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Notes and Comments

Stinkorns fungi in Central Brazil: expanding the geographic distribution of phalloid species (Phallales, Basidiomycota)

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Phalloid fungi (Phallales, Phallomycetidae), commonly defined as such, are characterized by the presence of the hypogeous or epigeous immature basidiomata, thick white rhizomorphs; mature basidiomata epigeous or partially hypogeous; receptacle carrying the mucilaginous to gelatinous, green olive to brown gleba; the gleba has a foul odor, which allows the dispersion of spores by insects (Tuno, 1998; Cortez et al., 2011; Sandoval-Leiva et al., 2014; Melanda et al., 2021). This group accommodates ecologically, and morphologically variable species distributed in the families Clathraceae, Claustulaceae, Gastrosporiaceae, Lysuraceae, Phallaceae, Protophallaceae and Trappeaceae (Hosaka et al., 2006; Melanda et al., 2021), which are widely geographically distributed, mainly in the tropics. In Brazil, approximately 51 species of phalloid fungi are known (Lima et al., 2019), however, the Midwest region is poorly studied and there is no reported species for the state of Goiás. Thus, we present the first record of the *Lysurus cruciatus* (Lepr. & Mont.) Henn for the Midwest region and *Phallus indusiatus* Vent. for the state of Goiás, Brazil.

The records reported here came from several collections in different environments, from urban areas to conservation units in the Cerrado biome (Table 1). The identification of species was carried out from the morphological analysis, in addition to consultations in the referenced literature, such as Miller and Miller (1988), Cortez et al. (2011) and Cabral et al. (2019), and phylogenetic analyses. Microscopic examinations and measurements were performed using an OLYMPCUS CX31 optical microscope, from the preparation of slides with the addition of 3% KOH, from which the micrographs presented here were also obtained. Color classification was based on Kornerup and Wanscher (1978). Basidiospores were measured using the Piximètre version 5.10 R 1541 (Henriot and Cheype, 2017) and the following abbreviations were used: Q = quotient between length and width, Qm = medium value of Q and, N = number of measured structures. The specimens were herborized and incorporated into the mycological collection of the HUEG Herbarium of the Universidade Estadual de Goiás.

Total DNA was extracted from small pieces of dried basidiomata ground with liquid nitrogen according to method CTAB (Goés-Neto et al., 2005; Hosaka, 2009). Unfortunately, due to both species having ephemeral basidiomata difficult to preserve, only specimen HUEG 14149 of *P. indusiatus* had quality DNA obtained. For this sample, the nuclear rDNA internal transcribed spacers (ITS) and nuclear rDNA large subunit (LSU) were amplified with primer pairs ITS5/ITS4 (White et al., 1990) and LR0R/LR5 (Vilgalys and Hester, 1990), respectively. The PCR products were purified and sequenced in Macrogen Inc., (Seoul, South Korea), with the same primers as in their amplifications. The same sequences used in the phylogenetic analysis of Cabral et al. (2019), were selected from GenBank (Table 2), and along with our sequences, under accession number MZ964946 (ITS) and MZ965048 (LSU), aligned in MAFFT v.7 (Katoh and Standley, 2013), and then manually inspected using MEGA v.6 (Tamura et al., 2013). Maximum likelihood (ML) and Baysian inference (IB) phylogenetic analyses were performed in W-IQ-TREE (Kalayaanamoorthy et al., 2017) and MrBayes 3.2 (Ronquist and Huelsenbeck, 2003), respectively. ML was determined with branch support (BS) inferred by 1000 bootstrap replications and Ultrafast bootstrap (UB), while IB was performed with 10 million generations, with convergence verified in TRACER v1.7.1 (Rambaut et al., 2018).

