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***Pseudocercospora palicoureae* sp. nov. associated with the toxic rubiaceous weed *Palicourea marcgravii* in Brazil, with observations on its mycobiota**

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A survey of the fungi associated with the toxic rubiaceous weed *Palicourea marcgravii* was conducted in Southeastern Brazil, and two phytopathogenic taxa were collected. *Pseudocercospora palicoureae* sp. nov. and *Puccinia palicoureae* found associated with rust symptoms on *P. marcgravii*, are described and illustrated. This is the second *Pseudocercospora* species reported on the genus *Palicourea* (*Rubiaceae*). *Pseudocercospora palicoureina* was previously described from the Dominican Republic and Mexico on *Palicourea dominguensis* and *P. galeottiana*. The other fungus collected on *P. marcgravii* is a known rust species: *Puccinia palicoureae*. The known mycobiota of *P. marcgravii* is surprisingly scarce considering its wide distribution in Brazil. The potential for using *Pseudocercospora palicoureae* as a biocontrol agent against this important weed is regarded as minimal since the fungus grew slowly in culture, did not sporulate and the damage to the weed in the field was of little significance. There is no potential for biological control of *P. marcgravii* in Brazil using the rust pathogen because it is a widespread biotrophic fungus (therefore not adequate for mass production) attacking a native weed in its center of origin (therefore the classical approach would not apply). A discussion about the taxonomy of *Puccinia palicoureae* is also provided.

**Key words:** Classical biological control, fungal survey, new species, plant disease, taxonomy

**Introduction**

*Palicourea marcgravii* St. Hil. (local names: cafezinho, café-bravo, erva-de-rato) is a small perennial shrub of the *Rubiaceae*, characterized by flowers with bright purplish-pink, tubular corollas, which contrast with its bright orange to yellow hypanthia. In Brazil, it occurs naturally in biomes ranging from the Atlantic Rain Forest (Lombardi and Gonçalves, 2000) to the Brazilian Cerrado (Durigan *et al.*, 2004; Harley and Giulietti, 2004) and the Amazon

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(Ribeiro *et al.*, 1999). This plant species is the poisonous weed most feared by cattle ranchers in Brazil, as it is able to cause an abrupt death when ingested by sheep, goats and especially bovines (Kissmann and Groth, 2000; Lorenzi, 2000). Since 2003, a systematic field survey of the mycobiota associated with this weed, together with several selected noxious weed species native to Brazil, was carried out in a part of its center of origin (Southern and Southeastern Brazil). The purpose of such a survey was to find potential biocontrol agents against those weeds. In the case of *P. marcgravii* the aim was to find necrotrophic phytopathogenic fungi that potentially could be used in future mycoherbicide development programs. A detailed account of the fungi found during this survey and their potential use as a biocontrol agent against *P. marcgravii*, as well as a brief discussion on phytopathogenic fungi of this species in general and their biocontrol potentials is given in the present paper.

## **Materials and methods**

Prior to the field survey a complete list of information contained in herbarium records of *P. marcgravii* in the following Brazilian herbaria was compiled: Herb. IAC, Herbário Fanerogâmico e Criptogâmico do Instituto Agrônômico; Herb. SP, Herbário Maria Eneyda P. K. Fidalgo; Herb. RB, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro; Herb. R, Herbário do Museu Nacional do Rio de Janeiro; Herb. VIC, Universidade Federal de Viçosa; Herb. MBM, Museu Botânico Municipal; Herb. OUPR, Herbário José Badini. The following Southern and Southeastern Brazilian states were visited, Minas Gerais, Rio de Janeiro, Espírito Santo, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul during the period between January 2003 and December 2005. Selected sites were visited in each state. For details of the survey methodology and the laboratory studies see Barreto and Evans (1994). Freshly collected samples were examined under a stereomicroscope. Hand free sections containing the fungal structures were mounted in lactophenol. Observations, measurements and line drawings were prepared using an Olympus BX 50 light microscope fitted with a camera lucida. The material examined was deposited in the herbarium at the Universidade Federal de Viçosa (Herbarium VIC). Additional collections previously deposited at VIC and representative specimens deposited at the Herbário Micológico e Uredinológico Victoria Rosseti (HMUVR) were also examined.

