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Original article

An overview on the mycoparasitic *Piptocephalis* (Zoopagomycota): taxonomic notes and geographic distribution with new occurrences for South America

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ABSTRACT

Piptocephalis includes mycoparasitic fungi, mainly targeting mucoralean species. Until now, there has been no compilation of data on the taxonomy and geographic distribution of *Piptocephalis*, which is a barrier to the proper identification of species of this genus by taxonomists. The present study provides an overview of *Piptocephalis* with taxonomic and occurrence data, in addition to reporting *P. graefenhanii* and *P. xenophila* for the first time in South America. Both species were observed parasitizing *Mucor* spp. The *P. graefenhanii* was observed growing on paca dung and *P. xenophila* was observed on guinea-pig dung in Recife, northeastern Brazil. Aspects of their morphology are discussed and a key for the genus is presented.

Keywords: Ecology, Haustorial fungi, Mycoparasitic fungi, Taxonomy, Zoopagales.

Introduction

Zoopagomycota comprises fungi parasitic of protozoa (e.g., amoebae), small animals (e.g., rotifers or nematodes) and other fungi, mostly of Mucorales (Mucoromycota) and Mortierellales (Mortierellomycota), rarely infecting species of Ascomycota (Tedersoo *et al*. 2018; Davis *et al*. 2019). This phylum includes the Zoopagales, with five families, among which Piptocephalidaceae is the best known (Reynolds *et al*. 2019). Piptocephalidaceae comprises both *Piptocephalis* and *Syncephalis* (Wijayawardene *et al*. 2022) that are merosporangipherous haustorial fungi that form merosporangia on terminal vesicles, with merospores remaining dry or released in a droplet of fluid at maturity. Zygospores are formed from the union of gametangia produced terminally by apposed or coiled progametangia (Benny *et al*. 2016).

Piptocephalis species form slender vegetative hyphae, with small appressoria which penetrate the host wall, giving

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rise to restricted, delicate, and branched haustoria (Benjamin 1959). Merosporangiophores are erect, septate, smooth or longitudinally striate, dichotomously branched several times where the ultimate branch ends in a head cell supporting few to many merosporangia containing one or more merosporangiospores (Ho 2004). Although *Piptocephalis* has a worldwide distribution (Fig. 1), the majority of species of this genus cannot be confirmed as cosmopolitan due to a lack of inventories of these fungi (Reynolds *et al*. 2019). For South America, records of *Piptocephalis* are restricted to Argentina, Brazil and Colombia (GBIF 2022).

Although sequences of *Piptocephalis* species are available in the GenBank database, identification of this genus has been mainly based on morphological characters due to the obligate parasitic nature of these fungi (Ho 2004; 2006). Although we recognize the importance of molecular biology to separate species of *Piptocephalis*, we believe that morphological identification is also a reliable tool for the identification of these species. Reynolds *et al*. (2019), after a morphological and phylogenetic (28S and ITS rDNA regions) study of *Piptocephalis* observed that the *Piptocephalis* strains formed a monophyletic group with several distinct clades corresponding to morphologically identified species, which indicates that morphological features are sufficient to identify species of *Piptocephalis*. However, the last morphological key for identification of *Piptocephalis* was provided by Gräfenhan (1998), not including species described in the last 24 years (until March 2023).

The present study aims to provide an overview of *Piptocephalis* with taxonomic, ecological and occurrence notes of all species, as well as to illustrate *P. xenophila* and *P. graefenhanii* (new records for South America), therefore expanding knowledge on geographic distribution of this still poorly studied genus. In order to fill a gap in time since the last identification key of *Piptocephalis* was produced, we provide a morphological identification key for the species known until March 2023.

Material and methods

Occurrence and substrates data - GenBank, UNITE and GBIF databases were used to access distribution and substrates information of *Piptocephalis* spp.

Observation and identification of *Piptocephalis* spp. – Samples of *Cuniculus paca* Linnaeus (paca) and *Cavia porcellus* Linnaeus (guinea-pig) dung were collected in the Parque Estadual de Dois Irmãos, an Ecological Reserve located in the city of Recife, state of Pernambuco, Brazil (Fig. 1). Dung samples were collected with a spatula previously sterilized in 70% alcohol, placed in plastic bags and taken to the laboratory where they were incubated in Petri dishes containing two filter paper sheets moistened with sterile distilled water at room temperature (± 28 °C) for seven days in alternate periods of light and dark. After four days, merosporangiophores of *Piptocephalis* were observed growing directly from hyphae of *Mucor* sp. Microscopic slides were prepared directly from samples using Amann's Cotton Blue containing both *Piptocephalis* and its host and observed under a light microscope (Leica MD 500). The identification was based on microstructural characters according to Gräfenhan (1998) and Ho (2003, 2006). Since *Piptocephalis* spp. could not be successfully grown on corn

