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Pilobolus species found on herbivore dung from the São Paulo Zoological Park, Brazil

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RESUMO – (Espécies de *Pilobolus* encontradas em fezes de herbívoros do Parque Zoológico de São Paulo, Brasil). Para o estudo de espécies de *Pilobolus*, foram coletadas 168 amostras de fezes de animais herbívoros no Parque Zoológico da cidade de São Paulo. Dez espécies foram verificadas, ilustradas e descritas e uma chave de identificação é apresentada.

Palavras-chave: fungos coprófilos, Mucorales, Zygomycota

ABSTRACT – (*Pilobolus* species found on herbivore dung from the São Paulo Zoological Park, Brazil). A study of *Pilobolus* species from 168 dung samples of various herbivorous animals, collected in the São Paulo Zoological Park, was carried out. Ten species were found, illustrated, described, and a key for their identification is provided.

Key words: coprophilous fungi, Mucorales, Zygomycota

Introduction

Pilobolus is a saprotrophic genus belonging to the Mucorales (Zygomycota), frequently found in herbivorous animals feces (Alexopoulos *et al*. 1996). The genus is characterized by coprophilous habit, positive phototropism and method of spore dispersal. In the dispersal process, the mature sporangium is thrown more than 2 meters by dehiscence of the mucilage found at the junction of the columella with the sporangium, caused by the rupture of the subsporangial vesicle. This is why it is called "spore-gun" dispersal (Ingold 1971). The group was studied and its taxonomy revised by Grove (1934). Since then there have been few works on the taxonomy of the group (Nand & Mehrotra 1968; Tandon 1968; Zycha *et al*. 1969). In Brazil, *Pilobolus* species have been recorded by Trufem (1984), Viriato & Trufem (1985) and Richardson (2001). The species of *Pilobolus* are attached to the substrate by an absorptive structure, the swollen trophocyst, semi-immersed in the substrate at the base of the sporangiophore. Generally the trophocysts are ovoid to globose, with a long-cylindrical, rhizoidal extension. The sporangiophores are straight, never branched, positively phototropic, with two rings of orange pigment, one at the base and the other at the base of the subsporangial vesicle. The orange carotenoid pigments act as light sensors which are lined up with each other by the expanded subsporangial vesicle acting as a lens, so that the sporangium is always aimed at the brightest light. The sporangia are black, sub-hemispherical and have resistant walls. The columellae are generally smooth and long-elliptical, and sometimes mammiform. The spores are spherical to ellipsoid, and generally smooth walled, hyaline or with carotenoid pigments. The diagnostic structure of the *Zygomycota* is the zygosporangium, containing the zygospore, formed by the conjugation and fusion of two gametangia. No zygospores were observed during the study. The objective of this research is to contribute to the knowledge of the biodiversity of this group of fungi and to learn about their distribution on herbivorous dung from different species of mammals.

Material and methods

One hundred and sixty-eight samples of animal feces [camel (*Camelus bactrianus* L.), horse (*Equus caballus* L.), "cotia" (*Dasyprocta fuliginosa* L.), "guanaco" (*Lama guanacoe* Muller), "orix" (*Orix gazela*

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L.), "veado-catingueiro" (*Mazama gouazoubira* Fischer), "veado-nobre" (*Cervus elaphus* L.), zebra (*Equus burchelli* Gray)] were collected monthly (from September 1994 to July 1995) from the Fundação Parque Zoológico de São Paulo by the breeder in the morning, using wooden spatulas, being careful to keep the samples intact. The samples were incubated in moist chambers, sterilized Petri-dishes, in a laboratory with ambient light and temperature conditions, for four or five days, when the observations were made. The morphology of each *Pilobolus* species was studied, measured with an eyepiece micrometer coupled to the optical microscope, and drawn with a drawing attachment/camera lucida. As *Pilobolus* specimens do not develop well in culture, they were removed end immediately examined.

