



Original Paper

Russula (Russulales, Agaricomycetes) associated with *Pinus* spp. plantations from northeastern Argentina

Natalia Andrea Ramírez^{1,2,4}, Lilian Katia Elizabeth Zacarias^{1,5}, Carlos Alberto Salvador-Montoya^{1,2,6}, Massimo Tasselli³, Orlando Fabián Popoff^{1,2,7} & Nicolás Niveiro^{1,2,8,9}

Abstract

Russula comprises more than 3,000 species worldwide and is a characteristic genus of the coniferous forests of the northern hemisphere. The forest plantations with non-native species in the northeastern Argentina, such as pine or eucalyptus, provide the biotic and environmental conditions for the establishment of ectomycorrhizal fungi associated with these forest plantations. Due to the complexity of identifying *Russula* at specific level, morpho-anatomical, scanning electron microscopy, and phylogenetic (ITS) analysis were used to identify the specimens. As result, three *Russula* species, *R. recondita*, *R. sardonias*, and *R. sororia*, are described in detail and illustrated, none previously known to Argentina. Also, two of them, *R. recondita* and *R. sororia*, represent new records for South America.

Key words: Basidiomycota; ectomycorrhizae; forestry; fungi; Russulaceae.

Resumen

Russula es un género que comprende más de 3.000 especies de distribución mundial, siendo un género característico de los bosques de coníferas del hemisferio norte. Las plantaciones forestales con especies no nativas en el Nordeste argentino, como por ejemplo pino o eucalipto, proporcionan las condiciones bióticas y ambientales para el establecimiento de hongos ectomicorrízicos asociados a dichos cultivos. Debido a la complejidad que presenta la determinación de *Russula* a nivel específico, se utilizaron métodos morfo-anatómicos, de microscopía electrónica de barrido y análisis filogenéticos (ITS) para la identificación de los especímenes. Como resultado, tres especies de *Russula*, *R. recondita*, *R. sardonias* y *R. sororia* son descritas en detalle e ilustradas. Ninguna de estas especies era conocida previamente para Argentina, y dos de ellas, *R. recondita* y *R. sororia*, representan nuevas citas para Sudamérica.

Palabras clave: Basidiomycota; ectomicorrizas; forestación; hongos; Russulaceae.

Introduction

Russula Pers. comprises more than 3,000 species worldwide (He *et al.* 2019; Wijayawardene *et al.* 2020). In a traditional sense, *Russula* is characterized by its middle to large size basidiomes, with brittle context due to the presence of sphaerocysts in conjunction with filamentous hyphae, basidiospores with amyloid ornamentations and ectomycorrhizal habit

(Schaeffer 1952; Romagnesi 1967; Largent & Baroni 1988; Sarnari 1998). However, with the use of phylogenetic tools, currently a broader generic concept is considered, which includes not only the traditionally agaricoid species, but also species of pleurotoid habit, and even truffle-like basidiome of hypogean habit (Buyck *et al.* 2018). Even though *Russula* is easy to recognize macroscopically to genus, most species are difficult to identify due to the complexity and subjectivity of many

¹ Universidad Nacional del Nordeste, Facultad de Ciencias Exactas y Naturales y Agrimensura, Micología, Av. Libertad 5060, CP 3400, Corrientes, Corrientes, Argentina.

² IBONE (UNNE-CONICET), Inst. Botánica del Nordeste, Sargento Cabral 2131, CC 209, CP 3400, Corrientes, Corrientes, Argentina.

³ Via della primula 5, 20090, Segrate, Milán, Italy. ORCID: <<https://orcid.org/0000-0003-2864-0360>>.

⁴ ORCID: <<https://orcid.org/0000-0002-5901-6571>>. ⁵ ORCID: <<https://orcid.org/0000-0002-5832-4353>>. ⁶ ORCID: <<https://orcid.org/0000-0002-2059-8201>>.

⁷ ORCID: <<https://orcid.org/0000-0001-9109-4118>>. ⁸ ORCID: <<https://orcid.org/0000-0002-3265-7061>>.

⁹ Author for correspondence: niconiveiro@gmail.com

characters (as odor and taste) that were utilized to delimit its species (Largent & Baroni 1988). Although its infrageneric classification was always complex (Kühner & Romagnesi 1953; Romagnesi 1967; Singer 1986), Buyck *et al.* (2018) proposed a classification based on morphoanatomic and multigene characters, concluding in seven well-defined subgenera: *Archaea* Buyck & V. Hofst., *Compactae* (Fr.) Bon, *Crassotunicata* Buyck & V. Hofst., *Heterophyllidia* Romagnesi, *Malodora* Buyck & V. Hofst., *Brevipes* Buyck & V. Hofst., and *Russula* Pers.

Russula is mainly represented in temperate cold forests associated with conifers (Bills *et al.* 1986; Schmit *et al.* 1989; Villeneuve *et al.* 1989; Ferris *et al.* 2000; Gómez-Hernández *et al.* 2011; O'Hanlon & Harrington 2012) and Angiosperms as Fagales (Halling & Mueller 2005; Gómez-Hernández *et al.* 2011; O'Hanlon & Harrington 2012) and Myrtaceae (Packham *et al.* 2002; Gates *et al.* 2005). Restricting our scope to South America, the main diversity of autochthonous *Russula* species is concentrated in Andino-Patagonic forests (Singer 1950; Gamundi & Horak 1994; Romano *et al.* 2017), lowlands forests in Amazonian region (Singer *et al.* 1983; Maia *et al.* 2015), Northeast Brazil (Sá & Wartchow 2016; Sá *et al.* 2018), and Guyana (Miller *et al.* 2012), being many of them endemic of these regions. Nevertheless, forestry with introduced species, pine and eucalyptus for example, provides biotic and environmental conditions for the establishment of allochthonous ectomycorrhizal fungi associated with these forest (Barroetaveña & Rajchenberg 2003; Diez 2005; Barroetaveña *et al.* 2007, 2012). In recent collections made in *Pinus* plantation from northeastern Argentina, three *Russula* species not previously known for Argentina were identified, two of them unknown to South America. These species are described and illustrated, and their distribution and phylogenetic position are discussed.

Materials and Methods

Collections and morphological analyses

The specimens here studied were collected under *Pinus taeda* L. and *P. elliottii* Engelm plantations of Misiones and Corrientes provinces, and deposited at CTES Herbarium. These samples were described according to Adamčík *et al.* (2019). Color codes follow Kornerup & Wanscher (1978). Microscopic characters were examined using light

microscope (LM), Leica model CME, and scanning electron microscope (SEM), JEOL 5800 LV, operating at 20 KV. The SEM images were obtained from herbarium samples, rehydrated in Triton aqueous solution, and dehydrated in an ethanol series, dried to critical-point, and then mounted on double-sided tape and coated with gold-palladium. All LM images were taken with Leica EC3 camera from material mounted in 5% KOH and stained with phloxine (1%) or treated with Melzer's reagent, Cresyl Blue and sulfobenzaldehyde (Adamčík *et al.* 2019). Ammonia was used to observe color changes in the basidiomes. The measurements of microstructures (basidiospores, basidia, hyphae in the lamellae, trama and pileipellis) were made directly in LM or through photographs using ImageJ software (Schneider *et al.* 2012). The basidiospore walls ornamentations were measured in SEM. For basidiospores' measurements, the following notations were used: \bar{x} = arithmetic mean of length and width; Q= quotient of length and width indicated as a variation range; Q_x = mean of Q values; n= number of basidiospores measured, N= number of basidiomata from which spores were measured. All GPS readings were taken on Garmin eTrex 10, hand held unit using WGS84 standard. Herbaria abbreviations follow Index Herbariorum (Thiers 2020) and the authors of species are according to Index Fungorum - Authors of Fungal Names (2020).

