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Foliicolous anamorphs of *Mycosphaerella* from South America

Pedro W. Crous[1], Ruth L. Benchimol[2], Fernando C. Albuquerque[2] & Acelino C. Alfenas[3]

[1] Department of Plant Pathology, University of Stellenbosch, P. Bag X1, Matieland 7602, South Africa
[2] Laboratório de Fitopatologia, Embrapa Amazônia Oriental, Caixa Postal 48, 66017-900, Belém, PA, Brazil
[3] Departemento de Fitopatologia, Universidade Federal de Viçosa, 36.570 Viçosa, MG, Brazil

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One species of *Mycosphaerella*, and four anamorph species are newly described in the present study. *Passalora crotoniphila* comb. nov. is described from *Croton* in Paraguay. New species from Brazil include: *Mycosphaerella bixae* sp. nov. and its *Passalora* anamorph from *Bixa*, *Pseudocercospora cordiana* sp. nov. from *Cordia*, *Pseudocercospora macrospora* stat. nov. from *Bertholletia*, and *Pseudocercospora xenoannonicola* sp. nov. from *Annona*.

Keywords: *Mycosphaerella*, anamorph, *Passalora*, cercosporoid fungi, leaf spot diseases.

Although there are numerous cercosporoid fungi causing leaf spot diseases of plants in South America, these organisms have as yet been poorly studied. Furthermore, hardly any cultures have been obtained of these anamorphs, and very few have been linked to their respective *Mycosphaerella* teleomorphs by means of cultural studies. As part of a larger project aiming to establish such links, and confirming the phylogeny and host range of these fungi by means of molecular techniques (Crous & al., 1999; Crous & al., 2000), a special effort is being made to culture these fungi so that these issues can also be addressed.

In recent years several groups have attempted to revise herbarium and type specimens of many of the cercosporoids described from South America (Pons & Sutton, 1988; Inacio & al., 1996; Crous, 1998; Braun & al., 1999; Crous & al. 1999). Unfortunately, however, most of these studies have dealt with dried, preserved material only, and given the paucity and quality of material, it is unlikely that these collections could aid in molecular studies aiming at addressing the

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issues outlined above. For this reason, new collections and subsequent cultural and molecular studies are essential if we are to attempt answering questions relating to phylogeny, teleomorph relationships and host ranges of these fungi. The present study is part of a revision of the cercosporoid species from South America, the cultures of which will also be included in subsequent molecular projects aimed at answering some of the questions outlined above.

Materials and methods

Specimens and isolates studied

Dilution plates were made on 2% malt-extract agar (Biolab, Midrand, Johannesburg) (MEA) from mycelial and conidial scrapes obtained from conidiomata sporulating on symptomatic leaves. After 24 h of incubation at 25 C, geminated conidia were transferred to MEA plates for a further incubation of 14 d at 25 C in the dark. Selected isolates were subcultured onto divided plates with one half containing carnation-leaf agar (CLA) (Fisher & al., 1982; Crous & al., 1992) and the other MEA, and incubated at 25 C under continuous near-ultraviolet light. Cultures were examined at weekly intervals for sporulation. Linear growth on MEA plates was determined for three single conidial isolates of each species after 14 d of incubation at 25 C in the dark. Two perpendicular measurements were obtained for each colony, and averages determined. Colony colours (top and bottom) were subsequently rated on MEA using the colour charts of Rayner (1970). Wherever possible, thirty measurements were made of structures mounted in lactophenol, and the extremes given in parentheses. Type specimens were lodged at the National Collection of Fungi in Pretoria (PREM), and ex-type cultures maintained in the culture collection of the Department of Plant Pathology at the University of Stellenbosch, South Africa (STE-U).

Results and discussion

Mycosphaerella bixae Crous & R. L. Benchimol, **sp. nov.** – Figs. 1–3.

Anamorph. – *Passalora* sp.