Results

The following key is for the species we observed.

Key for the identification of *Pilobolus* species

1. Spores spherical 2
1. Spores not spherical 7
   2. Spores with twin walls ..... 8. ***Pilobolus oedipus***
   2. Spores with single walls 3
      3. Spores 10-20 µm diam 4
      3. Spores < 10 µm diam 5
         4. Sporangiophores 0.4-0.5 mm high ..... 1. ***P. borzianus***
         4. Sporangiophores 4-6 mm high ..... 10. ***P. sphaerosporus***
            5. Sporangiophores 1-3 mm high ..... 5. ***P. longipes***
            5. Sporangiophores smaller 6
               6. Spores yellow, 5-7 µm diam ..... 6. ***P. morinii***
               6. Spores hyaline, 3.5-4 µm diam ..... 7. ***P. nanus***
                  7. Spores variously shaped ..... 3. ***P. heterosporus***
                  7. Spores ellipsoid to short-cylindrical 8
                     8. Sporangiophores 0.8-0.95 mm high; spores hyaline, short elliptical ..... 9. ***P. pullus***
                     8. Sporangiophores > 1.5 mm high; spores yellow, elliptical to short cylindrical 9
                        9. Columellae conical; trophocysts 500-700 µm long ..... 4. ***P. kleinii***
                        9. Columellae mammiform; trophocysts 350-400 µm long ..... 2. ***P. crystallinus***

1. ***Pilobolus borzianus*** Morini, Mem. Accad. Sci Inst. Bolgna ser. 6, 3: 126. 1906.
   Fig. 1-8

Trophocysts ovoid to globose, 250-300×150-250 µm; rhizoidal extensions up to 150 µm, little orange pigmentation; sporangiophores long-cylindrical, 400-500×90-110 µm; sporangia black, globose, 250-290×180-200 µm; columellae smooth, long-elliptical, 130-150×100-120 µm; subsporangial vesicles smooth, ovoid, 270-370×200-250 µm; spores smooth, yellow, with grainy contents, spherical, 10-20 µm diam. Collected from dung of horse (Oct., Nov., 1994, Jan., May, 1995), "guanaco" (Sep., 1994), "orix" (Sep., Dec.,1994, June, July, 1995), "veado-catingueiro" (October/1994), "veado-nobre" (February/1995, June/1995, July/1995), zebra (October/1994, May/1995). The observed material corresponds well with to the descriptions of Grove (1958), Naumov (1939) and Nand and Mehrota (1968). *Pilobolus borzianus* was recorded in the State state of São Paulo by Viriato and Trufem (1985) and the current material corresponds well with to their description. *P. borzianus* differs from the other species of the genus with spherical spores because of the spore size, which can reach 20 µm diam. As regards to the sporangiophore height, only *P. morinii* approaches that of *P. borzianus*, but its spores are much smaller (up to 7 µm diam.), and its columellae are semi elliptical, while those of *P. borzianus* are long-elliptical.

2. ***Pilobolus crystallinus*** (Wiggers) Tode, Schriften Naturforschenden Froukleich Berlin 5: 96. 1784.
   Fig. 9-15

Trophocysts ovoid to globose, 350-400×250-300 µm, with rhizoidal extensions up to 400 µm long, little orange