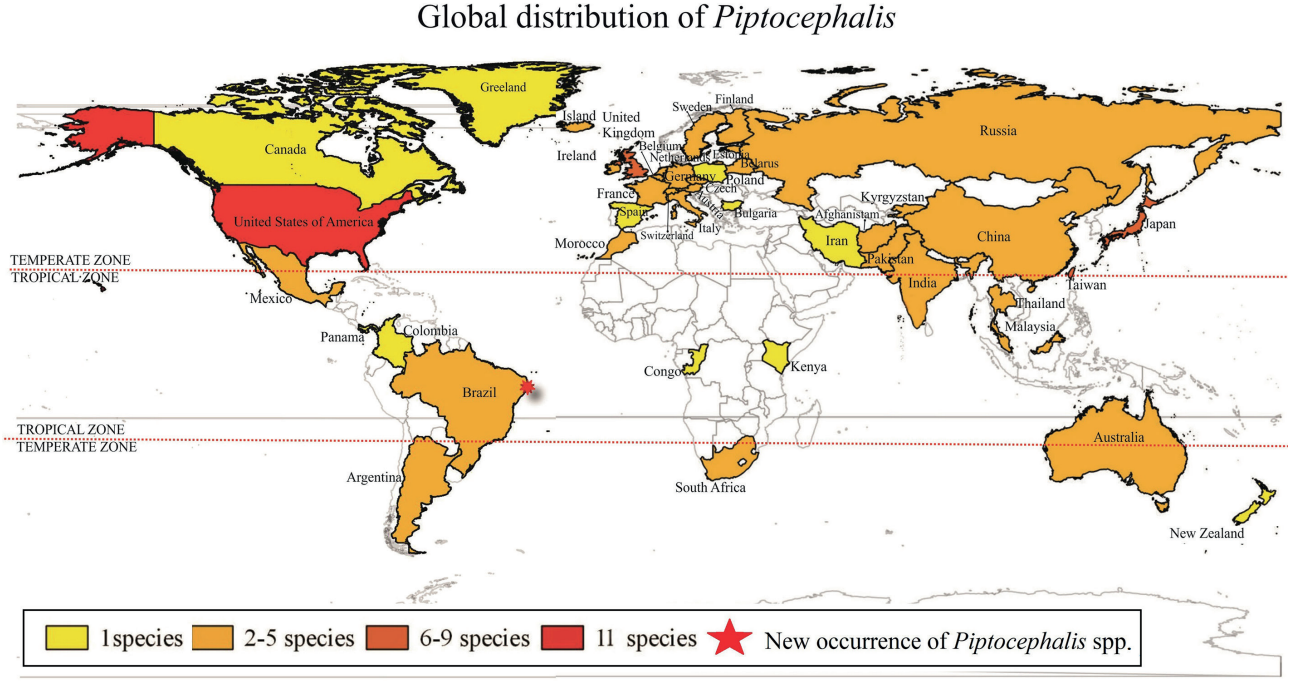

Figure 1. Geographical distribution of *Piptocephalis* species.

meal agar (CM), malt extract agar (MEA 2%), malt extract-yeast agar (MEYE) and potato dextrose agar (PDA) media, permanent slides prepared directly from the moist chambers using 50% glycerol containing a small amount of Amann's cotton blue (Benny 2008) were deposited in the URM Herbarium of the Universidade Federal de Pernambuco.

Results

New records for South America

Piptocephalis graefenhanii H.M. Ho, Botanical Studies 47(4): 453 (2006) Fig. 2

MycoBank number: 521964

Vegetative hyphae submerged, hyaline, thin, delicate. Rhizoids not observed. Merosporangiophores erect, hyaline, prostate and distantly septate with age, smooth-walled. Main stalks 1800–3600 µm long, 2–4 µm in diam. Merosporangiophores consisting of up to six successive dichotomies, not striate; primary branches 600–1800 × 2.5 µm, penultimate branches 7–27 × 1.5–2.5 µm, ultimate branches 6.5–15 × 1–2 µm. Head cells deciduous, hyaline, globose with conical projections, smooth-walled, 4.5–6 × 4–6 µm, supporting 4–14 merosporangia remaining dry at maturity. Merosporangia initially hyaline, ellipsoidal, erect, two-spored, while the apical merospore buds from the basal one, smooth-walled, 10–13 × 3–3.5 µm. Merospores hyaline, ellipsoidal, 5–6.5 × 2.5–3 um, smooth-walled. Zygospores not observed.

