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MYCOPARASITES OF *COCCODIELLA MICONIAE* (ASCOMYCOTA: PHYLLACHORACEAE) A POTENTIAL BIOCONTROL AGENT FOR *MICONIA* *CALVESCENS* (MELASTOMATACEAE)

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Mycoparasites of *Coccodiella miconiae* (Ascomycota: Phyllachoraceae) a potential biocontrol agent for *Miconia calvescens* (Melastomataceae)

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Abstract

Miconia calvescens is a devastating plant invader in Hawaii and French Polynesia. Exploratory surveys for pathogens associated with this small tree were performed in its neotropical native range as part of a classical biological control program. Observations made in the field in Brazil, Costa Rica and Ecuador suggested that *Coccodiella miconiae* (Ascomycota: Phyllachoraceae) has great potential as a biocontrol agent. It was also noted that in its native range populations of this fungus are often severely hyperparasitized by a series of fungi. The presence of such fungi is regarded as a serious threat to the potential efficacy of *C. miconiae* in the event of its introduction into the new ranges of distribution of the plant. In the present work the taxonomy of eight of these fungi was studied. Three new species and a new variety are described: *Sagenomella dimorphica*, *Cladosporium mycoparasiticum*, *Redbia annulata* and *Sagenomella alba* var.nov. *synematosa*. *Corynespora cassiicola* was found for the first time growing on another fungus. *Paranectriella juruana* was also recorded. In addition to those three fungal species were also found associated to *C. miconiae* stromata. These were tentatively identified as *Paecilomyces* sp., *Periconiella* sp. and *Pleospora/Lewia* and will be described and discussed in a later publication.

Key-words: hyperparasites, black pimple, weed biocontrol, biological control

Introduction

Miconia calvescens DC is a small tree or shrub native from wet tropical to subtropical areas of South and Central America. In alien situations where it was introduced, particularly in oceanic islands, it became a serious invader and threatens biodiversity and the survival of unique terrestrial ecosystems (3, 10, 15, 17, 18, 19, 20).

Classical biological control is the best, and usually the sole sustainable alternative for mitigating biological invasions, and particularly for that promoted by *M. calvescens* considering the widespread infestation of whole vegetation formations and the difficulty of access to many of the infested sites (18).

Surveys for pathogens associated with *M. calvescens* started in 1995 and covered areas within the natural range of its distribution in South and Central America. During this period, *Coccodiella miconiae* (Duby) Hino & Katumoto was found associated to a severe foliar disease of miconia in Brazil, Ecuador and Costa Rica. The disease cause by *C. miconiae* was named "black pimple" because of the protuberant black stromata produced abaxially on leaves accompanied by yellow and raised areas of the lamina adaxially. When observed with the unaided eye the fungus structures and disease symptoms resemble very closely those of rusts caused by microcyclic Uredinales. This fungus can be actually regarded as the ecological equivalent to a rust fungus - an obligate biotrophic plant pathogen that attacks growing leaves and provokes their distortion and reduction of photosynthetic area of infected leaves.

In natural conditions black pimple outbreaks can be very severe and since first observed this fungus has been considered a good candidate for biological control. That evaluation is made based on the observed damage it is capable of causing in its natural habitat despite the high level of arthropod feeding and mycoparasite incidence usually observed on *C. miconiae* stromata. Under particularly humid controlled conditions the commonly occurring white colony forming mycoparasite species, provisionally identified as *Acremonium* sp. was the principal impediment for attempts to keeping *C. miconiae* colonies on live plants in Hawaii (Hawaii Department of Agriculture Quarantine Lab). The potential of mycoparasites to limit the population of plant pathogenic fungi is well known for some associations and several studies have been made on their use in the biological control of plant diseases such as: gray mould of the Rosaceae (29, 30), witches' broom of cocoa (25), damping-off and root rots (2, 5, 13, 22, 32) and others. Those studies had always in perspective the useful aspect of mycoparasites. Perhaps the most common instance where mycoparasites are regarded as noxious is for the mushroom growing industry (28). No record could be found in the literature of studies of mycoparasites as a problem for the use of fungi in biological control. The presence of hyperparasitic fungi on *C. miconiae* is regarded here as noxious and as a threat to the manipulation and the potential efficacy of an introduction of this fungus as a classical biological control agent against miconia. Elucidating the taxonomy of those fungal species is a necessary step towards understanding the pathogenic mycobiota of *C. miconiae*.

Material and methods

Systematic sampling of *M. calvescens* leaves infected with *C. miconiae* was made during 12 months (October of 2000 to November of 2001), at a site known as "Balsa Velha" ou "Retirante" in an area belonging to the Companhia Agrícola Florestal

Santa Bárbara Ltda. (CAF) at the municipality of Dionísio, state of Minas Gerais (Brazil) at 3 week intervals. Thirty leaves were collected at each visit and incidence of mycoparasites associated with *C. miconiae* was recorded. Some *ad hoc* collections were also made in Brazil, Ecuador and Costa Rica and included in the present work.

Each leaf was carefully examined in the laboratory and every stroma on each leaf was observed under the dissecting microscope. Whenever a fungus was found growing on a stroma or an unrecognized, abnormally shaped or colored stroma was noted a sample was prepared for observation with a light microscope. Direct isolations from the fungal structures growing on the stromata were performed on plates containing Vegetable Broth Agar (VBA) (23). Plates were kept in an incubator at 25°C in the dark. Cultures were preserved in silica-gel as described in DHINGRA et al. (4).

Slide cultures were mounted from such cultures for a more detailed observation of conidial ontogeny and sporulating structures. Those slides together with slides prepared from fresh material were used for fungal descriptions and preparation of illustrations. Culture descriptions were made based on observation of cultures grown on VBA and PDA either in the dark or under a 12 hours light regime (combination of fluorescent daylight and BLB lamps).

