

MEIRIELE DA SILVA

**MICROBIOTA FOLÍCOLA DE *Dimorphandra wilsonii*, ESPÉCIE ARBÓREA
BRASILEIRA AMEAÇADA DE EXTINÇÃO**

Dissertação apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Fitopatologia, para obtenção do título de *Magister Scientiae*.

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Pós-Graduação em Fitopatologia, para
obtenção do título de *Magister Scientiae*.

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(Orientador)

*A Deus,
A minha mãe,
Por serem o meu apoio.*

Dedico!

“Contudo, seja qual for o grau a que chegamos o que importa é prosseguir
decididamente” Fi 3,16

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BIOGRAFIA

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RESUMO

SILVA, Meiriele da, M.Sc., Universidade Federal de Viçosa, fevereiro de 2012. **Micobiota folicola de *Dimorphandra wilsonii*, espécie arbórea brasileira ameaçada de extinção.** Orientador: Robert Weingart Barreto. Coorientador: Olinto Liparini Pereira.

Dimorphandra wilsonii Rizzini, Fabaceae, popularmente conhecida como “faveiro de Wilson”, é espécie endêmica de Minas Gerais, que ocorre na transição do Cerrado para a Mata-Atlântica, ao norte de Belo Horizonte. Consta do anexo I da lista da Flora Brasileira Ameaçada de Extinção, nas listas da flora ameaçada de Minas Gerais e da International Union for Conservation of Nature (IUCN), onde figura como criticamente ameaçada, o nível que antecede o de extinção. Em contrapartida, *Dimorphandra mollis* Benth., planta taxonomicamente muito próxima do faveiro de Wilson, popularmente conhecida como “faveira” e “falso barbatimão”, é comum e amplamente distribuída pelo Cerrado brasileiro principalmente nos estados de Goiás, Mato Grosso, Minas Gerais e São Paulo. Com o objetivo de contribuir para o conhecimento sobre a micobiota associada a plantas ameaçadas de extinção no Brasil e, por conseguinte, também possivelmente ameaçadas, efetuou-se levantamento da micobiota folicola associada a *D. wilsonii*, e, paralelamente, de fungos folicolas que colonizam *D. mollis*, encontrados nas proximidades de indivíduos remanescentes de *D. wilsonii*. Nesse tipo de estudo micológico visa-se à geração de informação tanto na área micológica quanto na contribuição na geração de uma lista preliminar de espécies fúngicas em risco de extinção. Pretendeu-se excluir da lista de espécies fúngicas em risco de extinção aquelas que, além de ocorrerem sobre *D. wilsonii*, também ocorrem sobre *D. mollis*. As coletas foram concentradas nos municípios de Caetanópolis, Paraopeba, Juatuba, Fortuna de Minas, Sete lagoas e Pequi. Quinze espécies fúngicas foram encontradas, descritas e ilustradas, dentre as quais oito tiveram a região ITS (Internal Transcribed Spacer) do rDNA e a região LSU (Large Subunit 28S) parcialmente sequenciadas e comparadas filogeneticamente a outras sequências disponíveis no GenBank a fim de se obter uma compressão mais completa das relações destes gêneros. Treze espécies fúngicas foram encontradas associadas a *D. wilsonii*: *Vesiculohyphomyces cerradensis*, *Johansonia chapadiensis*, *Trichomatomyces byrsonimae*, *Piricauda paraguayensis*, *Geastrumia polystigmatis*, *Phillipsiella atra*, *Stomiopeltis suttoniae*, *Microcalliopsis dipterygis*, *Janetia* sp. 1 (provável espécie nova), *Byssogene* sp. (provável espécie nova),

Pseudocercospora sp. (provável espécie nova), *Pseudocercosporella* sp. (provável espécie nova), cf. *Radulidium* sp. (provável gênero novo). Seis espécies foram encontradas associadas a *D. mollis*: *Trichomatomyces byrsonimae*, *Piricauda paraguayensis*, *Pseudocercospora* sp. (provável espécie nova), *Pseudocercosporella* sp. (provável espécie nova), *Janetia* sp. 2 (provável espécie nova) e *Alveariospora distoseptata* (proposto como novo gênero e espécie). As espécies *Vesiculohyphomyces cerradensis*, *Trichomatomyces byrsonimae*, *Piricauda paraguayensis*, *Geastrumia polystigmatis*, *Phillipsiella atra*, *Microcalliopsis dipterygis* e *Stomiopeltis suttoniae* já foram relatadas ocorrendo em outras espécies de plantas tendo ampla distribuição, portanto, não tem interesse conservacionista. Quatro fungos ocorreram em ambas as espécies de *Dimorphandra*: *Pseudocercospora* sp. (provável espécie nova), *Pseudocercosporella* sp. (provável espécie nova), *Piricauda paraguayensis* e *Trichomatomyces byrsonimae*. Conclui-se que há possibilidade de que os três novos taxa associados unicamente a *D. wilsonii*: *Janetia* sp., *Byssogene* sp. e cf. *Radulidium* sp., mereçam o status de espécies possivelmente ameaçadas de extinção.

ABSTRACT

SILVA, Meiriele da, M.Sc., Universidade Federal de Viçosa, February, 2012. **Foliicolous mycobiota of *Dimorphandra wilsonii*, an endangered Brazilian tree species.** Adviser: Robert Weingart Barreto. Co-Adviser: Olinto Liparini Pereira.

Dimorphandra wilsonii (Fabaceae), commonly known as “faveiro de Wilson”, is an endemic species of Minas Gerais, which occurs in the transition between the Cerrado and the Atlantic Forest, north of Belo Horizonte. It was included in the list of endangered species in the state of Minas Gerais, in the general list of endangered Brazilian flora, and in the list kept by the International Union for Conservation of Nature (IUCN), where it appears as critically endangered, the level that precedes extinction. In contrast, *Dimorphandra mollis* Benth., a plant that is taxonomically very close to the “faveiro de Wilson”, commonly known as “faveira” and “falso barbatimão”, is widely distributed in the Brazilian Cerrado mainly in the states of Goiás, Mato Grosso, Minas Gerais and São Paulo. This work represents a continuation of the investigations started some years ago aimed at contributing to the knowledge of the mycobiota associated with endangered Brazilian plants and, therefore, also possibly threatened with extinction. A survey of the foliicolous mycobiota of *D. wilsonii* was undertaken and parallel to that, also the mycobiota of *D. mollis* occurring in the same vicinity as *D. wilsonii* was investigated. Such a kind of mycological study was expected to yield both interesting mycological information and to contribute towards the generation of a preliminary list of fungal species in danger of extinction. The inclusion of the mycobiota of *D. mollis* in the surveys aimed at excluding the fungi occurring on both plants from such a list with regards to the risk of extinction because of host loss. The surveys were concentrated in the municipality of Caetanópolis and Paraopeba, Juatuba, Fortuna de Minas, Sete lagoas e Pequi. Fifteen fungal species were found, described and illustrated of which ten had the ITS region (Internal Transcribed Spacer) the rDNA and LSU (Large Subunit 28S) partially sequenced and phylogenetically compared with other sequences available in GenBank, in order to generate a more complete understanding of the taxonomic relationships. Thirteen fungal species were found associated with *D. wilsonii*: *Vesiculohyphomyces cerradensis*, *Johansonia chapadiensis*, *Trichomatomyces byrsonimae*, *Piricauda paraguayensis*, *Geastrumia polystigmatis*, *Phillipsiella atra*, *Stomiopeltis suttoniae*, *Microcalliopsis dipterygis*,

Janetia sp. 1 (probably new species), *Byssogene* sp. (probably new species), *Pseudocercospora* sp. (probably new species), *Pseudocercospora* sp. (probably new species), cf. *Radulidium* sp. (probably new genus). Six species were found associated with *D. mollis*: *Trichomatomyces byrsonimae*, *Piricauda paraguayensis*, *Pseudocercospora* sp. (probably new species), *Pseudocercospora* sp. (probably new species), *Janetia* sp. 2 (probably new species) and *Alveariospora distoseptata* (proposed as a new genus and species). The species *Vesiculohyphomyces cerradensis*, *Trichomatomyces byrsonimae*, *Piricauda paraguayensis*, *Geastrumia polystigmatis*, *Phillipsiella atra*, *Microcalliopsis dipterygis* and *Stomiopeltis suttoniae* are notoriously polyphagous and have been reported on a variety of substrates having a wide distribution and, therefore, have no interest for conservation. Four fungi occurred on both species of *Dimorphandra*: *Pseudocercospora* sp., *Pseudocercospora* sp., *Piricauda paraguayensis* and *Trichomatomyces byrsonimae*. In conclusion, there is a possibility that three of the taxa showed be described as new since they are found strictly in association with *D. wilsonii*, *Janetia* sp., *Byssogene* sp., cf. *Radulidium* sp. and, are considered to be endangered fungal species.

INTRODUÇÃO GERAL

O Cerrado é o segundo maior bioma do Brasil, depois da Amazônia, cobrindo 21% do território nacional em grande parte coincidente com o Planalto Central. O domínio original do Cerrado tem aproximadamente 2,0 milhões de km², dos quais apenas 400 mil km² não foram degradados (Coutinho, 2002; Klink & Machado, 2005). O Cerrado apresenta distribuição contínua na área nuclear, com cerca de 1,5 milhões de km², que se estende por toda a região Centro-Oeste e o oeste da Bahia e Minas Gerais, Tocantins, ocorrendo ainda no sul do Maranhão, norte do Piauí e sul de Rondônia (Eiten, 1972). No estado de Minas Gerais, o Cerrado ocupa toda a porção centro-ocidental, cobrindo mais de 300.000 Km² (Laca-Buendia & Brandão, 1995; Martins, 2000). Existem ainda áreas disjuntas de Cerrado encravadas entre domínios vizinhos e entre faixas de transição, como nos estados do Amapá, Amazonas, Pará, Roraima, São Paulo e norte do Paraná (Eiten, 1972; Ribeiro & Walter, 1998).

A flora do Cerrado é característica e diferenciada dos biomas adjacentes, embora muitas fisionomias compartilhem espécies com outros biomas. Além do clima, da química e física do solo, da disponibilidade de água e nutrientes, e da geomorfologia e topografia, a distribuição da flora é condicionada pela latitude, frequência das queimadas, profundidade do lençol freático, além de inúmeros fatores antrópicos (Ribeiro & Walter, 1998). A riqueza florística do domínio Cerrado compreende segundo Mendonça *et al.* (1998) um total de 6671 espécies nativas, distribuídas em 170 famílias e 1144 gêneros. As famílias mais representativas no bioma Cerrado são: Fabaceae (859 espécies), seguida por Compositae (559 espécies), Orchidaceae (493 espécies), Poaceae (373 espécies), Rubiaceae (257 espécies), Melastomataceae (238 espécies), Myrtaceae (212 espécies), Euphorbiaceae (195 espécies), Malpighiaceae (128 espécies) e Lytraceae (120 espécies).

Acredita-se que o Cerrado tenha se originado de formações vegetacionais do Cretáceo, antes da total separação entre a América do Sul e o continente Africano, há cerca de 70 ou 80 milhões de anos. A fragmentação das populações estaria fortemente relacionada com a grande especiação, apresentando alto grau de endemismo da flora e fauna (Ratter *et al.*, 1997; Mittermeier *et al.*, 2005; Mendonça *et al.*, 2008) conferindo

ao Cerrado biodiversidade estimada em 160.000 espécies de plantas, animais e fungos (Pennington *et al.*, 2000).

O bioma Cerrado é uma das 25 formações vegetacionais consideradas como prioritárias para a conservação da biodiversidade mundial, denominadas de *hot spots* (Myers *et al.*, 2000; Queiroz, 2009). Os remanescentes de vegetação primária do Cerrado correspondem a somente 20% da área original ocupada pelo bioma e somente 6,2% estão localizados em áreas de preservação. A diversidade florística do Cerrado é estimada em 10.000 espécies, sendo que 4.400 são endêmicas e respondem por 1,5% da biodiversidade vegetal mundial (Myers *et al.*, 2000).

No Brasil, a vegetação natural de Cerrado vem sendo substituída por culturas agrícolas, pastagens e espécies florestais de rápido crescimento (Sano *et al.* 2010; Lenza *et al.*, 2011). Se a exploração dessas áreas nativas continuar em forte ritmo de ampliação, a vegetação do Cerrado acabará restrita às áreas de Unidades de Conservação, terras indígenas e regiões impróprias à agropecuária, fazendo com que muitas espécies de plantas, assim como animais possam ser levados à extinção (Queiroz, 2009). Um exemplo de espécie endêmica do bioma Cerrado, criticamente ameaçada devido à degradação do seu habitat é a leguminosa arbórea *Dimorphandra wilsonii* Rizzini, conhecida popularmente como “faveiro de Wilson”. Um estudo publicado recentemente relata que apenas pouco mais de dez indivíduos desta espécie arbórea são conhecidos (Fernandes *et al.*, 2007). Todos esses exemplares ocorrendo em áreas de Cerrado já transformadas em pastagens nos municípios de Paraopeba e Caetanópolis, MG. A espécie foi incluída na lista da IUCN como estando criticamente ameaçada, o nível mais elevado de risco para a sobrevivência de uma espécie e que antecede o de extinta na natureza (Fernandes *et al.*, 2007; Fonseca *et al.*, 2010).

Mendonça & Lins (2000) publicaram uma lista de espécies vegetais ameaçadas de extinção em Minas Gerais com 537 espécies distribuídas em 77 famílias. Quando se discute sobre preservação de espécies ameaçadas de extinção no Brasil ou no exterior o enfoque é sempre dado a elementos da fauna e flora. No entanto, organismos pouco conhecidos e altamente diversificados e especializados como os fungos, não estão menos vulneráveis à extinção. Alguns trabalhos recentes vêm discutindo a importância em se preservar a microbiota, além de listarem algumas espécies que possivelmente estão ameaçadas de extinção (Thor, 1998; Moore *et al.*, 2001; Berlund & Jonsson, 2005; Griffith, 2011). A maior e mais importante rede de conservação no mundo lista

apenas três espécies fúngicas como ameaçadas de extinção, os líquens *Cladonia perforata* A. Evans e *Erioderma pedicellatum* (Hue) P.M. Jorg. e o cogumelo comestível *Pleurotus nebrodensis* (Inzenga) Quél. (IUCN, 2012).

A negligência com relação aos microrganismos deve-se principalmente ao fato dos mesmos não serem vistos a olho nu e pela falsa ideia de que microrganismos de modo geral possuem dispersão facilitada e, conseqüentemente, ampla distribuição geográfica (Griffith, 2011). Entretanto, especialmente após o uso de ferramentas moleculares no estudo da ecologia microbiana, sabe-se atualmente que populações microbianas podem ter estrutura populacional definida e endêmica (Rodriguez *et al.*, 2004). As dificuldades práticas de se reunir evidências que justifiquem o reconhecimento de espécies fúngicas como estando ameaçadas são em parte responsáveis pela virtual ausência de fungos nas listas de espécies ameaçadas e nas medidas de conservação. Minter (2010) se referiu aos fungos, neste contexto, como “the orphans of Rio”, por terem sido negligenciados na “Conferência das Nações Unidas sobre Ambiente e Desenvolvimento Sustentável”, também conhecida como ECO 92 ou Rio 92. Um grupo de fungos para os quais seria, em princípio, possível reunir evidências científicas sobre a condição de ameaça iminente de extinção é o dos fungos fitopatogênicos altamente específicos em suas relações com espécies de plantas hospedeiras ameaçadas de extinção (Rocha *et al.*, 2010).

A perda por extinção de uma espécie vegetal pode levar, num evento de coextinção, à extinção de um leque de organismos especializados que dependem desta planta. Eventos de coextinção são bem documentados para outros casos de interação parasitas-hospedeiro tais como: piolhos parasitas de pombos, diferentes parasitas em primatas (fungos do gênero *Pneumocystis*, nematóides e piolhos), vespas polinizadoras e insetos herbívoros (Koh *et al.*, 2004; Thacker *et al.*, 2006; Dunn *et al.*, 2009).

A emergência do reconhecimento da necessidade de conservação dos fungos é ilustrada pela realização, em Whitby - Reino Unido (26-30 de outubro de 2009) do evento “FUNGAL CONSERVATION science, infrastructure and politics” e, posteriormente em 6 de agosto de 2010 no Royal Botanic Garden Edinburgh, no âmbito do 9th International Mycological Congress, de onde foi fundada a International Society for Fungal Conservation (<http://www.fungal-conservation.org/>).

No Brasil, um trabalho pioneiro foi publicado por Rocha *et al.* (2010) sobre a microbiota possivelmente ameaçada de coextinção, associada a uma planta endêmica

da Floresta Atlântica, *Coussapoa flocosa* Akkermans & C.C. e listada como vulnerável (IUCN, 2012). Nesta publicação foram descritas seis novas espécies fúngicas, incluindo um gênero novo para a ciência.

Os objetivos do presente trabalho foram: I) Levantar e descrever a micobiota associada à folhagem de *Dimorphandra wilsonii*; II) Levantar e descrever a micobiota associada à folhagem de *Dimorphandra mollis*; III) Verificar a possível co-ocorrência dos fungos encontrados em *D. wilsonii* e em *D. mollis* e produzir evidências preliminares sobre a possível ameaça à sobrevivência de espécies fúngicas exclusivas de *D. wilsonii*.

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ARTIGO 1

According to the guidelines of Mycologia

Foliicolous mycobiota of *Dimorphandra wilsonii*, an endangered Brazilian tree species

Foliicolous mycobiota of *Dimorphandra wilsonii*, an endangered Brazilian tree species

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Abstract: A survey of foliicolous fungi associated with *Dimorphandra wilsonii* and *Dimorphandra mollis* (Fabaceae) was conducted in the state of Minas Gerais, Brazil. *Dimorphandra wilsonii* is a tree species native from the Brazilian Cerrado and listed as highly endangered of becoming extinct. Fungi strictly depending on this plant species as a substrate are probably also on the verge of co-extinction. The study of this mycobiota aimed at contributing towards the field of fungal conservation and generating preliminary lists of potentially endangered fungal species is a necessary step in such a study. Surveying the mycobiota of *D. mollis*, which is a common species with a broad geographical distribution, was also considered necessary as these two species are closely related and coexist. Therefore, fungi occurring on *D. wilsonii* might also occur on the non-endangered *D. mollis* and hence not be in danger of co-extinction. Fourteen fungal species were collected, identified, described and illustrated, including: five ascomycetes [*Phillipsiella atra*, *Johansonia chapadiensis*, *Stomiopeltis suttoniae*, *Microcalliopsis dipterygis*, *Byssogene* sp. nov.], eight hyphomycetes anamorphs [*Vesiculohyphomyces cerradensis*, *Trichomatomyces byrsonimae*, *Piricauda paraguayensis*, two *Janetia* sp. nov., *Pseudocercospora* sp. nov., *Pseudocercosporella* sp. nov., cf. *Radulidium* gen. nov.] and one coelomycete (*Geastrumia polystigmatis*). Three fungi were exclusive to *D. wilsonii* and possibly in endangered of extinction: *Byssogene* sp. nov., *Janetia* sp. nov. 1, and cf. *Radulidium* gen. nov.

Keywords: biodiversity, Cerrado, co-extinction, fungal conservation, phylogeny, taxonomic novelties.

INTRODUCTION

The Cerrado is a Brazilian biome that is second in surface area only to the Amazon. It covers 21% of the country (2 million km²) and is largely coincident with the central plateau (Klink and Machado 2005). In Brazil, the Cerrado natural vegetation is progressively being replaced by agricultural crops, pasture and cultivated exotic forest species (Sano et al. 2010, Lenza et al. 2011). One among the many plant species occurring in this biome that are now endangered is *Dimorphandra wilsonii* Rizzini (Fabaceae), a tree commonly known as “faveiro de Wilson”. A recently published study reports that fewer than ten individuals of this tree species are known

in nature (Fernandes et al. 2007). All these individuals occur in areas of Cerrado that have been turned into pastures and all occur in a small area in the neighboring municipalities of Paraopeba and Caetanópolis in the state of Minas Gerais. The species is listed in (<http://www.iucnredlist.org/>) as being critically endangered, the highest level of risk to the survival of a species and prior to extinction in nature.

The lack of knowledge and awareness about the fungi and the fact that most fungi are either invisible to the naked eye or produce ephemerals macroscopic fruit bodies has probably led to the mistaken impression that species belonging to the Fungi are capable of escaping environmental changes, are easily dispersed, ubiquitous and are broadly spread (Griffith, 2011). Nevertheless, investigations involving surveys of environmental DNA have been indicating that microbial communities may have a well defined structure, with the populations with a high level of endemism (Rodriguez et al. 2004). Nevertheless, the practical difficulties for gathering evidence that individual fungal species are actually threatened are in part responsible for their virtual absence from lists of endangered species and from policies aimed at preventing global loss of biodiversity. Minter (2010) has referred to fungi, considering that context as “the orphans of Rio”, for this large group of organisms having been left out of the agenda in the Earth Summit (United Nations Conference on Environment and Development - UNCED) that occurred in Rio de Janeiro in June 1992. Some years ago it was conjectured by Rocha et al. (2010) that it might be possible to gather convincing scientific evidence of threat of extinction by investigating highly host-specific plant pathogenic fungi associated with endangered plant species. The loss of one plant species may lead to events of coextinction threatening a range of specialized organisms depending strictly on that species for its survival. Such events are well documented for parasite-host interactions such as: pigeon lice, primate parasites, pollinizer wasps and herbivorous insects (Koh et al. 2004, Thacker et al. 2006, Dunn et al. 2009).

It is only recently that the need for conservation of fungi was embraced as a duty by mycologists. The first international conference on the issue took place in Whitby – UK in October 2009 “FUNGAL CONSERVATION science, infrastructure and politics” and later on 6th of August 2010, during the 9th International Mycological Congress, the International Society for Fungal Conservation was founded (<http://www.fungal-conservation.org/>).

In Brazil, the first work to be published addressing the issue of fungal conservation was published by Rocha et al. (2010) and involved the study of the foliage mycobiota of *Coussapoa flocosa* Akkermans & C.C. (Cecropiaceae) which may be in danger of coextinction because of its dependence on this rare endemic tree of the Brazilian Atlantic Forest. This study led to the discovery of six new fungal species, including a new fungal genus.

The present work aims at expanding the study started by Rocha *et al.* (2010) in order to encompass an additional endangered Brazilian plant species (*Dimorphandra wilsonii*) and its mycobiota - which may also be potentially endangered of coextinction. Additionally, *Dimorphandra mollis* Benth. which is a common species with a broad geographical distribution, closely related to *D. wilsonii* and which coexists with that plant in its remaining area of occurrence in nature also had its mycobiota studied. This was done in order to determine if fungi occurring on *D. wilsonii* also occur on the non-endangered *D. mollis* and hence should not be regarded as in potential danger of co-extinction.

Hence, the objectives of this study were: I) to survey and describe the foliicolous mycobiota associated with *Dimorphandra wilsonii*; II) to survey and describe the mycobiota of *D. mollis*; III) Check the possible co-occurrence of fungi found in *D. wilsonii* and on *D. mollis* and produce preliminary evidence about the possible threat to the survival of fungal species unique to *D. wilsonii*. The fungi that were collected are described and discussed below.

MATERIALS AND METHODS

Survey trips were conducted between 2009 and 2011, locally in the municipality of Paraopeba, Caetanópolis, Juatuba, Fortuna de Minas, Sete Lagoas and Pequi (TABLE 1). Existing information on localities of occurrence of *D. wilsonii* individuals in nature were provided by F. Fernandes (Fundação Zoo-Botânica de Belo Horizonte) who has been conducting regular surveys for the remaining individuals of that species since 2003. Whenever individuals belonging to the closely related species, *D. mollis*, were found growing in the vicinities of an individual of *D. wilsonii*, branches and foliage of individuals belonging to that species were also collected. *Dimorphandra wilsonii* is readily separated from *D. mollis* by having longer pods with a sweetish scent, paler

gray bark that is not easily detached as in *D. mollis*. Its leaflets are also larger than those in *D. mollis* (3-5 cm long) (FIG.1). Pictures were taken in the field with a SONY DSC-H9 digital camera, samples of branches bearing foliage were collected with a long-poled pruner and dried in a plant press.

