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Perisporiopsis lateritia, a new species on decaying leaves of *Hevea* spp. from the Amazon basin in Peru

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Abstract — The genus *Perisporiopsis (Ascomycota, Dothideomycetes, Parodiopsidaceae)* occurs on the underside of decaying leaves, mostly in tropical regions. A new species of *Perisporiopsis, P. lateritia*, is described that can be distinguished from other species in the genus by a combination of teleomorph and anamorph characteristics, such as ascospore size, size and shape of microconidia and macroconidia of the *Septoidium* anamorph, and the plant host (*Hevea*). This species is known only from the Peruvian Amazon.

Key words —leaf litter fungi, loculoascomycetes, systematics, taxonomy

Introduction

The plant genus *Hevea* (*Euphorbiaceae*) is known for the ability to produce latex that is processed to obtain natural rubber. The best-known species for production of natural rubber is *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. Other species in the genus include *H. benthamiana* Müll. Arg., *H. guianensis* Aubl., *H. nitida* Müll. Arg., and *H. pauciflora* (Spruce ex Benth.) Müll. Arg., as well as others that are rare (Schultes 1956). Although *Hevea* includes economically important species, the fungi associated with these hosts have not been explored (Araujo et al. 2004, Gazis & Chaverri 2010). As part of a study to characterize endophytic and ex planta fungi, e.g. saprophytes, of wild trees of *H. brasiliensis* and *H. guianensis*, ascomata of an unidentified species of *Perisporiopsis* Henn. (*Ascomycota, Dothideomycetes, Parodiopsidaceae*) were collected from decaying leaves in two locations in the Peruvian Amazon. Based on morphological data, this unidentified ascomycete is described here as a new species. A diagnostic sequence of the internal transcribed spacer region of the nuclear ribosomal DNA (ITS) has been deposited in Genbank.

Materials & methods

Source of specimens

Decaying leaves were collected near the base of wild *Hevea brasiliensis* and *H. guianensis* trees in old growth forests in two sites in the Peruvian Amazon, i.e. Los Amigos and Tambopata (Dept. Madre de Dios, Prov. Manu and Tambopata, respectively). Two specimens (P.C. 811 and P.C. 987) included ascomata of this unusual fungus. Ascospore germination was attempted by isolating asci and ascospores onto BBL™ cornmeal-dextrose-agar (CMD), supplemented with antibiotics (Sigma-Aldrich streptomycin-neomycin-penicillin). Plates were incubated at 25°C with alternating 12 h light/12 h darkness. However, ascospores did not germinate after one week. Specimens are preserved in the U.S. National Fungus Collection (BPI).

Morphological characterization

To observe internal and microscopic characteristics, the ascomata were rehydrated briefly in KOH, then supported by Tissue-Tek O.C.T. Compound 4583 (Miles Inc., Elkhart, Indiana, U.S.A.), and sectioned with a freezing microtome at a thickness of ca. 15 µm. Characteristics of asci and ascospores were observed by rehydrating the ascomata in 3% KOH, removing part of the centrum with a fine glass needle, and placing it on a glass slide. Characteristics of the anamorph on the natural substrata were also observed. Measurements of continuous characters such as length and width were made using Scion Image software beta version 4.0.2 (Scion Corporation, Frederick, Maryland, U.S.A.). Continuous measurements are reported as the extremes (maximum and minimum) in brackets separated by the 95% confidence interval. Color terminology is from Rayner (1970).

Source of ITS sequence

To obtain a representative ITS sequence, DNA was extracted from the ascomata of P.C. 811 by removing centrum contents with a fine glass needle and placing them in the bead-beating microtubes of the PowerPlant™ DNA isolation kit (MO BIO Laboratories, Inc., Carlsbad, California, U.S.A.). The primers used for ITS were ITS 5 and ITS 4 (White et al. 1990). PCR reactions were run in an Eppendorf Mastercycler EP using the parameters described in Gazis & Chaverri (2010). PCR products were cleaned using ExoSAP-IT® (USB Corporation, Cleveland, Ohio, U.S.A.) following the manufacturers instructions. Clean PCR products were sequenced at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland, U.S.A.). Sequences were assembled and edited with Sequencher 4.9 (Gene Codes, Madison, Wisconsin, U.S.A.). The ITS sequence was deposited in Genbank as accession number FJ884129.