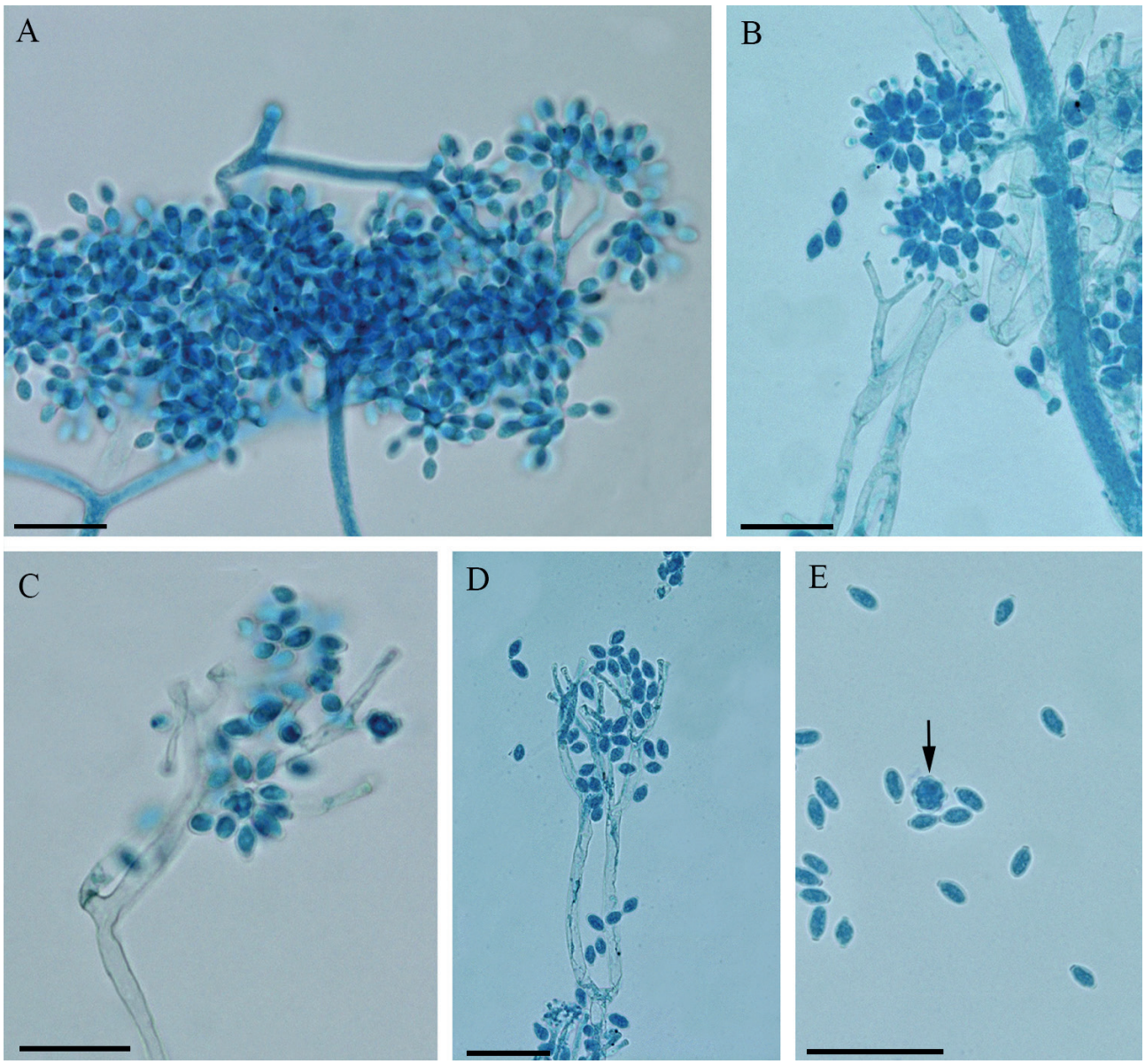

Figure 2. *Piptocephalis graefenhanii*. **A-D:** Branched fertile region of merosporangiophore with merosporangia. **E.** Merospores and one globose head cell (arrow). Bars = 25 µm.

Material examined: Brazil, Pernambuco: Recife, Parque Estadual Dois Irmãos (8°00'54.0"S 34°56'40.2"W), isolated from *Cuniculus paca* (paca) dung parasitizing *Mucor* sp., 10 Nov. 2021, M.O. Cruz (URM 94643).

Habitat and geographic distribution: This species was previously reported from soil in the United Kingdom and Taiwan (Gräfenhan 1998; Ho 2006; GBIF 2022). It has also been reported in Afghanistan and Australia without substrate indication (GBIF 2022). This species can parasitize *Cokeromyces recurvatus* (Gräfenhan 1998).

Notes: *Piptocephalis graefenhanii* is reported for the first time in South America from paca dung. Until now, *P. graefenhanii* had only been reported from soil. It is morphologically similar to *P. lepidula*. The main feature common to both species is the formation of apical merospores by budding from a basal merospore. However, unlike merosporangiophores of *P. graefenhanii*, which branch up to six times and are not striated, merosporangiophores of *P. lepidula* may branch up to eight times and exhibit striation. The primary branching of *P. graefenhanii* merosporangiophores is longer (from 1800 µm in length) than the primary branch of *P. lepidula* (up to 700 µm in length). Additionally, there are differences in the number and size of the merosporangia between both species. *Piptocephalis graefenhanii* head cells sustain 4–14 merosporangia, while head cells of *P. lepidula* sustain from 15–30 merosporangia.

Piptocephalis xenophila Dobbs & M.P. English, Transactions of the British Mycological Society 37 (4): 375 (1954)

Fig. 3

MycoBank number: 303660

Vegetative hyphae submerged, hyaline, thin, delicate. Rhizoids hyaline, poorly branched, up to 22 × 2.2 µm. Merosporangiophores hyaline, erect or ascending, prostate and distantly septate with age, longitudinally striate, smooth-walled. Main stalks 1250–3000 µm long, 3–4.5 µm in diam. Fertile branch system consisting of up to seven successive dichotomies; primary branches 300–1500 × 2.5 µm, penultimate branches 7–10 × 1.5–2 µm, ultimate branches 6.5–15 × 1–2 µm. Head cells deciduous, hyaline, heart-shaped, smooth-walled, 3–4 µm in diam., supporting 5–12 merosporangia remaining dry at maturity. Merosporangia hyaline, cylindrical, erect, with 3–10 merospores, 23–26 × 2–3 µm., smooth-walled. Merospores hyaline, cylindrical, 4–6 × 2.5–3 µm. Zygospores not observed.

Material examined: Brazil, Pernambuco: Recife, Parque Estadual Dois Irmãos (8°00'54.0"S 34°56'40.2"W), isolated from *Cavia porcellus* (guinea-pig) dung parasitizing *Mucor* sp., 10 Nov. 2021, M.O. Cruz (URM 94644).

Habitat and geographic distribution: This species was previously reported on animal dung from the United States of America; leaf litter from the United Kingdom; soil from Afghanistan, Canada, Estonia, Taiwan, the United Kingdom and the United States of America. It has also been reported in Afghanistan, Australia and Japan without substrate indication (Dobbs and English 1954; Gräfenhan 1998; GBIF 2022). This species can parasitize *Mucor* sp. (Gräfenhan 1998).

Notes: *Piptocephalis xenophila* is here reported for the first time to South America from guinea-pig dung. Until now, *P. xenophila* had only been reported from soil. Merosporangiophores of this species form heart-shaped head cells like ones of *P. microcephala* and *P. indica*. However, the merosporangiophores of *P. xenophila* form up to seven dichotomies, different from the ones of *P. microcephala* which form up to 11 and the ones of *P. indica* which form up to eight dichotomies. The head cells of *P. xenophila* sustain up to 12 merosporangia, while those of *P. microcephala* and *P. indica* support up to six merosporangia. Furthermore, unlike *P. xenophila*, merospores of *P. microcephala* remain in a drop of fluid at maturity. Additionally, *P. xenophila* forms cylindrical merospores, while merospores of *P. microcephala* and *P. indica* are doliform.

Taxonomic notes and geographic distribution of other ***Piptocephalis*** *species*

Piptocephalis arrhiza Tiegh. & G. Le Monn. Annls Sci. Nat. Bot. 5(17): 366 (1873)

MycoBank number: 202264

Notes: According to Gräfenhan (1998), this homothallic species is characterized by the formation of striate merosporangiophores with 5–7 successive dichotomies, head cell lobed, and merosporangia sustaining 2–5 cylindrical or doliform merospores, which are 3–11 × 3–5 µm; spore heads remain dry at maturity. According to Indoh (1962), zygospores are 38–40 µm in diam. with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from Austria, Germany, Ireland, the United Kingdom and the United States of America. *Piptocephalis arrhiza* has also been reported in Belgium and Sweden without substrate indication (Gräfenhan 1998; GBIF 2022). This species can parasitize *C. recurvatus* (Gräfenhan 1998).