## **Results**

Two fungal diseases were found associated with *P. marcgravii*: a leaf-spot and a rust. The fungi involved are described below.

***Pseudocercospora palicoureae*** O.L. Pereira & R.W. Barreto **sp. nov.** (Figs 1, 2)  
Mycobank 500719

*Etymology*: named in reference to the host genus.

Differt a *Pseudocercospora palicourea* lesionibus distinctis, stromatibus nullis, conidiophoris ex stomate oriundis 15-70  $\mu\text{m}$  longis, conidiis 13-115  $\mu\text{m}$  longis.

Leaf spots amphigenous, vein-delimited or irregular, yellowish to ochraceous, finally reddish with age, margin indistinct, 2-12 mm diam. or confluent and larger, covering large areas of the leaf surfaces. *Stromata* absent. *Conidiophores* hypophyllous, arising from internal hyphae, through stomata, in small, loose fascicles or solitary, cylindrical, erect to decumbent, flexuous, geniculate-sinuuous, simple or branched, thin-walled, sometimes reduced to conidiogenous cells only, 15-70  $\times$  3-4.5  $\mu\text{m}$ , 0-5-septate, smooth, medium to dark brown. *Conidiogenous cells* terminal, integrated, 5-15  $\times$  3.5-4.5  $\mu\text{m}$ , medium brown, smooth. *Conidiogenous loci* inconspicuous, not darkened, unthickened. *Conidia* solitary, obclavate-cylindrical, straight to slightly curved, 13-115  $\times$  2.5-3.5  $\mu\text{m}$ , apex obtuse or subacute, base obconically truncate or sometimes long obconically truncate, 0-5-septate, pale olivaceous to pale brown, smooth, thin-walled, hila unthickened, not darkened.

In culture: slow-growing, 1.8-2.5 cm after 30 days, stromatic and immersed in center, covered by a grayish white felty aerial mycelium, dark gray to black reverse, no sporulation.

*Teleomorph*: not seen.

*Habitat*: On living leaves of *Palicourea marcgravii*.

*Known distribution*: Minas Gerais (Brazil).

*Material examined*: BRAZIL, Minas Gerais, Marliéria, PERD (Parque Estadual do Rio Doce), on the margin of Lagoa Preta, on living leaves of *Palicourea marcgravii*, September 2004, O.L. Pereira (VIC 29390; **holotype**).

*Other material examined* (all on *Palicourea marcgravii*, Brazil, Minas Gerais): Ouro Preto, Parque Estadual do Itacolomi, June 2004, O.L. Pereira (VIC 29391); Marliéria, PERD (Parque Estadual do Rio Doce), Mumbaça, September 2004, O.L. Pereira (VIC 29392); Marliéria, PERD (Parque Estadual do Rio Doce), on the margin of Lagoa Preta, March 2004, O.L. Pereira (VIC 29393); Marliéria, PERD (Parque Estadual do Rio Doce), on the margin of Lagoa Preta, July 2004, O.L. Pereira (VIC 29395).