Pseudothecia epiphylla, atra, subepidermalia, postea leviter erumpentia, globosa, 50–90 µm diam.; paries compositus ex 2–3 stratis texturae angularis mediobrunneae. Asci sine paraphysibus, fasciculati, bitunicati, subsessiles obovoidei ad ellipsoidei, recti vel leviter incurvati, octospori, 25–35 × 7–10 µm. Ascosporae multiseriatae, hyalinae, guttulatae, parietibus tenuibus, rectae, fusoideo-ellipsoideae, extremis obtusis, latissimae ad medium cellulae apicalis, mediano 1-septatae, 8–10 × 2–3 µm. Caespituli fasciculati, amphigeni, brunnei, usque ad 50 µm lati et 70 µm alti. Conidiophora medio brunnea, levia, 1–4 septata, subcylindrica, recta ad

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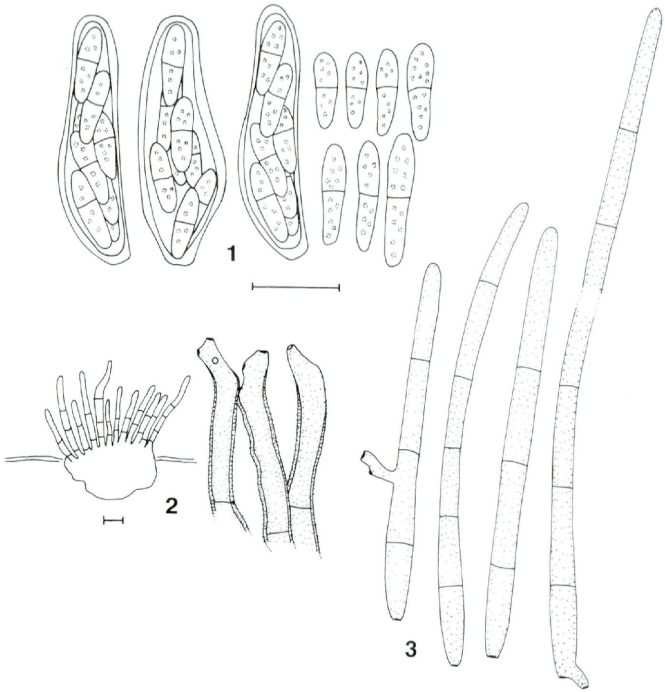

Figs. 1–3. – *Mycosphaerella bixae* and its *Passalora* anamorph (PREM 56550a & b, holotypes). – 1. Asci and ascospores. – 2. Fascicle of conidiophores. – 3. Conidiogenous cells and conidia. – Bars = 10 µm.

varie curvata, non ramosa, 20–70 × 3–4 µm. Cellulae conidiogenae terminales, non ramosae, medio brunneae, leves, sympodialiter proliferantes, 15–25 × 2.5–3.5 µm; cicatrices incrassatae, fuscatae et refractae. Conidia raro catenulata, catenis ramosis vel non ramosis, pallide brunnea, levia, anguste obclavata ad subcylindrica, apice obtuso, basis obconice truncata, recta ad leviter curvata, 3–7 septata, (25–)50–75 × (1.5–) 2.5–3.5(–4) µm; hila incrassata, fuscata et refracta.

Etymology. – named after its host, *Bixa*.

Leaf spots amphigenous, subcircular to irregular, 2–15 mm diam., coalescing with age, medium brown, surrounded by a red-purple border and a chlorotic zone. – Pseudothecia epiphyllous, black, subepidermal, becoming slightly erumpent, mostly under

