Analysis of morphological, ecological and molecular characters of Russula pectinatoides *Peck and* Russula praetervisa *Sarnari, with a description of the new taxon* Russula recondita *Melera & Ostellari*

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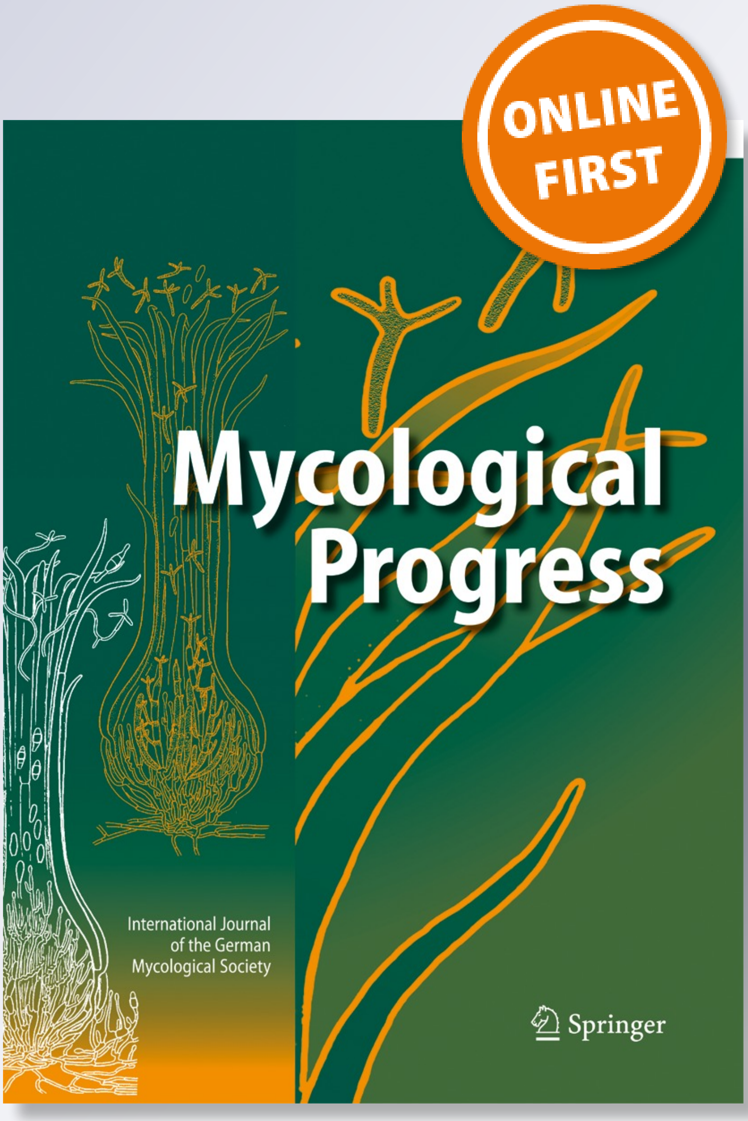

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ORIGINAL ARTICLE

Analysis of morphological, ecological and molecular characters of *Russula pectinatoides* Peck and *Russula praetervisa* Sarnari, with a description of the new taxon *Russula recondita* Melera & Ostellari

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Abstract Fetid Russulas belong to a homogeneous group including species with an unclear taxonomic classification. In particular, the correct interpretation of *Russula pectinatoides* Peck has remained unresolved, due to the reference collection deposited by C. H. Peck in 1906 consisting of poorly described specimens which were morphologically attributed to at least two different species by subsequent mycologists. Another species considered emblematic is *R. praetervisa* Sarnari, because it is closely related to *R. pectinatoides*, from which it was separated on the basis of the morphology of European samples found in Mediterranean habitats. In this paper, we review the interpretation of *R. pectinatoides* and *R. praetervisa* according to different authors, and we discuss the taxonomic status of these taxa in the light of molecular analyses carried out on Peck's reference specimens ($n = 3$) and 181 samples archived in European and American museums. Sequences of ITS1-5.8S ribosomal RNA-ITS2 regions indicated that *R. pectinatoides* reference samples deposited by Peck consisted of at least three different species. *R. praetervisa* was supported as an independent taxon, as it was molecularly distinct from other fetid Russulas. The analysis of museum samples revealed the necessity to describe the species to which in Europe the name *R. pectinatoides* is commonly applied as a new species, named *R. recondita* Melera & Ostellari.

Keywords Fetid Russulas · ITS · Molecular analysis · Scanning electron microscopy · Taxonomy

Introduction

Russula subsection *Foetentinae* (Melzer & Zvára) Singer (systematic concept of Romagnesi 1967) includes a morphologically characteristic and homogeneous group of species. Basidiomata of this group have ochre, brown-yellow cap colours, striate to tuberculate-striate cap margins, strong and unpleasant (fetid) odour, and intense (acrid, disagreeable) and often spicy taste. Although fetid Russulas can be very abundant and are commonly found worldwide, their taxonomy has long been a source of confusion and misidentification, and the number of autonomous species included in this group is not clear (Avis 2012).

A particular case is represented by *Russula pectinatoides* Peck (Romagnesi 1950; Kibby 2005; Avis 2012; Adamčík et al. 2013). In 1907, the American mycologist Charles Horton Peck described for the first time *R. pectinatoides* and deposited a collection of about 20 basidiomata, without

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designating a specific holotype (Peck 1907). His diagnosis lacked details, especially those features now known to be very informative for *Russula* identification, such as indications about the smell, the stipe base colour, and the spore ornamentation. In his notes, the species was classified as "edible but not very highly flavored", with a taste "mild or slightly and tardily acrid". Peck considered *R. pectinatoides* very similar to *R. sororia* Fr., from which "it may be separated by its milder taste". The lack of precise descriptions for the identification of *R. pectinatoides*, within a subsection that abounds with taxonomical problems, has progressively contributed to divergences in the interpretation of this species by successive authors, not only in North America but worldwide.

Subsequent authorities built more detailed descriptions and interpretations of *R. pectinatoides* but did not untangle the problem. Singer (1958) described *R. pectinatoides* as an independent species, having a variable smell, "usually of fresh fish or cod liver oil, but usually rather weakly so, taste mild but disagreeable, more rarely with a slight bitterish taste, and very rarely slightly and tardily acrid as an after-taste, stipe usually becoming early 'vinaceous russet' or 'vinaceous rufous' dotted at the base. Spores ornamentation rather low with ridges or chains never predominantly". In 1975, he included *R. pectinatoides* in the list of species belonging to the Pectinata series (including species of small dimensions, with cap margin long striate to tubercolate-striate, and small dermatocystidia; Singer 1975).

Romagnesi (1967) described different varieties and forms of *R. pectinatoides*. In the description of the typical var. *pectinatoides*, he mentioned a nauseating smell of caoutchouc, with a fruity component, and a mild to unpleasant taste including a disgusting bitter aftertaste. Spores of this species, he stated, had isolated spines, partially reticulate, while the stipe was red-spotted. His form *pseudoamoenolens* had a cap with a more amber, bistre, smoky colour, similar to *R. sororia* or *R. amoenolens* Romagn., and had reticulate–subreticulate spore ornamentation. The author also recognized a var. *brevispinosa* with a lower spore ornamentation, a form *pseudoconsobrina* with markedly spaced lamellae, and a form *dimorphocystis* with dysmorphic cystidia on lamellae (cheilocystidia), in part spindle-shaped, appendiculate and emerging, in part obtuse and more or less immersed.

In 1972, the American expert R. L. Shaffer carried out a revision on fetid Russulas and provided a description of *R. pectinatoides* on the basis of collections issued from Peck's herbarium and his own material. Because a formal type of *R. pectinatoides* had never been formally indicated, Shaffer designated the collection deposited at the New York State Museum (Albany, USA) as lectotype, formerly labelled as 'typus' and collected by Peck in Menands, New York, in August 1906. Moreover, among the samples included in the collection, he identified a specimen clearly different from the others that he determined as *R. laurocerasi* Melzer (Shaffer 1972). Shaffer described *R. pectinatoides* as follows: "Trama slightly spermatic or waxy and sometimes also faintly acrid in taste; with a slight to moderate, spermatic, waxy, or fetid odor". Lamellae had "slight to moderate taste having both spermatic to waxy and acrid components" and the stipe was "yellowish white, usually stained light olive brown to strong yellowish brown, or dark orange yellow to deep reddish orange, grayish red, or moderate to strong reddish brown basally, occasionally flushed yellowish gray basally or almost overall, unchanging when bruised or at most becoming light brown". Spores had "convex to cylindric or bluntly conic warts (…) and occasionally also a few connectives or short to long ridges; rarely forming a partial reticulum, never a complete one". He also stated: "The European fungus called *R. pectinatoides* by Romagnesi (1967) is certainly close enough to the North American one to bear the same name. It seems to be even less acrid and to have a different odor ('nauséeus de cautchouc, recouvrant un fond fruité plus ou moins distinct')". *Russula pectinatoides* in the sense of Singer (1958) also seems the same, except that he indicated that the spore ornamentation is never composed predominantly of isolated warts, but rather has common connectives and often forms an incomplete reticulum. The collections I studied have isolated warts as the usual situation. Due to the variability and the inconsistencies in the different descriptions of *R. pectinatoides*, in 1998, the Italian mycologist M. Sarnari came to a crucial decision, i.e. excluding the American taxon from his monography. To describe his samples, collected in Mediterranean habitats, he created the new species *R. praetervisa* (Sarnari 1998). According to Sarnari, "the creation of *R. praetervisa* resolves the problem of a name collectively used to describe two distinct species located in two different continents". However, the creation of a new species did not help clarify the taxonomy of the European continental collections that, as Sarnari himself declared, would deserve a deeper analysis. The author considered *R. praetervisa* macroscopically identical to *R. pectinatoides* Peck ss. Romagnesi. He based his species described from Italy on the Mediterranean biotype with subreticulate spores, opposed to a biotype of continental origin with tended warty-dotted spores. He emphasized that the spores of the lectotype designated by Shaffer (1972) were illustrated with isolated warts, and that this ornamentation type was usual in the North American collections. The typical form described in the monograph of Romagnesi (1967, p. 365, 'var. *pectinatoides*') shows conformity in this regard.

Adamčík et al. (2013) published the first modern revision exclusively based on the original material of Peck, with all measurements based on a single basidioma (labelled NYS2303.2 in this study) of the several basidiomata contained in the specimen box. They observed that *R. pectinatoides* is "one of the few North American Russula that has also been identified from Europe, where it was generally accepted as a common and widespread species, for which the principal character seems the absence of a distinctly acrid taste". Adamčík et al. also observed a "spore ornamentation composed of nearly always isolated, conical to almost cylindrical warts of variable size but, on average, relatively tall and stout". According to the authors, this type of ornamentation is rarely ever encountered in European collections identified as Peck's species.

Despite the substantial work carried out by these authorities, the problem of the correct interpretation of *R. pectinatoides* has therefore remained unresolved. The aims of this study were to clarify the taxonomic status of *R. pectinatoides* in Europe and North America, and to verify the autonomy of *R. praetervisa*. To achieve this, we combined morphological, ecological and molecular analyses based on ITS1-5.8S ribosomal RNA-ITS2 regions, currently considered a useful target for the specific classification within the genus *Russula* (Miller and Buyck 2002; Shimono et al. 2004; Miller et al. 2006; Buyck et al. 2008).

