

Extending the distribution of the hypogeous sequestrate genus *Reddellomyces* (Tuberaceae) to the American Continent

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Abstract: *Reddellomyces* is a genus distributed mainly in Australasia, Spain and Italy, growing in association with *Acacia*, *Eucalyptus*, and *Casuarina*. This genus is characterized mainly by having a glabrous to scabrous peridium, white to dark brown, smooth to tessellate-cracked or verrucose; paraphyses longer than the asci; white to pale brown, smooth to wrinkled peridium; and asci with 2–4 (–6) spores. In this work, the distribution of the genus is extended to the American continent based on its morphology, ecology, variation of ITS and LSU genes, Bootstrap (BT) support, and posterior probability (PP).

Keywords: diversity, false truffles, Mexico, phylogeny, truffles.

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Introduction

Reddellomyces is a genus described in 1992 by J.M. Trappe, M.A. Castellano, and N. Malajczuk, who segregated it from the genus *Labyrinthomyces*. *Reddellomyces* is characterized by having a smooth to wrinkled, white to pale brown peridium, and asci with 2–4 (–6) ascospores, features that differentiated it from *Labyrinthomyces* and *Dingleya*. Sbissi et al. (2020) recognize four species: *Reddellomyces donkii* (Malençon) Trappe, Castellano & Malajczuk; *R. parvulosporus* (Beaton & Malajczuk) Trappe, Castellano & Malajczuk; *R. magnisporus* Trappe, Castellano & Malajczuk; and *R. westraliensis* (Beaton & Malajczuk) Trappe, Castellano & Malajczuk, the latter being the type species. On the other hand, Index Fungorum recognizes a fifth species, *R. tunisiensis* Sbissi, Nouiou, Neffati, Boudabous & Gtari; however, this taxon requires more research to be clearly defined.

Although these species are native to Australasia, species of *Reddellomyces* have been recorded in Spain and Italy (Trappe et al. 1992, 2010; Sbissi et al. 2020), and molecular phylogenetic studies have confirmed this, also indicating a close relationship with *Labyrinthomyces* and *Dingleya* (Læssøe and Hansen 2007; O'Donnell et al. 1997). Furthermore, they have been associated with *Eucalyptus* and *Acacia* (Kope and Warcup 1986; Warcup 1988; Trappe et al. 2010; Sbissi et al. 2020).

In this work, the distribution is expanded, and the presence of the genus is reported for the first time on the American continent.

Materials and methods

Morphology

Ascoma collection was carried out in the Cuenca de la Esperanza Natural Protected Area (ANP, by its acronym in Spanish), Guanajuato, Mexico. The dominant vegetation is oak forest: *Quercus* sp., with seven species in association with madrone (*Arbutus* sp.) and pingüica (*Arctostaphylos pungens*). The elevation of the study area ranges from 2300 to 2750 m a.s.l.. Collection was conducted during the rainy season.

For the collection, the method of Castellano et al. (1989) was followed, digging around trees to a depth of 15 cm. All encountered fruit bodies were photographed, and macroscopic characters were described from fresh material; subsequently, the material was dehydrated at 50 °C. Manual sections were prepared, and the microscopic characters of the dried specimen were described; the specimen was mounted in 5% potassium hydroxide (KOH) to measure and observe the spores. For this purpose, a Carl Zeiss microscope equipped with an AxioCam ERc 5c Rev 2.0 camera was used. Finally, the herborized material was deposited in the José Castillo Tovar Herbarium of the Instituto Tecnológico de Ciudad Victoria (ITCV), Tamaulipas, Mexico.

DNA extraction, amplification and sequencing

DNA extraction, amplification, and sequencing of the ITS (Internal Transcribed Spacers) and LSU (Large Subunit) regions were performed at the Plant Pathology Laboratory of the University of Florida (UF).

The extraction method used was that described by Möller et al. (1992), based on CTAB for fungi and plants. PCR was carried out with a standard mixture of 2.5 µL of 10× buffer, 0.5 µL of dNTPs, 0.5 µL of forward primer, 0.5 µL of reverse primer, 0.25 µL of Taq polymerase, and nuclease-free water up to 25 µL, using primers ITS-1 and ITS-4 for the ITS region (White et al., 1990) and LR0R and LR5 for LSU (Vilgalys and Hester 1990).