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conidiomatal fascicles, globose, 50–90 µm diam., apical ostiole 5–10 µm wide; wall consisting of 2–3 layers of medium brown textura angularis. – Asci aparaphysate, fasciculate, bitunicate, subsessile, obovoid to ellipsoid, straight or slightly incurved, 8-spored, 25–35 × 7–10 µm. – Ascospores multiseriate, overlapping, hyaline, guttulate, thin-walled, straight, fusoid-ellipsoidal with obtuse ends, widest in the middle of apical cell, medianly 1-septate or basal cell slightly longer than apical cell, tapering towards both ends, but with more prominent taper towards lower end, 8–10 × 2–3 µm, apical cell 4–5 µm, basal cell 4–6 µm long. – Mycelium internal and external, light brown, consisting of septate, branched, smooth hyphae, 3–4 µm wide. – Caespituli fasciculate, amphigenous, brown, up to 50 µm wide and 70 µm high. – Conidiophores arising from superficial mycelium, or aggregated in loose to dense fascicles arising from the upper cells of a brown stroma up to 40 µm wide; conidiophores medium brown, smooth, 1–4-septate, subcylindrical, straight to variously curved, unbranched, 20–70 × 3–4 µm. – Conidiogenous cells terminal, unbranched, medium brown, smooth, tapering to flat-tipped apical loci, proliferating sympodially, 15–25 × 2.5–3.5 µm; scars thickened, darkened and refractive. – Conidia rarely catenulate, chains branched or not, pale brown, smooth, narrowly obclavate to subcylindrical, apex obtuse, base obconically truncate, straight to slightly curved, 3–7-septate, (25–)50–75 × (1.5–)2.5–3.5(–4) µm; hila thickened, darkened and refractive.

Cultural characteristics. – Colonies pale mouse grey (15""d) (surface), and olivaceous black (27""m) (bottom), with even margins and moderate aerial mycelium, obtaining 17 mm diam. after 14 d at 25 C in the dark.

Host. – *Bixa orellana* L. (Bixaceae).

Distribution. – Brazil.

Material examined. – Brazil, Amazonia, living leaves of *B. orellana*, R. L. Benchimol & F. C. Albuquerque, Apr. 1999, PREM 56550 (holotype), culture ex-type STE-U 2554.

Pseudocercospora bixae (Allesch. & F. Noack) Crous, Alfenas & R.W. Baretto was recently redescribed from *Bixa orellana* in Brazil (Crous & al., 1997). *P. bixae* is distinguished from the *Passalora* state of *M. bixae* by having narrower and shorter conidia (30–130 × (2–)3–4 µm), and having unthickened conidial loci. Thus far no species of *Mycosphaerella* has been described from *Bixa*. Although the anamorph connection was not established by means of single ascospore cultures, conidiomata were clearly observed to fruit on the subepidermal, erumpent pseudothecia.

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Passalora crotoniphila (Speg.) Crous, **comb. nov.** – Fig. 4.

Bas.: *Cercospora crotoniphila* Speg. (*crotonophila*), Bol. Acad. Nac. Ciencias, Cordoba (Arg.) 23: 528. 1919.

≡ *Pseudocercospora crotoniphila* (Speg.) Crous, U. Braun & Alfenas, Mycotaxon 72: 177. 1999.

Leaf spots hologenous, circular, 1–4 mm diam., brown throughout, or with a grey centre. – Mycelium internal and external, climbing leaf hairs, composed of smooth, branched, pale brown hyphae, 3–4 µm diam. – Caespituli amphigenous, brown, up to 50 µm wide and 40 µm high. – Conidiophores arising singly from superficial mycelium, or aggregated in dense fascicles, arising from the upper cells of a brown stroma up to 40 µm wide and 20 µm high; conidiophores pale brown, smooth, subcylindrical, 0–2-septate, straight to curved, unbranched, 10–30 × 4–5 µm. – Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering rounded apices with flattened conidial loci, proliferating sympodially, 10–20 × 4–5 µm. – Conidia solitary, rarely catenulate, pale brown, smooth, obclavate, apex subobtuse to obtuse, base obconically trun-