Lysurus cruciatus (Figure 1A-D) belongs to the Lysuraceae family and has cosmopolitan geographic distribution (Assyov and Gashtarov, 2007; Abrar et al., 2012; Sandoval-Leiva et al., 2014); in Brazil, it is reported in Paraná (Meijer, 2006) and Rio Grande do Sul (Cortez et al., 2011; Trierveiler-Pereira et al., 2018). The specimen examined has a basidioma 5-6 cm high, formed by a pseudostipe and a basal volva, pileus (fertile portion of receptacle) formed by arms presenting a viscid gleba. Fertile portion of receptacle with 3-4 light orange arms (5A4), measuring 2-3 cm long, united when young separating at maturity, inner face convex, showing striations to pronounced roughness in the center, light brown color when young; outer surface concave, smooth, dark reddish brown, with creamy white to light brown tips. Gleba viscous, dark brown (6E6), with unpleasant odor. Pseudostipe 4-5 × 1-2 cm, cylindrical or slightly sub-cylindrical, with a white, hollow, spongy surface. Volva present at base of pseudostipe, 2-2 × 1.6-3 cm, oval, hollow, white, basal rhizomorphs present. Basidiospores ellipsoid, hyaline, smooth, thin-walled,

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Table 1. Information on the collection area of the sampled phalloid fungi.

Species	Voucher	Collection date	Locality		
	HUEG		Latitude	Longitude	Area
Lysurus cruciatus	12990	12/10/2015	16°21′18″S	48°54′43″W	Urban area, Anápolis, Goiás, Brazil
Phallus indusiatus	11864	12/15/2015	16°23′40″S	37°57′32″W	REC/UEG
	12057	04/05/2019			
	11861	01/21/2019	16°38′0.7″S	48°39′14″W	FLONA
	14149	11/02/2020	16°20′5.34″S	48°56′15″W	Urban area, Anápolis, Goiás, Brazil
	10753	12/15/2020	13°41′27″S	46°18′48″W	PETER

PETER = Parque Estadual de Terra Ronca (São Domingos, Goiás, Brazil); FLONA = Floresta Nacional de Silvânia (Silvânia, Goiás, Brazil); REC/UEG = Reserva Ecológica da Universidade Estadual de Goiás (Anápolis, Goiás, Brazil).

Table 2. Species, origin, vouchers and GenBank accession numbers of sequences used in phylogenetic analyses. The sequences obtained in this study are marked in bold.

Taxa	Origin	Voucher	GenBank accession no.		Reference
			ITS	LSU	
Itajahya galericulata	India	KSRF 0014	MF506819	MH168327	GenBank
I. galericulata	South Africa	Sporocarp 3	-	KR071852	Marincowitz et al. (2015)
I. rosea	Brazil	UFRN Fungos 1001	MG678532	-	Cabral et al. (2019)
I. rosea	India	IT	MN135577	MN134400	GenBank
Mutinus sp.	-	KH TBG11 449	MF447820	-	GenBank
M. sp.	-	KH NC11 143	MF447824	KF783247	GenBank
Phallus atrovolvatus	Australia	MEL 2382787	KP012967	-	GenBank
P. atrovolvatus	Brazil	INPA Fungos 240016	MG678531	MG678470	Cabral et al. (2019)
P. cinnabarinus	Brazil	INPA Fungos 255835	KJ764821	MG678471	Cabral et al. (2019)
P. cinnabarinus	Brazil	INPA Fungos 255836	MG678533	MG678472	Cabral et al. (2019)
P. denigricans	Brazil	INPA Fungos 272382	MG678482	-	Cabral et al. (2019)
P. denigricans	Brazil	UFRN Fungos 2805	MG678485	MG678454	Cabral et al. (2019)
P. denigricans	Brazil	INPA Fungos 272383	MG678486	MG678455	Cabral et al. (2019)
P. indusiatus	Brazil	INPA Fungos 264929	MG678498	MG678461	Cabral et al. (2019)
P. indusiatus	Brazil	INPA Fungos 264930	MG678499	MG678462	Cabral et al. (2019)
P. indusiatus	Brazil	INPA Fungos 264931	MG678500	MG678463	Cabral et al. (2019)
P. indusiatus	Brazil	SP 416387	MG678503	-	Cabral et al. (2019)
P. indusiatus	Brazil	SP 416389	MG678505	-	Cabral et al. (2019)
P. indusiatus	Brazil	SP 416393	MG678507	MG678464	Cabral et al. (2019)
P. indusiatus	**Brazil**	**HUEG 14149**	**MZ964946**	**MZ965048**	**This study**
P. purpurascens	Brazil	UFRN Fungos 2808	MG678487	MG678456	Cabral et al. (2019)
P. purpurascens	Brazil	SINOP 28	MG678495	MG678459	Cabral et al. (2019)
P. purpurascens	Brazil	SINOP 30	MG678496	MG678460	Cabral et al. (2019)
P. squamulosus	Brazil	UFRN Fungos 2806	MG678497	-	Cabral et al. (2019)
Xylophallus clavatus	-	USJ 28095	-	MH020744	Crous et al. (2018)
X. xylogenus	-	CJL 12021408	-	KF783252	Trierveiler-Pereira et al. (2014)