PCR amplification included 35 cycles at 55 °C. PCR products were verified by electrophoresis for 1 h at 80 V on a 1% agarose gel and 1× TAE buffer (Tris-acetate-EDTA); the gel was stained with GelRed and subsequently visualized on a transilluminator. Amplified PCR products were purified using the Omega BIO-TEK E.Z.N.A. Cycle Pure kit, following the manufacturer's instructions. Nucleotide sequencing was performed using the Sanger method at UF.

Phylogenetic analyses

The phylogenetic analysis of the species included the use of two databases consisting of 70 sequences of the ITS and LSU regions (Table 1) obtained from GenBank. These were concatenated in MESQUITE 4.02 (Maddison and Maddison 2025), using *Choiromyces magnusii* as the root.

The ML tree was constructed using a TIM2+F+I+G4 evolutionary model with 100 000 bootstrap (bb) replicates in IQ-TREE (Nguyen *et al.* 2015). Meanwhile, Bayesian Inference (BI) employed the GTR + I + L evolutionary model with 10 000 000 generations, using MrBayes 3.2.7a (Ronquist *et al.* 2012). Tracer was used to observe the effective sample size (ESS) values and to verify data convergence (Rambaut *et al.* 2018). For the representation of the phylogeny, the ML tree was used due to its equal resolution to BI. Editing was performed in FigTree (Rambaut *et al.* 2018) and the Chiplot website (Xie *et al.* 2023), considering values of > 75% Bootstrap and > 0.90 posterior probability as significant.

Table 1. List of GenBank accession numbers used in phylogenetic analyses.

Species	ITS	LSU	Code	Location
<i>Choiromyces magnusii</i>	JF300143.1	JF300159.1	4359	Spain
	JF300144.1	JF300160.1	4460	Spain
<i>Dingleya geometrica</i>	JF300131.1	JF300149.1	3149	Australia
<i>Dingleya</i> sp	KP191974.1	KP191787.1	7487	New Zealand
	JQ925626.1		626	Australia
	KY697577.1	HM485334.1	7734	Australia
	JQ925628.1	GQ221457.1	2857	Australia
	JQ925627.1		627	Australia
	KP191975.1	KP191788.1	7588	New Zealand
	PX136882.1		882	Australia
	KP191972.1		972	New Zealand
	KY697578.1		578	Australia
	KY697579.1		579	Australia
	KY697580.1		580	Australia
<i>Labyrinthomyces</i> sp	JQ925633.1	MF503275.1	3375	Australia
	OL653022.1	PX220083.1	2283	New Zealand
	HM485335.1		335	Australia
	JQ925635.1		635	Australia
	JQ925634.1	MF503275.1	3475	Australia
	KP191973.1	KP191790.1	7390	New Zealand
	MF503288.1	JQ925670.1	8870	Australia
	MF503289.1	MF503274.1	8974	Australia
	KY697595.1		595	Australia
	MF503287.1	MF503273.1	8773	Australia
<i>Labyrinthomyces varius</i>	JF300129.1	JF300148.1	2948	Australia
<i>Nothojafnea</i> cf <i>thaxteri</i>	JQ925638.1	JQ925673.1	3873	Argentina
<i>Nothojafnea</i> sp	PX688662.1		662	Vietnam
	PX663128.1		128	Australia
	OR372824.1		824	Australia
	OR372827.1		827	Australia
	OR372828.1		828	Australia
	MZ150380.1		380	Chile
<i>Nothojafnea thaxteri</i>	MT366731.1		137	Chile