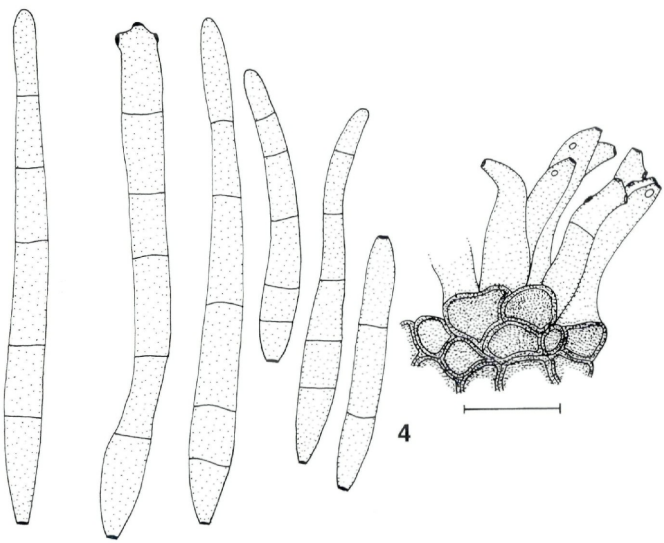

Fig. 4. – Conidia and conidiophores of *Passalora crotoniphila* (LPS 957, holotype). – Bar = 10 µm.

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cate, straight to slightly curved, 2–9-septate, (20–)45–70(–100) × (2.5–) 3–4(–5) µm; hila unthickened.

Cultural characteristics. – No cultures are available for study.

Host. – *Croton* sp.

Distribution. – Paraguay.

Material examined. – Paraguay, Trinidad, on leaves of *Croton* sp., 1892, J. D. Anisitz no. 258 & 259 = LPS 957 (holotype).

This species was recently treated by Crous & al. (1999), and redisposed to *Pseudocercospora*. As no type material could be obtained from LPS for examination, this decision was based on an examination of a Brazilian specimen (IACM 3721), which closely resembled the type description in general morphology. In the present study, the type of *C. crotoniphila* has been obtained and examined, and found to have smooth superficial mycelium climbing leaf hairs, and catenulate conidia with thickened hila, suggesting that this species would be better accommodated in *Passalora*. The separation of taxa in the *Passalora/Mycovellosiella/Phaeoramularia*-complex is not as clear as previously accepted (Crous & al., 2000), and is presently being revised (P. W. Crous, J. C. Kang & U. Braun, in prep.).

The collection treated as *Pseudocercospora crotoniphila* (IACM 3721) by Crous & al. (1999) is clearly a different taxon. While examining additional material from *Croton* collected by F. Freire in Brazil (U. Braun, pers. comm.), it was found that the material referred to as "*P. crotoniphila*" could be accommodated in *P. tiglii* (Henn.) Crous, U. Braun & Alfenas. The material collected by F. Freire had amphigenous fruiting, resembling the hypophyllous caespituli of *C. tiglii* (longer, looser, prominently branched conidiophores) and the epiphyllous caespituli of "*P. crotoniphila*" (shorter, denser conidiophores). It would seem, therefore, that IACM 3721 is only an epiphyllous form of *P. tiglii*, and that this collection can thus be treated as such.

Pseudocercospora cordiana Crous & R. L. Benchimol, **sp. nov.** – Figs. 5, 6.

Caespituli fasciculati, epiphylli, grisei, usque ad 70 µm lati et 35 µm alti. – Conidiophora aggregata in densis fasciculis exorientibus ex cellulis superioribus stromatis brunnei usque ad 45 µm lati et 35 µm alti; conidiophora pallide brunnea, levia, 0–2-septata, subcylindrica, recta ad sinuata, non ramosa, 15–25 × 2.5–4 µm. – Cellulae conidiogenae terminales, non ramosae, pallide brunneae, leves, in loca applanata decrescentes, sympodialiter proliferantes, 7–15 × 2–3 µm. – Conidia

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solitaria, pallide brunnea, levia, subcylindrica, apice anguste subobtuso, basis longa obconice truncata, varie curvata, raro recta, 1–3(–5)-septata, (30–)40–60 × 2–3 µm; hila inconspicua.

