MYCOTAXON

ISSN (print) 0093-4666 (online) 2154-8889 Mycotaxon, Ltd. ©2017

April–June 2017—Volume 132, pp. 459–470

https://doi.org/10.5248/132.459

Sporormiella longicolla sp. nov. and new *Sporormiella* records on herbivore dung from Brazil

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Abstract—Seven species of *Sporormiella* are reported from fresh dung collected in a survey including different vegetation areas throughout Pernambuco, Northeastern Brazil. An undescribed, long-necked, small-spored species is proposed as *Sporormiella longicolla*. *Sporormiella isomera* is a new record for the Neotropics; *S. australis*, *S. leporina*, and *S. pentamera* are new records for Brazil; and *S. herculea* (a rare species) and *S. minima* are also recorded. Descriptions, photographic plates and digital line drawings are provided, along with an identification key to all *Sporormiella* species recorded in Brazil. Substrate relationships and distribution data of these fungi are also discussed.

Key words—*Ascomycota*, coprophilous fungi, *Pleosporales, Sporormiaceae,* taxonomy

Introduction

Sporormiella Ellis & Everh. 1892 comprises ~80 pseudothecial species, usually with dark, 4-celled ascospores, commonly found on herbivore dung worldwide (Kirk et al. 2008). The genus is characterized by small, dark brown, glabrous or hairy uniloculate pseudothecia, bitunicate asci, cylindrical to clavate, bearing eight three–to many–septate, dark brown ascospores with conspicuous germ slits, surrounded by a hyaline gelatinous sheath (Ahmed & Cain 1972). Species of *Sporormiella* are predominantly coprophilous (Richardson 2001b), with occasional records as saprobes on

other substrata, and even as endophytes (Sun et al. 2006). Recently, the genus has received attention as a paleontological indicator to trace the wave of human invasion, habitat destruction, and subsequent megafaunal extinctions in Quaternary lake deposits (Comandini & Rinaldi 2004, Raper & Bush 2009).

The monophyly of *Sporormiella*, as well as other members of *Sporormiaceae* Munk, was studied by Kruys & Wedin (2009), who suggested a new generic classification of the family, in which *Preussia* Fuckel would include *Sporormiella* and *Sporormimula* Arx & Aa. Although Kruys & Wedin (2009) argued for the synonymy of *Preussia* and *Sporormiella*, they demonstrated no significant support for a joint monophyletic grouping and stated that resolution of the *Preussia–Sporormiella* complex required further study.

In Brazil, there have been few concentrated surveys on coprophilous mycobiota, and little is known about *Sporormiella*. After a visit to Brazil in 1998, Richardson (2001a) recorded *S. minima* and *S.* cf. *megalospora* (Auersw.) S.I. Ahmed & Cain in Bonito and Pantanal do Rio Negro, Mato Grosso do Sul State, providing short descriptions and discussing the importance of further investigations. Here, the *Sporormiella* species recorded on dung from Brazil are revisited, including revision of herbaria material, and new records and an undescribed species from fresh material collected in Pernambuco State.

Material & methods

Dung samples of camel (*Camelus bactrianus*), deer (*Cervus elaphus*), llama (*Lama glama*), and waterbuck (*Kobus ellipsiprymnus*) were collected from August 2009 to July 2010 in a zoological park in Recife (8°07′30″S 34°52′30″W), and samples of cattle (*Bos taurus*), goat (*Capra hircus*) and horse (*Equus caballus*) dung were collected from animal precincts on the campus of Universidade Federal Rural de Pernambuco, Recife (8°00′54″S 34°56′59″W), and from farms close to the Instituto Agronômico de Pernambuco (IPA) in Caruaru (8°01′59″S 36°06′59″W) and Serra Talhada (7°54′59″S 38°17′00″W), all located in Pernambuco, Northeastern Brazil. The samples were collected with sterilized spatulas, placed in clean plastic bags, taken to the laboratory in less than 24 hours and incubated in moist chambers at room temperature (28 ± 2 °C) for at least 60 days under alternating natural light and dark periods.