DNA extraction, amplification, and sequencing

Genomic DNA of specimens N. Niveiro 3274 CTES and N. Niveiro 3341 CTES were isolated from dried basidiomata tissue following standard protocols of the Canadian Centre for DNA Barcoding (CCDB) for fungi (Ivanova *et al.* 2006, 2016). The nuclear ribosomal internal transcribed spacer (ITS) region of the DNA was amplified using primers ITS1-F and ITS4-B (Gardes & Bruns 1993), which was suggested as the universal DNA barcode marker for Basidiomycetes fungi (Ivanova *et al.* 2008; Schoch *et al.* 2012). PCR products were purified and sequenced by the Canadian Center of DNA Barcoding (CCDB). The ITS sequences were deposited in the GenBank database (Tab. 1).

Phylogenetic analysis

The resulting sequences were assembled and manually edited using Geneious v. 6.1.8 (Kearse *et al.* 2012). The nrITS dataset was built with 61

Table 1 – Sequences used in the phylogenetical analysis. Sequences obtained in this study are in bold.

Species	Collection No	ITS genbank Accession No	Reference
<i>Lactifluus chrisocarpus</i>	LE<RUS>:253907	NR121551	Morozova <i>et al.</i> (2013)
<i>R. ahmadii</i>	LAH2101006	KU535608	Jabeen <i>et al.</i> (2017)
<i>R. ahmadii</i>	SJ54	KT834639	Jabeen <i>et al.</i> (2017)
<i>R. ahmadii</i>	LAH35006	KT834640	Jabeen <i>et al.</i> (2017)
<i>R. amerorecondita</i> (as <i>R. recondita</i>)	134	KJ834567	Adamčík <i>et al.</i> (2019), Melera <i>et al.</i> (2017)
<i>R. amerorecondita</i> (as <i>R. recondita</i>)	171	KJ530758	Adamčík <i>et al.</i> (2019), Melera <i>et al.</i> (2017)
<i>R. amerorecondita</i> (as <i>R. recondita</i>)	25	KJ834618	Adamčík <i>et al.</i> (2019), Melera <i>et al.</i> (2017)
<i>R. amerorecondita</i> (as <i>R. recondita</i>)	6271	KF318045	Adamčík <i>et al.</i> (2019), Melera <i>et al.</i> (2017)
<i>R. amerorecondita</i> (as <i>R. amoenolens</i>)	F:PRL7415	GQ166870	Adamčík <i>et al.</i> (2019), Avis <i>et al.</i> (unpubl.)
<i>R. amerorecondita</i> (as <i>R. pectinatoides</i>)	NAMA4998	EU819493	Adamčík <i>et al.</i> (2019), Palmer <i>et al.</i> (2008)
<i>R. aff amoenolens</i>	HMJAU37318	KY357332	Liu <i>et al.</i> (unpubl.)
<i>R. amoenolens</i>	nl27.9.95.6 (TUB)	AF418615	Eberhardt (2002)
<i>R. amoenolens</i>	MICH12838	KF245510	Bazzicalupo & Berbee (unpubl.)
<i>R. amoenolens</i>	110	KJ834594	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	91	KJ834607	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	115	KJ834590	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	76	KJ834609	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	PDD77763	GU222264	Johnston & Park (unpubl.)
<i>R. amoenolens</i>	110	KJ834594	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	91	KJ834607	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	115	KJ834590	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	76	KJ834609	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	111	KJ834593	Melera <i>et al.</i> (2017)
<i>R. amoenolens</i>	280	KY681463	Santolamazza Carbone & Calvino Cancela (unpubl.)
<i>R. amoenolens</i>	CDW122	JQ622327	Avis (2012)
<i>R. cerolens</i>	WTU_F_38361	KF245498	Bazzicalupo & Berbee (unpubl.)
<i>R. cerolens</i>	MICH9611	KF245486	Bazzicalupo & Berbee (unpubl.)
<i>R. cerolens</i>	OSC76727	KF245505	Bazzicalupo & Berbee (unpubl.)
<i>R. cerolens</i>	UBCF23015	KF245524	Bazzicalupo & Berbee (unpubl.)
<i>R. cerolens</i>	UBC_F18893	HQ604829	Berbee <i>et al.</i> (unpubl.)
<i>R. cerolens</i>	36	KF683922	Melera <i>et al.</i> (2017)
<i>R. cerolens</i>	29	KJ834617	Melera <i>et al.</i> (2017)
<i>R. foetens</i>	HMAS194245	JX425383	Li & Wen (unpubl.)
<i>R. foetens</i>	R32	LN714598	Větrovský <i>et al.</i> (2016)
<i>R. foetens</i>	VA14	AF478613	Yao & Zhang (2002)
<i>R. granulata</i>	PC BB2004-228	EU598189	Hughes & Buyck (unpubl.)

Species	Collection No	ITS genbank Accession No	Reference
<i>R. granulata</i>	PC BB2004-227	EU598188	Hughes & Buyck (unpubl.)
<i>R. granulata</i>	159	KJ834624	Melera <i>et al.</i> (2017)
<i>R. iliota</i>	MICH73719	KF245509	Bazzicalupo & Berbee (unpubl.)
<i>R. iliota</i>	UE26.07.2002-3	DQ422024	Eberhardt (unpubl.)
<i>R. iliota</i>		HQ677769	O'Hanlon & Harrington (2012)
<i>R. insignis</i>	94	KJ834606	Melera <i>et al.</i> (2017)
<i>R. insignis</i>	1223IS85	AY061700	Miller & Buyck (2002)
<i>R. laurocerasi</i>	E00290534	KF245532	Bazzicalupo <i>et al.</i> (unpubl.)
<i>R. laurocerasi</i>	nl1348 (TUB)	AF418614	Eberhardt (2002)
<i>R. laurocerasi</i> (as <i>R. grata</i>)	129	KJ834573	Melera <i>et al.</i> (2017)
<i>R. grata</i> (as <i>R. grata</i>)	100	KJ834604	Melera <i>et al.</i> (2017)
<i>R. grata</i> (as <i>R. grata</i>)	103	KJ834601	Melera <i>et al.</i> (2017)
<i>R. lilacea</i>	220135	KF318049	Melera <i>et al.</i> (2017)
<i>R. pectinatoides</i>	PC BB2004-206	EU598185	Hughes & Buyck (unpubl.)
<i>R. pectinatoides</i>	172	KU640187	Melera <i>et al.</i> (2017)
<i>R. pectinatoides</i>	168	KU640186	Melera <i>et al.</i> (2017)
<i>R. petinata</i>	6	KF318085	Melera <i>et al.</i> (2017)
<i>R. petinata</i>	79	KF318082	Melera <i>et al.</i> (2017)
<i>R. petinata</i>	82	KF318084	Melera <i>et al.</i> (2017)
<i>R. praetervisa</i>	39	KF303606	Melera <i>et al.</i> (2017)
<i>R. praetervisa</i>	124	KJ834578	Melera <i>et al.</i> (2017)
<i>R. praetervisa</i>	44	KF303600	Melera <i>et al.</i> (2017)
<i>R. pulverulenta</i>	PC BB2004-245	EU598186	Hughes & Buyck (unpubl.)
<i>R. recondita</i>	FH12-269	KF318064	Melera <i>et al.</i> (2017)
<i>R. recondita</i>	ZT Myc 1704	KF318063	Melera <i>et al.</i> (2017)
<i>R. recondita</i>	92	KJ530750	Melera <i>et al.</i> (2017)
<i>R. recondita</i>	Niveiro 3341 (CTES)	MT252520	This paper
<i>R. sororia</i>	Niveiro 3274 (CTES)	MT252521	This paper
<i>R. sororia</i>	2208ASI	KX574701	Lee <i>et al.</i> (2017)
<i>R. sororia</i>	ASIS15775	KX574702	Lee <i>et al.</i> (2017)
<i>R. sororia</i>	9	KF318053	Melera <i>et al.</i> (2017)
<i>R. sororia</i>	Nara_RuS01	AB211275	Nara (2006)
<i>R. sororia</i> (as <i>R. aff. amoenolens</i>)	HMJAU37318	KY357332	Liu <i>et al.</i> (unpubl.)

sequences of sect. *Ingratae* treated by Jabeen *et al.* (2017) and Melera *et al.* (2017), including our sequences (Tab. 1). *Lactifluus chrysocarpus* E.S. Popov & O.V. Morozova was used as outgroup (Wang *et al.* 2015). The nrITS sequences were initially aligned with MAFFT v.7 (under Q-INS-i criteria) (Kato & Standley 2013) and manually edited using MEGA5 (Tamura *et al.* 2011). The nrITS dataset was subdivided into three data partition (ITS1, 5.8S, and ITS2).

