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Broadening the distribution of *Lactarius denudatus* (Calonge & J.M. Vidal) P.M. Kirk, a rare truffle-like fungus from Mexico

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Abstract: *Lactarius denudatus* (Calonge & J.M. Vidal) P.M. Kirk is an hypogeous species characterized by the small, gasteroid basidiomata, exposed and anastomosed pale cream lamellae, large cystidia up to 90 µm in length, and small ellipsoid verrucose basidiospores. This species was described in 2005 and currently it is only known from the type collections in two locations in the Cofre the Perote region in the state of Veracruz, Mexico. Here we broaden the distribution of the species to the Mexican state of Oaxaca and a new location in Veracruz, growing in *Quercus* and *Quercus*-*Pinus* forests. Detailed descriptions, discussions and photographs are presented of this truffle-like fungus, so far only recorded from Mexican *Pinus patula* forests, and now also growing under *Quercus* spp.

Keywords: Fungal dversity, hypogeous fungi, mycorrhizal fungi, Russulaceae.

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Introduction

Russulaceae has a wide morphological diversity which goes from agaricoid, to pleurotoid and gasteroid species (Miller et al. 2000). Microscopically they are characterized by having basidiospores with amyloid spinose, verrucose or reticulate ornamentation. Additionally, the cystidia shapes, the arrangement of the pileipellis elements and the presence of laticiferous hyphae are also relevant as diagnostic features of Russulaceae to distinguish its species (Lebel and Trappe 2000). They are widely distributed in both temperate and tropical forests around the world, forming ectomycorrhizas with species of angiosperms and gymnosperms (Hackel et al. 2022). Some species are widely known for their gastronomic value (Guerin-Laguette et al. 2020). Most of the well-known Russulaceae are typically stipitate-pileate species but sequestrate forms are also observed in the genera *Russula* Pers., *Lactifluus* (Pers.) Roussel and *Lactarius* Pers. (Lebel et al. 2021).

One of the best known species within Russulaceae are those of the genus *Lactarius*, also known as milk-caps, due to the exuding latex when their basidiomata are cut. The species of this genus are of great

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cultural importance in Mexico due to their use as a food by various ethnic groups (Pérez-Moreno et al. 2021). They are abundant elements in the fungal communities of pine forests, mixed forests and oak forests in Mexico (Bandala et al. 2016). Despite the fact that epigeous species of *Lactarius* have been extensively explored, the hypogeous species of the genus have been rarely studied in Mexico. In contrast, these group of fungi have been explored in more detail in North America (Trappe et al. 2009), Oceania (Lebel and Trappe 2000), Africa and Europe (Miller et al. 2000; Beenken et al. 2016).

Lactarius denudatus (Calonge & J.M. Vidal) P.M. Kirk is a species originally described by Calonge and Vidal (2005) within the genus *Gastrolactarius* R. Heim ex J.M. Vidal, characterized by irregular, exposed, light brown hymenium, a depressed and reduced pileus in the apical area of the basidioma, ellipsoid basidiospores with an incomplete, amyloid reticulum, and elongated and capitate cystidia. This species was described from *Pinus patula* forests of Veracruz at more than 2000 m a.s.l. and so far is only known from two type provenances in this Mexican State. Recently, some specimens have been studied matching the description of this species. In this paper, the distribution of this species is expanded to other localities of the Mexican territory. Descriptions, photographs, and discussions of this fungus are presented.

Materials and methods

Mycological explorations were carried out in the Mexican states of Oaxaca, and Veracruz (Fig.1). Dominating vegetation in the sampling area were mixed forest with Fagaceae, Pinaceae and Ericaceae trees and shrubs. For sampling this sequestrate fungus, the methods proposed by Castellano et al. (1989) were followed. The method consisted in removing the upper layer of soil looking for hypogeous basidiomata near host trees, moist areas and burrows. The specimens were photographed, described and cured. Colour description was made according Kornerup and Wanscher (1979). Hand cuts sections were made and mounted using 5% KOH and Melzer reagent to observe and measure microstructures including basidiospores, basidia, cystidia and pileipellis elements. The next abbreviations were used: L= large, W=wide, Q= large/wide ratio, Qm= Q media. The microstructures and photographs were taken using a Motic BA310 microscope. The studied material is deposited in the herbaria from the Instituto Tecnológico de Ciudad Victoria (ITCV) and Instituto Tecnológico Superior de Zongolica (ZON).

Results and discussions

As a result of the mycological explorations, three collections of *L. denudatus* were found from locations in the states of Oaxaca and Veracruz. The specimens agree with the descriptions of Calonge and Vidal (2005) but the material from Oaxaca showed larger and very abundant cystidia. The species was only known from the collection type in Veracruz, and now extants its distribution to southeast and central Mexico. So far, the species remains as endemic from Mexico but more explorations are needed. A related species, *Lactarius rubriviridis* Desjardin, H.M. Saylor & Thiers, also lacks peridium and shows orange-reddish tones, nevertheless, it lacks stipe and grows under *Abies* spp. (Desjardin et al. 2003).