Piptocephalis benjaminii (Embree) R.K. Benj., Aliso 5(3): 284 (1963)

MycoBank number: MB337041

Notes: According to Benjamin (1963) and Gräfenhan (1998), this species is characterized by formation of striate merosporangiophores with 8–13 successive dichotomies, lack of head cells, merosporangia unisporate and arising from ultimate branch; merospores are ovate or obovate, 4–11 × 3–4 µm; spore heads remain dry at maturity. Zygospores are orangish-brown to brown, 45–64 µm in diam. with a reticulate exospore wall.

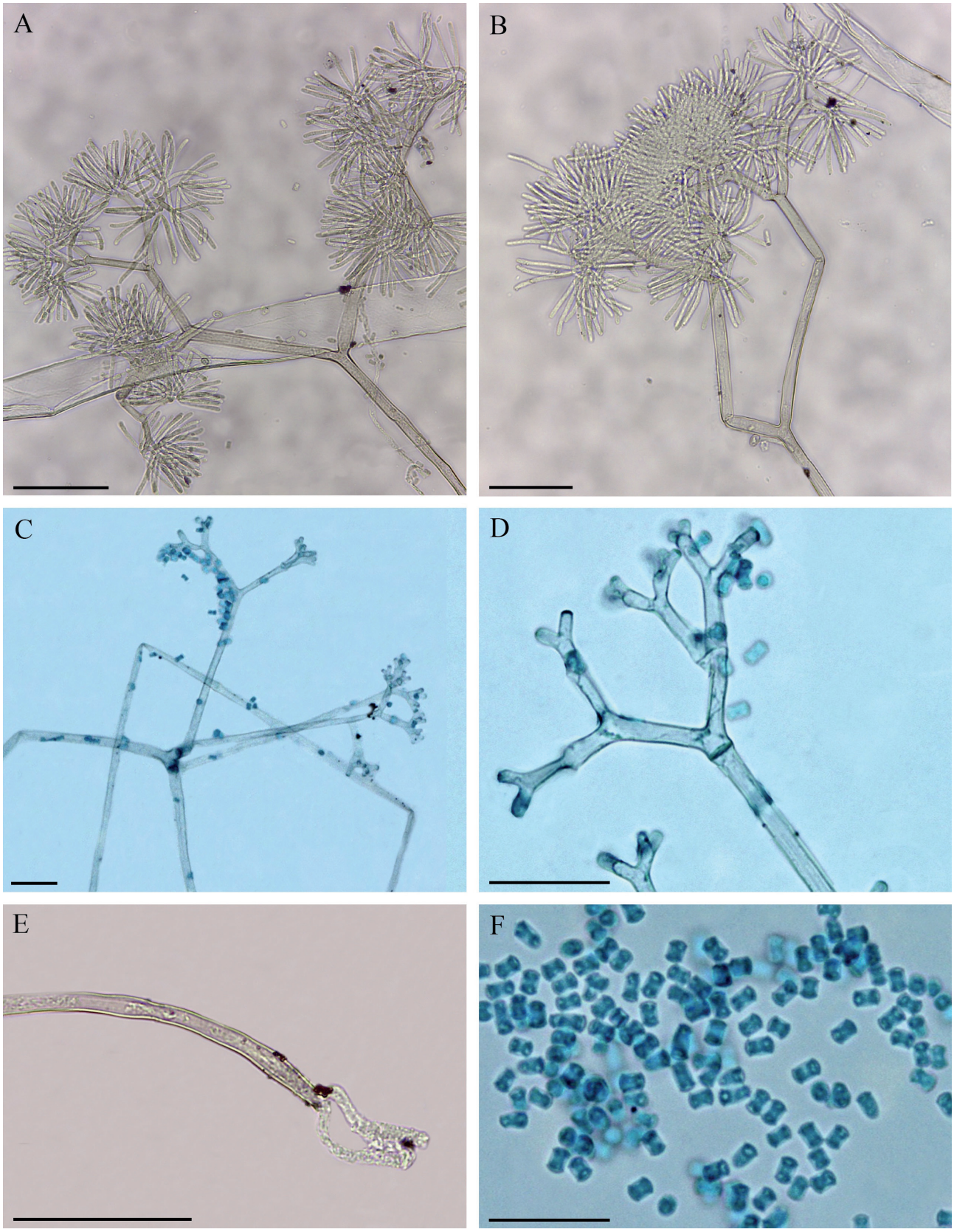

Figure 3. *Piptocephalis xenophila*. **A-B.** Branched merosporangiophore with merosporangia. **C.** Branched merosporangiophore after merospore release. **D.** Terminal branches of merosporangiophore after merospore release. **E.** Rhizoid. **F.** Merospores. Bars = 25

Habitat and geographic distribution: This species was previously reported on animal dung from the United States of America, old basidiome from the United Kingdon. *Piptocephalis benjaminii* has also been reported in Spain without substrate indication (Gräfenhan 1998; GBIF 2022). This species can parasitize *C. recurvatus* (Gräfenhan 1998).

Piptocephalis brijmohanii Mukerji, Mycologia 60(2): 326 (1968)

MycoBank number: 337042

Notes: According to Gräfenhan (1998), this homothallic species is characterized by formation of striate merosporangiophores with 4–6 successive dichotomies, subglobose head cells, and merosporangia sustaining 1–4 cylindrical to doliform merospores, which are 4–8 × 2.5–4 µm. Spore heads remain dry at maturity. Zygospores are yellowish-brown, 24–41 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from India and Pakistan (Gräfenhan 1998; Genbank 2022). *Piptocephalis brijmohanii* has also been reported in the Netherlands (from glass jar) and China without substrate indication (GBIF 2022). This species can parasitize *C. recurvatus* (Gräfenhan 1998).

Piptocephalis cruciata Tiegh., Annls Sci. Nat., Bot. 6(1): 149 (1875)

MycoBank number: 202411

Notes: According to Van Tieghem (1875) and Gräfenhan (1998), this species is characterized by formation of the striate merosporangiophores with 3–8 successive dichotomies, lobed head cells, and merosporangia sustaining 3–8 cylindrical or doliform merospores, which are 4–8 × 2.5–3 µm. Spore heads form a liquid droplet at maturity. Zygospores were not observed.