After screening in the lab, dried relevant specimens were deposited at the herbarium of Universidade Federal de Viçosa (VIC). Samples were carefully examined under a stereomicroscope (OLYMPUS SZX7), while still fresh and fungal structures were either scraped with a scalpel from tissue surfaces (when externally produced) or free-hand sections or sections prepared with the help of a freezing microtome (Microm HM 520) whenever necessary were prepared and mounted on in lactophenol or other mounting media. Observations and measurements were carried out with an OLYMPUS BX 51 light microscope fitted with a digital camera (OLYMPUS E330) and a drawing tube.

Isolations of fungi in pure culture were attempted by direct transfer of spores or other fungal structures onto plates containing VBA - vegetable broth-agar, as described by Pereira et al. (2003), with the help of a sterile fine pointed needle. Pure cultures were preserved in PCA slants or in silica-gel, as described by Dhingra and Sinclair (1996).

For scanning electron microscopy, the samples were prepared by the following protocol: samples were fixed in 2.5% glutaraldehyde + sodium cacodylate buffer (0.1mol L⁻¹, pH 7.2) (1:1) for 1h a room temperature; washed six times in 0.1mol L⁻¹ sodium cacodylate buffer (10 m each washing period); samples were postfixed by immersion in 1% O₅O₄ prepared in cacodylate buffer 0.1 mol L⁻¹ (1:1), and kept for 4 h at 4-8 °C then washed again six times in buffer; dehydrated by successive transfer in a graded alcohol solutions series (30, 50, 60, 70, 80, 95, 100%) and left 10min in each solution at room temperature; samples were placed in a critical point dryer (Baltec model 030) with CO₂ as transition fluid; after drying the samples were coated with gold (20 nm thick) with a sputter coater (Balzers® model FDU 010) and examined with a Carl-Zeiss Model LEO VP 1430 electron microscope.

Culture descriptions were based on observation of the colonies formed in plates containing potato dextrose-agar (PDA) or potato carrot-agar (PCA) incubated at 25°C and under a 12 h daily light regime (light provided by two white and one near-UV

lamps placed 35 cm above the plates) after 23 days. Color terminology followed Rayner (1970).

For the molecular phylogeny studies of five of the fungi (*Janetia* sp., *Radulidium-like* sp., *Piricauda* sp., *Pseudocercospora* sp. and *Pseudocercosporella* sp., pure cultures were grown on PDA at 25 °C for up to four weeks depending on their growth rate. Genomic DNA was extracted from the mycelium using Wizard® Genomic DNA Purification Kit (Promega corporation, WI, U.S.A.). For the sequencing of three of the fungal species (Mycosphaerellaceae, *Phillipsiella atra* and *Trichomatomyces byrsonimae*), DNA was extracted by removing fungal structures from the plant tissue with a fine glass needle and placing them in a microtube (1.5 ml) of the extraction kit. In this case, each fungal structure was carefully examined under the highest power of a stereomicroscope in order to check for possible contamination with other fungi or mycoparasites and to exclude any plant material from the sample. PCR reactions were set-up using the following ingredients for each 25 µl reaction: 12,5 µl of DreamTaq™ PCR Master Mix 2X (MBI Fermentas, Vilnius, Lithuania), 1 µl of 10 µM of each forward and reverse primer synthesised by Invitrogen (Carlsbad, USA), a maximum of 25 ng/µl of genomic DNA, and nuclease-free water to complete the total volume.

The primers ITS4 (5'- TCCTCCGCTTATTGATATGC -3') and ITS5 (5'- GGAAGTAAAAGTCGTAACAAGG -3') were used to amplify the internal transcribed spacer region (ITS) of the nuclear ribosomal RNA operon, including the 3' end of the 18S rRNA, the first internal transcribed spacer region, the 5.8S rRNA gene; the second internal transcribed spacer region and the 5' end of the 28S rRNA gene (White et al., 1990). The large subunit ribosomal were amplified with primer pairs LR0R (5'-ACCCGCTGAACTTAAGC-3') and LR5 (5'-TCCTGAGGGAACTTCG -3') (www.biology.duke.edu/fungi/mycolab/primers.htm). The amplifications were carried out starting with a BIO RAD C1000 (Thermal Cycler) with initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for ITS and at 53 °C for LSU for 45s, extension at 72 °C for 2 min and a final extension of 7 min at 72 °C. Amplified products were visualised on 1% agarose gel to check for product size and purity. PCR products were purified with the PEQLAB E.Z.N.A.® Cycle-Pure Kit following the protocol. The sequencing was carried out directly from purified PCR-amplified fragments using the automated sequencer

MegaBACE 500TM. The nucleotide sequences were read with Chromas lite 2.01 (http://www.technelysium.com.au/chromas_lite.html) and edited with the DNA Dragon software (<http://www.dna-dragon.com/index.php>). All sequences were checked manually and nucleotide arrangements at ambiguous positions were clarified using both primer direction sequences. New sequences will be deposited in GenBank (<http://www.ncbi.nlm.nih.gov>). Sequences of large subunit ribosomal of additional species were retrieved from GenBank (TABLE 2).

Consensus regions were compared against GenBank's database using their Mega BLAST program. In addition, sequences were selected from Arzanlou et al. (2007), Crous et al. (2009b, 2009c), Frank et al. (2010), Schoch et al. (2009) and Yang et al. (2010) to reveal the phylogenetic position of the strains within Capnodiales. The closest hit sequences were then downloaded in FASTA format and aligned using the multiple sequence alignment program MUSCLE® (Edgar 2004), with default parameters in operation. MUSCLE® was implemented using the program MEGA v.5 software (Tamura et al. 2011). Alignments were checked and manual adjustments were made where necessary. Gaps (insertions/deletions) were treated as missing data.

Bayesian inference (BI) analyses employing a Markov Chain Monte Carlo method were performed. MrMODELTEST 2.3 (Posada and Buckley 2004) was used to select the models of nucleotide substitution for the BI analysis. Once the likelihood scores were calculated, the models were selected according to the Akaike Information Criterion (AIC). The general time-reversible model of evolution (Rodriguez et al. 1990), including estimation of invariable sites and assuming a discrete gamma distribution with six rate categories (GTR+I+G) was used. BI analysis was done with MrBayes v. 3.1.2 (Rannala & Yang 1996, Mau et al. 1999, Huelsenbeck et al. 2001, Huelsenbeck et al. 2002). Four MCMC (Markov chain Monte Carlo) chains were run simultaneously, starting from random trees for 10 000 000 generations. Trees were sampled every 1000th generation for a total of 10 000 trees. The first 2 500 trees were discarded as the burn-in phase of each analysis. Posterior probabilities (Rannala and Yang 1996) were determined from a majority-rule consensus tree generated with the remaining 7 500 trees. Convergence of the log likelihoods was analysed with TRACER v. 1.4.1 (beast.bio.ed.ac.uk/Tracer); no indication of lack of convergence was detected. This analysis was repeated three times starting from different random trees to ensure trees from the same tree space were sampled during each analysis.

Trees were visualized in FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>) and exported to graphics programs. The species *Elsinoë eucalypticola* Cheewangkoon & Crous, *E. veneta* (Burkh.) Jenkins, *Myriangium duriaei* Mont. & Berk. and *M. hispanicum* J.B. Martínez were used as outgroup in these analyses.

RESULTS AND DISCUSSION

Phylogeny

The LSU region of the sequences was used to obtain additional sequences from GenBank, which were added to the alignment. Due to the inclusion of the shorter LSU sequences of *Stomiopeltis* sp. (AY598919) and *Stomiopeltis versicolor* (Desm.) Arx (FJ147163) in the alignment, it was not possible to subject the full length of the determined LSU sequences (TABLE 2) to the analysis. The manually adjusted LSU alignment contained 84 sequences (including the outgroup sequences) and, of all 461 characters used in the phylogenetic analysis, 172 were parsimony-informative, 193 were variable and 268 were conserved. Although the ITS sequences were not used in phylogenetic analyses, they were lodged in GenBank for future studies and DNA barcode purposes.

Taxonomy

Thirteen fungal species were found associated with *D. wilsonii*: *Byssogene* sp., *Geastrumia polystigmatis*, *Janetia* sp. 1, *Johansonia chapadiensis*, *Phillipsiella atra*, *Piricauda paraguayensis*, *Microcalliopsis dipterygis*, *Pseudocercospora* sp., *Pseudocercospora* sp., cf. *Radulidium* sp., *Stomiopeltis suttoniae*, *Trichomatomyces byrsonimae*, *Vesiculohyphomyces cerradensis*. Five fungal species were collected in association with *D. mollis*: *Piricauda paraguayensis*, *Pseudocercospora* sp., *Pseudocercospora* sp., *Trichomatomyces byrsonimae* and *Janetia* sp. 2. These fungi are described below.

Byssogene sp. (to be proposed as a new species)

FIG.2

Colonies on living leaves, hypophyllous, sparse, not aggregated, causing no disease symptoms. Internal mycelium indistinct. External mycelium hypogenous,

anastomosing, net-forming, slightly undulate, composed of pale brown, flattened, thin walled, septate hyphae, 2.5–5.0 μm , smooth. Ascomata hypophyllous, superficial, solitary, discoid, no ostiolate, black, margin raised, 175–225 μm diam., 100–117.5 μm high, outer gray wall of texture globulosa becoming hyaline, pseudoparenchymatose more internally, 6-cell thick, 12.5–20 μm , dark brown. Asci bitunicate, ovate, oblong to obclavate, 37.5–65 \times 25–35 μm , 8-spored. Ascospores cylindrical to ellipsoid, 20–27.5 \times 5–10 μm , dictyoseptate, subhyaline, guttulate, smooth.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 27 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31808).

Notes: The fungus described above belongs to the family Saccardiaceae by having ascomata superficially, discoid, asci parallel in a single layer, bitunicate, ascospores with two or many celled, hyaline or brown, and clearly belongs to the genus *Byssogene* Syd. in having dictyoseptate ascospores almost hyaline, ascomata discoid and superficial brown mycelium (von Arx and Müller 1975). *Byssogene amboinensis* Syd. is the single species in this genus and was previously known to occur on *Eugenia* sp. (Myrtaceae) in Amboina (Sydow 1922). *Byssogene amboinensis* differs from our species in having shorter and narrower asci 40–52 \times 18–25 μm and shorter ascospores 15–17 μm and, therefore, will be described as new. This is the first report of a member of *Byssogene* on *D. wilsonii*. The fungus was only found on *D. wilsonii* at a single location on a single plant.

Colonies on living leaves, adaxial, circular to irregular, sparse. Internal mycelium indistinct. External mycelium loose, branched, 2.5–3.0 µm diam, discrete and irregularly scattered over areas of the host, septate, medium brown to sub-hyaline, smooth. Conidiomata hypophyllous, superficial, scutellate, opening by an irregular rupture, hemispherical or subglobose, black, 125–140 µm diam., 52.5–85.0 µm in height, wall composed of one-celled layer, angular, 10–12.5 µm, dark brown, smooth. Conidiophores micronematous, restricted to conidiogenous cells, developing from the hyphae at the point of their transformation into the covering wall, terminal, holoblastic, narrowly elongate ampulliform, clavate to fusiform, 8–17 µm long, 2 µm wide at the base, cells swelling at the apex, hyaline and by more or less dichotomous forking produces a cluster of clavate to fusiform outgrowths which proceed to differentiate into conidial arms after the initial cluster has been cut off from the conidiogenous cell by a septum. Conidia dry, holoblastic, solitary, formed singly at the apex of each conidiogenous cell, cheiroid, fasciculate groups of 4–10 straight to slightly curved filiform arms closely united at long pedicellate basal cell, each arm, 15–50 × 2–4 µm, apex 2.5–4 µm, base 1.0–2.5 µm, 2–7 septate, hyaline when immature becoming light brown at maturity, smooth.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 13 Jul 2009, *M. Silva, O.L. Pereira & R.W. Barreto* (VIC 31771).

Notes: *Geastrumia polyastigmatis* Bat. & M.L. Farr is the only species in this genus and was previously known to occur on a member of the Fabaceae - *Andira jamaicensis* (W. Wright) Urb. in Brazil and Republica Dominicana, as well as on members of unrelated host species - *Costus afer* Ker Gawl. (Costaceae) in Tanzania and *Hymenocardia acida* Tul. (Phyllantaceae) in Tanzania (Pirozynski 1971). Our fungus fitted well within the description of *G. polyastigmatis*, and is reported here for the first time on *D. wilsonii*.

Janetia sp. 1 on *D. wilsonii* (to be proposed as a new species)

FIGS. 4–5

Colonies hypophyllous, dense, brown, forming dark heads on trichomes. Internal mycelium indistinct. External mycelium superficial, up to 4 µm diam, branched, septate, brown, smooth. Conidiophores forming sporodochial clusters on the apex of trichomes, micronematous, mononematous. Conidiogenous cells holoblastic, monoblastic, denticulate, cylindrical to narrowly ampulliform, straight to slightly curved, 7.5–37.5 × 2.5–5.0 µm, reddish brown to pale brown. Conidiogenous loci apical on conidiogenous cells, flat, truncate, not darkened, unthickened, conidial secession schizolytic. Conidia dry, solitary, obclavate to cylindrical, straight to slightly curved, occasionally slightly constricted at some of the septa, 12.5–75 × 5.0–7.5 µm, rounded at the apex up to 2.5 µm wide, rounded at the base 5.0 µm, 1–9 euseptate, reddish brown to pale brown, smooth, guttulate.

In culture: On PCA, slow-growing (3.7–4.5 cm diam, after 27 days), chrysanthemoid to subcircular, flat, to convex, cottonose aerial mycelium accompanied by immersed growth within medium, dense, or without aerial mycelium and internal mycelium irradiating and sinuose and raising from the medium on radial strands. On PDA greenish centrally glaucous, followed by a periphery of pale olivaceous buff thick ring of mycelium, or olivaceous grey, pronounced diurnal zonation, under alternating light but less pronounced in the dark no sporulation. Mycelium sparser and with less pronounced diurnal zonation, in the dark reverse blueish.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 13 Jul 2009, *M. Silva, O.L. Pereira & R.W. Barreto* (VIC 31772– HOLOTYPE); 19 Jul 2010, *M. Silva & O.L. Pereira* (VIC 31780); 19 Jul 2010, *M. Silva & O.L. Pereira* (VIC 31781).

Janetia sp. 2 on *D. mollis* (to be proposed as a new species – distinct from above)

FIGS. 6–8

Colonies hypophyllous, on trichomes, effuse, brown or dark brown, giving them a bottle brush appearance. Internal mycelium indistinct. Superficial mycelium growing on trichomes and foliar surface, 3–5 µm wide, branched, septate, brown, smooth. Conidiophores micronematous, restricted to fertile nearly undifferentiated

humped hyphal cells, $5\text{--}6 \times 3\text{--}4 \mu\text{m}$, brown, smooth. Conidiogenous cells holoblastic, monoblastic, denticulate. Conidiogenous loci a flat-topped hump on conidiogenous cells, $1.5\text{--}3.0 \mu\text{m}$, unthickened, dark brown. Conidia dry, holoblastic, solitary, cylindrical, straight to slightly curved, often slightly constricted at septae, $16\text{--}35 \times 6.5\text{--}9.5 \mu\text{m}$, apex rounded $4\text{--}5 \mu\text{m}$, base $4.5\text{--}6.0 \mu\text{m}$, 2–7 transversally distoseptate, eguttulate, pale brown to brown, smooth walled but faintly spirally sulcate along the conidial length.

Specimens examined: On living leaves of *Dimorphandra mollis*. BRAZIL: Minas Gerais: Paraopeba, Flona, 21 Jul 2010, *M. Silva & O.L. Pereira* (VIC 31812–HOLOTYPE).

Notes: The genus *Janetia* M. B. Ellis has 20 species characterized by producing euseptate or distoseptate, phragmosporous conidia with schizolytic secession from dematiaceous, denticulate, monoblastic or polyblastic, integrated conidiogenous cells (Calduch et al. 2002, Dornelo-Silva and Dianese 2003, Ellis 1976, Goh and Hyde 1996, Hughes 1983, Reddy et al. 2004) (TABLE 3). *Janetia* sp. 1 is similar to *Janetia salvertiae* Dornelo-Silva and Dianese, which occurs on *Salvertia convallariodora* St. Hil. (Vochysiaceae) and *Vochysia* sp. (Vochysiaceae), *Janetia euphorbiae* M. B. Ellis which colonizes stems of *Euphorbia tirulicallis* L. (Euphorbiaceae), and *J. cubensis* Matsush., described from leaves of *Roystonea regia* (Kunth) O.F. Cook (Arecaceae). *Janetia salvertiae* differs from *Janetia* sp. 1 by having clavate, shorter and narrower conidia ($15\text{--}30 \times 3\text{--}5 \mu\text{m}$) (Dornelo-Silva and Dianese 2003), while *Janetia euphorbiae* is distinguished from *Janetia* sp. 1 by having shorter and wider conidia ($18\text{--}36 \times 6\text{--}8 \mu\text{m}$), not forming sporodochial groups on host trichomes (Ellis 1976). Although *Janetia cubensis* has conidia of similar morphology to those of *Janetia* sp. 1, Goh & Hyde (1996) suggested that it is unlikely that *J. cubensis* belongs to *Janetia*, since it has rhexolytic conidial secession and its conidiogenous “denticles” do not appear to be bulbous (Goh and Hyde 1996; Reddy et al. 2004). *Janetia* sp. 1, therefore cannot be adequately placed in any known species of *Janetia* and will, be described as new.

Janetia sp. 2 is somewhat similar to *J. canensis* B. Sutton & Pascoe, described from stems of *Acacia fimbriata* A. Cunn. ex Don and *A. linifolia* (Vent.) Willd (Fabaceae), *Janetia bacilliformis* Gamundí, Arambi & Giaiotti found on leaves of *Nothofagus dombeyi* (Mirb.) Oerst. (Nothofagaceae) and *Janetia garryae* (Bonar) S.

Hughes described from leaves of *Garrya fremontii* Torr. (Garryaceae). *Janetia canensis* can be distinguished from *Janetia* sp. 2 by having polyblastic conidiogenous cells, conidial walls which are deeply invaginated at the distosepta, smooth conidial walls and longer conidia 16–57 μm (Sutton and Pascoe 1988). *Janetia bacilliformis* differs from *Janetia* sp. 2 by having, longer bacilliform conidia (60–156 μm long) as well as monoblastic or polyblastic, longer and wider conidiogenous cells (10–22 \times 3–5 μm) and smooth conidial walls. *Janetia garryae* differs from *Janetia* sp. 2 by having euseptate and longer conidia (25–70 μm long) (Hughes 1983). Hence, the introduction of a new species is also justified for *Janetia* sp. 2. The new species *Janetia* sp. 1 differs from *Janetia* sp. 2 by having longer conidiogenous cells (7.5–37.5 μm). It also has euseptate, smooth, longer and narrower conidia (12.5–75 \times 3–5 μm).

Only *Janetia* sp. 1 was successfully isolated in pure culture. The investigation of sequences obtained from that taxon has indicated that it groups closely to the genus *Zasmidium* Fr. in a clade that is highly supported in the family Mycosphaerellaceae. The lack of any other sequences of a member of *Janetia* in sequence databases and the lack of knowledge of the teleomorph connection for this genus limits a better understanding of the phylogenetic relationships for this taxon (FIG. 33).

Johansonia chapadiensis Crous, R.W. Barreto, Alfenas & R.F. Alfenas, *IMA Fungus* 1: 117-122 (2010) FIGS. 9–10

Colonies on living leaves, hypophyllous, sooty dots, sparsely distributed. Internal mycelium indistinct. External mycelium hypogenous, 3–5 μm diam., branched, net forming, composed of septate, pale brown, warty hyphae. Ascomata hypophyllous, superficial, solitary, discoid, sessile, non-ostiolate, 240–350 \times 85–115 μm , dark brown. Paraphyses intermingled among asci, filiform, 1.5–2 μm wide, septate, branched, hyaline becoming darkened and branched towards the apical region, forming a powdery epithecium. Exciple consisting of 3-4 layers of brown texture angularis. Asci bitunicate, parallel, ellipsoid to subcylindrical or clavate, 28–50.5 \times 14–16.5 μm , 8-spored, endotunica with ocular chamber. Ascospores inordinate, ellipsoidal, 7.5–10 \times 2.5–4 μm , 1-septate, constricted at the septum, eguttulate, hyaline, smooth. Setae separate and surrounding ascomata, needle-shaped, erect,

mostly straight to occasionally slightly curved, 110–385 × 4–5 µm, 5–14 septate, smooth, unbranched, brown, thick-walled dark brown, with rounded tips.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 19 Jul 2010, *M. Silva & O.L. Pereira* (VIC 31779); 25 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31795).

Notes: This genus was first described by Saccardo in 1889 with *Johansonia setosa* as the type species. The genus is characterized by having discoid ascoma surrounded by setose hyphae and containing numerous asci which contain eight hyaline, two-celled ascospores. The genus was placed in the family Saccardiaceae (von Arx and Müller 1975) There are about 13 species accepted within this genus but only four are reported in association with members of the Fabaceae. These are: *Johansonia amadelpa* (Syd.) Arx (on *Hymenaea* sp.), *Johansonia brasiliensis* Arx (on *Inga* sp.), *Johansonia setosa* (G. Winter) Sacc. (on *Andira* sp. and *Dipteryx alata* Vog.) and *Johansonia chapadiensis* Crous, R.W. Barreto, Alfenas & R.F. Alfenas (on *D. mollis*). The fungus found during the survey on *D. wilsonii* has a morphology which is equivalent to that described for *J. chapadiensis* (Crous et al. 2010), hence we decided to place it within this taxon. Therefore, this is the first record of *J. chapadiensis* on *D. wilsonii*.

Phillipsiella atra Cooke, *Grevillea* (1878)

FIGS. 11–12

Colonies on living leaves, hypophyllous, sparsely spread, not associated with disease symptoms on host. Internal mycelium not observed. External mycelium hypogenous, 2.5–4 µm diam, branched, net forming, slightly undulate, composed of septate, pale brown, smooth hyphae. Ascomata apothecoid, hypophyllous, superficial, solitary, discoid, non-ostiolate, 445–550×75–105 µm high, pseudoparenchymatous basal stroma 12.5–20 thick, walls of brown texture powdery, ill-differentiated, dark grey centrally with raised pale gray margins,. Pseudoparaphyses filiform, up to 2 µm wide, septate, unbranched between asci, hyaline, at maturity pseudoparaphyses branching above the asci, becoming inflated and pigmented at apices to form an epithecium. Asci bitunicate, parallel, cylindrical, 30–40 × 7.5–11µm, 8-spored, endotunica flattened apically at a distance from apical exotunica but extending as a

narrow column towards the conspicuously domed ascus apex. Ascospores biserial to inordinate, fusiform to ellipsoid, $7.5\text{--}10 \times 2.5\text{--}4 \mu\text{m}$, 1-septate, upper cell slightly broader than lower cell, eguttulate, hyaline, smooth.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 14 Jul 2011, *M. Silva, O.L. Pereira & R.W. Barreto* (VIC 31773); 27 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31805); on living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Caetanópolis, Fazenda São Bento, 27 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31807).

Notes: The genus *Phillipsiella* was proposed by Cooke (1878) with *Phillipsiella atra* as the type species, reported on *Quercus virginiana* Mill (Fagaceae) in Georgia (Saccardo 1882), based on the following features: ascoma discoid, asci bitunicate, containing numerous asci, ascospores hyaline with two cells. There are twelve species accepted within this genus but none is reported in association with members of the Fabaceae (Farr et al. 2011). Our specimens collected on *D. wilsonii* are the first to be reported growing on a member of the Fabaceae and they fit well within the description of *P. atra*. Müller and von Arx (1962) placed the genus *Phillipsiella* within Schizothyriaceae. Later, von Arx & Müller (1975) transferred *Phillipsiella* to the Saccardiaceae. However, Barr (1979) revived the family Phillipsielaceae of von Höhnelt (1909) and Eriksson (1981) also discussed and accepted the Phillipsiellaceae (Katumoto 1986). Based on the analysis of large subunit ribosomal DNA gene sequences and resulting phylogeny generated in the present study (FIG. 33), it is finally clarified that *Phillipsiella* belongs to the Dothideomycetes (Capnodiales) and is a member of Schizothyriaceae in a clade highly supported in connection with *Schizothyrium pomi* (Mont. ex Fr.) v. Arx, the type species of the family Schizothyriaceae. In the same way, based on our DNA phylogeny, the genus *Johansonia* also belongs to Schizothyriaceae, since *Phillipsiella* and *J. chapadiensis* grouped in the same highly supported clade (FIG. 33).