pigmentation; sporangiophores long cylindrical, 1.5-5.0 mm×115-150 µm, black sporangia, hemispherical to ovoid, 250-350×240-340 µm, resistant and smooth walled; smooth columellae, mammiform, 180-200× 160-175 µm, subsporangial vesicles with smooth walls, without pigmentation, ovoid to globose 500-700× 400-600 µm, yellow spores, homogeneous content, smooth, short-cylindrical, 8.0-10×7.0-9.0 µm. Collected from dung of horse (December/1994, June/1995), "guanaco" (October/1994), "orix" (December/1994), "veado-catingueiro" (November, December/1994, May/1995), zebra (September/1994, October/1994, December/1994, January/1995, April/1995). The characteristics of the material well with the descriptions of Naumov (1939), Boedjin (1958), Grove (1958), Tandon (1968), and Richardson & Watling (1969). *Pilobolus crystallinus* was also mentioned by Viriato and Trufem (1985) in the of São Paulo. It differs from the other species of the genus due to the short-cylindrical spores, up to 10 µm long. The only species with similar characteristics is *Pilobolus pullus*, but it differs in sporangiophore height, because *P. crystallinus* is about 1,0 mm larger. *P. pullus* spores are short-elliptical and can reach up to 12 µm long, bigger than in *P. crystallinus*.

2. ***Pilobolus heterosporus*** Palla, Zur Kenntniss der Pilobolus Arten, Österreichische Botanische Zeitschrift 50: 349. 1900.
Fig. 16-22

Trophocysts ovoid to globose, short-ellipsoid, 300-400×250-300 µm, with rhizoidal extensions up to 150 µm long, little yellow pigmentation; sporangiophores long-cylindrical, 2.0-3.0 mm×100-120 µm, black sporangia, hemispherical and ovoid, 350-400× 250-350 µm, resistant wall; conical columellae, 140-150×120-135 µm, little pigmented subsporangial vesicles, ovoid and ellipsoid, 450-550×350-400 µm; smooth spores, soft yellowish grainy pigmentation, globose, oval, piriform to irregular, (8.5)10.0-18.0(20.0)×(5.0)7.0-9.0(11.0) µm. Collected from zebra's dung (October/1994). The observed material shows similarities with the descriptions of Naumov (1939), Grove (1958), Tandon (1968), and Nand & Mehrotra (1968). *Pilobolus heterosporus* was cited by Viriato & Trufem (1985) in the State of São Paulo. The referred taxon differs from the other species of the genus due to its irregular spores with grainy contents.

4. ***Pilobolus kleinii*** van Tieghem, Trois. Mém. Annales des Sciences Naturalles, ser 6. 4: 337-338. 1876.
Fig. 23-29

Trophocysts ovoid to globose, with yellow pigments, globose to elliptical, 550-700×300-450 µm, with rhizoidal extensions up to 250 µm; sporangiophores long-cylindrical 3.0-5.0 mm× 100-135 µm; black sporangium, hemispherical to lowered, 350-400× 150-300 µm diam., smooth and resistant wall, smooth columellae, conical, 200-205×150-190 µm; smooth subsporangial vesicles, hyaline, ovoid to subellipsoid, 500-700×450-550 µm; yellow spores, refringent and with slightly grainy contents, smooth, ellipsoid, 11-9.0× 8.0-5.0 µm. Collected from dung of camel (April/1995), horse (November/1994, February/1995, March/1995, May/1995), and zebra (September/1994, January/1995, February/1995, March/1995, June/1995). The characters of the are similar to the descriptions of Nand and Mehrotra (1968), Richardson and Watling (1969), and Liu and Yang (1973). For the state of São Paulo, the species was described and illustrated by Trufem (1984) and Viriato and Trufem (1985), and both descriptions correspond to the current material.

