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New records of Brazilian hypogeous sequestrate fungi

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Abstract—Examination of specimens held in three Brazilian herbariums (UFRN, URM, ICN) for the genus *Rhizopogon* revealed that one collection represented *Alpova* cf. *austroalnicola,* a first record of the genus for Brazil. *Rhizopogon angustisepta* from South Brazil represents a new record for the Western Hemisphere; *R. verii* is a new record for Southeast and Northeast Brazil; *R. nigrescens* is tentatively reconfirmed from South Brazil, based on a poorly preserved specimen; and *R. marchii* is identified from a specimen with confused label information that does not indicate the country of origin.

Key words —*Basidiomycota, Boletales, Paxillaceae*, *Rhizopogonaceae,* taxonomy

Introduction

Species of *Rhizopogon* Fr., known as "false truffles," have hypogeous and semi-hypogeous habits. The globose to subglobose basidiomata possess a simple structure: an external sterile peridium that protects the interior gleba, containing fertile chambers. The species are ectomycorrhizal, preferably with members of *Pinaceae* (Trappe 1962, Martín 1996, Grubisha & al. 2002), and distributed worldwide (Kirk & al. 2008).

Rhizopogon can be distinguished from other hypogeous sequestrate fungi by its lacunar gleba without columella and its smooth elliptical spores (Martín 1996); however, the genus has few reliably diagnostic morphological characters. Smith & Zeller (1966), using morphological characteristics and chemical tests ($FeSO_4$, 5% KOH, and Melzer's reagent), described 121 new species for the genus. However, as discussed in Martín (1996) and Martín & al. (1998), chemical reactions together with peridium and gleba color generally distinguish only basidiome developmental stages and not distinctions between species. In general, morphological examination combined with molecular analyses is the preferable taxonomic approach for determining *Rhizopogon* species (Johannesson & Martin 1999). Smith & Zeller (1966) divided *Rhizopogon* into two subgenera: *R.* subg. *Rhizopogonella* A.H. Sm., based on whitish streaks observed in the gleba, and *R.* subg. *Rhizopogon*, based on the absence of these stretch marks. Trappe & Guzmán (1975), re-evaluating the species of *R.* subg. *Rhizopogonella,* regarded this subgenus as synonymous with *Alpova* C.W. Dodge, due to features of the hymenium and basidia.

Rhizopogon encompasses 150 species (Kirk & al. 2008). Although the genus is cosmopolitan, most species have been described from Pacific coastal coniferous forests of the United States of America (Grubisha & al. 2002) and on the European continent (Martín 1996). For Brazil, only eight species have been recorded, from the Southern and Southeastern regions and generally associated with introduced *Pinus* species: *R. fuscorubens* A.H. Sm., *R. luteolus* Fr., *R. nigrescens*, *R. roseolus* (Corda) Th. Fr., *R. rubescens* (Tul. & C. Tul.) Tul. & C. Tul., *R. verii*, *R. vulgaris* (Vittad.) M. Lange, and *R. zelleri* A.H. Sm. (Giachini & al. 2000, Baseia & Milanez 2002, Cortez & al. 2011, Sulzbacher & al. 2016). Index Fungorum (2018) and MycoBank (2018) list 26 species of *Alpova,* most described from the United States and Europe; they cite no records for *Alpova* in Brazil.

For this paper our intention was to increase the knowledge of *Alpova* (*Paxillaceae*) and *Rhizopogon* (*Rhizopogonaceae*) in Brazil by reviewing collections located in different Brazilian herbaria.

Materials & methods

Studied specimens are located in the herbaria of Universidade Federal do Rio Grande do Norte, Natal, Brazil (UFRN); Universidade Federal de Pernambuco, Recife, Brazil (URM); and Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil (ICN). Additional specimens from the Herbarium of Universidade Federal de Santa Catarina, Florianópolis, Brazil (FLOR) were lost in transit. Herbaria are abbreviated according to Thiers (2016). All descriptions are based on dried material.

Macroscopic studies follow methods in Martín (1996) and Baseia & al. (2014). We noted for each basidioma, the shape (globose, subglobose, and irregular), size, consistency and resistance of the mushroom to cutting, and the presence/absence of rhizomorphs. To determine color changes in the peridium and gleba, dried specimens were tested with standard reagents: 2.5% potassium hydroxide (KOH), 10% iron sulphate ($FeSO_4$), and Melzer's reagent (Largent & al. 1977). Colors were coded according to Kornerup & Wanscher (1978).