Habitat and geographic distribution: This species was previously reported from soil in Japan (Gräfenhan 1998; Genbank 2022; UNITE 2022). *Piptocephalis cruciata* can parasitize *Mycotypha microspora* (Gräfenhan 1998).

Piptocephalis cylindrospora Bainier, *Étud. Mucor.*, (Thèse, Paris) (Paris): 92 (1882)

MycoBank number: 202030

Notes: According to Mangin (1899) and Gräfenhan (1998), this species is characterized by the formation of striate merosporangiophores with up to 9 successive dichotomies, globose head-cells, merosporangia sustaining 2–7 cylindrical merospores, wich are 3.5–9 × 2–2.5 µm. Spore heads remain dry at maturity. Zygospore are yellow-orange to yellow-brown, 19–37 µm diam. with coarsely rugulose-reticulate exospore wall.

Habitat and geographic distribution – This species was previously reported on dung, material vegetable and soil from the United States of America, the United Kingdom and France (Gräfenhan 1998; GBIF 2022). *Piptocephalis cylindrospora* has also been reported in Argentina (from soil) and from Belgium, the Netherlands, South Africa and Switzerland without substrate indication (GBIF 2022). This species can parasitize *Absidia glauca, Lichtheimia ramosa, Mucor hiemalis* and *M. racemosus* (Gräfenhan 1998; GBIF 2022).

Piptocephalis curvata Baijal & B.S. Mehrotra, Zentbl. Bakt. ParasitKde, Abt. 2 (122): 181 (1968)

MycoBank number: 337043

Notes: According to Baijal & Mehrotra (1968), Gräfenhan (1998) and Ho (2004), this species is characterized by the formation of non-striate merosporangiophores with 3–8 successive dichotomies, in which the ultimate branch is curved, head cells lobed, and merosporangia sustain two cylindrical or doliform merospores, which are 4–8 × 2.5–3 µm. Spore heads form a liquid droplet at maturity. According to Reynolds *et al*. (2019), zygospores are hyaline, 20–30 µm diam., with a smooth-walled exospore.

Habitat and geographic distribution – This species was previously reported from soil in France, Japan, Malaysia (Baijal & Mehrotra 1968; Gräfenhan 1998; Genbank 2022) and Taiwan (Ho 2004). *Piptocephalis curvata* can parasitize *C. recurvatus* (Gräfenhan 1998) and *Backusella circina* (Ho 2004).

Piptocephalis debaryana B.S. Mehrotra [as 'de-baryana'], Proc. Natl. Acad. Sci. India, Sect. B, Biol. Sci. 30: 371 (1960)

MycoBank number: 337044

Notes: According to Mehrotra (1960) and Gräfenhan (1998), this homothallic species is characterized by the formation of the striate merosporangiophores with up to seven successive dichotomies, in which the ultimate branches have a slightly swollen apex. Head cells are lobed and merosporangia sustain 2–5 cylindrical merospores, which are 3–9 × 2.5–3 *µm*. Spore heads form a liquid droplet at maturity. Zygospores are hyaline, 17–34 µm in diam., with a faintly rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from India, Ireland, Taiwan, the United Kingdom and the United States of America (Ho 2006; GBIF 2022; Genbank 2022). This species can parasitize *Cokeromyces recurvatus*.

Piptocephalis fimbriata M.J. Richardson & Leadb., Trans. Br. mycol. Soc. 58(2): 206 (1972)

MycoBank number: 320532

Notes: According to Gräfenhan (1998) and Ho (2004), this homothallic species is characterized by the formation of striate merosporangiophores with up to 6 successive dichotomies, subglobose to lobed head cells falling at maturity leaving a truncate tip on the ultimate branch from merosporangiophores. Merosporangia sustain 2–6 cylindrical merospores, which are 4–7.5 × 2–3 µm. Spore heads form a liquid droplet at maturity. Zygospores are hyaline, 16–26 µm in diam., with a smooth-walled exospore.

Habitat and geographic distribution: This species was previously reported from soil, leaf litter and animal dung from the United Kingdom and Ireland; soil from Japan, Taiwan and the United States of America (Gräfenhan 1998; Ho 2004; GBIF 2022; Genbank 2022). It has also been reported in Argentina, Australia, Austria, Belarus, Belgium, Bulgaria, China, Congo, Colombia, Czechia, Finland, Germany, Greenland, Iceland, Iran, Italy, Kenya, Kyrgyzstan, Mexico, Morocco, South Africa, Thailand, Russia, Sweden and Switzerland without substrate indication (GBIF 2022). *Piptocephalis fimbriata* can parasitize *C. recurvatus* (Gräfenhan 1998) and *Mucor* sp. (Ho 2004).

Piptocephalis formosana H.M. Ho & P.M. Kirk, Bot. Studies (Taipei) 50(1): 69 (2009)

MycoBank number: 540746

Notes: According to Ho & Kirk (2009), this species is characterized by the formation of striate merosporangiophores with up to four successive dichotomies, lobed head cells, and merosporangia sustaining 2–4 cylindrical merospores, which are 2.5–4 × 1–2 µm. Spore heads form a liquid droplet at maturity. Zygospores are light brown, 42–47 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported from soil in the United States of America and Taiwan (GBIF 2022; Ho & Kirk 2009). It has also been reported in Afghanistan without substrate indication (GBIF 2022). It can parasitize *Mucor* sp. (Ho & Kirk 2009).

Piptocephalis freseniana de Bary, Abh. senckenb. naturforsch. Ges. 5: 356 (1865)

MycoBank number: 202311

Notes: According to Zycha *et al*. (1969) and Gräfenhan (1998), this homothallic species is characterized by the formation of striate merosporangiophores with 4–9 successive dichotomies, lobed head cells, merosporangia in a feather-like arrangement, sustaining 3–7 cylindrical to doliform merospores, which are 4–9 × 2.5–4 µm. Spore heads form a liquid droplet at maturity. Zygospores are yellowish-brown to reddish-orange, 20–37 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from Brazil, the United Kingdom and the United States of America; soil from the United States of America (Gräfenhan 1998; GBIF 2022; Genbank 2022). It has also been reported in Germany and Morocco without substrate indication (GBIF 2022). *Piptocephalis freseniana* can parasitize *C. recurvatus* and *Mucor* sp. (Gräfenhan 1998).