Results and discussion

Six fungal species were found colonizing *C. miconiae* stromata during the survey at Dionísio. Additionally, two fungal species were found parasitizing it in *ad hoc* collections made respectively in Ecuador and Costa Rica. This fungus assemblage included six hyphomycete anamorphic fungi and two ascomycetes. Those species are described below:

***Sagenomella alba* Gams & Söderström var.nov. *synematosa* (Figures 1 and 2)**

Colonies white to gray initially sparse and later becoming dense and completely covering the stromata of *C. miconiae* and also spreading on the surrounding surface of the leaf and forming feathery strands. *External mycelium* composed of branched, septate, hyaline hyphae with abundant phialides, each holding a terminal drop of mucilagenous conidia (on slides mounted from freshly collected material) or long conidial chains (in slide cultures), on mature colonies feathery synematous mycelial strands are typically formed. *Stromata* absent. *Conidiophores* absent. *Conidiogenous cells* phialidic laterally produced on the external mycelium; enteroblastic-phialidic; filiform, wider at the base tapering towards the apex; 27-57 x 1-3µm; hyaline. *Conidiogenous loci* terminal indistinct. *Conidia* mucilaginous; catenulate forming basipetal chains; fusiform to oval; 3-4.5 x 1µm, apex and base acute, aseptate, hyaline, conidia scars indistinct, eguttulate, smooth.

In culture: Slow growing (2.5 to 2.8cm diam. after 13 days), aerial mycelium generally cottony to floccose, dense, periphery humid and flat, colonies sometimes showing dense mucilagenous mycelial strands, white to pink with similarly colored reverse; sporulation abundant dry, faintly zonated.

Material examined: VIC 22193; Dendrologia, Universidade Federal de Viçosa, Viçosa, state of Minas Gerais, Brazil, 17th Aug 2001, C.D.S. Seixas.

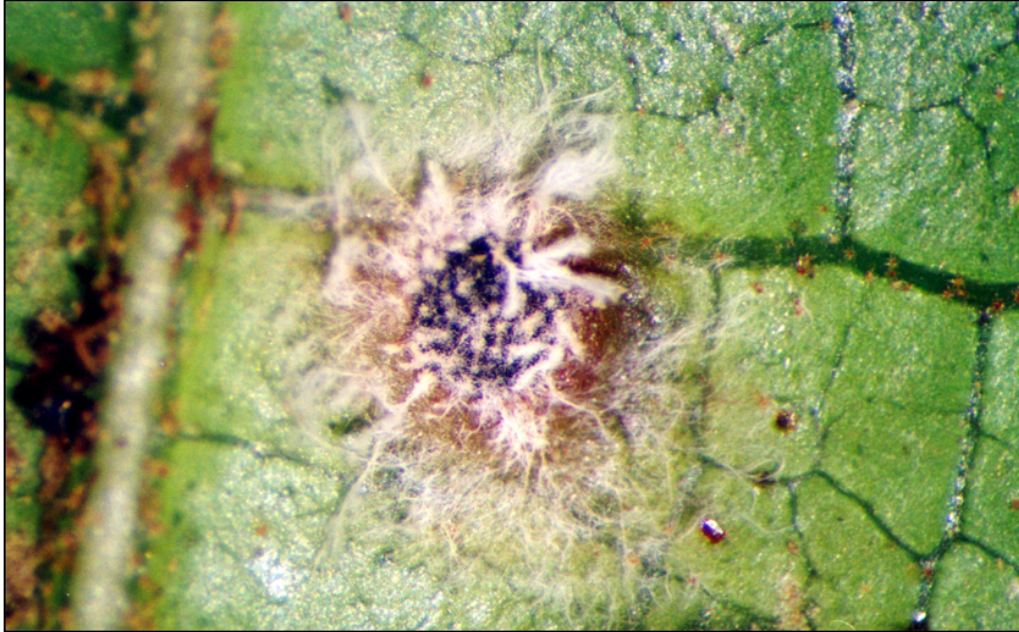


Fig. 1. Stromata of *Coccidiella miconiae* parasitized by *Sagenomella alba* var. *synematoso* (19x). Note white feathery synematoso strands.

Sagenomella dimorphica C.D.S. Seixas & R.W. Barreto **sp.nov.** (Figures 3 and 4)

Ab *Sagenomella diversispora* (van Beyma) W. Gams, cellulae conidiogenae singularia, 25.0-65.0 x 2µm, hyalinae; a) conidia fusiformia, 3.0-6.0 x 1.5µm, hyalinae, laeves; b) conidia observe-ovalis, 3.0-6.0 x 2.0-3.0µm, hyalinae, laeves, differens.

Etim.: referring to the production by the fungus of conidia having two different shapes.

Colonies white to gray initially sparse and later becoming dense and completely covering the stromata of *C. miconiae* and also spreading on the surrounding surface of the leaf. *External mycelium* mostly flattened on the substrate surface but occasionally forming upright threads composed of hyaline hyphae, septate, branched, smooth. *Stromata* absent. *Conidiogenous cells* phialidic, formed singly from the external mycelium, filiform, 25.0-65.0 x 2.0µm, hyaline; smooth. *Conidiogenous loci* terminal, minute *Conidia* of two kinds: a) mucilaginous, catenulate, forming basipetal chains, fusiform, 3.0-6.0 x 1.0-1.5µm, aseptate, hyaline, eguttulate, smooth; b) dry, isolate, obovoid, 3.0-6.0 x 2.0-3.0µm, aseptate, hyaline, eguttulate, smooth (observed only in slide cultures).