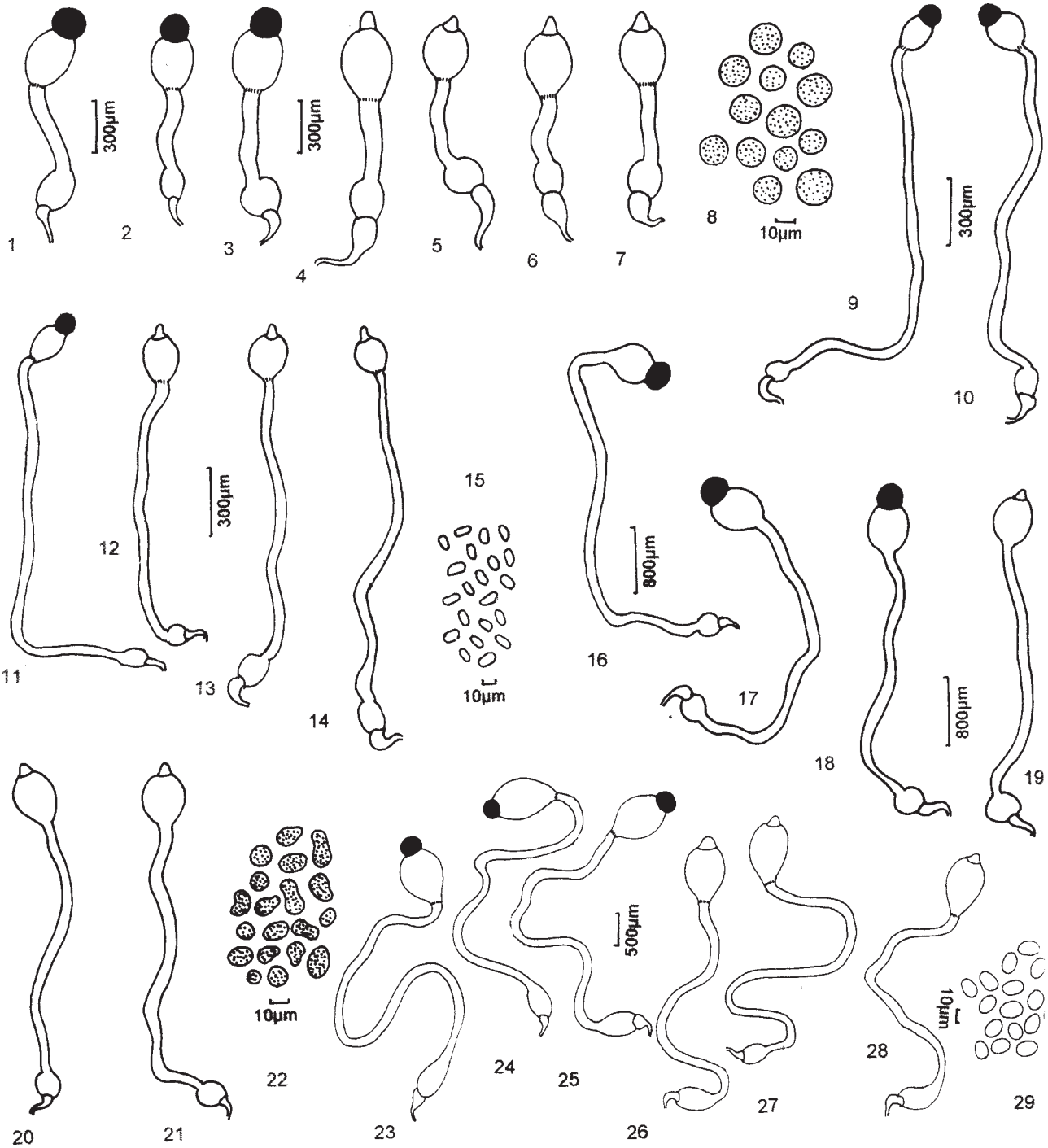
5. ***Pilobolus longipes*** van Tieghem, Trois. Mém. Annales des Sciences Naturalles, ser. 6. 4: 338-340. 1876.
Fig. 30-36

Trophocysts ovoid to globose, long-cylindrical, 400-750×200-300 µm, with rhizoidal extensions up to 950 µm, with a soft yellow pigmentation; sporangiophores 1.0-3.0 cm×90-110 µm; black sporangium, globose, ovoid to slightly levelled, 300-350×250-200 µm, resistant wall, with up to 1.0 µm high thorns, smooth, conical columellae, 155-170× 140-150 µm; subsporangial vesicles with smooth walls, little pigmentation, ovoid, 650-700×500-600 µm; yellow spores, grainy content, smooth, spherical, 6.0-15 µm diam. Isolated from horse (July/1995), and zebra (February/1995, June/1995, August/1995). The characters of the are similar to the descriptions of Grove (1958), Nand & Mehrotra (1968), and Tandon (1968). Spores of *Pilobolus sphaerosporus* and *P. oedipus* are almost the same size as those of *P. longipes*, but those of the former are lightly pigmented and reaches 14 µm diam., while the latter has spores with twin walls, which was not seen in the material being discussed.