Etymology. – named after its host, *Cordia*.

Leaf spots hologenous, irregular to subcircular, 2–10 mm diam., dark brown. – Mycelium predominantly internal, pale brown, consisting of branched, smooth hyphae, 2–3 µm wide. – Caespituli fasciculate, epiphyllous, grey, up to 70 µm wide and 35 µm high. – Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 45 µm wide and 35 µm high; conidiophores pale brown, smooth, 0–2-septate, subcylindrical, straight to sinuous, unbranched, 15–25 × 2.5–4 µm. – Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering to flat-tipped loci, proliferating sympodially, 7–15 × 2–3 µm. – Conidia solitary, pale brown, smooth, subcylindrical, apex narrowly subobtuse, base long obconically truncate, variously curved, rarely straight, 1–3(–5)-septate, (30–)40–60 × 2–3 µm; hila unthickened.

Cultural characteristics. – Colonies pale mouse grey (15""d) (surface), and olivaceous black (27""m) (bottom), with even margins and moderate aerial mycelium, obtaining 25 mm diam. after 14 d at 25 C in the dark.

Host. – *Cordia goeldiana* Huber (Boraginaceae).

Distribution. – Brazil.

Material examined. – Brazil, Pará, Belém, living leaves of *C. goeldiana*, R. L. Benchimol & F. C. Albuquerque, Mar. 1998, PREM 56552 (holotype), culture ex-type STE-U 2552.

Three cercosporoid taxa have been described from *Cordia*, namely *Mycovellosiella cordiicola* (J. Yen) R. K. Verma & Kamal from Singapore, *Stenella myxa* (Syd.) J. Yen, Kar & Das from India, and *Cercospora cordiae* Chupp from Venezuela. The latter species was omitted from the monograph of *Cercospora* (Chupp, 1954), but was published in the series Monographs of the University of Puerto Rico (Chupp, 1934). It was described as not being associated with definite leaf spots, having hypophyllous fruiting, with superficial mycelium climbing leaf hairs, no fascicles, cylindrical to sometimes obclavate 1-septate conidia, with rounded bases and blunt apices, 12–20 × 2.5–4 µm, thus being clearly distinct from *P. cordiana*.

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Pseudocercospora luzardii Furlanetto & Dianese, Mycol. Res. 103: 1207. 1999. – Fig. 7.

Leaf spots hologenous, confined by leaf veins, spots stretching in bands from midrib to margin, 3–10 mm diam., brown with dark brown margins. – Mycelium internal and external, medium brown, consisting of branched, smooth to finely verruculose hyphae, 3–4 µm wide. – Caespituli sporodochial, mainly epiphyllous, dark brown to black, up to 100 µm wide and 50 µm high. – Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 90 µm wide; conidiophores pale brown, smooth, 0–2(–4)-septate, subcylindrical, straight to slightly curved, unbranched, 10–45 × 4–5 µm. – Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering to flat-tipped loci, proliferating sympodially, occasionally percurrently, 10–20 × 4–6 µm. – Conidia solitary, pale brown, smooth, shape variable, mostly subcylindrical to obclavate, apex obtuse, base truncate to obconically truncate, straight to slightly curved, (1–)3–6-septate, (17–) 30–50(–65) × (3–)4–5 µm; hila unthickened.

Cultural characteristics. – Colonies pale mouse grey (15""d) (surface), and olivaceous black (27""m) (bottom), with even margins and moderate aerial mycelium, obtaining 23 mm diam. after 14 d at 25 C in the dark.

Host. – *Hancornia speciosa* Gomes (Apocynaceae).

Distribution. – Brazil.

Material examined. – Brazil, Pará, Belém, living leaves of *H. speciosa*, A. C. Alfenas, 28 Apr. 1999, PREM 56553, culture STE-U 2556.