Piptocephalis fusispora Tiegh., Annls Sci. Nat., Bot. 6(1): 146 (1875)

MycoBank number: 199810

Notes: According to Van Tieghem (1875), this homothallic species is characterized by the formation of striate merosporangiophores with 4–8 successive dichotomies, globose head-cells, merosporangia sustaining 3–5 fusiform merospores, which are 2–4 × 2 µm. Zygospores are blackish-brown, 42–50 µm in diam., with an exospore wall sculptured with large papillae. Spore heads were not described.

Habitat and geographic distribution: It was found by Van Tieghem (1875) growing on *Mucor* sp. mycelium associated with *Helvella crispa* in France. According to Gräfenhan (1998), *P. fusispora* has been also reported in China, Italy, the Netherlands and Poland without substrate indication.

Piptocephalis indica B.S. Mehrotra & Baijal, Sydowia 17(1-6): 171 (1964)

MycoBank number: 337045

Notes: According to Mehrotra & Baijal (1964), Zycha *et al*. (1969), Gräfenhan (1998), and Ho (2003), this homothallic species is characterized by the formation of striate merosporangiophores in whorls with 2–4 primary branches and 3–8 successive dichotomies. Head cells are heart-shaped, and merosporangia sustain 4–8 cylindrical merospores with membranes derived from sporangial wall that remain on the surface of the merospores forming fringes at both ends. Merospores are 3–6 × 2–4 µm. Spore heads remain dry at maturity. Zygospores are orangish-brown, 11–14 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from India (Mehrotra & Baijal 1964; Gräfenhan 1998); soil from Malaysia, the United States of America and Taiwan (Ho 2003; Genbank 2022). It has also been reported in Afghanistan without substrate indication (GBIF 2022). *Piptocephalis indica* can parasitize of *C. recurvatus* (Gräfenhan 1998) and *Cunninghamella* sp. (Ho 2003).

Piptocephalis lemonnieriana Vuill., Bull. Séanc. Soc. Sci. Nancy, 3(3): 47 (1902)

MycoBank: 199706

Notes: According Vuillemin (1902), Zycha *et al*. (1969) and Gräfenhan (1998), this species is characterized by forming striate merosporangiophores. Head cells are globose, and merosporangia sustain two ellipsoidal merospores, which are 5 × 2.5 µm. Spore heads form a liquid droplet at maturity. Zygospores were not observed, and the formation of successive dichotomies on merosporangiophores were not informed in the protolog.

Habitat and geographic distribution: *Piptocephalis lemonnieriana* has been reported in France as a parasite of *Mucor fragilis* Bainier, which was found growing on a birch stump (Vuillemin 1902). It has also been reported on animal dung from Brazil (Santiago *et al*. 2011).

Piptocephalis lepidula (Marchal) Sacc., Syll. fung. (Abellini) 12: 571 (1897)

MycoBank number: 199590

Notes: According to Gräfenhan (1998), this homothallic species is characterized by the formation of striate merosporangiophores with 4–8 successive dichotomies, globose head cells, and merosporangia sustaining 1–3 ellipsoidal merospores, which are 3–8 × 2–3.5 µm. Spore heads remain dry at maturity. Zygospores are yellowish-orange, 36–68 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from Belgium, Brazil, Germany, Japan, the Netherlands, New Zealand, Sweden and Taiwan (Gräfenhan 1998; Santiago *et al*. 2011; GBIF 2022; Genbank 2022); tree bark from the United States of America (Gräfenhan 1998; GBIF 2022); cereal grains from the United Kingdom (Gräfenhan 1998); soil from Argentina, Austria, Estonia, Iceland, the United Kingdom and the United States of America (Gräfenhan 1998; GBIF 2022; Genbank 2022). It has also been reported in Afghanistan, China and India without substrate indication (GBIF 2022). *Piptocephalis lepidula* can parasitize *C. recurvatus*, *Mucor plumbeus* Bonord., and rotting mushrooms (Gräfenhan 1998).

Piptocephalis macrocephala J.H. Mirza, S.M. Khan, S. Begum & Shagufta, *Mucor*. Pakistan, (Univ. Agric. Faisalabad) (Faisalabad): 123 (1979)

MycoBank number: 115005

Notes: According to Gräfenhan (1998) this species is characterized by forming striate merosporangiophores with up to 4 successive dichotomies, lobed head cells, and merosporangia sustaining 6–9 cylindrical merospores, which are 6–8 × 2–3 µm. Spore heads form a liquid droplet at maturity. Zygospores were not observed.

Habitat and geographic distribution: This species was previously reported on animal dung from Pakistan (Gräfenhan 1998).

Piptocephalis microcephala Tiegh., Annls Sci. Nat., Bot. 6(1): 147 (1875)

MycoBank number: 199737

Notes: According to Gräfenhan (1998), this homothallic species is characterized by the formation of striate merosporangiophores with up to 11 successive dichotomies, heart-shaped head cells, merosporangia sustaining up to three cylindrical, 2.5–9 × 2.5–3.5 µm merospores. Spore heads form a liquid droplet at maturity. Zygospores are orangish-brown to brown, 19–40 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from France and the United Kingdom (Gräfenhan 1998; GBIF 2022; Genbank 2022). *Piptocepalis microcephala* can parasitize *C. recurvatus* (Gräfenhan 1998).

Piptocephalis minuta Kuzuha, J. Jap. Bot. 51(4): 123 (1976)

MycoBank number: 320533

Notes: According to Benjamin (1985), this heterothallic species is characterized by the formation of non-striated merosporangiophores with five successive dichotomies, lacking head cells, and merosporangia borne singly on each apex of the ultimate branch, occasionally branched, sustaining 2–5 cylindrical merospores, which are 5–8 × 2–3 µm. Spore heads form a liquid droplet at maturity. Zygospores brown to dark brown, 22–38 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported from soil in Japan (Gräfenhan 1998; GBIF 2022). It has also been reported in Australia, Belarus, Finland, Kyrgyzstan, Russia and the United States of America without substrate indication (GBIF 2022). This species can parasitize *Mortierella humilis* (Gräfenhan 1998).

