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New sequestrate *Russula* (Russulaceae, Basidiomycota) from a Mexican tropical lowland forest

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Abstract: A new sequestrate *Russula* from the Yucatán peninsula, Mexico, is presented. *Russula kongii* is characterized by the hypogeous basidiomata with whitish pileus, whitish hymenia that turns brown with age and globose to sub globose basidiospores of 7-13 µm, with partial amyloid reticulum. This new species is putatively associated with *Gymnopodium floribundum* in lowland forest.

Keywords: mycorrhizal fungi, Polygonaceae, truffles, truffle-like fungi, Yucatán peninsula.

Introduction

The family Rusulaceae Lotsy (Basidiomycota, Agaricomycotina) is characterized by large, fleshy pileate-stipitate basidiomata, usually with chalky context due to the presence of sphaerocytes, the presence of lactificerous hyphae and the basidiospores with amyloid ornamentation (Singer 1986). Nevertheless, its members may also include sequestrate taxa, which share microscopic features such as the spore ornamentation and tissue structure (Lebel and Trappe 2000). This family is composed of the genera *Lactarius* Pers., *Lactifluus* (Pers.) Roussel, *Russula* Pers. and *Multifurca* Buyck & V. Hofst (Looney et al. 2018). Most of its species form ectomycorrhizal associations with several members of Pinaceae, Fagaceae, Dypterocarpaceae and Polygonaceae (Pegler 1983). Some species are edible and appreciated in gourmet gastronomy and have high cultural importance in groups worldwide (Pérez-Moreno et al. 2021).

In classic taxonomy, the sequestrate species related to *Russula* were assigned to several genera such as *Cystangium* Singer & A.H. Sm, *Elasmomyces* Cavara, *Gymnomyces* Massee & Rodway, *Macowanites* Kalchbr., and *Martellia* Mattir. Nevertheless, molecular evidence placed those genera within *Russula* (Miller et al. 2001, 2006) and then were renamed or recombined by Elliot and Trappe (2018). The sequestrate members of *Russula* have been studied mainly in North America, Europe, and Australasia,

growing mainly in temperate forest in association with Angiosperms and Gymnosperms (Montechi and Sarassini, 2000). These species contribute to the nutrient circulation cycle within the forest being a food resource for mammals and invertebrates, also can translocate nutrients such as nitrogen and phosphorus to their host tree (Sulzbacher et al. 2016). To this day, several new species of sequestrate *Russula* have been described worldwide in both temperate and tropical ecosystems (Vidal et al. 2019; Lebel et al. 2022; Miller and Van T Cotter 2025).

The taxonomic arrangement of *Russula* and their relatives has changed over the years. Aside from the progress made with molecular techniques in recent years, an affinity between epigeous and sequestrate Russulaceae has always been recognized (Singer and Smith 1960). Some authors separated the species with hypogeous habits within the Family Elasmomycetace and the pileated ones in Russulaceae (Pegler and Young 1979). Other authors theorized about the phylogenetic relationships of *Macowanites*, *Gymnomyces*, and *Russula*, based on the presence of spherocytes in the sterile tissues and the lack of latex when cutting, and placed them within the same family Russulaceae (Castellano et al. 1989; Vidal et al. 2019). Subsequently, a more detailed diagnosis was made to be able to separate the different genera of sequestered Russulaceae (Lebel and Trappe 2000) in order to define the limits between these genera. Currently, with phylogenetic studies, all these sequestrate genera have been transferred to relative epigeous genera such as *Russula* and *Lactarius*, leading to many nomenclatural changes (Miller et al. 2001; Lebel and Tonkin 2007; Elliot and Trappe 2018; Lebel et al. 2022).

