



New records of coprophilous ascomycetes (Fungi: Ascomycota) from Brazil and Neotropical Region

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Abstract: This study presents the first records from three genera *Hypocopa*, *Pyxidiophora*, *Zopfiella* and the second of the rare species, *Cercophora coronata* (Cailleux) Udagawa and T. Muroi, for Brazil. It also presents the first record of *H. stercoraria* (Sowerby) Sacc., *P. arvernensis* (Breton and Faurel) N. Lundq. and *Z. latipes* (N. Lundq.) Malloch and Cain for the Neotropical region. Specimens were obtained from dung collected in areas of the Brazilian Savannah cultured in moist chambers. Distribution data, taxonomic descriptions and new substrate records for are presented.

Key words: Cerrado; dung fungi; fungal diversity; Neotropics

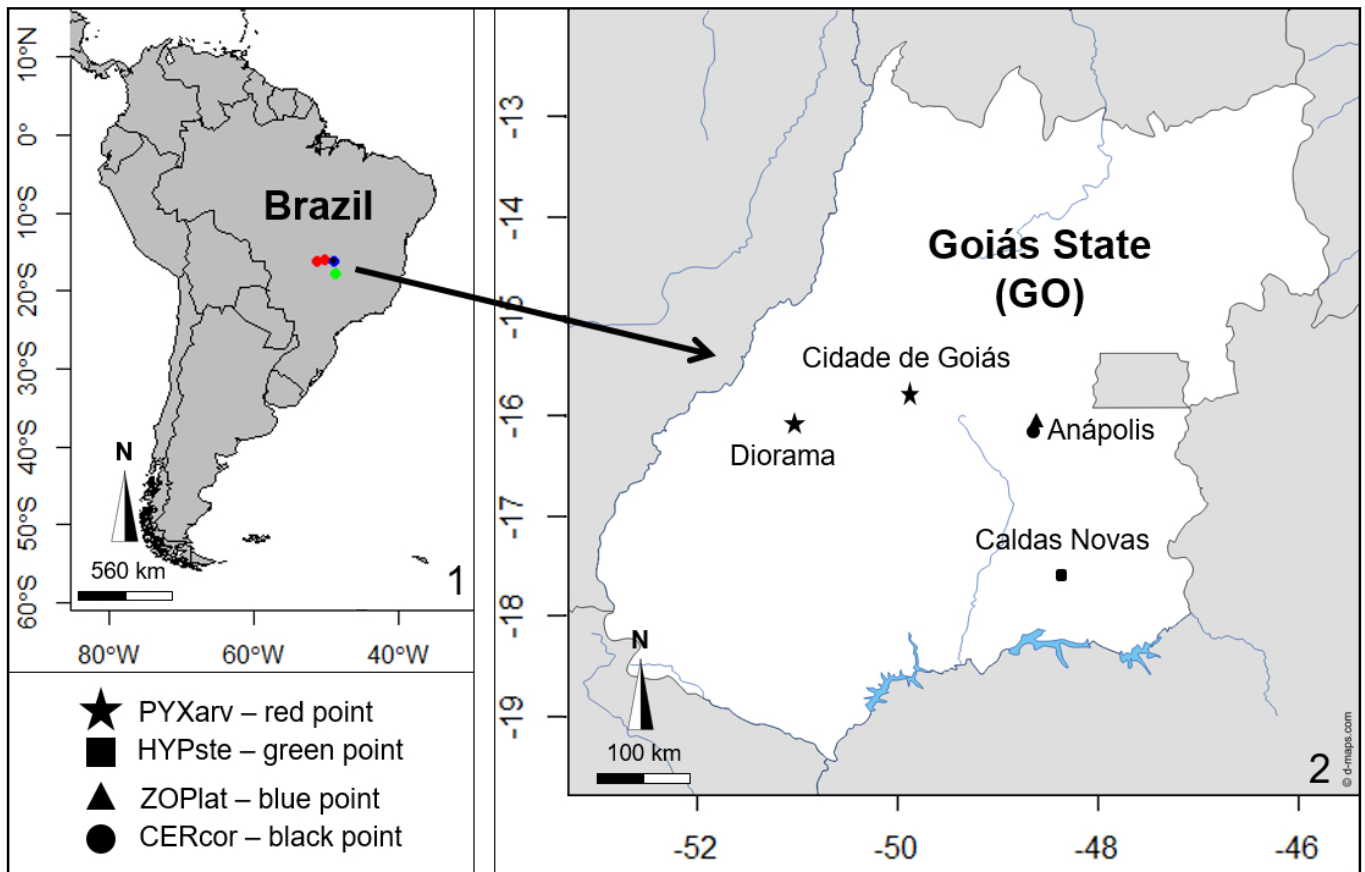
Coprophilous fungi are organisms ecologically and physiologically specialized for living in dung, they can assimilate nutrients that were not absorbed during the passage of food through the digestive tract of the animals, thereby taking part in the decomposition and recycling processes of these nutrients in the environment (Harrower and Nagy 1979; Ávila et al. 2001; Richardson 2001a, 2003; Krug et al. 2004; Masunga et al. 2006).