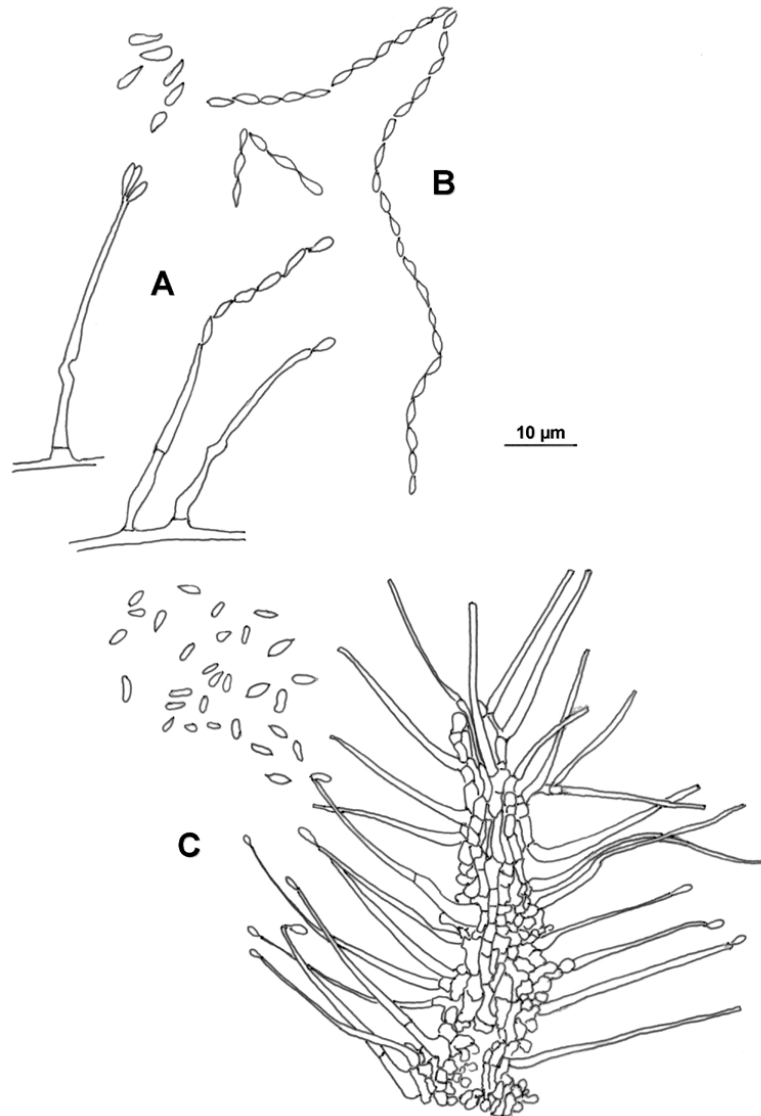


Fig. 2. Sagenomella alba var. *synematosa*: phialides forming conidia (A), conidial chain (B), mature and immature phialides on the apical surface of a synematous mycelial strand (C).

In culture: Slow growing (1.8 to 3.5cm diam. after 13 days); central area of relatively sparse aerial mycelium followed by a relatively wide zone without aerial mycelium and composed only of humid superficial mycelium; medium appressed by wrinkles that form a wide concentric zone from the central area where aerial mycelium is present to the periphery with sparse to absent aerial mycelium; white to pink with a similarly colored reverse; sporulation abundant, faintly zonated.

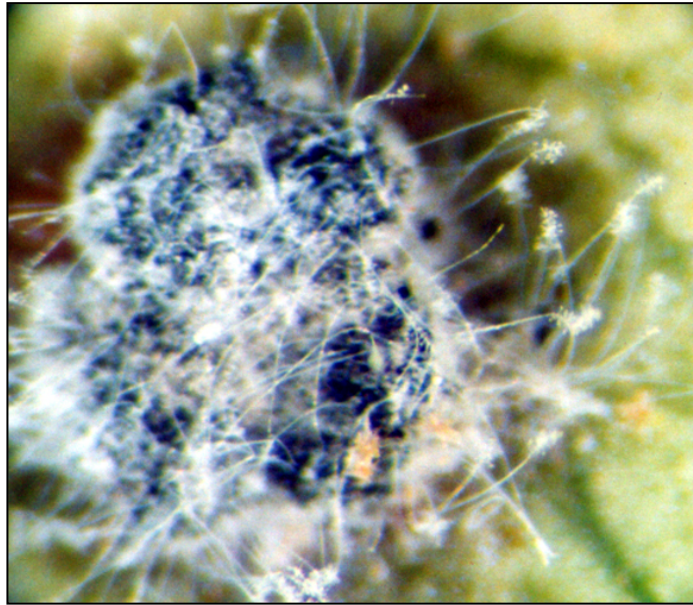


Fig. 3. A stroma of *Coccidiella miconiae* parasitized by *Sagenomella dimorphica*. Note the long conidiophores formed on this growth form of the fungus (53x).

The two fungal species that were at first identified as *Acremonium* sp. and later recognized as belonging to *Sagenomella* were by far the most common and seemingly damaging to *C. miconiae*. *S. alba* var. *synematosa* predominates in the municipality of Viçosa whereas *S. dimorphica* appears to be the dominant mycoparasite of *C. miconiae* in Dionísio. Microclimatic conditions appear to be critical for determining the severity of outbreaks of *S. alba* var. *synematosa* in Viçosa. It was noticed that a group of plants growing along the banks of a reservoir on the campus of the Universidade Federal de Viçosa often had the vast majority of *C. miconiae* stromata parasitized by *S. alba* var. *synematosa*.

Sagenomella was proposed by Gams (11) and is morphologically similar to *Acremonium*. Until now this genus included six species. The material collected in Viçosa (VIC 22193) fits well within the description provided by Gams (11) for *S. alba*. Nevertheless, the fungus occurring in Viçosa has synematous mycelial strands that were not described for *S. alba*. Although obvious, this morphological difference represents a single difference between this fungus and *S. alba* and was not regarded here as sufficient for the proposal of a new species of *Sagenomella*. Recognition of distinction at the variety level was considered as more appropriate. In the event other relevant differences are found this taxon should then be raised to the rank of species.