The dataset was analyzed with Maximum Likelihood (ML) and Bayesian Inference (BI) approaches. Maximum Likelihood searches were conducted with RaxML-HPC v.8 (Stamatakis 2014), searching for the best scored trees with GTRGAMMA model for the dataset with all the default parameters estimated by the software. The analysis first involved 1000 ML independent searches each one starting from one randomized stepwise addition parsimony tree. Only the best-scored ML tree was kept, and the confidence of nodes was accessed through non-parametric Bootstrapping (BS) pseudoreplicates under the same model, allowing the program to stop bootstrapping automatically by the autoMRE option. An additional alignment partition file was used to force RAXML software to search for a separate evolution model for each partition. The best fit model of nucleotide evolution to the dataset was selected using AIC (Akaike Information Criterion) as implemented in jModelTest2 v.1.6 (Guindon & Gascuel 2003; Darriba *et al.* 2012). Bayesian Inference analyses were performed with MrBayes 3.2.6 (Ronquist & Huelsenbeck 2003), and implemented with two independent runs, each one beginning from random trees with four simultaneous independent chains. A total of 2×10^7 generations were carried out, sampling one tree every 1×10^3 generations. The initial 25% of the sampled trees was discarded as burn-in and checked by the convergence criterion (frequencies of average standard deviation of split < 0.01) in Tracer v.1.6 (Rambaut *et al.* 2014), while the remaining ones were used to reconstruct a 50% majority-rule consensus tree and to estimate Bayesian posterior probabilities (BPP) of the branches. J Model Test2 v.1.6, MrBayes 3.1.2 and RaxML-HPC v. 8.2.3 were used in CIPRES Science Gateway (Miller *et al.* 2010; <http://www.phylo.org/>). A node was considered to be strongly supported if it showed a BPP ≥ 0.95 and/or BS $\geq 90\%$, while moderate support was considered BPP ≥ 0.9 and/or BS $\geq 70\%$. Only the topology from the best ML tree

is shown, indicating support values (BPP/BS) of each node.

Results

Molecular phylogenetic inferences

The nrITS dataset included 61 sequences belonging to 15 species of *Russula* plus the outgroup, resulting in an alignment with 631 characters, of which 396 are constant sites, 235 variable uninformative and 135 parsimony-informative.

The phylogenetic inference from nrITS dataset (Fig. 1), shows that *Russula* specimens from *Pinus* forest plantation in the northeastern Argentina are distributed in two strongly supported clades. The specimen N. Niveiro 3274 (CTES) is closely related to Asian and North American *R. sororia* (BPP=1/BS=98), and the specimen N. Niveiro 3341 (CTES) with European specimens of *R. recondita* (BPP=1/BS=100). Both species are described below.

Taxonomy

Russula recondita Melera & Ostellari. Mycological Progress 16 (2): 128 (2017). Figs. 2-4

Pileus 30–70 mm diam., flattened-globose when young, then convex to flat, depressed when mature; margin tuberculate-corrugated, incurved to plane-upturned; cuticle viscid when young, turning dry, easily separable from the context at approximately one third of the diameter of the pileus, orange white (6A2), pale orange (6A3), light orange (6A4) to grayish orange (6B3-5) surface, occasionally with deep orange (5A6-7) stains, dark brown (7F4-8) in the center. Lamellae up to 3 mm broad, adnate to sinuate, crowded at first, turning close, thick, whitish (5A1), orange white (5A2) to orange gray (5B2), occasionally with brownish orange (5C5) to brownish yellow (5C7) stains when mature; entire and concolorous edge. Stipe 30–80 × 10–20 mm, thick, cylindrical, rough, bright, white (5A1) when young, turning pale gray (5B1) when old, occasionally with reddish brown (7B7) to brown (7E7) stain in lower half. Context fleshy, up to 8 mm thickness, whitish (1A1) to orange white (6A2), brittle. Taste mild, odor fruity. Spore-print cream color (2A2 “yellowish white” to 2B2 “yellowish gray”). Negative reaction with potassium hydroxide and ammonia.

Basidiospores 6.5–8.5 × 4.5–7 μm; $x = 7.5 \times 6.0$ μm; Q= 1.15–1.35; $Q_x = 1.25$; n= 22; N= 1; ovoid to broadly ellipsoidal, hyaline,



Figure 1 – Molecular phylogeny carried out by Maximum Likelihood based on dataset of ITS sequences. Bayesian posterior probability above 0.7 (BPP \geq 0.7) and Bootstrap value above 70% (BS \geq 70%) are shown. Sequences obtained from this work are indicated in bold type. Clade with taxa of interest is shown in grayish box.



Figure 2 – Macroscopic characters of *R. recondita* [*N. Niveiro 3341* (CTES)]. Scale bar = 5 cm.

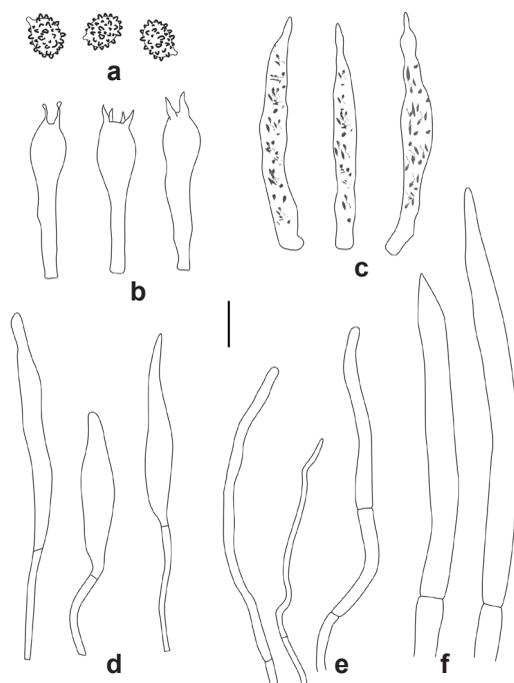


Figure 3 - Microscopic characters of *R. recondita* - a. basidiospores - b. basidia - c. hymenial cystidia - d. pileipellis terminal hyphae - e. pileocystidia - f. pileocystidia originated by gloeopleural hyphae [*N. Niveiro 3341* (CTES)]. Scale bars = 10 μ m.

ornamented with isolated warts (2–4 warts in a 3 μ m diam. circle), or occasionally with thin-lines interconnections (0–1 fusion in the circle), warts up to 0.8–1.3 μ m high, amyloid; smooth suprahilar spot, inamyloid. Basidia 43–46 \times 9–11 μ m, clavate, 4-spored, thin-walled, hyaline. Hymenial cystidia moderately numerous, ca. 600–1200/mm², (30–)37–55(–70) \times 5–8 μ m, fusiform,

rarely cylindrical, apically obtuse, mucronate, with an appendage 5–9 μ m long, occasionally moniliform, mainly originating slightly below the level of the basidia, a few longer originating in subhymenium, thin-walled, smooth, content completely heteromorphous, grayish reaction in sulfovanillin. Hymenophoral trama with globose to subglobose sphaerocysts, 11–21 μ m diam., thin-walled, intermixed with cylindrical hyphae up to 5 μ m diam. Gloeopleural hyphae up to 8.5 μ m diam., turning black with sulphobenzaldehyde. Pileipellis in an ixotrichoderm up to 280 μ m deep, orthochromatic in Cresyl Blue; suprapellis 90–150 μ m deep, made up ascending cylindrical hyphae, 1–3 μ m diam., septate, hyaline, intermixed, embedded in a gelatinized matrix; subpellis 70–130 μ m deep, composed of horizontally oriented, intermixed and strongly gelatinized hyphae. Hyphal termination near the pileus margin composed of 2–3 unbranched cells, thin-walled, terminal cells 30–35 \times 2–3 μ m, cylindrical, similar to subterminal cells. Hyphal termination near the pileus center similar to the margin, slightly longer, terminal cells 30–40 \times 2–3 μ m, cylindrical to narrowly lageniform. Pileocystidia dispersed near the pileus margin, composed of 1 cell, 38–50 \times 3–5 μ m, fusiform to narrowly lageniform, thin-walled, content undifferentiated; pileocystidia near the pileus center, 25–40 \times 4–5 μ m, fusiform to narrowly lageniform, thin-walled, content undifferentiated. Oleiferous hyphae frequent in context, with yellowish pigments, terminal cells occasionally formed larger pileocystidia, 45–80 \times 4–5 μ m, cylindrical to lanceolate, thin-walled.