Piricauda paraguayensis (Speg.) R. T. Moore, *Mycologia* 50: 691 (1959) FIGS. 13–16

Colonies hypophyllous, effuse, black, on trichomes. Internal mycelium indistinct. External mycelium superficial, $2.5\text{--}4 \mu\text{m}$, branched, septate, grayish brown, smooth. Conidiophores on trichomes, micronematous along the hyphae, sometimes

macronematous terminally at the apex of trichomes, mononematous, cylindrical, 5.0–62.5 × 5.0–7.5 µm, 0–5 septate, occasionally branched, brown, smooth. Conidiogenous cells terminal or intercalary, integrated, monotretic, cylindrical, 10–25.0 × 5.0–7.5 µm, light brown. Conidiogenous loci often with conspicuous dark scars with a well-defined pore in the middle, 1–6 per cell, up to 2.5 µm diam. Conidia dry, solitary, ovoid becoming pyriform, beaked at maturity, 15–20 × 10.0–12.5 when immature becoming 32.5–60.0 × 17.5–30 µm (body) when mature, dictyoseptate, 5–6 transversally and 4–7 longitudinal septa, pale brown to dark brown, rugose, beak, 40.0–125 × 2.5–4.5 µm, brown to pale brown to subhyaline terminally, tapering to 1.5–2.5 diam at apex, 3–5 septate.

In culture: On PCA, slow-growing (0.6–1.6 cm diam, after 27 days), either of scanty floccose aerial mycelium and growing very poorly or of mostly immersed lobate, flat, dendritic mycelium, colony composed of monilioid and filamentous hyphae, striate and granulose, dark mouse gray, to iron gray, reverse dark olivaceous with slight yellow pigmentation of medium, no sporulation.

Specimens examined: On living leaves of *Dimorphandra mollis*. BRAZIL: Minas Gerais: Paraopeba, Flona, 20 Jul 2010, *M. Silva & O.L. Pereira* (VIC 31782); on living leaves of *Dimorphandra wilsonii*. Brazil, Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 21 Jul 2010, *M. Silva & O.L. Pereira* (VIC 31785).

Notes: The morphology typical of fungi in the genus *Piricauda* Bubák following the generic concept of Hughes (1960) and Ellis (1971) is: micronematous conidiophores developing on superficial hyphae, conidiogenous cells monotretic and tretic conidia arising singly from a pore on the conidiogenous cell. There are eight species in the genus *Piricauda*: *P. cochiniensis* (Subram.) M. B. Ellis, *P. cubensis* Hol.Jech. & Mercado, *P. longispora* Mercado, Guiné & Guarro, *P. mexicana* Mercado, Heredia & Mena, *P. paraguayensis* (Speg) R.T. Moore, *P. pseudarthriae* (Hansf.) M.B. Ellis, *P. taiwanensis* Matsush. and *P. vulcanensis* in several hosts (Ellis 1971, 1976, Sierra et al. 2005). Our species fits well with the description of *P. paraguayensis* characterized by having spherical to ovoid, pale brown to dark brown conidia, with hyaline to subhyaline apical appendage 20–40 × 18–30 µm (Ellis 1971). This species was previously reported on *Bignonia* sp., *Citharexylum* sp. and *Duranta* sp. in Brazil, Cuba and Paraguay (Ellis 1971; Sierra et al. 2005). This is the first report of this fungus on *D. wilsonii* and *D. mollis*. Phylogenetically *P. paraguayensis*

grouped in a clade in Dothideomycetes, Capnodiales, Mycosphaerellaceae. This is the first information to become available on the phylogenetic position of this obscure genus (FIG. 33).

Pseudocercospora sp. (to be proposed as a new species)

FIGS. 17–18

Lesions on living leaves amphigenous, starting as chlorosis that later develop into necrosis in the oldest parts of leaves, irregular, brown, 1.5–8.0 mm diam, coalescing to cover the whole surface of the leaflets and leading to leaflet blight. Internal mycelium indistinct. External mycelium absent. Stromata well developed, substomatal, irregular to convex, 25–40 × 30–62.5 µm. Conidiophores hypophyllous arising from stromata, in sporodochium, cylindrical–obclavate, straight to curved or sinuous, 10.0–27.5 × 2.5–5.0 µm, 0–3 septate, unbranched, light brown, smooth, mostly restricted to the conidiogenous cells. Conidiogenous cells terminal, holoblastic, integrated, mostly cylindrical, light brown. Conidiogenous loci terminal, inconspicuous, truncate, 1–2.5 µm diam, neither thickened nor darkened. Conidia dry, solitary, cylindrical, mostly curved, 17.5–87.5 × 3.0–4.0 µm, truncate at the base, tapering at the end to a subacute apex, 1–12 septate, hilum unthickened and not darkened, subhyaline to olivaceous, guttulate, smooth.

In culture: slow-growing (2.1–2.8 cm diam, after 27 days), slightly to pronouncedly lobate at edges, flat to low convex and pale olivaceous grey cerebriform centrally, surrounded with a ring of honey colored immersed mycelium followed by a narrow ring of olivaceous mycelium, followed by a periphery of pale olivaceous grey mycelium, diurnal zonation either pronounced or subtle, reverse followed by a centre of mycelium leaden black, followed by a periphery of leaden gray mycelium, no sporulation.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 14 Jul 2009, *M. Silva, O.L. Pereira & R.W. Barreto* (VIC 31774–HOLOTYPE); 25 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31797); on living leaves of *Dimorphandra mollis*. BRAZIL: Minas Gerais: Paraopeba, Flona, 15 Jul 2009, *M. Silva & O.L. Pereira* (VIC 31775).

Notes: *Pseudocercospora* is one of the largest genera of anamorphic fungi including more than 1.200 species (Kirk et al. 2008). Approximately 200 are parasitic on members of the Fabaceae. Besides host-association, conidiogenous loci and hila, presence or absence of pigmentation in conidiophores and conidia are the main morphological characters used in the taxonomy of genus *Pseudocercospora* (Crous and Braun 2003). The fungus on *Dimorphandra* was compared with species of *Pseudocercospora* reported on hosts phylogenetically close to *Dimorphandra* - the *Dimorphandra*-group (Banks and Lewis 2009) including *Burkea* Benth., *Erythrophleum* Afzel. Ex R. Br., *Mora* Schomb. ex Benth., *Pachyelasma* Harms, *Stachyothyrsus* Harms and *Sympetalandra* Stapf. Only one species of *Pseudocercospora* is known to occur on a member of this group, *Pseudocercospora erythrophlei* Z.Q. Yuan reported on leaves of *Erythrophleum chlorostachys* Baill (Yuan 1996). This is the first report of *Pseudocercospora* on a member of *Dimorphandra* and the fungus found in this study clearly differs from *P. erythrophlei* in having shorter conidiophores and conidia (10–27 µm, 17.5–87.5 µm respectively), and because of the absence of geniculate conidiogenous cells with short “denticles”, thus justifying the proposition of the new species. Morphology of *Pseudocercospora* on *D. wilsonni* and on *D. mollis* are identical and sequence analysis has confirmed that isolates obtained from the two hosts belong to the same species which will be proposed as new (FIG. 33). The *Pseudocercospora* on *Dimorphandra* spp. grouped very closely to *Pseudocercospora bixae* (Allesch. & F. Noack) Crous, Alfenas & R.W. Barreto in the phylogenetic analysis, nevertheless, *P. bixae* is a parasite of a distantly related host (*Bixa orellana* L.) belonging to a different host-family (Bixaceae) and has a clearly distinct morphology - longer and narrower conidiophores (15–60 × 2.5–3.5 µm), longer conidia (25–60 µm long) (Chupp 1954) and is clearly not conspecific with the fungus on *Dimorphandra*.

Pseudocercospora sp. (to be proposed as a new species)

FIGS. 19–20

Lesions on living leaves amphigenous, starting as minute dark dots becoming circular to irregular, necrotic, coalescing and expanding towards the leaf, leading to leaf blight, 1.5–5.0 mm diam, brown, covered with white foamy fungal colonies, coalescing to cover the whole surface of the leaflet and leading to leaflet blight.

Internal mycelium intercellular, 1.5–2.5 µm, branched, septate, pale brown. External mycelium absent. Stromata superficial, 17.5–30 x 37.5–55 µm., composed of subhyaline textura angularis cells. Conidiophores mostly restricted to conidiogenous cells, hypophyllous arising from stromata, in dense fascicles, cylindrical, straight to slightly sinuous, 10–20 × 2.5–5.0 µm, 0–1 septate, unbranched, hyaline, smooth. Conidiogenous cells integrated, terminal, holoblastic, cylindrical, hyaline. Conidiogenous loci, truncate, up to 2.5 µm diam, neither thickened nor darkened. Conidia dry, solitary, cylindrical, slightly curved to curved, 22.5–57.5 × 4–6 µm, truncate at the base, apex rounded, 2–7 septate, hilum unthickened and not darkened, hyaline to olivaceous, guttulate, smooth.

In culture: slow-growing (2.0-3.0 cm diam, after 27 days), slightly lobate edges, flat, immersed on media at periphery, aerial mycelium buff cottonous either followed with an outer ring with irregular portions of buff or honey cottonose mycelium (on PCA) or followed with a narrow ring of white cottonous mycelium over a layer of olivaceous gray colony, followed by a periphery of pale olivaceous gray mycelium (on PDA), reverse either rosy buff (on PCA) on prinrose alternating with with leaden black (on PDA), no sporulation.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 26 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31788–HOLOTYPE); 26 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31798, VIC 31804); on living leaves of *Dimorphandra mollis*. BRAZIL: Minas Gerais: Paraopeba, Flona, 15 Jul 2009, *M. Silva & O.L. Pereira* (VIC 31776); 20 Jul 2010, *M. Silva & O.L. Pereira* (VIC 31783); 08 Feb 2011, *M. Silva* (VIC 31789); on living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Juatuba, 07 Feb 2011, *M. Silva* (VIC 31786); on living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Fortuna de Minas, 07 Feb 2011, *M. Silva* (VIC 31787); 08 Feb 2011, *M. Silva* (VIC 31793); on living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Sete Lagoas, 09 Feb 2011, *M. Silva* (VIC 31790); 09 Feb 2011, *M. Silva* (VIC 31791); 09 Feb 2011, *M. Silva* (VIC 31792).

Notes: The cercosporoid fungus found on *D. wilsonii* and *D. mollis* clearly belongs to the genus *Pseudocercospora* Deighton. It bears the key morphological characters of the genus - hyaline septate conidiophores, unthickened and not darkened scars, hyaline, septate, conidia with unthickened hila and schizolytic secession (Deighton 1973). Four *Pseudocercospora* species are known to occur on members of the Fabaceae (Farr et al. 2011): *P. astragali* (Rostr.) U. Braun, *P. cystisi* (Jaap) U. Braun, *P. ougeniae* M.D. Mehrotra & R.K. Verma and *P. tephrosiae* (Hansf.) U.

Braun. *Pseudocercospora astragali* and *P. cystisi* are easily distinguished from *Pseudocercospora* on *Dimorphandra* by having longer and narrower conidia (15–70 × 2.5–4 µm, 50–125 × 1–4 µm respectively) while *P. ougeniae* has longer conidia (35)60–90(115) µm and narrower conidiophore 3–4 µm. *Pseudocercospora tephrosiae* has longer conidiophores 50–100(125) µm and smaller conidia 15–45 µm than the fungus on *Dimorphandra* (Braun 1995, Chupp 1954) (TABLE 4). Additionally, considering the usual significant levels of host-specificity found for this group of fungi, it is hence considered adequate to place the fungus on *Dimorphandra* in a species to be proposed as new. The overlapping in DNA sequence analysis (FIG. 33) together with the common morphology found for *Pseudocercospora* specimens on *D. wilsonii* and on *D. mollis* clearly shows that they belong to the same specimens.

cf. *Radulidium* (to be proposed as a new genus)

FIGS. 21–23

Colonies hypophyllous, effuse, brown, on trichomes. Internal mycelium absent. External mycelium superficial, 3–4 µm, branched, septate, pale brown, smooth. Conidiophores formed apically on trichomes, in loose groups, semi-macronematous, cylindrical, 12.5–37.5 × 4.0–5.0 µm, 0–2 septate, occasionally branched, light brown, smooth. Conidiogenous cells holoblastic, terminal or intercalary, integrated, cylindrical, 10.0–15.0 × 4.5–5.0 µm, light brown. Conidiogenous loci with protuberant dark scars 3–8 per cell, up to 1 µm diam, darkened 1 µm. Conidia dry, solitary, fusiform to cylindrical, 15–35 × 5.0–7.5 µm, 0–3 septate, hilum unthickened, not darkened, eguttulate, light brown, verruculose.

In culture: slow-growing (3.7–4.0 cm diam, after 27 days), circular, low convex, floccose aerial mycelium centrally with faint diurnal zonation, followed by narrow periphery of dense rosy buff immersed mycelium, ochraceous reverse, no sporulation.

Specimen examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 26 Jul 2011, M. Silva & O.L. Pereira (VIC 31803–HOLOTYPE).

Notes: According Arzanlou et al. (2007) the anamorphic genus *Ramichloridium* Stahel ex de Hoog accommodates a wide range of species which differs in morphology and also in life-style. These authors have used information on morphological characters, conidial ontogeny and DNA sequences to reallocate some

species belonging to this genus and also to segregate some new genera from *Ramichloridium*. The fungus on *D. wilsonii* is morphologically and phylogenetically closer to species belonging to the *Ramichloridium* segregates *Radulidium* Arzanlou, W. Gams & Crous, *Veronaea* Cif. & Montemart. and *Veronaeopsis* Arzanlou & Crous (Arzanlou et al. 2007). *Radulidium* differs from the fungus on *D. wilsonii* by having conidiophores with an apical part forming a pale brown, generally straight axis, crowded, prominent, blunt denticles, unthickened scars and hila on conidia predominantly aseptate. In *Veronaea*, differently from the fungus on *D. wilsonii* conidiophores are long, conidiogenous cells are faintly pigmented, scars are unthickened and conidia are smooth. *Veronaeopsis* differs from the fungus on *D. wilsonii* by having conidiogenous cells with densely crowded, prominent denticles and smooth conidia (Arzanlou et al. 2007). Hence, the introduction of a new genus seems to be justified.

Stomiopeltis suttoniae (J. M. Mend.) Luttrell, *Mycologia* 38:572 (1946) FIG. 24

Colonies on living leaves, hypophyllous, sparse, fuliginous, covering part of the leaf. Internal mycelium not observed. External mycelium hypogenous, anastomosing, net-forming, slightly undulate, composed of pale brown, flattened, thin walled, septate hyphae, 2.5–5.0 μm , smooth. Ascumata hypophyllous, superficial, solitary, scutellate, ostiolate, 100–350 μm diam., composed of meandrically interwoven hyphae (textura epidermoidea), black. Pseudoparaphyses filiform, 2–2.5 μm , septate, unbranched, hyaline. Asci bitunicate, radially arranged, clavate to ovate-oblong, 35–55 \times 10–15 μm , 8-spored. Ascospores obovoid to ellipsoid, 10–17.5 \times 4–5.0 μm , 1-septate, with the upper cell slightly broader, guttulate, hyaline, smooth.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 14 Jul 2009, M. Silva, O.L. Pereira, R.W. Barreto (VIC 31809, VIC 31810); on living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Caetanópolis, Fazenda São Bento, 15 Jul 2009, M. Silva, O.L. Pereira, R.W. Barreto (VIC 31811).

Notes: The genus *Stomiopeltis* was established for fungi having the following morphological features (Luttrell 1946): brownish superficial hyphae; superficial, shield-like, ostiolate ascumata, composed of meandrically interwoven hyphae (textura epidermoidea); bitunicate, radially arranged asci mixed with pseudoparaphyses, two

celled, hyaline ascospores (von Arx and Müller 1975). The fungus on *D. wilsonii* fits well with the description of *S. suttoniae* (Mendonza) Luttrell (Batista 1959, Luttrell 1946). This species was previously reported on *Suttonia lessertiana* (A. DC.) Mez in Hawaii and on *Erythroxyllum* sp. in association with *Micropeltis gravataensis* Bat. & Vital and *Hymenopeltis erythroxylii* Bat. & Vital in Brazil (Batista 1959). This is the first report of this fungus on *D. wilsonii* (Fabaceae). Although recorded sporadically, the few existing records of this easily overlooked species suggest that this is a broadly spread species having a wide host-range.

Trichomatomyces byrsonimae Dornelo-Silva & Dianese, *Mycologia* 96: 879–884 (2004) FIGS. 25–27

Colonies minute, hypophyllous, dense, fuliginous, dark brown to black, forming spirally arranged heads on the apex of trichomes. Internal mycelium absent. External mycelium superficial, 2.5–5.0 μm , branched, septate, light brown, smooth. Conidiophores crowded on apex of trichomes, single, micronematous, straight to curved, 11.0–21.5 \times 4.0–5.0 μm , 0–2 septate, unbranched, dark brown, smooth. Conidiogenous cells integrated, polyblastic, sympodial, 7.5–15.0 \times 3.5–5.0 μm , 0–2 septate, brown. Conidiogenous loci indistinct. Conidia dry, solitary, elliptic-fusiform, slightly unilaterally curved, 12.5–25 \times 7.5–12.5 μm , walls thinner and smooth on one side and thicker and roughened on the other, apex acute and base truncate, 1–4 septate, constricted at the septa on one (thinner-walled) side but not on the other (thicker-walled), brown, eguttulate, striated.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 13 Jul 2009, *M. Silva, O.L. Pereira & R.W. Barreto* (VIC 31769); on living leaves of *Dimorphandra mollis*. BRAZIL: Minas Gerais: Paraopeba, Flona, 15 Jul 2009, *M. Silva & O.L. Pereira* (VIC 31777); on living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Caetanópolis, Fazenda São Bento, 19 Jul 2010, *M. Silva & O.L. Pereira* (VIC 31778); on living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 26 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31799); 26 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31800); 26 Jul 2011, *M. Silva & O.L. Pereira* (VIC 31801).

Notes: *Trichomatomyces byrsonimae* (Bat. & Peres) Dornelo-Silva & Dianese is recorded here for the first time on *D. wilsonii* and *D. mollis*. This species was originally described as *Piricauda byrsonimae* Bat. & Peres, on *Byrsonima basiloba* A. Juss (Malpighiaceae), and later transferred to a new genus - *Trichomatomyces* - by Dornelo-silva and Dianese (Batista et al. 1962, Dornelo-Silva and Dianese 2004). Our fungus fitted well within the description of *T. byrsonimae* given by Dornelo-Silva and Dianese (2004). The material examined by latter authors was growing on foliar trichomes of *Qualea grandiflora* Mart. (Vochysiaceae). Although poorly known and only recorded three times, it is already clear that this fungus is not host-specialized since for each record the host-genus belonged to a distinct family. All records are from the Cerrado and it is possible that this fungus is restricted to this biome. The analysis of DNA sequences placed *Trichomatomyces byrsonimae* in the proximity of *Stomiopeltis* in Micropeltidaceae (FIG. 33), nevertheless, as there is little information available in DNA sequence banks for this and related fungi, it is early for conjecturing on the phylogenetic affinities of *Trichomatomyces*.

Vesiculohyphomyces cerradensis Armando, Pereira-Carvalho & Dianese, Mycol. Res. 113: 261-274 (2009) FIGS. 28–29

Colonies hypophyllous, brown, sooty. Internal mycelium absent. External mycelium superficial, 4–5 μm , branched, septate, brown, smooth. Conidiophores hypophyllous, solitary, macronematous, cylindrical, 255–362.5 \times 5.0–7.5 μm , 9–16 septate, unbranched, dark brown, smooth. Conidiogenous cells in terminal clusters of verticillate rings on conidiophores, discrete, polyblastic, an inflated subspherical vesicle-like head on short and somewhat crooked stalk, 5.0–12.5 \times 4.25–7.5 μm , light brown. Conidiogenous loci 2–5 per cell, 1.5–2.0 μm diam, black. Conidia dry, grouped, fusiform to cymbiform, 12.5–39.5 \times 5–6.5 μm , conidial apex acute, 2–2.5 μm , base rounded, 4–5.0 μm , 1–4 euseptate, hilum slightly protuberant, dark brown, eguttulate, striate when mature.

Specimens examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL: Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 26 Jul 2011, M. Silva & O.L. Pereira (VIC 31802).

Notes: The monotypic genus *Vesiculohyphomyces* is characterized by the presence of discrete vesicle-shaped conidiogenous cells, arranged in verticillate rings apically on conidiophores and conidia that are solitary, pigmented and wrinkly when mature. The fungus collected on *D. wilsonii* fits well within the description of *V. cerradensis*. This species was previously known only from trichomes of *Caryocar brasiliense* Cambess. (Caryocaraceae) also from the Brazilian Cerrado (Pereira-Carvalho et al. 2009). The occurrence of this fungus on two unrelated hosts suggests that it is a polyphagous epiphyte on Cerrado plants. *Vesiculohyphomyces cerradensis* was recorded here for the first time colonizing *D. wilsonii*.

Microcalliopsis dipterygis Batista, Peres & Bezerra, Brotéria Série de Ciências Naturais, 31: 1-26 (1962) FIGS. 30–32

Colonies on living leaves, adaxial, sooty, irregular, sparse. Internal mycelium indistinct. External mycelium hypogenous, 2.5–3.0 μm diam, branched, net forming, slightly undulate, composed of septate, brown, smooth hyphae. Ascomata pseudothecial, hypophyllous, superficial, solitary, ostiolate, irregularly scattered over the surface of the colonies, spherical to somewhat flattened, oblate spheroidal, 120–170 \times 58–90 μm , walls of brown textura angularis, 10–17.5 μm thick, smooth. Setae 6–9 per ascoma, evenly arising mostly from the lower half of the pseudothecia, needle-shaped mostly straight to slightly curved, 140–600 \times 3.0–5.0 μm , multiseptate (up to 22), smooth, dark brown, unbranched, tips rounded. Asci bitunicate, parallel, obclavate, 57.5–62.5 \times 12.5–15.0 μm , 8-spored, endotunica extending as a narrow column into the conspicuously domed ascus apex. Ascospores inordinate, fusiform to ellipsoid, with rounded ends, 17.5–22.5 \times 4–5 μm , 1-septate, eguttulate, hyaline, smooth.

Material examined: On living leaves of *Dimorphandra wilsonii*. BRAZIL, Minas Gerais: Paraopeba, Fazenda Tabuleiro Grande, 27 Jul 2011, M. Silva & O.L. Pereira (VIC 31806– HOLOTYPE).

Notes: A examination of its morphology led to the conclusion that the fungus on *D. wilsonii* belong to genus *Microcalliopsis*. There are only four species of *Microcalliopsis* recorded, and our specimens fitted well within the description of *M. dipterygis*. This species was previously related on leaves of *Dipterys alata* Vog. in

Brazil. This genus sits in a highly supported clade in Mycosphaerellaceae although strikingly different morphologically from other genera of Mycosphaerellaceae (FIG. 33). This genus morphologically resembles the family Chaetothyriaceae.

A high diversity was found in our exploration of the foliicolous mycobiota of *D. wilsonii* (thirteen species). This is significantly higher than the estimated average of six fungal species expected for each plant species roughly suggested by Hawksworth (2001). Additionally, this list is clearly only partial as a whole range of fungi occupying other niches in the plant remain unexplored in this work. It is also of interest to note that, despite their taxonomic relatedness *D. wilsonii* and *D. mollis* shared few fungi, as indicated in our study (*Pseudocercospora* sp., *Pseudocercospora* sp., *P. paraguayensis* and *T. byrsonimae*). Certainly these results are not waterproof. For instance, *J. chapadiensis*, which was described recently from *D. mollis*, in the context of this study was only found on *D. wilsonii* – a mistaken suggestion of specificity to *D. wilsonii* which reminds us of the inherent limitations of this kind of study and of the need of complementary studies to allow for more reliable conclusions. Several of the fungi were recognized as polyphagous organisms of little relevance in terms of conservation. Interestingly, several of these fungi appeared to be particularly rare in our sampling. Several occurred in only very few leaves (*Vesiculohyphomyces cerradensis*, *Piricauda paraguayensis*, *Geastrumia polystigmatis*), whereas others were either abundant, frequent or both (*Johansonia chapadiensis*, *Trichomatomyces byrsonimae*, *Phillipsiella atra*, *Microcalliopsis dipterygis* and *Stomiopeltis suttoniae*). The discovery of three new fungal taxa seemingly restricted to *Dimorphandra wilsonii*, including two which will be described as new genera (*Janetia* sp. 1, *Byssogene* sp., cf. *Radulidium* sp. – gen. nov.) mirrors the results of the pioneering work on the mycobiota of *C. floccosa* where six novel fungal taxa were discovered, including a new genus (Rocha et al. 2010). This is, perhaps, the best indication that the loss of a single plant species such as *D. wilsonii* can have disastrous consequences for a unique portion of the mycosphere. Additional studies are needed to confirm that conjecture and to demonstrate, for those taxa that were only found on *D. wilsonii*, that these are strictly host-specific and not capable of surviving on other substrates (as saprophytes, endophytes on other hosts, etc) and should be conducted to test the hypothesis of risk of co-extinction.