Materials and methods

Samples

Samples morphologically determined as *R. pectinatoides* and *R. praetervisa*, as well as other closely related taxa (including *R. amoenolens* Romagn., *R. foetentula* Peck, *R. fragrantissima* Romagn., *R. illota* Romagn., *R. inamoena* Sarnari, *R. insignis* Quél., *R. grata* Britzelm., *R. ombrophila* Mortón Gómez & Monedero, *R. pectinata* Fr., *R. sororia* Fr.), were obtained from museums or personally collected by the authors (Table 2). Specimens were selected to obtain a representative distribution from different geographical areas (Europe: Belgium, England, France, Germany, Ireland, Italy, Norway, Spain, Switzerland; North America: California, Connecticut, Florida, Illinois, Indiana, Iowa, Kansas, Minnesota, New Jersey, New York, North Carolina, Pennsylvania, Tennessee, Texas, Washington, West Virginia, Wisconsin; Canada: Ontario, Quebec; North Africa: Morocco), a wide variety of habitats (coniferous, hardwood and mixed forests; lawns; sandy soils; anthropic environments) and potential host plants (22 genera: *Abies*, *Arbutus*, *Arctostaphylos*, *Betula*, *Carpinus*, *Carya*, *Castanea*, *Cedrus*, *Cistus*, *Corylus*, *Fagus*, *Helianthemum*, *Juniperus*, *Kalmia*, *Ostrya*, *Picea*, *Pinus*, *Populus*, *Quercus*, *Salix*, *Tilia*, *Tsuga*).

Three samples consisting of stipe and cap, deposited by Peck (1907) as holotype of *R. pectinatoides* at the New York State Museum, labelled as NYS2303.1, NYS2303.2 (specimen selected by Adamčík et al. 2013) and NYS2303.3, were also obtained.

Morphological analysis

Freshly collected samples and dry museum samples lacking a complete morphological description were (re)characterised macro- and microscopically.

Macroscopic parameters examined (on fresh tissue if available) included pileus colour (encoded according to the code of Séguy 1936), colour of the stem base, reaction of the flesh to 10% iron sulphate (on the fresh stipe tissue, reaction ranges from salmon pink to blue-green, differentiating some taxonomic groups), and to guaiac tincture (positive reaction if a drop on fresh stipe tissue turns blue-green, grey-green; negative if it does not change colour or becomes yellow, brown-yellow). Smell and taste were checked only for fresh specimens collected by the authors and the colour of spore deposits was made according to Dagron (1989) and Romagnesi (1967). For samples received from various herbaria (dried material), these data were reported if present in the description accompanying the sample.

For microscopic parameters, samples were analysed using a Laborlux Leitz and Carl Zeiss KF 2 microscope. The cuticle was observed in Congo red for fresh samples and Congo red ammoniacal for dried samples. Spores were observed in Melzer's reagent which highlights ornamentation (Melzer 1924). The size of the basidiospores (excluding their ornamentation) was established through 30 measurements (in Melzer), taking into account the minimum and maximum measurement calculated on the basis of the standard deviation. To describe the spore ornamentation, the following categories were made: 0 = isolated warts; 1 = rare connections; 2 = few connections; 3 = some connections; 4 = connected warts; 5 = reticulate warts.

Scanning electron microscopy (SEM) pictures were taken for some samples that were considered to be particularly representative for each taxon. For SEM, samples were directly mounted on 12-mm carbon conductive adhesive tabs (Electron Microscopy Sciences) coated with gold, and examined with a field emission scanning electron microscope (FE-SEM; JEOL JSM-7001FA).

Molecular analysis

Specimens (about 5–40 mg, depending on the available material) were ground to a fine powder using a pestle. DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen), according to the 'DNA Purification from Tissues' protocol, with the following modifications: samples were incubated at 98 °C in 400 μL lysis solution (10 μL NaOH 1 M, 40 μL SDS 5%, 350 μL sterile water) for 10 min; 400 μL HCl 25 mM were added; 400 μL AL buffer were added and samples were incubated at 95 °C for 10 min; 400 μL pure ethanol were added and samples were centrifuged at 20,000*g* for 5 s; 600 μL supernatant were loaded in a DNA Mini Spin column and centrifuged at 6000*g* for 1 min; 500 μL AW1 buffer was added and centrifuged at 6000*g* for 1 min; 500 μL AW2 buffer was added and centrifuged at 20,000*g* for 3 min; a further centrifugation at full speed for 1 min was performed to avoid buffer carryover. In the final step, DNA was eluted in 50 μL AE buffer after incubation at room temperature for 2 min and centrifugation at 6000*g* for 1 min. A consistent part of the samples were old and some of them damaged. Therefore, to optimize PCR conditions, DNA was quantified using the PicoGreen dsDNA Quantitation Kit before the amplification.

Polymerase chain reaction (PCR) was performed with the HotStar Taq Master Mix Kit (Qiagen), by testing about 0.5–5 ng DNA in a solution of 25 μL total volume, with a final concentration of 1.5 mM MgCl2, 200 μM each dNTP, 2.5 units of HotStar Taq DNA Polymerase, and 0.5 μM of each primer. Amplification of ITS1-5.8S rRNA-ITS2 region was carried out with the forward primer ITS5 and the reverse primer ITS4 (White et al. 1990). The touchdown thermal cycle consisted of 1 cycle at 95 °C for 15 min; 11 cycles at 95 °C for 1 min, 60 to 50 °C ($\Delta = 1$ °C) for 1 min and 72 °C for 1 min; 30 cycles at 95 °C for 1 min, 50 °C for 1 min and 72 °C for 1 min; and a final extension cycle at 72 °C for 10 min. Two internal primers, specific for *Russula* sp. ITS1-5.8S rRNA-ITS2 region, were designed and used for particularly degraded DNA samples. For specimens contaminated by other fungal organisms, the reverse primer Ru_Int_rev (5’-GGGKGTGCCYYTCGGRATRCCAA-3’) was used with primer ITS1 (White et al. 1990) and the forward primer Ru_Int_For (5’-CTTGCGCCYYTTGGYATTCCGA-3’) was used with primer ITS4 (White et al. 1990). The touchdown thermal cycle for the first primer set consisted of 1 cycle at 95 °C for 15 min; 15 cycles at 95 °C for 1 min, 64 °C to 50 °C ($\Delta = 1$ °C) for 1 min and 72 °C for 1 min; 30 cycles at 95 °C for 1 min, 54 °C for 1 min and 72 °C for 1 min; and a final extension cycle at 72 °C for 10 min. The touchdown thermal cycle for the second primer set consisted of 1 cycle at 95 °C for 15 min; 15 cycles at 95 °C for 1 min, 61 to 47 °C ($\Delta = 1$ °C) for 1 min and 72 °C for 1 min; 30 cycles at 95 °C for 1 min, 47 °C for 1 min and 72 °C for 1 min; and a final extension cycle at 72 °C for 10 min. Amplicons were visualized on 0.8% agarose gel stained with GelRed (Biotium), and purified using Sephadex G-100 (GE Healthcare), according to the manufacturer’s instructions. Sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems) and Sephadex G-50 plates for purification (Sigma-Aldrich), followed by screening in an automatic ABI PRISM 3130xl Genetic Analyzer (Applied Biosystems).

Electropherograms were checked and the resulting sequences assembled using BioEdit v.7.0.9 (Hall 1999) then submitted to BLAST search (Altschul et al. 1990). Sequences of *Russula* species showing ≥98% homology over the whole length to the isolates were selected from GenBank and Unite databases; all suitable entries were initially included in the phylogenetic analysis, but those with uncertain determination (i.e. not identified to the species level) or containing heterozygous peaks were discarded from the final phylogenetic tree. In cases of redundant data (i.e. sets of identical sequences of the same species belonging to a same study), only one representative sequence was included in the analysis. The selected public available sequences are listed in Table 1. Data were aligned by MUSCLE (Edgar 2004), and phylogenetic trees [Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods] and genetic distances (within group and between groups average calculations; Kimura-2 parameters) were obtained using MEGA v.6.0.6 (Tamura et al. 2013). The ML tree was based on the GTR+G+I model (Nei and Kumar 2000; bootstrap test: 1000 replications). Initial trees for the heuristic search were obtained automatically by applying NJ and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.9406)].

Nucleotide sequences data reported in this paper are available in the EMBL, GenBank and DDJB databases under the accession numbers: KF303597 to KF303606, KF318045 to KF318088, KF971694, KJ530747 to KJ530760, KJ476888 to KJ476890, KF683922, KF683923, KJ834553 to KJ834631, KU640186 to KU640189.

Results

From a total of 184 samples analysed molecularly (Table 2), 173 ITS sequences were obtained, 17 of which yielded partial data (forward or reverse primer sequence only). Eleven samples could not be analysed because of insufficient DNA quality (‘no DNA’ in Table 2; n = 5) or due to contaminations (‘mixed seq’ in Table 2; *n* = 6). Peck’s type specimens generated sequences with baseline noise and some heterozygous peaks that were referred to double or triple bases according to the IUPAC nucleotide code. These samples were reanalysed by PCR several times, but the templates were of poor quality and did not allow determining the identity of all positions.

For most samples, the original identification of the species did not correspond to the molecular identification produced by this study (Fig. 1). Overall, the original identification matched sequences deposited in public databases with the same identification and/or clades likely to be these species for 48 specimens (28%), while the remaining 125 (72%) did not.

Forty-five target samples were analysed morphologically by observing spore ornamentation and spore size. For the other morphological parameters considered, including smell, taste and colour of the stipe base, we were not able to gather information for all specimens (Table 3). However, these

Table 1 Public sequences retrieved from GenBank and Unite databases, selected for phylogenetic analysis of the target Russula species and their most closely related taxa

Sequence Acc. no.	Species	Database	Reference
DQ422026	*R. pectinatoides*	GenBank	U. Eberhardt, unpublished
JX679371	*R. pectinatoides*	GenBank	M. Kavkova, unpublished
JF908639	*R. pectinatoides*	GenBank	Osmundson et al. 2013
EU819493	*R. pectinatoides*	GenBank	Palmer et al. 2008
EU819500	*R. pectinatoides*	GenBank	Palmer et al. 2008
KF245514	*R. pectinatoides*	GenBank	A. L. Bazzicalupo and M. L. Berbee, unpublished
JX434670	*R. pectinatoides*	GenBank	D.-D. Chai, unpublished
AB211276	*R. pectinatoides*	GenBank	Nara 2006
EU598185	*R. pectinatoides*	GenBank	K. W. Hughes and B. Buyck, unpublished
UDB011156	*R. pectinatoides*	Unite	V. Liiv and M. Vaasma, unpublished
UDB015983	*R. pectinatoides*	Unite	J. Vauras and I. Saar, unpublished
UDB019331	*R. praetervisa* (holotype)	Unite	M. Sarnari and U. Eberhardt, unpublished
UDB019333	*R. praetervisa*	Unite	U. Eberhardt, unpublished
AB211275	*R. sororia*	GenBank	Nara 2006
EU598184	*R. laurocerasi* (*R. grata*)	GenBank	K. W. Hughes and B. Buyck, unpublished
AF418614	*R. laurocerasi* (*R. grata*)	GenBank	U. Eberhardt 2002
JF908651	*R. laurocerasi* (*R. grata*)	GenBank	Osmundson et al. 2013
KF245533	*R. laurocerasi* (*R. grata*)	GenBank	A. L. Bazzicalupo et al., unpublished
UDB000004	*R. grata*	Unite	J. Heilmann-Clausen and R. Kjöller, unpublished
UDB016090	*R. grata*	Unite	J. Vauras and I. Saar, unpublished

Acc. no. accession number

parameters seemed to be highly variable even within the same species. A full morphological analysis of the three samples issued from *R. pectinatoides* Peck's reference collection was not possible due to the very small portions received and their poor conditions. Nevertheless, spores of sample NYS2303.2 could be observed by optical microscopy and SEM.