The morphology of the present collection correlates well with that of the holotype specimen. As cultures were obtained in the present study, a full cultural description is also provided. Furlanetto & Dianese (1999) discuss the morphological similarities of *P. luzardii* to other species in the Apocynaceae.

Pseudocercospora macrospora (Bat. & Peres) Crous & R. L. Benchimol, **stat. nov.** – Figs. 8, 9.

Bas.: *Cercospora bertholletiae* var. *macrospora* Bat. & Peres, Publ. Inst. Micol. Univ. Recife 450: 4. 1965.

Leaf spots hologenous, circular to irregular, 2–15 mm diam., medium brown, surrounded by a chlorotic margin. – Mycelium

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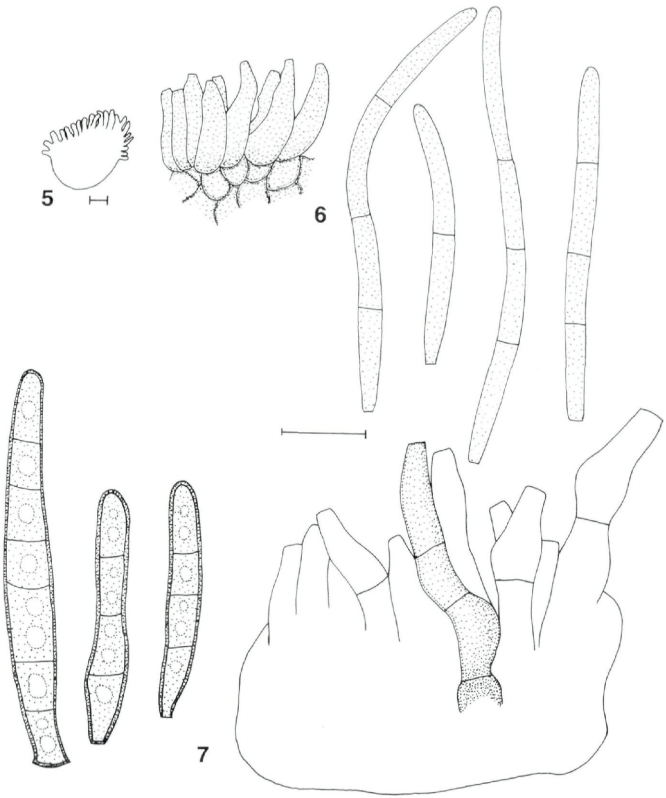

Figs. 5–7. – Fascicles of conidiophores and conidia. – 5, 6. *Pseudocercospora cordiana* (PREM 56552, holotype). – 7. *Pseudocercospora luzardii* (PREM 56553). – Bars = 10 µm.

mostly internal, medium brown, consisting of branched, smooth hyphae, 2–3 µm wide. – Caespituli sporodochial, initially subepidermal, becoming erumpent at maturity, amphigenous, medium brown, up to 150 µm wide and 70 µm high. – Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 120 µm wide; conidiophores pale brown, smooth, 0–1-septate, subcylindrical, straight to variously curved, unbranched, 15–35 × 4–6 µm. – Conidiogenous cells terminal, unbranched,