Taxonomy

Perisporiopsis lateritia P. Chaverri & Gazis, **sp. nov.** PLATE A–H

MYCOBANK — MB518067

Perisporiopsis melioloides *similis. Ascospores (65.0–)66.0–75.5(–78.0) × 18.0–21.5(–23.0) µm.* Septoidium *macroconidia ovoideus fusiformes ad cymbiformes, (59.0–)61.5–69.0 (–80.0) × (15.7–)16.5–18.0(–19.3) µm, longitudo/crassitudo 3.7–3.8(–4.2). Microconidia*

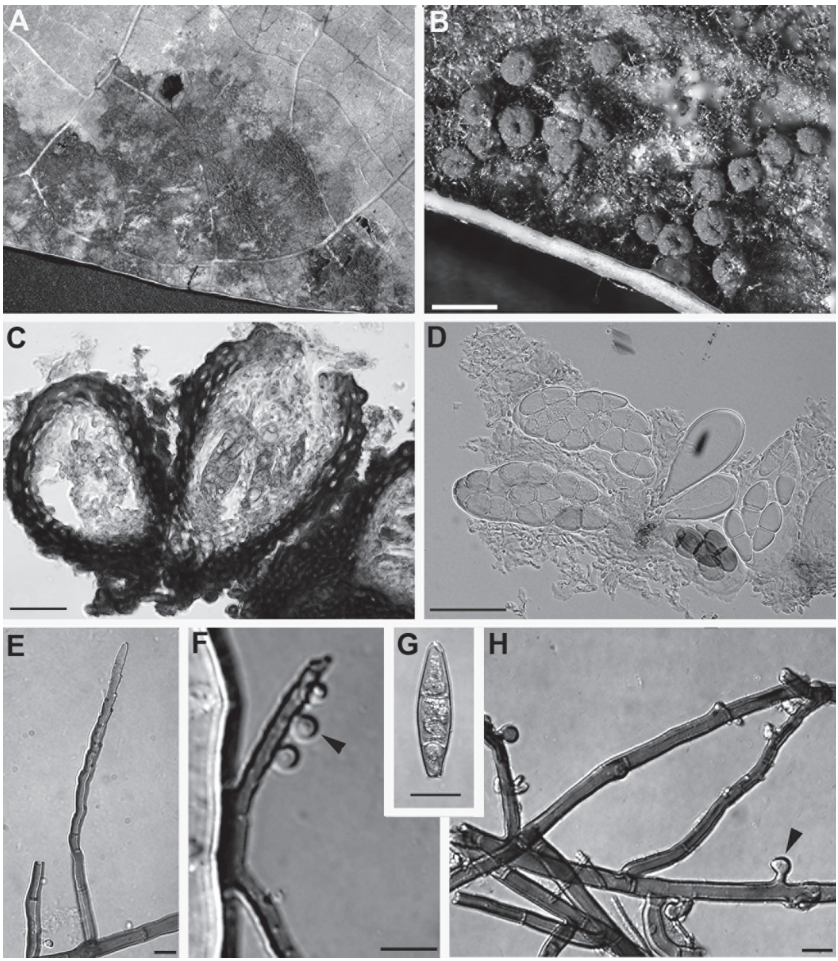

Plate. *Perisporiopsis lateritia* Holotype P.C. 811 = *BPI 880185*. A, B. Ascomata and dark mycelium on the underside of leaves. C. Longitudinal section of ascomata. D. Asci and ascospores. E, F. Denticulate conidiophore of the microconidial anamorph. F. Arrow indicates globose microconidia. G. *Septoidium* macroconidium. H. Simple stomatopodia indicated by arrow.

Bars: B = 1 mm; C, D = 100 µm; E–H = 10 µm.

globosae ad subglobosae, 4.5–5.5 × 4.8–5.5 µm, longitudo/crassitudo 1.0–1.1. Stomatopodii simplex.

Type: 17 June 2007, coll. R. Gazis, H.C. Evans, P. Chaverri; (**Holotype** BPI 880185, *P.C. 811*) on underside of decaying leaves of *Hevea brasiliensis*, Picaflor Research Station, near Tambopata River, Prov. Tambopata: Dept. Madre de Dios, Peru. GenBank accession number FJ884129.

Etymology: The name is Latin for brick red, in reference to the color of the ascomata.