Although they are cosmopolitan, the occurrence records of this group of organisms are more frequent from Europe, North America and a number of Neotropical countries (Brummelen 1967; Lundqvist 1972; Eliasson and Lundqvist 1979; Jahn 2000; Doveri 2004; Bezerra et al. 2008; Calaça and Xavier-Santos 2012). Studies involving coprophilous fungi have recently been conducted in Brazil (Richardson 2001b; Bezerra et al. 2008; Viriato 2008; Melo et al. 2011; 2012, 2014, 2015a, 2015b; Calaça and Xavier-Santos 2012; Calaça et al. 2013, 2014, 2015), but there are still few data about the distribution of this group in the country, and there are a large number of unexplored areas that could provide

essential knowledge of their diversity. This study adds new records of genera and species for Brazil and for the Neotropical Region, with taxonomic descriptions, characterization and geographic distribution of these species.

Between 2012 and 2014, dung of horse (*Equus caballus* L.), cattle (*Bos taurus* L.), white-lipped peccary (*Tyassu pecari* Link) and domestic pig (*Sus domesticus* L.) were collected in different localities of Goiás State, Brazil: Vale das Antas' Farm Sector (Chácara Céu Azul), Anápolis (16°16'27.26" S, 048°54'27.13" W); Agroecological Technology Center for Small Farmers (AGROTEC), Diorama (16°14'52" S, 051°16'55" W); Banks of the Bacalhau River, Goiás City (15°55'37" S, 050°08'22.9" W); and Serra de Caldas Novas State Park (PESCAN), Caldas Novas (17°46'11.8" S, 048°39'33.5" W) (Figures 1 and 2). Goiás state is located in the Midwest Region of Brazil. This region comprises the states of Goiás, Mato Grosso, Mato Grosso do Sul and the Federal District. The major part of Midwest Region coincides with the Cerrado biome domain, which is characterized by the largest savanna in the Neotropical Region, the most diverse in the world. The Cerrado is a biodiversity hotspot and one of the 25 most important terrestrial regions for conservation according to Myers et al. (2000).

The collected dung samples were incubated in moist chambers, in accordance with the methodology proposed by Lundqvist (1972), Bell (1983, 2005), Richardson (2001a), and Doveri (2004). The emergence of fungal fruiting bodies was observed every two days using stereoscopic and compound microscopes. The material was characterized, through of macro- and microscopic characters, observed under a light microscope using semi-permanent slides, prepared with a drop of sterile water, Melzer's reagent (when necessary) and fixed using polyvinyl-lacto-glycerol. The taxonomy follows



Figures 1 and 2. Location of study areas in Brazil and Goiás state. **1.** Map of South America, showing the distribution of new records of coprophilous fungi for Brazil. **2.** Location of areas in Goiás state where substrates were collected. PYXarv: *Pyxidiophora arvernensis*; HYPste: *Hypocropa stercoraria*; ZOPlat: *Zopfiella latipes*; CERcor: *Cercophora coronata*.

Lundqvist (1969, 1972, 1980), Bell (1983, 2005), Richardson and Watling (1997), and Doveri (2004, 2011). The nomenclature adopted follows the Index Fungorum Partnership proposal (IFP 2015). Voucher specimen as well as their permanent slides were deposited in the Herbarium of Universidade Estadual de Goiás (HUEG). Maps were produced using Software R (2015) and maps available at www.d-maps.com.

Pyxidiophoraceae

Pyxidiophora arvernensis (Breton & Faurel) N. Lundq. Bot. Notiser 133(2): 134 (1980). Figures 3–9.