***Puccinia palicoureae*** Mains, Carnegie Inst. Washington Publ. 461: 102 (1935). (Figs 3, 4)

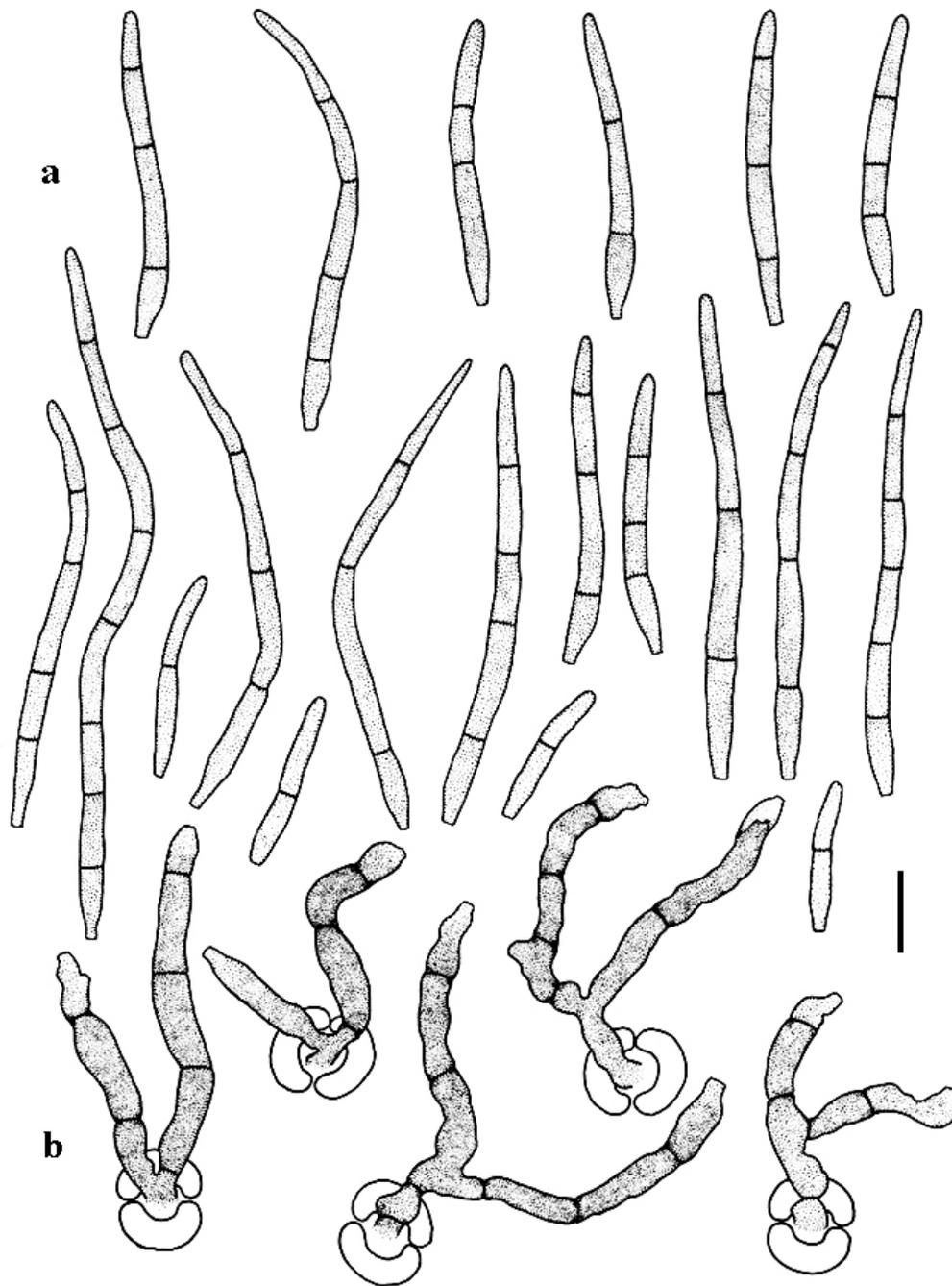
Lesions on living leaves, chlorotic adaxially, somewhat reddish-brown abaxially, 5-20 mm diam., sometimes vein-limited. *Spermogonia* and *aecia* unknown. *Uredinia* and *urediniospores* on abaxial leaf surfaces. *Uredinia* subepidermal, elliptical to circular, 150-500  $\mu\text{m}$  diam., scattered or grouped. *Urediniospores* 18-25.0  $\times$  21-32  $\mu\text{m}$  diam., broadly ellipsoid, obovoid or subgloboid, mostly hyaline to subhyaline, sometimes yellowish, pores obscure,