Examined material: ARGENTINA. CORRIENTES: Dpto. Capital, Santa Ana de los Guácaras, 27°27'42.01"S, 058°40'04.85"W, 70 m a.s.l., 2.VI.2019, in soil between pines at the edge of the road, former pine plantation area, *N Niveiro 3341* (CTES); 11.VIII.2019, in soil between isolated pines at the roadside, former pine plantation area, *A Somrau et al. 3* (CTES).

The species is distributed in Europe, North America, and Asia (Lee *et al.* 2017; Melera *et al.* 2017). *Russula recondita* was found in different habitats (deciduous forest, coniferous forest, grasslands, plantations, anthropic habitats) associated with numerous potential symbionts (e.g. *Pinus* sp., *Quercus* sp., and *Salix* sp.) on sandy and airy soils (Melera *et al.* 2017).

Previous records were not found for South America.

Russula pectinatoides Peck is currently considered a species complex, that is still not resolved (Sarnari 1998; Adamčík *et al.* 2013;

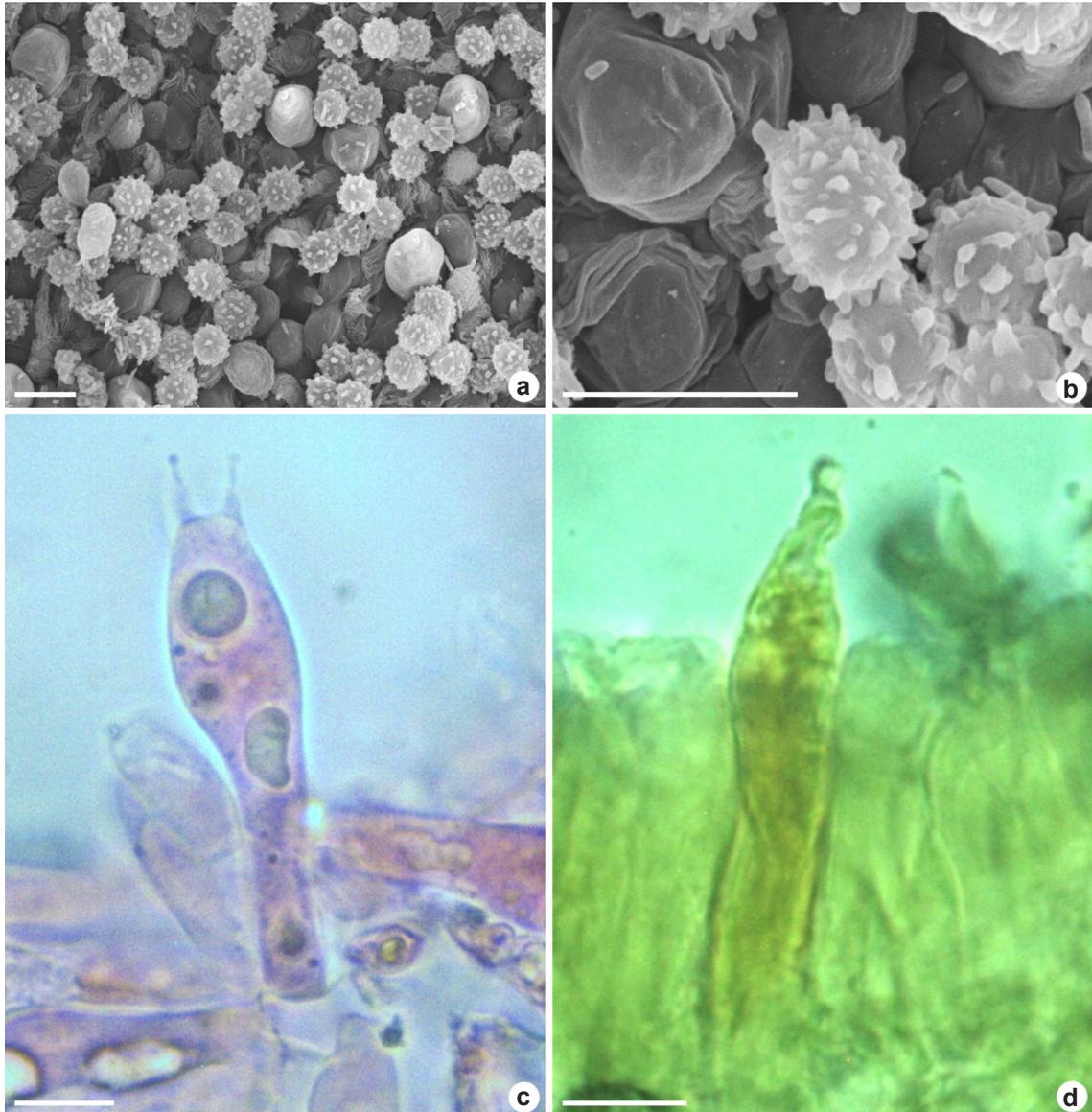


Figure 4 - Microscopic characters of *R. recondita* - a-b. basidiospores on SEM - c. basidia - d. hymenial cystidia. [*N. Niveiro 3341* (CTES)]. Scale bars = 10 μ m.

Melera *et al.* 2017). Firstly, Sarnari (1998), separated many European collections of *R. pectinatoides* as an independent species, *R. praetervisa* Sarnari, emphasizing that both species are macroscopically identical. Later, Melera *et al.* (2017) analyzed this species complex, concluding that the original concept of *R. pectinatoides* (Peck 1907) corresponds to at least three different species: *R. pectinatoides*, *R. praetervisa*, and a new species, *R. recondita*, differentiating them mainly by molecular data. *Russula recondita* is described

as a wide and very diverse species, with variability in spore size (average range of $6\text{--}10 \times 5\text{--}7 \mu\text{m}$), ornamentation (from isolated warts to sometimes with some connections), habitat, and potential symbionts (Melera *et al.* 2017). In addition, recent phylogenetic studies (Lee *et al.* 2017; Melera *et al.* 2017) have shown the concept of *R. recondita* (*vide* Melera *et al.* 2017) or *R. aff. pectinatoides* (*vide* Lee *et al.* 2017) comprises three geographical distinct clades: i- European specimens, ii- Asian specimens and, iii- North American specimens. Further

studies of *R. recondita* are needed to determine if this phylogenetic arrangement indicates a middle stage of speciation or a discrete species (Lee *et al.* 2017). Recently, Adamčík *et al.* (2019), propose to *R. amerorecondita* Avis & Barajas as the North American sister species of *R. recondita*, restricting the latter to Europe and Asia. The South American specimens described here are closely related to the clade of European specimens, named *R. recondita* s.s. by Melera *et al.* (2017).

The completely mild taste, without any bitterness, and its fruity smell distinguish *R. recondita* from other morphologically similar species (Melera *et al.* 2017). *Russula ahmadii* S. Jabeen, A. Razaq., A.R. Niazi, I. Ahmad & A.N. Khalid is a similar species of the *R. pectinatoides* species complex, but is differentiated from *R. recondita* by having partially reticulate basidiospores, with crests that are mostly forked (Jabeen *et al.* 2017). *Russula catillus* H. Lee, M.S. Park & Y.W. Lim is microscopically similar to *R. recondita*, but differs in its paler pileus coloration, with pale yellow to light yellow surface (Lee *et al.* 2017). *Russula hortensis* Sarnari and *R. pseudopectinatoides* G.J. Li & H.A. Wen are two other macroscopically similar species, but are differentiated by their crested ornamented basidiospores, forming complete reticles (Sarnari 1998; Li *et al.* 2015). The recently described *R. amerorecondita* is another similar species to *R. recondita* but has larger basidiospores (8.5–10.7 × 7.5–9.5 µm) that the European and Argentinean specimens (Adamčík *et al.* 2019). In relation to this complex of species, Wright & Albertó (2002) described *R. pectinatoides* for introduced pine forests on Pampean region of Argentina. *Russula pectinatoides* could be confused with *R. recondita*, but further morphological studies and molecular analyses should be performed to confirm their identity and relationship. "*Russula recondita* was described as part of the section *Foetentinae* Melzer & Zvára (Lee *et al.* 2017; Melera *et al.* 2017), currently included in subgenus *Heterophyllidae* (Byuck *et al.* 2018; Adamčík *et al.* 2019).