*Target species (***R. pectinatoides** *and* **R. praetervisa***)*

Based on the ITS sequences examined in this study, samples originally identified as *R. pectinatoides* (*n* = 107) belonged to different clades without a clear correspondence to publicly available sequences referred to this species (Fig. 1 and Table 2). We could assign them to the following taxa: *R. amoenolens* (*n* = 2), *R. cerolens* Shaffer (*n* = 3 + 3 uncertain), *R. foetens* Pers./*subfoetens* W.G. Sm. (*n* = 4 + 2 uncertain), *R. foetentula* (*n*= 4), *R. granulata* Peck (*n* = 3), *R. insignis* (*n* = 2), *R. grata* (*n* = 4), *R. lilacea* Quél. (*n* = 1), the group *odorata* Romagn./*versicolor* Jul. Schäff. (*n* = 1), *R. praetervisa* (*n* = 2), *R. pulverulenta* Peck (*n* = 4), and *R. sororia* (*n* = 5).

Peck's specimens clustered in three genetically different groups (Fig. 1; Table 2; Supplementary material 1). Sample NYS2303.3 clustered with *R. grata*, supporting previous observations made by Shaffer (1972), Adamčík et al. (2013) and others. Sample NYS2303.2 was included in a North American group sister to the only available sequence (AB211275) referred to *R. sororia* long enough to be inserted in the nucleotide alignment, in addition to two European sequences (nos. 78 and 122; which were also determined as *R. sororia* by morphological characters). On SEM observation, its spores showed mostly isolated and prominent warts (Fig. 2). Unfortunately, we did not have indications about smell or taste for the mentioned sample (original Peck's description lacked this detail). Sample NYS2303.1 fell into a third group, together with samples no. 168, 172 and 174 (originally identified as *R. pectinatoides*), well supported by bootstrap value (99), to which we assigned the name *R. pectinatoides* (see "Discussion"). Estimates of evolutionary divergence over sequence pairs within this clade reached 99.9% similarity (d = 0.001). R. *pectinatoides* showed the same ornamentation type as *R. sororia* (0 = isolated warts, according to the category of Table 3) and intercarpic range of absolute values for spore size 4.8–8 × 4.2–6 μm (*R. sororia*: 6–8 × 5–6.5 μm, slightly larger).

Samples originally identified as *R. praetervisa* (*n* = 24) matched *R. praetervisa* sequences retrieved from GenBank and Unite databases in 14 cases, while 10 samples were

Table 2 Samples analysed in this study, including results of morphological (original identification) and molecular identification

ID	Original identification (label included in sample collection)	Molecular identification (ITS)	Symbiont	Origin	Collection date	GenBank accession number
1	*R. pectinatoides* (legit: Shaffer)	*R. recondita complex* clade 1	*Tsuga* sp.	North Carolina - USA	21 Jul 1970	KF318045
2	*R. pectinatoides* (legit: Shaffer)	*Russula* sp. clade 3	Broad-leaved trees	Indiana - USA	22 Aug 1970	KF318086
3	*R. pectinatoides*	*R. recondita s.s.*	*Betula* sp., *Fagus* sp., *Quercus* sp.	France - EU	08 Aug 1986	KF318071
4	*R. pectinatoides*	*R. recondita s.s.*	*Abies* sp., *Fagus* sp.	Switzerland - EU	08 Aug 1986	KF318063
5	*R. pectinatoides*	*R. recondita complex* clade 1	nd	Connecticut - USA	16 Sep 1989	KF318046
6	*R. pectinatoides*	*R. pectinata* complex clade 4	nd	New Jersey - USA	16 Sep 1989	KF318085
7	*R. pectinatoides*	*R. recondita complex* clade 1	*Quercus* sp., *Tilia* sp.	Washington - USA	01 Aug 1988	KF318047
8	*R. pectinatoides*	*R. subfoetens*? (partial seq)	Wooded ravine	Kansas - USA	21 Jun 1951	
9	*R. pectinatoides*	*R. sororia* complex clade 7	Wooded lawn	Pennsylvania - USA	21 Feb 1931	KF318053
10	*R. pectinatoides* (legit: Shaffer)	*Russula* sp.	Wooded ravine	Kansas - USA	27 Sep 1951	
11	*R. pectinatoides* (legit: Shaffer)	*R. foetens* (partial seq)	Wooded ravine	Kansas - USA	25 Jul 1951	KF318087
12	*R. pectinatoides* (legit: Shaffer)	*R. pulverulenta*	*Juniper* sp.	Kansas - USA	02 Apr 1951	KJ530747
13	*R. pectinatoides*	*R. grata* complex clade 10 (partial seq)	*Castanea*, *Kalmia*, *Quercus* sp.	New York - USA	nd	KJ834585
14	*R. pectinatoides*	*R. grata* complex clade 10	Wood	New York - USA	nd	KF318051
15	*R. pectinatoides* (legit: Shaffer)	*R. subfoetens*? (partial seq)	Wooded ravine	Kansas - USA	09 Jul 1951	
16	*R. pectinatoides*	*Russula* sp.	*Castanea*, *Kalmia*, *Quercus* sp.	New York - USA	09 Jul 1951	
17	*R. pectinatoides* (legit: Shaffer)	*R. pulverulenta*	Wooded ravine	Kansas - USA	04 Jul 1951	KJ834619
18	*R. pectinatoides*	nd (mixed seq)	*Quercus hemisphaerica*, *Quercus laurifolia*	Florida - USA	25 Oct 1950	
19	*R. pectinatoides*	*Russula* sp.	nd	Pennsylvania - USA	08 Jul 1950	KF318088
20	*R. pectinatoides*	*R. granulata*	nd	Pennsylvania - USA	09 Aug 1949	KF318054
21	*R. pectinatoides*	*R. granulata* (partial seq)	nd	Pennsylvania - USA	12 Jul 1950	KJ834584
22	*R. pectinatoides*	nd (mixed seq)	Mixed wood	Pennsylvania - USA	03 Jul 1939	
23	*R. pectinatoides*	nd (no DNA)	nd	Pennsylvania - USA	nd	
24	*R. pectinatoides*	*R. odorata/versicolor*	nd	Quebec - CAN	nd	KF318048
25	*R. pectinatoides*	*R. recondita complex* clade 1	Mixed wood	Pennsylvania - USA	15 Sep 1937	KJ834618
26	*R. pectinatoides*	nd (mixed seq)	nd	North Carolina - USA	18 Aug 1961	
27	*R. pectinatoides*	nd (mixed seq)	Mixed wood	Pennsylvania - USA	19 Jul 1937	
28	*R. pectinatoides*	nd (mixed seq)	nd	Florida - USA	nd	
29	*R. pectinatoides*	*R. cerolens*	*Pinus radiata*	California - USA	10 Dec 1982	KJ834617
30	*R. pectinatoides*	*R. granulata* (partial seq)	*Fagus* sp., *Tilia* sp., *Tsuga* sp.	New York - USA	08 Jul 1910	KJ834583
31	*R. pectinatoides*	nd (no DNA)	nd	Florida - USA	nd	
32	*R. pectinatoides* (legit: Shaffer)	*R. pulverulenta*	*Quercus* sp.	Kansas - USA	01 Sep 1951	KF318055
33	*R. pectinatoides* (legit: Shaffer)	*R. pulverulenta* (partial seq)	nd (sample label: *Ulmus* sp. and *Celtis* sp.)	Kansas - USA	28 Aug 1951	
34	*R. pectinatoides* (legit: Shaffer)	*R. recondita complex* clade 1	Grassland	Kansas - USA	26 May 1951	KJ834616
35	*R. pectinatoides* (legit: Shaffer)	*R. recondita complex* clade 1	*Quercus* sp.	Kansas - USA	25 Jun 1951	KJ530748
36	*R. pectinatoides*	*R. cerolens*	*Pinus radiata*	California - USA	07 Jun 1985	KF683922
37	*R. pectinatoides*	*R. cerolens*	*Arbutus* sp., *Arctostaphylos* sp.	California - USA	05 Dec 1984	KF683923
38	*R. pectinatoides*	*Russula* sp.	*Quercus* sp.	New York - USA	20 Jul 1975	KJ834615
39	*R. praetervisa*	*R. praetervisa*	*Quercus suber*, *Pinus pinaster*	Spain - EU	25 Nov 2011	KF303606
40	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*, *Quercus suber*	Italy - EU	28 Nov 2011	KF303605
41	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*	Italy - EU	28 Nov 2011	KF303604
42	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*	Italy - EU	28 Nov 2011	KF303603
43	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*, *Quercus suber*	Italy - EU	28 Nov 2011	KF303602
43a	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*, *Quercus suber*	Italy - EU	28 Nov 2011	KF303601
44	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*, *Quercus suber*	Italy - EU	28 Nov 2011	KF303600
45	*R. praetervisa*	*R. recondita s.s.*	*Quercus* sp., *Tilia cordata*	Italy - EU	26 Oct 2003	KF318072
46	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*	Italy - EU	05 Dec 2003	KJ834614
47	*R. pectinatoides*	*R. lilacea*	*Corylus avellana*, *Pinus* sp.	Norway - EU	10 Aug 2001	KF318049
48	*R. pectinatoides*	*R. recondita s.s.*	Conifers, *Corylus avellana*, *Quercus* sp.	Norway - EU	25 Sep 2004	KF318070
49	*R. pectinatoides*	*R. recondita s.s.*	*Tilia* sp.	Norway - EU	24 Sep 2004	KF318062
50	*R. pectinatoides*	*R. recondita s.s.*	*Corylus avellana*	Norway - EU	10 Sep 2010	KF318073
51	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*	Italy - EU	21 nov 2009	KF303599
52	*R. pectinatoides*	*R. recondita s.s.*	*Tilia cordata*	Switzerland - EU	26 Jul 2009	KF318069
53	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*	Italy - EU	16 Oct 2008	KF303598
54	*R. praetervisa*	*R. recondita s.s.*	*Quercus cerris*	Italy - EU	nd	KJ834613
55	*R. pectinatoides*	*R. recondita s.s.*	*Cedrus* sp.	Switzerland - EU	28 Oct 1976	KJ834612
56	*R. pectinatoides*	*R. recondita s.s.*	*Cedrus* sp.	Switzerland - EU	12 Jun 1980	KJ834611
57	*R. pectinatoides*	*R. recondita s.s.*	*Cedrus* sp.	Switzerland - EU	15 Jul 1981	KF318061
58	*R. pectinatoides*	*R. recondita s.s.*	Conifers	Switzerland - EU	28 Oct 1981	KJ834610
59	*R. pectinatoides*	*R. recondita s.s.*	Plantation	Switzerland - EU	19 Jun 2000	KF318074
60	*R. praetervisa*	*R. recondita s.s.*	Plantation	Switzerland - EU	28 Sep 1999	KF318068

Table 2 (continued)