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TABLES AND FIGURES

TABLE 1- Localities of survey of *D. wilsonii* and *D. mollis*

Localities	Date/month/year	Coordinates in degrees-min-seg
Paraopeba- Fazenda Tabuleiro Grande	13 jul 2009; 19 jul 2010; 21 jul 2010; 27 jul 2011;	44W 25' 57" , 19S 15' 24"
Paraopeba Flona	15 jul 2009; 20-21 jul 2010;	
Caetanópolis Fazenda São Bento	15 jul 2009; 27 jul 2011	44W 25' 27" , 19S 19' 51"
Juatuba	07 fev 2011	44W 24' 42" , 19S 56' 52" 44W 24' 52" , 19S 56' 57"
Fortuna de Minas	07 fev 2011	44W 27' 55" , 19S 33' 28" 44W 27' 58" , 19S 33' 29"
Sete lagoas	09 fev 2011	44W 12' 18" , 19S 31' 10" 44W 12' 24" , 19S 31' 00"

TABLE 2- Genbank accession numbers of LSU rDNA sequences derived from strains used in the phylogenetic analysis

Species	LSU	References
<i>Capnobotryella renispora</i> CBS 214.90	EU019248	Crous et al., 2007
<i>Capnobotryella renispora</i> CBS 215.90	GU214399	Crous et al., 2009b
<i>Capnodium coffeae</i> CBS 147.52	DQ247800	Schoch et al., 2006a
<i>Capnodium salicinum</i> AFTOL-ID 937	DQ678050	Schoch et al., 2006b
<i>Cladosporium uredinicola</i> CPC 5390	EU019264	Crous et al., 2007
<i>Conidioxyphium gardeniorum</i> CPC 14327	GU301807	Schoch et al., 2009
<i>Cymadothea trifolii</i> strain 133	EU167610	Simon et al., 2009
<i>Davidiella macrospora</i> CBS 138.40	DQ008148	Avila et al., 2005
<i>Davidiella tassiana</i> CBS 723.79	GU214410	Crous et al., 2009b
<i>Dissoconium aciculare</i> CBS 204.89	GQ852587	Crous et al., 2009c
<i>Dissoconium australiensis</i> CBS 120729	GQ852588	Crous et al., 2009c
<i>Dissoconium commune</i> CBS 114239	GQ852590	Crous et al., 2009c
<i>Elsinoe eucalypticola</i> CBS 124765	GQ303306	Cheewangkoon et al., 2009
<i>Elsinoe veneta</i> AFTOL-ID 1853	DQ767658	Schoch et al., 2006b
<i>Johansonia chapadiensis</i>	HQ423450	Crous et al., 2010
<i>Mycosphaerella aleuritidis</i> CBS 282.62	EU167594	Simon et al., 2009

<i>Mycosphaerella bixae</i> CBS 111804	GQ852630	Crous et al., 2009c
<i>Mycosphaerella bixae</i> CPC 2554	GU214455	Crous et al., 2009b
<i>Mycosphaerella cruenta</i> CBS 462.75	GU214473	Crous et al., 2009b
<i>Mycosphaerella ellipsoidea</i> CMW 5166	DQ246254	Hunter et al., 2006b
<i>Mycosphaerella elongata</i> CBS 120735	JF700942	Quaedvlieg et al., 2011
<i>Mycosphaerella endophytica</i> CBS111519	DQ246255	Hunter et al., 2006b
<i>Mycosphaerella fori</i> CMW 9095	DQ204748	Hunter et al., 2006a
<i>Mycosphaerella gracilis</i> CBS 243.94	DQ204750	Hunter et al., 2006a
<i>Mycosphaerella graminicola</i> CBS 110744	EU019298	Crous et al., 2007
<i>Mycosphaerella gregaria</i> CBS 110501	EU167580	Simon et al., 2009
<i>Mycosphaerella intermedia</i> CMW7164	DQ246248	Hunter et al., 2006b
<i>Mycosphaerella madeirae</i> CBS 112895	DQ204756	Hunter et al., 2006a
<i>Mycosphaerella marksii</i> CBS 110942	GQ852612	Crous et al., 2009c
<i>Mycosphaerella parkii</i> CBS 387.92	GQ852616	Crous et al., 2009c
<i>Mycosphaerella pseudoellipsoidea</i> CBS 114709	EU167585	Simon et al., 2009
<i>Mycosphaerella pseudomarksii</i> AGI 126.2	EU882137	Cheewangkoon et al., 2009
<i>Mycosphaerella punctiformis</i> CBS 113265	NG_027571	Spatafora et al., 2006
<i>Mycosphaerella rosigena</i> CBS 330.51	GU214413	Crous et al., 2009b
<i>Mycosphaerella sphaerulinae</i> CPC 4314	GU214451	Crous et al., 2009b
<i>Mycosphaerella stromatosa</i> CBS 101953	EU167598	Simon et al., 2009
<i>Mycosphaerella vietnamensis</i> CBS 119974	JF700944	Quaedvlieg et al., 2011
<i>Myriangium duriaei</i> CBS 260.36	NG_027579	Schoch et al., 2006b
<i>Myriangium hispanicum</i> CBS 247.33	GU301854	Schoch et al., 2009
<i>Penidiella strumelloidea</i> CBS 114484	EU019277	Crous et al., 2007
<i>Phaeothecoidiella illinoisensis</i> CMG TN1_2.4E1d	GU117902	Yang et al., 2010
<i>Phaeothecoidiella missouriensis</i> AHE7c	AY598917	Batzer et al., 2005
<i>Phaeothecoidiella missouriensis</i> CBS 118959	GU117903	Yang et al., 2010
<i>Polychaeton citri</i> CBS 116435	GU214469	Crous et al., 2009b
<i>Pseudocercospora basitruncata</i> CBS 111280	DQ204760	Hunter et al., 2006a
<i>Pseudocercospora crousii</i> CBS 119487	GQ852631	Crous et al., 2009c
<i>Pseudocercospora eucalyptorum</i> CMW 5228	DQ204762	Hunter et al., 2006a
<i>Pseudocercospora natalensis</i> CBS 111069	DQ267576	Hunter et al., 2006b
<i>Pseudocercospora platani</i> CBS 110755	GQ852635	Crous et al., 2009c
<i>Pseudocercospora robusta</i> CMW 5151	DQ204767	Hunter et al., 2006a
<i>Pseudocercospora sphaerulinae</i> CBS 112621	GQ852652	Crous et al., 2009c
<i>Pseudocercospora vitis</i> CPC 11595	DQ073923	Ayala-Escobar et al., 2006
<i>Pseudovirgaria grisea</i> CPC 19130	JF957612	Braun et al., 2011
<i>Pseudovirgaria hyperparasitica</i> CPC 10702	EU041822	Arzanlou et al., 2007
<i>Pseudovirgaria hyperparasitica</i> CPC 10704	EU041823	Arzanlou et al., 2007
<i>Radulidium epichloes</i>	AF050287	Untereiner and Naveau, 1999
<i>Radulidium epichloes</i> CBS 361.63	EU041842	Arzanlou et al., 2007
<i>Radulidium subulatum</i> CBS 287.84	EU041844	Arzanlou et al., 2007
<i>Ramichloridium cerophilum</i> CBS 103.59	EU041855	Arzanlou et al., 2007
<i>Schizothyrium pomi</i> CBS 406.61	EF134949	Batzer et al., 2008

<i>Schizothyrium pomi</i> CBS 486.50	EF134948	Batzer et al., 2008
<i>Schizothyrium pomi</i> CBS 228.57	EF134947	Batzer et al., 2008
<i>Stomiopeltis</i> sp. CCRS 8.20.96 4R Gp002	AY598919	Batzer et al., 2005
<i>Stomiopeltis versicolor</i> GA3_23C2b	FJ147163	Diaz-Arias et al., 2010
<i>Teratosphaeria fibrillosa</i> CBS 121707	GU323213	Schoch et al., 2009
<i>Teratosphaeria macowanii</i> CPC 1488	FJ493199	Crous et al., 2009a
<i>Teratosphaeria stellenboschiana</i> CPC 10886	EU019295	Crous et al., 2007
<i>Teratosphaeria toledana</i> CBS 115513	FJ493225	Crous et al., 2009a
<i>Zasmidium cellare</i> CBS 146.36	EU041878	Arzanlou et al., 2007
<i>Zasmidium nocoxi</i> CPC 14044	GQ852735	Crous et al., 2009c
<i>Zasmidium xenoparkii</i> CBS 111185	JF700966	Quaedvlieg et al., 2011

TABLE 3 -Conidial size and septation of *Janetia* species recorded on members of the *Fabaceae*

Species	Conidial size μm	Septation
<i>J. bacilliformis</i> Gamundi, Arambi & Giaiotti	60-156 x 5-9	5-9 distoseptate
<i>J. canescens</i> B. Sutton & Pascoe	16-57 x 5.5-9	1-7 distoseptate
<i>J. refugia</i> B. Sutton & Pascoe	31-37 x 7-8	4-6 distoseptate
<i>J. synnematososa</i> Sivan. W.H. Hsieh	80-115 x 10-12.5	9-22 distoseptate
<i>Janetia</i> sp. on <i>D. mollis</i>	16-35 x 6.5-9.5	2-7 distoseptate
<i>J. bonarii</i> (M.B. Ellis) S. Hughes	55-95 x 10-12	5-12 euseptate
<i>J. capnophila</i> S. Hughes	58-145 x 10.8-6.2	7-16 euseptate
<i>J. cubensis</i> Matsush.	16-77 x 5-8	2-8 euseptate
<i>J. curviapis</i> Goh & K.D. Hyde	65-100 x 5.5-7.5	6-12 euseptate
<i>J. euphorbiae</i> M.B. Ellis	18-36 x 6-8	3-6 euseptate
<i>J. faureae</i> (Piroz.) M.B. Ellis	50-120 x 4-5	3-9 euseptate
<i>J. garryae</i> (Bonar) S. Hughes	25-70 x 6-8.5	2-6 euseptate
<i>J. indica</i> S.R.Bussa	65-105 x 9-15	5-9 euseptate
<i>J. interna</i> H.J. Swart	57-128 x 10-11	5-8 euseptate
<i>J. leprosa</i> (Piroz.) S. Hughes	10-17 x 3.5-4	2-3 euseptate
<i>J. longispora</i> P.M. Kirk	90-285 x 10-15	6-12 euseptate
<i>J. mangiferae</i> S. Hughes & Cavalc.	8.5-23 x 4.3-6	1-5 euseptate
<i>J. matsushimae</i> Subram.	20-31.5 x 5-6	4-7 euseptate
<i>J. obovata</i> M. Caldusch, Gené, Abdullah & Guarro	22.5-33.5 x 12-15	3-5 euseptate
<i>J. salvertiae</i> Dorn.-Silva & Dianese	15-30 x 3-5	1-6 euseptate
<i>J. salicis</i> Li Xu & Y.L. Guo	34-91 x 2.5-4	multiseptate
<i>Janetia</i> sp. on <i>D. wilsonii</i>	12.5-75 x 5-7.5	1-9 euseptate

TABLE 4 - Conidial and conidiophore size of *Pseudocercospora* species recorded on members of the *Fabaceae*

Species	Conidia size (μm)	Conidiophore size (μm)
<i>P. astragali</i> (Rostr.) U. Braun	15-70 x 2.5-4	4-10 x 3-4
<i>P. cystisi</i> (Jaap) U. Braun	50-125 x 1-4	10-40 2-5
<i>P. ougeniae</i> M.D. Mehrotra & R.K. Verma	(35)60-90(115) x 5-6.5	10-20 x 3-4.5(4)
<i>P. tephrosiae</i> (Hansf.) U. Braun	(15)15-45 x (3)4-5(6)	50-100(125) x 2.5-5.5
<i>Pseudocercospora</i> sp. nov.	22.5–57.5 × 3.75–6.25	10–20 × 2.5–5.0

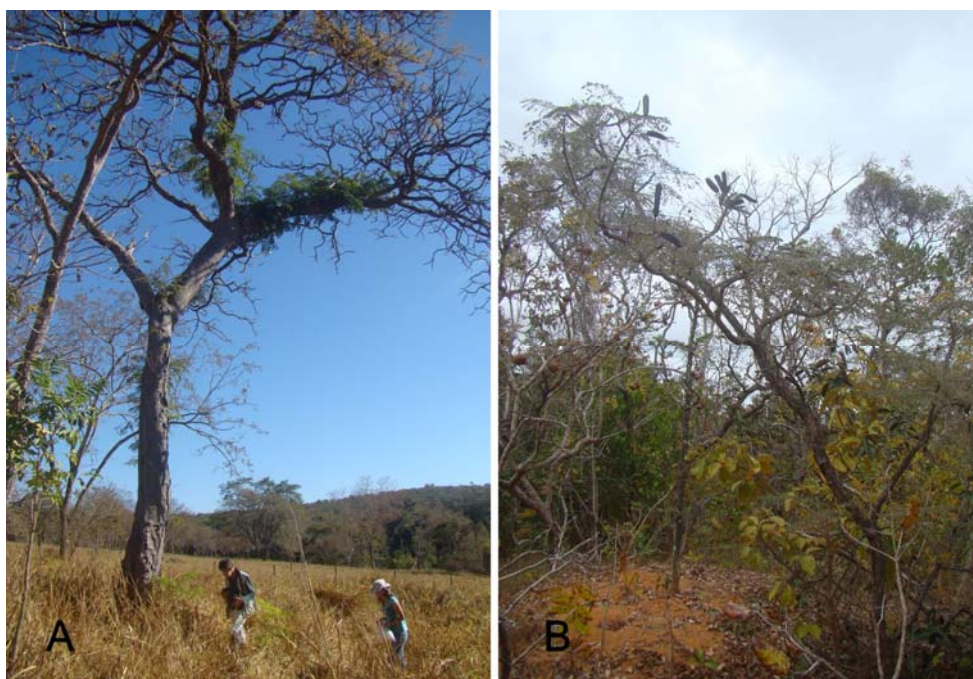


FIG. 1 Plants surveyed during the work. *Dimorphandra wilsonii* on pasture (A) and *Dimorphandra mollis* on preserved fragment of Brazilian Cerrado (B).

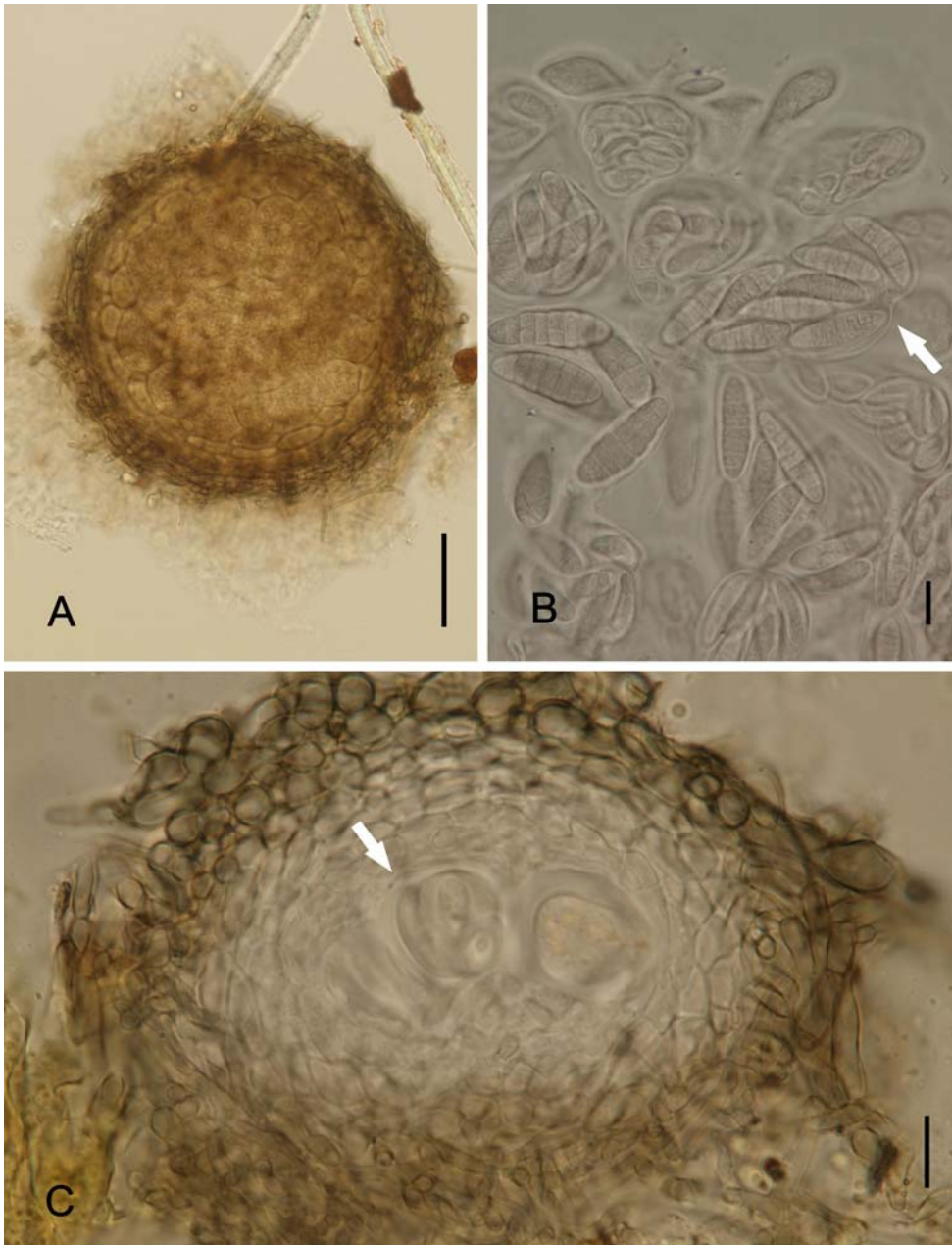


FIG. 2 *Byssogene* sp. on *Dimorphandra wilsonii*. (A) Discoid, non ostiolate ascoma surrounded by a net forming external mycelium. (B) Bitunicate, 8-spored asci with cylindrical to ellipsoid, dictyoseptate, subhyaline ascospores – arrowed. (C) Cross section of superficial ascoma showing asci – arrowed. Bars: 50 μm (A); 10 μm (B); 20 μm (C).

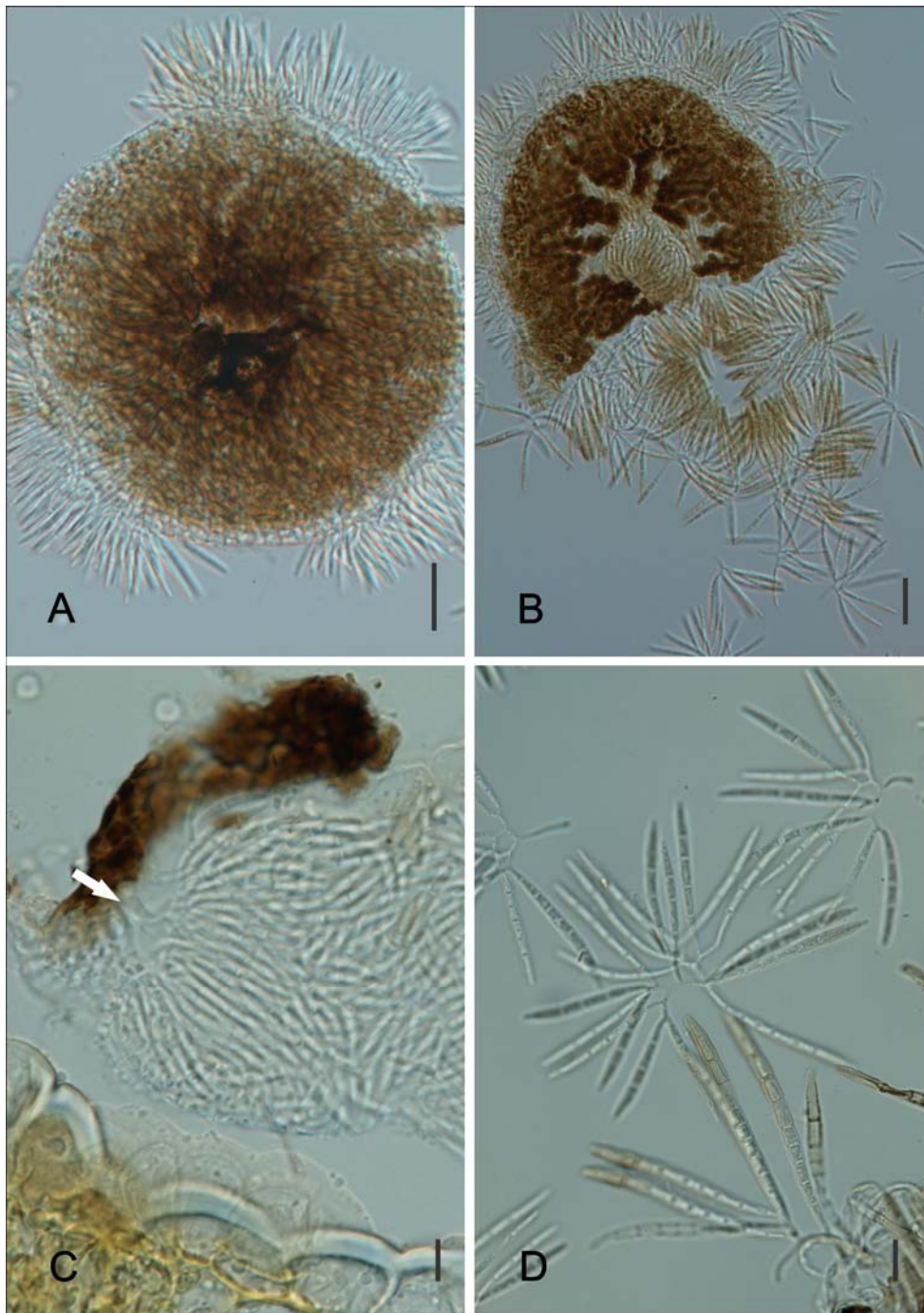


FIG. 3 *Geastrumia polystigmatis* on *Dimorphandra wilsonii*. (A) Dark shield-like conidioma. (B) Conidioma opening by irregular rupture, releasing groups of conidia. (C) Cross section of conidiomata showing conidiogenous cells developing from the hyphae into the upper wall – arrowed. (D) Cheiroid conidia or fasciculate group of filiform arms attached to a common, branched, long pedicellate basal stalk cell . Bars: 20 μm (A, B); 10 μm (C, D).