Russula sardonica Fr., *Epicrisis Systematis Mycologici*: 353 (1838). Figs. 5-7

Pileus 70–120 mm diameter, convex to flattened, depressed on the central region when mature; striated to subsulcate at the margin, incurved; subviscid cuticle when wet, turning dry, glabrous, red-purple (11A6), brownish-violet (11D6) to brownish-red (9A7-9C7), turning darker

in the center, darker violet (11F5-11F8). Lamellae up to 5 mm, deep, attached, sinuated, crowded to close, smooth to finely crenulated margin, whitish (1A1) when young, turning yellowish white (1A2) to pale yellow (1A3) when mature, frequently with brownish orange (6C6-6C8) stained; entire and concolorous edge. Stipe 60–82 × 10–18 mm, cylindrical or more attenuated towards the base, central, slightly rough surface, fibrillose, whitish (9A1) when young, with a red-pinkish stained when mature, pale red (9A3) to grayish red (9B4). Context fleshy, up to 10 mm thickness, whitish (1A1), slightly reddish brown (9D6-9E6) towards the cuticle, firm, brittle, with watery line at the level of the lamellae. Taste hot spicy, odor fruity. Spore-print cream color (2A2 “yellowish white” to 2B2 “yellowish gray”). Positive reaction with potassium hydroxide and ammonia coloring salmon red (near 9B5 “grayish red”).

Basidiospores 6.4–9.8 × 5.5–8 µm; $x = 8 \times 6.4 \mu\text{m}$; $Q = 1.05\text{--}1.44$; $Q_x = 1.23$; $n = 21$; $N = 2$; ellipsoidal, hyaline, with subreticulated warts [(4–) 5–7 warts in a 3 µm diam. circle] frequently fused in short to long ridges (0–3 fusions in the circle), connected by occasional thin-lines (0–4 fusions in the circle), warts of 0.75–1.22 µm high, amyloid; smooth suprahilar spot (wrinkled under SEM), inamyloid. Basidia 38–51 × 5.8–7 µm, cylindrical-subclavate, 4-spored, thin-walled, hyaline. Hymenial cystidia numerous, ca. 1800–2600/mm², 57.5–90 × 7.5–12.5 µm; cylindrical-fusoid, apically obtuse, mucronate, with an appendage 1–9 µm long, originating in subhymenium, thin-walled, smooth, content completely heteromorphous, grayish reaction in sulfovanillin, more numerous near to the lamellae edges, usually smaller, 35–50 × 5.5–7.5 µm, cylindrical-fusoid to fusoid, occasionally claviform. Hymenophoral trama irregular, with polyhedral sphaerocysts up to 20 µm diameter intermixed with cylindrical hyphae, up to 6 µm diam. Gloeopleural hyphae up to 7.8 µm thick, turning black when stained with sulphobenzaldehyde.

Pileipellis in an ixotrichoderm, 160–200 µm deep, orthochromatic in Cresyl Blue; suprapellis 90–120 µm deep, made up ascending cylindrical hyphae, 1–4 µm diam., septate, hyaline, intermixed, embedded in a gelatinized matrix; subpellis 70–100 µm deep, composed of horizontally oriented, intermixed and strongly gelatinized hyphae. Hyphal termination near the pileus margin composed of 2-3 unbranched cells, thin-walled, terminal cells 35–45 × 2.5–3.5 µm, cylindrical



Figure 5 – Macroscopic characters of *R. sardonía* - a. general aspect - b. pileus surface detail - c. longitudinal section of basidiome - d. lamellae detail. [*O. Popoff 5601* (CTES)]. Scale bars = 5 cm.

to narrowly lageniform, subterminal cells wider and shorter, unbranched. Hyphal termination near the pileus center similar to the margin, slightly shorter, terminal cells $20\text{--}40 \times 2\text{--}3 \mu\text{m}$, cylindrical to narrowly lageniform. Pileocystidia dispersed near the pileus margin, composed of 1–2 cells, thin or slightly thick-walled, often flexuous and occasionally slightly moniliform; terminal cells $23\text{--}43 \times 4\text{--}5 \mu\text{m}$, conic to narrowly

fusiform, content with dispersed granulations; pileocystidia near the pileus center similar to the latter in shape and size, slightly wider, $38\text{--}45 \times 4\text{--}6 \mu\text{m}$. Oleiferous hyphae frequent in context, with yellowish pigments.

Examined material: ARGENTINA. CORRIENTES. Dpto. Gral. San Martín, La Cruz, Paraje Tres Cerros, $29^{\circ}04'09.15''\text{S}$, $057^{\circ}03'13.52''\text{W}$, 88 m a.s.l., 19.V.2017, associated with *Pinus elliottii* plantation, abundant. *O. Popoff 5601* (CTES).

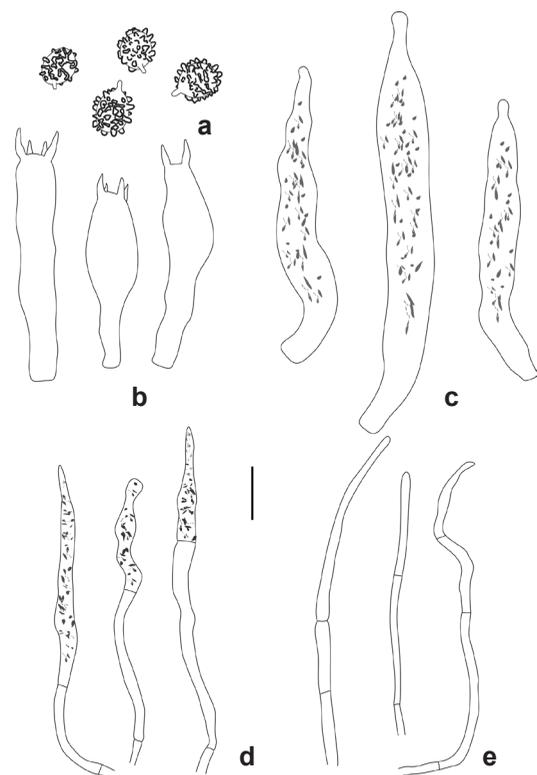


Figure 6 – Microscopic characters of *R. sardoniana* - a. basidiospores - b. basidia - c. hymenial cystidia - d. pileipellis terminal hyphae - e. pileocystidia. [O. Popoff 5601 (CTES)]. Scale bars = 10 µm.

The species is known from Europe (Kühner & Romagnesi 1953; Moser 1978; Moreno *et al.* 1986; Sarnari 1998). In South America, described from center and south Chile, associated with pine plantation (Garrido 1982; Furci 2008). *Russula sardoniana* grows associated with pine forest or plantation, on sandy or loose soils, with a great abundance of basidiomes (Moreno *et al.* 1986; Furci 2008). Previous records were not found for Argentina.

Russula sardoniana is characterized by its large pileus up to 18 cm diameter, with glabrous, purple-reddish to purplish surface, yellowish lamellae, subglobose basidiospores, 7–9 × 6–8 µm in size, ornamented with subreticulate amyloid warts, context and lamellae with hot spicy taste, and by reacting to potassium hydroxide or ammonia turning salmon red color. This species forms ectomycorrhizal associations with several *Pinus* species (Garrido 1982; Valenzuela *et al.* 1996, 1998). Its basidiomes with purple-violet coloration are morphologically similar to *R. queletii* Fr. and

R. torulosa Bresad; however, these species can be distinguished from *R. sardoniana* by their context and lamellae immutable to ammonia and potassium hydroxide test, smaller basidiomes (up to 6 and 8 cm wide respectively), and basidiospores with isolated warts (Kühner & Romagnesi 1953; Moser 1978). Other similar species is *R. exalbicans* (Pers.) Melzer & Zvára, which is differentiated by having basidiospores, 7.5–8.5 × 6–7 µm, with isolated warts (Moser 1978). *Russula cavipes* Britzelm. is other species with yellowish-toned lamellae, which turns salmon red with ammonia as observed in *R. sardoniana*, but it has smaller basidiomes with variable colorations and basidiospores with more marked warts (Kühner & Romagnesi 1953).