The specimens were observed directly from the substrata under a Leica EZ4 stereomicroscope. Pseudothecia were mounted in tap water and/or lactophenol with cotton blue for measurements and identification, and in Polyvinyl–Lacto–Glycerol resin for preservation. Specimens were identified based on morphology according to Ahmed & Cain (1972), Bell (1993, 2005), Richardson & Watling (1997), and

Doveri (2004). A survey of representative national collections of fungi (acronyms following Thiers 2016) was also conducted, including the herbaria Pe. Camille Torrend (URM), Dárdano de Andrade Lima (IPA), Maria Eneyda P. Kaufmann Fidalgo (SP), Dimitri Sucre Benjamin (RB), Instituto Nacional de Pesquisas da Amazônia (INPA), Museu Paraense Emilio Goeldi (MG), and Instituto de Biociências, Universidade Federal do Rio Grande do Sul (ICN). High quality images were captured with a QImaging QColor 3 digital camera mounted on an Olympus BX51 compound microscope using differential interference or phase contrast microscopy. Methods for the digital line drawing illustrations of both pseudothecia and microscopic structures were adapted from Barber & Keane (2007). Permanent slides were mounted and deposited in Herbário Padre Camille Torrend, Departamento de Micologia, Universidade Federal de Pernambuco, Recife, Brazil (URM).

Taxonomy

Sporormiella australis (Speg.) S.I. Ahmed & Cain,
Canad. J. Bot. 50: 434 (1972) Fig. 1a–d

Pseudothecia scattered, immersed when young, becoming partially immersed to superficial on dung when mature, subglobose to obpyriform, dark brown to black, 185–210 × 145–175 µm diam. Neck small, papilliform, glabrous, black. Peridium membranaceous, semitransparent, composed of angular cells. Asci bitunicate, 8-spored, cylindrical to clavate, broader near the apex, rounded above, tapering abruptly downwards to form a short stipe, 125–140 × 14.5–18 µm. Ascospores obliquely triseriate, less commonly biseriate, 4-celled, cylindrical, rounded at the ends, light brown to brown, 37.5–40 × 6–7.5 µm, smooth, with easily separable segments in all septa, germ slits oblique, surrounded by a gelatinous sheath.

Material examined: **BRAZIL**. **Pernambuco**, Caruaru, Instituto Agronômico de Pernambuco (IPA), on cattle dung, 6.VI.2012, R.F.R. Melo (URM86773); Recife, Horto Zoobotânico do Parque Dois Irmãos, on goat dung, 12.IX.2011, R.F.R. Melo (URM86775).

Notes: *Sporormiella australis* can be easily mistaken for other common *Sporormiella* species based on ascospore shape, which are smaller in *S. minima* (27.5–30 × 4–5 µm) and larger in *S. intermedia* (Auersw.) S.I. Ahmed & Cain (48–59 × 9.5–11.5 µm) (Ahmed & Cain 1972). The ascospore cells of *S. australis* do not easily separate in all septa.

Sporormiella herculea (Ellis & Everh.) S.I. Ahmed & Cain,
Canad. J. Bot. 50: 442 (1972) Fig. 1e–i

Pseudothecia scattered, immersed on dung, obpyriform, dark brown to black, 210–275 × 130–155 µm diam. Neck cylindrical, glabrous, black.

Peridium membranaceous, semitransparent to opaque, composed of angular cells. Asci bitunicate, 8-spored, cylindrical-clavate, with somewhat narrowed apex, tapering abruptly below to form a short stipe, 235–270 × 45–50 µm. Ascospores obliquely uni- to biseriate, 6–12-celled, cylindrical to fusiform, with a wide morphological diversity, rounded or narrowed at the ends, with some cells larger and thicker than the others, usually with the uppermost spore in the ascus bearing a greatly enlarged and pigmented cell, dark brown at maturity, 87.5–112 × 15–20(–25) µm, smooth, with transverse germ slits, surrounded by a gelatinous sheath.

Material examined: **BRAZIL**. **Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on horse dung, 9.I.2012, R.F.R. Melo s.n. (URM86777); Serra Talhada, Instituto Agronômico de Pernambuco, on horse dung, R.F.R Melo s.n. (URM86776).

Notes: *Sporormiella herculea* is distinguished by its 6–16-celled ascospores, with the second to fifth cell sometimes greatly enlarged in the uppermost spore in the ascus. Ahmed & Cain (1972) discussed morphological differences of Brazilian records.

