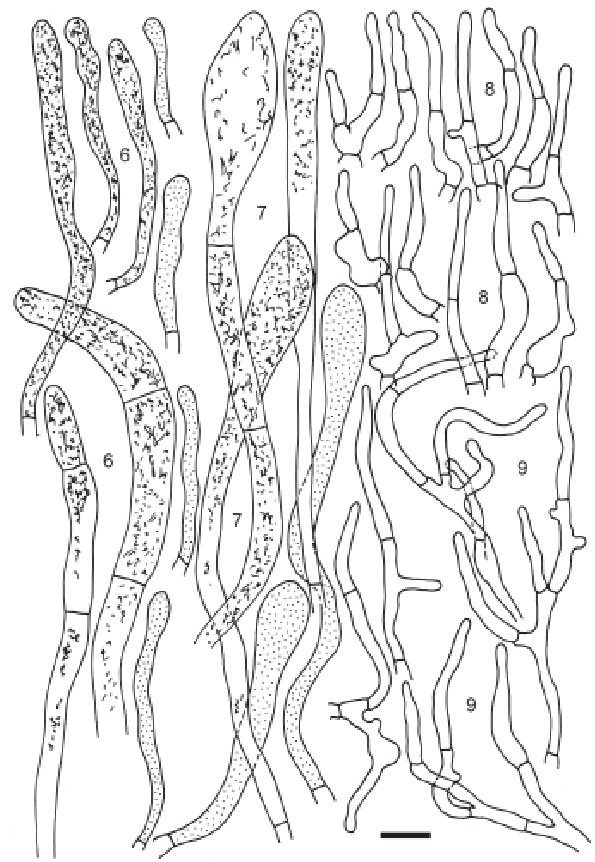
field description

MORPHOLOGY

PHYLOGENY

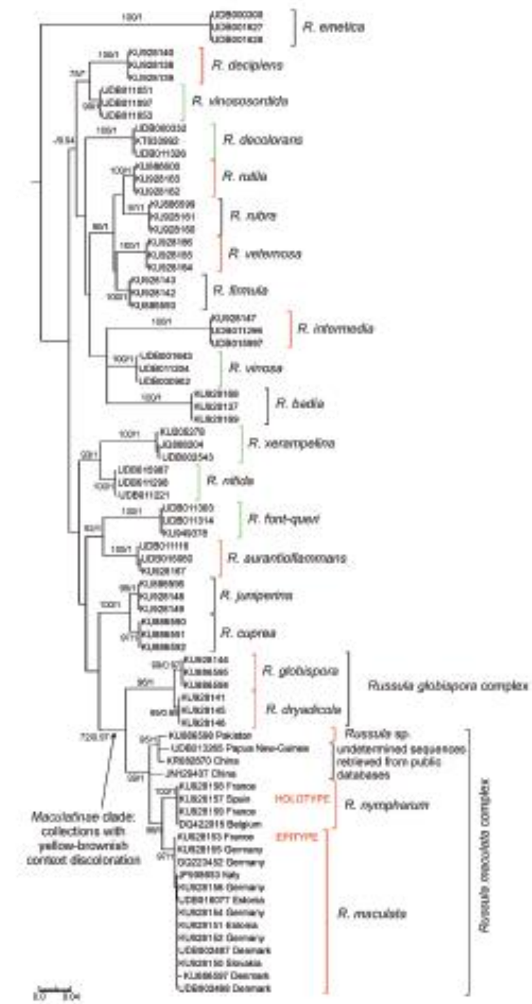
microscopy

sequencing



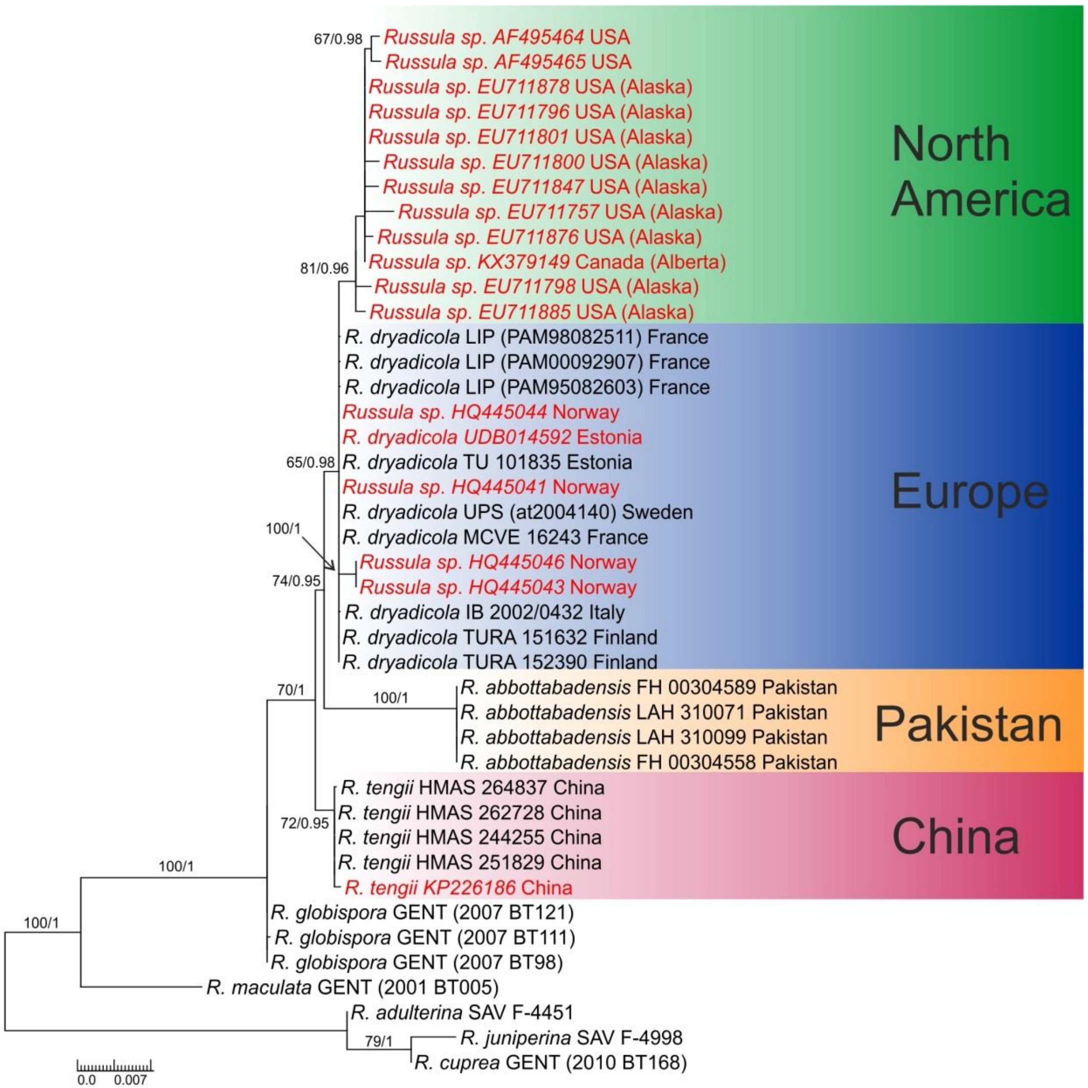
FIGURES 6–9. *Russula nympharum* (holotype). **6.** Pileocystidia near the pileus centre. **7.** Pileocystidia near the pileus margin. **8.** Hyphal terminations in the pileus centre. **9.** Hyphal terminations near the pileus margin. Contents of cystidia are represented as observed in Congo Red for some elements only, the others are simply filled with dots to indicate their cystidial nature. Scale bar equals 10 µm. Drawings by: S. Jančovičová.

80 • *Phytotaxa* 270 (2) © 2016 Magnolia Press ADAMČÍK *ET AL.*



Taxonomy's changing

Sanger sequencing is not dominating any more
it is replaced by 2nd generation sequencing (Illumina, Pack Bio, MinION, ...)



prior to our study, most published sequences in *R. dryadicola* lineage were environmetal

phylogeny, ecology and biogeography offer more reliable arguments for species delimitation

=

morho-species is replaced by phylogentic species and MOTUs

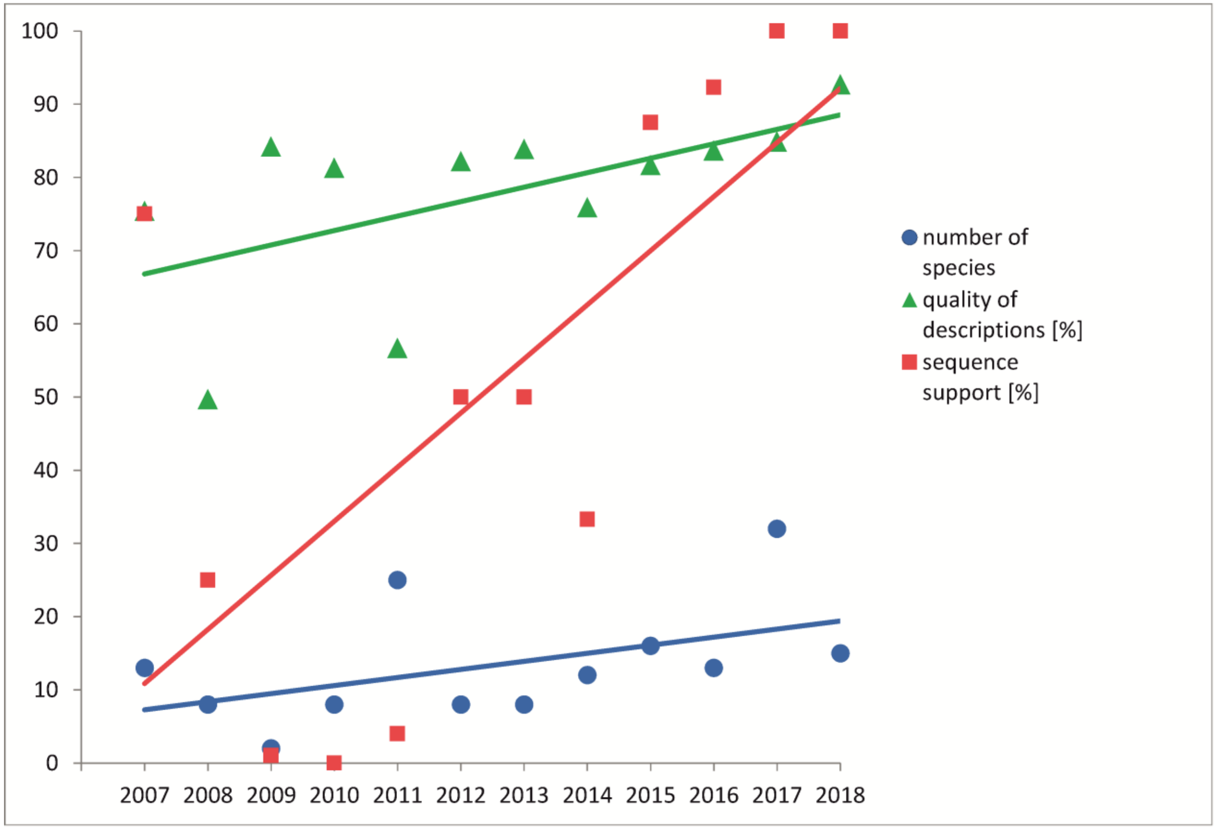
Decreasing role of morphology

Russula

ECM symbiont

More then 2000 species worldwide

ca. 160 species described since 2007 to 2018



Morphology is becoming only a formal necessity

Mycologists rely on molecular identifications, expecially in ecological studies

The traditional view of morphology

type studies and species concepts

species identification

- 1 Spores with isolated prominent spines
- 2 Pileus with predominantly pale red, pink, ochre and cream colours; spores mainly up to 8.5 µm long; usually associated with *Fagus* .... ***R. veternosa***
- 2* Pileus with predominantly wine-red, blue-red, purple and red-brown colours; spores mainly longer than 8.5 µm; usually associated with coniferous trees .... ***R. firmula***
- 1* Spores with warts merged in chains and connected by occasional lines
- 3 Basidiomata medium sized to large (60–100 mm), with thick context turning slowly grey; pileus cuticle velutinous or matt; spore print ochre (IIIb–IIIc) .... ***R. rubra***
- 3* Basidiomata small to medium sized (30–70 mm); context soon becoming fragile, thin and not turning grey; pileus cuticle shiny at least near the pileus margin; spore print yellow (IVb–IVd)........................ ***R. rutila***

species descriptions and delimitations

Russula nympharum F. Hampe & Marxm., *sp. nov.* Figs. 4, 6–15
MycoBank no.:—MB 816289.