Few species of sequestrate Russulaceae have been studied in Mexico. The first record of a sequestrate russuloid was made by Singer, describing *Cystangium pineti* from Central Mexico (Singer et al. 1985). Later, Guzmán (1988) described two secotioid species, which he named *Macowanites mexicanus* and *M. durangensis,* from *Pinus* forests of northern Mexico. Calonge and Vidal (2005) described *Lactarius denudatus* from Veracruz, associated with *Pinus patula* and recently reported in other mexican states (de la Fuente et al. 2023). Kong et al. (2008) pointed out the similarities between *C. pineti* and *R. mexicanus* and concluded that both species belonged to the same taxon. García-Jiménez et al. (1998) and Guevara-Guerrero et al. (2014) made some notes on sequestrate Russulaceae from central and northeast Mexico but without deep taxonomic analyses. So far, three species of sequestrate Russulaceae are recognized from Mexico.

During mycological explorations in the Mexican state of Campeche, in the Yucatán peninsula, some sequestrate specimens were found and are described here as new species. The new species is characterized by the hypogeous basidiomata with whitish pileus, whitish hymenia turning brown with age and globose to sub globose basidiospores of 7-13 µm, with partial amyloid reticulum. This new species is putatively associated with *Gymnopodium floribundum* in lowland forest. Herein, morphological information, and a key for the Mexican sequestrate members of Russulaeae is presented.

Materials and methods

The study area is located in the municipality of Calakmul, within the Mexican state of Campeche, in the Yucatán Peninsula (Fig. 1). The vegetation of the studied area, a lowland forest, is represented by *Gymnopodium floribundum*, *Haematoxylon campechianum*, *Metopium brownei*, *Coccoloba diversifolia*, *Terminalia buceras* and *Sabal yapa*. Soils in this type of vegetation have poor drainage, hence they flood during the rainy season. Sampling of macrofungi was according to Castellano et al. (1989). Colour

description was made according to Kornerup and Wansher (1978). Temporal slides were made using 5% KOH and Melzer's reagent to observe and measure the peridium hyphae, basidia, and basidiospores. In addition, the following values were obtained: average length (L), average width (W), Q index (length/width), and total of observations (n). The studied material was deposited in the herbarium "José Castillo Tovar" of the Instituto Tecnológico de Ciudad Victoria (ITCV).