Sporormiella isomera S.I. Ahmed & Cain, Canad. J. Bot. 50: 445 (1972) Fig. 1j–l

Pseudothecia scattered, immersed when young, becoming partially immersed on dung when mature, subglobose, dark brown to black, 190–245 µm in diam. Neck small, papilliform, glabrous, black. Peridium membranaceous, semitransparent, composed of angular cells. Asci 8-spored, cylindrical-clavate, broader near the apex, tapering gradually downwards to a short stalk, 130–150 × 12.5–15 µm. Ascospores obliquely biseriate, 4-celled, cylindrical, rounded at the ends, light brown when young, becoming dark brown at maturity, 35–40 × 5–7 µm, smooth, with easily separable segments in all septa, with germ slits parallel to oblique, with a crook near the middle, surrounded by a gelatinous sheath.

Material examined: **BRAZIL**. **Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on camel dung, 5.IV.2010, R.F.R. Melo s.n. (URM82397, 82398).

Notes: *Sporormiella isomera* is similar to *S. leporina*, except it possesses easily separable rounded terminal ascospore cells with a germ slit crooked near the middle. *Sporormiella minima* differs in having smaller ascospores (27.5–30 × 4–5 µm) and pseudothecia (115–165 × 90–125 µm) and asci that are abruptly constricted to form a stipe at their base.

Sporormiella leporina (Niessl) S.I. Ahmed & Cain,
Canad. J. Bot. 50: 447 (1972) Fig. 1M,N

Pseudothecia isolated to scattered, immersed when young, becoming partially immersed on dung when mature, subglobose, dark brown to black, 180–195 × 150–170 µm. Neck cylindrical, glabrous, black. Peridium membranaceous, semitransparent, composed of angular cells. Asci 8-spored, cylindrical–clavate, with a short stalk, 95–125 × 12–17.5 µm. Ascospores obliquely biseriate, 4-celled, cylindrical, narrowed at the ends, light brown when young, becoming dark brown at maturity, 30–36.5 × 5–6.5 µm, smooth, equally separable in all segments, with germ slits strongly oblique, surrounded by a gelatinous sheath.

Material examined: **BRAZIL**. **Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on llama dung, 02.II.2010, R.F.R. Melo s.n. (URM82395, 82396).

Notes: *Sporormiella leporina* can be distinguished by its cylindrical-clavate asci that are wider near the apex and gradually narrow toward the base and its ascospore cells with oblique germ slits.

Sporormiella longicolla R.F.R. Melo, **sp. nov.** Figs 2, 3

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Differs from 4-celled *Sporormiella* species by its longer neck, longer and narrower asci, and ascospores with persistently united cells.

Type: Brazil. Pernambuco: Recife, Horto Zoobotânico do Parque Dois Irmãos, on horse dung, 29.II.2012, R.F.R. Melo s.n. (**Holotype,** URM87584).

Etymology: *longicolla* = with a long neck.

Pseudothecia isolated to gregarious, immersed to semi–immersed on the substrate, subglobose to obpyriform, dark brown to black, 450–650 × 140–220 µm, glabrous. Neck cylindrical, long, glabrous, carbonaceous, black, 250–460(–550) × 40–65 µm, straight or flexuous. Peridium pseudoparenchymatous, membranaceous, composed by angulated, thick–walled cells, 6–12 µm long. Asci bitunicate, 8-spored, cylindrical-clavate, tapering slightly downwards to form a long stipe, twice the length of the sporiferous part, (72–)95–105(–137.5) × 7.5–10 µm. Ascospores obliquely biseriate, 4-celled, slightly apiculate on both ends, hyaline when young, becoming brown to dark brown when mature, 17.5–20.5 × 3–3.5 µm, smooth, usually with the cells remaining united even after the spore liberation, with longitudinal germ slit, surrounded by a thin hyaline gelatinous sheath. Anamorph unknown.

Additional material examined: **BRAZIL**. **Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on horse dung, 15.II.2012, R.F.R. Melo s.n. (URM87585).

Notes: *Sporormiella longicolla* has pseudothecia with long prominent necks, long slender asci with stipes c. 2× the length of the sporiferous part, and small stout ascospores with cells that remain united after spore liberation. This combination of morphological characters supports separation of a new species.