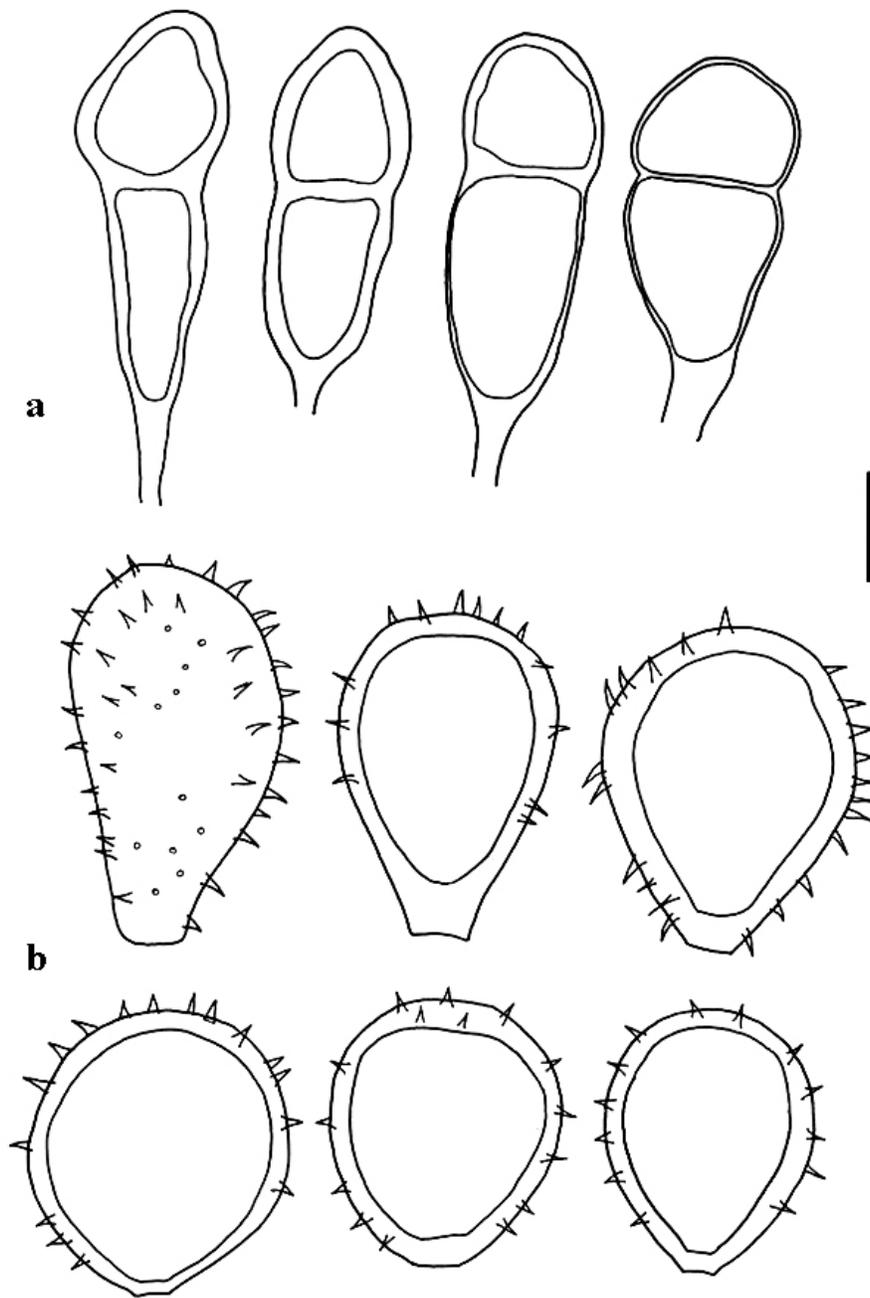
**Fig. 1.** *Pseudocercospora palicoureae* O.L. Pereira & R.W. Barreto (VIC 29390). Faint chlorotic symptoms on infected leaves.

thick-walled, wall 1.5-2  $\mu\text{m}$  wide, sparsely echinulate, spines very prominent, 2-2.5  $\mu\text{m}$  long, wide at the base. *Teliospores* not observed.