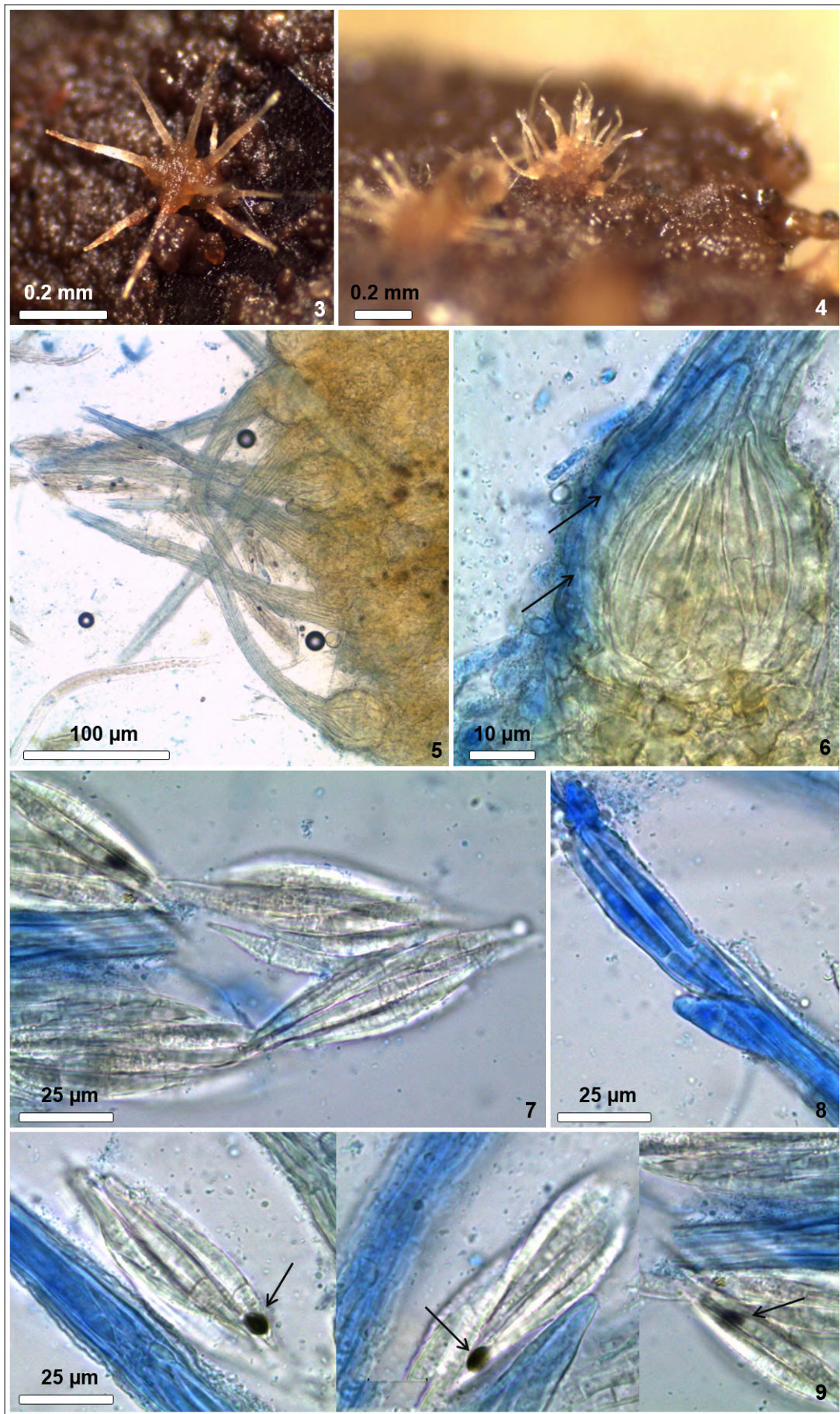
Perithecia yellowish, non-stromatic, glabrous, superficial, with long necks (up to 100 μm) and small bodies up to 100 μm in diam. (Figures 3 and 4), gregarious. Neck cells long; peridium with globular texture, 15 μm on average (Figures 5 and 6). Asci very conspicuous, small, cylindrical to clavate, up to 65 μm long. Ascospores fusiform, 45–60 μm \times 3–5 μm (Figures 7–9), hyaline, apiculate, with a transverse septum, some with a blackish adhesive disc (Figure 9), located on one of the apices.

Habitat and distribution: Coprophilous species, occurring in Europe [France: on cow and horse dung; Finland: on hare (*Leporidae*) dung; Sweden: on reindeer

(*Rangifer tarandus* L.) and roe deer (*Capreolus capreolus* L.) dung] (Lundqvist 1980) and Australia, on undefined dung substrate (Bell 2005). This is the first record of the genus for Brazil, and the first of the species for the Neotropical Region.

Material examined: Brazil, Goiás, Diorama, perithecia obtained from white-lipped peccary feces (*T. pecari*), stored in a moist chamber, collected at the AGROTEC, 30-VI-2012, Souza, RCS and Calaça FJSC30. In Goiás City, perithecia obtained from cow dung (*Bos taurus*), stored in a moist chamber, collected on the banks of the Bacalhau River, 30-VI-14, Xavier-Santos, S and Calaça SXS 6173. HUEG-10045.