Teleomorph – Mycelium superficial, hypophyllous, extensive, appearing black, anastomosing to form a close network, almost subiculum-like, with simple, knob-shaped stomatopodia. Ascomata superficial on mycelium, aggregated, associated with a hyphomycetous dematiaceous anamorph (i.e. *Septoidium*). Ascomata dark brown to black, almost completely covered with a sienna to brick tomentum, except near the apex where they appear black, subglobose to obovoid, 300–310 × 420–450 µm (n = 5), non-ostiolate, irregularly dehiscent at apex; ascomatal wall composed of one region of 2–3 layers of thick-walled cells, textura angularis. Asci few, generally less than 5, 200–220 × 80–90 µm (n = 10), obovoid, sessile to short stalked, somewhat thickened at apex, eight-spored. Ascospores 1-septate, strongly constricted at septum, initially hyaline, later pale brown or fawn, smooth to slightly spinulose, broadly fusiform to ovoid, somewhat inequilateral, with apical cell slightly larger than basal cell, (65.0–) 66.0–75.5(–78.0) × 18.0–21.5(–23.0) µm (average = 70.5 × 20 µm, n = 30).

Anamorph – Both macro- and microconidia of the hyphomycetous anamorph observed on natural substrata. For the macroconidial anamorph (i.e. *Septoidium*) no conidiogenous cells observed. Macroconidia ovoid, fusiform to cymbiform, truncate at base, smooth, pale brown, sometimes with tinges of pale grayish rose, 2-septate, (59.0–)61.5–69.0(–80.0) × (15.7–) 16.5–18.0(–19.3) µm (average = 65.2 × 17.2 µm, n = 10), length/width ratio 3.7–3.8(–4.2) (average = 3.8, n = 30). Microconidial anamorph with erect conidiophores, brown near base, pale brown almost hyaline near tip, simple, not branching, septate, with scattered denticles on upper part; conidiogenous cells polyblastic, sympodial, with small denticles; microconidia borne on denticles, globose to subglobose, unicellular, almost hyaline, sometimes apiculate at base, 4.5–5.5 × 4.8–5.5 µm (average = 5 × 5.2 um), length/width ratio 1.0–1.1 (average = 1.0, n = 8).

Habitat – On the underside of decaying *Hevea* spp. leaves in old growth forests. Known only from Peru.

Additional specimen examined: PERU. Dept. Madre de Dios: Prov. Manu, Los Amigos Research Station, near Los Amigos River, on underside of decaying leaves of *Hevea guianensis*, July 2007, coll. R. Gazis *BPI 880186 (= P.C. 987)*.

Notes – *Perisporiopsis* includes 19 species, all of them occurring on decaying leaves in tropical regions; most are described in Sivanesan (1984). A manuscript under review (Chaverri & Gazis) shows that *Perisporiopsis* is also a common endophyte and soil inhabitant. *Perisporiopsis lateritia* is most similar to *P. melioloides* (Berk. & M.A. Curtis) Arx in having a reddish tomentum covering the ascomata and relatively large ascospores. *Perisporiopsis melioloides* has conidia that are significantly wider than those of *P. lateritia*. In addition, the stomatopodia of *P. melioloides* are lobed while in *P. lateritia* they are simple. Other species with a reddish covering on the ascomata are *P. brasiliensis*

(Bat. & Nascim.) Arx, *P. cecropiae* (R.E.D. Baker) Arx, *P. fusispora* (Pat.) Arx, *P. kwangensis* (Henn.) Arx, and *P. megalospora* (Sacc. & Berl.) Arx. Most of these species have smaller ascospores than *P. lateritia*, and *P. fusispora* has multiseptate, fusiform ascospores. In addition, *P. brasiliensis* has *Septoidium* macroconidia that are generally 3-septate, *P. cecropiae* has macroconidia generally 1-septate, *P. fusispora* has 3-septate macroconidia, *P. kwangensis* has smaller microconidia, and *P. megalospora* has lobed stomatopodia and larger microconidia than *P. lateritia*.

Among species of *Perisporiopsis,* host preferences seem to exist, i.e. most species are from plants of close taxonomic affinity (Sivanesan 1984). For example, *Perisporiopsis brachystegiae* (Henn.) Arx and *P. fusispora* are known only from legumes in Africa and Tropical America, respectively. *Perisporiopsis megalospora* is known from various genera in the *Malpighiales* such as *Banisteriopsis, Hiraea, Mascagnia*, and *Tetrapteris*; and *P. melioloides* from the *Myrtaceae*. Only two other species have been found on *Euphorbiaceae*, namely *P. hurae* (R.E.D. Baker & Dale) Arx ["*urae*"] and *P. kwangensis*; these two species are morphologically distinct from *P. lateritia*.

All species of *Perisporiopsis*, except *P. lantanae* (F. Stevens) R.W. Barreto, have *Septoidium* macro- and microconidial anamorphs. In Barreto et al. (1995), *P. lantanae* is described as having a pycnidial anamorph, more typical of a *Leptosphaeria*. In addition, the ascospores illustrated in Barreto et al. (1995), resemble *Leptosphaeria.* Therefore, it is likely that this species may not belong in *Perisporiopsis*.