Wright & Albertó (2002) described *R. amethystina* Quélet from Argentina, a species with similar dimensions and lilaceous colorations that resembles *R. sardoniana*, nevertheless, it is differentiated by its darker and velvety pileus surface, whitish colored lamellae, and sweet taste.

Russula sardoniana is part of the subgenus *Russula* (Byuck *et al.* 2018).

Russula sororia (Fr.) Romell, Öfvers. K. Förh. Kongl. Svenska Vetensk.-Akad. 48 (3): 177 (1891). Figs. 8-10

Pileus 30–70 mm diam., globose at first, then convex to flat, broadly depressed when mature; margin corrugated, incurved to plane-upturned; viscid cuticle when young, turning dry, easily separable from the context at approximately one third of the pileus diameter; light brown surface (6A2 “orange white” to 6A3 “pale orange”), darker in the center, dark brown (6F4-8), with ferruginous (6C5 “brownish orange” to 6D5 “light brown”) stains in mature specimens. Lamellae up to 3 mm deep, adnate to sinuate, crowded at first, turning close, thick, whitish (1A1) when young, turning yellowish white (1A2) when mature, with brownish orange (6C6-6C8) stained when bruised. Stipe 30–60 × 10–15 mm, thick, cylindrical or slightly attenuated at the base, rough, bright, white (1A1) when young, turning pale gray (1B2) when old, stained with ochre (6B2-3, “orange-gray”, “grayish orange”) at the base or when is bruised. Context fleshy, up to 6 mm thick, whitish (1A1), brittle. Taste hot spicy, odor spermatic or artichoke. Spore-print cream color (near to 2A2 “yellowish white”). Negative reaction with potassium hydroxide and ammonia.

Basidiospores 7–9.2 × 5.4–7.2 µm; $x = 8 \times 6.2 \mu\text{m}$; $Q = 1.13\text{--}1.45$; $Q_x = 1.3$; $n = 20$; $N =$

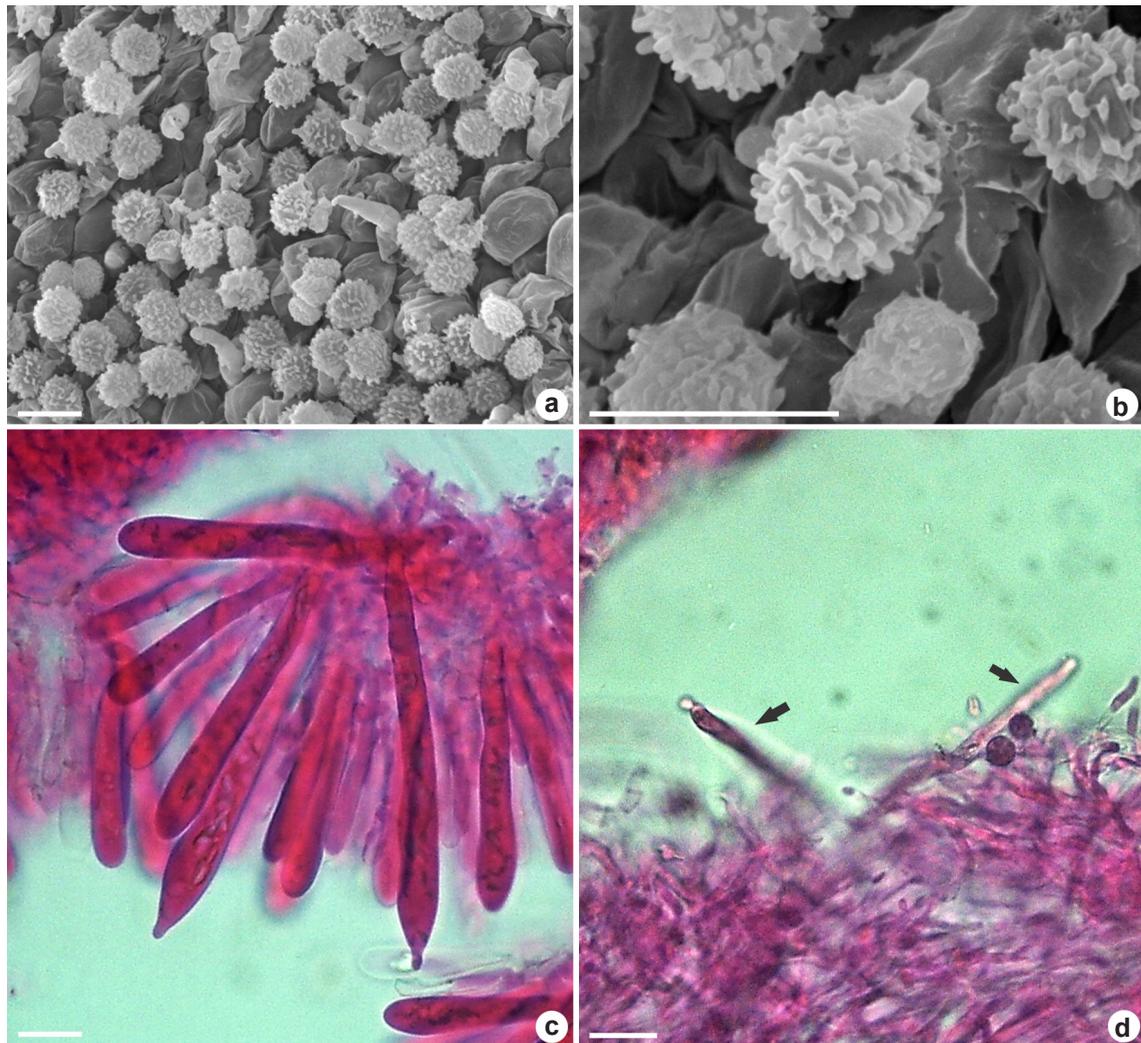


Figure 7 - Microscopic characters of *R. sardonía* - a-b. basidiospores on SEM - c. hymenial cystidia - d. pileipellis with pileocystidia (marked with arrows) [*O. Popoff 5601* (CTES)]. Scale bars = 10 μ m.

2; broadly ellipsoidal to subglobose, hyaline, ornamented with isolated warts [3–5 (–7) warts in a 3 μ m diam. circle], or occasionally with thin interconnections [0–3 (–5) fusions in the circle], warts 0.8–1.0 (–1.2) μ m high, amyloid; smooth suprahilar spot, inamyloid. Basidia 38–47 \times 5–9 μ m, clavate, 4-spored, thin-walled, hyaline. Hymenial cystidia numerous, ca. 1100–1700/mm², (38–)45–69 \times 5–9 μ m, fusiform, rarely cylindrical, apically obtuse, mucronate, with an appendage 2–12 μ m long, usually moniliform, occasionally bifurcate, mainly originating slightly below the level of the basidia, a few longer originating deeply in subhymenium, thin-walled, smooth, content completely heteromorphous,

grayish reaction in sulfovanillin; Hymenial cystidia near the lamellae edges smaller, 38–45 \times 4.5–7.5 μ m, fusiform to narrowly lageniform. Hymenophoral trama with globose to subglobose sphaerocysts, 12–34 \times 11–22 μ m, thin-walled, intermixed with cylindrical hyphae up to 5 μ m diam. Gloeopleural hyphae dispersed, up to 5 μ m diam., black with sulphobenzaldehyde. Pileipellis in an ixotrichoderm up to 150 μ m, orthochromatic in Cresyl Blue; suprapellis 60–90 μ m deep, made up ascending cylindrical hyphae, 1–3 μ m diam., septate, hyaline, intermixed more or less parallel, embedded in a gelatinized matrix; subpellis up 40–70 μ m deep, composed of horizontally oriented, intermixed and strongly