Notes: No differences were observed with characters of the original description (Lundqvist 1980). Species of *Pyxidiophora* are essentially coprophilous (Lundqvist 1980; Bell 2005) and depend on arthropod dispersers to complete their life cycle. Few somatic or reproductive structures, such as ascospores, were observed in samples found in white-lipped peccary dung, because these specimens are delicate, with perithecioid fruiting bodies, often damaged by mycophagous insects [primarily Aphodiinae beetles (*Scarabaeidae*)] and other small arthropods. However, more details were observed in specimens found in cow dung, seen at the onset of fungal



Figures 3–9. *Pyxidiphora arvernensis*. **3–5.** Perithecia on dung. **6.** A perithecium under optical microscope; arrows point to the exoperidial wall. **7.** Ascospores, the arrow points to the acute apex. **8.** The ascospore stained with cotton blue. **9.** Adhesive discs present in the region near the apex of mature ascospores (arrows).

succession, (Figure 3–9). *Pyxidiophora* is rare (Blackwell et al. 1989; Blackwell and Malloch 1989), and is recorded for the first time in Brazil, and *P. arvernensis* for the first time in the Neotropical Region. This is also the first record of the species in white-lipped peccary feces.

Lasiosphaeriaceae

Cercophora coronata (Cailleux) Udagawa & T. Muroi, *Trans. Mycol. Soc. Japan* 20(4): 459 (1979). Figures 10–18.

Perithecia sparse to gregarious (Figure 10), with few individuals, olive brown in color, pyriform and elongated, with short neck, 600–800 μm \times 300–400 μm , covered with papillae formed by groups of long and inflated cells, primarily around the neck (Figure 15). Peridium (exoperidium) semitransparent, membranaceous, pseudoparenchymatous, for which two types of texture were identified: angular, on the body of the perithecium and epidermoid, in the neck region (Figures 11–13). Asci long, 100–185 \times 15–20 μm , cylindrical and slightly clavate, unitunicate, with truncated apex, apical ring very conspicuous, with large (5 μm diam.) and verrucous subapical globule, 8-spored (Figure 16). Ascospores hyaline and vermiform when immature, 40–50 \times 5–8 μm , with a whip-like appendage. At maturity, the pigmented cell (blackish) of ascospores is ellipsoid (17–20 \times 10–13 μm), with a truncated base and whip-like gelatinous tail; the superior apex exhibits a germinative pore, from which a thin filiform gelatinous appendage emerges (Figure 17).

Habitat and distribution: It has been recorded in Africa (Central African Republic), on buffalo (*Syncerus caffer* Sparrman) dung (Cailleux 1971), Japan, on cow dung (Udagawa and Muroi 1979), Martinique (French Lesser Antilles), on cow dung (Delpont 2011) and Brazil, on capybara (*Hydrochoerus hydrochaeris* L.) dung (Jahn 2000). This is the second record of this species for Brazil, the first for the Brazilian Savannah and the Midwest Region.

Material examined: Brazil, Goiás, Anápolis, perithecia obtained from horse dung (*Equus caballus*), collected on the Vale das Antas' Farm Sector (Chácara Céu Azul), 25-II-2012, Calaça FJSC28, HUEG-8955.

Notes: No differences were observed with characters of the original description (Udagawa and Muroi 1979). This species is mainly characterized by the presence of agglutinated hairs on the neck of the perithecia, formed by long and inflated cells, resembling small tufted papillae. This does not occur in other members of this genus, whose hairs are not agglutinated and rigid. *Cercophora mirabilis* Fuckel is a closely related species, but differs from *C. coronata* by the absence of papillae on the perithecium. *Cercophora mirabilis* shows size variation in the black ascospores; 16–21 \times 9–12.5 μm (Udagawa and Muroi 1979) and (18–) 20–23.4 \times (10–) 10.8–13.5 μm , according to Doveri (2004). In addition, the perithecia of *C. mirabilis* are immersed in the substrate, while those

of *C. coronata* are partially immersed (Delpont 2011; Delpont, pers. comm.).

Zopfiella latipes (N. Lundq.) Malloch & Cain, *Can. J. Bot.* 49: 876 (1971). Figures 19–25.

Cleistothecia superficial, sparse to gregarious, blackish, globose, 0.5–1 mm diam., covered with hyaline, flexuous and thick hairs, opening by dehiscence of the peridium (Figures 19–20). Peridium pseudoparenchymatous (Figure 25), texture angular, olivaceous brown, with cells measuring \pm 15 μm diam. Paraphyses very evanescent, asci clavate, 8-spored, measuring 100 \times 15–20 μm (Figure 22). Ascospores biseriate, hyaline when immature, unicellular, cells pigmented at maturity (olivaceous), 15–20 \times 10–12 μm , truncated at the base, guttulate, where a flat, wide, evanescent pedicel is located, mamilliform at the apex, with a subapical germinative pore (Figures 21–24).