ID	Original identification (label included in sample collection)	Molecular identification (ITS)	Symbiont	Origin	Collection date	GenBank accession number
61	*R. pectinatoides*	*R. recondita s.s.*	Plantation	Switzerland - EU	29 Jul 1996	KF318060
62	*R. praetervisa*	*R. recondita s.s.*	Plantation	Switzerland - EU	11 Oct 2000	KF318075
63	*R. praetervisa*	*R. praetervisa*	*Quercus ilex*	Italy - EU	05 Nov 2011	KF303597
64	*R. pectinatoides* fo. *amarescens*	*R. praetervisa*	*Pinus pinea*	Morocco - AFR	19 Dec 1970	KJ530749
65	*R. pectinatoides* var. *pseudoamoenolens* (holotype)	*R. praetervisa* (partial seq)	*Pinus halepensis*	Morocco - AFR	09 Dec 1967	
66	*R. pectinatoides*	*R. recondita s.s.*	*Fagus* sp., *Castanea* sp.	Switzerland - EU	13 Jun 2011	KF318067
67	*Russula* cf. *praetervisa*	*R. recondita s.s.*	*Carpinus* sp., *Quercus* sp., *Tilia* sp.	Germany - EU	15 Jun 2002	KF318059
68	*R. pectinatoides*	*R. recondita s.s.*	*Quercus* sp.	Germany - EU	08 Sep 2006	KF318076
69	*R. pectinatoides*	*R. recondita s.s.*	*Fagus* sp., *Quercus* sp.	Germany - EU	27 Aug 2007	KF318066
70	*R. pectinatoides*	*R. recondita s.s.*	*Quercus* sp.	Germany - EU	22 Aug 2008	KF318058
71	*R. pectinatoides*	*R. recondita s.s.*	*Betula* sp., *Quercus* sp.,*Tilia* sp.	Germany - EU	19 Jul 2008	KF318077
72	*R. insignis*	*R. insignis*	*Quercus* sp.	Germany - EU	01 Jul 2009	KF318057
73	*R. pectinata*	*R. pectinata* complex clade 6	Mixed deciduous forest	Germany - EU	31 Jul 2010	KF318081
74	*R. pectinatoides*	*R. recondita s.s.*	*Carpinus* sp., *Quercus* sp.	Germany - EU	08 Aug 2010	KF318065
75	*R. amoenolens*	*R. amoenolens*	*Quercus* sp.	Germany - EU	12 Aug 2010	KF318080
76	*R. pectinatoides* fo. *alba*	*R. amoenolens*	*Fagus* sp., *Pinus* sp.	Germany - EU	03 Sep 2010	KJ834609
77	*R. pectinata*	*Russula* sp.	*Fagus* sp., *Quercus* sp., *Tilia* sp.	Germany - EU	14 Aug 2010	KF318050
78	*R. sororia*	*R. sororia* complex clade 8	*Carpinus* sp., *Fagus* sp., *Quercus* sp., *Tilia* sp.	Germany - EU	14 Aug 2010	KF318052
79	*R. pectinata*	*R. pectinata* complex clade 6	*Castanea* sp., *Quercus* sp., *Populus* sp.	Germany - EU	22 Aug 2010	KF318082
80	*R. pectinata*	*R. pectinata* complex clade 6	*Quercus* sp.	Germany - EU	13 Jun 2011	KF318083
81	*R. pectinatoides*	*R. recondita s.s.*	*Helianthemum* sp.	Germany - EU	16 Jul 2011	KF318078
82	*R. pectinatoides*	*R. pectinata* complex clade 6	*Quercus* sp.	Germany - EU	03 Jul 2012	KF318084
83	*R. pectinatoides*	*R. recondita s.s.*	*Tilia* sp.	Belgium - EU	10 Oct 2012	KF318064
84	*R. praetervisa*	*R. insignis* (partial seq)	*Quercus ilex*, *Pinus pinea*	Spain - EU	12 Dec 2012	KF318056
85	*R. pectinatoides* (legit: Romagnesi)	*R. recondita s.s.*	Wood	France - EU	19 Aug 1991	KF318079
86	*R. ombrophila* (holotype)	*R. ombrophila*	*Corylus avellana*, *Salix atrocinerea*	Spain - EU	17 Jul 2006	KF971694
91	*R. pectinata*	*R. amoenolens*	*Carpinus* sp., *Pinus strobus*	Switzerland - EU	02 Jul 2010	KJ834607
92	*R. pectinatoides*	*R. recondita s.s.* (designed as holotype)	*Corylus maxima purpurea*	Switzerland - EU	25 Aug 2013	KJ530750
93	*R. pectinatoides*	*R. recondita s.s.*	*Cedrus deodara*	Switzerland - EU	26 Sep 2013	KJ530752
94	*R. insignis*	*R. insignis*	*Quercus ilex*, *Pinus* sp.	France - EU	06 Sep 2006	KJ834606
95	*R. praetervisa*	*R. recondita s.s.*	*Castanea sativa*	Italy - EU	13 Sep 2013	KJ530754
96	*R. pectinatoides*	*R. recondita s.s.*	*Castanea sativa*	Italy - EU	22 Aug 2013	KJ530753
97	*R. illota*	*R. illota*	*Castanea sativa*	Italy - EU	18 Jul 2002	KJ834605
98	*R. fragrantissima*	*R. fragrantissima*	*Quercus cerris*, *Quercus pubescens*	Italy - EU	09 Nov 2012	KJ530751
99	*R. sororia*	*R. grata* complex clade 11	*Quercus pubescens*	Italy - EU	04 Oct 2003	
100	*R. sororia*	*R. grata* complex clade 11	*Quercus ilex*, *Pinus* sp.	Italy - EU	05 Dec 2003	KJ834604
101	*R. insignis*	*R. insignis*	*Castanea sativa*	Italy - EU	20 Jul 2002	KJ834603
102	*R. insignis*	*R. insignis*	*Quercus cerris*	Italy - EU	27 Aug 2005	KJ834602
103	*R. grata*	*R. grata* complex clade 11	*Fagus* sp.	Italy - EU	29 Jul 2002	KJ834601
104	*R. grata*	*R. grata* complex clade 11	*Betula* sp., *Castanea* sp.	Italy - EU	28 Sep 2003	KJ834600
105	*R. grata*	*R. grata* complex clade 11	*Fagus sylvatica*	Switzerland - EU	05 Oct 2013	KJ834599
106	*R. grata*	*R. grata* complex clade 11	*Castanea sativa*	Italy - EU	25 Sep 2001	KJ834598
107	*R. inamoena*	*R. inamoena*	*Quercus ilex*	Italy - EU	26 Oct 2002	KJ834597
108	*R. fragrantissima*	*R. fragrantissima*	*Quercus pubescens*	Italy - EU	15 Aug 2002	KJ834596
109	*R. inamoena*	*R. inamoena*	*Quercus ilex*	Italy - EU	14 Oct 2006	KJ834595
110	*R. amoenolens*	*R. amoenolens*	*Quercus peduncolata*	Italy - EU	27 Aug 2006	KJ834594
111	*R. amoenolens*	*R. amoenolens*	*Quercus* sp.	Italy - EU	02 Nov 2003	KJ834593
112	*R. amoenolens*	*R. grata* complex clade 11	*Castanea sativa*	Italy - EU	08 Nov 2003	KJ834592
113	*R. amoenolens*	*R. recondita s.s.*	*Picea pungens*	Switzerland - EU	06 Oct 2013	KJ530755
114	*R. amoenolens*	*R. amoenolens*	*Quercus pubescens*	Italy - EU	04 Oct 2003	KJ834591
115	*R. amoenolens*	*R. amoenolens*	*Fagus sylvatica*	Switzerland - EU	05 Oct 2013	KJ834590
116	*R. insignis*	*R. recondita s.s.*	Grassland	Switzerland - EU	06 Oct 2010	KJ834589
117	*R. insignis*	*R. insignis* (partial seq)	*Quercus* sp.	Switzerland - EU	31 Jul 1987	
118	*R. pectinatoides*	*R. recondita s.s.*	*Quercus* sp.	Italy - EU	03 Oct 2012	KJ834588
119	*R. pectinatoides* var. *brevispinosa*	*R. recondita s.s.*	Mixed deciduous forest	Switzerland - EU	19 Oct 1985	KJ530756
120	*R. illota*	*R. illota*	*Abies alba*, *Fagus* sp.	Switzerland - EU	16 Aug 1987	KJ834587
121	*R. illota*	*R. illota*	*Picea abies*	Switzerland - EU	17 Aug 2012	KJ834586
122	*R. sororia*	*R. sororia* complex clade 8	*Quercus* sp.	Italy - EU	08 Oct 2012	
123	*R. sororia*	*R. parazurea*	nd	Switzerland - EU	21 Nov 1996	
124	*R. sororia*	*R. praetervisa*	*Pinus pinaster*	Italy - EU	08 Nov 1997	KJ834578

Table 2 (continued)