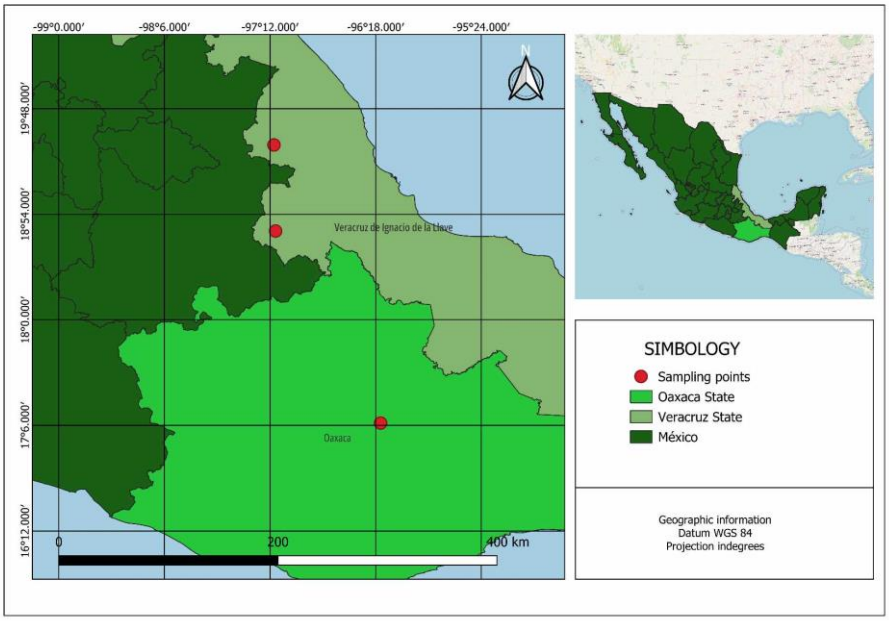


Figure 1. Current known distribution of *Lactarius denudatus* in Mexico.

Lactarius denudatus (Calonge & J.M. Vidal) P.M. Kirk (Figs. 2 and 3).

Basidiomata 14 × 11 mm, subhypogeous, with reduced pileus located at the top of the basidiomata, orange white to pale orange (5A2-3), smooth to sligthly tomentose. Hymenia composed of irregular, elongate to angular locules, filled with withish spore-mass, with light orange (5A5) to reddish orange (7A7) trama, latex not observed when cut; columella dendriform, reddish orange (7A7). Stipe 3 mm long, dark orange (5A8) to brownish yellow (5C8), sligthly excentric. Odor and taste fungoid, mild.

Pileus composed of two layers: epicutis 80-110 µm thick, composed of erect or interwoven hyphae of 3-5 µm in diameter, branched, with septa, with terminal cells cylindrical, with rounded tips, hyaline, thin-walled. Subcutis 60-150 µm composed of subglobose to irregularly globose hyphae of 12-30 µm in diameter, hyaline, with thick, yellowish cell walls. Hymenophoral trama 30-60 µm composed of tubulose, sinouse hyphae of 2-6 µm in diameter, with septa, with abundant subglobose sphaerocytes up to 10 µm near the subhymenium, laticiferous hyphae present, 6-10 µm in diameter, with yellowish content in Melzer, rarely branched, thin-walled. Cystidia 80-110 × 6-12 µm, hialine or with granular content, setae-like, some with capitate tips, wide at the base, thin-walled, abundant. Basidia 20-34 × 8-12 µm, clavate, mostly 2-spored, rarely 4-spored, hyaline or with abundant intracelular content, with slender sterigmata up to 3 µm, thin-walled. Basidiospores 6-11 × 5-7 µm (L=8.03, W=6.06, Q=1-1.8, Qm= 1.33, n=30), mostly ellipsoid, globose, subglobose, to rarely elongate, with abundant amyloid small spines up to 1 µm, sometimes coalescing forming a partial reticulum, with small hylar appendage up to 2 µm, hyaline or gutulated in KOH%, thin-walled.

Material revised: Mexico. Oaxaca, Santo Domingo Xagacia, growing in *Quercus* - *Pinus* forests. 20/08/2023, de la Fuente (JIF-625-ITCV). Veracruz, Soledad Atzompa, Tepaxapa, growing under *Quercus* spp., 13/08/2023, Wendy Rosales-Rosales (75-ZON).



Figure 2. Basidiomata of *Lactarius denudatus* (Calonge & J.M. Vidal) P.M. Kirk sampled in Santo Domingo Xagacia, Oaxaca, Mexico (JIF-625-ITCV). Bar: 10 mm.