Sporormiella minima (Auersw.) S.I. Ahmed & Cain,
Pakistan J. Sci. Industr. Res. 12: 241 (1970) Fig. 1o–s

Pseudothecia isolated to gregarious, immersed to semi–immersed, subglobose to obpyriform, dark brown, 115–165 × 90–125 µm. Neck papilliform, glabrous, black, 45–50 × (35–)40–50 µm. Asci bitunicate, 8-spored, cylindrical, with the lower portion abruptly constricted to form a short stipe, 90–97.5 × 12.5–15 µm. Ascospores obliquely biseriate to triseriate, 4-celled, rounded at the ends, hyaline when young, becoming brown to dark brown, 27.5–30 × 4–5 µm, smooth, easily separable at the central septum, with germ slits parallel to the spore axis, with a crook at the central portion on each cell, surrounded by a gelatinous sheath.

Material examined: **BRAZIL**. **Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on llama dung, 13.X.2009, R.F.R. Melo (URM82389); 2.II.2010, R.F.R. Melo (URM82390); 1.III.2010, R.F.R. Melo (URM82391); 25.III.2010 (URM82392); 5.IV.2010 (URM82393); 2.VI.2010 (URM82394); on guinea pig dung, 1.XII.1947 (IPA37901).

Notes: *Sporormiella minima* is the most common *Sporormiella* species on herbivore dung from Brazil. Its striking features include the short ascus stipe formed by an abrupt end at its base, small ascospores (27.5–30 × 4–5 µm in the examined material) with a tendency to separate only at the central septum (even inside the asci), and parallel germ slits that form a distinct crook at the middle. Exsiccata IPA 37901 contained, along with the holotypus of *Ascobolus cuniculorum* Bat. & Pontual, several pseudothecia of *S. minima*, well preserved on dry guinea pig dung.

Sporormiella pentamera (Oudem.) S.I. Ahmed & Cain,
Canad. J. Bot. 50(3): 454 (1972) Fig 1t–v

Pseudothecia scattered to isolated, immersed to partially immersed on dung, subglobose, dark brown to black, 190–215.5 × 30–50 µm. Neck small, papilliform, glabrous, black. Asci 8-spored, clavate, with rounded apex, broader near the middle, tapering slightly downwards to form a short stipe, 190–210 × 15–17 µm. Ascospores biseriate, 5-celled, cylindrical, rounded to slightly narrowed at the ends, light brown when young, becoming dark