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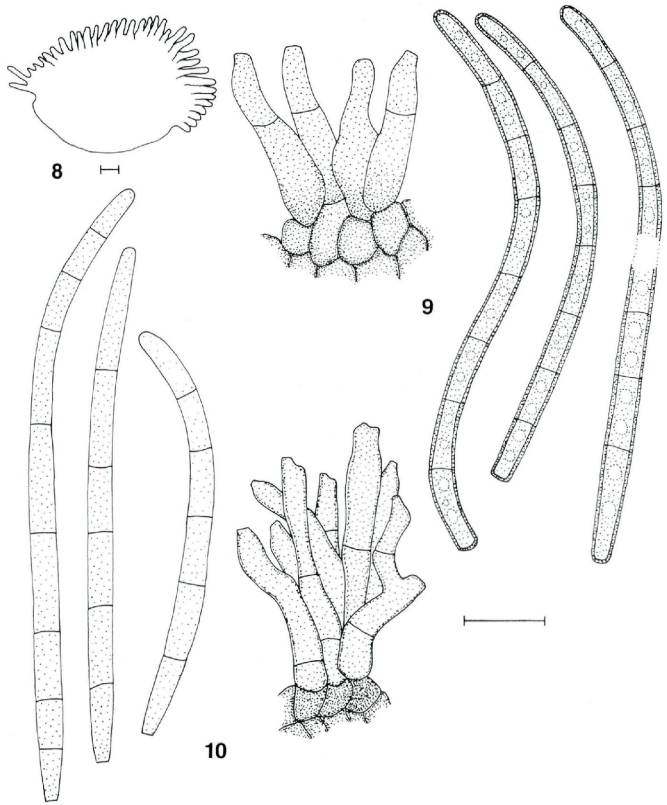

Figs. 8–10. – Fascicles of conidiophores and conidia. – 8, 9. *Pseudocercospora macrospora* (PREM 56554). – 10. *Pseudocercospora xenoannonicola* (PREM 56555, holotype). – Bars = 10 µm.

pale brown, smooth, tapering to flat-tipped loci, proliferating sympodially, 5–10 × 3–4 µm. – Conidia solitary, pale brown, smooth, guttulate, thick-walled, subcylindrical, apex obtuse, base truncate, straight to curved, 5–10-septate, (40–)50–75(–90) × 3–4 µm; hila unthickened.

Cultural characteristics. – Colonies mouse grey (15""i) (surface), and olivaceous black (27""m) (bottom), with slightly irre-

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gular margins and moderate aerial mycelium, reaching 17 mm diam. after 14 d at 25 C in the dark.

Host. – *Bertholletia excelsa* H.B.K. (Lecythidaceae).

Distribution. – Brazil.

Material examined. – Brazil, Amazonia, living leaves of *B. excelsa*, R. L. Benchimol & F. C. Albuquerque, Apr. 1999, PREM 56554, culture STE-U 2553.

Two cercosporoid species are known from *Bertholletia* in Brazil, namely *C. bertholletiae* Albuquerque and *C. bertholletiae* var. *macrospora* Bat. & Peres (Albuquerque, 1960; Batista & al., 1965). These two taxa are chiefly distinguished by *C. bertholletiae* having distinctly obclavate conidia, 28-64 × 4 µm (Albuquerque, 1960), while those of *C. bertholletiae* var. *macrospora* are cylindrical-obclavate, thick-walled, with unthickened, truncate bases, 35.5-85 × 3-3.5 µm. Although attempts to obtain the type specimen have proven unsuccessful, the description and illustration of the latter species (Batista & al., 1965) clearly resemble the specimen obtained in the present study. The pigmented conidia and unthickened hila determine that this collection would be better accommodated in *Pseudocercospora*.

Pseudocercospora xenoannonicola Crous & R. L. Benchimol, **sp. nov.** – Fig. 10.

Caespituli fasciculati, praecipue hypophylli, grisei ad medio brunnei, usque ad 60 µm lati et 35 µm alti. Conidiophora aggregata in densis fasciculis exorientibus ex cellulis superioribus stromatis brunnei usque ad 30 µm lati; conidiophora medio brunnea, levia, 0–2-septata, subcylindrica, recta ad geniculata sinuosa, non ramosa vel supra ramosa, 10–30 × 3–4 µm. Cellulae conidiogenae terminales, non ramosae, pallide brunneae, leves, in loca applanata decrescentes, sympodialiter proliferantes, 7–20 × 3–4 µm. Conidia solitaria, pallide brunnea, levia, anguste obclavata ad subcylindrica, apice obtuso ad subobtuso, nunquam acuto, basis anguste obconice truncata, recta ad curvata, 5–7-septata, (30–)50–80(–100) × 2–3 µm; hila inconspicua.