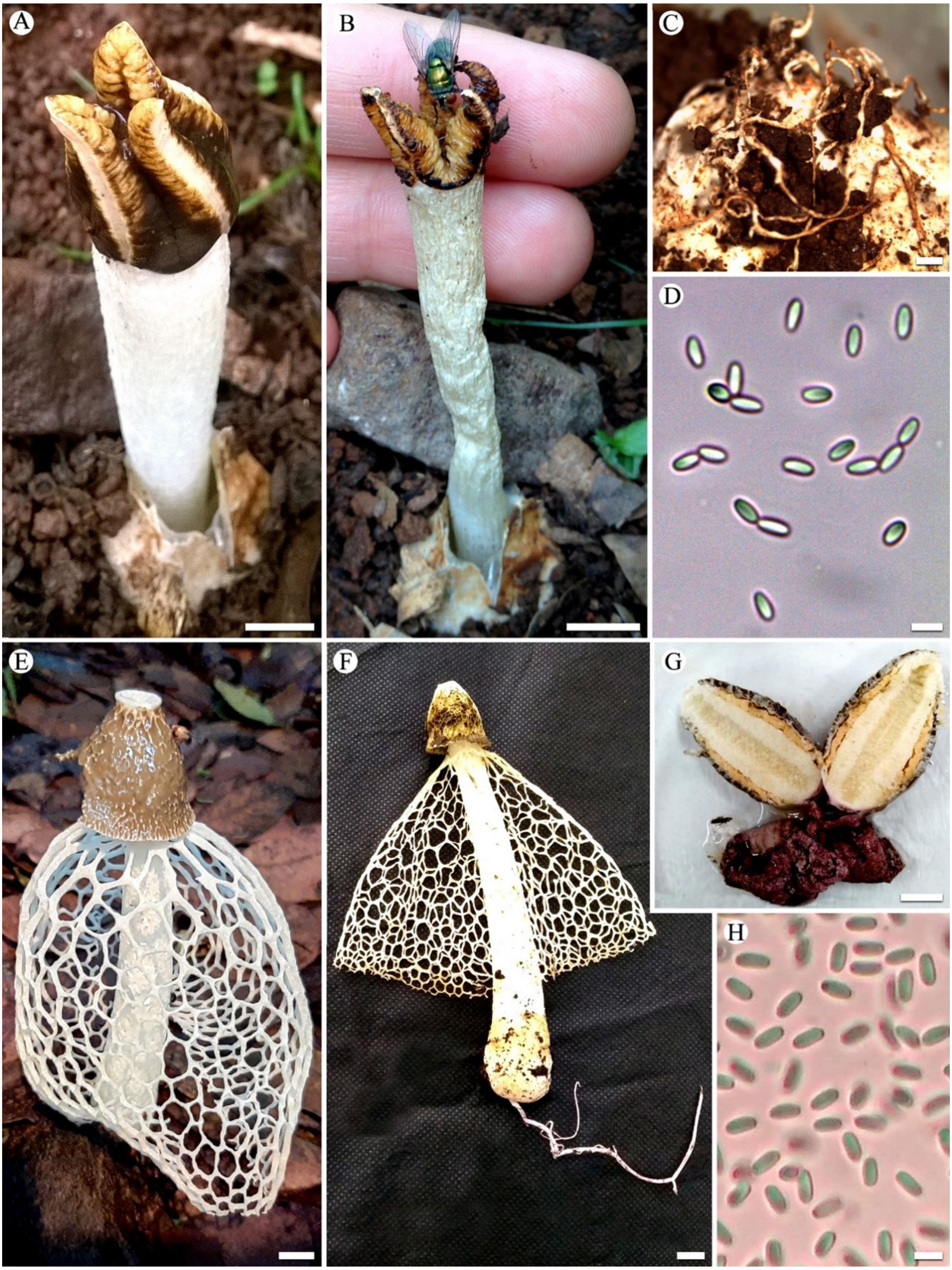

Figure 1. *Lysurus cruciatus*. (A, B) Mature Basidioma; (C) Rhizomorphs; (D) Basidiospores. *Phallus indusiatus*; (E, F) Mature basidioma; (G) Immature basidioma; (H) Basidiospores. Bars = 1 cm (A, B, E-G); 1 mm (C); 3 um (D, H).

(3) 3.1 - 3.7 (3.9) × (1.3) 1.5 - 1.9 (2.2) µm [Q = (1.5) 1.8 - 2.3 (2.5), Qm = 2, N = 50].

Phallus indusiatus (Figure 1E-H) is a member of the Phallaceae family, presenting geographic distribution restricted to South America (Cabral et al., 2019). For Brazil, it is reported in the States of Amazonas, Ceará, Espírito Santo, Mato Grosso do Sul, Pará, Paraíba, Paraná, Rio de Janeiro, Rio Grande do Norte, Rio Grande do Sul, Rondônia,