ID	Original identification (label included in sample collection)	Molecular identification (ITS)	Symbiont	Origin	Collection date	GenBank accession number
125	*R. amoenolens*	*R. amoenolens*	*Castanea* sp., *Quercus* sp.	Switzerland - EU	19 Oct 2012	KJ834577
126	*R. amoenolens*	*R. amoenolens*	*Picea* sp.	Switzerland - EU	21 Oct 2000	KJ834576
127	*R. grata*	*R. grata* complex clade 11	*Betula* sp., *Castanea* sp., *Quercus* sp.	Switzerland - EU	08 Aug 2008	KJ834575
128	*R. grata*	*R. foetentula* complex	*Castanea* sp., *Tilia* sp.	Switzerland - EU	31 Jul 2009	KJ834574
129	*R. laurocerasi* var. *fragrans*	*R. grata* complex clade 11	Broad-leaved trees, *Castanea* sp.	Switzerland - EU	27 Jul 1978	KJ834573
130	*R. laurocerasi* var. *fragrans*	*R. grata* complex clade 11	Mixed deciduous forest	Switzerland - EU	23 Jul 1979	KJ834572
131	*R. pectinatoides*	*R. recondita complex* clade 1	*Quercus rubra*	Quebec - CAN	20 Jul 2010	KJ834571
132	*R. pectinatoides*	*R. foetentula complex*	*Quercus* sp.	Quebec - CAN	07 Aug 1995	KJ834570
133	*R. pectinatoides*	*R. recondita complex* clade 1	*Carya* sp., *Fagus* sp., *Quercus* sp., *Ostrya* sp.	Quebec - CAN	12 Jul 2006	KJ834569
134	*R. pectinatoides*	*R. recondita complex* clade 1	*Carya* sp., *Quercus* sp.	Quebec - CAN	19 Jul 1990	KJ834567
135	*R. pectinatoides*	*R. recondita complex* clade 1	*Quercus* sp., *Salix* sp.	Quebec - CAN	10 Jul 2008	KJ834566
136	*R. pectinata*	nd (no DNA)	nd	California - USA	nd	
137	*R. pectinata*	*R. cerolens* (partial seq)	nd	California - USA	nd	KJ834582
138	*R. pectinata*	*R. illota*	nd	Ontario - CAN	nd	KJ834565
139	*R. pectinata*	*Russula* sp.	nd	Ontario - CAN	nd	KJ834564
140	*R. pectinatoides*	*R. recondita complex* clade 1	*Quercus borealis*, *Quercus gambelii*, *Quercus robur*	Ontario - CAN	02 Aug 1973	KJ834562
141	*R. pectinatoides*	*R. recondita complex* clade 1	nd	Iowa - USA	08 Sep 1924	KJ834560
142	*R. praetervisa*	*R. praetervisa*	*Cistus* sp., *Pinus pinea*	Italy - EU	27 Oct 2013	KJ530760
143	*R. pectinatoides*	*R. recondita s.s.*	*Pinus sylvestris*	Italy - EU	17 Nov 2013	KJ834559
144	*Russula* cf. *praetervisa*	*R. recondita s.s.*	*Quercus robur*	Italy - EU	27 Oct 2013	KJ834557
145	*Russula* cf. *praetervisa*	*R. recondita s.s.*	*Betula* sp., *Castanea sativa*, *Corylus avellana*	Italy - EU	06 Oct 2013	KJ834556
146	*Russula* cf. *praetervisa*	*R. praetervisa*	*Pinus pinea*	Italy - EU	05 Oct 2013	KJ834555
147	*R. amoenolens*	*R. amoenolens*	*Quercus robur*	Italy - EU	02 Nov 2013	KJ834554
148	*R. pectinatoides*	*R. insignis*	*Quercus robur*	Italy - EU	02 Nov 2013	KJ834553
149	*R. pectinatoides*	*R. insignis* (partial seq)	*Quercus robur*	Italy - EU	02 Nov 2013	
150	*R. pectinatoides*	*R. grata* complex clade 10	*Quercus* sp.	Florida - USA	09 Jul 1984	KJ834620
151	*R. pectinatoides*	*R. subfoetens* (partial seq)	*Quercus* sp.	USA	15 Jul 2013	
152	*R. pectinatoides*	*R. foetens* (partial seq)	Mixed forest	Tennessee - USA	30 Jun 2011	
153	*R. pectinatoides*	*R. recondita complex* clade 1	*Quercus* sp.	Florida - USA	15 Jul 2013	KJ834621
154	*R. pectinatoides*	*R. sororia* complex clade 7	*Quercus phellos, Pinus strobus*	Tennessee - USA	16 Oct 2009	
155	*R. pectinatoides*	*R. recondita complex* clade 1	*Fagus* sp., *Quercus* sp., *Pinus* sp.	North Carolina - USA	08 Sep 2012	KJ834622
156	*R. foetentula*	*R. foetentula complex*	Mixed forest	Tennessee - USA	26 Jun 2011	KJ834623
157	*R. pectinatoides*	*R. sororia* complex clade 7	*Quercus* sp., ferns	Minnesota - USA	11 Jul 2002	
158	*Russula* cf. *pectinatoides*	*R. amoenolens* (partial seq)	*Quercus* sp.	Minnesota - USA	15 Jul 2002	
159	*R. foetentula*	*R. granulata*	*Fagus grandifolia*, *Quercus* sp., *Tsuga canadensis*	West Virginia - USA	03 Aug 2002	KJ834624
160	*R. foetentula*	*R. foetentula complex*	*Carpinus caroliniana*, *Tsuga canadensis*	West Virginia - USA	06 Aug 2002	KJ834626
161	*R. foetentula*	*R. granulata*	*Quercus* sp., *Tsuga canadensis*	West Virginia - USA	06 Aug 2002	KJ834625
162	*R. pectinatoides*	*Russula* sp. clade 3	Broad-leaved trees, *Tsuga canadensis*	West Virginia - USA	07 Aug 2002	KJ834627
163	*R. pectinatoides*	*Russula* sp.	*Betula lenta*, *Fagus grandifolia*, *Tsuga canadensis*	West Virginia - USA	10 Aug 2005	KJ834628
164	*R. pectinatoides*	*R. subfoetens* (partial seq)	*Fagus grandifolia*, *Quercus* sp., *Tsuga canadensis*	West Virginia - USA	24 Aug 2005	
165	*R. foetentula*	*R. foetentula complex*	*Carpinus* sp., *Carya* sp.	Quebec - CAN	20 Aug 2004	KJ834629
166	*R. foetentula*	*R. granulata* (partial seq)	*Pinus* sp., *Quercus* sp., *Tsuga* sp.	Quebec - CAN	31 Jul 1980	KJ834580
167	*R. pectinatoides*	*R. foetentula complex*	Mature river floodplain forest	Texas - USA	09 Jun 1990	KJ834630
168	*R. pectinatoides*	*R. pectinatoides*	*Quercus* sp. dominated woodland	Indiana - USA	19 Aug 1996	KU640186
169	*R. pectinatoides*	*R. foetentula complex*	River bottomland forest (hardwoods)	Texas - USA	10 Jul 1991	
170	*R. pectinatoides*	*R. recondita complex* clade 1	Mixed deciduous forest	Illinois - USA	22 Aug 1996	KJ530759
171	*Russula* cf. *pectinatoides*	*R. recondita complex* clade 1	nd	Wisconsin - USA	05 Sep 1978	KJ530758
172	*R. pectinatoides*	*R. pectinatoides*	*Quercu*s sp.	Indiana - USA	02 Aug 1995	KU640187
173	*R. pectinatoides*	*Russula* sp.	Mixed deciduous forest	Illinois - USA	03 Sep 1996	
174	*R. pectinatoides*	*R. pectinatoides*	*Quercu*s sp.	Indiana - USA	16 Aug 1995	KU640188
175	*R. pectinatoides*	*R. recondita s.s.*	Ornamental conifer in garden	Spain - EU	23 Oct 2010	KJ530757
176	*R. praetervisa*	*R. parazurea*	*Quercus* sp.	England - EU	06 Nov 2005	
177	*R. praetervisa*	*R. praetervisa*	Quercus sp. in parkland	Ireland - EU	14 Oct 2000	
178	*R. pectinatoides*	nd (no DNA)	*Tilia* sp.	England - EU	05 Oct 1998	
179	*R. pectinatoides*	nd (no DNA)	*Betula* sp., *Cedrus* sp., *Pinus* sp.	England - EU	30 Oct 1996	
180	*R. pectinatoides*	nd (mixed seq)	*Tilia* sp.	England - EU	03 Oct 2000	
181	*R. pectinatoides*	*Russula* sp.	Mixed deciduous forest	Iowa - USA	22 Jul 2006	
182	*R. pectinatoides*	*R. pectinata* complex clade 4	Mixed deciduous forest	Iowa - USA	22 Aug 2006	

Table 2 (continued)

ID	Original identification (label included in sample collection)	Molecular identification (ITS)	Symbiont	Origin	Collection date	GenBank accession number
183	*R. pectinatoides*	*R. foetentula complex*	*Carya* sp., *Quercus* sp.	Iowa - USA	31 Aug 2009	
184	*R. pectinatoides*	*R. sororia* complex clade 7	Upland wood	Iowa - USA	13 Jul 2004	
186	*R. pectinatoides* (Peck's reference collection NYS2303.1)	*R. pectinatoides*	Grassy ground in groves or pastures	New York - USA	Aug 1906	KU640189
187	*R. pectinatoides* (Peck's reference collection NYS2303.2)	*R. sororia* complex clade 7	Grassy ground in groves or pastures	New York - USA	Aug 1906	KJ476888
188	*R. pectinatoides* (Peck's reference collection NYS2303.3)	*R. grata* complex clade 10	Grassy ground in groves or pastures	New York - USA	Aug 1906	KJ476889

nd no data; *seq* sequence

assigned to other species (Table 2). Conversely, specimens morphologically identified as *R. pectinatoides* fo. *amarescens* (no. 64, from Morocco), *R. pectinatoides* var. *pseudoamoenolens* (no. 65, from Morocco, not shown in the tree because of partial ITS sequence) and *R. sororia* (no. 124) were included in *R. praetervisa* (Fig. 1). This species appeared to be genetically well-characterised, with high bootstrap support (99) and 100% homology among the sequences, with the exception of the Moroccan samples that had five nucleotide substitutions compared to the European ones (G↔A at position 11; C↔T at position 134, G↔A at position 480, C↔T at position 519, C↔T at position 568), resulting in 99.6% homology with the main haplotype (mean distance d = 0.004). Estimates of evolutionary divergence over sequence pairs between *R. praetervisa* and the other clades in the tree (Fig. 1) showed always <96.5% similarity (d = 0.035), which is consistent with this species being distinct from the others. Morphological and ecological characters were also consistent in this taxon. Spore ornamentation was characterised by warts mostly connected by ridges (intercarpic variation range 3–5, according to the category of Table 3; Fig. 3). As described by Sarnari (1998), *R. praetervisa* is common in the Mediterranean habitat. All samples identified in this study were found under evergreen oaks (*Quercus ilex* and *Quercus suber*), Mediterranean pines (*Pinus pinaster*, *Pinus pinea* and *Pinus halepensis*) and/or *Cistus* spp. One sample (no. 177) was found in a parkland in Ireland under *Quercus* sp. (see “Discussion”).

The largest clade in Figure 1 included 38 samples originally identified as *R. pectinatoides* (n = 28, including a specimen of var. *brevispinosa*), *R. praetervisa* (n = 8), *R. amoenolens* (n = 1) and *R. insignis* (n = 1). However, it did not contain any sequences generated from the Peck type elements we examined. This group appears to be the same as clade 15 in Avis (2012; supported by bootstrap = 93 vs.66 in this paper) and largely corresponds to what has commonly been identified as *R. pectinatoides* in Europe. Based on this, we opted to describe this clade as a new species, named *R. recondita* Melera & Ostellari (see description below). Spores of *R. recondita* had variable ornamentation, normally with warts from isolated to briefly connected, more rarely with obvious connections (intercarpic variation range 1–4, according to the category of Table 3). The intercarpic range of absolute values for spore size was 6–10 × 5–7 μm (see Table 3). *R. recondita* was found in a variety of habitats, from deciduous and coniferous forests to grasslands, from wild to anthropic habitats, in plantations and also in association with ornamental plants. Potential symbionts include deciduous and evergreen plants, such as *Abies* sp., *Betula* sp., *Carpinus* sp., *Carya* sp., *Castanea* sp., *Cedrus* sp., *Corylus* sp., *Fagus* sp., *Helianthemum* sp., *Ostrya* sp., *Picea* sp., *Pinus* sp., *Quercus* sp., *Salix* sp., *Tilia* sp., *Tsuga* sp.

Sister to the proposed *R. recondita* are a set of geographically divergent clades found on different continents (Fig. 1). One clade, labelled ‘*R. recondita* complex clade 1 (NA)’, composed exclusively of North American specimens (99.98% homology within the sequences; 98.8% homology with the European *R. recondita*), was supported by a consistent bootstrap value (90) and is comparable to clade 13 in Avis (2012; supported by bootstrap = 92). Morphologically, the intercarpic range of absolute values for spore size in this group was 6.5–8.5 × 5–6.5 μm, with spore ornamentation characterised by generally isolated warts (0–3, according to the category of Table 3). A smaller clade, labelled ‘*R. recondita* complex clade 2 (Asia)’, was composed by two sequences from Estonia (UDB015983) and China (JX434670), showing 99% homology with the European haplotypes. Another clade, labelled ‘*Russula* sp. clade 3’ contained North American samples nos. 2 and 162 (highly supported by bootstrap = 97; 100% homology between the samples). Estimates of evolutionary divergence over sequence pairs within the complex composed by *R. recondita* (EU), clade 1 (‘*recondita* NA’) and clade 2 (‘*recondita* ASIA’) reached 99.8% similarity (d = 0.0018); homology of this group with clade 3 was <98% and <96.7% (d = 0.033) with all the other clades. These results indicate that *R. recondita* is either an individual species with geographically determined subspecies or, alternatively, a species complex with a set of