Piptocephalis moniliformis (R.K. Benj.) N.K. Reynolds, H.M. Ho, Benny & M.E. Sm., in Reynolds, Benny, Ho, Hou, Crous & Smith, Mycologia 111(1): 61 (2019)

MycoBank number: 823737

Notes: According to Benjamin (1985) and Gräfenhan (1998), this homothallic species is characterized by formation of the non-striated merosporangiophores with 2–3 successive dichotomies, lacking head cells. Merosporangia form irregular chains of merospores produced by acropetal budding, and the chains show a zigzag appearance. Merospores are globose, 3.5–4 µm diam., or doliform with rounded ends, 4–9 × 3.5–6 µm. Spore heads remain dry at maturity; zygospores are orangish-brown, 20–33 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution – This species was previously reported from soil in Japan (Gräfenhan 1998; GBIF 2022). This species has also been reported in Panama and Thailand without substrate indication (GBIF 2022).

Piptocephalis pseudocephala P.M. Kirk, Trans. Br. mycol. Soc. 70(3): 337 (1978)

MycoBank number: 320534

Notes: According to Kirk (1978) and Gräfenhan (1998), this species is characterized by the formation of striate merosporangiophores with 5–7 successive dichotomies, lacking head cells. Merosporangia are borne from subglobose or lobed terminal enlargements on the ultimate merosporangiophore branches, sustaining 8–14 cylindrical merospores, which are 3–6 × 2–2.5 µm. Spore heads remain dry at maturity. Zygospores were not observed.

Habitat and geographic distribution: This species was previously reported on leaf litter from the United Kingdom (Gräfenhan 1998; GBIF 2022). *Piptocephalis pseudocephala* can parasitize *C. recurvatus* (Gräfenhan 1998).

Piptocephalis sphaerospora Tiegh., Annls Sci. Nat., Bot., sér. 6 1: 150 (1875)

MycoBank number: 199789

Notes: According Van Tieghem (1875), this species is characterized by the formation of striate merosporangiophores with 2–3 successive dichotomous branches, globose head cells, merosporangia containing 5–8 globose merospores, which are 2–3 µm in diam. Zygospores were not observed. Spore heads were not indicated in the protolog.

Habitat and geographic distribution: *Piptocephalis sphaerospora* has been reported on animal dung from France (Van Tieghem 1875).

Piptocephalis tieghemiana Matr., Bull. Soc. mycol. Fr. 16: 58 (1900)

MycoBank number: 232201

Notes: According to Gräfenhan (1998), this homothallic species is characterized by formation of striate merosporangiophores with 4–8 successive dichotomies, globose head cells, and merosporangia sustaining 2–3 cylindrical to doliform merospores, 3.5–7 × 2–3 µm. Spore heads remain dry at maturity. Zygospores are orangish-brown to brown, 24–41 µm in diam., with a rough–walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from India, Pakistan, Taiwan and the United States of America; beetle larvae from Japan; hog feed and lizard eggs from the United States of America and soy sauce from China (Gräfenhan 1998, GBIF 2022, Genbank 2022). It has also been reported in the Netherlands and South Africa without substrate indication (Gräfenhan 1998). It can parasitize *C. recurvatus*, *Lichtheimia ramosa*, *Mucor hiemalis*, *M. racemosus* and *Rhizopus arrhizus* (Gräfenhan 1998; GBIF 2022).

Piptocephalis unispora R.K. Benj., Mycologia 58(1): 23 (1966)

MycoBank number: 337047

Notes: According to Benjamin (1966) and Gräfenhan (1998), this homothallic species is characterized by formation of striate merosporangiophores with 2–6 successive dichotomies, globose head cells, merosporangia with one obovate or ovate merospore, which is 6–9 × 2–4 µm. Spore heads remain dry at maturity. Zygospores are yellowish-brown to orangish-brown, 5.5–12 µm in diam., with a rough-walled exospore.

Habitat and geographic distribution: This species was previously reported on animal dung from Mexico (Gräfenhan 1998; GBIF 2022). *Piptocephalis unispora* can parasitize *C. recurvatus* (Gräfenhan 1998).

Key for species of genus **Piptocephalis**

1. Head cell not formed 2
1. Head cell formed 5
2. Merosporangia unbranched 3
2. Merosporangia regularly branched or occasionally branched 4
3. Merosporangiophores with terminal enlargements on the ultimate branches ..... *P. pseudocephala*
3. Merosporangiophores without terminal enlargements on the ultimate branches ..... *P. benjaminii*
4. Merospores moniliform ..... *P. moniliformis*
4. Merospores cylindrical ..... *P. minuta*
5. Merospores fusiform ..... *P. fusispora*
5. Merospores not fusiform 6
6. Merospores globose ..... *P. sphaerospora*
6. Merospores not globose 7
7. Spore head forming a liquid droplet at maturity 8
7. Spore head remaining dry at maturity 16
8. Head cells with multiple lobes 9
8. Head cells without multiple lobes 15
9. Ultimate branches of merosporangiophores curvate ..... *P. curvata*
9. Ultimate branches of merosporangiophores not curvate 10
10. Head cells falling at maturity leaving a truncate tip on the ultimate branch of the merosporangiophore .... *P. fimbriata*
10. Head cells collapsing at maturity but not leaving a truncate tip on the ultimate branch of the merosporangiophore 11