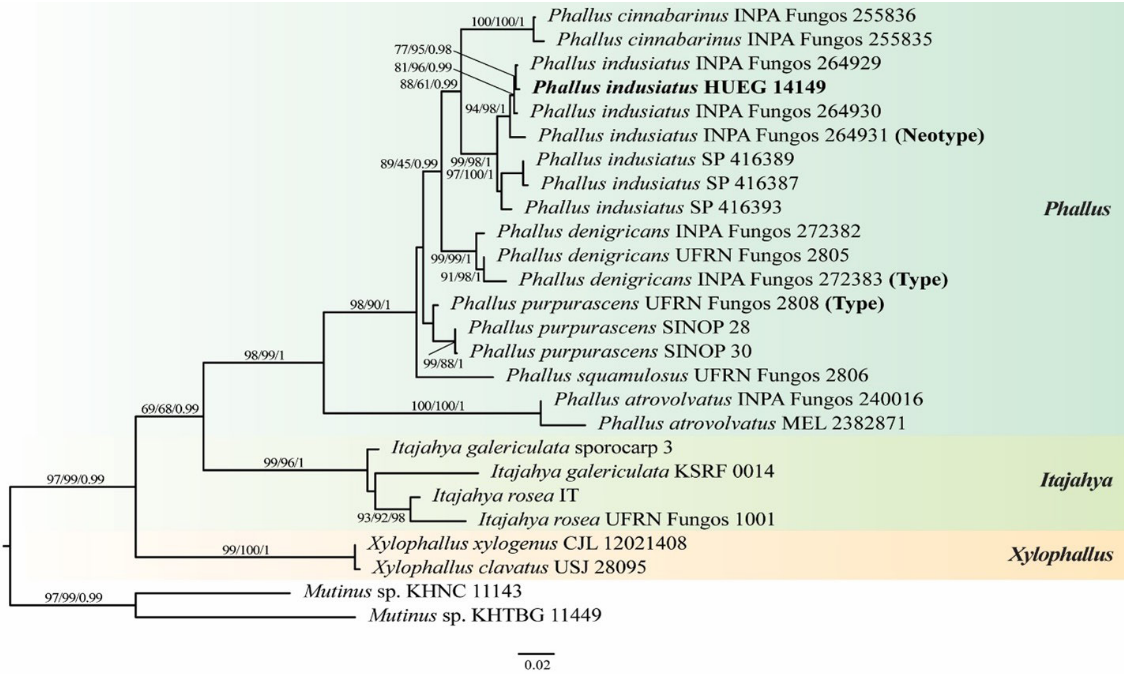

Figure 2. Phylogenetic tree obtained using ITS + LSU. The tree topology is from ML analysis and the species of *Phallus indusiatus* recorded in this study is indicated in bold. Numbers at branches indicate the values of bootstrap frequency (BS)/ultrafast bootstrap (UB)/bayesian posterior probability (PP).

Santa Catarina and São Paulo (Trierveiler-Pereira and Baseia, 2009, 2011; Trierveiler-Pereira et al., 2009; Magnago et al., 2013; Cabral et al., 2014; Bononi et al., 2017; Lima et al., 2019; Santana et al., 2019).

The specimens observed presented mature expanded basidiomata 120 mm high. Pseudostipe 95 mm long, cylindrical, spongy, white (1A1). Receptacle 25 mm in diam., campanulate, reticulated surface, with a well-defined apical pore. Indusium extending from the base of the receptacle to the ground, polygonal, white (1A1). Volva hypogeous and fragile. Rhizomorphs resistant, slightly pinkish (11C4). Gleba mucilaginous, foul-smelling, grayish green to olive (1D5-1E4). Basidiospores elongated, smooth, hyaline, (3.1) 3.6 - 4.2 (4.4) × (1.6) 1.7 - 2.1 (2.3) µm [Q = (1.6) 1.8 - 2.3 (2.5), Qm = 2, N = 50].

The phylogenetic tree (Figure 2) based using sequences from 26 specimens formed 3 clades, with the Phallus clade being strongly supported (BS = 98%, UB = 99%, PP = 1). Our species of *P. indusiatus* formed a strong group with other specimens from Brazil, including the neotype of the species (BS = 94%, UB = 98%, PP = 1).