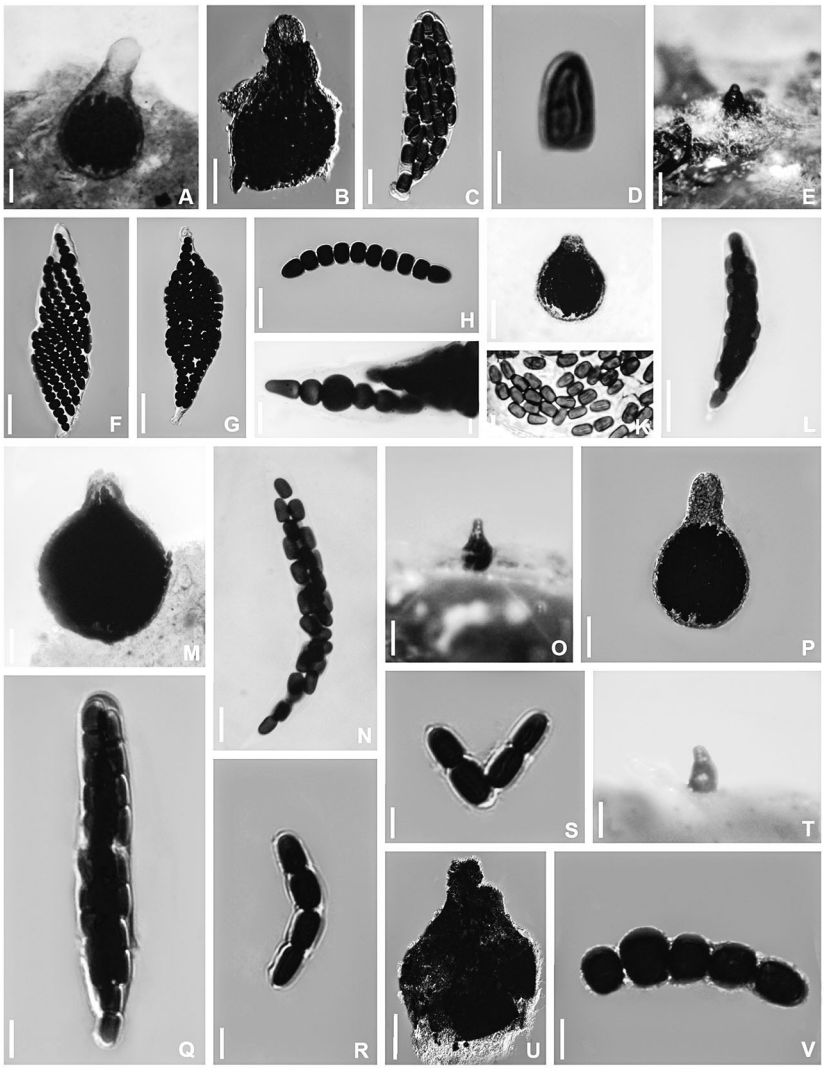

Fig. 1. *Sporormiella* species recorded on herbivore dung from Brazil. ***S. australis*** (URM86773): A. pseudothecium on dung; B. pseudothecium in mountant; C. mature ascus with cells united or equally separated on each septum; D. ascospore cell with a visible germ slit. ***S. herculea*** (URM86777): E. pseudothecium on dung; F, G. mature asci; H, I. ascospores. ***S. isomera*** (URM82398): J. pseudothecium in mountant; K. ascospores equally separated in all septa; L. mature ascus. ***S. leporina*** (URM82395): M. pseudothecium in mountant; N. mature ascus. ***S. minima*** (URM82391): O. pseudothecium on dung; P. pseudothecium in mountant; Q. mature ascus; R, S. ascospores, usually separated in the middle septum. ***S. pentamera*** (URM86786): T. pseudothecium on dung; U. pseudothecium in mountant; V. ascospores. Scale bars: A, B, F, G, U = 50 µm; C = 25 µm; D, R, S = 5 µm; E = 300 µm; H, I, L, M, P = 40 µm; J, O = 100 µm; K, Q = 10 µm; N, V = 15 µm; T = 150 µm.