Etymology.—The species epithet refers to the collection site (Val des Nymphes) of two of the paratypes one of which was illustrated in Marxmüller (2014).

Holotype (designated here).—SPAIN. Mallorca: Bunyola, associated with *Quercus ilex* and *Arbutus unedo*, 15 December 2011, *FH11121505* (GENT).

Short diagnosis.—Basidiomata relatively large and with firm, thick context, surface of stipe, pileus and lamellae with yellow-brownish spots, pileus cuticle red or orange and discolouring to cream, taste acrid, spore print yellow, spore ornamentation with low (up to 0.6 µm), amyloid warts often merged or connected by line connections, hymenial cystidia relatively numerous, hyphal terminations in pileipellis near the pileus margin mainly cylindrical, pileocystidia near the pileus margin 6–12 µm wide (on average wider than 7 µm).

Does bad morphology help?

80y old lady Cecilia Chimenez said:

- With nothing but good intentions I did what I believed was the right thing.
- The priest knew it.
- We used always repair things in our church ourself.

Mycology often goes as far as changing species concept using morphology

What can we do better?

sampling

field descriptions

photography

drawings

sequencing

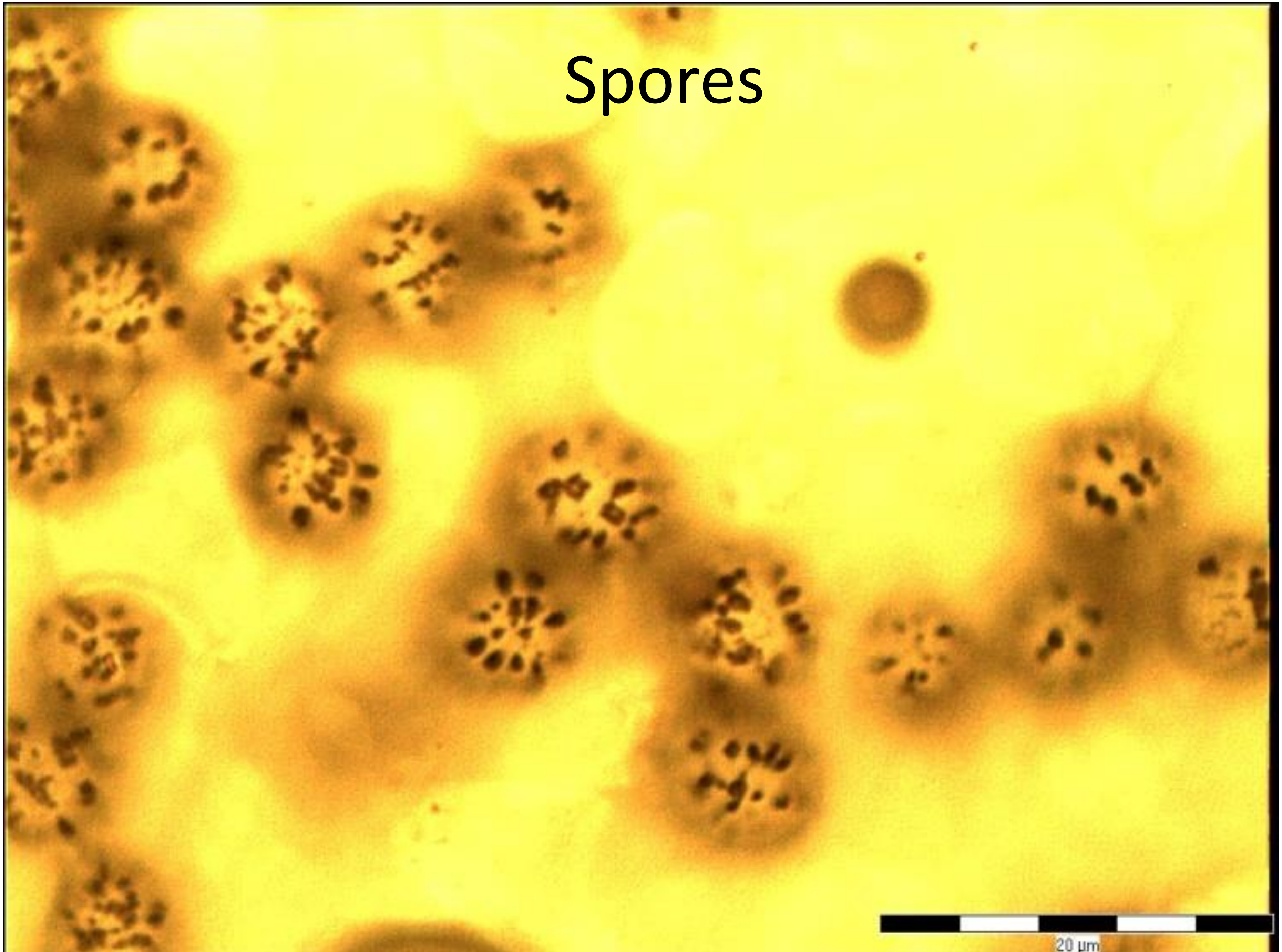
type studies

phylogentic analyses

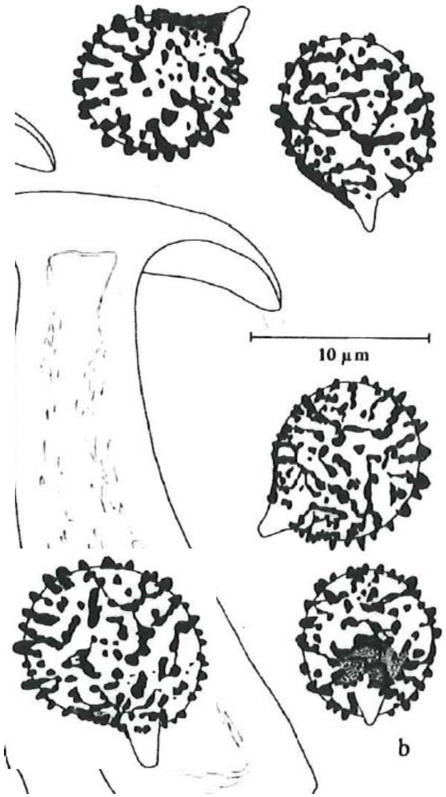
MICROSCOPY !

Quality of descriptions depends on region and author and is very variable

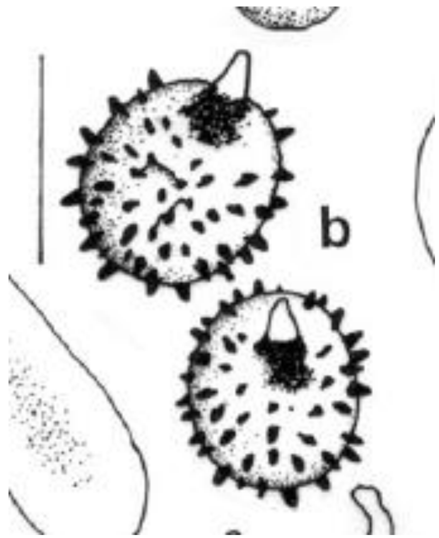
Spores



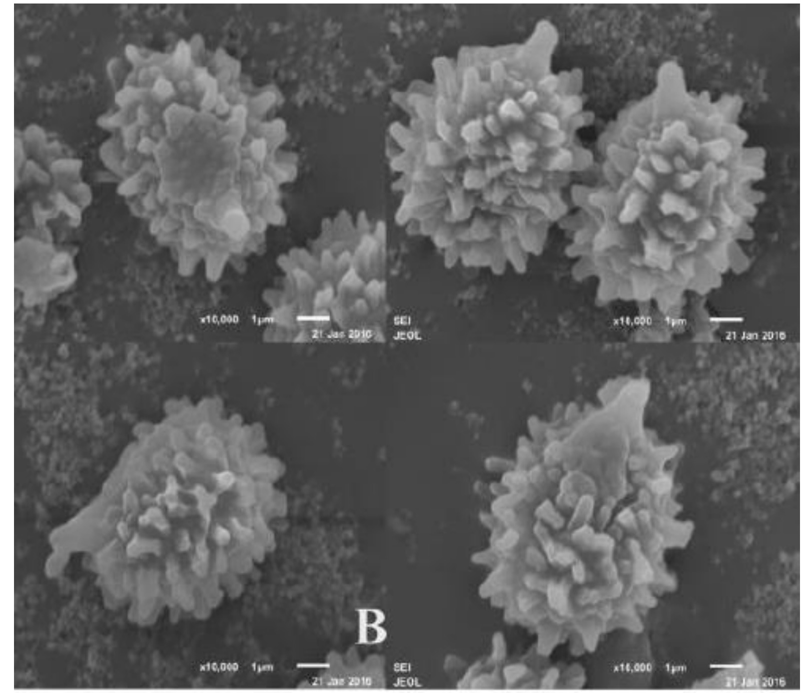
Examples



Europe



India



China

ratio of length and width – prominence of ornamentation – structure of ornamentation

Spores in mass ochre (Romagnesi IIIa–IVa), (8.3–)8.5–9.4–10.5(–11.0) × (7.0–)7.3–8.1–9.1(–9.5) µm, total range of mean values 8.9–9.8 × 7.6–8.6 µm, Q = 1.1–1.16–1.2(–1.3), total range of mean Q values 1.14–1.19 (120 spores from 6 collections); subglobose, amyloid, with partial reticulum, warts up to 1.5 µm high, blunt to aculeate, plage amyloid. *Basidia* 39–48–56 ×

Basidiospores 7.7–11.5 x 6.2–9 µm, globose, subglobose, broadly ellipsoid to ellipsoid (Q = 1.05–1.4); ornamentation amyloid, composed of numerous conic warts, up to 1.75 µm high, rarely connected by fine ridges.