The fungus from Dionísio (VIC 22194) is also morphologically similar to *S. alba* and when the key (11) is used the name *S. alba* is the closest match. Nevertheless, in *S. alba* conidia can be either fusiform or lemon-shaped but conidia of both shapes are not produced simultaneously and contrarily to what described here for the new species *S.*

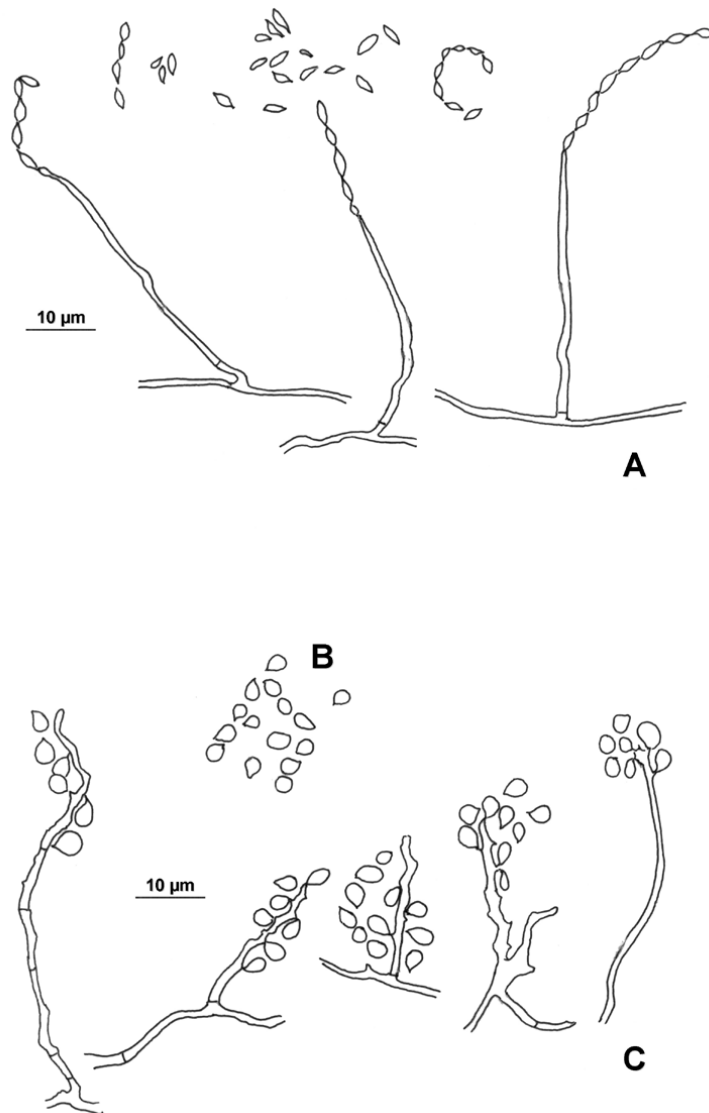


Fig. 4. *Sagenomella dimorphica*: phialides forming chains of fusiform conidia (A), obovoid conidia (B), phialides with obovoid conidia (C).

dimorphica not obpiriform. One species of *Sagenomella* is dimorphic. *S. diversispora* (11) but conidia in this species are ornamented and not smooth as in the specimens from Dionísio. This fungus doesn't therefore match properly within the concept for any of the known species of *Sagenomella* and therefore represents a previously unknown species.

Cladosporium mycoparasiticum Berk & Curt J. Linn. Soc. 10(46): 362 (1868) (Figures 5 and 6)

Colonies on *C. miconiae* stromata, associated to the complete destruction of the host ascomata centrum, dry, brown. *External mycelium* branched, 2.5-5µm diam., septate, smooth. *Stromata* absent. *Conidiophores* cylindrical irregularly inflated along the axis, occasionally geniculate, often with percurrent enteroblastic proliferations, 210.0-440.0 x 5.0µm, 4-10 septate, unbranched, chestnut brown, smooth. *Conidiogenous cells* terminal and intercalary, integrated, holoblastic, pale brown, smooth. *Conidiogenous loci* conspícuos, 1-6/cell, protuberant, thickened, darkened. *Conidia* dry, forming branched and unbranched acropetal chains, elipsoidal, lemon-shaped, fusiform, ovoid, cylindrical or subcylindrical, 3.0-17.0 x 2.0-3.0µm, 0-1 septate, conidial scar prominent, slightly thickened, darkened, 1-4/conidium ; pale brown to subhyaline, egutulate, smooth.

In culture: Slow growing (2.6 to 4.9cm diam. after 13 days); colonies powdery with sparsely distributed raised areas of hairy tufts, medium surface appressed centrally and concentrically waved from the centre outwards, grayish-brown with white to gray tufts, dark green to black reverse, sporulation abundant and dry.

Material examined: VIC 22195, municipality of Dionísio, state of Minas Gerais, Brazil, 17th Oct. 2001, C. D. S. Seixas

Comparison of the mycoparasitic *Cladosporium* on *Coccidiella miconiae* (VIC 22195) with the common species described in literature (7, 8) has indicated that the known species appearing to be closest to the specimen from Dionísio is *C. oxysporum*. Nevertheless, conidiogenous cells of *C. oxysporum* are not inflated, conidia are larger than those found in VIC 22195 and *C. oxysporum* is a common and widespread species normally associated with dead plant tissue and not known to occur as a mycoparasite. It is therefore considered here to be a new species for the genus *Cladosporium*.

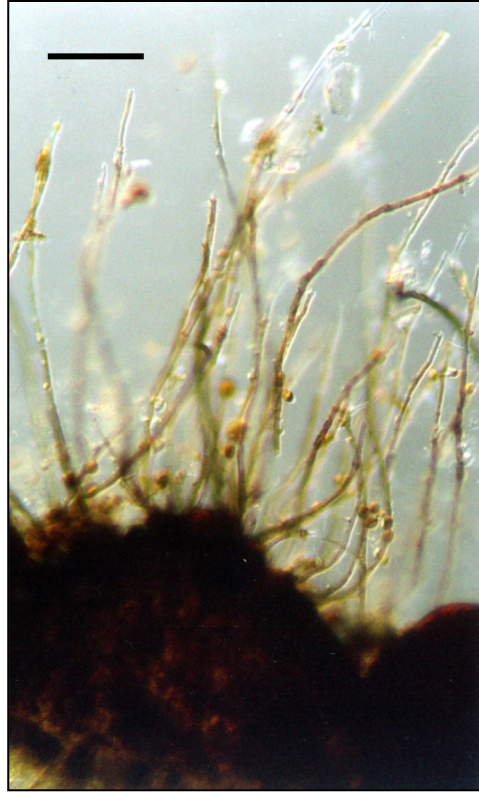


Fig. 5. Conidiophores of *Cladosporium mycoparasiticum* on stromata of *Coccidiella miconiae* (Bar = 20 μ m).