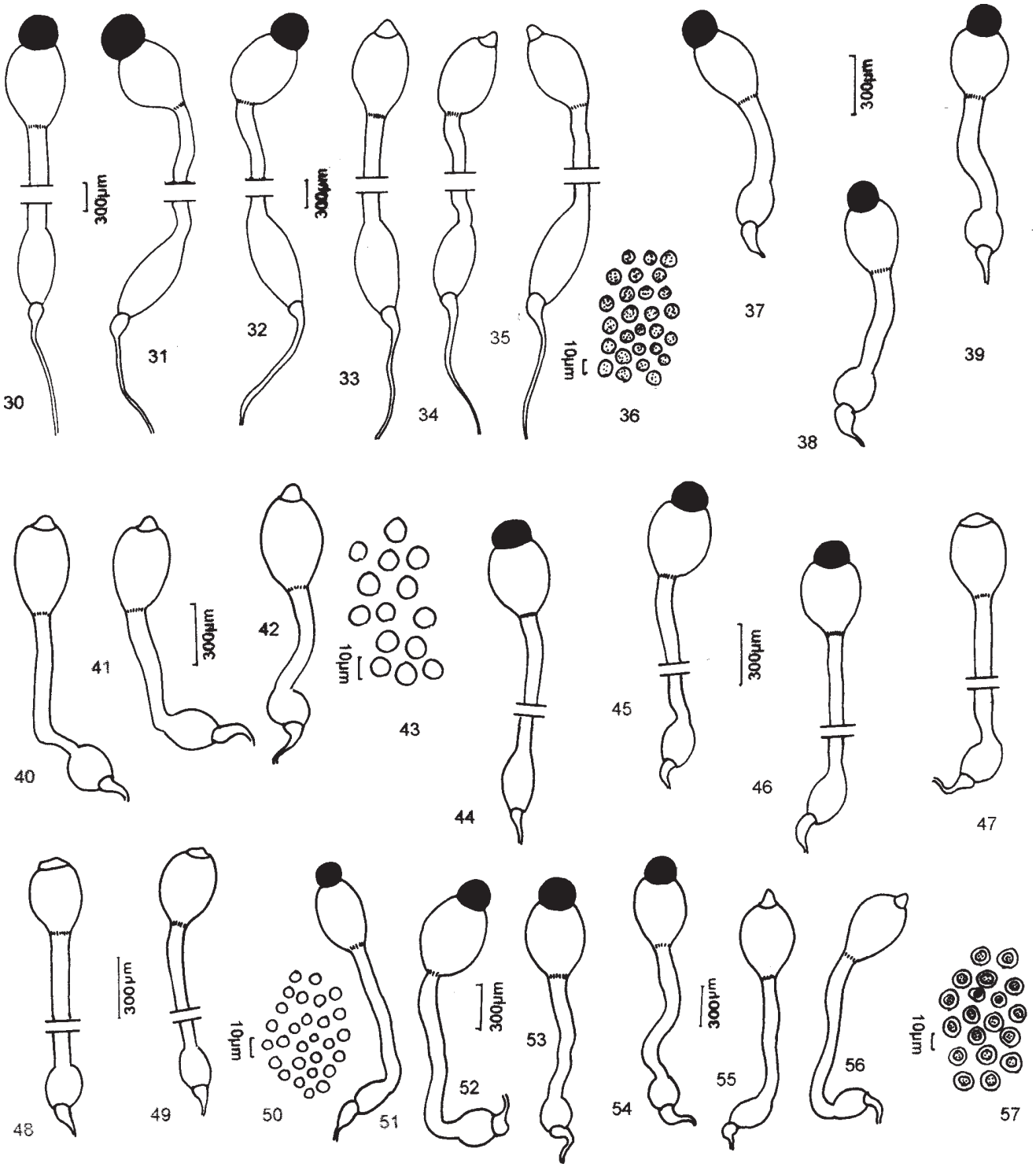
6. ***Pilobolus morinii*** Saccardo, Sylloge Fungorum 17: 505. 1905.
Fig. 37-43