–Basidiospores (Fig. 2B) [52/2/2] (6.1) 6.2–8.5 (8.8) × (5.1) 5.3–7.1 (7.8) µm, [Q = (1.0) 1.03–1.31 (1.43), Q_{av} = 1.18 ± 0.09], subglobose to broadly ellipsoid, rarely globose or ellipsoid; ornamentation amyloid; warts bluntly conical to subcylindrical 0.7–0.9 µm in height, isolated or connected at base or ridges, not forming a reticulum; plage distinctly, amyloid; hyaline in 5% KOH. –Basidia

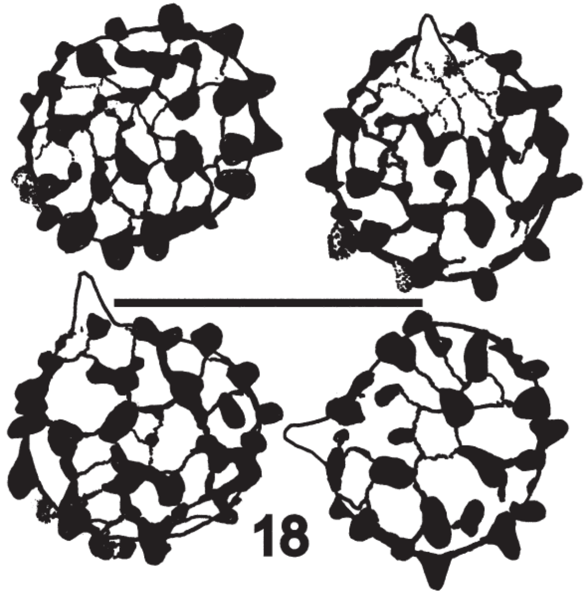
with partial reticulum

warts rarely connected by fine ridges

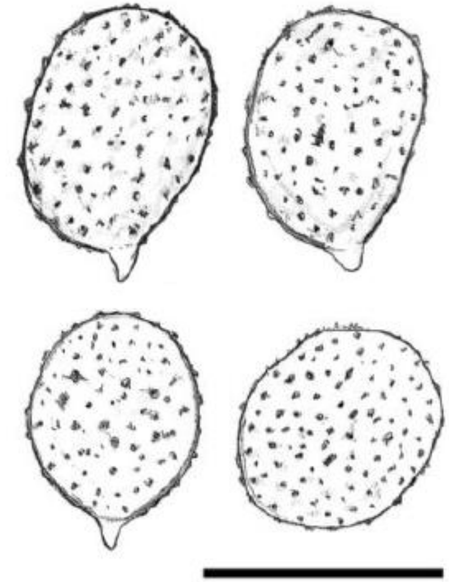
isolated or connected at base or ridges

Examples

Africa



South America



North America

ratio of length and width – prominence of ornamentation – structure of ornamentation

Spores subglobuleuses à ellipsoïdes, 6-*6,95*-8 × 5-*6,11*-7 µm, (Q = 1,00-*1,15*-1,40, n = 30), à ornementation à peine visible, même dans le Melzer, très densément ; plage non amyloïde.

très densement

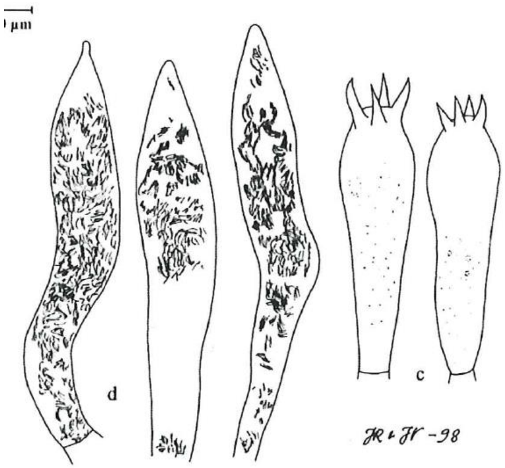
Basidiospores (6.8–)7.2–7.46–8 × 6–6.8(–7.2) µm (Q = 1.06–1.13–1.27), subglobose to broadly ellipsoidal; ornamentation reticulate or incompletely reticulate; ornamentation consisting of partial crests with short lateral diverticulations and large broadly conical or multiplex blunt spines, 1.6–2.8 µm high, connected by fine lines or verrucae, strongly but often partially amyloid; suprahilar plage moderately large, verruculose, barely decurrent on apiculus.

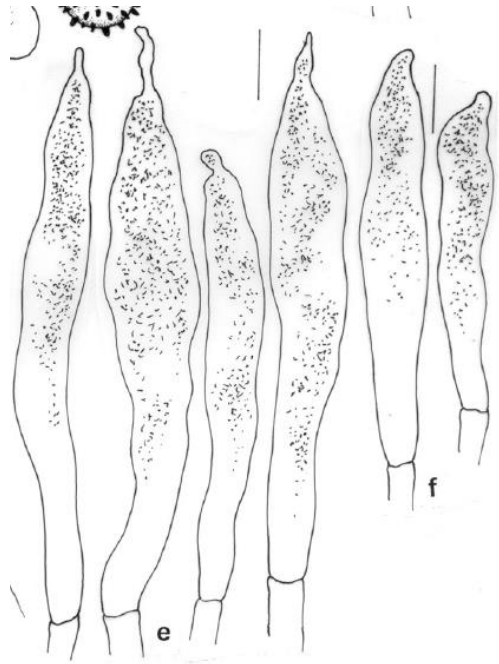
Spores deposit white (Codice Romagnesi Ia), spores 7–10 × 6–8 µm (avQ=1.2, *n*=30), subglobose, broadly ellipsoid to ovoid, weakly ornamented with low (<0.5 µm) amyloid warts connected in some places by very fine lines (the amyloid warts are so small and close together that the whole spore appears weakly amyloid).

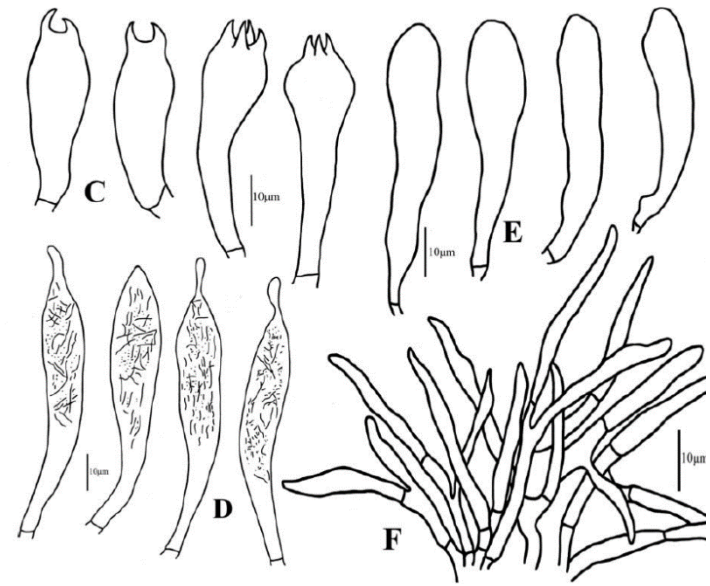

hymenium
20 µm

basidia and basidiola – hymenial cystidia on sides – hymenial cystidia on edges – marginal cells

Examples







China

Europe

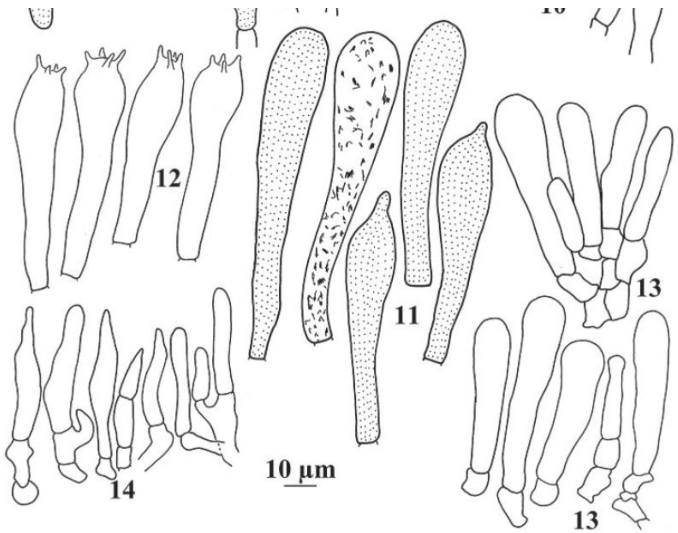
India

Basidia 39-48-56 x 12- 14-16 µm (n = 31), clavate, 4-spored. **Hymenial cystidia** 10-15 µm broad, blunt to appendiculate.

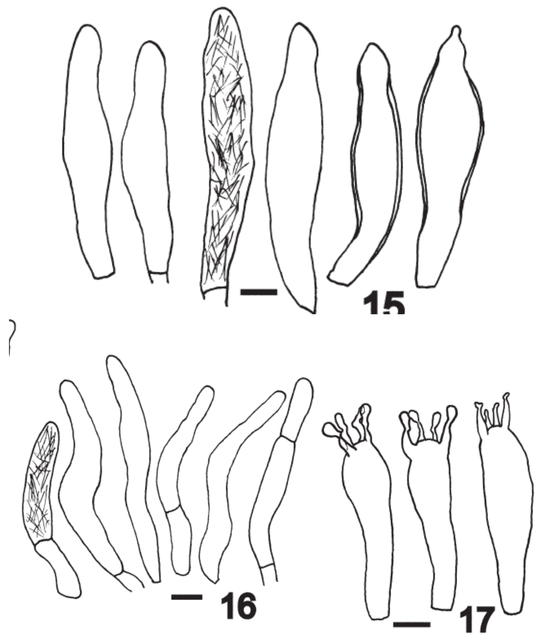
Basidia 40–50 x 7–9 µm, subclavate to clavate, 4-spored; sterigma up to 6 µm long. **Pleurocystidia** 65–125 x 7.7–15 µm, emergent up to 40 µm, abundant, fusiform or with acute, acuminate to narrowly moniliform apex; contents dense. **Lamellae edge** sterile with few cystidia. **Cheilocystidia** 46–70 x 6–9 µm, fusiform; contents dense. Subhymenium layer up to 20 µm thick, cellular.

Basidia 14 (Fig. 2C) 32–46 (52) × 10–13.5 µm, narrowly clavate to clavate, inflated towards 15 upper half, 4-spored, rarely 2-spored, hyaline in KOH, sterigmata about 2.5–4.5 µm 16 long. – **Pleurocystidia** (Fig. 2D) 18 54–89 × 9.0–13µm, abundant, narrowly clavate to clavate, often apex with papillate 19 appendage, with abundant granular contents in the upper part, red to a slightly 20 purplish red with weakly grey in SV. – **Cheilocystidia** (Fig. 2E) 43–82×7–13 21 µm, rare, clavate with rounded or indistinctly mucronate apex, few with granular contents.