Corynespora cassiicola (Berk. & Curt.) Wei *Mycological Papers* 34: 5 (1950) (Figures 7 and 8)

Colonies composed of sparse grayish growth on degraded *C. miconiae* stromata. External mycelium absent. Stromata absent. Conidiophores emerging from *C. miconiae*, isolate, straight to slightly curved, unbranched, cylindrical with a relatively inflated base and often inflated into somewhat bulbous parts above loci where percurrent enteroblastic proliferations occurred, 0-2 bulbous expansions and up to four percurrent proliferations per conidiophore, 99.0-199.0 x 4.0-10,5 μ m, 1-5 septate, brown becoming pale brown close to the apex, smooth. Conidiogenous cells tretric with enteroblastic percurrent proliferations occasionally with constrictions along the axis and commonly with a constriction at the base, cylindrical, 64.0-121,5 x 6,5-11,5 μ m, pale brown. Conidia dry,

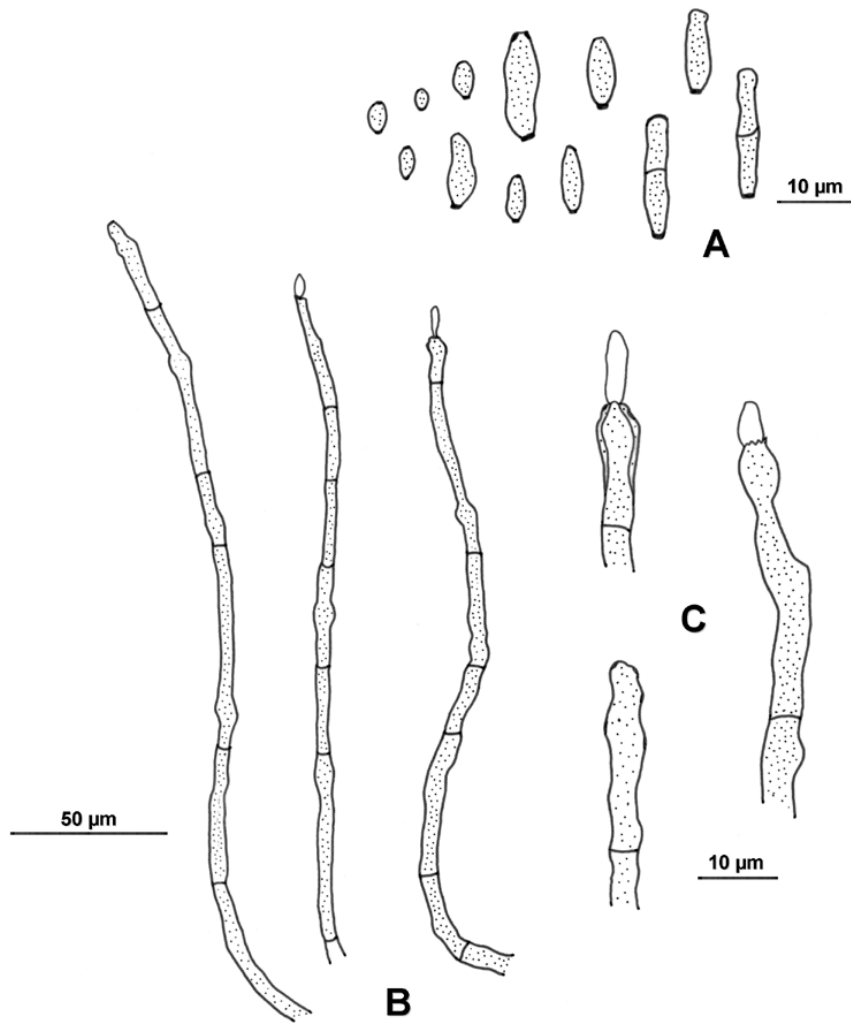


Fig. 6. *Cladosporium mycoparasiticum*: conidia (A), conidiophores (B), conidiophore apices (C).

either isolate (on fresh material mounts) or catenulate, forming acropetal conidial chains with 2-5 conidia (in slide cultures), obclavate to cylindrical, 54.0-141.0 x 10,5-19.0 μ m; apex rounded, base truncated, 5-5.0 μ m; 4-11 pseudoseptate, scar thickened, darkened, pale brown to subhyaline, eguttulate, smooth.

In culture: relatively fast growing (9 cm diam after 10 days), aerial mycelium felty on PDA and wooly on VBA, sparse when growing under 12 hours photoperiod and dense when growing in the dark, pale gray to gray with dark gray to black reverse, humid, sporulation abundant.

Material examined: VIC 22196, municipality of Dionísio, state of Minas Gerais, Brazil, 25th Sept 2001, C. D. S. Seixas