Habitat and distribution: Europe (Denmark), isolated from soil from a greenhouse, and North America (United States of America), isolated from submerged balsa wood blocks (Lundqvist 1969; Malloch and Cain 1971), and Asia, on sambar deer (*Rusa unicolor* Kerr) dung (Jeamjitt et al. 2007). This is the first record of the genus for Brazil, and the first record of the species for the Neotropical Region.

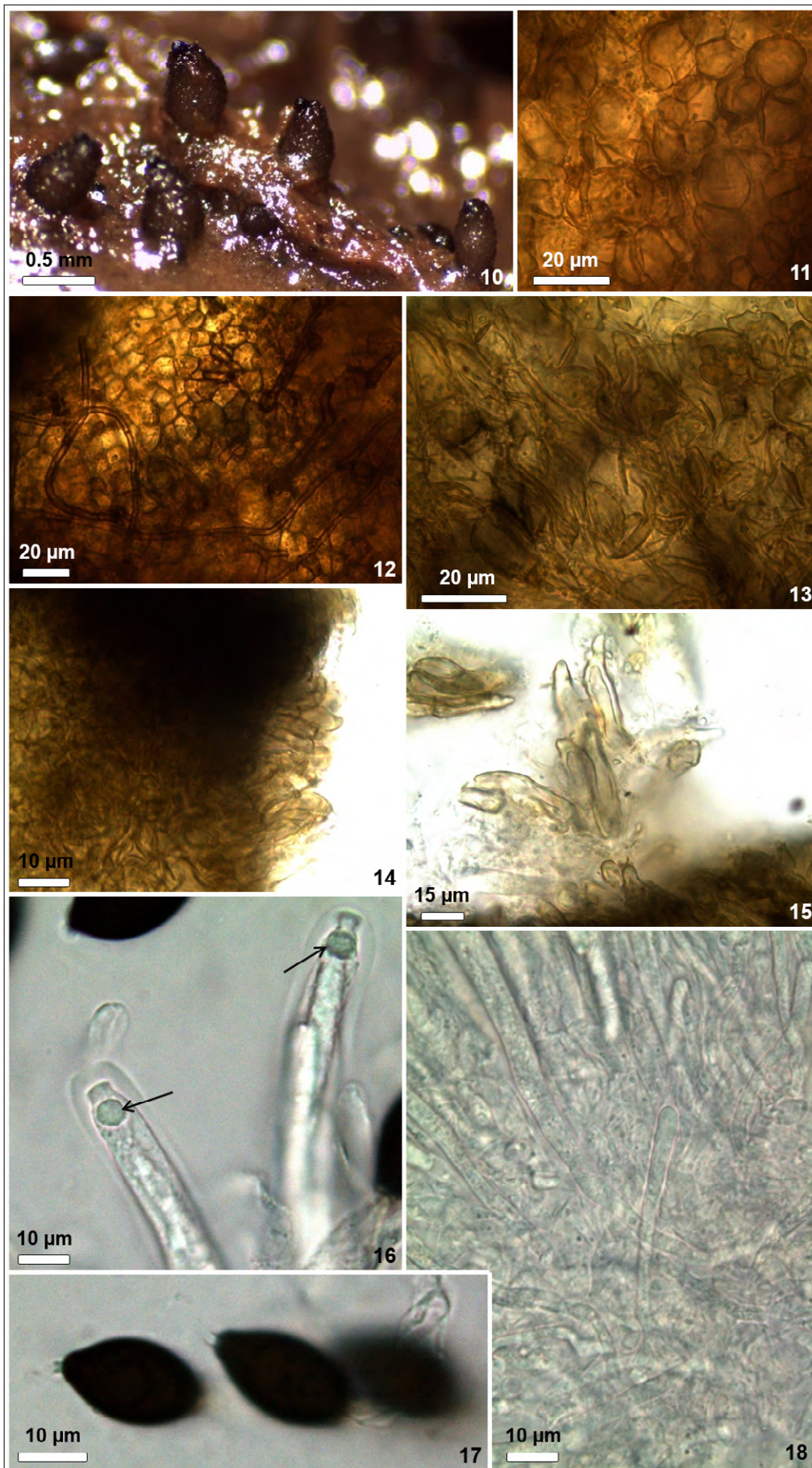
Material examined: Brazil, Goiás, Anápolis, cleistothecia obtained from pig dung (*Sus domesticus*), collected on the Vale das Antas' Farm Sector (Chácara Céu Azul), 01-IV-2012, Calaça FJSC29, HUEG-8952.