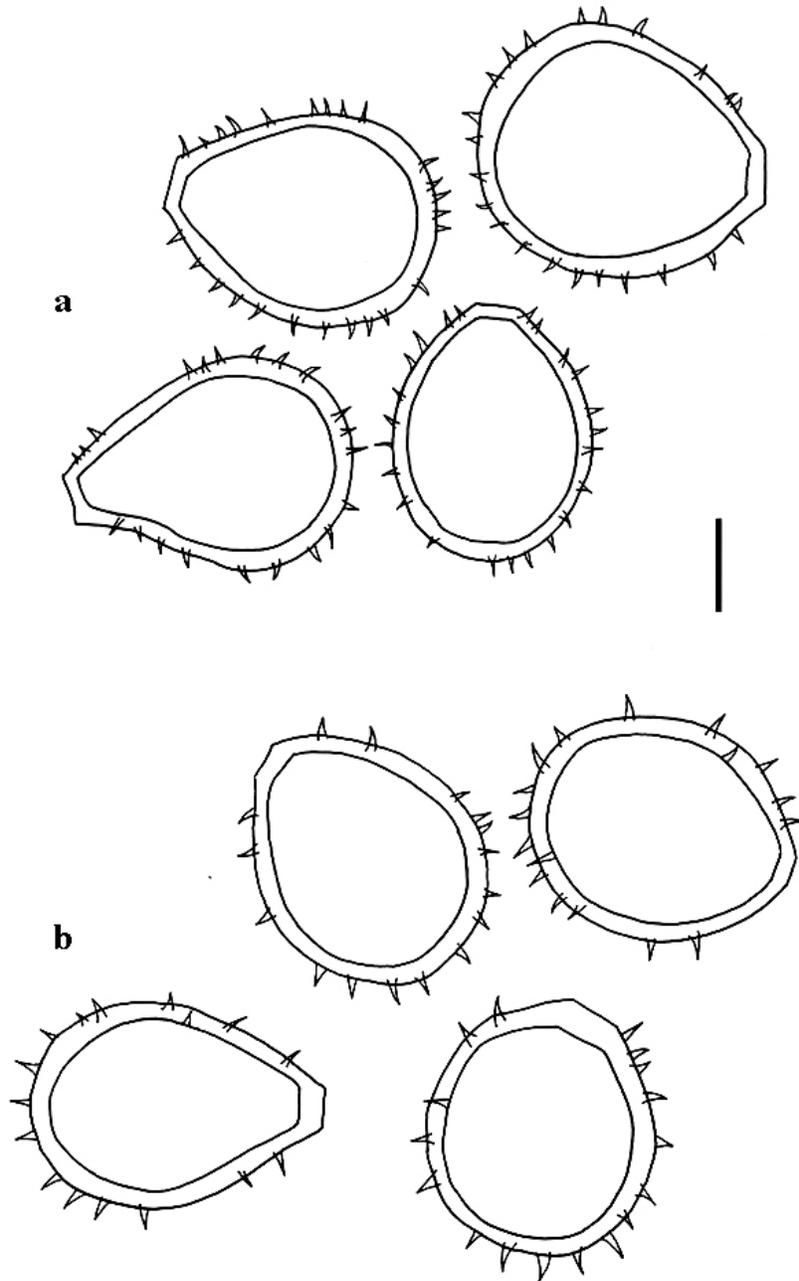
*Material examined* (all on *Palicourea marcgravii*, BRAZIL): Minas Gerais, Ouro Preto, Parque Estadual do Itacolomi, April 2004, O.L. Pereira (VIC 29412); Minas Gerais, Barbacena, May 2004, O.L. Pereira (VIC 29416); Minas Gerais, Mariana, April 2004, O.L. Pereira (VIC 29413); Marliéria, Parque Estadual do Rio Doce, Mumbaça, September 2004, O.L. Pereira (VIC 29414); Brazil, Minas Gerais, Tiradentes, May 2004, O.L. Pereira (VIC 29415); Minas Gerais, Marliéria, Parque Estadual do Rio Doce, on the margin of the Lagoa Preta, September 2004, O.L. Pereira (VIC 29417); Minas Gerais, Viçosa, campus UFV, May 1999, R.W. Barreto (VIC 29418); Paraná, Ponta Grossa, Parque de Vila Velha, on the Lagoa Dourada, January 2001, R.W. Barreto (VIC 29419).



**Fig. 2.** *Pseudocercospora palicoureae* O.L. Pereira & R.W. Barreto (VIC 29390). Conidiophores (a) and conidia (b). Bar = 10  $\mu$ m.



**Fig. 3.** *Puccinia palicoureae* on *Palicourea* sp. from Amapá (HUMVR 16012). Hyaline teliospores (a) and sparsely echinulated urediniospores (b). Bar = 10  $\mu$ m.