Trophocysts ovoid to globose, with little yellowish pigmentation, spherical to ovoid, 250-300×150-250 µm, rhizoidal extensions up to 150 µm; sporangiophore long-cylindrical, 400-800×80-110 µm; black sporangium, smooth and resistant walls, globose to hemispherical, 150-200×100-150 µm; columellae with smooth wall, hyaline, half-elliptic, 110-140×70-100 µm; hyaline subsporangial vesicles, smooth wall, oval, 250-550× 200-

300 µm; yellow spores, smooth, little grainy content, globose, 5.0-7.0 µm diam. Isolated from zebra (September/1994). The observed material corresponds to that described by Naumov (1939). The descriptions provided by Boedjin (1958) and Tandon (1968) differ in sporangiophore height, mentioned as up to 3.0 mm. The taxon differs from the other species of the genus, with spherical spores, because of spore size that can reach 7.0 µm diam., and also due to the half-elliptical columella form which is seen only in this species.



Figures 1-8. *Pilobolus borzianus* Morini: 1-3. Sporangiophores. 4-7. Columellae. 8. Spores. Figures 9-15. *Pilobolus crystallinus* (Wiggers) Tode: 9-11. Sporangiophores. 12-14. Columellae. 15. Spores. Figures 16-22. *Pilobolus heterosporus* Palla: 16-18. Sporangiophores. 19-21. Columellae. 22. Spores. Figures 23-29. *Pilobolus kleinii* van Tieghem: 23-25. Sporangiophores. 26-28. Columellae. 29. Spores.



Figures 30-36. *Pilobolus longipes* van Tieghem: 30-32. sporangiophores. 33-35. Columellae. 36. Spores. Figures 37-43. *Pilobolus morinii* Saccardo: 37-39. Sporangiophores. 40-42. Columellae. 43. Spores. Figures 44-50. *Pilobolus nanus* van Tieghem: 44-46. Sporangiophores. 47-49. Columellae. 50. Spores. Figures 51-57. *Pilobolus oedipus* Montagne: 51-54. Sporangiophores. 55-56. Columellae. 57. Spores.

7. ***Pilobolus nanus*** van Tieghem, Trois. Mém. In Annales des Sciences Naturalles, ser 6 4: 340-342. 1876.
Fig. 44-50

Trophocysts ovoid to globose, elliptical, 200-250× 155-195 µm, with rhizoidal extension up to 120 µm, little yellowish pigmentation; sporangiophores long-cylindrical, 0.5-1.0 mm×55-65 µm; black sporangia, resistant wall, hemispherical to ovoid, 170-200× 130-150 µm; columellae with smooth walls, convex, 75-95×120-140 µm; subsporangial vesicles with smooth wall, hyaline, little pigmentation, slightly ovoid, 290-310×270-290 µm; hyaline spores, smooth, globose, 3.5-4.0 µm diam. Collected from dung of horse (October/1994). The isolated material corresponds well with the description of Grove (1958), except for columella format. There have been no previous reports from Brazil. *P. nanus* differs from the other species of the genus with spherical spores because its spores are smaller, up to 5.5 µm diam. Additionally, it has convex columellae, which do not occur in any other species of the genus.