Notes: No differences were observed with characters of the original description (Lundqvist 1969). *Zopfiella latipes* develops under plant material and soil (Lundqvist 1969) and, more rarely, on dung (Doveri 2004). This is the first report of the species in pig feces. The main trait of this species is its ascospore morphology, which differentiates it from others of the genus. In *Z. latipes*, ascospores are conical and elliptical, with a subapical germinative pore and a large number of oily vesicles (guttula). Moreover, it exhibits a wide and relatively short pedicel at the truncated base of the ascospore. This species is very similar to *Z. pleuropora* Malloch & Cain, but differs in its aseptate ascospores and dark cleistothecia. Furthermore, *Z. latipes* is considered a fimicolous species (generalist), occurring in soil and submersed plant material (Lundqvist 1969) as well as in dung (Jeamjitt et al. 2007), while *Z. pleuropora* has only been found in deer dung (Malloch and Cain 1971), differentiating them by their ascospores and habitat.

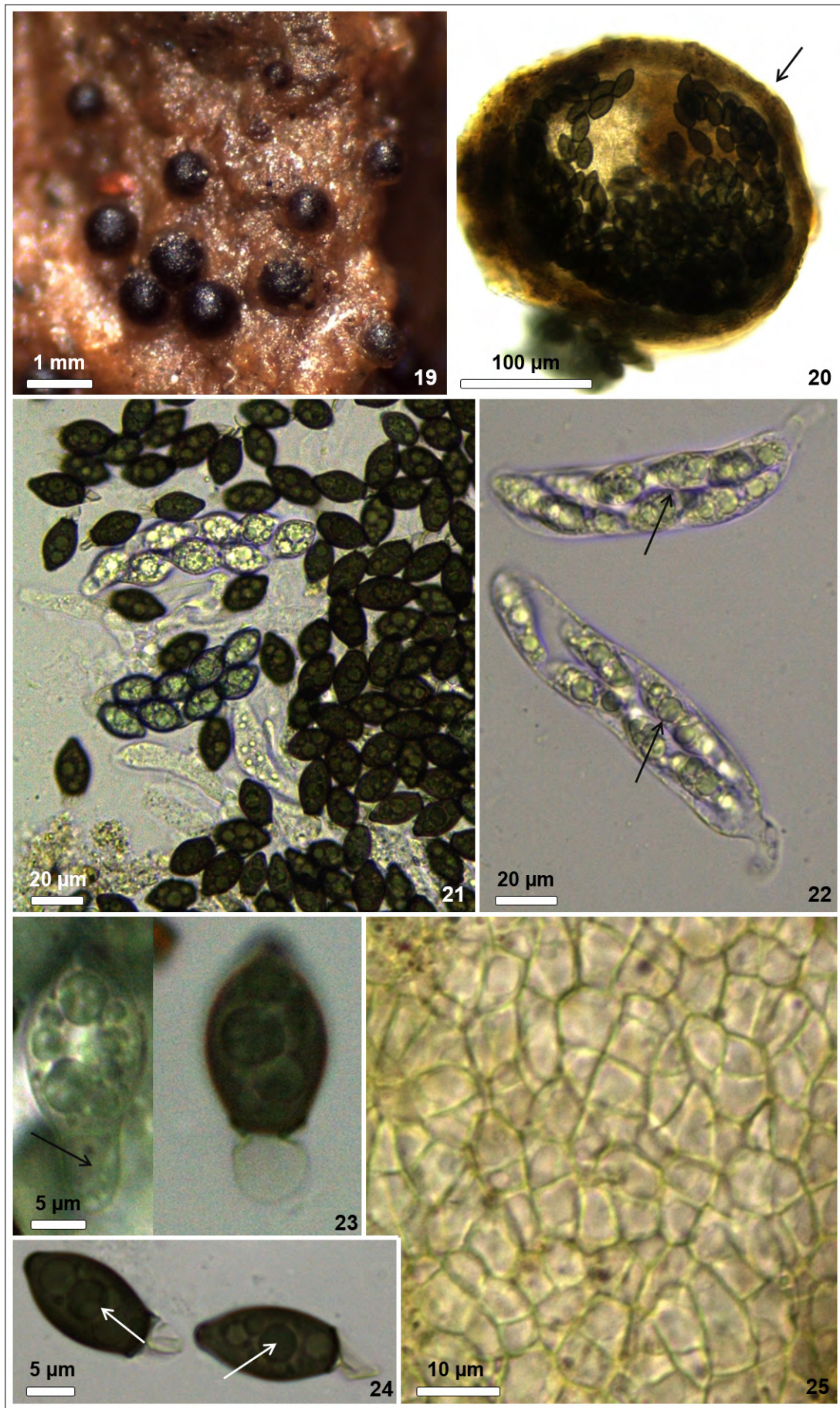
Xylariaceae

Hypocopra stercoraria (Sowerby) Sacc., *Syll. fung. (Abellini)* 1: 244 (1882). Figures 26–36.