11. Merosporangia may contain more than six merospores each 12
11. Merosporangia never containing more than six merospores each 14
12. Ultimate branches often > 40 µm long ..... *P. cruciata*
12. Ultimate branches usually < 40 µm long 13
13. Fertile branch system consisting of up to 4 successive dichotomies ..... *P. macrocephala*
13. Fertile branch system consisting of more than 4 successive dichotomies ..... *P. freseniana*
14. Merospores up to 4 µm long ..... *P. formosana*
14. Merospores up to 9 µm long ..... *P. debaryana*
15. Head cells globose ..... *P. lemonnieriana*
15. Head cells not globose ..... *P. microcephala*
16. Head cells heart-shaped 17
16. Head cells not heart-shaped 18
17. Primary branches of merosporangiophores in whorls of 2–4 ..... *P. indica*
17. Primary branches of merosporangiophores never in whorls ..... *P. xenophila*
18. Merospores ellipsoidal 19
18. Merospores never ellipsoidal 20
19. Merosporangiophores with longitudinal striations ..... *P. lepidula*
19. Merosporangiophores lacking longitudinal striations ..... *P. graefenhanii*
20. Merosporangia unispored ..... *P. unispora*
20. Merosporangia multispored 21
21. Primary branches of merosporangiophores less than 500 µm in length 22
21. Primary branches of merosporangiophores more than 500 µm in length 23
22. Fertile branch system consisting of up to 6 successive dichotomies, merospores doliform to cylindrical ..... *P. brijmohanii*
22. Fertile branch system consisting of more than 6 successive dichotomies, merospores cylindrical ..... *P. cylindrospora*
23. Penultimate branches of branch system with 7 – 20 µm in length, merosporangia 6–11 µm in length ..... *P. tieghemiana*
23. Penultimate branches of branch system with 15 – 40 µm in length, merosporangia 13–25 µm in length ..... *P. arrhiza*

Discussion

In this study we considered 24 *Piptocephalis* species as valid. Gräfenhan (1998) considered *P. dichotomica*, *P. fusispora, P. lemonnieriana*, *P. monospora* and *P. sphaerospora* as doubtful. In Benjamin (1959), Gräfenhan (1998) and in this work, *P. monospora* was not considered as valid because the morphological description by Mangin (1899) was not informative enough to differentiate this species from other *Piptocephalis* species. In the same way, *P. dichotomica* described by Krzemieniewska & Badura (1954) was not considered valid in this study. However, morphological features of *P. fusispora*, *P. lemonnieriana* and *P. sphaerospora* are enough to differentiate these species, which is why they were recognized as valid species by Benjamin (1959) and Zycha *et al.* (1969). In this study we also considered those species as valid despite the fact that Gräfenhan (1998) did not.

According to Reynolds *et al.* (2019), species of *Piptocephalis* formed a well-supported monophyletic group with 25 clades including five still unpublished species. Although they concluded that morphological characters are not phylogenetically informative at the generic level, they are sufficient to separate species of *Piptocephalis*. Among the main morphological characters for identification of *Piptocephalis* are the following: spore heads (if they are wet- or dry-spored), head cell shape, striation and ramification

of merosporangiophores, including pigmentation, diameter, and texture of zygospores (Benjamin 1959; Curtis *et al*. 1978; Gräfenhan 1998). Among the *Piptocephalis* species it is known that 10 species form wet spore heads. The spore head are easily observed macroscopically in cultures. This structure was not cited in the descriptions of *P. fusispora* and *P. sphaerospora* (Gräfenhan 1998).

Most species of *Piptocephalis* form striate merosporangiophores, except *P. curvata*, *P. graefenhanii*, *P. minuta* and *P. moniliformis*. The presence or absence of stolons and rhizoids was used by Van Tieghem (1875) to classify *Piptocephalis* species. Nevertheless, absence, presence, shape and size of both structures vary according to the substrate, growth temperature and host (Curtis *et al*. 1978), thus they should not be used to differentiate *Piptocephalis* species (Benjamin 1959; Gräfenhan 1998).

Leadbeater and Mercer (1957) and Benjamin (1959) also indicated zygospore morphology as an important characteristic for the identification of *Piptocephalis* species. Nonetheless, this structure has not been observed in *P. cruciata*, *P. lemonnieriana*, *P. macrocephala*, *P. pseudocephala* and *P. sphaerospora*. In addition, zygospore formation may require specific conditions, thus we decided not to include zygospore characteristics in our taxonomic key.

To the best of our knowledge, species of *Piptocephalis* have been reported in 43 countries, in temperate (most species) and tropical zones, with the highest number of species reported in the United States of America (10 species), the United Kingdom (eight), Japan (six), and Taiwan (eight). For 12 countries only one species of *Piptocephalis* has been documented (Fig. 3). Reynolds *et al*. (2019) considered that some *Piptocephalis* species have a worldwide distribution, while also highlighting the possible endemism of some species. For example, *P. cruciata*, *P. macrocephala*, *P. pseudocephala*, *P. sphaerospora* and *P. unispora* have only been reported once and from just one country (Gräfenhan 1998; GBIF 2022), and the fact that these species have not been found again may suggest that they are endemic in their countries. However, it is difficult to predict whether the majority of the known species belonging to this genus are endemic or not, because inventories of *Piptocephalis* are rare. This is probably due to the insufficient number of taxonomists specialized in Zoopagales. What we know so far is that all species of *Piptocephalis* described until March 2023 occur in the temperate zone (Fig. 1), and *P. arrhiza*, *P. benjaminii*, *P. cruciata*, *P. fusispora*, *P. macrocephala*, *P. microcephala*, *P. sphaerospora*, *P. pseudocephala* and *P. tieghemiana* have exclusively been reported in this zone. Fourteen species also occur in the tropical zone but there is no species exclusively observed in the tropics.

Piptocephalis species are mostly reported in soil and dung. However, some species have been reported in leaf litter and other substrates (Gräfenhan 1998). According to Richardson & Leadbeater (1972), the frequent occurrence of *Piptocephalis* species on dung does not correspond to susceptibility of this substrate to mycoparasites, but to the high prevalence of mucoralean fungi that are common hosts of *Piptocephalis* on dung, which may also be valid for soil and leaf litter.

The low number of species reported in South America is probably related to the scarcity of studies focused on zoopagalean fungi. Herein we report the first occurrence of two *Piptocephalis* species in South America, both parasitizing *Mucor* sp. on herbivore dung, contributing to knowledge of these fungi in the tropical zone.

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