8. ***Pilobolus oedipus*** Montagne, Ann. Soc. Linn. de Lyon p. 1-7. 1828.
Fig. 51-57

Trophocysts ovoid to globose, globose to subglobose, 200-250×145-190 µm, with rhizoidal extension up to 300 µm, little yellowish pigmentation; sporangiophores long-cylindrical, 1.5-2.0 mm× 100-110 µm; black sporangia, wall with cuticle, resistant, spherical to hemispherical, 350-450×240-300 µm; columellae smooth walled, 200-250×130-150 µm; subsporangial vesicle smooth walled, little pigmentation, ovoid to elliptical, 550-650×450-500 µm; yellow spores, grainy content, smooth wall, twin, globose, 9.0-16 µm diam. Collected from dung of horse (January/1995), and zebra (November/1994, March/1995, June/1995). The observed material corresponds well to the description of Nand & Mehrotra (1968). *Pilobolus oedipus* was registered in the state of São Paulo by Viriato & Trufem (1985). It differs from the other species of the genus with spherical spores because of the specific twin wall of the spores.

9. ***Pilobolus pullus*** Massee, Kew Bulletin p. 160. 1901.
Fig. 58-64

Trophocysts ovoid to globose, hyaline, globose to ovoid, 110-155×60-100 µm, with rhizoidal extension up to 300 µm; isolated sporangiophores long-cylindrical, 800-950×80-100 µm; black sporangia, with resistant wall, hemispherical to lowered, 200-300×170-210 µm; smooth columellae, mammiform, 150-200×120-140 µm; subsporangial vesicle with smooth wall, no pigmentation, ovoid to globose, 550-650×350-4500 µm; hyaline spores, smooth, short elliptical, 8.5-12×6.5-7.5 µm. Isolated from dung of horse (November/1994) and "guanaco" (September/1994). The isolated material has characters that agree with the description of Naumov (1939). *Pilobolus pullus* was recorded in the state of São Paulo by Viriato & Trufem (1985). It differs from the other species of the genus because it has short-elliptical spores, up to 12 µm. The columellae are typically mammiform. *Pilobolus crystallinus* is the only species whose characteristics come close to *P. pullus*, but the sporangiophores of *P. crystallinus* are larger, reaching 1.9 mm high.

10. ***Pilobolus sphaerosporus*** Palla, Oesterr. Bot. Zeitschr. 50: 400. 1900.
Fig. 65-71

Trophocysts ovoid to globose, long-ovoid, 550-700×250-275 µm, with rhizoidal extension up to 600 µm, with little pigmentation; sporangiophores long-cylindrical, 4.0-6.0 mm×130-150 µm; black sporangia, with resistant wall, hemispherical to ovoid, 185-270× 295-400 µm; smooth columellae, mammiform, 95-200×65-150 µm; subsporangial vesicles smooth walled, no pigmentation, ovoid to globose, 650-800× 625-695 µm; yellow spores, smooth, grainy content, globose, 10-20 µm diam. Observed on dung of horse (October/1994, November/1994, March/1995, July/1995), "guanaco" (November/1994, December/1994, January/1995), and zebra (October/1994, December/1994). The studied material is similar to the descriptions of Naumov (1939), Grove (1958) and Nand & Mehrotra (1968). *Pilobolus sphaerosporus* was isolated and described in the state of São Paulo by Viriato & Trufem (1985). It differs from the other species of the genus due to sporangiophores height, up to 4-6 mm high.