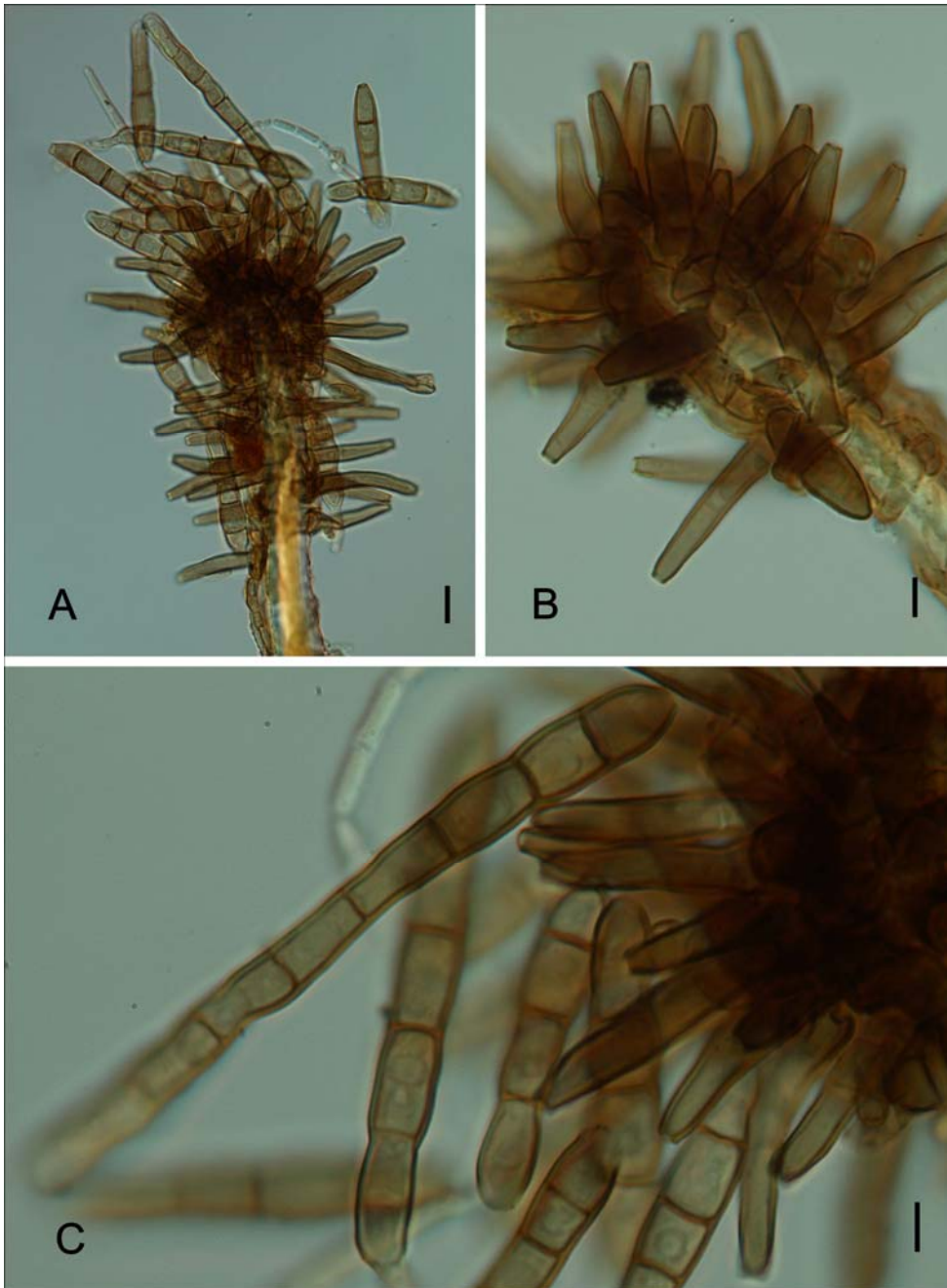


FIG. 4 *Janetia* sp. 1 on *Dimorphandra wilsonii*. (A) Colonies on apex of trichome. (B) Denticulate, monoblastic conidiogenous cells. (C) Euseptate, reddish brown conidia. Bars: 10 μ m (A); 5 μ m (B,C).

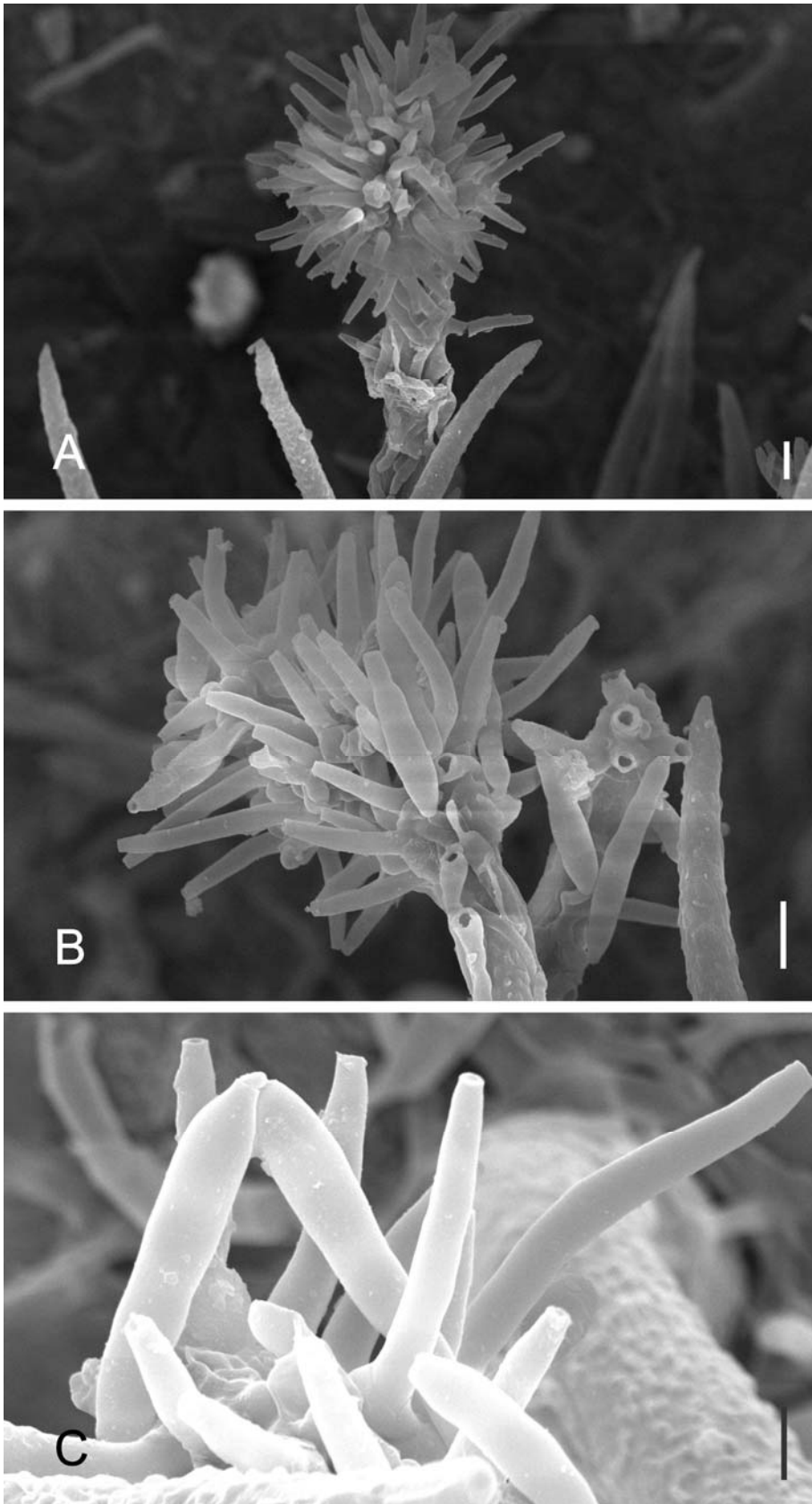


FIG. 5 SEM of *Janetia* sp. 1 on *Dimorphandra wilsonii*. (A) Dense colonies on apex of trichome. (B) Denticulate conidiogenous cells. (C) Schizolytic conidial secession. Bars: 10 μ m (A, B); 5 μ m (C).

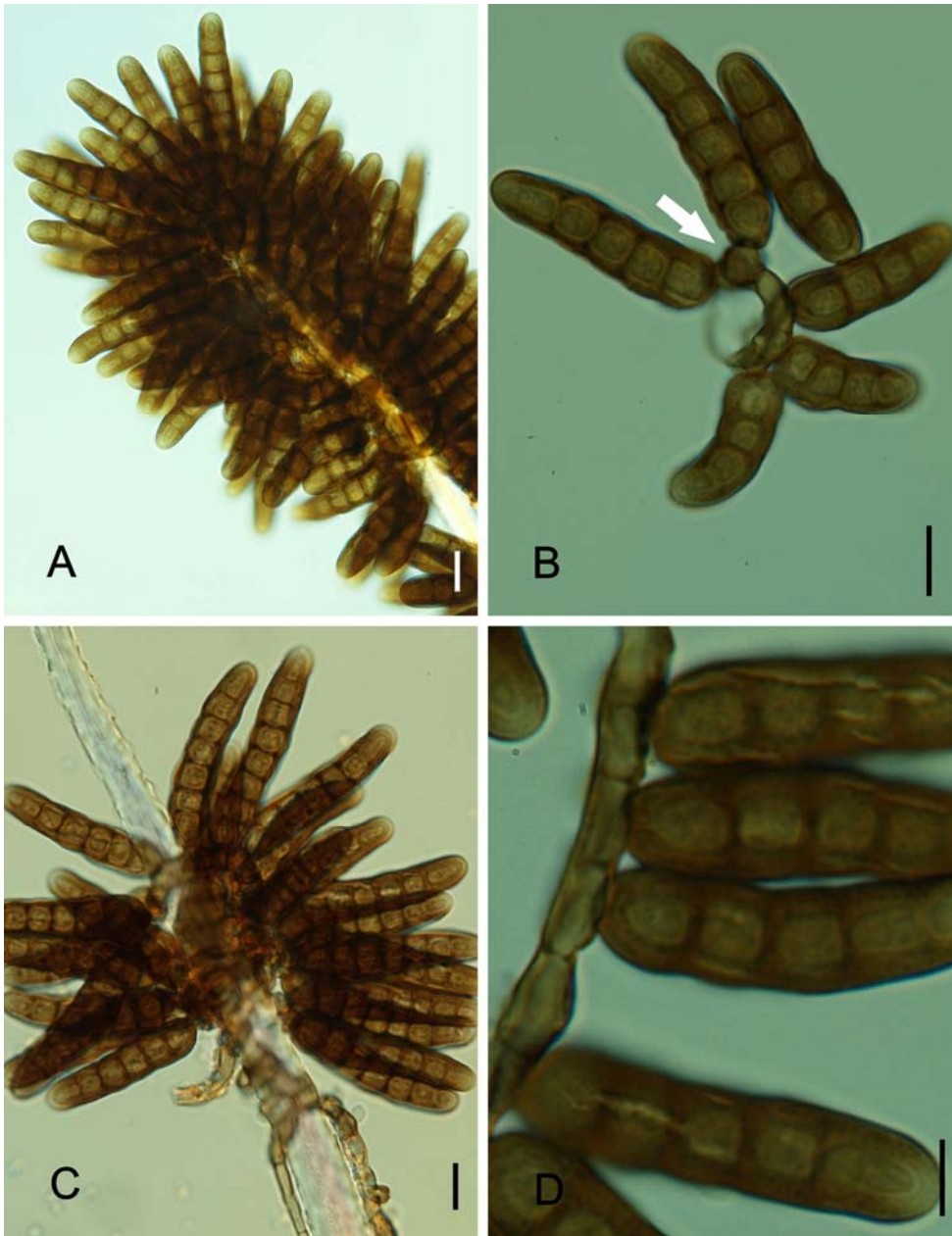


FIG. 6 *Janetia* sp. 2 on *Dimorphandra mollis*. (A) Dense colonies on upper portion of trichome. (B) Conidiophores consisting only of ill-differentiated conidiogenous cells – arrowed. (C) Cylindrical distoseptate conidia. (D) Close-up of distoseptate conidia. Bars: 10 μm (A, B, C); 5 μm (D).

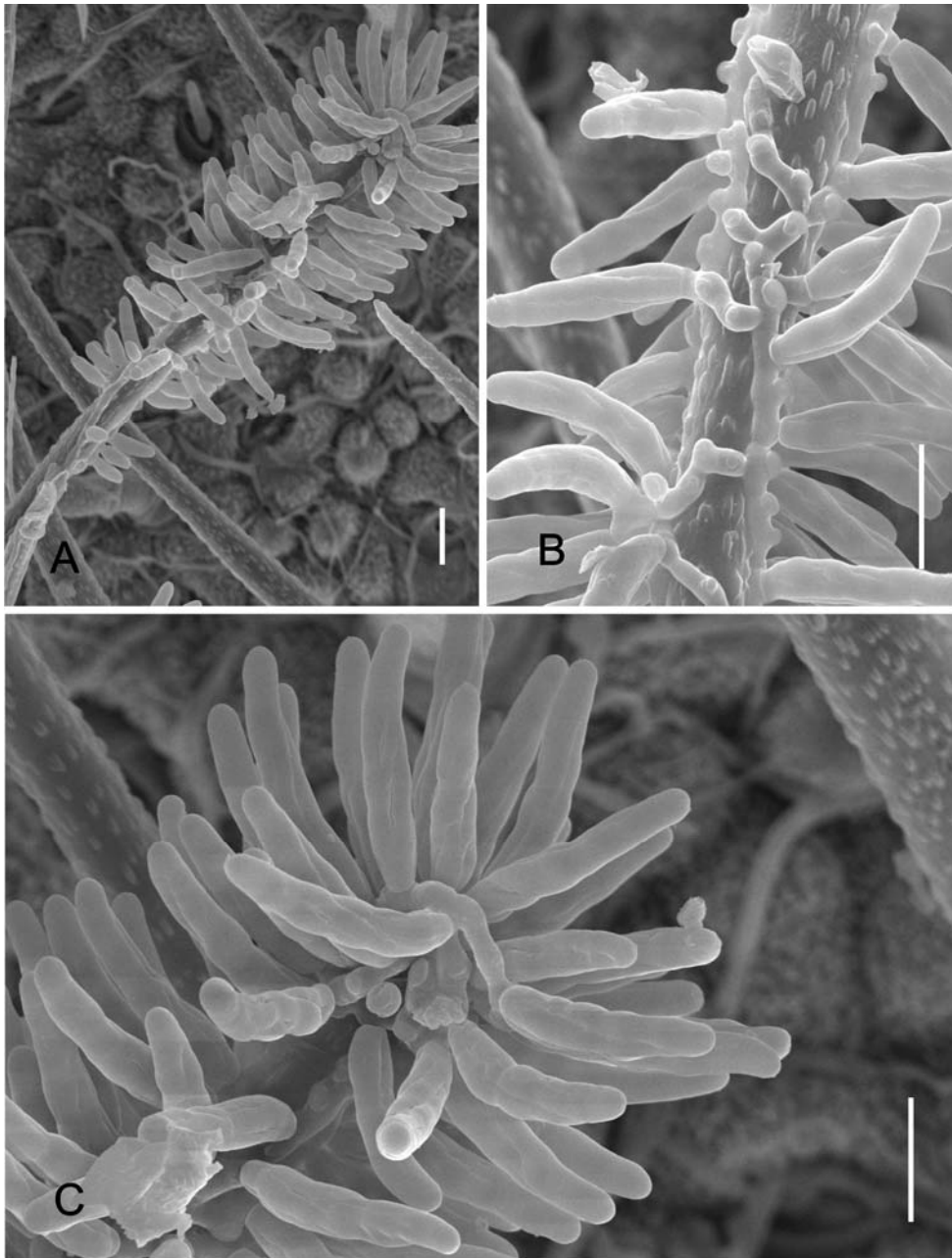


FIG. 7 SEM of *Janetia* sp. 2 on *Dimorphandra mollis*. (A) Dense bottle brush-like colonies on trichome. (B) Denticulate micronematous conidiogenous cells. (C) Close-up of group of conidia. Bars: 10 μ m.

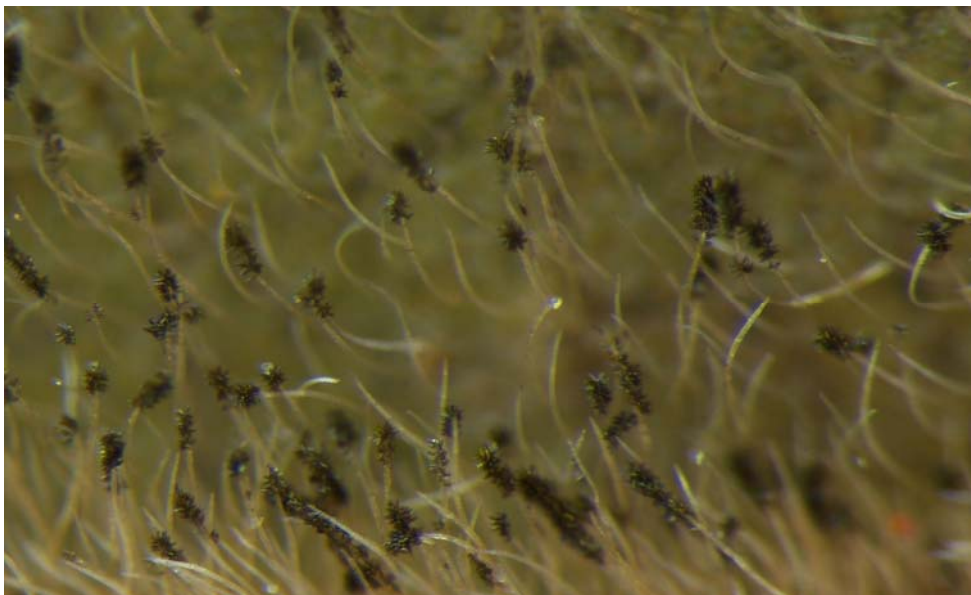


FIG. 8 *Janetia* sp. 2 on *Dimorphandra mollis*. (A) Black colonies on trichomes.

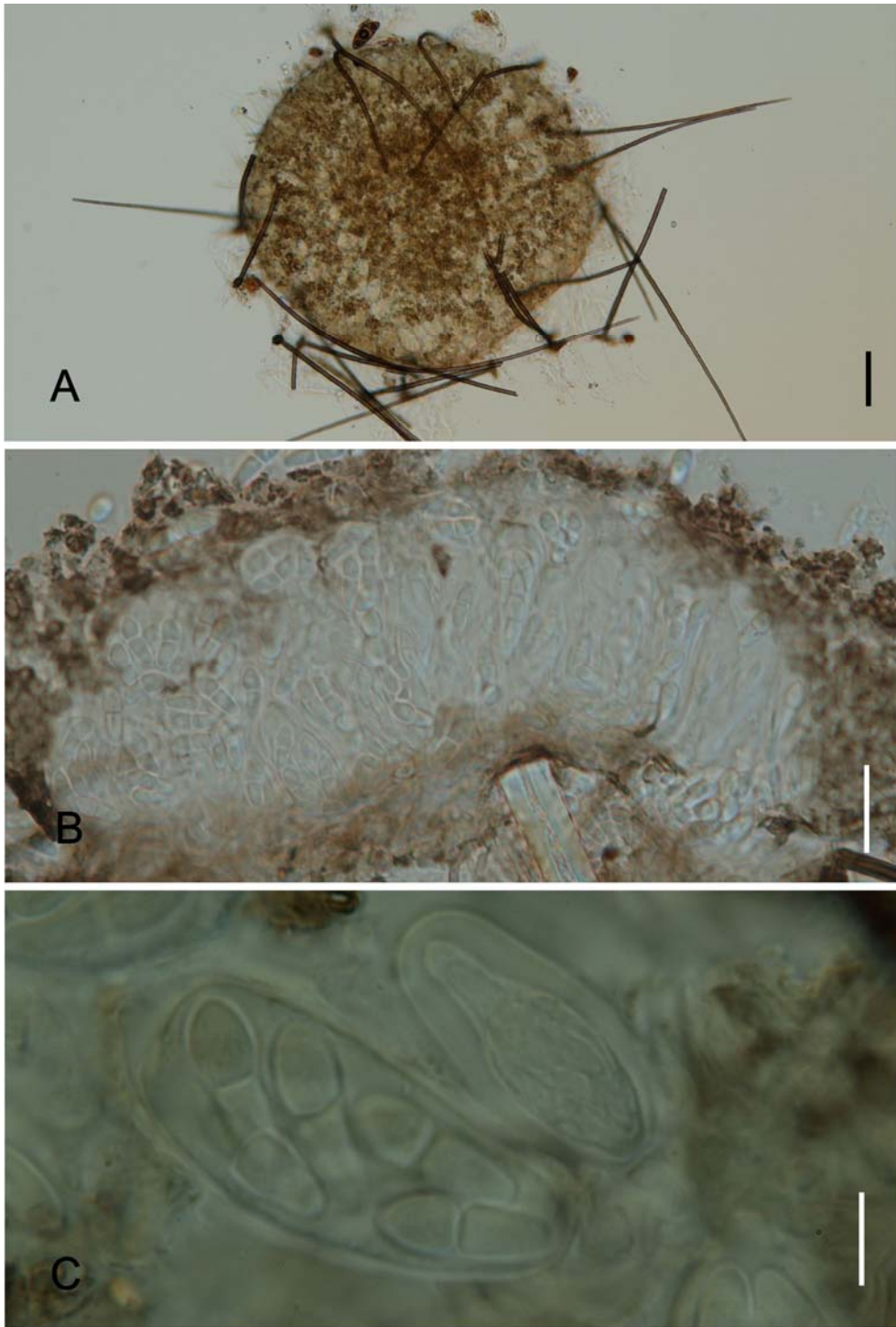


FIG. 9 *Johansonia chapadiensis* on *Dimorphandra wilsonii*. (A) Upper view of setose, discoid, non-ostiolate ascoma. (B) Cross section showing paraphyses intermingled with bitunicate, parallel asci. (C) Close-up of immature ascus and mature ascus with hyaline, 1-septate ascospores. Bars: 50 μm (A); 20 μm (B); 5 μm (C).

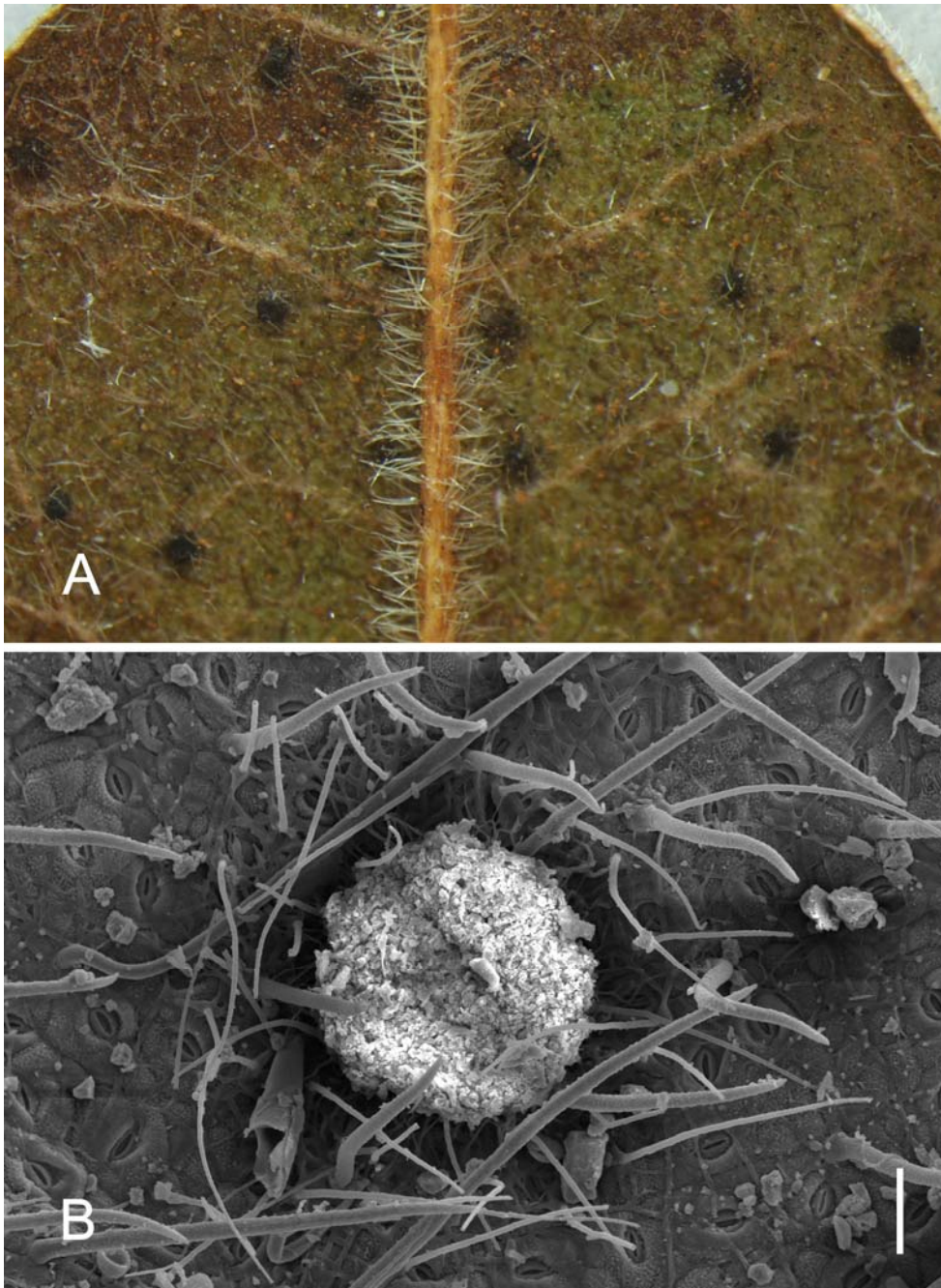


FIG. 10 *Johansonia chapadiensis* on *Dimorphandra wilsonii*. (A) Sooty dots on abaxial leaf surface of *D. wilsonii*. (B) SEM of ascomata surrounded by setae. Bar: 50 μm .