Etymology. – resembling *P. annonicola*, but morphologically distinct.

Leaf spots hologenous, subcircular to irregular, 7–40 mm diam., initially chlorotic yellow, becoming grey to pale brown in centre, dark brown in outer region, surrounded by a chlorotic margin. – Mycelium internal and external, pale brown, consisting of branched, smooth hyphae, 2–3 µm wide. – Caespituli fasciculate, mainly hypophyllous, grey to medium brown, up to 60 µm wide and

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35 µm high. – Conidiophores aggregated in dense fascicles arising from the upper cells of a brown stroma up to 30 µm wide; conidiophores medium-brown, smooth, 0–2-septate, subcylindrical, straight to geniculate-sinuous, unbranched or branched above, 10–30 × 3–4 µm. – Conidiogenous cells terminal, unbranched, pale brown, smooth, tapering to flat-tipped loci, proliferating sympodially, 7–20 × 3–4 µm. – Conidia solitary, pale brown, smooth, narrowly obclavate to subcylindrical, apex obtuse to subobtuse, never acute, base narrowly obconically truncate, straight to curved, 5–7-septate, (30–)50–80(–100) × 2–3 µm; hila unthickened.

Cultural characteristics. – Colonies olivaceous grey (25""i) (surface), and olivaceous black (27""m) (bottom), sectoring, with uneven, smooth margins and sparse aerial mycelium, obtaining 18-20 mm diam. after 14 d at 25 C in the dark.

Host. – *Annona montana* Macf. (Annonaceae).

Distribution. – Brazil.

Material examined. – Brazil, Pará, Belém, living leaves of *A. montana*, R. L. Benchimol & F. C. Albuquerque, Mar. 1998, PREM 56555 (holotype), culture ex-type STE-U 3137, 3138.

Several *Cercospora* species with pigmented conidia have been reported from Annonaceae. Two of these have subsequently been treated and placed in genera other than *Pseudocercospora*, namely *Stenella annonaceae* (Henn.) U. Braun and *Stigmina oblecta* (Syd.) U. Braun. Other species such as *Cercospora anonae* Muller & Chupp (50–150 × 5–6 µm) and *Pseudocercospora scitula* (Syd.) Deighton (50–110 × 5–8 µm), have conidia much wider than those of *P. xenoannonicola*. Three species, however, have conidia of similar dimensions, namely *Pseudocercospora annonicola* Goh & Hsieh (20–75 × 2.5–3 µm), *Cercospora annonifolii* Bat. & Peres (23–241 × 3.5–5 µm), and *Cercospora caracasensis* Chupp & Muller (15–75 × 2.5–5 µm). *P. annonicola* is clearly distinct from *P. xenoannonicola* by having much smaller leaf spots (1–8 mm diam.), and conidia that have acute apices. *C. annonifolii* has much longer, multiseptate, verrucose conidia, and is illustrated by Batista & Peres (1964) to have thickened conidial hila, suggesting that it is probably a species of *Stenella*. *C. caracasensis* has wider conidia, angular leaf spots, and mostly epiphyllous fruiting.

None of the cultures of cercosporoid species studied here proved to be fertile on MEA or CLA. Given this fact, as well as their rather nondescript grey colonies, these cultures proved to be of limited value in providing additional features for species separation. However,

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as reported by Crous (1998), this is not the case with all anamorphs of *Mycosphaerella*. Furthermore, the added advantage of molecular studies still provide sufficient motivation to obtain cultures of these fungi for future studies aiming to clarify their host range and phylogeny. In the present study we have also refrained from describing the *Passalora* anamorph of *Mycosphaerella bixae* as new. The description was, however, included as part of the holomorph. The reason for this is partly to move towards a system of integrating anamorph and teleomorph names, and partly because molecular data (Crous & al., 2000) have shown *Mycosphaerella* to be monophyletic, including these cercosporoid anamorphs.

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