	MT366722.1		722	Chile
<i>Reddellomyces donkii</i>	JF300140.1	JF300151.1	4051	Spain
	JF300139.1	JF300152.1	3952	Spain
<i>Reddellomyces parvulosporus</i>	KX879781.1	KX879783.1	8183	Tunisia
<i>Reddellomyces</i> sp	HM485336.1		336	Australia
	JF300141.1	JF300153.1	4153	Spain
	HM485337.1	JQ925677.1	3777	Australia
	KC494013.1	KC494010.1	1310	Tunisia
	KC494012.1	KC494008.1	1208	Tunisia
	KC494011.1	KC494009.1	1109	Tunisia
	KC494014.1		14	Tunisia
	JQ925641.1	JQ925676.1	4176	Australia
	JQ925642.1	JQ925678.1	4278	Australia
<i>Reddellomyces</i> sp	PZ441387	PZ448934	ITCV_1912_MX	Mexico
<i>Reddellomyces westraliensis</i>	JF300135.1	GQ231748.1	3548	Australia
	KX879782.1	KX879784.1	8284	Tunisia
	JF300134.1	JF300150.1	3450	Spain
<i>Rubroboletus pulchrotinctus</i>	JF908771.1		711	Italy
<i>Tuber aereum</i>	PP425946.1	PP425963.1	4663	Greece
	PQ336161.1	PP425961.1	6161	Greece
	PP425948.1	PP425969.1	4869	Greece
	PQ336045.1	PP425966.1	4566	Greece
<i>Tuber aestivum</i>	KC934930.1	KC934931.1	3031	Israel
	PQ310597.1	KF523368.1	9768	Israel
	FM205679.1	FJ809843.1	7943	Serbia
<i>Tuber magnameshanum</i>	OM256767.1	PX125313.1	6713	China
<i>Tuber neoexcavatum</i>	OM256763.1	OM366184.1	6384	China
	OM256766.1	KY013647.1	6647	China
	OM256762.1	OM366169.1	6269	China
<i>Tuber pseudofulgens</i>	OM256757.1	OM366194.1	5794	China
<i>Tuber</i> sp	PX892948.1	OM416921.1	4821	Unites States
	PX892951.1	OM416928.1	5128	Unites States
	KM576676.1	PQ427319.1	7619	Spain
	MZ616377.1	FJ809849.1	7749	Spain
	JQ925636.1	JQ925671.1	3671	Australia
<i>Tuber torulosum</i>	LC774460.1	LC637317.1	6017	Japan
<i>Underwoodia</i> sp	KY462388.1		388	Chile

Results

Molecular phylogeny

The phylogenetic tree obtained by concatenating the ITS and LSU ribosomal regions demonstrated a topology with high support values in both Maximum Likelihood and Bayesian Inference. In general, the main clades present Bootstrap values ≥ 90 and posterior probabilities close or equal to 1.0, indicating high topological stability. The tree is structured into several clades that include *Tuber*, *Nothojafnea*, *Labyrinthomyces*, *Dingleya*, and *Reddellomyces*. *Choiromyces magnusii* was used as the root (Fig. 1).

At the upper part of the tree, a robust clade corresponding to the genus *Tuber* is observed, representing multiple subclades with good support. Subsequently, a clade grouping species of *Nothojafnea*

Figure 1. Maximum likelihood phylogeny of *Reddellomyces* based on DNA sequences of two loci (ITS, LSU). Branching supports with > 75% bootstrap value or > 95% Bayesian posterior probability based of maximum-likelihood and Bayesian phylogeny, respectively, are shown at the nodes.



The clade of greatest interest in this study corresponds to the genus *Reddellomyces*, which appears well supported within the tree, with support values close to 100/1 (BB/PP). Within this clade, several species previously reported in the Southern Hemisphere, particularly from Australasia and Europe, are grouped, which coincides with biogeographical patterns described for hypogeous fungi associated with ecosystems with mycorrhizal tree species.

The sample ITCV_1912_MX is clearly nested within the *Reddellomyces* clade, forming part of a subclado that groups sequences close to species such as *Reddellomyces parvulosporus* and other taxa of this genus. The node delimiting this group presents high values of phylogenetic support, suggesting that the taxonomic affinity of the sample with the genus is statistically robust (Fig. 1).

Although a clustering between *Reddellomyces* and the boletaceous *Rubroboletus* is observed, it is believed to be due to contamination or misidentification, since both genera belong to distinct evolutionary lineages (Ascomycota and Basidiomycota) whose divergence is deep.

Taxonomy

Reddellomyces sp. Figs. 2A–D

MycoBank no.: PZ441387/ PZ448934

Ascoma hypogeous, solitary, $10 \times 8 \times 7$ mm in size, subglobose to ellipsoidal, cream-colored when fresh, yellowish when dry, surface with light brown to dark brown patches, as well as with various elongated and ellipsoidal depressions.

Gleba spongy with irregular cavities, globose to elongated, bright cream-colored, with reddish-brown areas and dark red spots near the peridium or at the margins. The fertile zone is located in the reddish-yellow, spongy veins. Odor not recorded.