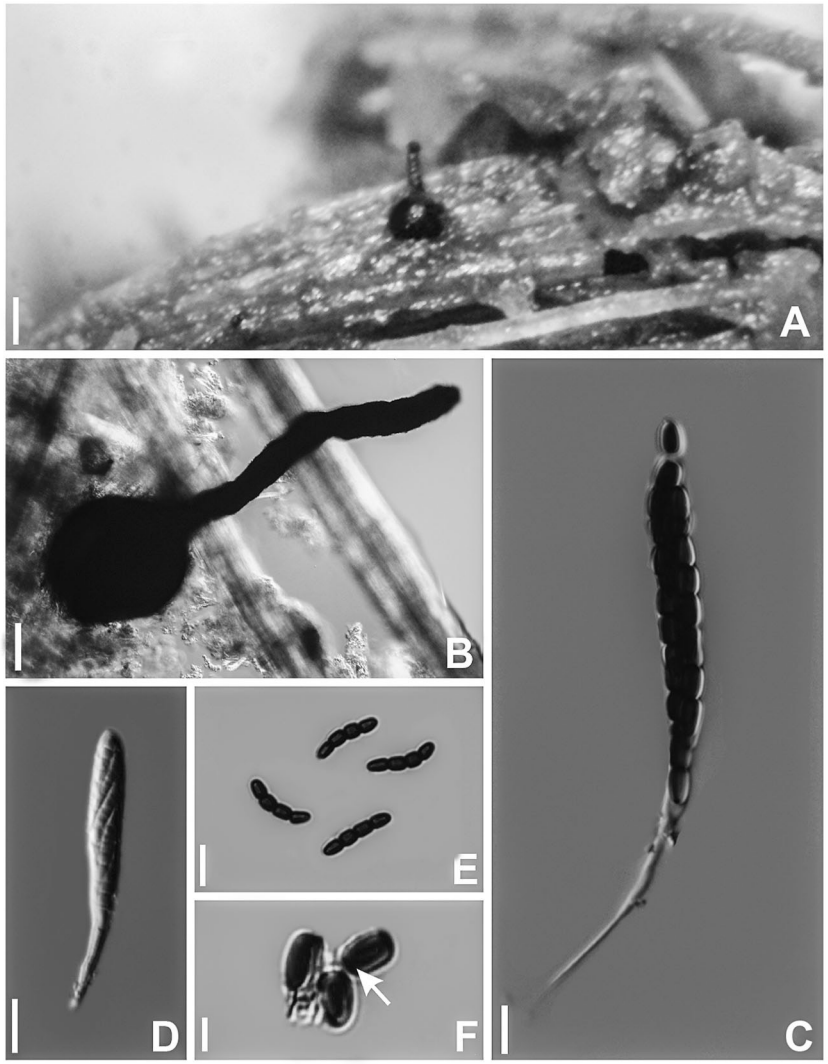

Fig. 2. ***Sporormiella longicolla*** (holotype, URM87584): A. pseudothecium on dung; B. pseudothecium in mountant; C. mature ascus; D. young ascus; E. ascospores with united cells even after their liberation; F. ascospore cells with visible germ slits (arrow). Scale bars: A = 300 µm; B = 60 µm; C = 7.5 µm; D = 15 µm; E = 10 µm; F = 2.5 µm.

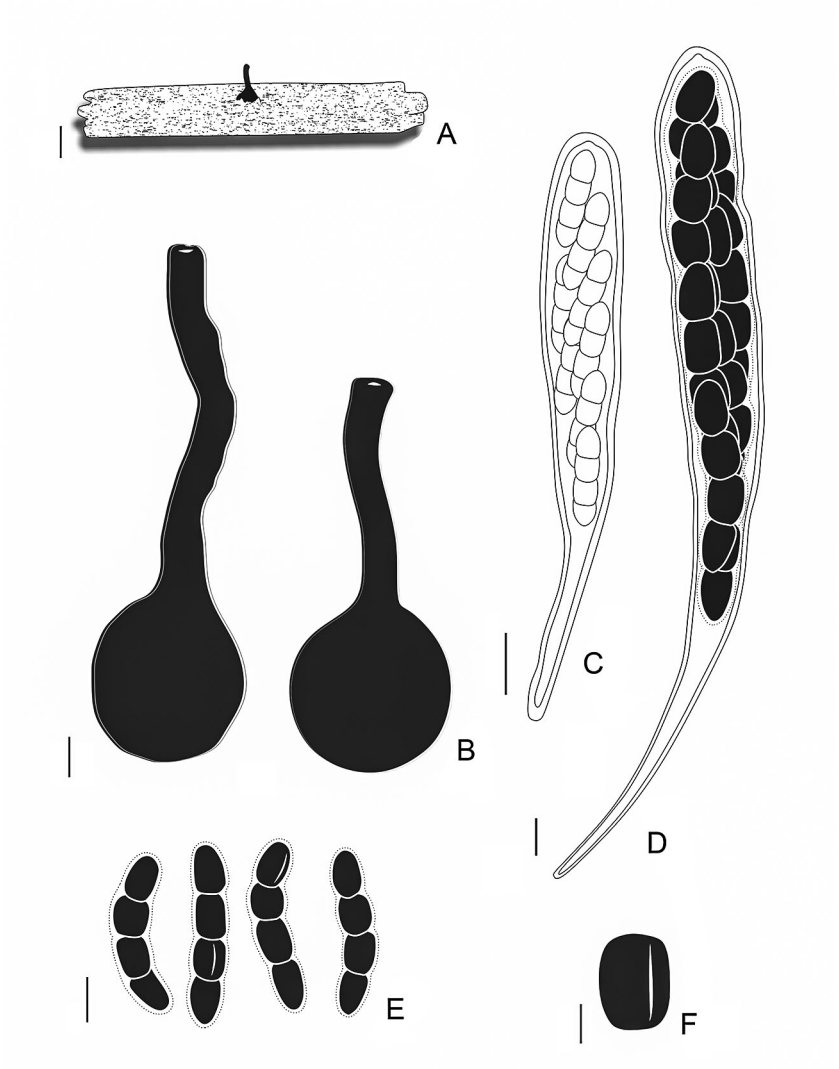

Fig. 3. ***Sporormiella longicolla*** (holotype, URM87584): A. pseudothecium on dung. B. long–necked pseudothecia. C. young ascus . D. mature ascus. E. ascospores with united cells even after their liberation. F. ascospore cell with a visible germ slit. Scale bars: A = 1 mm; B = 50 µm; C = 10 µm; D, E = 5 µm; F = 2 µm.