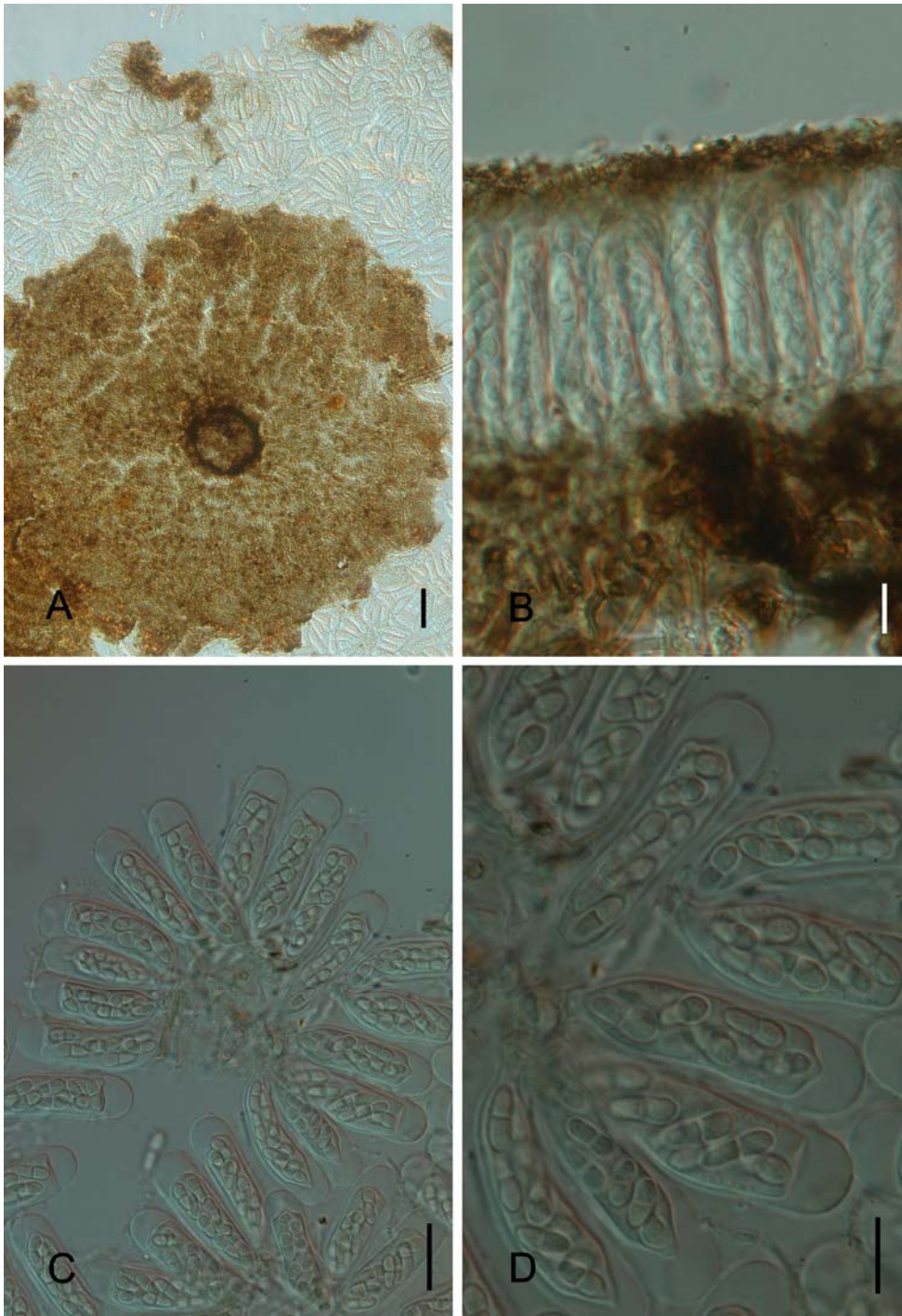


FIG. 11 *Phillipsiella atra* on *Dimorphandra wilsonii*. (A) Discoid, superficial ascoma. (B) Cross section of fertile ascoma showing parallel asci. (C) Numerous bitunicate asci intermixed with filiform pseudoparaphyses. (D) Close-up of asci bearing eight, 1-septate, hyaline ascospores. Bars: 50 μ m (A); 10 μ m (B, D); 20 μ m (C).

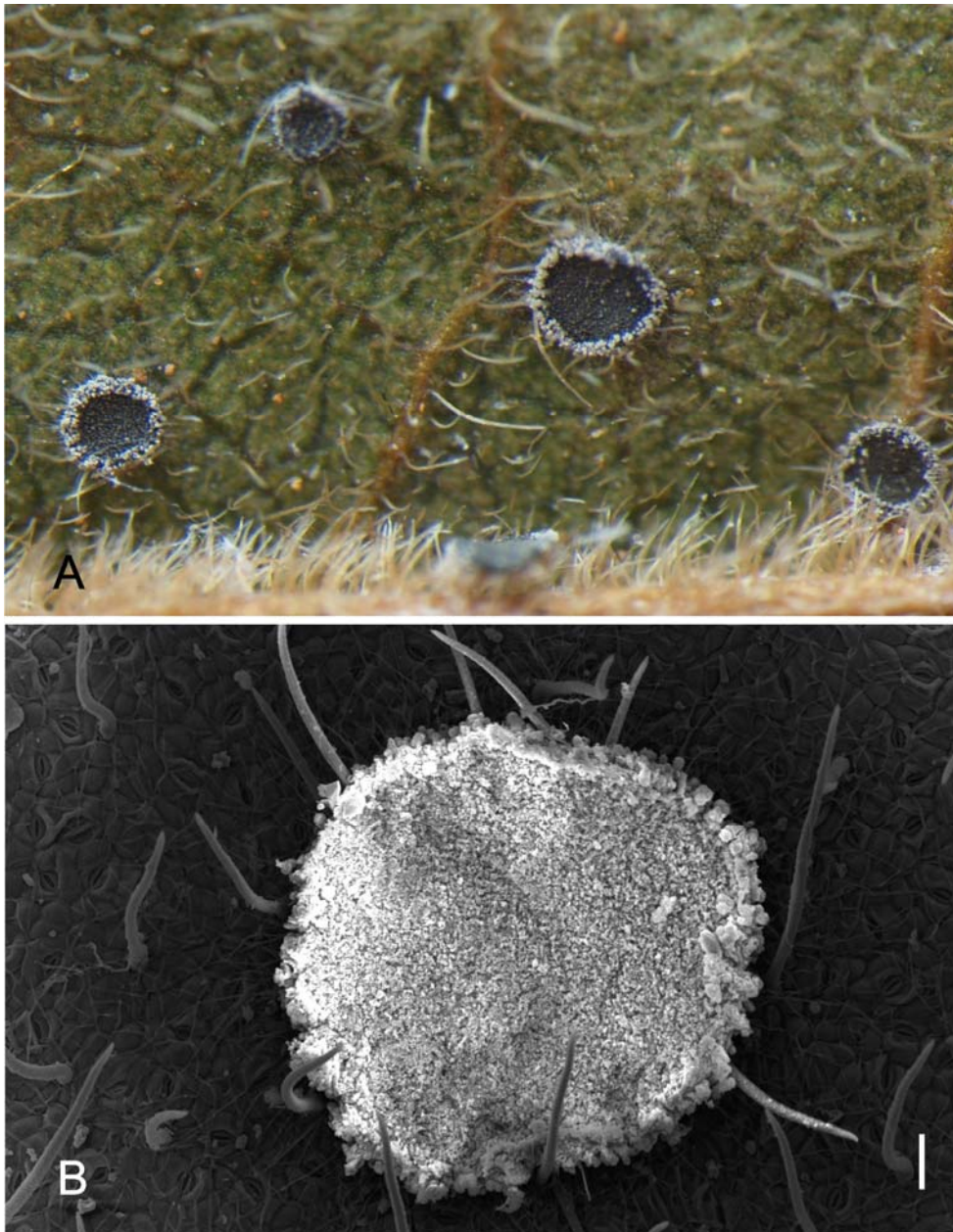


FIG. 12 *Phillipsiella atra* on *Dimorphandra wilsonii*. (A) Apothecioid ascomata on the abaxial side of a leaf (B) SEM of detail of apothecioid ascoma. Bar: 50 μm (B).



FIG. 13 *Piricauda paraguayensis* on *Dimorphandra wilsonii*. (A) Colony with immature conidia (notice the absence of beaks) on trichomes. (B) *Ibid* at different plane of focus showing tretic conidiogenous scars – arrowed. (C) Dictyoseptate conidium developing beak. (D) Same as C at different plane of focus showing rugose surface. Bars: 20 μ m (A,B); 10 μ m (C,D).

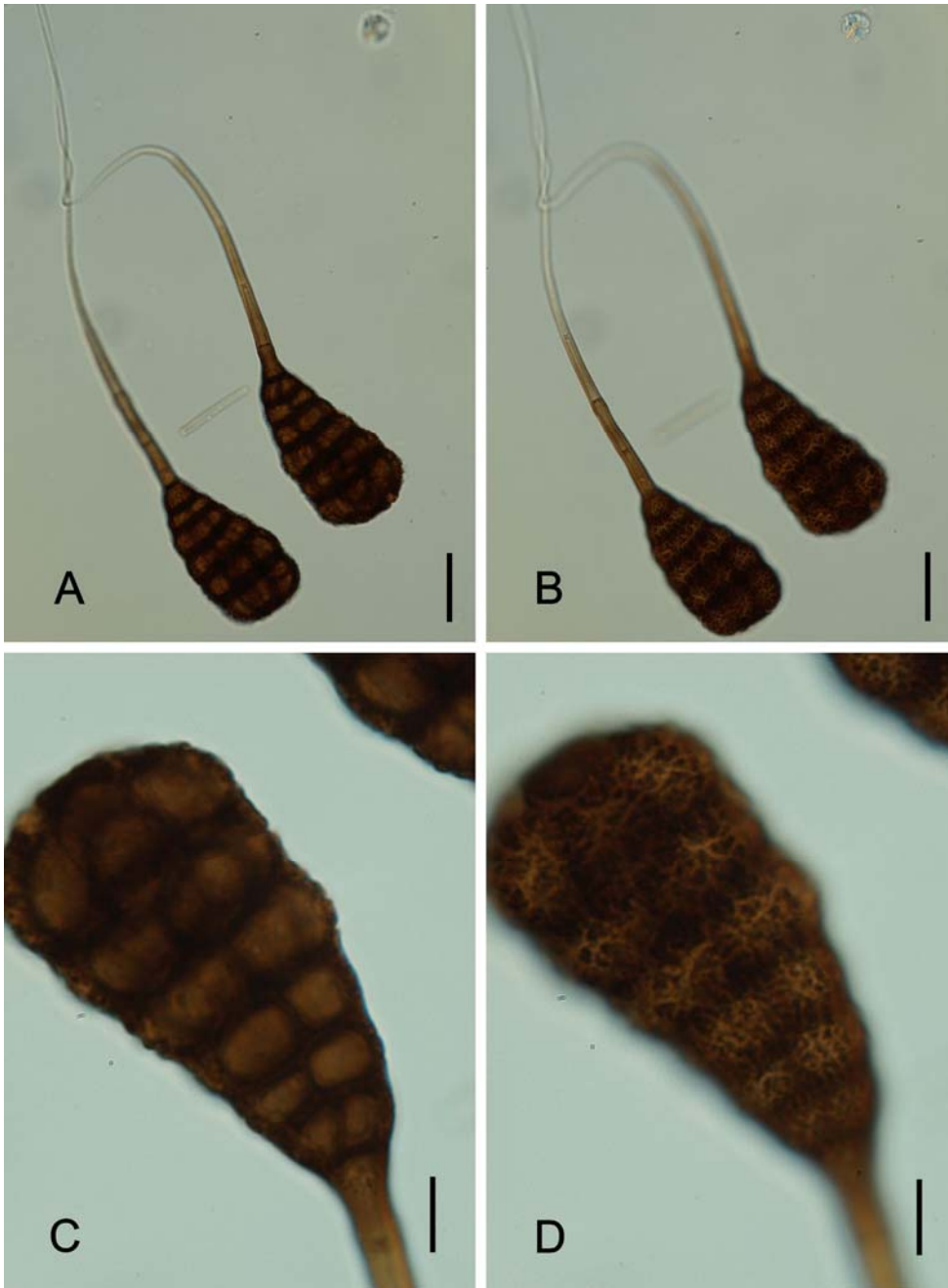


FIG. 14 *Piricauda paraguayensis* on *Dimorphandra wilsonii*. (A) Mature conidia with fully developed beak. (B) *Ibid* at different plane of focus. (C) Close-up of mature conidium. (D) *Ibid* at different plane of focus showing irregular rugose (seemingly reticulate pattern) on surface. Bars: 20 μm (A,B); 10 μm (C,D).

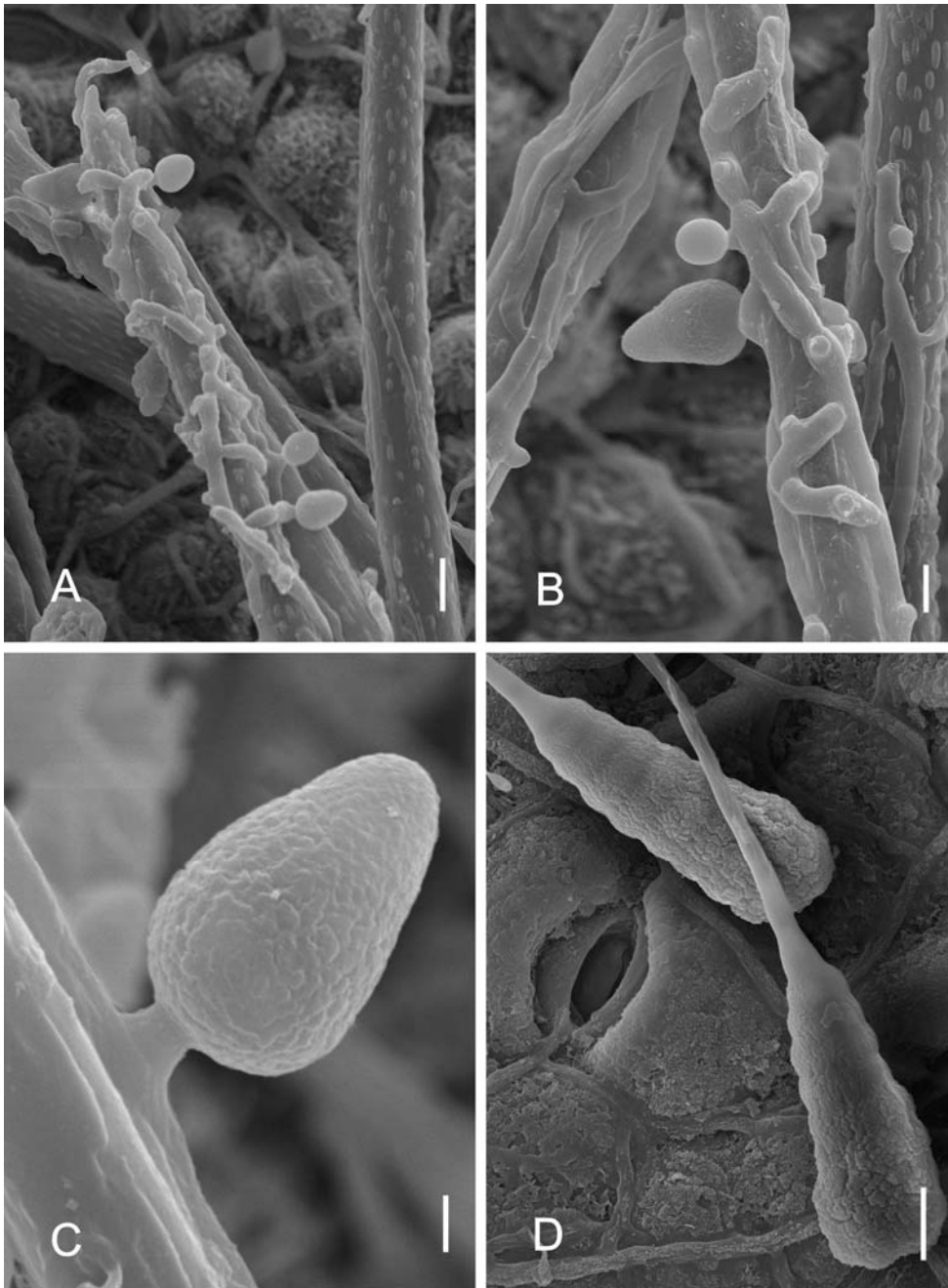


FIG. 15 SEM of *Piricauda paraguayensis* on *Dimorphandra* spp. (A) Colonies on trichome. (B) *Ibid* closer view with conidiogenous cell and conidia at early stages of development. (C) Close-up of immature conidia on conidiogenous cell. (D) Mature conidia on surface leaf (note smooth beaks and irregularly rugose bodies). Bars: 10 μm (A, D); 5 μm (B); 2 μm (C).

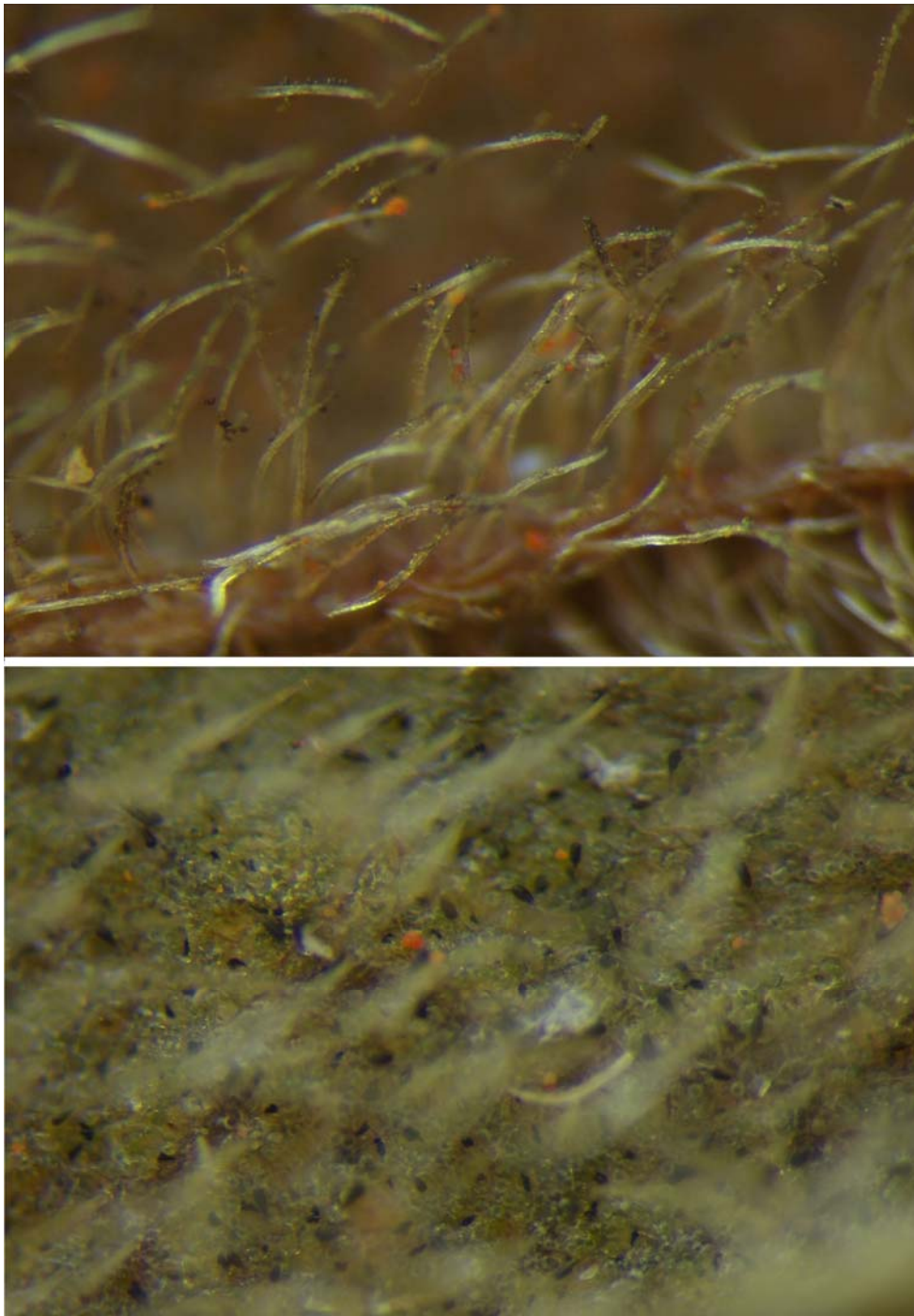


FIG. 16 *Piricauda paraguayensis* on *Dimorphandra* spp. (A) Brown colonies of conidiophore and immature conidia on trichomes. (B) Mature conidia fallen on foliar surface.

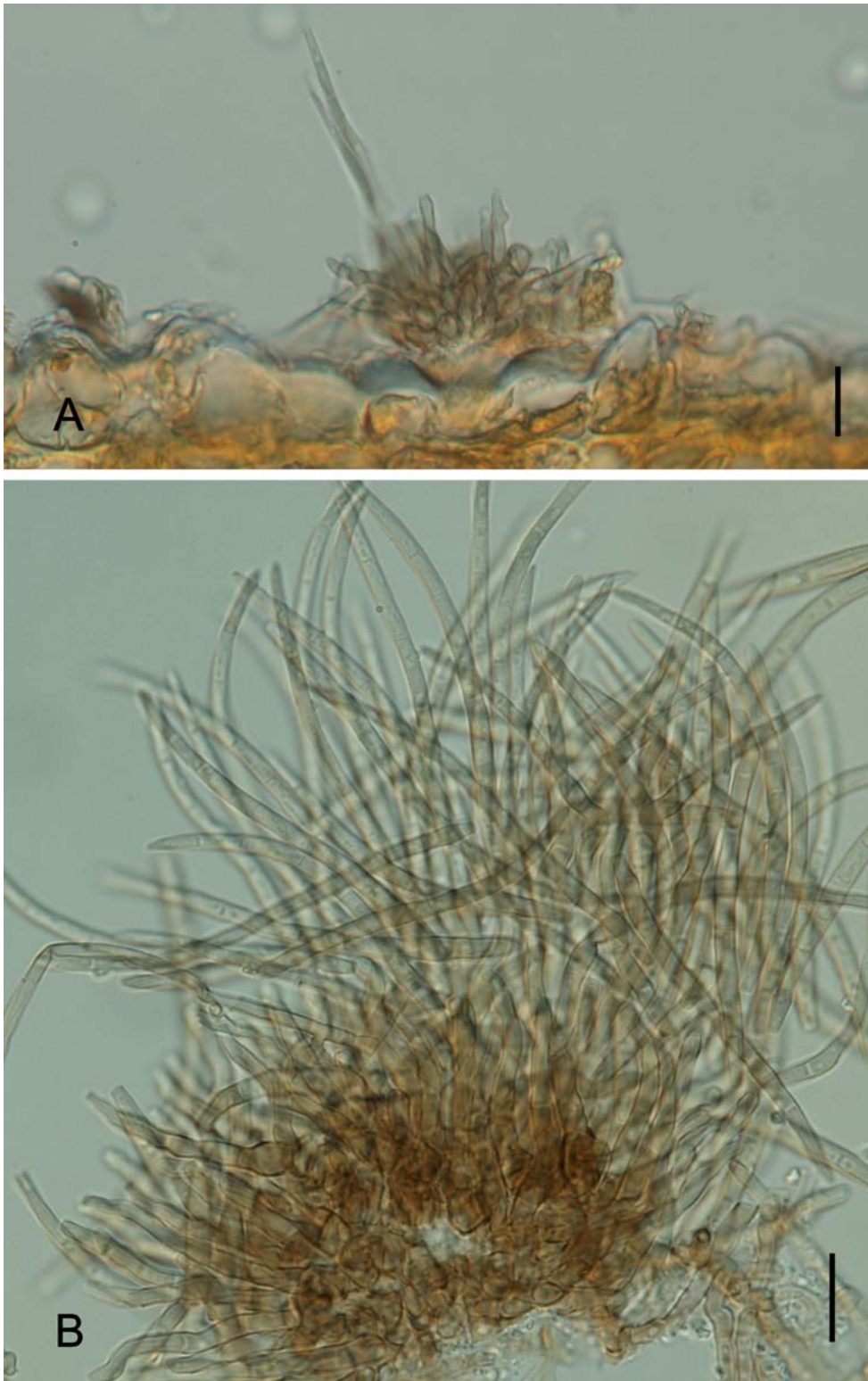


FIG. 17 *Pseudocercospora* on *Dimorphandra* spp. (A) Cross section of substomatal cavity from which conidiophores emerge. (B) Conidiophores in sporodochium and solitary, multiseptate, subhyaline to olivaceous conidia. Bars: 20 μ m.