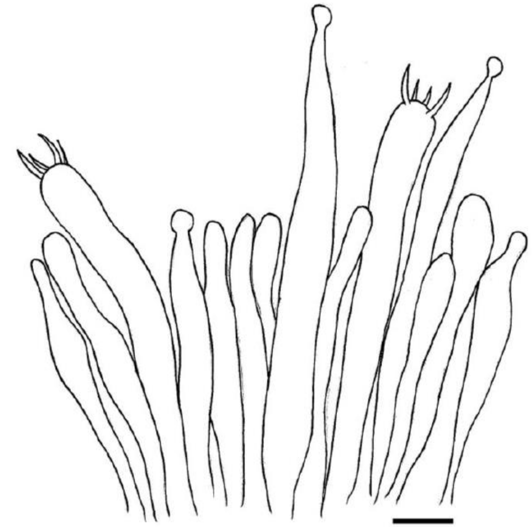
Examples



Africa



South America



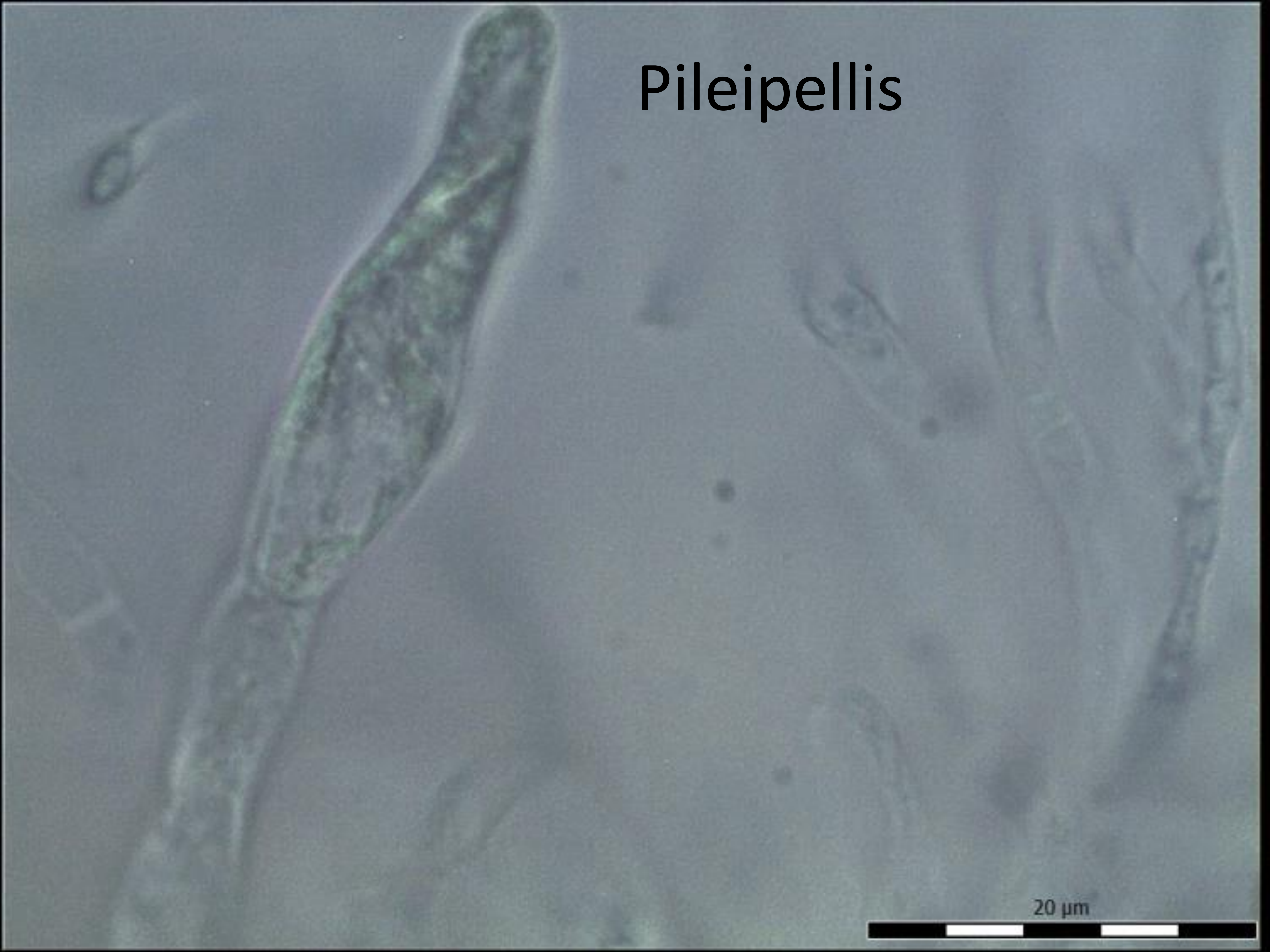
North America

basidia and basidiola – hymenial cystidia on sides – hymenial cystidia on edges – marginal cells

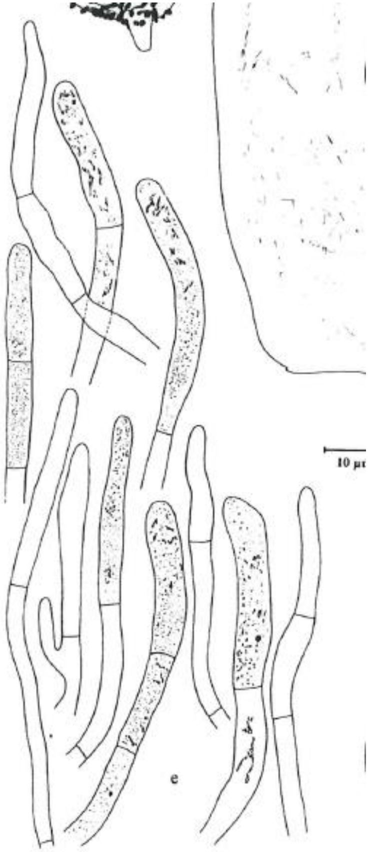
Basides 64-92 × 16-22 µm, clavulées, bi – à tétrasporiques; stérigmates assez petits, 5-7 × 1,5-3 µm. **Cystides** nombreuses, 90-140(155) × 18-26 µm, très apparentes et très volumineuses, à paroi légèrement épaissie, obtuses-arrondies à mucronées, à contenu variable, allant d'optiquement vide à pailleté très abondant. **Cellules marginales** petites, étroites, s'amincissant fréquemment vers le haut.

Basidia 55–62 × 12–15 µm, subclavate to nearly cylindrical, 4–spored; sterigmata stout 7–10 × 2–3 µm. **Cystidia** 80–95 × 15–20 µm, subclavate to subfusiform, thin or thick walled, emergent for ca. 20–30 µm, numerous, arising from gloeopleurous elements, with refringent to crystalline contents, SV+, thick walled lamprocystidia present but not numerous. **Marginal cells** 55–105 × 8–15 µm, narrowly subclavate, tortuous, thin-walled, optically empty, abundant. Subhymenium distinct, a gelatinous layer composed of interwoven cylindrical flattened and variously swollen hyphae of 2–5 µm diam. on apiculus.

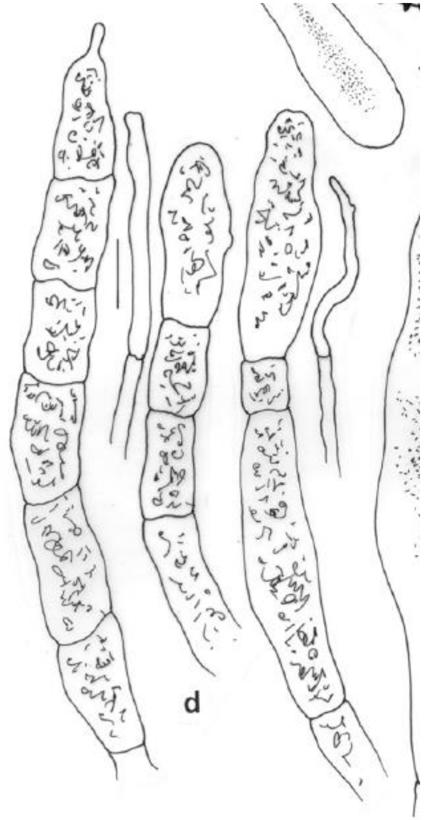
Pleurocystidia and **cheilocystidia** 50–90 × 6–12 µm, elongate-fusoid. **Basidia** 48–63 µm long, 5–7.5 µm thick; sterigmata 5–6.3 µm long.


Pileipellis
20 µm

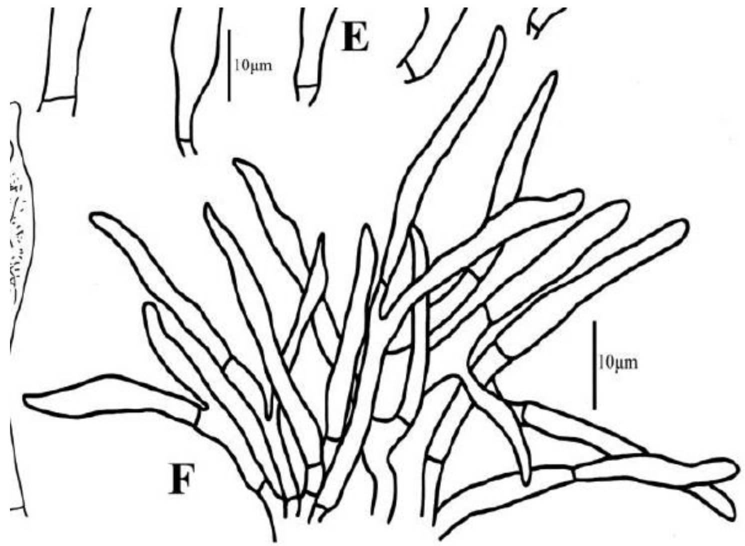
Examples



Europe



India



China

vertical structure – hyphal terminations – pileocystidia – macrochemical reactions

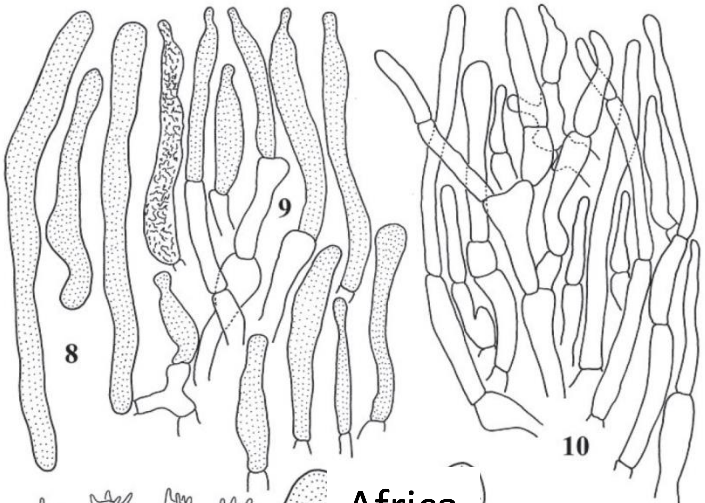
diculate. *Pileipellis*: *apical cells* 3–6 µm broad, cylindrical or tapering to apex, some with knobs, *dermatocystidia* 4–11 µm broad, 1–3 -septate, cylindrical to subclavate, without encrusted elements.

Pileipellis up to 100 µm thick, composed of erect to suberect hyphae and abundant pileocystidia; pileocystidia up to 12 µm, broad, fusiform to cylindrical or acuminate-rostrate, 3–6 septate.

Pileipellis (Fig. 2F) composed of hyaline hyphae, often branched and interwoven, septate; terminal cells 16–37 × 2.2–4.1 µm, cylindrical, with obtuse apex, sometimes attenuate; – Pileocystidia absent.