Microscopic observations followed Martín (1996); the four basic patterns of the peridium anatomy based on hyphal arrangement observed in *Rhizopogon* are: *luteolus*-type (hyphal packages arranged in different directions); *corsicus*-type (hyphal packages arranged in two different directions, intermixed or not with globose cells); *abietis*-type (hyphae forming a loosely interwoven weft); and *roseolus*-type (hyphae running parallel to the surface). We also recorded the presence of pigments and oleiferous hyphae in the peridium and gleba; size of the trama plates and diameter and arrangement of trama plate hyphae; subhymenium height; size and shape of basidioles, brachybasidioles, and basidia; and basidiospore size and shape.

All tissues were prepared with the aid of a Leica stereoscopic microscope and microstructures observed in a Nikon EclipsiNi optical microscope. The taxonomic concepts were based on specialized *Rhizopogon* literature and articles.

Table 1. *Alpova* and *Rhizopogon* spp. identified in this study

Species	Herbarium identification	Occurrence record
A. austroalnicola	*R. luteolus* (UFRN-Fungos 144) *R. roseolus* (URM 10733)	First record for Brazil
R. angustisepta	*R. roseolus* (ICN 154594; 168975) *R. provincialis* (URM 9403)	First record for Western Hemisphere
R. marchii	*R. briardii* (URM 9468)	No location data
R. cf. *nigrescens*	*R.* sp. (UFRN-Fungos 2787)	Tentative second record for South Brazil
R. verii	*R. luteolus* (UFRN-Fungos 380; 1853) *R. roseolus* (UFRN-Fungos 2786)	First records for SE & NE Brazil

Results & discussion

One *Alpova* and four *Rhizopogon* species were identified and described (Tab. 1). *Alpova* cf. *austroalnicola* represents the first record of *Alpova* for Brazil, while *Rhizopogon angustisepta* represents the first record from the Western Hemisphere and *R. verii* the first record for Southeast and Northeast Brazil. *Rhizopogon nigrescens* is tentatively reconfirmed from South Brazil, based on a poorly preserved specimen (labeled as *R.* cf. *nigrescens*). *Rhizopogon marchii* is identified from a specimen with confused label information that does not indicate the country of origin.

Taxonomy

Alpova* cf. *austroalnicola L.S Domínguez, Mycologia 97: 599, 2005 Fig. 1A–C

Basidiomata subglobose to irregular, 1.4–2.7 × 0.9–2 cm. Peridium clay (5D5) to dark brown (6F5). Rhizomorphs covering all basidiomata, but some basidiomata are not covered by rhizomorphs on the upper surface. Gleba dark brown (6F5) and firm in dried material.

Macroscopic chemical reactions—No color changes observed for dried peridium or gleba in KOH, $FeSO_4$, and Melzer's.

Peridium ≤395 µm high, hyphae <4.5 µm diam., septate; in young material the peridium hyphal packets are arranged in different positions (*luteolus*-type), in mature specimens, forming a loosely interwoven weft similar to the *abietis*-type peridium. Trama plates <66 µm, hyphae 4–5.1 µm diam., hyaline, intertwined, refractive; some globose and subglobose cells between tramal plate hyphae, 14.8–24.7 × 15–22.3 µm. Spores 6–7.8 × 2.5–3.2 µm, hyaline and ellipsoidal.

> Material examined—**BRAZIL**. **Pernambuco**: **Cabo Santo Agostinho**, Gurjaú Ecological Reserve, 24 March 2013, I.G. Baseia (UFRN–Fungos 144; as *R. luteolus*). **URUGUAY**. **Montevideo**: 15 May 1957, A.C. Batista & J.E Wright (URM 10733; as *R. roseolus*).

Comments—The blackened gleba in the dried collections (Fig. 1A) is characteristic for *Alpova*. Nouhra & al. (2005) described *A. austroalnicola* from Argentina associated with *Alnus.* The Argentina species and our specimens shared similar trama plate and subhymenium cells, such as the globose cells in the gleba (Fig. 1B) and the basidiospore size and shape (Fig. 1C). However, the abundant dermocystidia noted for *A. austroalnicola* were not observed in our specimens. Furthermore, *A. austroalnicola* associates exclusively with *Alnus,* not found in Brazil, while our Brazilian specimen was collected in the native Brazilian Atlantic rainforest. There is

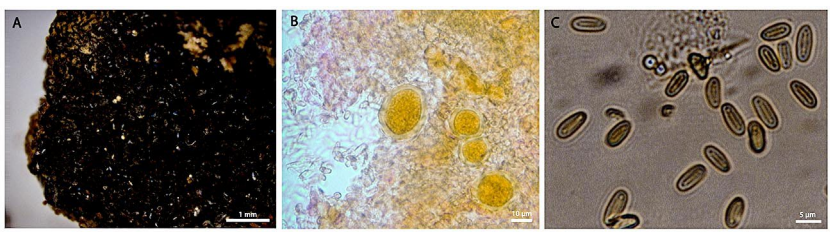

Fig. 1. *Alpova* cf. *austroalnicola* (UFRN–Fungos 144 as *R. luteolus*). A. Transverse section showing the peridium and gleba. B. globose and subglobose cells between trama plates. C. Spores.

no reference to the plant associate for the Uruguayan specimen. Our report represents the first records of *Alpova* for Brazil and Uruguay.