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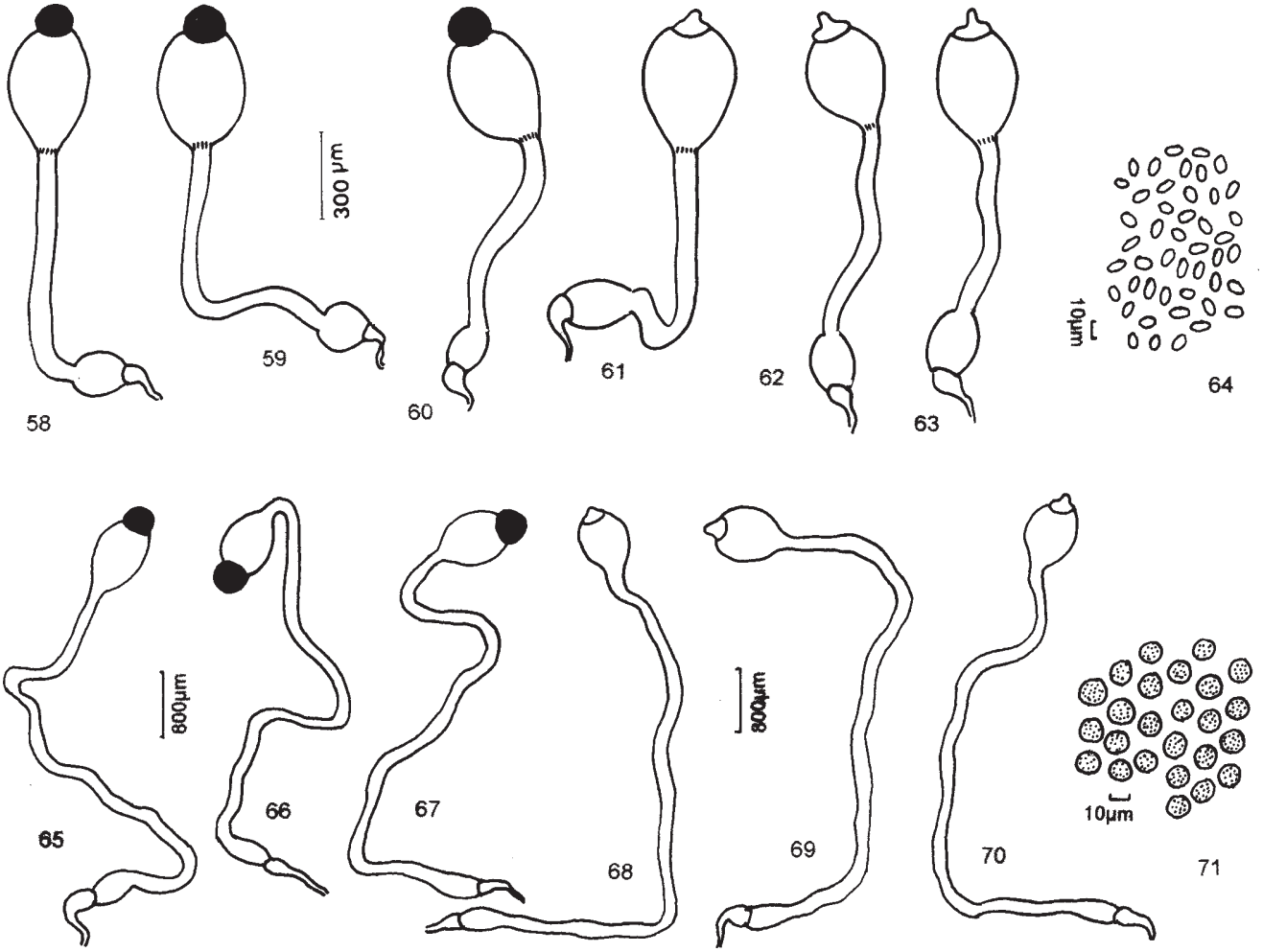

Figures 58-64. *Pilobolus pullus* Massee: 58-60. Sporangiophores. 61-63. Columellae. 64. Spores. Figures 65-71. *Pilobolus sphaerosporus* Palla: 65-67. Sporangiophores. 68-70. Columellae. 71. Spores.

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