Peridium easily peelable, up to $141 \mu\text{m}$ thick, composed of two layers : Epicutis $57\text{--}75 \mu\text{m}$ thick, formed by thin, compact, and loose hyphae with spaces between them, $4\text{--}10 \mu\text{m}$, wall $1 \mu\text{m}$, septum up to $1 \mu\text{m}$, hyaline in 5% KOH; Mesocutis $50\text{--}66 \mu\text{m}$ thick, delimited from the epicutis, formed by thick hyphae $5\text{--}11 \mu\text{m}$ wide, prostrate to intertwined down to the gleba, giving the appearance of one layer in some areas and two layers in others, septum up to $1 \mu\text{m}$ thick, hyaline in 5% KOH. Gleba hyphae parallel, thick, $5\text{--}10 \mu\text{m}$, septum up to $2.5 \mu\text{m}$ and wall less than $2.5 \mu\text{m}$. Hyaline in 5% KOH. Asci with 2 to 4 spores, uniseriate, mostly with 3 spores, ventricose, constricted, clavate to subclavate, $98.4\text{--}231.4 \times 41.6\text{--}62.4 \mu\text{m}$ wide, excluding the pedicel. Pedicel $13\text{--}122.2 \mu\text{m}$ long, walls $2.6\text{--}5.8 \mu\text{m}$ thick, hyaline in 5% KOH.

Ascospores globose. 2-spored asci: $33.8\text{--}46.8 \times 33.8\text{--}46.8 \mu\text{m}$ including ornamentation, $26\text{--}31.2 \times 26\text{--}33.8 \mu\text{m}$ excluding ornamentation. 3-spored asci: $36.4\text{--}49.4 \times 31.2\text{--}49.4 \mu\text{m}$ including ornamentation, $20.8\text{--}33.8 \times 26\text{--}33.8 \mu\text{m}$ excluding ornamentation, 4-spored asci: $33.8\text{--}49.4 \times 33.8\text{--}46.8 \mu\text{m}$ including ornamentation, $23.4\text{--}31.2 \times 20.8\text{--}31.2 \mu\text{m}$ excluding ornamentation. Ornamentation up to $5 \mu\text{m}$, robust, blunt, brown to reddish-brown at maturity. Immature ascospores smooth, inamyloid, hyaline in 5% KOH. Melzer's reaction negative.

Habitat and habit : Solitary, hypogeous, growing under *Eucalyptus* sp. in an oak forest with interspersed eucalyptus, at 2328 m a.s.l.

Material examined: Mexico, Guanajuato; ANP Cuenca de la Esperanza, locality. 21°2'49" N; 101°14'55" W, at 2328 m.a.s.l., collected on November 7, 2021. Collection 1912 ITCV.