The basidioma of *L. cruciatus* was found early in the morning in a residential garden after a period of continuous rain. The basidioma remained fresh and attracting flies until early afternoon, when it began to show signs of dehydration, and was then collected. We also observed flies in the receptacle of *L. cruciatus* and *P. indusiatus* (Figure 1B and E, respectively), due to the intense odor of putrefying meat exhaled by the gleba, which attracts insects that disperse their spores (Tuno, 1998).

Cabral et al. (2019) concluded that the worldwide distribution of *P. indusiatus* may be mistaken, and suggest that the species is restricted to South America. Besides that, due to the high plasticity of the diagnostic characters of the genus *Phallus*, several species were wrongly identified as *P. indusiatus* in Brazil. The authors also neotyped *P. indusiatus* (due to lack of holotype), and according to the description of the neotype, and by our specimen identified by phylogenetic analysis, the other reported samples show remarkable morphological characteristics of this species, such as indusium extending to the soil, presence of pinkish rhizomorphs, and campanulate receptacle.

Our results show the first occurrence of *P. indusiatus* for the state of Goiás, and the first record of *L. cruciatus* for the Midwest region of Brazil, in addition to expanding the knowledge of the distribution of phalloid fungi in the country, contributing to filling the gaps in knowledge of mycological diversity in these areas.

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