Rhizopogon angustisepta Zeller & C.W. Dodge,
Ann. Missouri Bot. Gard.5: 24, 1918 Fig. 2A–C

Basidiomata subglobose to irregular 0.9–2 × 0.7–1.5 cm. Rigid fruiting body but crumbling away when cut. Peridium dark brown (7F4) to reddish brown (6F5). Scarce rhizomorphs. Gleba brownish brown (7E4).

Macroscopic chemical reactions—No color changes observed for dried peridium or gleba in KOH, $FeSO_4$, and Melzer's.

Peridium *roseolus*-type, <300 µm high, hyphae brownish (Congo red), 7.7 µm (some reaching 12.6 µm) diam., arranged parallel to surface. Trama plates <46 µm broad, formed by interwoven hyphae, hyaline, refractive, branched and <5 µm diam. Subhymenium <28.1 µm diameter formed by subglobose (17.1–8.6 µm) and elongated (12.7–19.6 × 6-8.4 µm) cells. Brachybasidioles clavate, 17.9–22.9 × 7–7.2, wall <2.2 µm diameter. Basidia cylindrical, 4.5–14.2 × 5.6–7.3 µm, thin walled (<0.5 µm); Spores 7.0–10.5 × 3–4.5 µm, naviculate, biguttulate and with a basal scar.

Material examined–**BRAZIL**. **Rio Grande do Sul**: **Guaíba**, Fazenda São Maximiano, 21 August 2010, L. Trierveiler-Pereira 93, under *Pinus* sp. (ICN 168975; as *R. roseolus*); **Itaara**, Pinhal Park, 21 April 2007, V.G. Cortez 069/07, under *Pinus* sp. (ICN 154594; as *R. roseolus*). **PORTUGAL**. **Setúbal**: 3 December 1957, C. Torrend (URM 9403; as *R. provincialis*).

Comments—In *Rhizopogon angustisepta* neither the dried peridium nor the dried gleba changed color when tested with 5% KOH (Fig. 2A, B), whereas the dried peridium of *R. roseolus* changes to a reddish color. *Rhizopogon angustisepta* is characterized by naviculate spores (Fig. 2C). This species was known only from the type locality, Tilsit, East Prussia, Germany (now Sovetsk, Kaliningrad Oblast, Russian Federation), as indicated by Zeller & Dodge (1918), the only work on *Rhizopogon* included in GBIS (2016). Our newly identified specimens extend the distribution of *R. angustisepta* to western Europe, besides serving as the first record for to the Western Hemisphere.

Rhizopogon marchii (Bres.) Zeller & C.W. Dodge,
Ann. Missouri Bot. Gard. 16: 121, 1929 Fig. 2D –F

Basidiomata subglobose to irregular, 11.5–13.1 mm. Peridium brown leather (6E6), occasional blackish rhizomorphs present. Gleba orange gray (5B4) to yellowish brown (5E8), crumbly, irregularly chambered.

Macroscopic chemical reactions—No color changes observed for dried peridium or gleba in KOH, $FeSO_4$, and Melzer's.

Peridium *abietis*-type, <290 µm high. Trama plates <83 µm broad, hyphae septate, branched, hyaline, refractive, <4.5 µm diam. Subhymenium <33.8 µm diam.; cells 15–22 × 6.2–8 µm. Brachybasidioles clavate, 12–16 ×7–6.5 µm. Basidia cylindrical 27.6–41.1 × 5.6–6.9 µm. Spores 6.4–9.2 × 3.5–5 µm, smooth, hyaline, elongated.

Material examined–No location, 3 December 1957, J. Bresadola (URM 9468; as *R. briardii*).