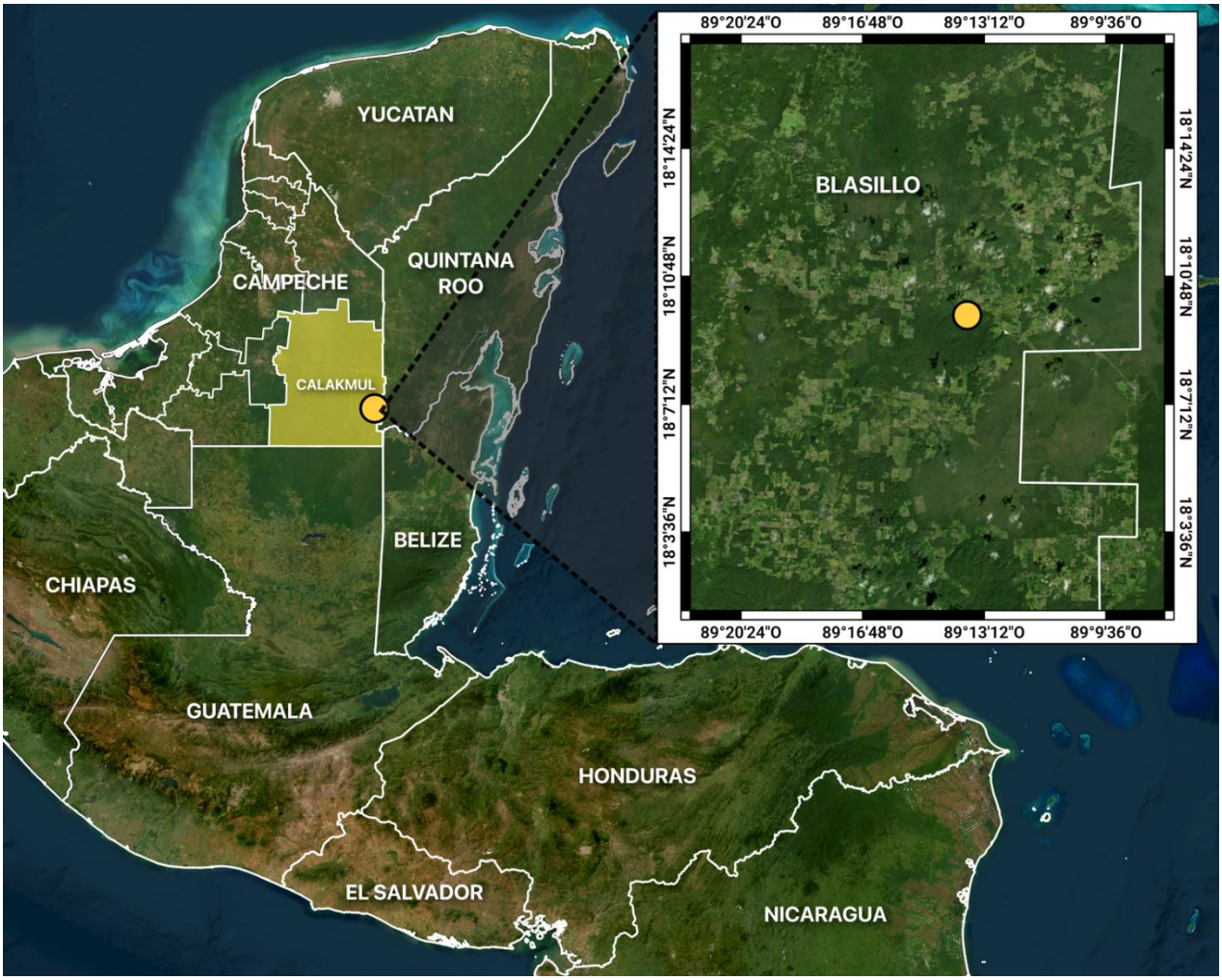

Figure 1. Study area. Type locality of *Russula kongii* is depicted in yellow.

Results

Russula kongii de la Fuente, Sánchez-Flores, Cruz-Campuzano & J. García, sp nov (Figs. 2 and 3).

Index Fungorum Identifier: 906075

Holotype: Mexico, Campeche, Calakmul municipality, Blasillo town, 18°09'42.22" N, 89°13'42.22" W, July 09 2018, Collected by J. I. de la Fuente, JIF-402 (ITCV).

Etymology: This species is dedicated to mycologist and ethnomycologist Alejandro Kong (Universidad Autónoma de Tlaxcala, Mexico), in recognition of his outstanding contributions to the study of the

Russulaceae, with particular emphasis on white and red *Russula* species. The epithet honors his more than 30 years of research, his commitment to advancing mycological knowledge, and his inspiring role in mentoring and training future generations of mycologists.

Diagnosis: *Russula kongii* differs from other species by the following combination of characteristics: small sub globose basidiomata up to 5 mm, whitish pileus, brownish hymenium when mature, globose to subglobose basidiospores of 7-13 µm and the habit under *Gymnopodium floribundum*.

Description: Basidiomata hypogeous, 3-5 mm, sub globose, sessile, pileus orange white (5A2) to orange gray (5B2), greyish orange in some areas (5B4), smooth to sligthly velvety (*sub lente*), absent near the base of the basidioma; hymenium sequestrate, with irregular locules, withish when young, brown (6E6, 7E6) when aged, without columella, sterile base nor pseudostipe; odor and taste indistinc. Pileipellis 45-70 µm wide, composed of strongly interwoven hyphae, with cystidia-like cells of 18-23 × 2-5 µm, erected, hyaline, thin-walled. Hymenophoral trama 10-25 µm wide, composed of strongly interwoven hyphae of 4-7 µm in diameter, hialine or gutulate, thin-walled. Cystidia absent. Basidia 21-30 × 5-10, clavate to cylindrical, hyaline or gutulate, 2 or 4-spored, with small sterigma up to 2 µm, thin-walled. Basidiospores globose to sub globose of 7-13 µm (L=10.79, W= 10.14, Q=10.07, n=30), hyaline in KOH %5, amyloid in Melzer's reagent, with crowded small verrucae of 0.5-1 µm and complete to incomplete reticulum, with hylar appendix short, upt to 2 µm, thick-walled.