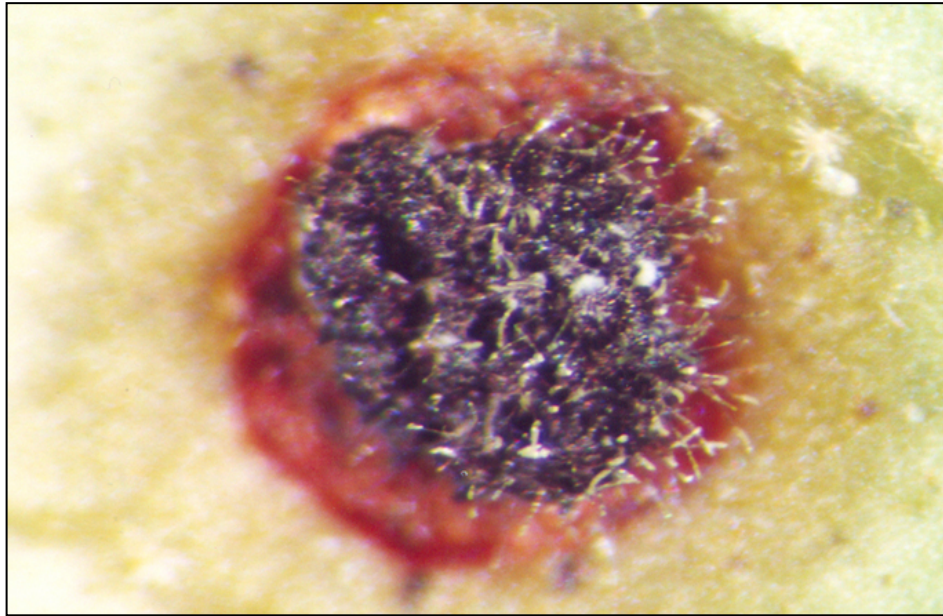


Fig. 7. Stroma of *Coccidiella miconiae* parasitized by *Corynespora cassiicola* (48x).



Fig. 8. Corynespora cassiicola: basipetal chain of conidia formed on a slide culture (Bar = 50 μ m).

Corynespora was found only once and was the rarest among the hyperparasites found on *Coccodiella miconiae* during the sampling period in Dionísio-MG.

The fungus found growing on stromata of *C. miconiae* has a morphology that is equivalent to that described for *C. cassiicola* (7) and differs from that described for other species in the genus (6, 9, 16, 31). The length range for conidia and conidiophores recorded for VIC 22196 is smaller than that described for *C. cassiicola*. This, nevertheless is a very variable character for most anamorphic taxa and usually regarded as not having taxonomic significance. Additionally, *C. cassiicola* is regarded as a very variable species within the genus. A comparison of *C. cassiicola* collected on 20 different host plants from various parts of the world has shown a great variation of conidial size for this species (21). Another study, involving the comparison of genetic differences among different isolates of *C. cassiicola*, using PCR-based techniques, has shown that there was no detectable genetic differences among such isolates (26,27). Therefore, care needs to be taken while analyzing morphological differences in this highly variable taxon in order to avoid inadequate proposition of new taxa. It is, nevertheless, surprising to find a mycoparasitic strain of *C. cassiicola* since until now this species has been generally considered a cosmopolitan non-specific plant pathogen (7, 21).

Redbia annulata C.D.S. Seixas & R.W. Barreto *ad interim* (Figures 9 and 10)

Similar to *Redbia puccinicola* Deighton & Pirozynski, but differs in that the conidiophores are 15.5-61 x 2.0-4.0µm, conidia 1-4 denticulate, rounded at the apex, obconical to rounded at the base, 6.5-15.5 x 3µm, verruculose.

Etim. Referring to the protuberant ring appearing on conidial septa, particularly on dried specimens.

Colonies dense, powdery, brown, covering the stromata of *C. miconiae*. *External mycelium* branched, 1.5-3.0 µm diam, septate, pale brown, smooth. *Stromata* absent. *Conidiophores* fasciculate or produced singly from the external mycelium, slightly curved, sometimes sinuose and geniculate, unbranched, cylindrical, tapering abruptly towards the apex, 15.5-61.0 x 2.0-4.0 µm, 0-6 septate, pale brown, sometimes becoming almost hyaline near the apex, mostly smooth but sparsely denticulate near the apex, pale brown becoming nearly hyaline at the apex, smooth. *Conidiogenous cells* integrate, terminal and intercalary, holoblastic with enteroblastic percurrent proliferations, raduliform, 1-4 denticulate. *Conidia* dry, subcylindrical to fusiform, 6.5-15.5 x 3.0 µm, apex rounded, base obconic to rounded, 1-3 septate (predominantly 2), subhyaline to grayish, conidial scar slightly thickened and darkened, eguttulate, thinly verruculate.

In culture: very slow growing (1.6 to 1.8 cm diam. in 13 days), wooly with setae-like and occasionally also synematous structures distributed over the colony, gray, dark gray, greenish-gray or chestnut-brown and equivalently colored at colony reverse, diurnal zonation occasionally noticeable, no sporulation.

Material examined: VIC 22197, municipality of Dionísio, MG, Brazil, 17 Oct 2001.

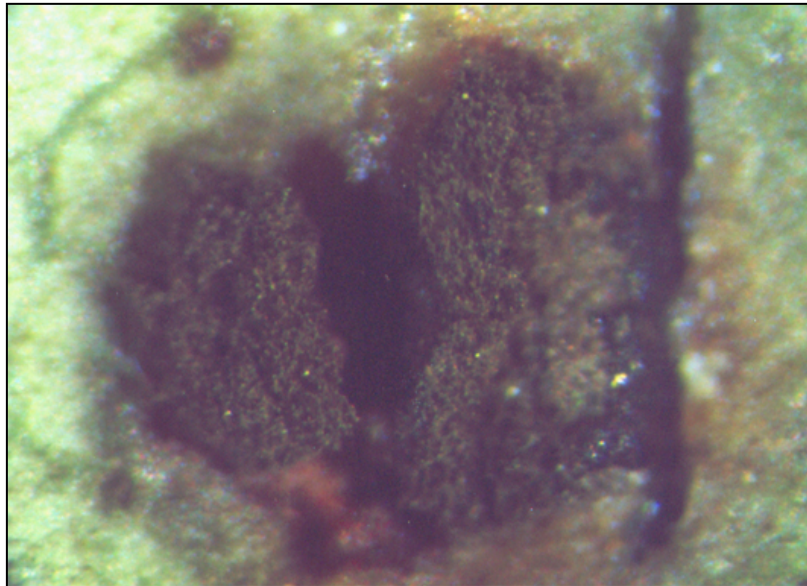


Fig. 9. *Redbia annulata* growing on stroma of *Coccidiella miconiae* (62x).