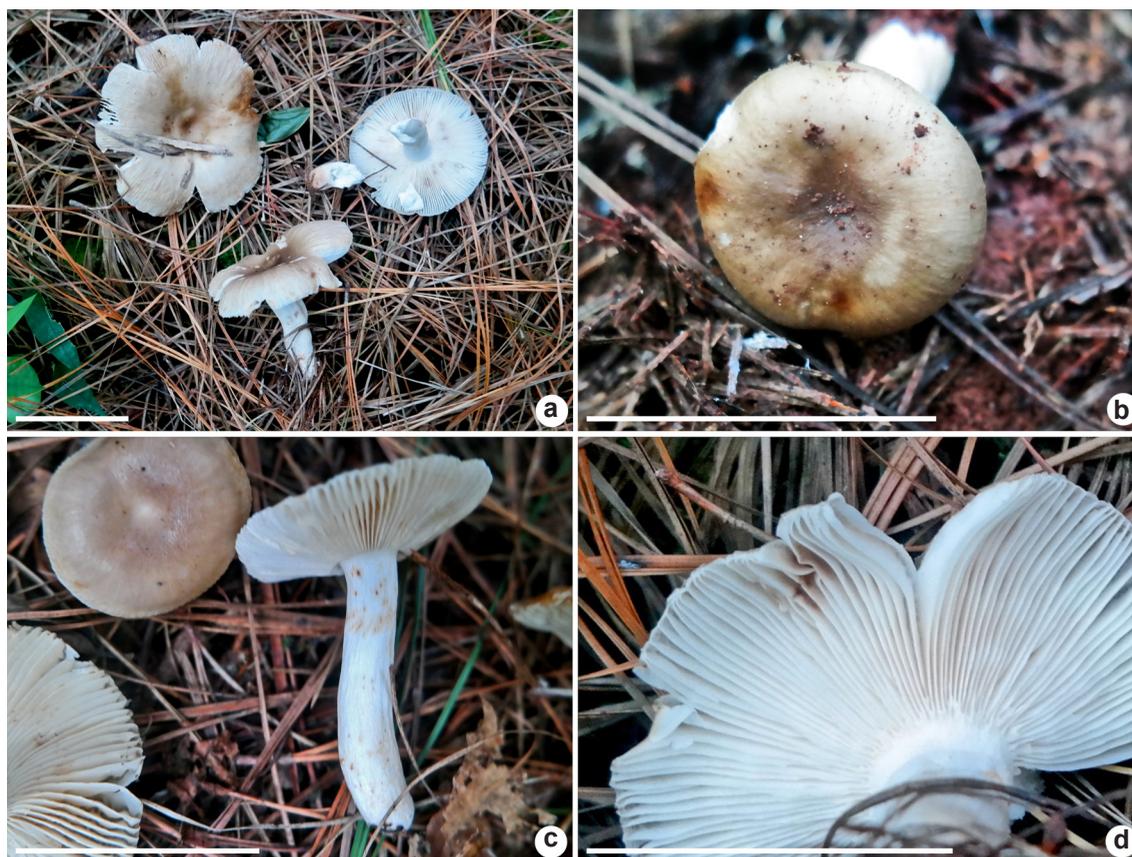


Figure 8 - Macroscopic characters of *R. sororia* - a. general aspect - b. pileus surface detail from a young basidiome - c. stipe surface detail - d. lamellae detail [*N. Niveiro* 3274 (CTES)]. Scale bars = 5 cm.

gelatinized hyphae. Hyphal termination near the pileus margin composed of 2-3 unbranched cells, thin-walled, terminal cells $38\text{--}68 \times 1.5\text{--}2.5 \mu\text{m}$, cylindrical, thin-walled, occasionally flexuose, slightly moniliform. Pileocystidia near the pileus margin dispersed, composed of one apical cell, $35\text{--}45 \times 4\text{--}6 \mu\text{m}$, fusiform, narrowly lageniform to cylindrical, thin-walled, with heteromorphous content; pileocystidia near the pileus center longer, $43\text{--}58 \times 5\text{--}6 \mu\text{m}$, fusiform to lageniform, thin-walled, with heteromorphous content. Oleiferous hyphae not observed in the context.

Examined material: ARGENTINA. MISIONES: Dpto. Iguazú, Puerto Libertad. Arauco S.A., San Jorge plantation, $25^{\circ}48'09.84''\text{S}$, $054^{\circ}30'43.70''\text{W}$, 271 m a.s.l., 2.V.2017, in 10 years old *Pinus taeda* plantation, *N Niveiro* 3274, *N Niveiro* ARA-P01-00.; ARA-P01-03; ARA-P03-01; ARA-P04-02 (CTES); 10.IV.2018, *N Niveiro* 2018.10.152 (CTES); *N Ramirez* 2018.10.88 (CTES). Dpto. Libertador General San Martín, Garuhapé, $26^{\circ}48'36.16''\text{S}$, $54^{\circ}55'57.20''\text{W}$, 163 m a.s.l., 6.X.2018, in *Pinus taeda* plantation, *N Niveiro et al.* 3350 (CTES).

Dpto. San Ignacio, San Ignacio, $27^{\circ}16'55.46''\text{S}$, $55^{\circ}33'07.68''\text{W}$, 149 m a.s.l., in 15 years old *Pinus taeda* plantation, 29.IX.2018, *N Niveiro* 3351 (CTES).

Russula sororia grows scattered, solitary or gregarious, in soil associated with pinus and deciduous forest in Europe (Kühner & Romagnesi 1953; Moser 1978) and North America (Shaffer 1972; Chou & Wang 2005). Previous records were not found for South America.

Russula sororia is morphologically difficult to distinguish from related species (Sarnari 1998). However, characters as the light brown pileus that does not exceed 8 cm in diameter, the white stipe that turns brown when is bruised, and the broadly ellipsoidal to subglobose basidiospores with subreticulated warts (Kühner & Romagnesi 1953; Sarnari 1998; Chou & Wang 2005; Kränzlin 2005; Mir *et al.* 2017) are useful to recognize it.

Russula sororia constitutes a complex of morphologically similar species and commonly confused with *R. amoenolens* Romagn., *R. cerolens*

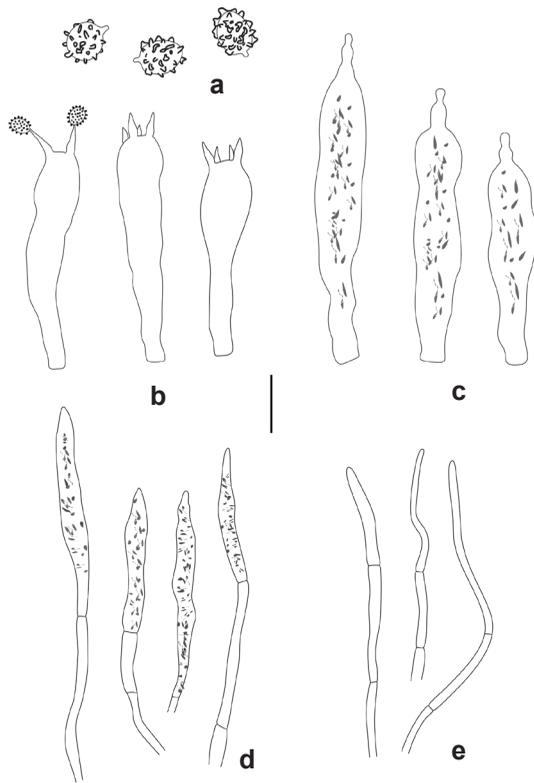


Figure 9 – Microscopic characters of *R. sororia* - a. basidiospores - b. basidia - c. hymenial cystidia - d. pileipellis terminal hyphae - e. pileocystidia. [N. Niveiro 3274 (CTES)]. Scale bars = 10 μ m.