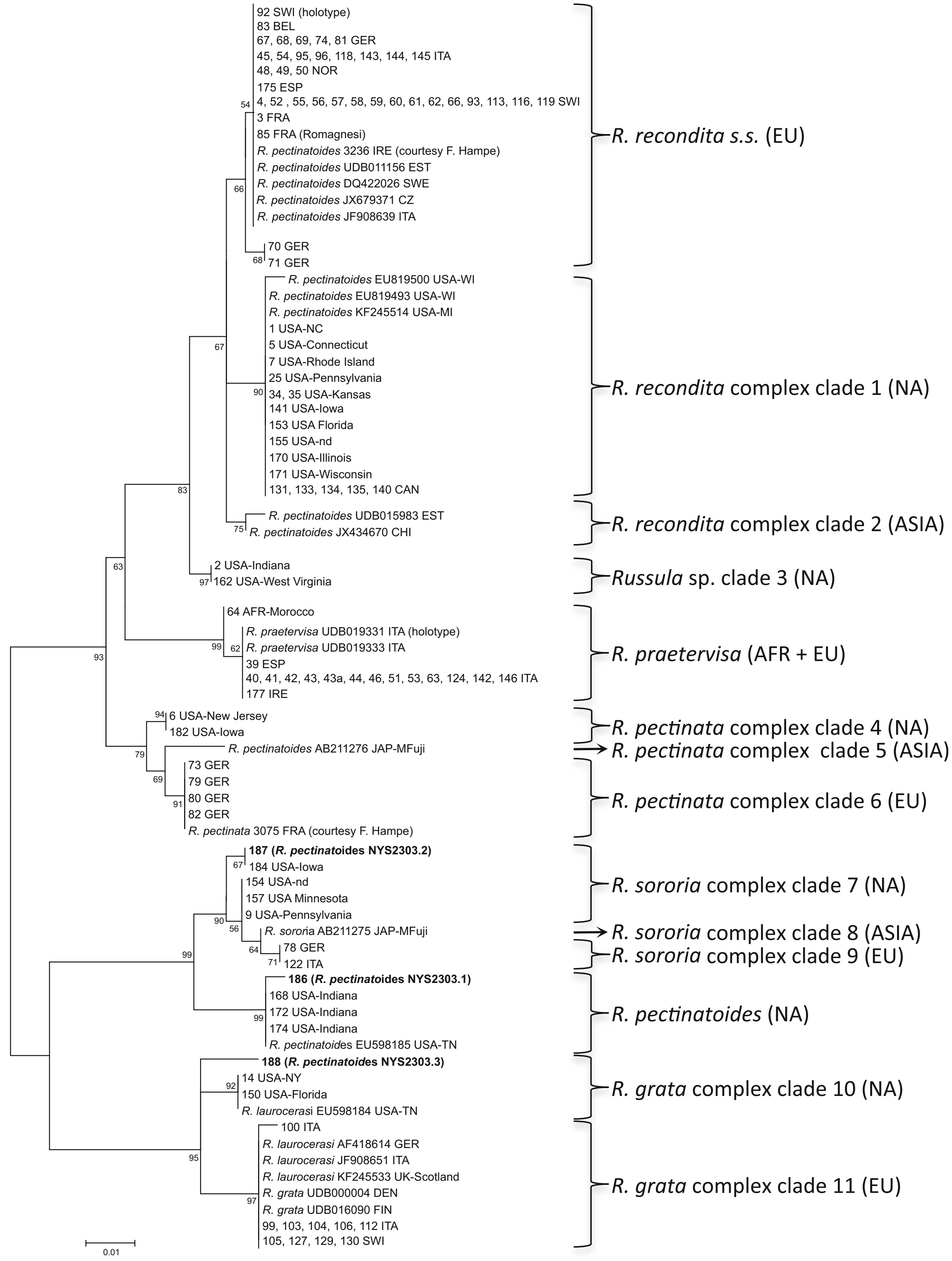

Fig. 1 Maximum Likelihood tree based on 643-bp fragment of ITS1-5.8S rRNA-ITS2 region of samples of *Russula pectinatoides*, *R. praetervisa* and the most closely related taxa analysed in this study. The tree with the highest log likelihood (−1005.8333) is shown. Bootstrap values are shown next to the branches. EU Europe (BEL Belgium, CZ Czech Republic, DEN Denmark, ESP Spain, EST Estonia, FIN Finland, FRA France, ITA Italy, IRE Ireland, NOR Norway, SWE Sweden, SWI Switzerland, UK United Kingdom); USA United States (FL Florida, IA Iowa, IN Indiana, MI Michigan, MN Minnesota, NC North Carolina; NJ New Jersey, NY New York, PA Pennsylvania, TN Tennessee, WI Wisconsin, WV West Virginia); AFR Africa; CAN Canada; CHI China; JAP Japan; NA North America. NJ and ML approaches yielded trees with the same topology.

Table 3 Morphological data on fresh samples and selected dried specimens

ID	Origin	Spore ornamentation	Spore size (μm)	Spore average length (μm)	Spore average width (μm)	Stipe	Smell	Taste
R. pectinatoides:								
168	Indiana - USA	0 (OM)	6–7 × 5–6	6.45	5.55	Reddish staining at the base of stipe	nd	nd
172	Indiana - USA	0 (OM)	7–8 × 5.5–6	7.2	5.85	nd	Inocybe odor	nd
174	Indiana - USA	0 (OM)	4.8–6 × 4.2–4.8	5.4	4.5	Orange-brown staining	SFightly oily unpleasant	Mild
	RANGE:	0	4.8–8 × 4.2–6	6.35	5.3			
R. praetervisa:								
39	Spain - EU	5 (OM)	7–9 × 6–7	8	6.5	nd	nd	nd
40	Italy - EU	4 (OM)	7–8.5 × 5.5–7	7.75	6.25	nd	nd	nd
41	Italy - EU	4 (OM + SEM)	7–8.5 × 6–7	7.75	6.5	nd	nd	nd
42	Italy - EU	4 (OM)	7.5–9 × 6.5–7.5	8.45	6	nd	nd	nd
43	Italy - EU	4 (OM)	7–8 × 6–7	7.5	6.5	nd	nd	nd
43a	Italy - EU	4 (OM)	7–8 × 5.5–6.5	7.5	6	nd	nd	nd
44	Italy - EU	4 (OM)	7–8.5 × 6–7	7.75	6.5	nd	nd	nd
46	Italy - EU	4 (OM)	7–8 × 6–6.5	7.5	6.25	nd	nd	nd
51	Italy - EU	3 (OM + SEM)	7–9 × 5.5–7.5	8	6.5	nd	nd	nd
53	Italy - EU	3 (OM + SEM)	7–8 × 6–7	7.5	6.5	nd	nd	nd
63	Italy - EU	4 (OM + SEM)	7–8.5 × 5.5–6.5	7.75	6	nd	fruity to *R. foetens*	Unpleasant, not peppery
64	Morocco - AFR	4 (OM)	7–9 × 6–6.5	8	6.5	nd	nd	nd
	RANGE:	3-5	7–9 × 5.5–7.5	7.79	6.33			
R. recondita s.s. (EU):								
3	France - EU	3 (OM)	7.5–8.5 × 5.5–6.5	8	6	nd	nd	nd
4	Switzerland - EU	3 (OM)	6–7.5 × 5–6.5	6.75	5.75	nd	nd	nd
45	Italy - EU	3 (OM + SEM)	8–10 × 6–6.5	9	6.25	nd	nd	nd
48	Norway - EU	4 (OM + SEM)	7–8 × 5.5–6.5	7.5	6	nd	nd	nd
49	Norway - EU	4 (OM + SEM)	6.5–8 × 5–6.5	7.25	5.75	nd	nd	nd
50	Norway - EU	3 (OM + SEM)		7.25	6.25	nd	nd	nd
52	Switzerland - EU	1 (OM)	7–8 × 6–6.5	7.5	6.25	nd	nd	nd
54	Italy - EU	3 (OM)	6.5–7.5 × 5–6	7	5.5	nd	nd	nd
55	Switzerland - EU	2 (OM)	7–8 × 5–7	7.5	6	nd	Unpleasant, similar foetens	Unpleasant but not acrid
56	Switzerland - EU	1 (OM)	7–8 × 5.5–6.5	7.5	6	nd	nd	nd
57	Switzerland - EU	2 (OM + SEM)	7–8.5 × 6	7.5	5.5	nd	nd	nd
58	Switzerland - EU	1 (OM)	7–8 × 5.5–6.5	7.5	6	nd	nd	nd
59	Switzerland - EU	1 (OM)	7–7.5 × 5–6	7.25	5.5	nd	nd	nd
60	Switzerland - EU	1 (OM)	7 × 5.5–6	7	5.75	nd	nd	nd
61	Switzerland - EU	1 (OM)	7–8 × 6	7.5	6	nd	nd	nd
62	Switzerland - EU	3 (OM + SEM)	7–9 × 6–7	8	7	nd	nd	nd
66	Switzerland - EU	3 (OM + SEM)	6–7.5 × 5–6	6.5	5.5	nd	Similar *R. foetens*, also fruity or similar *R.fellea*	Unpleasant, slightly peppery
92	Switzerland - EU	1 (OM + SEM)	7–8.5 × 5.5–7	7.75	6.25	Purple red touched at the base	Smell fruity-acid, unpleasant	Mild then disgusting, rubbery
	RANGE:	1-4	6–10 × 5–7	7.46	5.96			
R. recondita clade 1 (NA):								
1	North Carolina - USA	1 (OM)	6.5–8 × 5–6.5	7.25	5.75	nd	nd	nd
5	Connecticut - USA	1 (OM; spines 0.5-0.8 μm)	7–8 × 5.5-6	7.5	5.75	nd	nd	Mild
7	Washington - USA	1 (OM + SEM)	7–7.5 × 5.5–6	7.25	5.75	Buff	Nil to slowly sweet	

Table 3 (continued)

ID	Origin	Spore ornamentation	Spore size (µm)	Spore average length (µm)	Spore average width (µm)	Stipe	Smell	Taste
								Mild, then slowly bitter, then hot peppery
25	Pennsylvania - USA	1 (OM)	7–8 × 5.5–6.5	7.5	6	nd	nd	nd
34	Kansas - USA	3 (OM)	7.5–8.5 × 6–6.5	8	6.25	nd	nd	nd
35	Kansas - USA	1 (OM)	7–7.5 × 5.5–6	7.25	5.75	nd	nd	nd
134	Quebec - CAN	0 (OM; spines 0.5-0.75 µm)	7.5–8.5 × 6–6.5	8	6.25	nd	Slightly nauseating	Mild
	RANGE:	0-3	6.5–8.5 × 5–6.5	7.54	5.93			
R. sororia complex:								
9	Pennsylvania - USA	0 (OM)	6–7.5 × 5–6	6.75	5.5	nd	nd	nd
78	Germany - EU	0 (OM)	7–8 × 5.5–6.5	7.3	5.9	nd	nd	nd
122	Italy - EU	0 (OM)	7–8 × 5.5–6.5	7.3	6	Rust staining	Spermatic	Strongly peppery
184	Iowa - USA	0 (OM)	6.5–7 × 5.5–6	6.9	5.5	nd	nd	nd
187	New York - USA	0 (OM)	6–6.5 × 5–6.5	6.3	5.6	nd	nd	nd
	RANGE:	0	6–8 × 5–6.5	6.91	5.7			

Categories of spore ornamentation: *0* = isolated warts; *1* = rare connections; *2* = few connections; *3* = some connections; *4* = connected warts; *5* = reticulate warts

Nd no data; *OM* optical microscopy; *SEM* scanning electron microscopy.

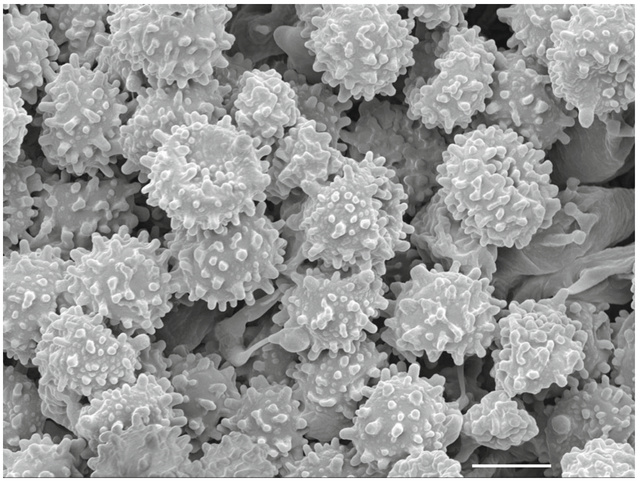

Fig. 2 Spores of Peck's holotype of *R. pectinatoides* NYS2303.2 observed by SEM (*Scale bar* 5 µm; picture: M.-L. Chappuis).

species, most of which have diverged by geography. This study was not designed to select between these alternative explanations, but we encourage future research to do so. This group, as well as other groups (clades nos. 4, 5 and 6 for *R. pectinata*; clades nos. 7, 8 and 9 for *R. sororia*; clades nos. 10 and 11 for *R. grata*), were not assigned a specific name because no sufficient complementary data or reference sequences were available to describe them more exhaustively.

Description of new species of Russula

Russula recondita Melera & Ostellari, sp. nov. – Mycobank MB 815676; Figs. 4 and 5.