Comments—The basidiospores (Fig. 2F), subhymenial elements, and peridium type (Fig. 2E) support our identification of *R. marchii* for this collection, even though the extremely thin peridial layer (Fig. 2D) and the material conservation status made thorough microscopical observation difficult. The collection label provides very little reliable information: there are no location data, and 1957 is an unlikely collection date for the Italian collector(?), Bresadola (1847–1929). If this was collected by Bresadola, it is probably a European specimen, and the 1957 date may refer to its deposition in herb. URM. It seems more likely that the name of the collector is missing and that Bresadola's name was written (wrongly) as the authority of *R briardii* Boud. We contacted URM curators to learn more about the origin of this material, but no new information was available.

Rhizopogon* cf. *nigrescens Coker & Couch, Gasteromycetes East. U.S: 30, 1928 Fig. 2G –I

Basidiomata irregular, 2.1–1.6 cm diam. Peridium grayish brown (9E2-8F2). Gleba brown (6E4), irregular, labyrinthiform, firm, resistant to cutting.

Macroscopic chemical reactions—No color changes observed for dried peridium or gleba in KOH, $FeSO_4$, and Melzer's.

Peridium <480 µm high, hyphae darkened, <3 µm diam. Trama plates <45 µm broad, hyphae hyaline, <3.5 µm diam. Subhymenium <30 µm diam., cells subglobose, 11–15.1 × 6.8–9.7 µm. Spores 7.7–8.4 × 3–5.2 µm with truncate bases, biguttulate.

Material examined—**BRAZIL. Rio Grande do Sul: Dom Pedrito,** 10 May 2009, M.A. Sulzbacher (UFRN-Fungos 2787; as *Rhizopogon* sp.).

Comments—Material not preserved in good condition so that we were unable to determine the peridium type (Fig. 2G); only the trama plates

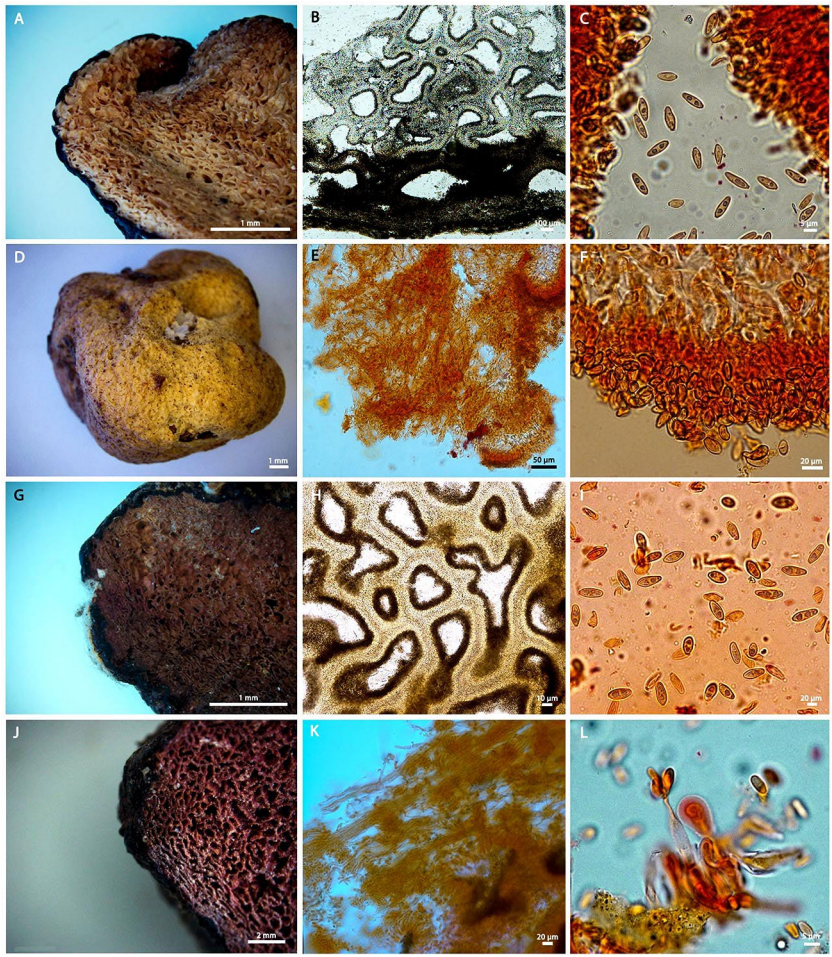

Fig. 2. Basidiomata and micromorphology of *Rhizopogon* species. ***R. angustisepta*** (URM 9403): A, B. Transverse section showing the peridium (darker region) and gleba (brighter region); C. Spores; ***R. marchii*** (URM 9468): D. Basidiomata; E. Peridium of *abietis*-type; F. Spores; ***R* cf. *nigrescens*** (UFRN-Fungos 2787): G. Transverse section showing the peridium and gleba; H. Trama plates; I. Spores; ***R. verii*** (UFRN-Fungos 380): J. Transverse section showing the peridium and gleba; K. Peridium of *luteolus*-type; L. Lageniform basidia.

and spores were well observed (Fig. 2H, I). Giachini & al. (2000) recorded *R. nigrescens* from South Brazil, but the material requested from herb. FLOR was lost in transit.