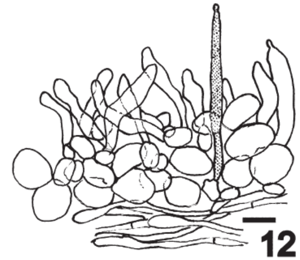
Examples

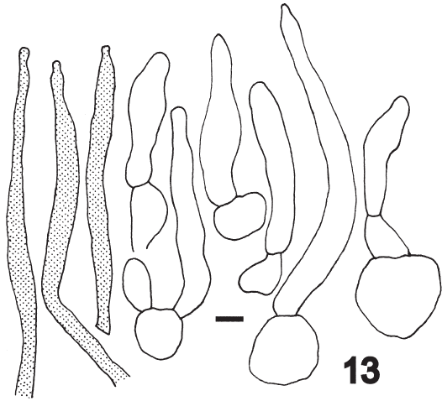
Africa



Revêtement piléique à subpellis formé d'hyphes de 4-7 µm de large, parcouru par peu de dermatocystides, celles-ci mesurant 3-5 µm de large, obtuses-arrondies, à contenu pailleté, abondant et à paroi légèrement épaisse; surmonté d'un suprapellis composé d'un chevelu d'extrémités courtes, verticales ou en oblique, 3-8(10) µm de diam. avec l'article terminal cylindracé ou plus irrégulier, tortueux, obtus ou subcapité, à paroi légèrement épaisse, et mêlées aux piléocystides assez nombreuses et très apparentes, 40-140 × 8-13 µm, coniques à subulées, minusculement boutonnées-capitées, substituant l'article terminal des extrémités, à contenu pailleté, et avec la paroi légèrement épaissie, nettement plus longues encore dans le subpellis.

South America





Pileipellis orthochromatic in Cresyl Blue, two-layered; subpellis gelatinized, forming a dense mat close to the underlying trama, of tightly interwoven hyphae; hyphae 2–5 µm diam, thin-walled, frequently septate, with scattered strongly refringent gloeopleurous elements of 5 µm diam, frequently terminating with cylindrical to swollen or mucronate embedded dermatocystidia; suprapellis composed of 2–5 strongly inflated, spherical cells, often gradually smaller towards the terminal cell, the latter cylindrical to narrowly subclavate, ampullaceous, or mucronate, resembling an epithelium; pileocystidia dispersed, terminal, more or less the same diam. As other terminal elements, 50–90 × 10–13 µm, contents granular-refringent in KOH.

vertical structure – hyphal terminations

pileocystidia – macrochemical reactions

North America
- no illustration

Pileocystidia not observed. Pileipellis 200–334 µm thick, embedded in a clear layer of gluten up to 250 µm thick; Epicuticular hyphae with free tips, 1.6–4.7 µm thick, interwoven and interspersed with thick-walled hyphae 3.1–4.7 µm.

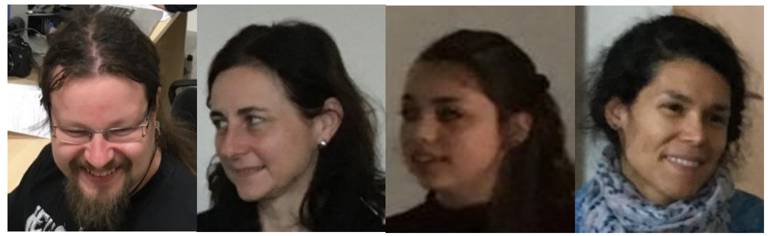
Russula workshop on microscopy “Quest for a globally comprehensible *Russula* language” 23. – 28. February 2018 in Slovakia.

agreement to use a standard morphological description for the genus *Russula* with defined minimal requirements

template measurements table
glossary of *Russula* terms
description template

Every participant prepared during the workshop a description of a new or an interesting *Russula*

Russula workshop on microscopy "Quest for a globally comprehensible *Russula* language" 23. – 28. February 2018 in Slovakia.

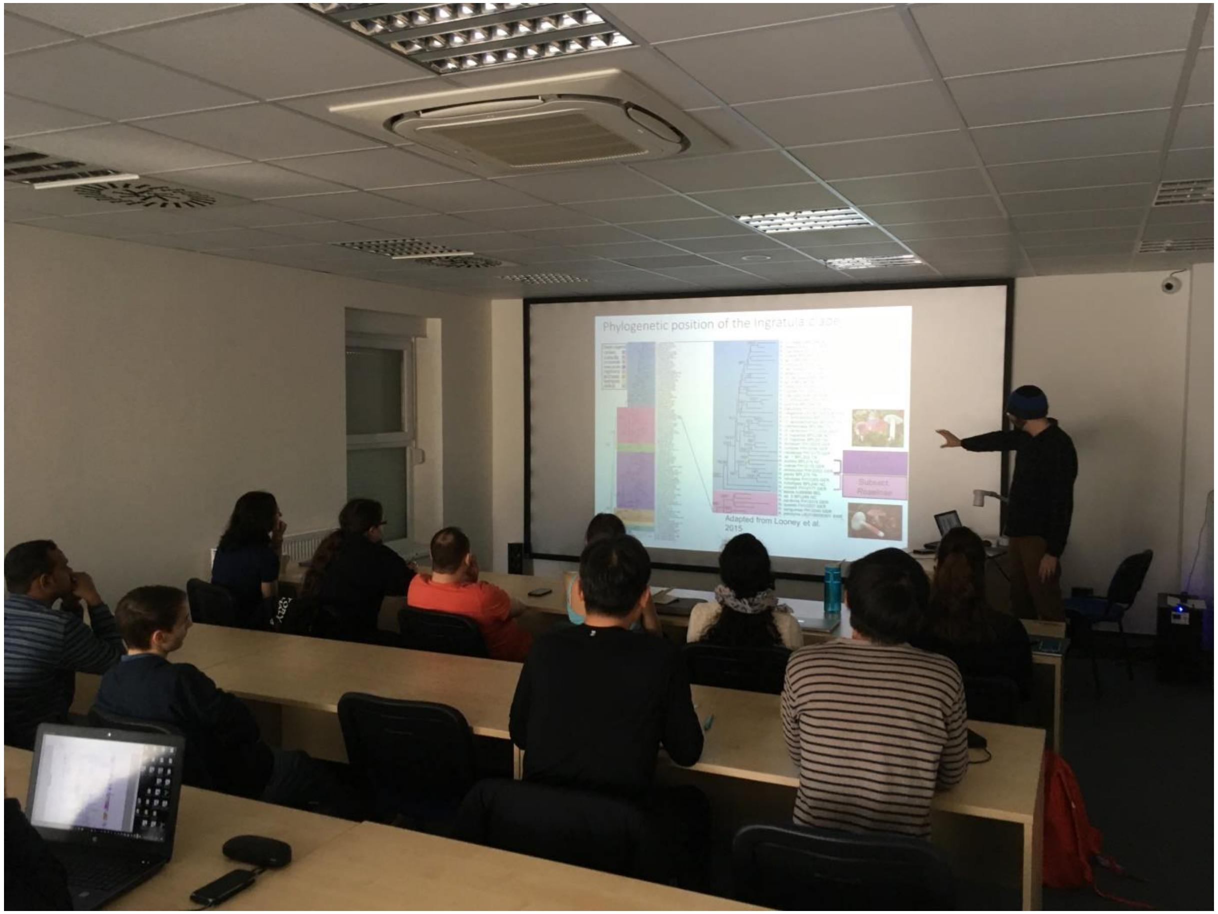


Slavomír Adamčík, Miroslav Caboň, Soňa Jančovičová, Magdalena Barajas, Adriana Corrales, Ruben De Lange, Aniket Ghosh, Felix Hampe, Ville Kälviäinen, Huyn Lee, Brian Looney, Cathrin Manz, Tero Taipale, Komsit Wisitrassameewong and Bart Buyck

Quest for a globally comprehensible *Russula* language

Bart Buyck (Museum Histoire National Naturelle Paris, France) speaking about phylogenetic significance of morphological traits at higher-rank Russula taxonomy

Quest for a globally comprehensible *Russula* language

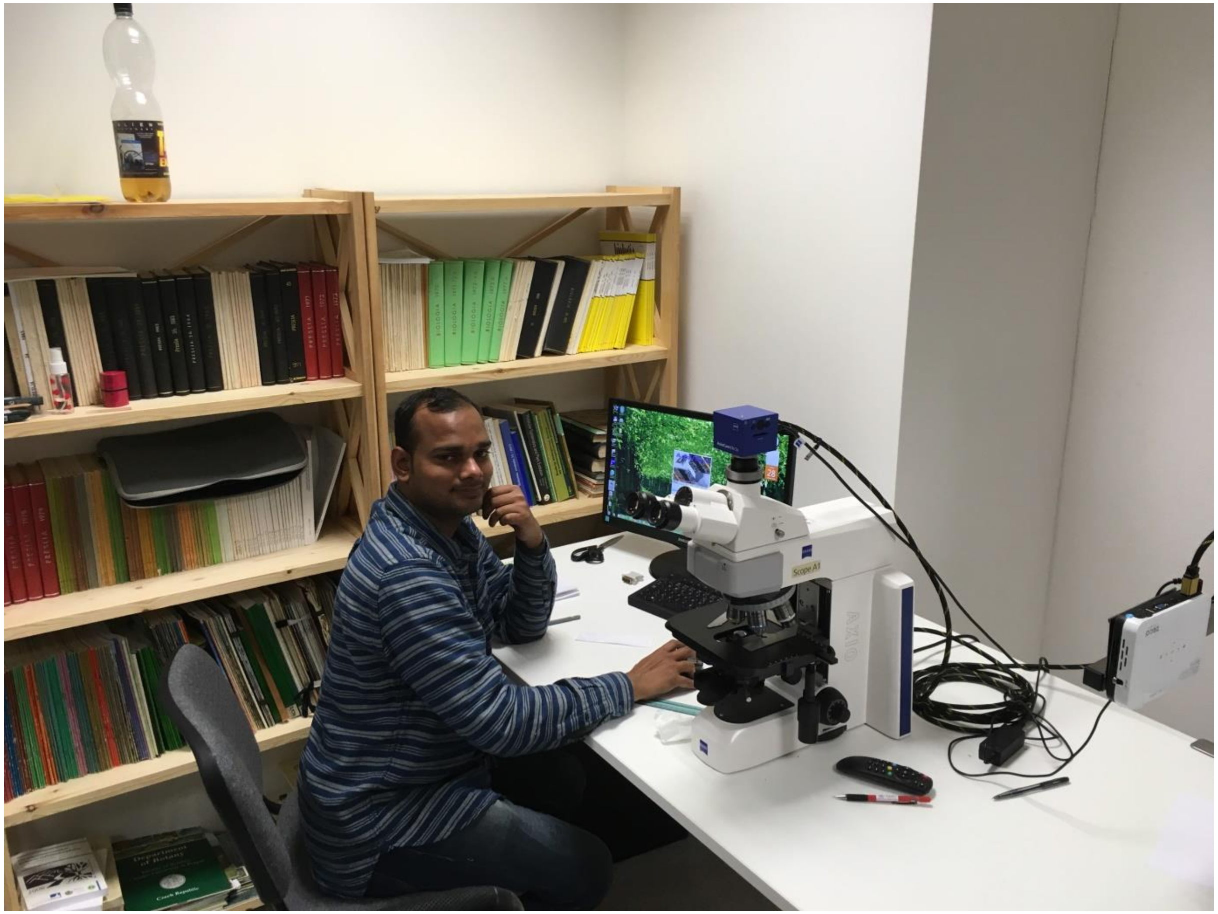


Brian Looney (INRA Nancy, France, now Duke university, USA) showing his nominate species from the lineage of *R. subtilis* (*Lilaceinae*)