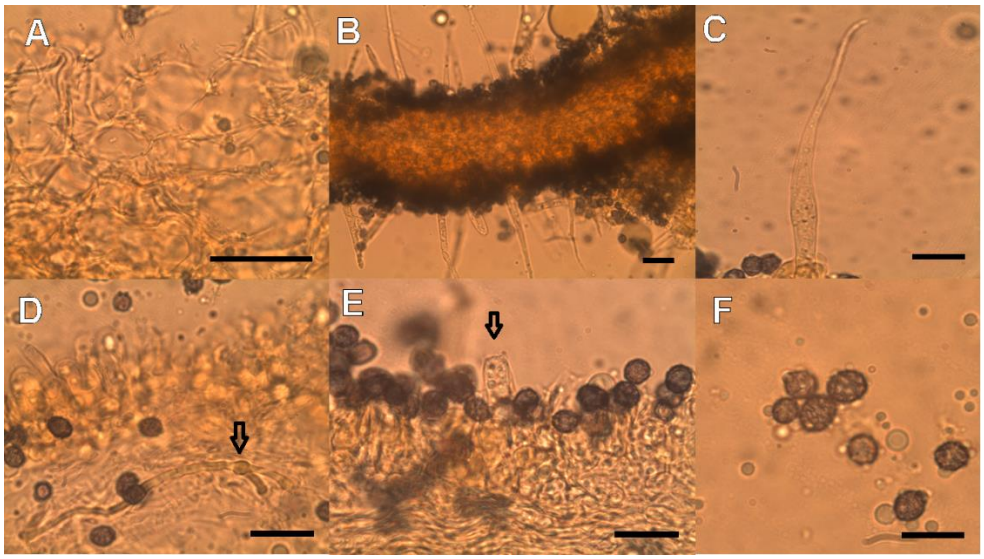


Figure 3. Microstructures of *Lactarius denudatus* (Calonge & J.M. Vidal) P.M. Kirk. A) Details of epicutis. B) Saeta-like cystidia emerging from hymenophoral trama. C) Cystidia. D) Laticiferous hyphae. E) Basidia (black arrow). F) Basidiospores. Bars: 20 µm.

The study of sequestrate members of Russulales started in North America with the studies of Zeller and Dodge (1919, 1935) with the delimitation of several genera associated with *Russula* and *Lactarius*. Due to the morphological and microscopic similarity with other genera of Agaricales and Boletales, they

were placed within the Astrogastracea series (Singer and Smith 1960). Despite the similarities in certain microscopic characteristics among genera belonging to sequestrated genera of Russulales, there some characteristics such as the peridium morphology, presence of spherocysts, spore ornamentation or laticiferous hyphae which distinguish them separate them (Pegler and Young 1979; Lebel and Trappe 2000). *Gastrolactarius* was described with a wide geographical distribution, ocurring in America, Africa and Australasia. The genus is morphologically similar to *Arcangeliella* Cavara, however, they are separated by the presence of a more developed columella, wich is absent or reduced to a sterile base in the latter (Vidal 2004; Trappe et al. 2009). However, with the advent of molecular studies, these genera have changed their nomenclatural status based on their phylogenetic relations and all of the species previously classified within *Arcangeliella*, *Zelleromyces* Singer & A.H. Sm. and *Gastrolactarius*, currently belong to *Lactarius* (Miller et al. 2000; Verbeken et al. 2014; Vidal et al. 2019).

Lactarius denudatus is a noteworthy species characterized by the efused hymenium, short stipe and the saeta-like cystidia. This features strickingly differ from other sequestrate *Lactarius* in North America (Trappe et al. 2009; Guevara-Guerrero et al. 2014). Due to the secotioid form, it can resemble some *Russula* from Mexico such as *R. duranguensis* (Guzmán) Trappe & T.F. Elliott and *R. pineti* (Singer) Trappe & T.F. Elliott, but they differ mainly by the absence of latex and the well developed pileus (Guzmán 1988; Kong et al. 2008). Due to the exposed and anastomosed hymenia, the species resembles a species of the genus *Gymnopaxillus* E. Horak, nevertheless, this genus belongs to the order Boletales (Claridge et al. 2001).

Lactarius is a well-studied genus in Mexico due to its cultural importance as a food source (Pérez-Moreno et al. 2021) and its taxonomic diversity (Montoya and Bandala 1996, 2005). However, sequestrate members of *Lactarius* are poorly known in Mexico with a few taxa reported in the state of Veracruz (Calonge and Vidal et al. 2003) and Northeast Mexico, where there are generic records without identification at species level (Guevara-Guerrero et al. 2014). The gasterization process of basidiomycetes has been discussed by Thiers (1986) who considers that changes in temperature and humidity in areas with very marked temporalities favored the progression to a gasteroid state and an adaptation to disperse by zoochory (Beenken et al. 2016). However, other theories have been proposed for the evolution of these forms such as paedomorphosis and neoteny (Kuhar et al. 2023). The expansion of the geographic distribution of *L. denudatus*, contributes to the knowledge of sequestrate Russulales in Mexico and aims to encourage the study of the this scarcely studied group of fungi.

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