**Fig. 4.** *Puccinia palicoureae* on *Palicourea marcgravii* from Minas Gerais. Urediniospores from material collected in Ouro Preto (VIC 29391) (a) and Monte Verde (HMUVR 14571) (b). Note the prominent echinulations with irregular smooth areas on the urediniospores wall. Bar = 10  $\mu$ m.

*Other material examined:* on *Palicourea marcgravii*, BRAZIL, Minas Gerais, Monte Verde, J.F. Hennen & MBF (HMUVR 14571); on *Palicourea* sp., Brazil, Amapá, Reserva Monte Dourado, November 1987, J.F. Hennen & MBF (HMUVR 16012).

## Discussion

Only one cercosporoid fungus was known to occur on a member of the genus *Palicourea* (*Rubiaceae*), viz. *Pseudocercospora palicourea* (Petr. & Cif.) U. Braun (Braun, 2001), reported on *Palicourea dominguensis* (Jacq.) DC. and *P. galeottiana* M. Martens from the Dominican Republic and Mexico (Chupp, 1954; Crous and Braun, 2003). *Pseudocercospora palicoureae* differs from *P. palicourea* in producing distinct lesions, having no stromata and producing shorter conidiophores arising exclusively from stomata, and longer conidia (Table 1). *Pseudocercospora palicoureae* is the first cercosporoid fungus reported on a member of *Palicourea* in South America. There are numerous *Pseudocercospora* species on hosts of other genera of the *Rubiaceae*, but only *P. borrieriae* (Ellis & Everh.) Deighton (conidiophores usually fasciculate and unbranched) and *P. ixoricola* (J.M. Yen) J.M. Yen (conidia wider, 3.5-5 µm) are morphologically very close (Chupp, 1954; Yen and Lim, 1980). Other species are easily distinguishable in having well-developed stromata (*Pseudocercospora cephalanthi* Goh & W.H. Hsieh, *P. coprosmae* U. Braun & C.F. Hill, *P. crossopterygis* Deighton, *P. genipicola* U. Braun & Freire, *P. hameliae* (Chupp) U. Braun & Crous, *P. houstoniae* (Ellis & Everh.) U. Braun & Crous, *P. ixoriae* (Solheim) Deighton, *P. ixoriana* (J.M. Yen & Lim) U. Braun & Crous, *P. morinae* (Syd.) Sarbajna, *P. morindicola* (Jenkins & Chupp) U. Braun & McKenzie, *P. richardsoniicola* Crous & M.S.P. Câmara, *P. sarcocephali* (Vienn.-Bourg.) Deighton, *P. tenuis* (Peck) U. Braun & Crous and *P. ubatubensis* (Chupp & Viégas) Crous, Alfenas & R.W. Barreto), superficial hyphae with solitary conidiophores (*P. adinicola* (A.K. Kar & M. Mandal) Deighton, *P. hymenodictyi* (Petr.) Y.L. Guo & X.J. Liu, *P. randiae* (Thirum. & Govindu) Y.L. Guo & X.J. Liu) or numerous conidiophores in dense fascicles, mostly unbranched (*P. carveriana* (Sacc. & D. Sacc.) Crous & M.S.P. Câmara, *P. cinchonae* (Ellis & Everh.) U. Braun & Crous, *P. hemidiodiae* (Toro) Deighton, *P. paederiae* Goh & W.H. Hsieh, *P. psychotriicola* (Chupp & Doidge) Deighton) [Chupp, 1954; Yen and Lim, 1980; Deighton, 1983; Hsieh and Goh, 1990; Guo and Hsieh, 1995; Braun and Freire, 2002; Braun *et al.*, 2003]. *Pseudocercospora mitracarpicola* (J.M. Yen & Gilles) U. Braun & Crous, *P. meynae-laxiflorae* Kamal, C. Gupta & A. N. Rai and *P. mycetiae* Y.L. Guo possess very long, pluriseptate conidiophores, up to 600 µm (Guo, 2001; Kamal *et al.*, 1985; Yen, 1975), and *P. xeromphina* H.S.G. Rao *et al.* (Rao *et al.*, 1996) has conidia 3-6.5 µm wide.