Quest for a globally comprehensible *Russula* language

The youngest participant Magdalena Barajas from Indiana University (USA) with her quest to describe new North American members of *Foetentinae*

Quest for a globally comprehensible *Russula* language



Aniket Ghosh from Garhwal University (Uttarakhand, India) operating the microscope equipped by camera, measuring software and digital projector for drawing

Quest for a globally comprehensible *Russula* language

Microscopy room at the first day – spore observations

Quest for a globally comprehensible *Russula* language

... all microscopes were occupied and used a lot

Quest for a globally comprehensible *Russula* language

Miroslav Caboň was very willing and provided technical support for microscopes and software, he was also responsible for preparing of microscopy room with all tools and equipement

Quest for a globally comprehensible *Russula* language

Participants watching presentations of results with species descriptions almost ready to be published

The quest for a globally comprehensible *Russula* language

submitted in Fungal Diversity

- 26 Russula species described, 22 as new
- collections from 9 countries and 4 continents
- 27 authors from 11 countries

Slavomír Adamčík, Brian Looney, Miroslav Caboň, Soňa Jančovičová, Katarína Adamčíková, Peter G. Avis, Magdalena Barajas, Rajendra P. Bhatt, Adriana Corrales, Kanad Das, Felix Hampe, Aniket Ghosh, Genevieve Gates, Ville Kälviäinen, Abdul Nasir Khalid, Munazza Kiran, Ruben De Lange, Hyun Lee, Young Woon Lim, Alejandro Kong, Cathrin Manz, Clark Ovrebo, Malka Saba, Tero Taipale, Annemieke Verbeken, Komsit Wisitrassameewong, Bart Buyck

Standards: description template

Table 2. The description template used in this study. More details explaining character stages and observation styles are in Electronic Supplementary Table S6, numbering of characters (here in parenthesis) is consistent between both Tables and descriptions in the Taxonomy part.

Spores (1) shape and Q value, (2) size; (3) shape of elements in the spore ornamentation, (4) their density [in a 3 μm circle], and (5) prominence (6) general appearance of spore ornamentation, (7) frequency of line connections and fusions [in a 3 μm circle]; (8) size, amyloidy and surface of the suprahilar spot.

Basidia (9) size, shape and number of sterigma; (10) estimated size and shape of basidiola. **Hymenial cystidia on lamellae sides** (11) density at 1 mm^2, (12) size, (13) shape, terminations, presence and length of an appendage, emergence above basidium level, origin and thickness of walls, (14) contents observed in Congo Red and sulfovanillin; **cystidia on lamellae edges** (15) size and (16) relative differences [compared to lamellae sides]. **Lamellae edges** (17) presence and frequency of different cell types; **marginal cells** (18) size, (19) shape, contents and thickness of walls.

Pileipellis (20) colour reaction in Cresyl Blue, (21) delimitation from context, (22) depth, (23) distinction and delimitation of supra- and subpellis, (24) gelatinization and presence of extra gelatinous matter; (25) suprapellis depth and arrangement of hyphal terminations; (26) subpellis depth, structure and hyphal width. **Hyphal terminations** near the pileus margin (27) general aspect; (28) terminal cells size, (29) shape and general appearance of the terminal cells; (30) subterminal cells width and relative differences [compared to the terminal cells]. Hyphal terminations near the pileus centre (31) size of terminal cells and (32) relative differences in general aspect, terminal and subterminal cells shape [compared to the pileus margin].

Pileocystidia in suprapellis near the pileus margin (33) number of cells, shape, insertion of basal part, thickness of cell walls and irregularities, (34) size of terminal cells, (35) their shape and terminations, (36) contents observed in Congo Red and sulfovanillin, (37) presence of acid-resistant incrustations and incrustations observed in Congo Red and sulfovanillin. Pileocystidia in suprapellis near the pileus centre (38) size of the terminal cells and (39) relative differences in general aspect and terminal cells shape [compared to the pileus margin]. **Cystidioid or oleiferous hyphae** (40) presence in subpellis and trama and contents.

Standards: character list

Supplementary Table S6. List of characters with explanations of character stages or observation styles
SPORES
1. Shape based on Q values calculated from average of minimum 20 measurements
Subglobose: Q=1.05–1.15
broadly ellipsoid: Q=1.16–1.30
ellipsoid: Q=1.31–1.45
narrowly ellipsoid: Q=1.46–160
oblong: Q>1.60
2. Size: length and width based on 20 measurements minimum (optimum on 3 specimens)
given as (minimum) average minus stand. dev. (SD) – average – average plus SD (maximum)
...
HYMENIUM
9. Basidia
size represented by length and width, based on 20 measurements and given as minimum (optimum on 3 specimens) given as(minimum) average minus stand. dev. (SD) – average – average plus SD (maximum)
shape (e.g. subcylindrical, fusiform, clavate, …)
number of sterigmata
10. Basidiola
shape and estimated width
...
PILEOCYSTIDIA
33. General aspect of pileocystidia in the suprapellis near the pileus margin
number of cells
general shape (e.g. clavate, cylindrical, fusiform, lanceolate, lageniform, ...)
insertion of pileocystidia (in suprapellis, in upper or lower part of subpellis)
thickness of walls (also adding maximum thickness in µm if relevant)
irregularities (nodes, diverticules, lateral projections or branches ...)
40. Cystidioid or oleiferous hyphae
presence in subpellis and trama
character of their contents

Standards: terminology

Table 3. Explanation of selected terms used for descriptions of *Russula*

Terms	Explanation
Acid-resistant incrustations	incrustations that turn red in carbolfuchsin and retain the colour after being exposed to a weak acid for few seconds
Acute apical part	cell walls at the terminal part shaped in a sharp angle (narrowing tip)
Appendage	apical constriction of cystidium, resulting in a vermiform, capitulate, fusiform or moniliform 'appendage', which often easily breaks off and allows discharge of cystidial contents
Chains in spore ornamentation	more than two warts or spines aligned very closely
Cystidioid hyphae	hyphae (or portions of hyphae) in subpellis or trama with cystidia-like heteromorphous contents
Essential number of measurements	at least 20 measurements per specimen, optimum number 3 and more specimens measured per species
Essential statistics	length, width and for spores also ratio of length and width (Q value); always provide the average value and the range estimated as the average+/- standard deviation
Fusions in spore ornamentation	when two warts or spines adhere to each other (twinned elements)
Heteromorphous contents	contents of hyphae or pileocystidia, they may have granular, crystalline, banded components or refringent bodies, sometimes they are disconnected or limited to a part of the cystidium
Hymenial cystidia	sterile elements in hymenium defined by their contents or shape
Hyphal terminations	free terminations of hyphae that end in transition between suprapellis or subpellis; they can be represented by one or multiple cells
Incrustations	droplets, crystals or glutinous coatings on the surface of pileocystidia and hyphal terminations
Line connections	thin and low, amyloid lines that connect warts or spines of spore ornamentation
Marginal cells	sterile cells on lamellae edges that clearly differ from basidiola by their shape and size and have optically empty contents; mostly they are similar to hyphal endings of the pileipellis
Metachromatic reaction	colour change to blood red in Cresyl Blue, the negative reaction is orthochromatic
Mucronate apical part	having an abruptly projecting point
Number of cells of pileocystidia	number of cells that are separated by septa and have heteromorphous contents or a specific shape typical for pileocystidia
Obtuse apical part	rounded tips of hymenial elements
Oleiferous hyphae	hyphae in subpellis or trama with homogeneous, refractive, oily contents, sometimes pigmented
Pileipellis	cuticle on upper surface of pileus delimited from the pileus trama by its specific structure, often also with conspicuous pigmentations and gelatinisation
Pileocystidia	hyphal structures in pileipellis with specific contents or shape
Primordial hyphae	pileocystidia with acid-resistant incrustations staining red after carbolfuchsin treatment
Ridges	linear elements made up of aligned warts or spines that are interconnected by amyloid 'walls' of more than half their height
Spines on spores	elements of spore ornamentation with pointed (acute) tips
Spore shape	defined by ratio of length and width of spores
Spore size	length and width of spores excluding spore ornamentation
Subpellis	hyphal structure in pileipellis between trama and suprapellis; many species have a separable pileipellis under the suprapellis
Subterminal cells	single cell next to the terminal cell
Suprahilar spot	area above the hilum with nearly smooth or smooth surface that is in some species completely or partly amyloid
Suprapellis	the upper part of pileipellis that is near the surface and is composed of hyphal terminations and usually also pileocystidia
Terminal cells	single hyphal cells at the terminal position in suprapellis
Warts on spores	elements of spore ornamentation with obtuse tips
Wings	same as ridges, but much higher (> 2 µm)

Standards: measurements table

Supplementary Table S5. Template table for measurements of micromorphological characters. The grey-shaded rows at the bottom of the table are automatically calculated statistical values.

	spores						TC margin							TC centre			PC margin			PC centre			basidia		cystidia on lamellae sides				marginal cells		cystidia on lamellae edges			
	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	1	2	3	4	5	6	7	8	9	10	11	12
	length	width	Q	spines	lines	fus	length	w max	w tips	dif	binf	cyl	tinf	length	width	binf	length	width	cells	length	width	cells	length	width	length	width	acute	apx	length	width	length	width	acute	apx
1			####							0	0	0	0			0											0						0	
2			####							0	0	0	0			0											0						0	
3			####							0	0	0	0			0											0						0	
4			####							0	0	0	0			0											0						0	
5			####							0	0	0	0			0											0						0	
6			####							0	0	0	0			0											0						0	
7			####							0	0	0	0			0											0						0	
8			####							0	0	0	0			0											0						0	
9			####							0	0	0	0			0											0						0	
10			####							0	0	0	0			0											0						0	
11			####							0	0	0	0			0											0						0	
12			####							0	0	0	0			0											0						0	
13			####							0	0	0	0			0											0						0	
14			####							0	0	0	0			0											0						0	
15			####							0	0	0	0			0											0						0	
16			####							0	0	0	0			0											0						0	
17			####							0	0	0	0			0											0						0	
18			####							0	0	0	0			0											0						0	
19			####							0	0	0	0			0											0						0	
20			####							0	0	0	0			0											0						0	
21			####							0	0	0	0			0											0						0	
22			####							0	0	0	0			0											0						0	
23			####							0	0	0	0			0											0						0	
24			####							0	0	0	0			0											0						0	
25			####							0	0	0	0			0											0						0	
26			####							0	0	0	0			0											0						0	
27			####							0	0	0	0			0											0						0	
28			####							0	0	0	0			0											0						0	
29			####							0	0	0	0			0											0						0	
30			####							0	0	0	0			0											0						0	
min	0	0	####	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
st. dev.	####	####	####	####	####	####	####	####	####	0	0	0	0	####	####	0	####	####	####	####	####	####	####	####	####	####	0	####	####	####	####	####	0	####
av-sd	####	####	####	####	####	####	####	####	####	0	0	0	0	####	####	0	####	####	####	####	####	####	####	####	####	####	0	####	####	####	####	####	0	####
average	**####**	**####**	**####**	**####**	**####**	**####**	**####**	**####**	**####**	**0**	**0**	**0**	**0**	**####**	**####**	**0**	**####**	**####**	**####**	**####**	**####**	**####**	**####**	**####**	**####**	**####**	**0**	**####**	**####**	**####**	**####**	**####**	**0**	**####**
av+sd	####	####	####	####	####	####	####	####	####	0	0	0	0	####	####	0	####	####	####	####	####	####	####	####	####	####	0	####	####	####	####	####	0	####
max	0	0	####	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Standards: macrochemical reactions