Habitat: Scattered, hypogeous under *G. floribundum*, in lowland forest, so far only known from the type collection.

Figure 2. Basidiomata of *Russula kongii* (Holotype). Bar = 10 mm.

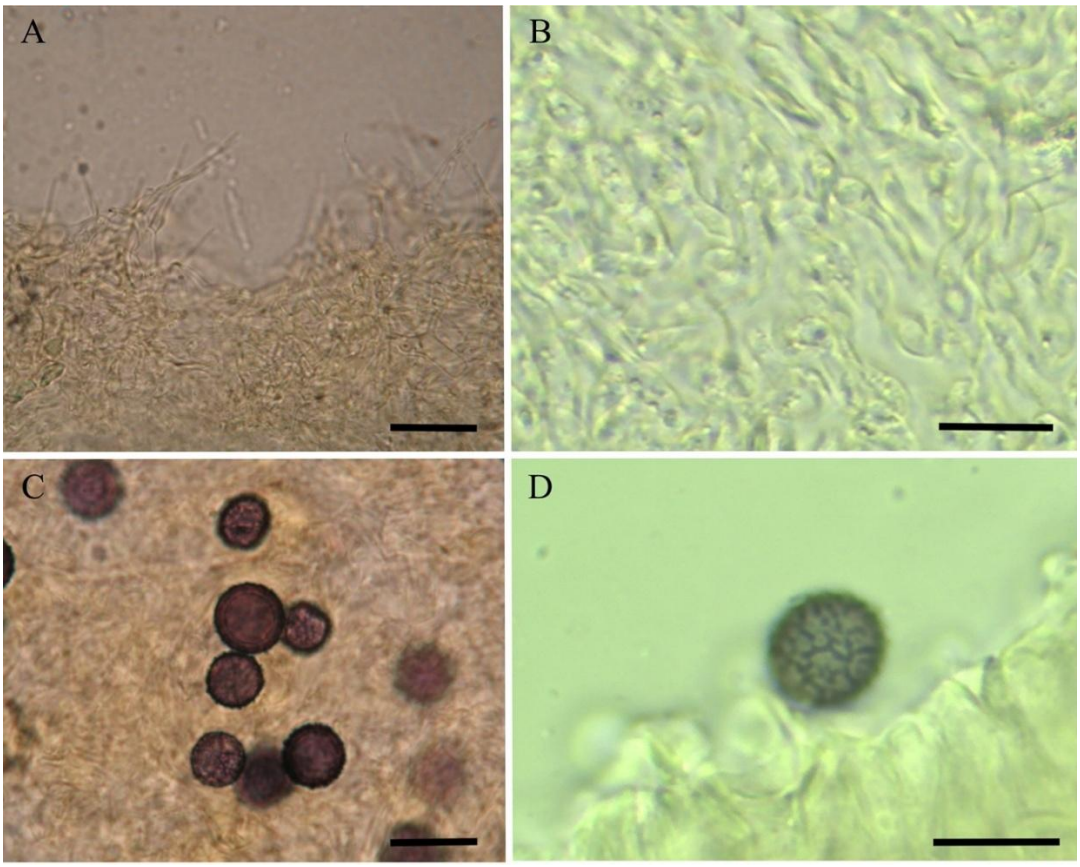

Figure 3. *Russula kongii*. A) Elements of the pileus. B) Detail of hymenophoral trama. C) Basidiospores. D) Details of the basidiospore ornamentation. Bar= 10 µm.