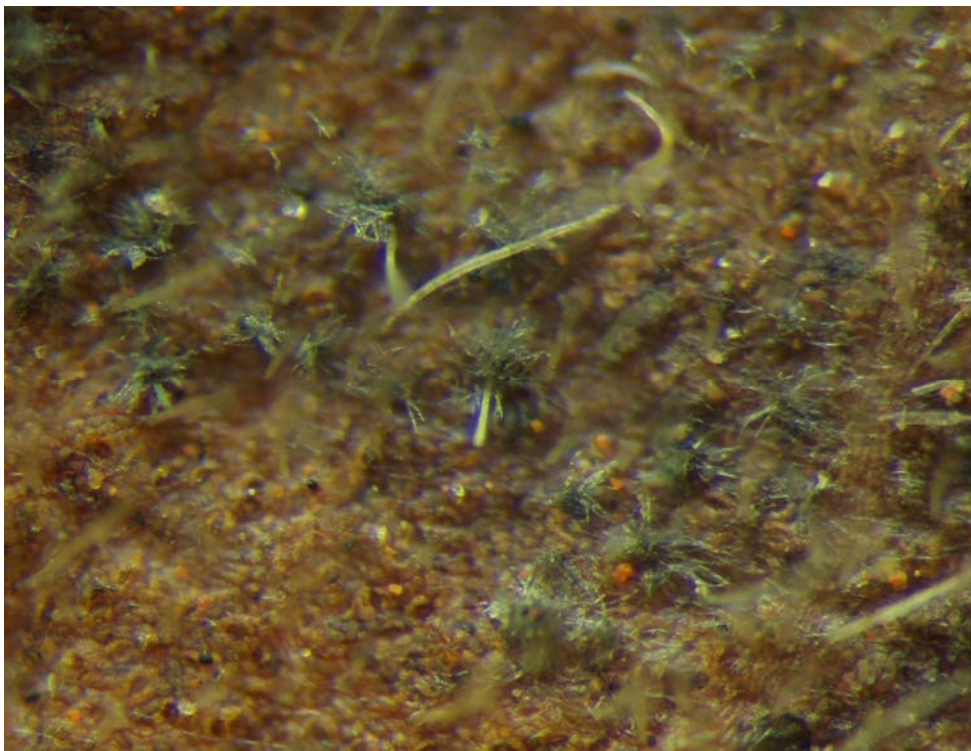


FIG. 18 *Pseudocercospora* on *Dimorphandra* spp. (A) Tufts of conidiophores and conidia on leaf surface.

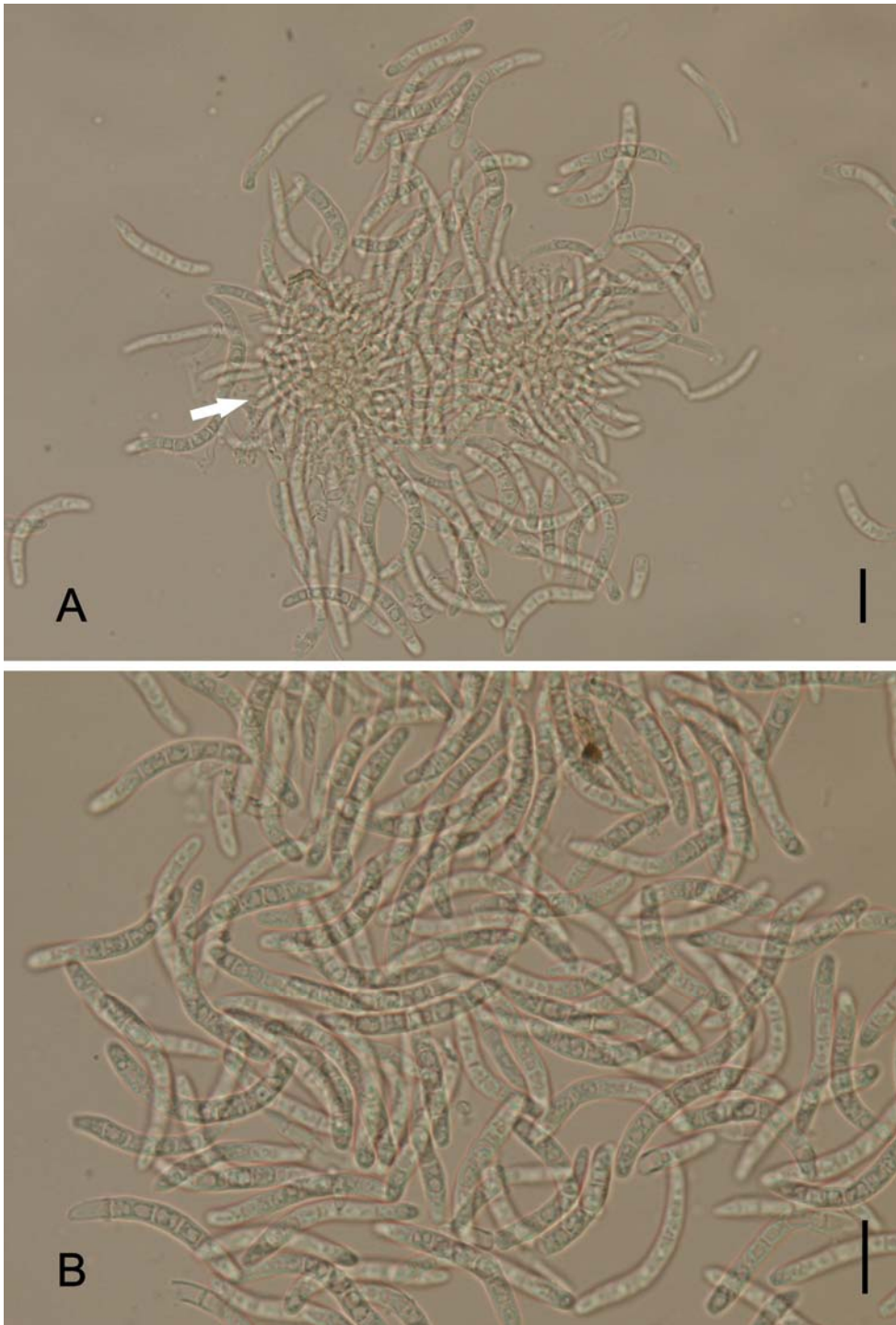


FIG. 19 *Pseudocercospora* on *Dimorphandra* spp. (A) Unbranched, hyaline conidiophores – arrowed and hyaline conidia. (B) Close-up of dry, cylindrical, slightly curved to curved conidia. Bars: 20 μ m.

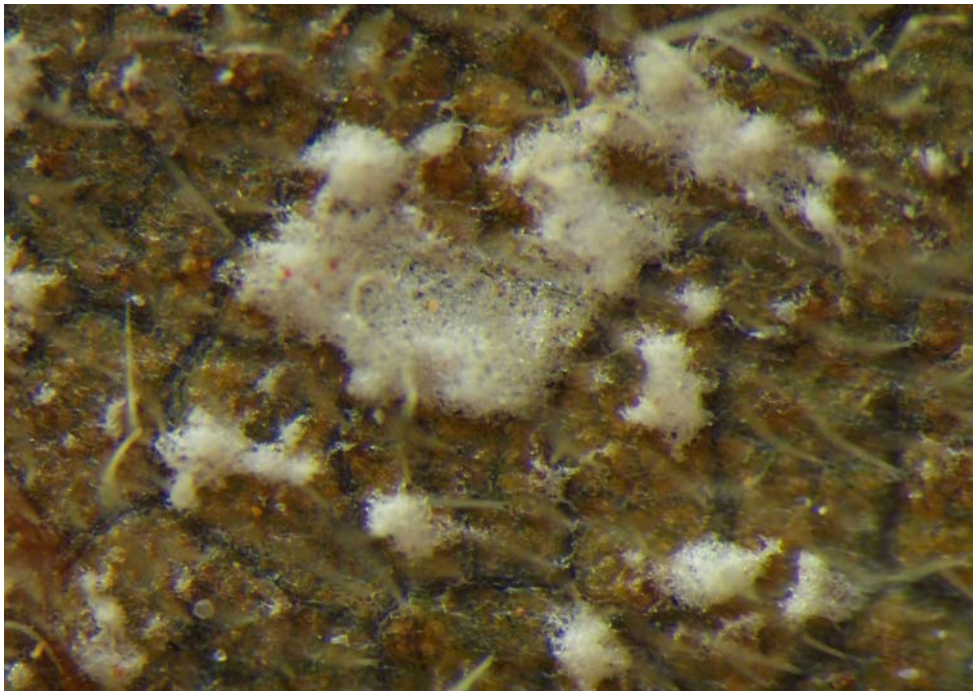


FIG. 20 *Pseudocercospora* on *Dimorphandra* spp. (A-B) Foamy colonies on leaf surface.



FIG. 21 Hyphomycete cf. *Radulidium* sp. on *Dimorphandra wilsonii*. (A) Dense colony on trichome. (B) Terminal conidiogenous cell bearing protuberant dark scars – arrowed. (C) Close-up of fusiform to cylindrical, verruculose, septate conidia. Bars: 20 μ m (A); 10 μ m (B, C).

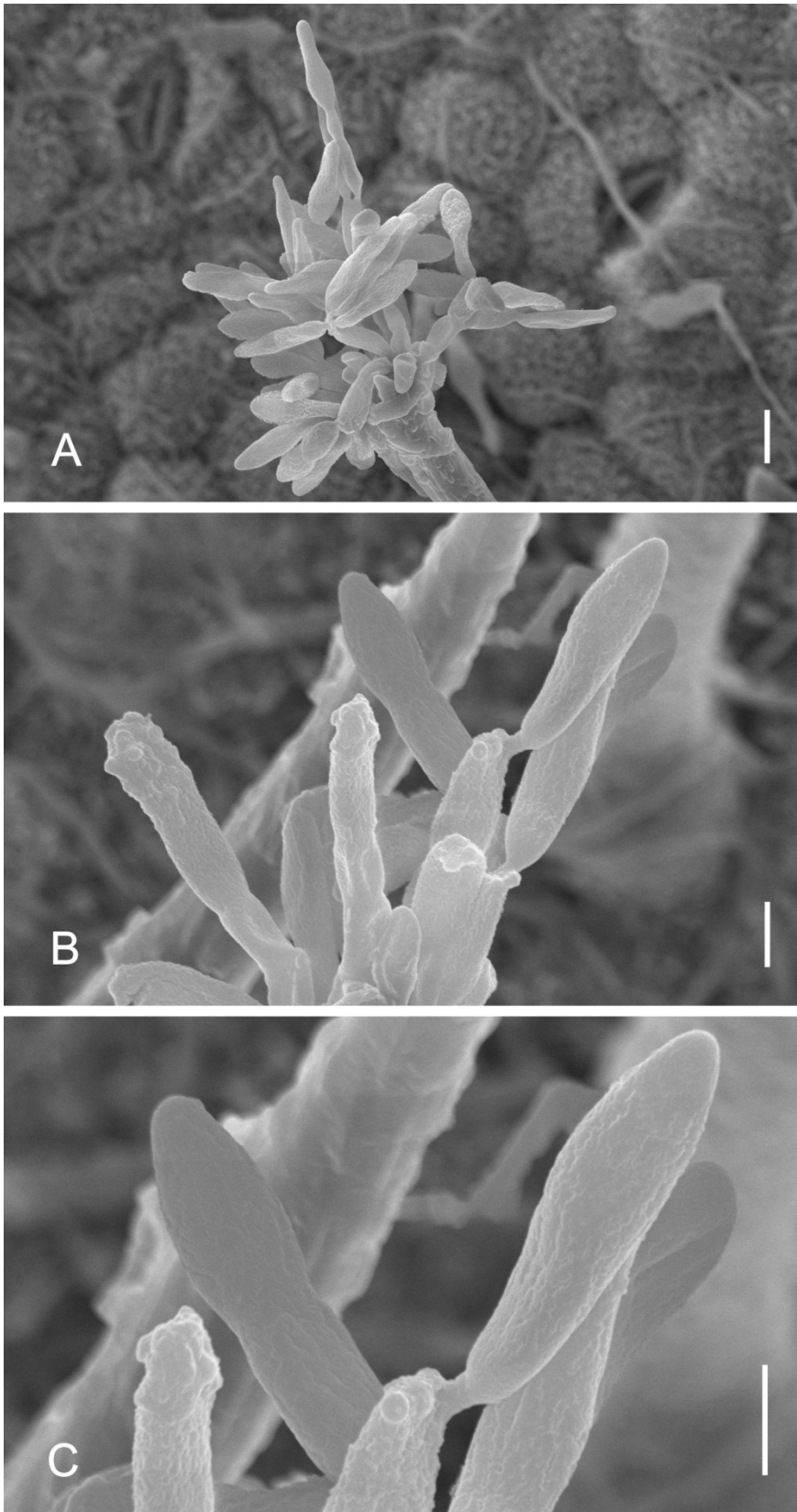


FIG. 22 SEM of Hyphomycete cf. *Radulidium* sp. on *Dimorphandra wilsonii* (A) Colony on trichome (B) Terminal polyblastic, conidiogenous cells. (C) Close-up of verruculose conidia attached to conidiogenous cell. Bars: 10 μ m (A); 5 μ m (B, C).



FIG. 23 Hyphomycete cf. *Radulidium* sp. on *Dimorphandra wilsonii*. (A) Colonies on the apex of trichomes.

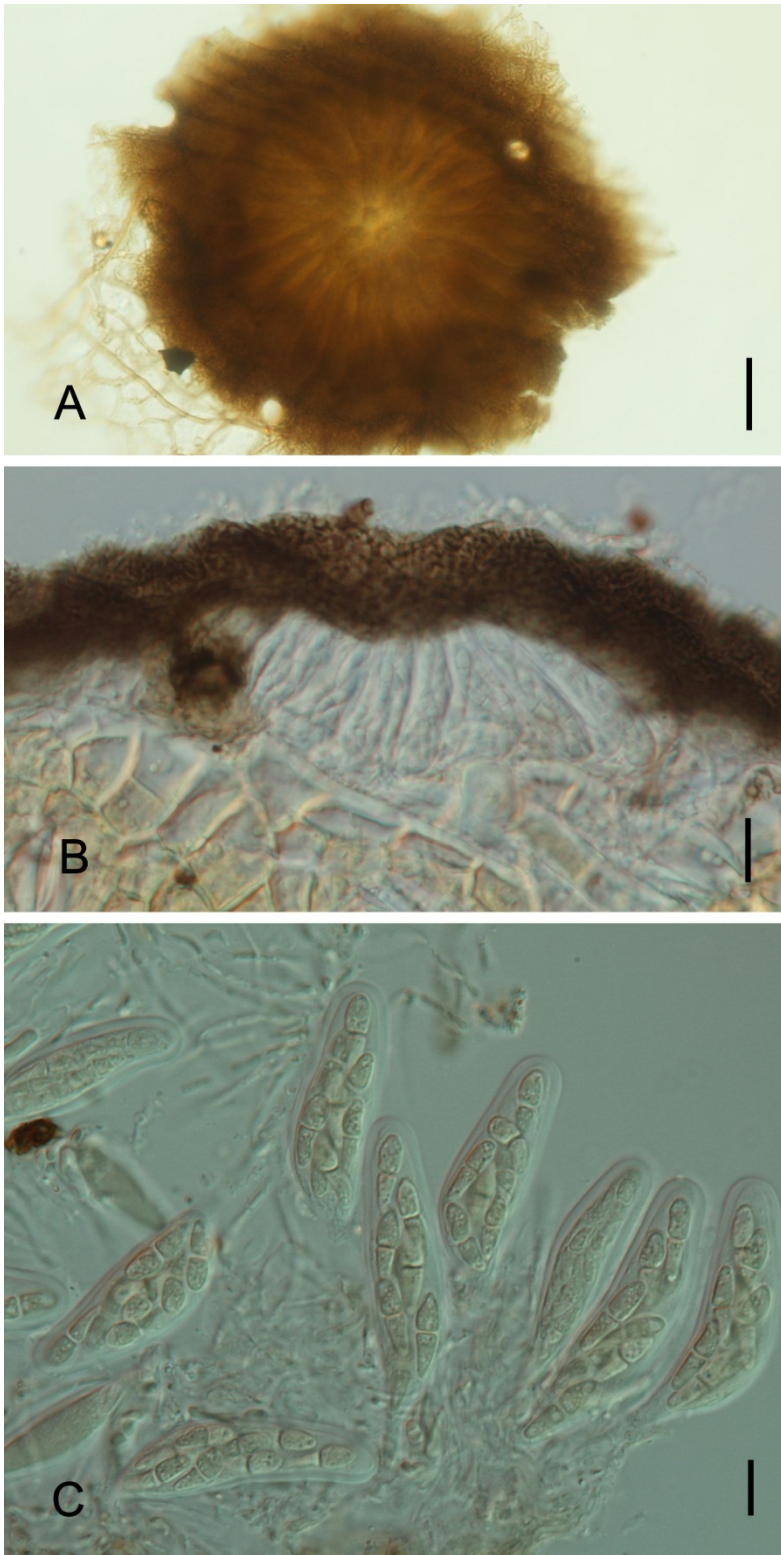


FIG. 24 *Stomiopeltis suttoniae* on *Dimorphandra wilsonii*. (A) Upper view of shield-like ascoma. (B) Cross section of an ascoma. (C) Bitunicate asci with 1-septate hyaline ascospores. Bars: 50 μm (A); 20 μm (B); 10 μm (D).



FIG. 25 *Trichomatomyces byrsonimae* on *Dimorphandra* spp. (A) Dense, dark brown to black colony spirally arranged on trichome apex. (B) Polyblastic, condigenous cell. (C) Conidia constricted at the septa at one side. Bars: 20 μm (A); 10 μm (B, C).

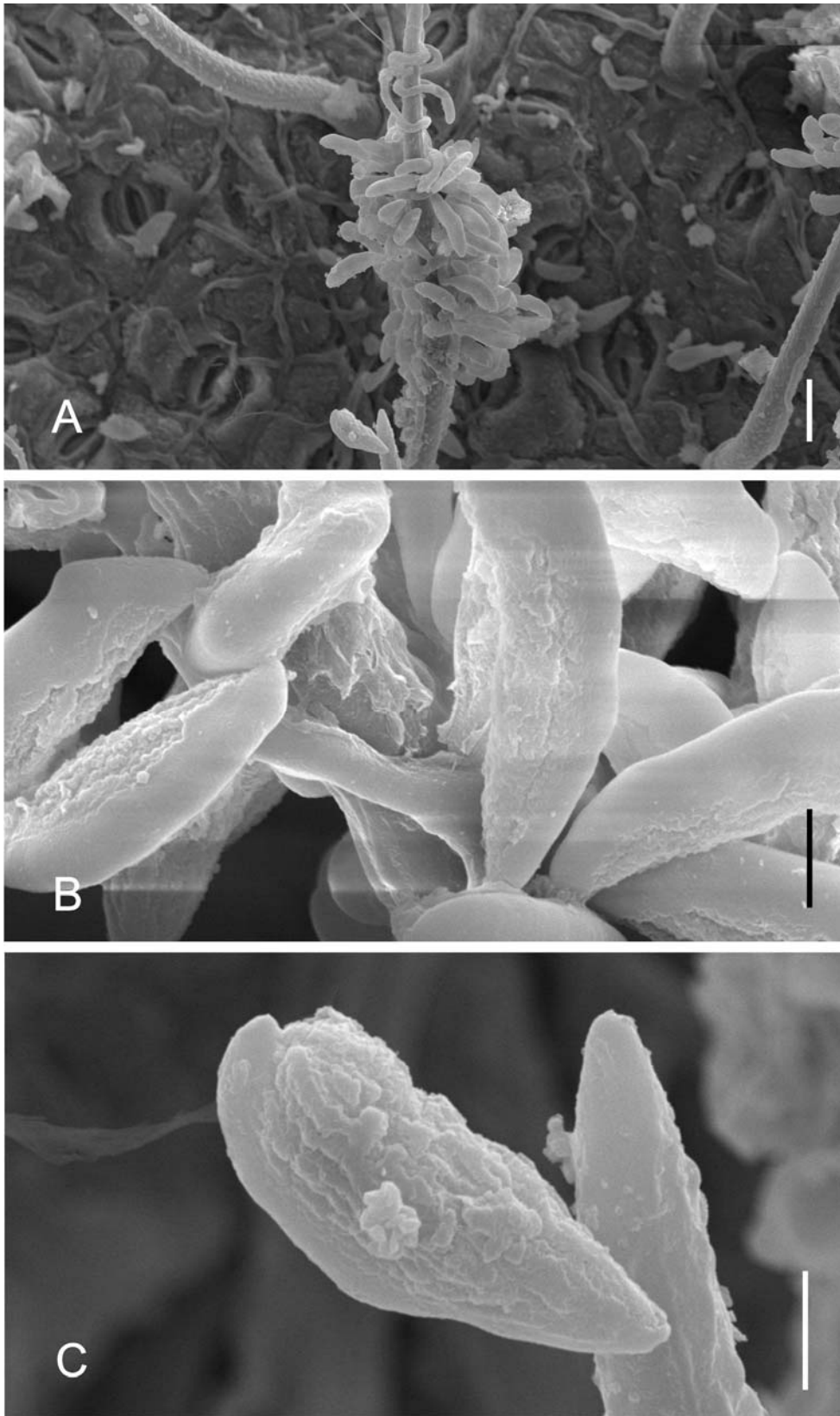


FIG. 26 SEM of *Trichomatomyces byrsonimae* on *Dimorphandra* spp. (A) Colony on trichome. (B) Conidia attached to conidiogenous cell. Note one side smooth and one side roughened. (C) Close-up of conidia. Bars: 20 μm (A); 5 μm (B, C).