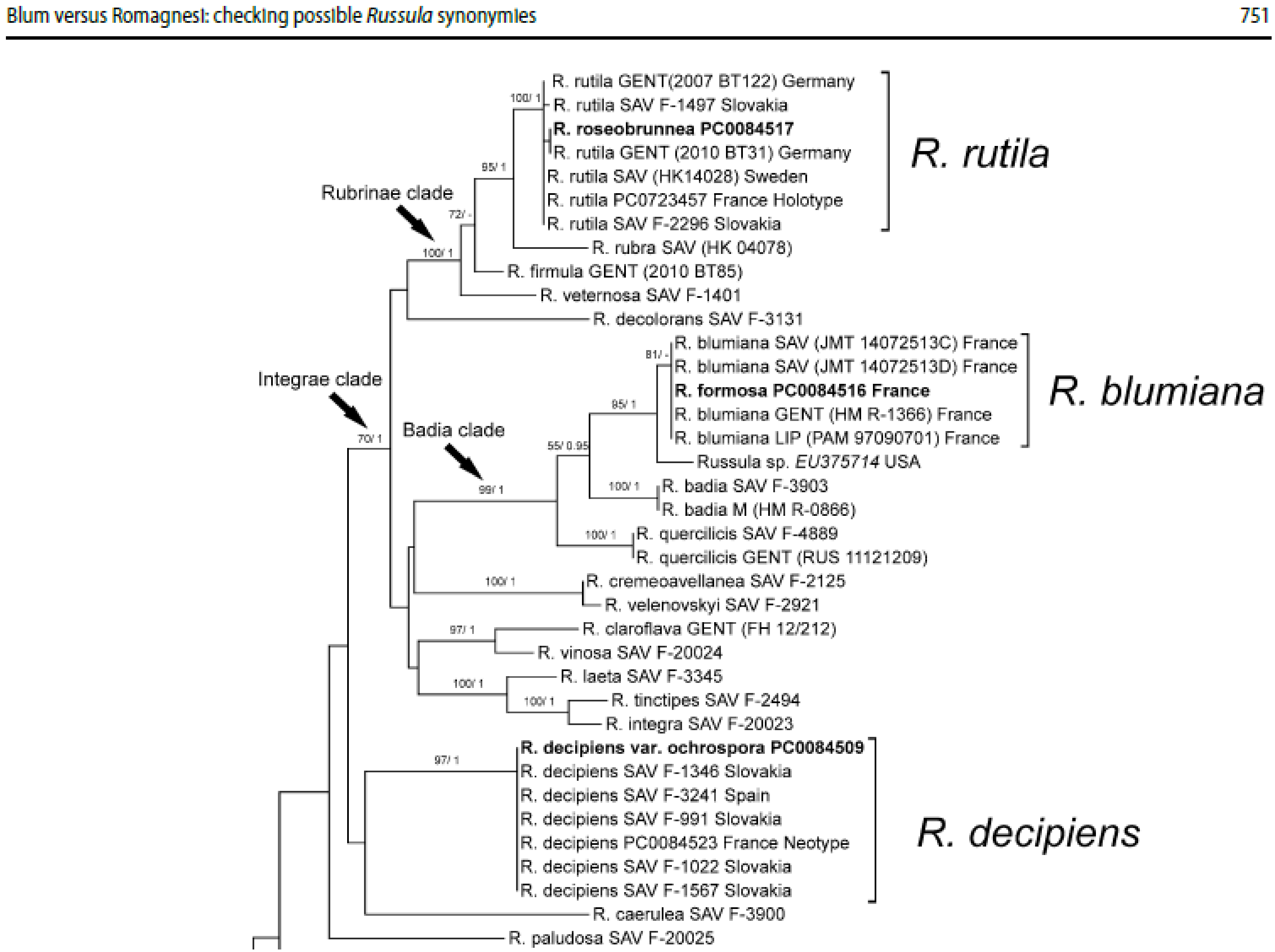
Table 1. List of reagents and tissue preparations used for micro-morphological observations

Reagent	Composition / manner of use	Purpose of use	References
carbolfuchsin	5 g phenol + 84 ml H_2O + 1 g fuchsin + 10 ml ethanol / stained with carbolfuchsin, washed and observed in distilled water after incubation for a few seconds in a 10% solution of HCl	incrustations on primordial hyphae	Romagnesi (1967)
Congo Red	1 ml 25% NH_3 dissolved in filtrated solution of 1.5 g Congo Red and 50 ml H_2O / used after short treatment in 10% KOH solution	contrast improvement of elements in hymenium and pileipellis	Heilmann-Clausen et al. (1998)
Cresyl Blue	2 ml Cresyl Blue + 1.3 ml glycerin + 2 ml ethanol + 4.2 ml H_2O / used dirently	presence of metachromatic incrustations in pileipellis	Buyck (1989)
Melzer´s reagent	1.5 g I + 5 g KI + 100 ml $C_2H_3Cl_3O_2$ + 100 ml H_2O / used directly	colouring of spore ornamentation	Melzer and Zvára (1927)
sulfovanillin	1 g of vanillin dissolved in 6 ml H_2O and 5 ml concentrated $H_2SO_{4,}$ / used directly, observed after 5 min and 30 min staining	colouring of cystidia contents	Caboň et al. (2017)

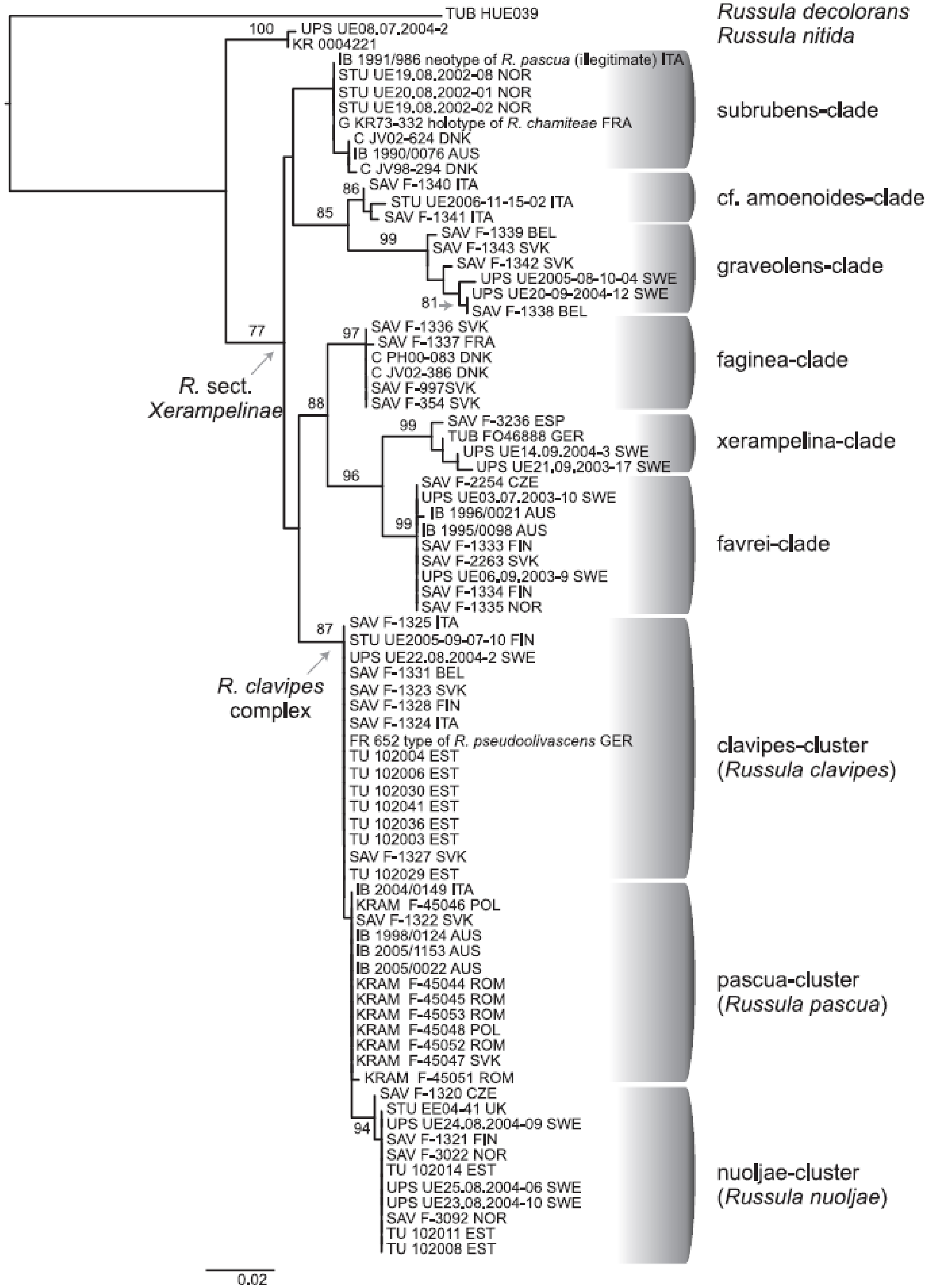
Standards: what is the minimum for descriptios?