Shaffer, *R. pectinata* (Bull.) Fr., *R. pectinatoides* (Shaffer 1972; Melera *et al.* 2017), and *R. ahmadii* (Jabeen *et al.* 2017). *Russula amoenolens* is distinguished by having larger (up to 10 cm) and firmer basidiomes, darker pileus surface (dark yellowish brown), more strongly acrid lamellae and a strong artichoke odor (Shaffer 1972). *Russula cerolens* is characterized by the red-brown stains on the stipe base and the ornamentation's pattern of its basidiospores, with warts up to 0.8 μ m high, isolated or with occasional interconnections forming a partial reticle (Shaffer 1972). *Russula pectinata* has more yellowish pileus surface, fish-like odor and is related to deciduous trees (Moser 1978; Thiers 1997). *Russula pectinatoides*, originally described for North America, is differentiated by its mild taste, of oil or fruity, and smaller basidiospores, 6–8.5 \times 5.7–7.2 μ m, with well-marked warts (Singer & Digilio 1951; Schaeffer 1952; Kühner & Romagnesi 1953; Moser 1978; Adamčík *et al.* 2013). *Russula ahmadii*, recently described from

Pakistan, differs by its smaller pileus (up to 4.5 cm), darker colorations and a more reticulated spore ornamentation (Jabeen *et al.* 2017).

Russula sororia and related species were traditionally considered within the subgenus *Ingratula* Romagnesi, section *Foetentinae* (Lee *et al.* 2017), but are currently considered in the subgenus *Heterophyllidae* (Byuck *et al.* 2018; Adamčík *et al.* 2019).

Discussion

Russula is one of the most diverse and well-known genus worldwide with more than 3,000 species (He *et al.* 2019). However, the identification of its species is difficult due to the subjectivity of many diagnostic characters, and by having species complex morphologically very similar among them, such as *R. recondita* and *R. sororia* case. To understand the diversity of this group, comprehensive studies are necessary, covering morphological, ecological, and phylogenetic analysis based on molecular data. Furthermore, the identification of species associated with introduced environments, such as pine forest plantations, is challenging because in many cases there is no prior knowledge of their biodiversity, neither the relationships that may exist among their components.

The diversity of ectomycorrhizal fungi associated with forest plantations in northern Argentina is little known (Niveiro *et al.* 2009; Campi *et al.* 2017). Studies on diversity of ectomycorrhizal fungi associated with *Pinus elliotii* in southern Brazil (Giachini *et al.* 2000, 2004; Sulzbacher *et al.* 2013, 2018; Silva-Filho *et al.* 2020), and center of Argentina (Urcelay *et al.* 2017), *Pinus radiata* D. Don. (Barroetaveña & Rajchenberg 2003), and Pinaceae (Hayward *et al.* 2015) in southern Argentina, did not record *Russula* species.

In South America there is a mixture of forest species of different biogeographic origins, with their respective associated ectomycorrhizal fungi, which favors the conditions for hosts switch (Silva-Filho *et al.* 2020). Thus, it is probable that most non-native ectomycorrhizal fungi have been introduced together with the seeds or seedlings of exotic *Pinus* species, coming from North America and Europe with ornamental or industrial purposes (Sulzbacher *et al.* 2013; Silva-Filho *et al.* 2020). Specimens of *R. recondita* studied here, in spite of being related to the European lineage of this

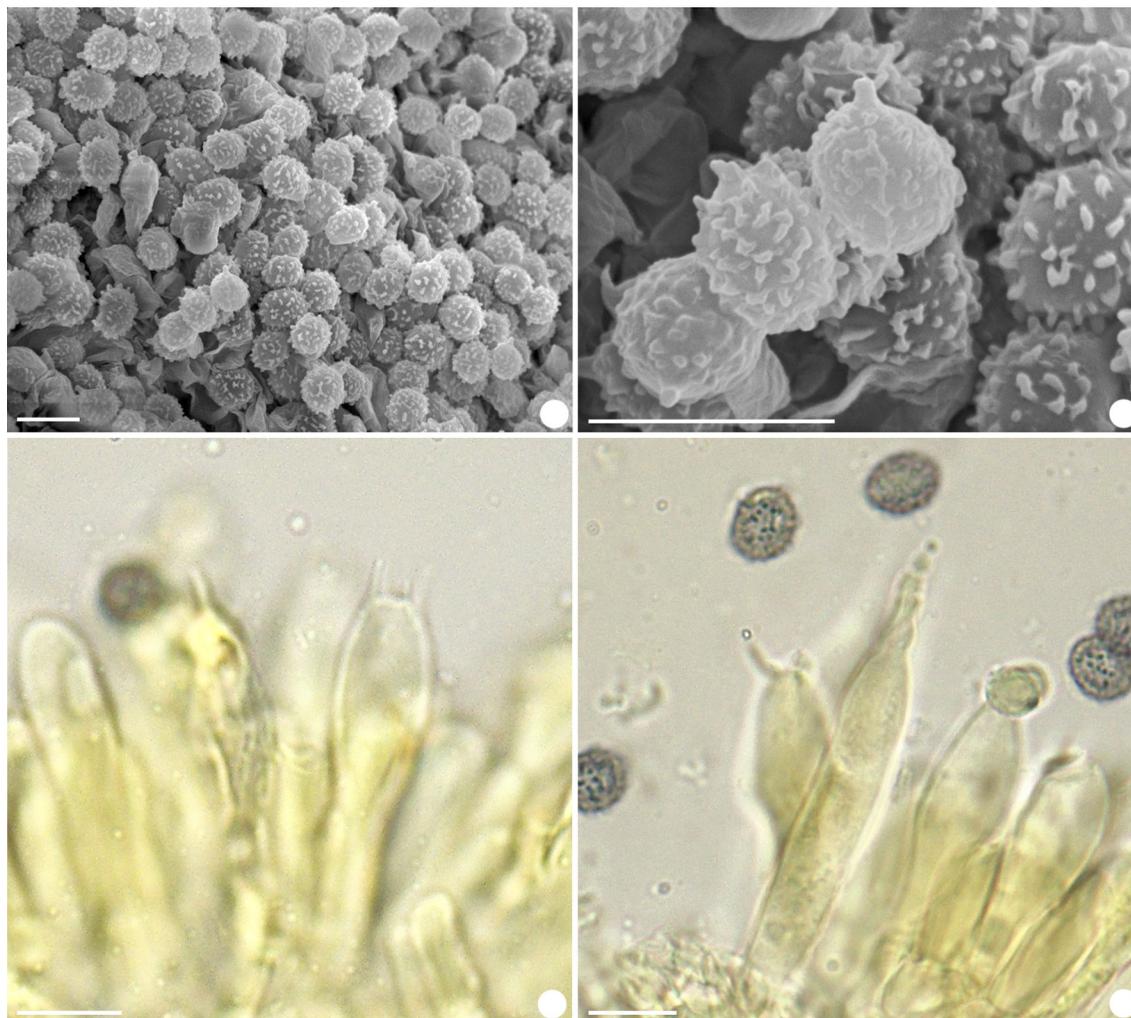


Figure 10 - Microscopic characters of *R. sororia* - a-b. basidiospores on SEM - c. basidia - d. hymental cystidia [*N. Niveiro* 3274 (CTES)]. Scale bars = 10 μ m.

species, were found associated with symbionts originating from southeastern North America such as *P. taeda* and *P. elliottii*. This was also previously found in *Lactarius quieticolor* Romagn., which was originally described associated to the European *P. sylvestris* L. and in South America found growing under *P. taeda* in Brazil and *P. radiata* in Chile, both species of North American flora (Silva-Filho *et al.* 2020).

The presence of ectomycorrhizal fungi is a fundamental requirement for the normal growth of pine species (Barroetaveña & Rajchenberg 2003), therefore knowledge about its distribution and ecology is important for the species selection for use in forest nurseries (Castellano & Molina 1989; Giachini *et al.* 2000), or to establish control

mechanisms on introduced species that may have effects as invasive (Hayward *et al.* 2015; Urcelay *et al.* 2017).

The presence of *R. recondita*, *R. sardonica*, and *R. sororia* represents the first records for the subtropical region of South America, allowing us to have a more complete knowledge about the diversity of ectomycorrhizal fungi associated with pine forest plantations.

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