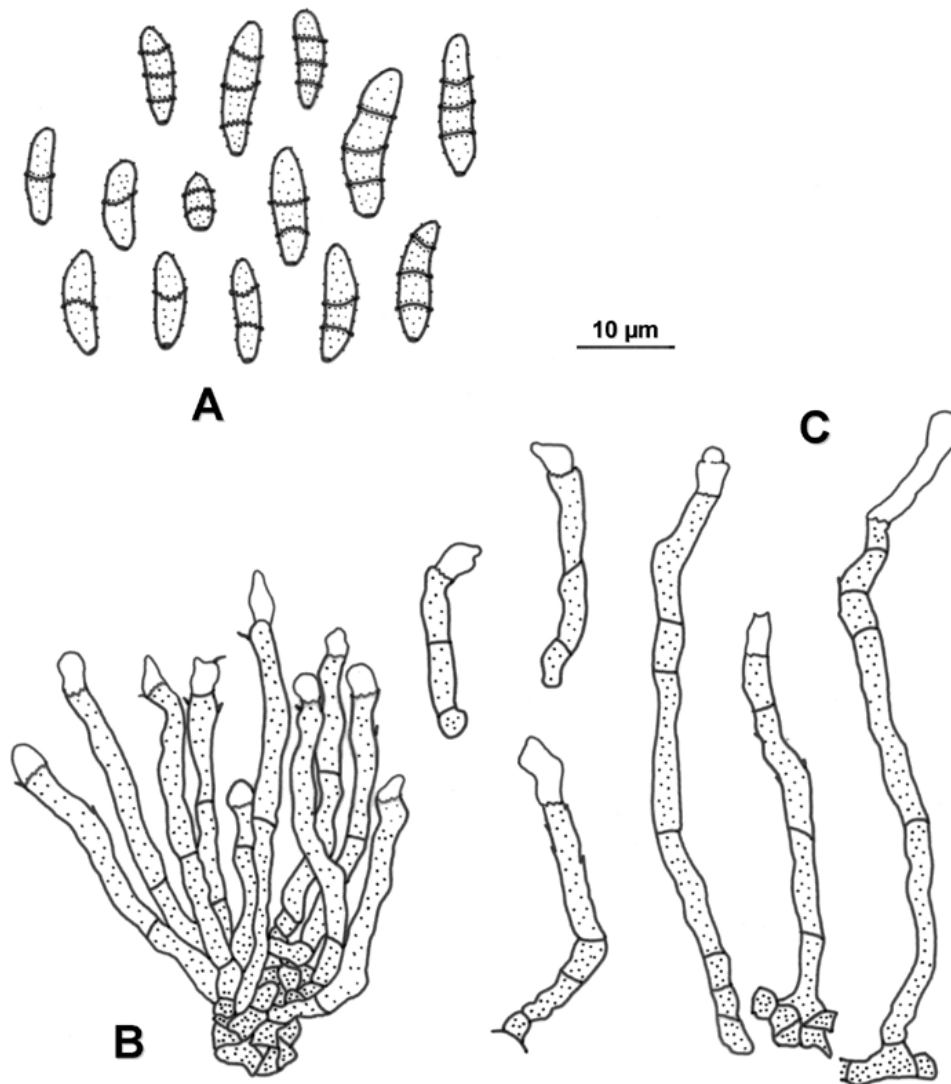


Fig. 10. *Redbia annulata*: conidia (A), conidiophore fascicle (B), isolated conidiophores (C).

There are three species of *Redbia* described in the literature: *Redbia puccinicola* Deighton & Pirozynski, described from telia of *Puccinia holosericea* Cooke; *Redbia elegans* Pirozynski & Hodges (8) and *Redbia trichomambusta* R.W. Barreto (1).

Relevant morphological features helping to separate species in this genus are given in Table 1.

Tabela 1. Morphological features of fungi in the genus *Redbia*.

Species	Conidial size (µm)	Conidiophore size (µm)	Conidial surface
<i>R. puccinicola</i>	10.0-22.0 x 3.0-4.5	>230.0 x 4.0-5.0	smooth
<i>R. elegans</i>	15.0-35.0 x 4.0-5.0	>600.0 x 5.0-6.0	verruculose
<i>R. trichomambusta</i>	8.0-21.0 x 1.0-6.0	22.0-66.0 x 1.0-4.0	smooth
<i>R. annulata</i>	6.5-15.5 x 3.0	15.5-61.0 x 2.0-4.0	verruculose

Redbia annulata is easily distinguished from *R. puccinicola* and *R. trichomanbusta* by its verruculose conidial surface and differs from *R. elegans* by having smaller conidia and conidiophores. Another distinctive feature of *R. annulata* is the projection that can be observed along each conidial septate periphery, particularly on microscopic mounts made from dried specimens. *R. annulata* and *R. puccinicola* are of mycoparasitic habit whereas *R. elegans* is a saprophyte and *R. trichomambusta* was found associated with trichomes of *Chromolaena odorata* (L.) King & Robinson, sometimes associated with *Mycovellosiella perfoliata* (Ellis & Everh.) Munt.-Cvetk. It is, nevertheless, uncertain whether *R. trichomambusta* is a mycoparasite or a weak plant pathogen. *R. annulata* was the second most frequent mycoparasite of *C. miconiae* found during the survey.

Paranectriella juruana (Henn.) Henn. ex Piroz Kew Bull 31: 598 (1977) (Figures 11 and 12) Syn. *Paranectria juruana* Henn. Hedwigia 43: 245 (1904)

Anamorphic stage: *Titaea acarifera* (Höhnelt) Damon J. Wash. Acad. Sci. 42: 367 (1952)

Colonies white, waxy, completely covering decomposed stromata of *C. miconiae*. *External mycelium* absent. *Ascomata* pseudothecial, partially immersed on a stroma composed of loose white mycelium covering the carbonaceous stroma of host fungus, subglobose, deformed when dry, 150.0-310.0 x 50.0-300.0 µm, walls of textura angularis, 10-13 µm thick, wall cells elongated in longitudinal sections, 15.0 x 6.0 µm, white to pale yellow, pseudothecial hairs abundant, hyphoid, cylindrical, curved to sinuous, 0-2 septate, with obtuse terminal portion, 10.0-24.0 x 4.0-6.0 µm. *Intercial filaments* filiform pseudoparaphyses, longer than 80.0 µm, branched, anastomosed. *Asci* bitunicate, subclavate, 60.0-80.0 x 9.0-10.0 µm, eight spored. *Ascospores* bisseriate, ellipsoidal to fusiform, 11.0-15.0 x 5.0-6.0 µm, 3 septate, slightly constricted at septae, hyaline, smooth, equally apiculate at both ends, 5.0-6.5 x 1.5 µm.