Discussion

Some basidiomata were found under *Gymnopodium floribundum* (Polygonaceae), a common shrub from the lowland forest. Several studies have confirmed the ectomicorrizal asociation of Polygonaceae with several members of Russulaceae (Pegler and Young 1979; Pegler 1983; Bullaín-Galardis et al. 2024) but as far a we know, this is the first sequestrate *Russula* described from Mexico, asociated with Polygonace members.

Sequestrate Russuloid fungi have been widely studied in the Americas, especially in the United States mainly by Singer and Smith (1960) and Zeller and Dodge (1919). Similarly, some species have been reported in South America, associated with *Nothofagus* forests (Sulzbacher et al. 2016). In the case of the temperate forests of Mexico, there are some species of both *Russula* and *Lactarius* described from coniferous forests and mixed pine and oak forests (de la Fuente et al. 2023). Furthermore, there are several undescribed species from the temperate forests of northern and central Mexico, which have not been formally described (García-Jiménez et al. 1998). So far there are no records of sequestrate Russulaceae in tropical forests, as occurs in South Asia and Australia, where species associated with tropical eucalyptus and dipterocarp forests are frequently described. However, more studies focused on tropical lowlands are required in order to broaden the knowledge of the mexican tropical sequestrate Russulaceae.

Russula kongii represents the first record of a sequestrate *Russula* in Mexican lowland tropical forests, associated with Polygonaceae. Most previous records of this fungus type have been from temperate or subtropical forests associated with Pinaceae, Betulaceae, and Fagaceae (Guevara-Guerrero et al. 2014). The hypogeous habit easily distinguishes this species from other *Russula* described for the Caribbean, and also associated with Polygonaceae, mainly with trees of the genus *Coccoloba* (Pegler 1983). It also differs from other sequestrate species such as *Russula mexicana* in that the latter has reddish caps and it is associated with species of *Pinus* in mixed forests of northern Mexico (Guzmán 1998). *Russula pineti* also exhibits sequestrate habits, but it also has pileus with reddish or pinkish tones, in addition to having cystidia, which are absent in *Russula kongii* (Kong et al. 2008).

Molecular data could not be obtained for this species due to the limited material available; however, it is proposed here as a new species based on morphological, microscopic, and ecological evidence. Although the members of Polygonaceae host a wide diversity of Russula species (Pegler et al. 1983), no other sequestrate Russulaceae species associated with *G. floribundum* exists in the Caribbean. This ecological association also distinguishes it from sequestrate species in North America, which are typically associated with conifers and broad-leaved trees, primarily *Quercus* (Trappe et al. 2009) and from South American species, which usually associate with *Nothofagus* (Treiveiller-Pereira et al. 2015, Sulzbacher et al. 2016). It is hoped that further sampling will yield additional material, allowing for the elucidation of this species' phylogenetic relationships within the Russulaceae.

The tropical forests of the Yucatán Peninsula are under anthropogenic pressure due to land-use changes, accelerating to the loss of many fungal species associated with these forests. In the case of *R. kongii*, this species has only been observed in the type locality in Blasillo, Campeche, in southeastern Mexico. Unfortunately, the collection site has been degraded by human disturbance related to agriculture, and no further specimens have been located since 2018. However, the distribution of *G. floribundum* is broad, encompassing southern Mexico and Central America (Ancona et al. 2019), so it is possible that this fungal species also has a wider distribution. Further mycological studies are recommended to obtain more information about this rare fungal species.

Key to the sequestrate Russulaceae from Mexico

1a. Basidioma secotioid, stipe visible……..2

1b. Basidioma globose, stipe absent…….……...…*Russula kongii*

2a. Hymenium anastomosed, exposed, with slender long cystidia……*Lactarius denudatus*

2b. Hymenium enclosed within the peridium, without long slender cystidia……3

3a. Pileus brown……..…..*Russula durangensis*

3b. Pileus pinkish, pale red……...*Russula pineti*

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