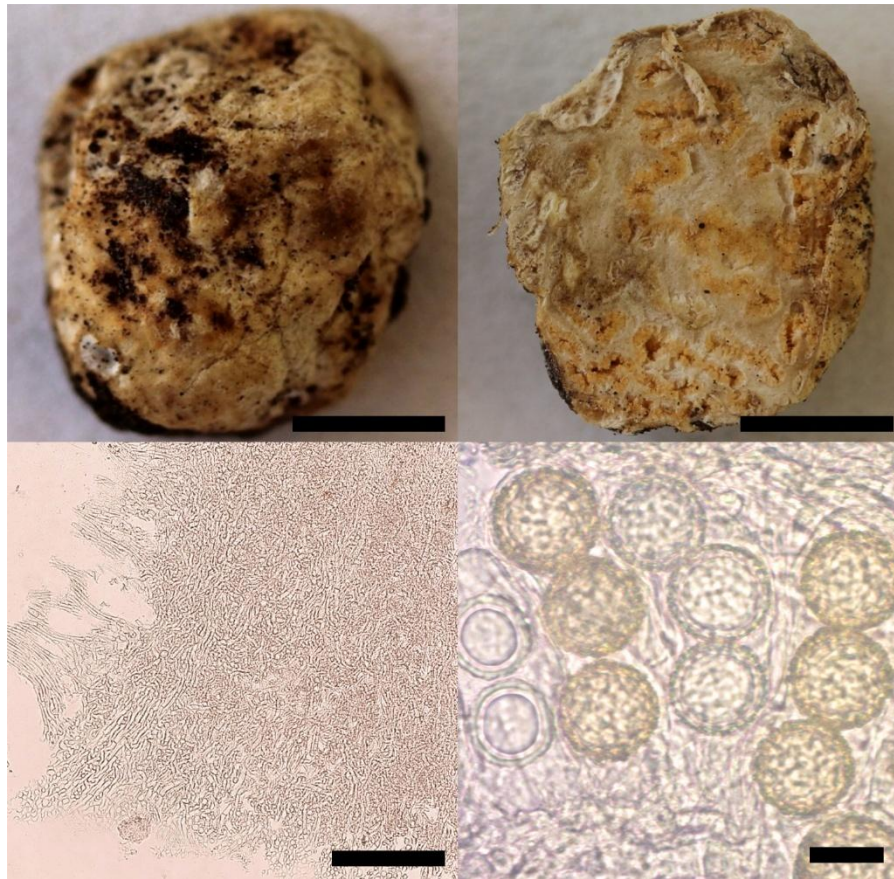


Figure 2. A) Ascomata. B) Gleba. C) Peridium cells. D) asci with 3 ascospores, mature ascospores. Scale bars: A-B= 5mm; C=60 μ m; D= 30 μ m.

Discussion

The molecular phylogenetic studies obtained in this work support the presence of the genus *Reddellomyces* on the American continent, specifically in Guanajuato, Mexico. This represents a relevant finding from both a biogeographical and taxonomic perspective. This research expands the known distribution of a genus that previously authors such as Trappe et al. (1992, 2010) and Sbissi et al. (2020) had reported solely from Australasia and Europe.

The presence of *Reddellomyces* sp. in an area dominated by deciduous trees (*Quercus* sp., *Arbutus* sp.) within this protected area prompted us to return to the study site to confirm the hosts reported by previous authors. Isolated patches of *Eucalyptus* sp. were found, which, judging by their large size (height and stem diameter), were introduced before this area was declared a Natural Protected Area (ANP) in

1998. This is particularly notable since, in past years, exotic species such as *Eucalyptus*, among others, were introduced into the country through reforestation programs.

It is known that most *Reddellomyces* species form ectomycorrhizal associations with host plants primarily native to Australasia, such as species of *Acacia*, *Eucalyptus*, and *Casuarina* (Kope and Warcup 1986; Warcup, 1988; Trappe et al. 2010; Sbissi et al. 2020). The collection in Mexico under *Eucalyptus* sp. strongly suggests that this represents an allochthonous introduction, reflecting invasion patterns observed in the Iberian Peninsula and other Mediterranean regions. e.g. *R. donkii* and *R. westraliensis* have been recorded in Spain and Portugal associated with introduced plantations of *Eucalyptus globulus* and *E. camaldulensis* (Diez 2005; Moreno et al. 2012). Similarly, in Tunisia and Turkey, *Reddellomyces* has been found under introduced species of *Acacia saligna* and *Eucalyptus*, indicating a generalized pattern of human-mediated dispersal along with their specific host plants (Sbissi et al. 2020). These cases highlight the role of commercial forestry and tree planting in expanding the range of ectomycorrhizal fungi globally (Vellinga et al. 2009).

The morphological characteristics observed in the Mexican specimen—such as its subglobose to ellipsoidal ascoma, easily peelable peridium, cream to beige coloration, solid gleba with distinctive veins, and globose ascospores (including size ranges and ornamentation) agree with the general descriptions of *Reddellomyces* (Trappe et al. 1992; Sbissi et al. 2020). Although variations in peridium thickness (141 μm), asci (231 μm), and ascospores (33.8–49.4 μm) were observed, they differ from those of the Australasian species, which show peridium (50 μm), asci (250 μm), and ascospores (20–27 μm), respectively. These measurements agrees most with *R. parvulosporus*. Nevertheless, to determine whether or not it represents the same species, a more detailed comparative review combining molecular and morphological analyses is still required.

The nesting of *Rubroboletus pulchrotinctus* (an epigeous mushroom) with *Reddellomyces* is incongruent taxonomically. Since *Reddellomyces* belongs to Ascomycota (Pezizales: Tuberaceae) and *Rubroboletus* to Basidiomycota (Boletaceae), suggesting a possible contamination of the sample or a misidentification of *Rubroboletus*.

This first report of *Reddellomyces* on the American continent highlights the ongoing process of biotic exchange facilitated by human activities, such as reforestation and horticulture with non-native host plants (Vellinga et al. 2009), as well as the importance of continuous research in hypogeous mycology in Mexico.

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