Stroma present, diffusely spread over the substrate, whitish in color, becoming yellow to cream-colored with



Figures 10–18. *Cercophora coronata*. **10.** Perithecia in dung. **11.** Angular cells of the exoperidium. **12.** Angular exoperidium, with visible perithecial hair. **13.** Epidermoid cells in the neck region. **14.** Ostiole region. **15.** Papilla formed by elongated cells with acute and inflated peaks, located in the neck region. **16.** Apex of the ascus; arrows show the verrucous globule below the apical ring. **17.** Mature ascospores. **18.** Hyphae of the centrum, a number of paraphyses are visible.



Figures 19–25. *Zopfiella latipes*. **19.** Cleistothecia in dung. **20.** A cleistothecium sectioned transversely, exhibiting ascospores inside. The arrow points to the exoperidium. **21.** Ascospores. **22.** Complete asci with eight ascospores, still immature inside. The arrow shows the guttulas in the ascospores. **23.** Comparison between immature and mature ascospores. The arrow shows the pedicel in the basal region. **24.** Oily guttula inside the mature ascospores. **25.** Peridium, formed by angular pseudoparenchymatous tissue.

age (Figures 26 and 27), formed by yellowish, thick-walled, polygonal cells up to 10 µm in diam. (Figure 36). Perithecia immersed in stromatic tissue, pyriform, measuring up to 1 mm, blackish, with peridium coriaceous, smooth, neck short, papillaceous and ostiolate, growing sparse to gregarious in the stroma (Figures 26–28). Guttulate paraphyses present (Figure 35). Asci cylindrical, pedicellate, 250–300 × 35–40 µm, dextrinoid, with apical apparatus becoming blue in iodine (I+), 8-spored (Figures 29–31). Ascospores uniseriate, elliptical, 38.6–48(–50) × (18)18.9–22.2(–24) µm, dark olivaceous at maturity, with an easily visible gelatinous sheath and a central longitudinal germ slit measuring an average of 30 µm (Figures 32–34).

Habitat and distribution: It is distributed across Europe (Sweden), on horse and human dung (Saccardo 1882), and New Zealand, on herbivore dung (Bell 1983). This is the first record of the genus for Brazil, and the first of the species for the Neotropical Region (possibly for the entire Americas).

Material examined: Brazil, Goiás, Caldas Novas, perithecia obtained from cow dung, collected in the Serra de Caldas Novas State Park (PESCAN), 18-XII-2013, Calaça FJSC49, HUEG-10044.

Notes: *Hypocopra* (Fr.) J. Kickx f. differs from *Podosordaria* Ellis & Holw., and *Poronia* Willd. (closely related genera) in its sessile stroma, reduced, appearing as a ring of mycelial tissue around the perithecial ostioles (in many species, although the stroma is indistinct) and partially immersed in the substrate, containing few perithecia (Krug and Cain 1974; Bell 1983; Doveri 2004). All known *Hypocopra* species are coprophilous (Cain 1934; Krug and Cain 1974). Krug and Cain (1974) described 14 new species in one of the first studies on this genus and Doveri (2004) has provided a key for all known species. There are few literature reports on the taxonomy of this genus; therefore, more studies are needed to understand its biology and ecology. Our material showed longer ascospores than in Saccardo's (1882) original description (1882), which reported a spore length of 30 µm. No further differences from characters of the original description (Saccardo 1882) or other material such as Krug and Cain (1974), Bell (1983), and Doveri (2004) were observed.

This study enhances knowledge of the Neotropical (mainly Brazilian) mycobiota and heightens understanding of the geographical distribution of coprophilous fungi. It presents the first records of three genera (*Pyxidiophora*, *Zopfiella* and *Hypocopra*) for Brazil and the first report of *P. arvernensis*, *Z. latipes* and *H. stercoraria* for the entire Neotropical Region, supporting the hypothesis of their cosmopolitan distribution. Moreover, it increases knowledge of the microhabitats of some species, because *P. arvernensis* and *Z. latipes* are recorded for the first time on white-lipped peccary dung (and likely

on cow dung) and pig feces, respectively.