**Table 1.** Morphological features of *Pseudocercospora* spp. occurring on *Palicourea* (*Rubiaceae*).

Fungus	Conidiophore	Conidia length x width (µm)	Reference
<i>P. palicoureina</i>	10-150 x 3.0-5.0	10-75 x 2.5-4.0	Braun, 2001
<i>P. palicoureae</i>	15-70 x 3.0-4.5	13-115 x 2.5-3.5	This publication

*Puccinia palicoureae* Mains was first reported on *Palicourea triphylla* DC. from Belize and its probably anamorph is considered to be *Uredo psychotriicola* Henn. (Hennen *et al.*, 2005). In Brazil, it is reported on *Palicourea coriacea* (Cham.) K. Schum., *P. guianensis* Aubl., *P. marcgravii*, *P. rigida* Kunth, *Palicourea* sp. and *Psychotria* sp. (Sotão *et al.*, 2001; Hennen *et al.*, 2005). Only two *Puccinia* species occur on hosts of the genus *Palicourea* in Brazil: *Puccinia palicoureae* and *P. fallax* Arthur (Hennen *et al.*, 2005). Both species are closely related and Cummins (1941) distinguished both by differences in the urediniospore spines. On *P. palicoureae* the urediniospores have more prominent spines and irregular smooth areas on the wall, whereas on *P. fallax* the spines are less prominent and uniformly distributed on the urediniospore wall surfaces. Additionally, Hennen *et al.* (2005) reported that the teliospores of *P. palicoureae* are larger. No teliospores were observed in any of the *P. marcgravii* samples collected with rust disease during our survey, however, the characteristic urediniospores fit well with those reported for *P. palicoureae* by other authors (Cummins, 1941; Sotão *et al.*, 2001; Hennen *et al.*, 2005). During the examination of the original material on which the first report of *P. palicoureae* on *P. marcgravii* in Brazil was based (HMUVR 14571), no teliospores were found as well, only the characteristic urediniospores (Fig. 4a). Teliospores were observed only on the material of *Palicourea* sp. from Amapá (HMUVR 16012) (Fig. 3a). Hennen *et al.* (2005) described teliospores of *P. palicoureae* from the latter material, as well as Sotão *et al.* (2001). Possibly, *P. palicoureae* needs warmer climatic conditions such as those found in Northern Brazil in order to be produced.

Curiously, the phytopathogenic mycobiota of this plant species is restricted until now to only these two species. A possible explanation for this low number of phytopathogenic fungi infesting this plant is that *P. marcgravii* is known to form associations with an endophytic species of *Xylaria* that produces compounds with high antifungal activity (Cafêu *et al.*, 2005). It is possible that the expansion of this survey to include additional areas in Brazil will increase this list. A damaging scab disease, possibly caused by a species of *Elsinoë* Racib., was collected once by R.W. Barreto in the State of Paraná

(Ponta Grossa, Parque Estadual de Vila Velha) but attempts to isolate the fungus were unsuccessful and attempts to recollect the fungus also failed.

Cercosporoid fungi have been studied in more than one occasion as potential weed biocontrol agents either by following the classical approach or as possible mycoherbicides. An example is *Cercospora caricis* Oudem. which was studied as a possible mycoherbicide for purple nutsedge *Cyperus rotundus* L. (Ribeiro *et al.*, 1997). It causes severe necrotic symptoms and defoliation on *C. rotundus* (Ribeiro *et al.*, 1997; Inglis *et al.*, 2001). Field observations of the damage caused by the new species *P. palicoureae* on its host indicated that symptoms are not very pronounced. No necrotic areas can be observed and only faint chlorotic symptom results from infection. Even when large areas of a leaf surface are colonized, no leaf death or defoliation resulted. Additionally, *P. palicoureae* grew very slowly in culture and did not sporulate. So, at this stage it seems that this fungus has little potential as a mycoherbicide. There is no potential for biological control of *P. marcgravi* in Brazil using the rust pathogen because it is a widespread biotrophic fungus (therefore not adequate for mass production) attacking a native weed in its center of origin (therefore the classical approach would not apply).

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