Etymology: 'recondita' (Latin), 'hidden, forgotten' meaning that, despite the species being widespread in Europe, it has been overlooked and not properly interpreted or identified.

Holotype: Switzerland, Canton Ticino, Claro, Coord. WGS 84 (lat/lon): 46°15'5'N, 9°1'19E (46.25130N, 9.02188E),

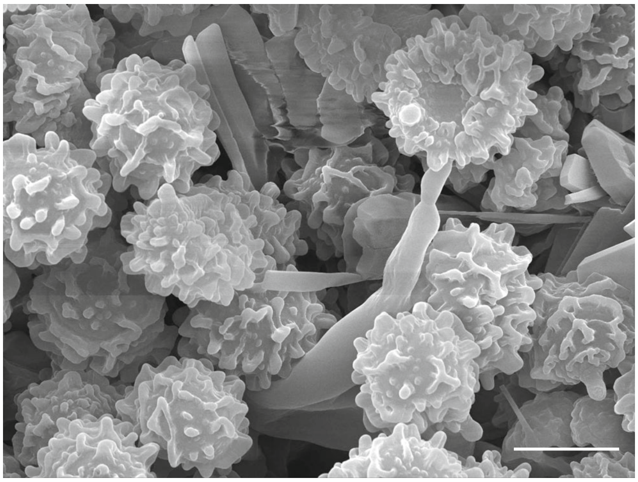

Fig. 3 Spores of *Russula praetervisa* observed by SEM (sample no. 51, — scale bar = 5 µm; picture: M.-L. Chappuis).

Fig. 4 *Russula recondita* (LUG19058, holotype; *scale bar* 10 mm; picture: S. Melera).

under *Corylus maxima*, 25 Aug. 2013, collector Sacha Melera. Herbarium number LUG 19058, Switzerland, Canton Ticino, Lugano, Museo cantonale di storia naturale (Fig. 4). GenBank accession number KJ530750.

Diagnosis: Compared to other fetid Russulas, *R. recondita* is distinguished by its completely mild flavour, without any bitterness, and its smell composed of fruity (pelargonium-like) and rubbery (*R. foetens*-like) notes. The spores of *R. recondita* have variable ornamentation, normally with warts from isolated to briefly connected, more rarely with obvious connections. In contrast, *R. praetervisa* has warts with numerous connections, forming an incomplete mesh. As the intercarpic variation for spore ornamentation ranges overlap for these two species (3–5 vs. 1–4, according to the category of Table 3), as well as intercarpic range of absolute values for spore size (6–10 × 5–7 μm vs. 7–9 × 5.5–7.5 μm), the distinction of the two species based on these characters could be problematic. *R. insignis* is distinguished from *R. recondita* by having faint smell, vaguely fruity and pleasant taste, the yellowish remains of veil at the base of the stipe, the immediate reaction to KOH (the base of the stipe turns red), and subreticulate spores. *R. hortensis* is distinguished from *R. recondita* by the metallic gray, grey-beige or gray-brown pileus, the smell of fish or pickled anchovies, a sweet taste, the stipe not purple-red touched at the base, and reticulated spores. *R. fuscodiscoidea* is distinguished from *R. recondita* by having a pale-coloured pileus with white-cream, brown or grey-orange at the centre, the smell of lye or soap, mild to slightly acrid taste of the lamellae, and the stipe not purple-red touched at the base.

Description

Pileus: Quite fleshy, 35–70 mm in diameter, first convex then flattened to deeply depressed. Margin thin, first slightly involuted then straight, at maturity striated-sulcate up to half of the radius or over. Cuticle easily removable towards the centre of the cap, relatively dry but viscid, especially in the centre, when wet. Colour ochraceous, fawn, ochre honey, ochre-grey-bistre, almost always darker in the centre, often rusty spotted; spots usually limited, seldom extensive.

Lamellae: Quite regular, brittle, rarely with lamellulae, adnate or slightly subdecurrent, in some cases slightly anastomosing, cream-coloured, sometimes rust touched.

Stipe: Cylindrical or slightly tapered downwards, 30–55 × 8–16 mm, sometimes slightly flared at the top, first firm but soon spongy and then lacunose. Whitish, sometimes with bistre nuances, often purple red touched at the base, or in the absence of this pigment, often rust or rusty-brown touched.

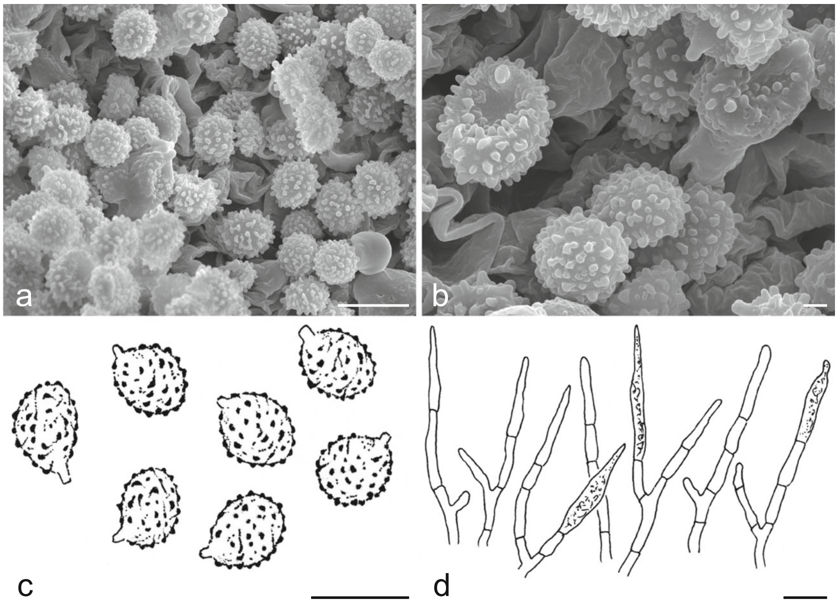

Fig. 5 *Russula recondita* (LUG19058, holotype). **a, b** Spores observed by SEM (picture: M.-L. Chappuis); **c** spores observed by optical microscopy (illustration: Carlo Ostellari); **d** cuticle (illustration: Carlo Ostellari). *Scale bars* (**a, c, d**) 10 μm; (**b**) 1 μm.

Flesh: Not very firm, soggy and soon saggy, whitish but often rusty touched. Smell fruity-acid, overall unpleasant. Taste mild everywhere, initially little perceptible but soon decidedly nauseating and disgusting, reminiscent of rubber and caoutchouc.

Chemical reaction: Guaiac: quickly blue-green; $FeSO_4$: weak pink-grey.

Spore print: Dark cream, Code Romagnesi IIc–IId.

Spore: Ovoid, elongated, 7–8.5 × 5.5–7 μm (Q: 1.14–1.46 μm), clearly amyloid, echinulate, with obtuse conical warts, some of them sometimes pronounced and up to 1 μm high, completely isolated or more or less connected by short ridges or fine connections, in some cases partially cross-linked (reticulate). Hilar plage evident by SEM observation (Fig. 5a–c).

Cuticle: Cuticle hairs cylindrical and septate, branched, up to 4–5 μm in width, with end obtuse or attenuated. Dermatocystidia few in number and with poorly reflective content, measuring 4–6 μm, usually conical-elongated but sometimes with end attenuated or capitate (Fig. 5d).

Habitat: Common from summer to late autumn, growth in groups of many specimens at the edge of deciduous or coniferous forests, preferably on not heavy or clay soils. This species seems to prefer open grassy areas on sandy and airy soils, but it is particularly reported from parks and highly frequented places. Not observed under evergreen oaks (*Quercus ilex* and *Quercus suber*), Mediterranean pines (*Pinus pinaster* and *Pinus pinea*) and *Cistus* spp.

Distribution: Europe. Haplotypes of sister clades found in North America and Asia, provisionally described as a species complex, need to be better described to establish whether they represent distinct taxa or geographic variants of *R. recondita*.

Additional material examined: See Table 2 (samples nos. 3, 4, 45, 48, 49, 50, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 66 and 92).

Other fetid Russulas

The original identification of three of the nine samples of *R. pectinata* was supported. One sample could not be analysed, two were identified only to genus level, and the others appeared to be more similar to *R. amoenolens*, *R. cerolens* and *R. illota*. A major problem about the molecular interpretation of *R. pectinata* was that no reliably identified sequences referred to this species were available in public databases. To this purpose, a sequence of *R. pectinata* (sensu Romagnesi 1967) was kindly provided by F. Hampe (named '*R. pectinata* 3075' in Fig. 1). Three specimens morphologically determined as *R. pectinatoides* (nos. 6, 82, 182) fell into the cluster including this sequence. Estimates of evolutionary divergence over sequence pairs within the cluster we named as *R. pectinata* reached 98.7% similarity (d = 0.013).

Samples originally identified as *R. grata* and *R. laurocerasi* var. *fragrans* (*n* = 8) matched public sequences in all cases, except sample no. 128 which fell in a clade containing specimens nos. 156, 160 and 165 morphologically determined as *R. foetentula* (see Supplementary material 1). Estimates of evolutionary divergence over sequence pairs within the cluster *R. grata* reached 99.1% similarity (d = 0.009).

At the time of our study, no ITS sequences identified as *R. foetentula* existed in public databases. This complicated the identification of our samples originally identified as this species (*n* = 6; see Supplementary material 1). As a result of our study, ITS sequences for what we believe to be this taxon (possibly a species complex) were generated and accessioned for the first time. Specimens nos. 159, 161 and 166 were 100% homologous with GenBank sequences of *R. granulata* (EU598188, EU598192 and JQ272365). All samples molecularly included in the *R. foetentula* complex originated from North America (nos. 132, 156, 160, 165, 167, 169 and 183; Supplementary material 1), except from sample no. 128 that was sampled in Switzerland, in the immediate surroundings of a college attended exclusively by American students (possibly introduced).

Samples originally identified as *R. sororia* (n = 6) matched public sequences in two cases (nos. 78 and 122), while two specimens matched *R. grata* (nos. 99 and 100), one specimen *R. parazurea* (no. 123) and one specimen *R. praetervisa* (no. 124). Estimates of evolutionary divergence over sequence pairs within the cluster identified as *R. sororia* reached 98.2% similarity (d = 0.018). Spore size and ornamentation of samples included in this group (nos. 9, 78, 122, 184 and 187; Table 3) were comparable to sample 187 (Peck's type collection NYS2303.2).

Samples originally identified as *R. amoenolens* (n = 10; see Supplementary material 1) matched public sequences in eight cases, while one specimen matched *R. grata* (no. 112) and another *R. recondita* (no. 113). Sequences of three specimens morphologically determined as *R.* cf. *pectinatoides* (no. 158), *R. pectinatoides* fo. *alba* (no. 76) and *R. pectinata* (no. 91) had ITS sequences homologous to *R. amoenolens* public sequences AF418615, GU222264, KF245510 and UDB000343.

All samples originally identified as *R. fragrantissima* (*n* = 2; see Supplementary material 1) matched public sequences, as did samples identified as *R. illota* (*n* = 3). Also included in this latter taxon was sample no. 138 collected from Canada, which was originally labelled as *R. pectinata*. This specimen is of interest since it is unknown in North America (where *R. illota* is present but rare) and therefore probably often wrong determined in this area.

Samples originally identified as *R. insignis* (*n* = 6; see Supplementary material 1) matched public sequences in five cases, while one specimen matched *R. recondita* (no. 116). Also, two samples originally labelled as *R. pectinatoides* (nos. 148 and 149) and one *R. praetervisa* (no. 84) matched what could be considered *R. insignis*.

Samples originally identified as *R. inamoena* (n = 2; see Supplementary material 1) showed 100% identity with GenBank sequence JF908679, deposited as *R. foetens* (Italy). At the time of publication, no molecular data were available in public databases for *R. inamoena* ITS regions, but numerous other sequences referred to *R. foetens* were available. Sequence JF908679 was therefore presumably incorrectly identified, as the other deposited sequences of *R. foetens* fell in other groups (see Supplementary material 1).