Whether the phenotypic characteristics used to separate species of *Perisporiopsis,* i.e. ascospores, conidia, and host, have phylogenetic significance remains unknown. This genus has not been included in phylogenetic studies of the *Dothideomycetes* (Schoch et al. 2009). Its relationship with other genera in the *Parodiopsidaceae* is unclear. Given the small ascomata, few asci, lack of interthecial elements and occurrence on leaves, one would suspect a relationship with the *Mycosphaerellaceae* sensu lato. However, in a recently submitted manuscript by Chaverri & Gazis, phylogenetic analyses of nuclear ribosomal DNA suggest a close relationship with *Leptosphaeriaceae* and *Phaeosphaeriaceae*.

Key to species of *Perisporiopsis*

Modified from Sivanesan (1984)

1. Ascospores always one-septate, conidia transversely multiseptate or staurosporous 2

1. Ascospores with one or more septa, conidia transversely multiseptate 9

2. Conidia staurosporous 3

2. Conidia straight 4

3. Ascospores 25–33 x 7–12 µm, conidia 50–100 x 10–16 µm, on *Lophira (Ochnaceae)* ..... *P. lophirae*

3. Ascospores 30–52 x 10–15 µm, conidia 50–140 x 28–56 µm, on legumes ..... *P. brachystegiae*

4. Conidia 1–2-septate 5

4. Conidia 2–3-septate 8

5. Ascomata in shades of orange or red, not brown or black 6

5. Ascomata brown or black. 7

6. Ascospores 40–50 × 11–15 µm, conidia 57–68 × 12–15 µm, microconidia 5–9 × 2–3 µm, on *Alchornea*, *Pera*, *Sapium* and other *Euphorbiaceae* ..... *P. kwangensis*

6. Ascospores 36–51 × 12–15 µm, conidia 36–45 × 15–20 µm, microconidia 5–7.5 × 3–4 µm, on *Cecropia (Cecropiaceae)* ..... *P. cecropiae*

6. Ascospores 40–55 × 12–16 µm, conidia 60–80 × 12–15 µm, microconidia 7–10 × 5–7 µm, on *Banisteriopsis, Hiraea, Mascagnia*, and *Tetrapteris,* and other *Malpighiales* ..... *P. megalospora*

6. Ascospores 30–75 × 16–21 µm, conidia 55–79 × 12–16 µm, microconidia 4–7 × 3–5 µm, on *Myrtaceae* ..... *P. melioloides*

6. Ascospores 66–76 × 18–22 µm, conidia 61–69 × 16–18 µm, microconidia 4.5–5.5 × 4.5–5.5 µm, on *Hevea (Euphorbiaceae)*. ..... *P. lateritia*

7. Ascospores 35–45 × 20–24 µm, conidia 40–63 × 17–21 µm, microconidia absent, on *Mauria (Anacardiaceae)* ..... *P. escharoides*

7. Ascospores 40–45 × 10–14 µm, conidia 40–56 × 18–20 µm, microconidia 3–5 × 1.5–2.5 µm, on *Buddleja (Scrophulariaceae)* ..... *P. torrendii*

7. Ascospores 36–52 × 13–20 µm, conidia 60–65 × 18–20 µm, microconidia 9–12 × 8–10 µm, on *Oryctanthus (Loranthaceae)* ..... *P. sydowii*

7. Ascospores 55–66 × 17–21 µm, conidia 56–80 × 23–38 µm, microconidia 2.5–4 × 2–2.5 µm, on *Hura (Euphorbiaceae)*. ..... *P. hurae* [“*urae*”]

8. Ascospores 27–38 × 12–15 µm, conidia 50–72 × 12–14 µm, microconidia 5–7.5 × 4–6.5 µm, on *Tapirira*. ..... *P. brasiliensis*

8. Ascospores 45–60 × 12–16 µm, conidia 62–80 × 16–22 µm, microconidia 6–8 × 2–3 µm, on *Clusia (Clusiaceae)* ..... *P. clusiae*

9. Ascospores 1–5-septate, 52–86 × 11–15 µm, conidia 65–100 × 14–17 µm, on *Struthanthus* and other *Loranthaceae*. ..... *P. struthanthi*

9. Ascospores 1–3-septate 10

10. Ascospores 50–70 × 9–12 µm, conidia 50–77 × 12–14, on legumes. ..... *P. fusispora*

10. Ascospores 60–82 × 9–14 µm, conidia 80–100 × 15–18 µm, on *Calophyllum (Clusiaceae)* ..... *P. portoricensis*

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