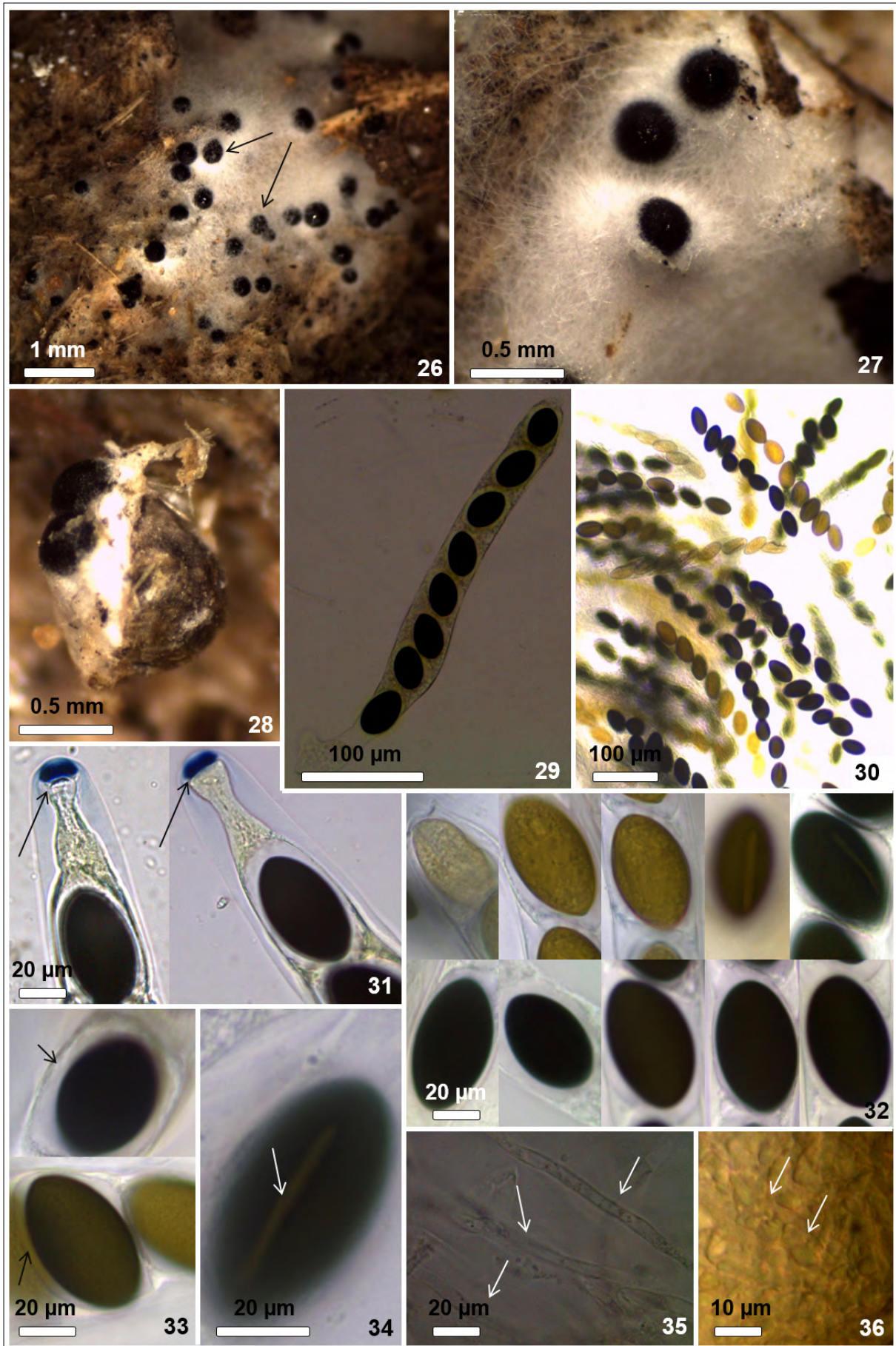
These results, added to the recent findings reported by Melo et al. (2014, 2015a, 2015b) and Calaça et al. (2015), increases the number of species of coprophilous fungi and other fungi from Brazil recorded on dung from 210 (Calaça et al. 2014) to 225.

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Figures 26–36. *Hypocopra stercoraria*. **26.** Stroma in dung. The arrows show the emerging perithecia. **27.** Stroma, shared by three perithecia. **28.** A perithecium with two necks. **29.** 8-spored ascus, stained from the reaction with Melzer's reagent. **30:** Mass of asci and ascospores at different stages of maturation. **31.** Positive iodine reaction (blue) of the apical ring. **32.** Ascospores and their maturation process. **33.** Gelatinous sheath (black arrows). **34.** Germination slit (arrow). **35.** Paraphyses. **36.** Stromal cells.

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