We obtained a specimen of *R. ombrophila* (no. 86) from the author of the species, C. Monedero Garcìa, which was molecularly analysed for the first time. Its sequence fell alone in a well separated lineage (see Supplementary material 1). Estimates of evolutionary divergence over sequence pairs between *R. ombrophila* and the other clusters showed ≤96.7% (d = 0.0333), supporting its status as an independent taxon.

Discussion

Based on the comparison between morphological and molecular results from *R. pectinatoides*, *R. praetervisa* and the related fetid Russulas considered in this study, most of the samples provided by museums and private herbaria had been inaccurately determined at the species level when diagnosed only on the basis of morphological characters. Moreover, molecular analysis carried out in this study raised further problems. For instance, ITS sequences available in public databases turned out to be only partially reliable. In some cases, the sequences referred to a given species fell in different clades. In other cases, no publicly available ITS sequences existed for a species (such as for *R. pectinata* ss. Romagnesi, *R, foetentula*, *R. inamoena*, and *R. ombrophila*). The situation was further complicated by the fact that very few specific type collections had been sequenced, so that standard references, universally valid, were lacking. To overcome this problem, we tried to obtain the holotypes of some taxa. Unfortunately, the epitype of *R. sororia* and the holotype of *R. pectinatoides* var. *pseudoamoenolens* Romagn., requested from the Herbier des Plantes Cryptogames del Muséum National d'Histoire Naturelle (Paris), were not found by the curator; and the sample of *R. pectinatoides* sensu Romagnesi was sent after several months and could not be sequenced due to low DNA quality. The complete reference collection of *R. pectinatoides* itself, deposited in New York State Museum, was impossible to obtain; therefore we could analyse only three small fragments. Last but not least, new *Russula* species have been recently described on the basis of morphological observations of a single collection found locally, without genetic confirmation (see, e.g., Sarnari 1998; Gómez Arenaza and Monedero Garcìa 2011; Pidlich-Aigner 2014, who described *R. fuscodiscoidea*).

It is not difficult to understand how all these factors could have led to the misidentification of many taxa included in the Foetentinae, with *R. pectinatoides* being the iconic representative of the confusion dominating the subsection. The description of this species is problematic under many aspects, starting from the contents of Peck's type specimen box which contains eight samples composed of stipe and cap, four samples with cap only, various pieces including nine stipes, three stipe fragments, four cap fragments of 3–4 cm, ten cap fragments of 1–2 cm, approximately ten cap fragments of one cm or less, and additional fragments. In addition, Peck's notes give vague description of these specimens and make no mention as to how or why so many samples were combined into a single collection. Only when subsequent mycologists, much later, pointed out the presence of other species within the collection were any attempts made to intensively study one basidioma as representative of *R. pectinatoides*, to define its characteristics (see Adamčík et al. 2013) Based on our molecular analysis of three of Peck's samples, the collection likely consists of at least three distinct species. Sample NYS2303.1 may correspond to *R. pectinatoides* as intended by Peck, because of its homology with the only available deposited sequence that refers to a precise species (*R. pectinatoides* EU598185, identified by a world authority on Russulas, B. Buyck). The terminal clade containing this sample is very highly supported (bootstrap value = 99) and includes only North American specimens (nos. 168, 172 and 174; for GenBank sequences see Supplementary material 1). Moreover, this group corresponds to the (robust) clade 12 in Avis (2012) that contains many collections from the Eastern part of the United States, including some relatively close to the type location in New York. Given the potential that this clade represents *R. pectinatoides*, we propose NYS2303.1 as the single, definitive lectotype of *R. pectinatoides* among all fragments contained in Peck's box—although we do not formally designate it here as such, due to the lack of requirements according to the International Code of Nomenclature (see http://www.iapt-taxon.org/nomen/main.php?page=art7; McNeill et al. 2012). This is an arbitrary, yet useful, proposal, because it would resolve the nomenclatural uncertainty surrounding this species. However, due to the scarcity of tissue available (Table 3), we were not able to provide a more extensive morphological characterisation of this taxon. Thus, we encourage more in-depth observations and study of Peck's collection to better define the characteristics of this species and definitively support or refute our proposal. Using similar logic, we assigned sample NYS2303.2 to the *R. sororia* complex, even if the concept of this species also deserves to be deepened with further research. Given the quite low homology within sequences included in the clade (98.2%), and possible morphological differences (e.g. spores of European samples were slightly larger than the American ones, with intercarpic ranges of absolute values for spore size of 7–8 × 5.5–6.5 μm vs. 6–7 × 5–6.5 μm, respectively), further analyses should be carried out to clarify if Asian, European and American lineages represent separate taxa. At the time of this study, only five sequences deposited as *R. sororia* were available in GenBank (and none in the Unite database), with only one of them long enough to be inserted in our alignment. It is interesting to note that American samples (nos. 9, 154, 157 and 184) had been morphologically determined as *R. pectinatoides*, while European samples (nos. 78 and 122) had been identified as *R. sororia*. This fact seems to indicate an American concept of *R. pectinatoides*, recognized by European mycologists as *R. sororia*. Indeed, Peck himself, in his original description, wrote that "'It (*R. pectinatoides*) closely resembles *Russula sororia* in its general appearance, but may be separated from it by its milder taste". (Peck 1907). On the other hand, the problem linked to Peck's collection has been well known since 1972, when Shaffer noted the presence of *R. laurocerasi* (syn. *R. grata*), later confirmed by morphological observations by subsequent mycologists, and now very well supported by our molecular data (holotype NYS2303.3).

The second taxon investigated in this study, *R. praetervisa*, was also supported as a separate species by the results of our study. Interestingly, the Moroccan samples, although having few nucleotide substitutions compared to European ones, were originally determined as *R. pectinatoides* form *amarescens* (no. 64) and *R. pectinatoides* var. *pseudoamoenolens* (no. 65) which are varieties discussed by Sarnari, who was yet not sure of their autonomy in his description of *R. praetervisa. R. praetervisa* is easily recognizable because it is typically found in the Mediterranean area and is linked to specific host plants. We were therefore surprised to find a sample belonging to this species originating from Northern Ireland (no. 177, Fig. 1). More investigation revealed that the fungus was collected in a tourist parkland, where a *Quercus ilex* was mentioned in a floristic inventory made by the authorities. Potential introduction events by humans are known in fetid Russulas (Avis 2012), as well as ectomycorrhizal fungi in general (Bonito et al. 2013; Dickie et al. 2016). Bonito et al. (2013) showed, at least for *Tuber* spp., that continental disjuncts with <1% ITS variation could be considered introduced. If this same criterion applies to *Russula*, then the Northern Ireland sample is introduced.

Our analyses revealed the need to describe a new taxon, *R. recondita*, and apply it to the many European collections previously and commonly considered *R. pectinatoides*. This clarifies the status of European continental collections, even though it does not definitively solve the problem of using the name '*pectinatoides*' on the North American collections, for which this name is also frequently misapplied, or in Asia (terminal clade parallel to the European taxon, containing the Chinese sequence JX434670 together with the Estonian sequence UDB015983). Given the role of geography in the speciation of Russulas (Looney et al. 2016) and many other ectomycorrhizal fungi (Dentinger et al. 2010; Feng et al. 2012; Bonito et al. 2013), these groups probably evolved separately in different continents. This hypothesis is supported by the morphological data: spore ornamentation of the European *R. recondita* is variable, whereas the American sister clade shows mostly isolated warts (intercarpic variation range of spore ornamentation 1–4 vs. 0–3, according to the category of Table 3). The smell of American specimens varies from sweet to almonds, fetid and nauseating, while the smell of European ones ranges from fruity to unpleasant. The taste of American specimens is mild to slightly bitter; but the taste of European ones is mild, then unpleasantly bitter to peppery. Another clade containing two samples from North America (nos. 2 and 162) clustered independently from the others. Due to the lack of complementary data to describe them exhaustively, these terminal clades were generically labelled as 'clade 1', 'clade 2' and 'clade 3'. We recommend that they should be better studied before stating new species status.

A similar geography-related clustering was also observed in *R. pectinata* and *R. grata*, for which American, European and Asian (for *R. pectinata*) sequences fell in separate subgroups. These findings are consistent with Avis (2012), who found strongly supported separate clades that corresponded to the geographical origin of the samples. However, again, new species should only be proposed on the basis of more consistent data, through focused studies using additional sequences, ideally more genes (e.g. EF1α; Leacock et al. 2016) as well as thorough and detailed morphological analyses.

Our study highlights the persistent challenges in reconciling identification for fungal species in general and especially for fetid Russulas. As mentioned, most species lacked well-curated and extensively studied public reference sequences, such as for *R. foetentula*, *R. inamoena* and *R. ombrophila* that were molecularly analysed for the first time in this study. In addition, new species have been described but have been based solely on morphology. For instance, *R. fuscodiscoidea* was recently proposed as a new species (Pidlich-Aigner 2014), but to date has not been sequenced. Although morphological evidence suggests this taxon is unique, until a molecular comparison is made to other fetid Russulas, synonymy cannot be excluded. Other species, such as *R. amoenolens*, are well represented in public databases, but their sequences do not cluster together (Supplementary material 1). However, one clade containing four sequences identified as *R. amoenolens* by different authors (AF418615, GU222264, UDB000343 and KF245510), as well as a set of samples (nos. 75, 110, 111, 114, 115, 125, 126 and 147) all originally determined as *R. amoenolens* by morphological characters is most likely, at least based on the consensus of these data, this taxon. As expected based on the strong morphological similarity, this clade was found between *R. pectinatoides* and the group containing *R. cerolens*, but until a sequence from the *R. amoenolens* type can be analysed, a definitive answer on this species will remain unknown. In our phylogenetic tree (Supplementary material 1), it was not possible to insert samples originally labelled as *R. foetens* and *R. subfoetens* (nos. 8, 11, 15, 151, 152 and 164), because sequences obtained from these specimens were partial; we could only add some sequences retrieved from public databases, which were scattered throughout the cladogram, making sample identification uncertain. For this reason, we deposited only one sequence in public databases (sample no. 11). Further studies are also needed to clarify the status of *R. foetens* and *R. subfoetens*.

A major problem encountered in this study was the molecular analysis of old (>50 years) museum samples that in some cases had been subjected to chemical preservatives. Such factors, together with exposure to UV radiation, temperature, pH, and salt concentration of the environment, are known to affect DNA quality. Due to DNA degradation, PCR amplification of Peck's specimens (>100 years old) turned out to be particularly difficult. Possible consequences of this degradation could have influenced the sequence position in the phylogenetic tree (e.g. NYS2303.3 did not cluster perfectly with the other American *R. grata*). In other cases, samples yielded mixed sequences because they were contaminated by other fungi, mainly moulds and mildews. In most cases, this issue was resolved by using internal primers specifically designed by the authors for the genus *Russula*.

Perspectives

Our study underlines the importance of a polyphasic approach for the identification of fetid Russulas. Morphological characters (smell, taste, spore ornamentation) currently represent the basis for classification for field mycologists, as well as ecological factors (geographical origin, habitat), but genetic analysis should be carried out in case of doubtful or inconclusive findings, and should be especially requested when new taxa are described.

Public databases, in their current state, do not offer reliably and consistently identified reference sequences, at least for fetid Russulas. A goal of great importance for the future is to ensure that for each species, selected reference sequences (one for each DNA region) of a holotype (ideally a single individual designed as type strain) is available. This would then allow for and encourage better bioinformatic (i.e. BLAST) and phylogenetic analyses to be conducted before submitting new sequences, and could help to ensure the accuracy of species identification.

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