described species / compared species and description source	spores								hymenial elements					pileipellis										
	size	ratio of length and width	prominence of ornamentation	structure of ornamentation	number of elements	line connections	fusions	suprahilar spot	basidia	hymenial cystidia on sides size	hymenial cystidia on sides shape, contents, terminations	hymenial cystidia near edges size	marginal cells	depth	terminal cells near the pileus margin size	terminal cells near the pileus margin shape	subterminal cells	terminal cells near the pileus centre size	pileocystidia near the pileus margin size	pileocystidia near the pileus margin number of cells	pileocystidia near the pileus margin shape	pileocystidia near the pileus centre size	pairwise sequence identity	morphological difference
R. aurantioflava / R. xantho (Buyck 2005)	1	0	0	0	-	-	-	0	1	0	0	-	1	-	-	-	-	-	-	-	-	-	NA	33,3%
R. brunneocystidiata / R. subsordida (Adamčík & Buyck 2014)	1	1	1	1	0	0	0	0	1	1	0	1	0	1	0	0	0	1	1	1	1	1	NA	59,1%
R. laevis / R. brevipes (Buyck & Adamčík 2013)	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	NA	18,2%
R. purpureogracilis / R. albida (Adamčík & Buyck 2012)	0	1	0	0	1	1	0	0	0	0	1	-	0	0	0	0	0	1	0	0	0	0	NA	23,8%
R. seperina / R. cinerascens (Adamčík & Buyck 2011)	1	1	1	0	0	1	0	0	1	1	0	-	0	1	0	0	0	0	1	0	1	-	NA	45,0%
R. subtilis/ R. uncialis (Adamčík et al. 2018)	0	1	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	-	NA	23,8%
R. amarissima / R. indoarmeniaca (Ghosh et al. 2016)	1	0	-	0	-	-	-	-	0	1	-	0	-	0	-	-	-	-	-	-	-	-	81	28,6%
R. fortunae / R. eccentrica (Adamčík et al. 2018)	0	1	1	0	1	0	0	0	0	0	1	1	1	0	1	1	1	1	1	0	0	0	82	50,0%
R. aurantiopectinata / R. rufobasalis (Song et al. 2018)	0	0	0	0	-	-	-	0	1	0	0	0	0	1	0	0	0	-	1	-	1	1	84	29,4%
R. wielangtae / R. flavida (Adamčík et al. 2018)	1	1	1	0	1	0	1	0	0	0	0	-	1	0	1	0	1	1	1	0	1	1	86	52,2%
R. abietiphila / R. nympharum (Adamčík et al. 2016)	1	0	1	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	86	22,7%
R. tenuihyphata / R. abietiphila (this study)	1	0	1	0	1	0	0	0	1	1	0	0	0	0	0	1	0	0	1	0	0	1	88	36,4%
R. amerorecondita / R. pectinatoides (Adamčík et al. 2013)	1	0	0	1	0	0	1	0	0	0	1	-	0	0	0	0	0	0	1	0	1	1	88	33,3%
R. gemmata / R. subtilis (this study)	1	0	0	0	1	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0	0	0	89	27,3%
R. echidna / R. amerorecondita (this study)	0	0	0	0	0	1	0	1	0	1	1	-	-	1	0	0	1	1	1	0	1	1	91	50,0%
R. caesarea / R. aurantioflava (this study)	1	0	1	0	0	0	0	1	1	0	0	1	0	0	0	1	0	0	-	-	-	-	92	33,3%
R. castanopsidis / R. purpureogracilis (this study)	0	1	1	0	0	1	0	0	0	1	0	0	0	0	0	1	1	1	1	1	1	1	92	50,0%
R. olivaceohimalayensis / R. seperina (this study)	1	0	0	1	0	1	1	0	1	1	0	1	1	1	1	1	1	0	0	1	0	0	94	59,1%
R. tlaxcalensis / R. nuoljae (Adamčík et al. 2016)	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	1	0	0	1	95	40,9%
R. garyensis / R. amerorecondita (this study)	0	0	1	0	0	0	0	0	0	1	1	0	0	1	-	-	-	1	-	-	-	1	95	37,5%
R. spinuloconnata / R. globispora (Adamčík & Jančovičová 2013)	0	0	1	1	0	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	-	95	28,6%
R. magica / R. olivaceohilamayensis (this study)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	95	13,6%
R. fluvialis / R. foetentula (Adamčík et al. 2013)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	96	9,1%
R. madrensis / R. xerampelina (Adamčík 2002)	0	0	1	0	0	0	0	0	0	0	0	-	-	-	0	0	1	0	0	0	0	0	98	10,5%
R. sancti-pauli / R. madrensis (this study)	0	0	0	0	1	0	1	0	0	0	0	1	1	1	0	1	1	0	0	0	0	1	99	36,4%
R. flavobrunnescens / R. katarinae (Adamčík et al. 2015)	1	0	0	0	0	0	0	0	1	0	0	-	0	1	0	0	1	0	-	-	-	-	99	23,5%
differences [%]	50,0%	30,8%	48,0%	19,2%	26,1%	30,4%	21,7%	8,0%	34,6%	34,6%	32,0%	26,3%	30,4%	45,8%	26,1%	39,1%	43,5%	43,5%	52,4%	15,0%	36,4%	50,0%		

What can we do better?



Dont publish new species we have already enought !



What can we do better?

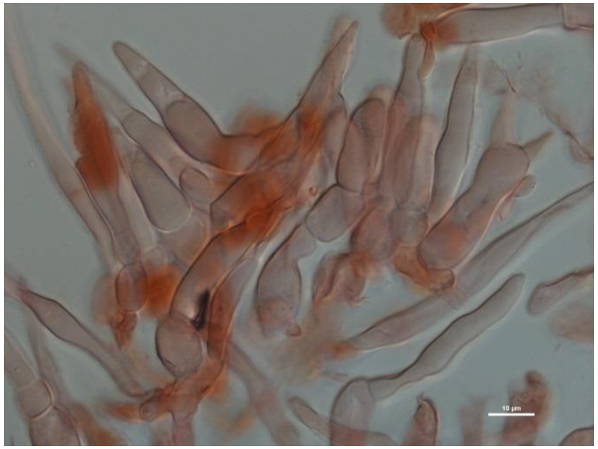
Focus on lineages and deep sampling to estimate diversity of phylogentic species

What can we do better?

Let’s do good morphology

Why should we do morphology?

regulation



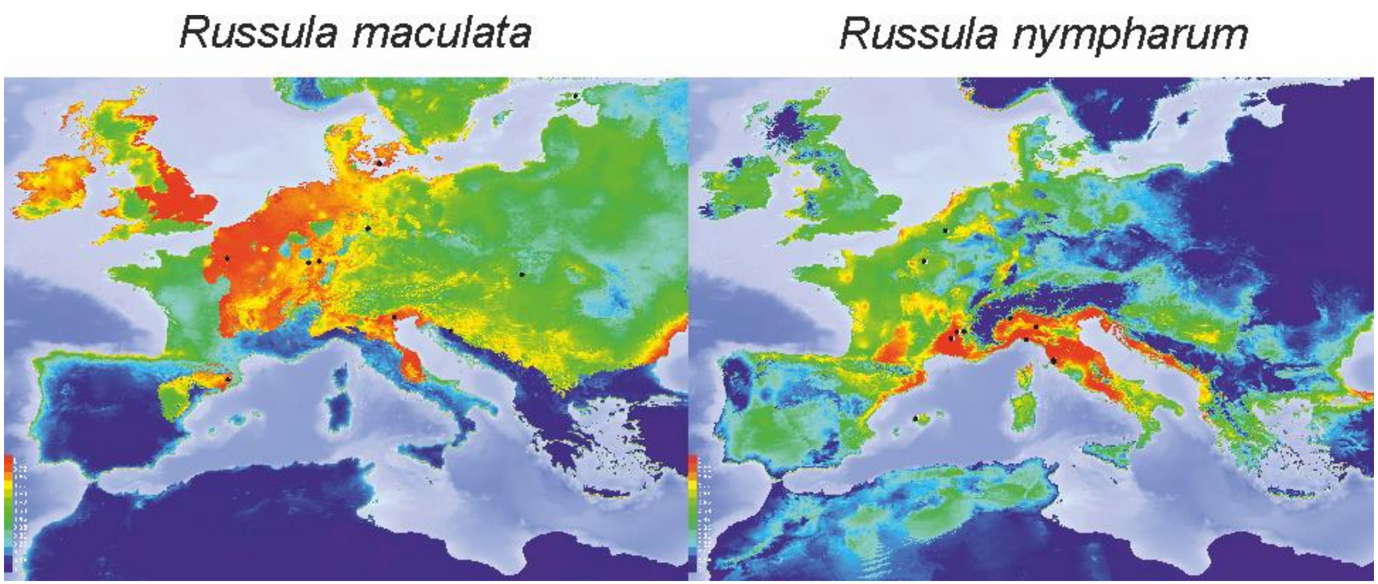
phenotype

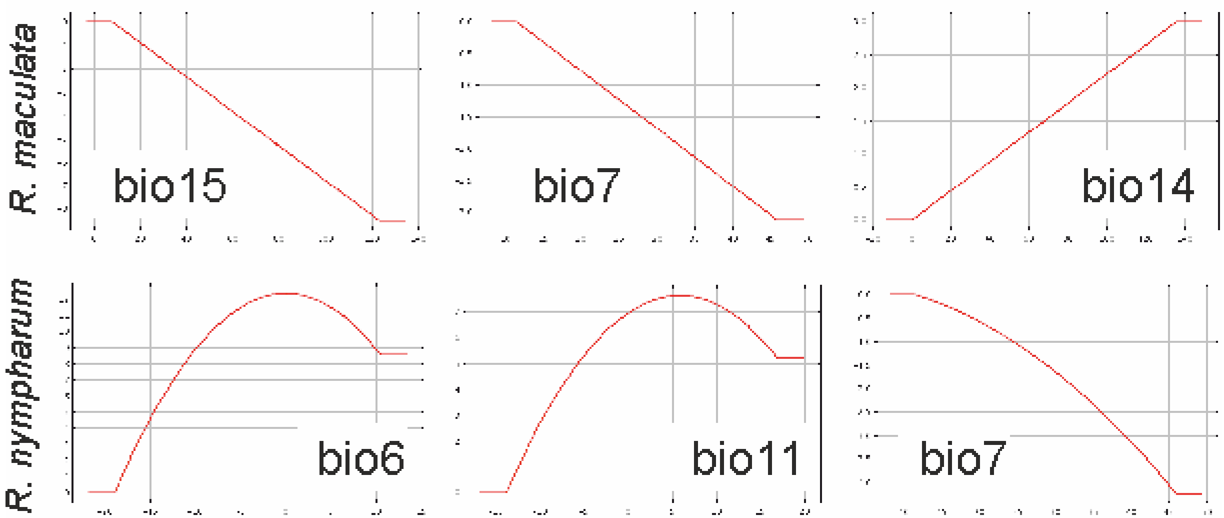
as result of evolutionary adaptation to climate and ecological factors

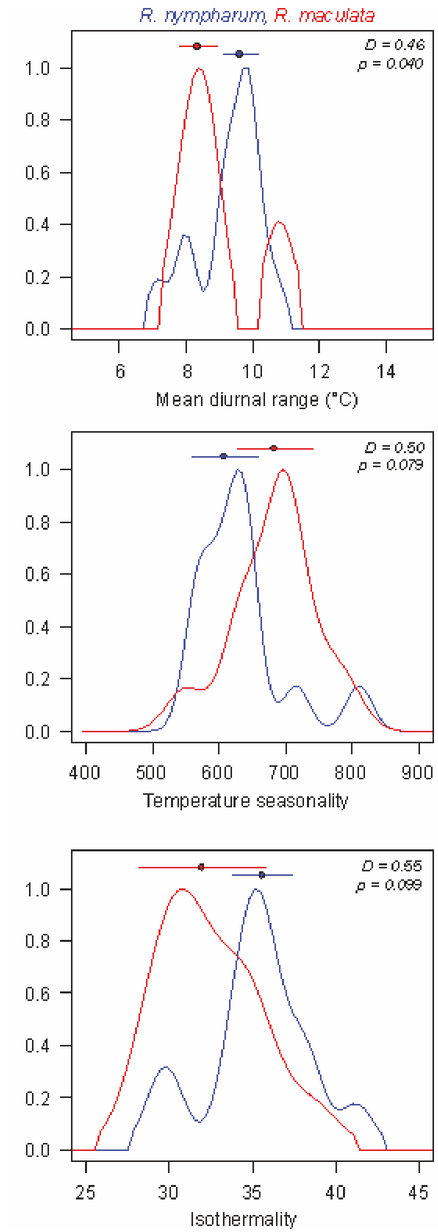
Biogeography

Evolution

Adaptations







Future chalenge

Pileipellis as shield
to protect against
harsh conditions

Is structure of
pileipellis depending
on adaptation ?

Thank you for the attention