Rhizopogon verii Pacioni, Bull. Soc. Myc. Fr. 100: 119, 1984 Fig. 2J–L

Basidiomata subglobose to irregular 1–1.6 cm diameter. Peridium with fibrous brown surface (6E4), black at the base. Rhizomorphs brown (6E4) covering the entire surface of the peridium; under magnifying glass the wine-colored spots can be easily seen. Gleba firm when dry, bone hard, labyrinthiform, brown coloring (6F4) to grayish brown (6E3).

Macroscopic chemical reactions—No color changes observed for dried peridium or gleba in KOH, $FeSO_4$, and Melzer's

Peridium *luteolus*-type, <337 µm high, hyphae brownish in 5% KOH, prostrate, interwoven, 4–6 µm diam.; some hyphae had brown pigments, and were arranged in packets that then become loose. Trama plates hyphae interwoven, 2–5.1 µm diam., septate, branched. Subhymenium <20–23 µm broad, cells globose to subglobose, 12.9–17.8 × 7.5–11.2 µm. Brachybasidioles clavate, 6–32 × 3–4.5 µm, thick-walled (<2.5 µm thick). Basidia lageniform, thick-walled (<1.5 µm thick); base ventricose, 17.8–24.7 × 4.2–5.6 µm; beak thin-walled (<0.5 µm thick), 5–8.6 × 2–3 µm; 4–8 spores developing in the apical portion. Spores 5.7–8.4(–9.2) × 3.0–3.9 µm, ellipsoid, without visible guttule; some with a scar at the base.

Material examined–**BRAZIL. São Paulo:** Instituto de Botânica, 24 April 1999, I.G. Baseia (UFRN-Fungos 380; as *R. luteolus*); **Rio Grande do Sul**: **Santa Maria**, Centro de Educação Física, 21 May 2009, M.A. Sulzbacher (UFRN-Fungos 2786; as *R. roseolus*); **Ceará**: **Tianguá**, APA de Ibiapaba, 20 April 2012, D.S. Alfredo & B.T. Goto (UFRN-Fungos 1853; as *R. luteolus*).

Comments—*Rhizopogon verii* is very close to *R. luteolus* but can be distinguished by the type of basidium: lageniform in *R. verii* (Fig. 2L) vs. cylindrical in *R. luteolus*. Pacioni (1984) considered *R. verii* close to *R. angustisepta;* however, in *R. angustisepta* the peridium is *roseolus*-type, and the spores are naviculate, while in *R. verii* the peridium is *luteolus*-type (Fig. 2J, K) and the spores are oblong-ellipsoid with a truncate base.

Rhizopogon verii was described from Tunisia (Pacioni 1984) and subsequently reported from Italy and Spain (Martín 1996). Sulzbacher & al. (2016) reported the first record for the Western Hemisphere from Rio Grande do Sul State, South Brazil. Our newly identified specimens extend the Brazilian distribution to São Paulo (Southeast Region) and Ceará (Northeast Region).

Discussion

All collections examined were initially misidentified, mainly as *R. luteolus* and *R. roseolus*. This is understandable because for a long time *Rhizopogon*

species were identified based only on color changes in the peridium: absence of change identified *R. luteolus*, while a change to red identified *R. roseolus*. However, as indicated in Martín (1996), other characteristics such as the arrangement of the peridial hyphae and the basidium type can help to distinguish species, even dried specimens in herbaria. Until now, it has not been not possible for us to confirm the presence of *R. luteous* or *R. roseolus* in Brazil, since all the material examined corresponds to other species.

If confirmed, *Alpova* cf. *austroalnicola* (also in *Boletales* but in a different family, *Paxillaceae*) was also initially labeled as representing *R. roseolus* or *R. luteolus*. Even in dried specimens, however, the gleba is a good diagnostic character to separate genera.

The importance of accurate herbarium labels became apparent when studying the specimen of *R marchii*, since it was not possible to confirm the occurrence of this species in Brazil.

Our research has made it possible to expand the knowledge of taxonomy for *Alpova* and *Rhizopogon* as well as species distributions. However, many habitats have not yet been explored for hypogeous fungi, and new fieldwork will obviously further amplify the knowledge surrounding the taxonomic richness and species distribution of this group.

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