Material examined: VIC 22198, Rio Pedra Fina, Ecuador, 10th May 2000, R. W. Barreto.

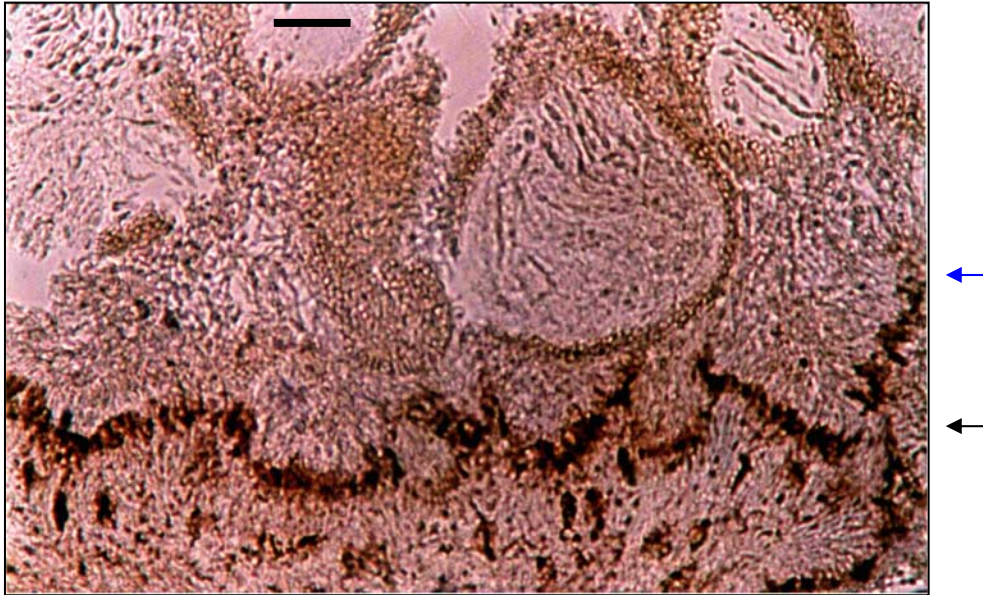


Fig. 11. *Paranectriella juruana* (→) on stromata of *C. miconiae* (→) (Bar = 50 μ m).

The genus *Paranectriella* includes several mycoparasitic species (24, 14). The comparison of the morphological features of the hyperparasite of *C. miconiae* from Ecuador and the other species of *Paranectriella* indicate that it was an isolate of *P. juruana*. There were only small biometric discrepancies that were regarded as being too small to be of taxonomic significance.

Specimens of *M. calvescens* attacked by *C. miconiae* collected at highland situations in Ecuador were often so highly hyperparasitised by *P. juruana* that they were regarded as useless for inoculation works on miconia. This fungus was found only in Ecuador under this environmental situation and it was never found in Brazil or Costa Rica.

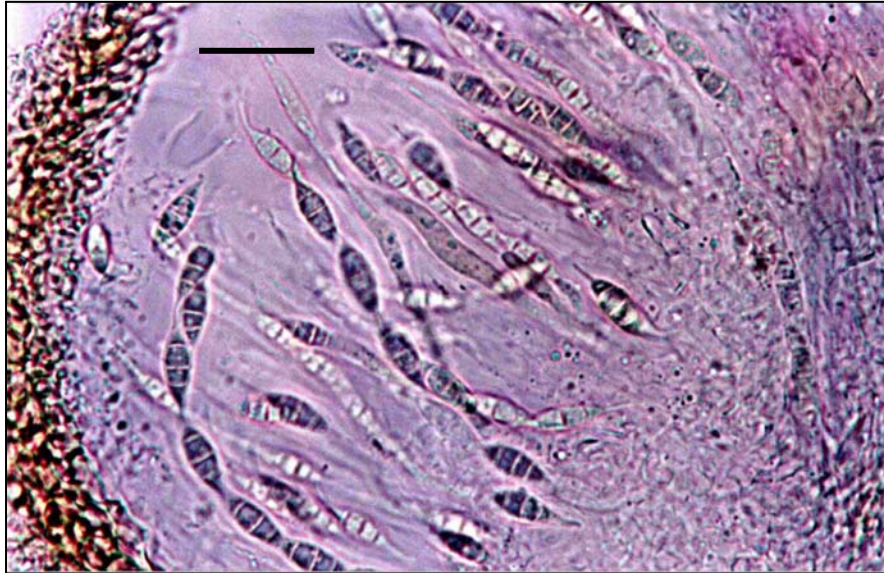


Fig. 12. *Paranectriella juruana*: asci and ascospores (Bar = 20 μ m).

Additional species

More recently some additional fungal species were found in association with *C. miconiae*. These were tentatively identified as belonging to *Paecylomyces*, *Periconiella* and *Pleospora/Lewia*. Their identity is still under investigation and these taxa will be dealt with in a later publication.

An unexpectedly diverse and novel mycobiota was found using *C. miconia* as substrate. At least four among the eight mycoparasites described here represent new taxa. All, except for *P. juruana* were recorded here for the first time on *C. miconiae*. This illustrates the lack of information available on hyperparasitic fungi as a whole. Unfortunately, with few exceptions, these fungi had attracted little attention from mycologists, perhaps because of a lack of practical motivation for this. The intent to use *C. miconiae* as a classical biocontrol agent for *M. calvescens* offered a good opportunity for a contribution in this field.

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