brown at maturity, 65–72.5 × 18–20.5 µm, usually with the second cell from the upper end of the spore larger than the others, smooth, with segments not easily separable, germ slits oblique, surrounded by a gelatinous sheath.

Material examined: **BRAZIL**. **Pernambuco**: Recife, Horto Zoobotânico do Parque Dois Irmãos, on horse dung, 13.XI.2011, R.F.R. Melo (URM86786).

Key to species of *Sporormiella* on herbivore dung from Brazil

1. Ascospores 4-celled 2
   Ascospores >4-celled 3
2. Neck >250 µm long; ascospores 17.5–20.5 µm long ..... *S. longicolla*
   Neck <200 µm; ascospores >25 µm long 7
3. Asci tapering abruptly below to form a short stipe 4
   Asci tapering gradually from the broadest part into an elongated stipe 5
4. Ascospores 27.5–30 × 4–5 µm; germ slit parallel, forming a crook near the middle, with cells readily separable only at the central septum ..... *S. minima*
   Ascospores 37.5–40 × 6–7.5 µm; germ slit slightly to strongly oblique and cells easily separable at all segments ..... *S. australis*
5. Ascospores 80–90 × 19 µm; germ slits parallel to the long axis ..... *S.* cf. *megalospora*
   Ascospores shorter, <30 µm long; germ slits parallel to oblique 6
6. Germ slit parallel to oblique, with a crook near the middle ..... *S. isomera*
   Germ slit oblique, without a crook near the middle ..... *S. leporina*
7. Ascospores 5-celled ..... *S. pentamera*
   Ascospores 10–16-celled ..... *S. herculea*

Discussion

Due to their small size and frequent immersed habitat, *Sporormiella* pseudothecia can easily be overlooked in surveys of coprophilous fungi. Among the herbaria consulted, only two exsiccatae contained material collected in Brazil, one (IPA37901) representing a good specimen of *S. minima* and the other (URM1086) lacking a specific identification and not well preserved enough to allow accurate revision. Our study shows the flexibility regarding substrate preference and vegetation region by *Sporormiella* species. No clear substrate and/or regional preference could be detected. *Sporormiella minima,* with the highest number of records, was the most common *Sporormiella* species in herbivore dung in Brazil. These fungi usually specialize in exploiting herbivore dung and only occasionally have been recorded on other substrates due to stresses or disturbances affecting the community structure over the time.

According to Kruys & Wedin (2009), at one time *Sporormiella* circumscription was restricted to coprophilous species. Despite being controversial, the presence of the ostiole on the ascomata (traditionally used to separate *Preussia* from *Sporormiella*) appears to follow substrate preferences of species for both genera: *Preussia* species are non-ostiolate and found on plant debris, wood, or soil (Cain 1961), while *Sporormiella* species are ostiolate and coprophilous. This statement is supported by the polyphyly observed between the genera by Kruys & Wedin (2009) and by the inconsistent ostiole developmental pattern observed in *Sporormiaceae* by Guarro et al. (1997). The importance of active spore liberation is known in the study of coprophilous fungi (Ingold 1965, Webster 1970, Krug et al. 2004). However, development of the active method of liberation as an adaptation to coprophilous substrates in *Sporormiaceae* has yet to be studied.

Common species, like *S. minima* and *S. australis*, were found throughout the year alongside less common species. All exsiccatae containing representatives of *Sporormiella* in Herbarium Pe. Camille Torrand (URM) were collected in other countries under the name *Sporormia* and thus were not included in this treatment.

Acknowledgments

The authors thank Profa. Marcela Caceres (Universidade Federal de Sergipe) and Prof. Jadergudson Pereira (Universidade Estadual de Santa Cruz, Ilhéus) for expert presubmission reviews.

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