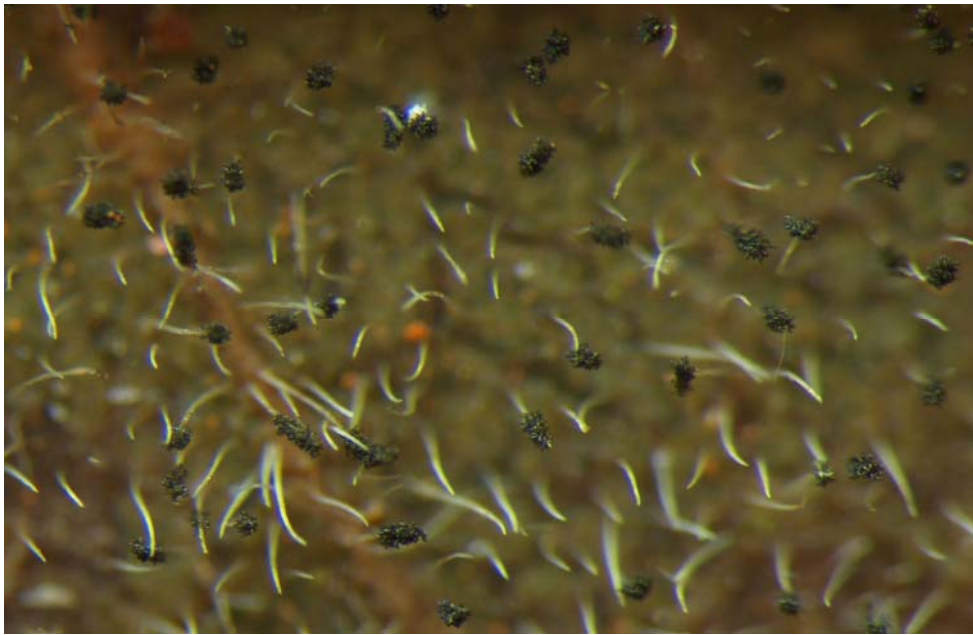


FIG. 27 *Trichomatomyces byrsonimae* on *Dimorphandra* spp. (A) Black colonies at apices of trichomes.

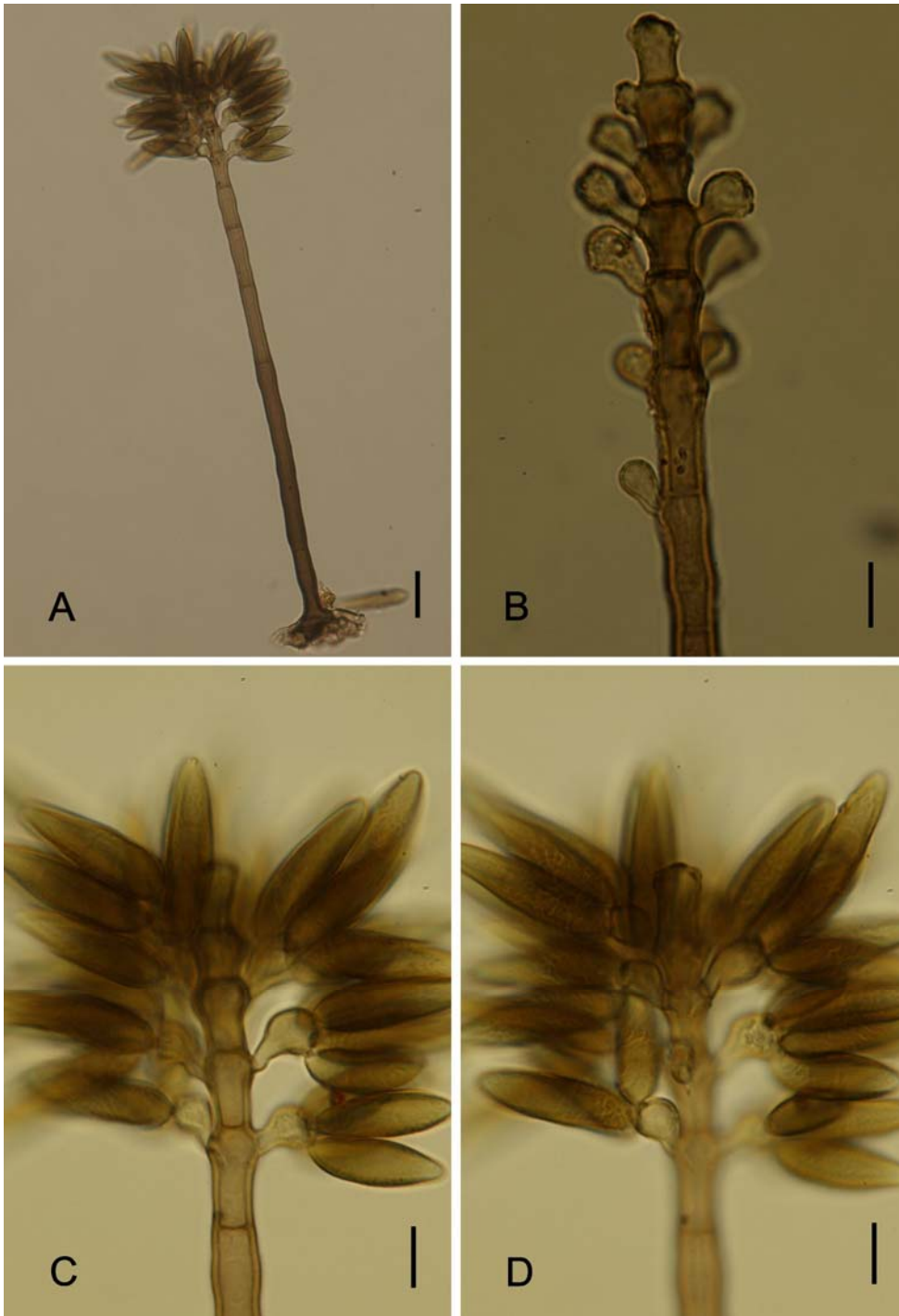


FIG. 28 *Vesiculohyphomyces cerradensis* on *Dimorphandra wilsonii*. (A) Macronematous, cylindrical, solitary conidiophore. (B) Whorls of polyblastic, vesicle-shaped, conidiogenous cells at apex of conidiophore. (C) Fertile conidiophores bearing numerous fusiform, conidia. (D) *Ibid* at different plane of focus showing wall sculpturing. Bars: 20 μm (A); 10 μm (B, C, D).

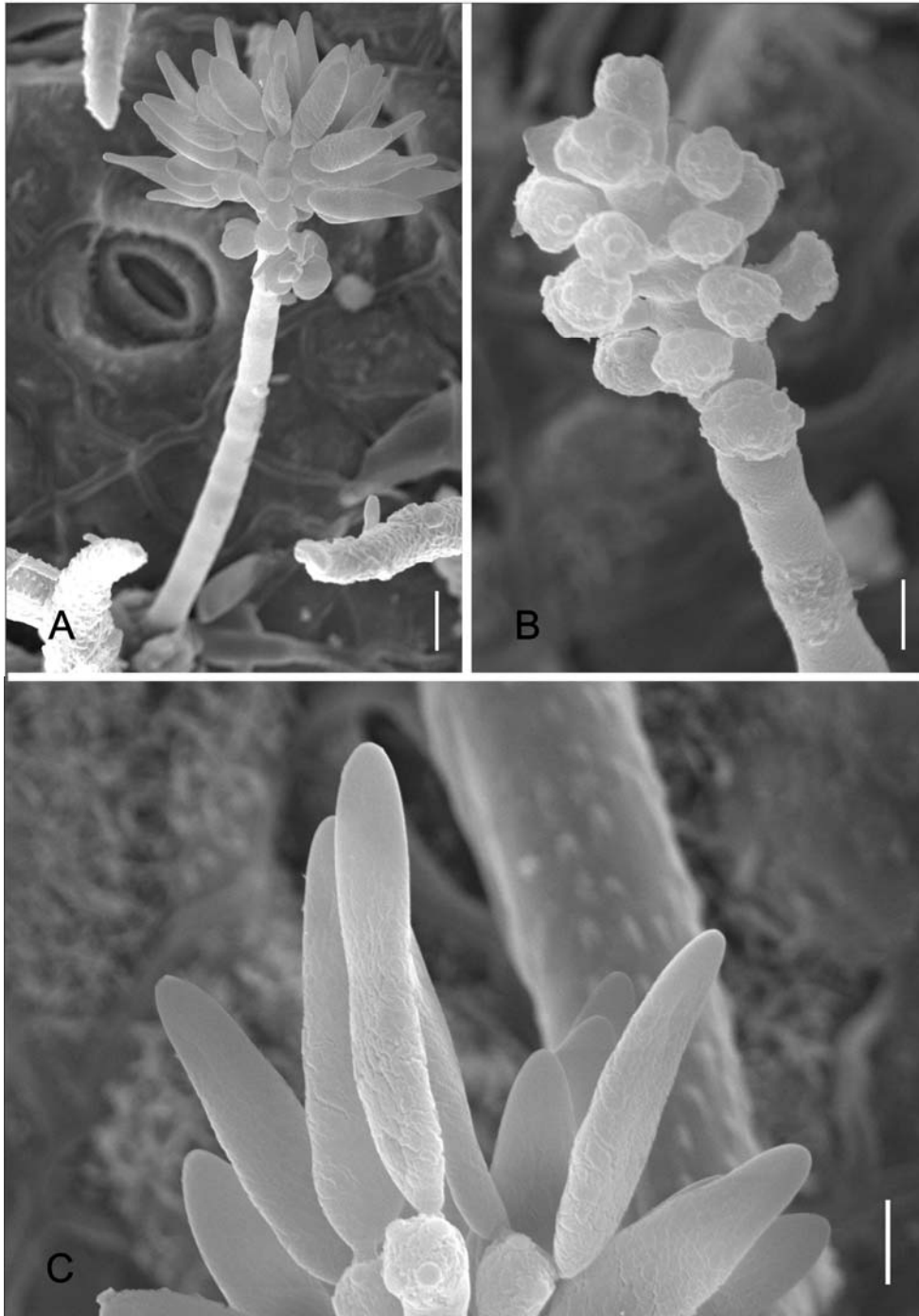


FIG. 29 SEM of *Vesiculohyphomyces cerradensis* on *Dimorphandra wilsonii*. (A) Macronematous conidiophore. (B) Close-up of conidiogenous cells at upper, fertile part of conidiophore. (C) Close-up of striate conidia. Bars: 10 μm (A); 5 μm (B, C).

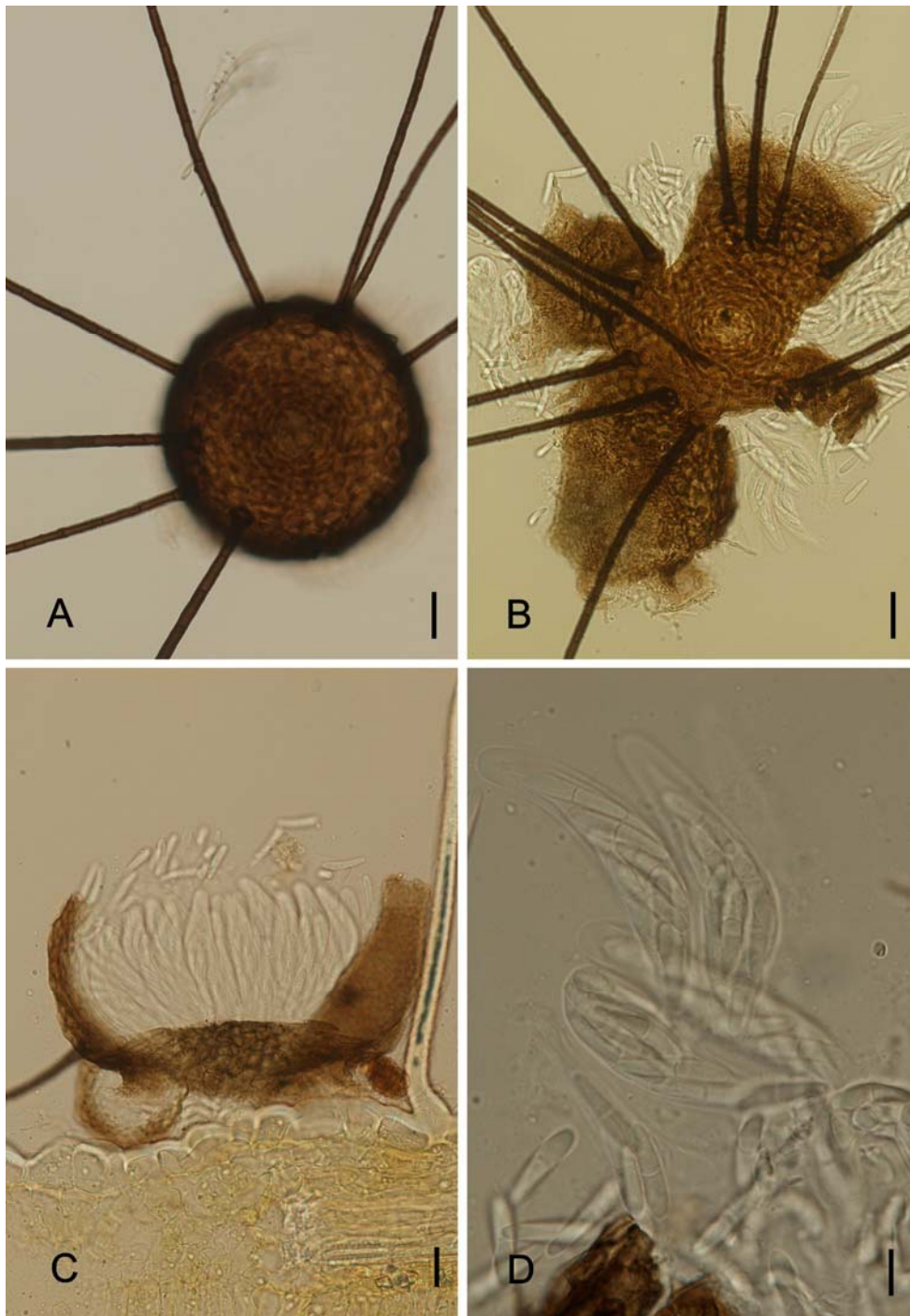


FIG. 30 *Microcalliopsis dipterygis* on *Dimorphandra wilsonii*. (A) Upper view of pseudothecial ascoma bearing needle-shaped setae. (B) Squashed ascoma releasing asci and ascospores. (C) Cross section of pseudothecium showing parallel asci. (D) Bitunicate asci with 1-septate hyaline ascospores. Bars: 20 μm (A, B, C); 10 μm (D).

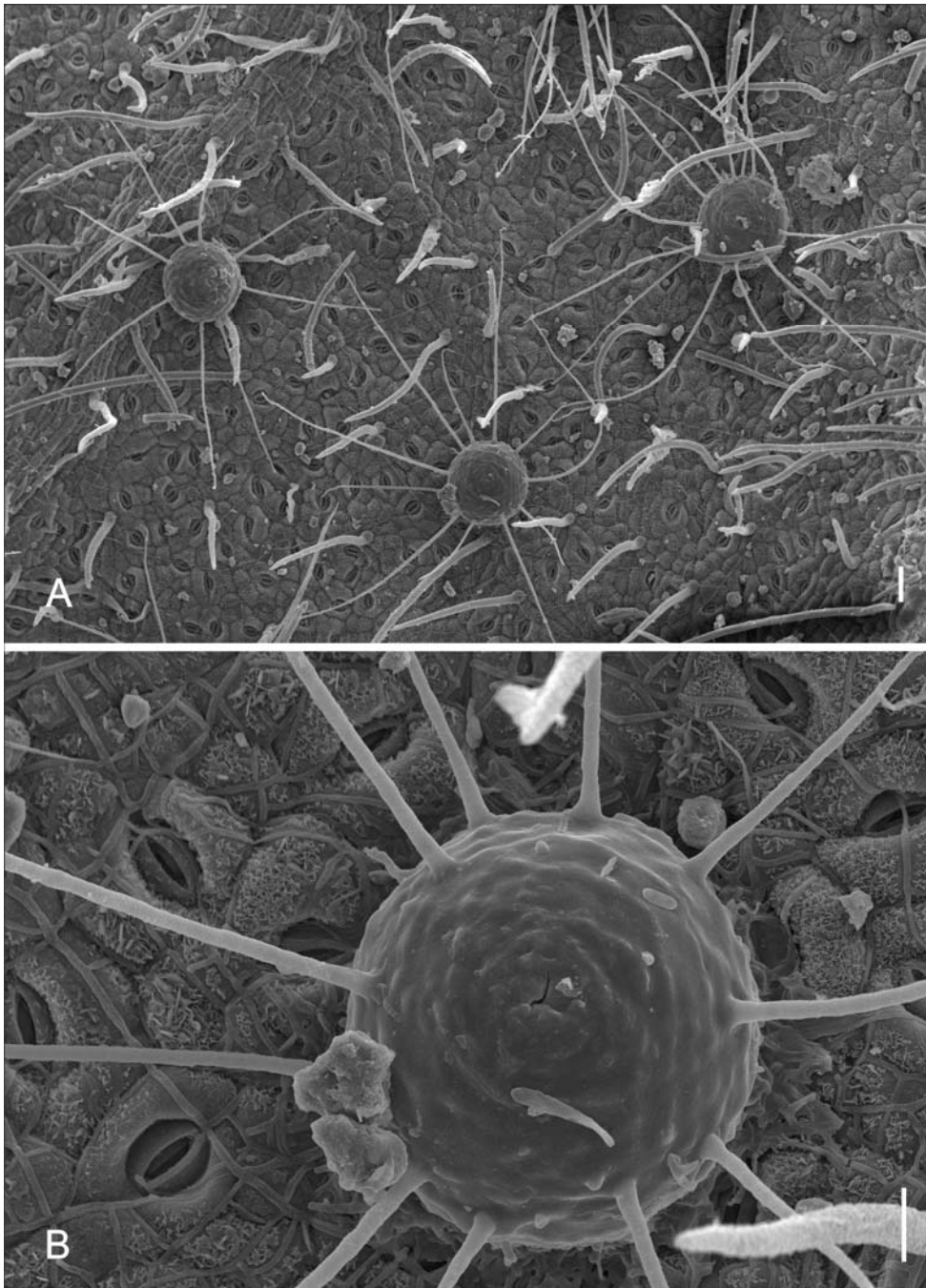


FIG. 31 SEM of *Microcalliopsis dipterygis* on *Dimorphandra wilsonii*. (A) Ascomata formed on leaf surface of *D. wilsonii*. Note sputnik-like shape of pseudothecia (B) Detail of inconspicuous ostiole, protuberances on external wall and setae on individual ascoma. Bars: 50 μm (A); 20 μm (B).



FIG. 32 *Microcalliopsis dipterygis* on *Dimorphandra wilsonii*. Black pseudothecia on leaf surface.

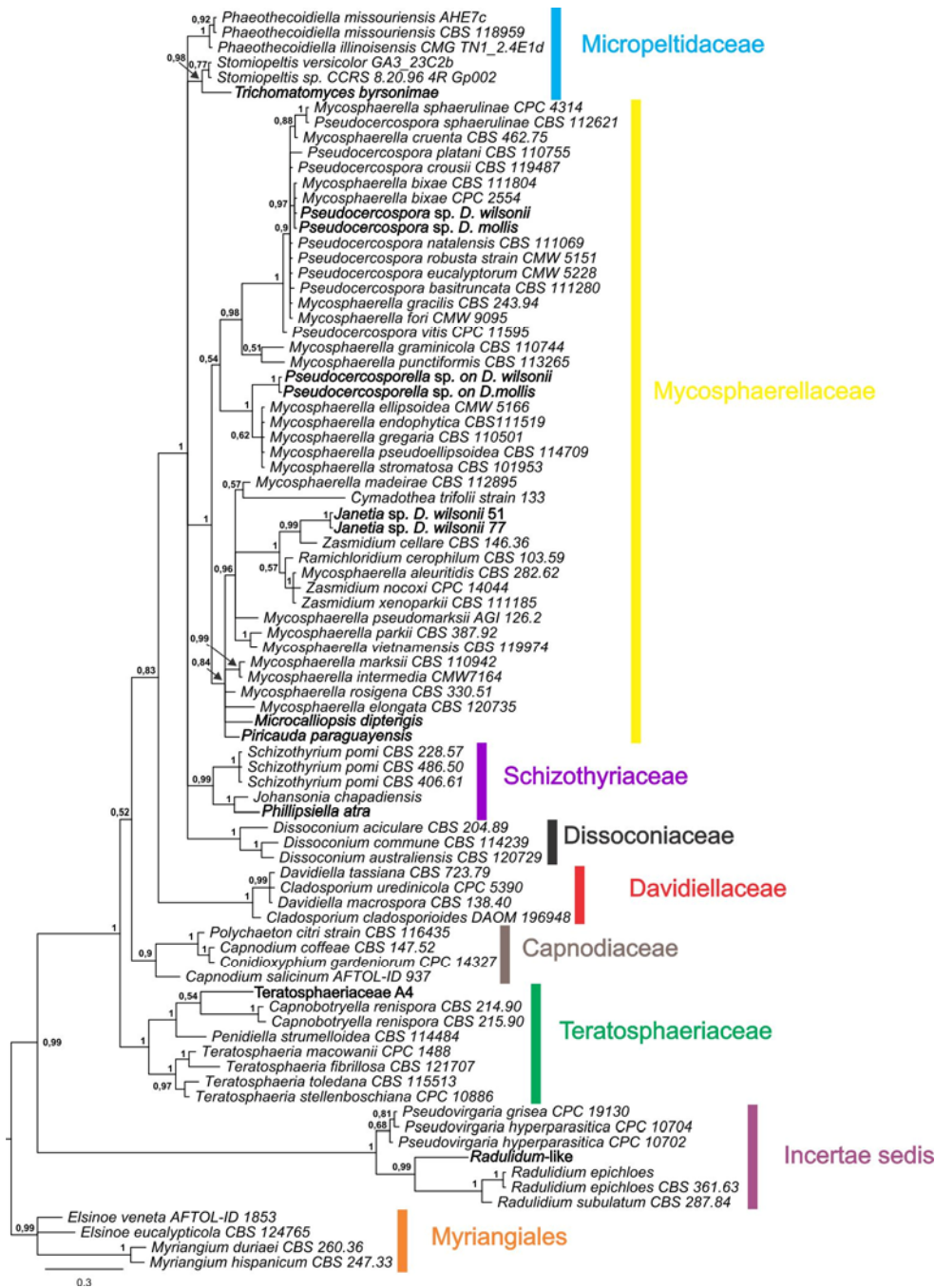


FIG. 33 Hypothesis about the phylogenetic placement of the fungi collected on *Dimorphandra wilsonii* and *Dimorphandra mollis* in connection with related fungi derived from Bayesian analysis of partial nuclear large subunit ribosomal DNA gene sequences. Bayesian posterior probabilities are indicated at the nodes. The tree was rooted with members of the Myriangiales. Isolates with newly obtained sequences for this study are given in bold.

ARTIGO 2

Mycotaxon (In Press)

***Alveariospora*, a new anamorphic genus from trichomes of *Dimorphandra mollis* in Brazil**

***Alveariospora*, a new anamorphic genus from trichomes of *Dimorphandra mollis* in Brazil**

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ABSTRACT — A dematiaceous fungus was found associated with trichomes on leaflets of *Dimorphandra mollis* during a survey of the fungi associated with the genus *Dimorphandra* (*Fabaceae*). The host plant is a tree, endemic to the Brazilian cerrado. Although widely distributed there, this plant has been neglected in mycological studies, since no fungus has ever been recorded in association with it. The new fungus produces large alveariform (skep or beehive-shaped), muriform, dictyosporous, distoseptate, verruculose conidia from large, thickened, dark washer-like conidiogenous loci. Among the genera of anamorphic fungi hitherto described, this fungus is somewhat similar to *Annellophragmia*, *Annellosympodia*, *Briansuttonia*, *Dictyospiropes*, and *Veracruzomyces* as well as *Spiropes dictyosporus*, but has some significant differences from those taxa. Hence, the new genus, *Alveariospora* (type species: *A. distoseptata* sp. nov.) is proposed for this fungus.

KEY WORDS — fungal survey, taxonomy, tropical fungi

Introduction

Dimorphandra mollis Benth. (*Fabaceae*: faveira, faveiro-do-cerrado, falso barbatimão) is a plant native to the cerrado. This species is adapted to conditions of low rainfall, developing foliage, flowering and fruiting in the wet season and losing its leaves in the dry season (Lorenzi 2002). The fruits are utilized for extraction of rutin and other glycoside flavonoids, used in the pharmaceutical industry (Ferreira et al. 2001; Lorenzi 2002). It is also regarded as having potential for exploitation as a source of gum for the food industry (Panegassi et al. 2000). Although *D. mollis* is a common component of the cerrado flora, an ecosystem that has been explored by Brazilian mycologists more intensively in recent decades (e.g.: Rezende & Dianese 2003; Dornelo-Silva & Dianese 2004; Hernández-Gutiérrez & Dianese 2009; Pereira-Carvalho et al. 2010), no attention was paid to the mycobiota of this host plant and no fungus have ever been recorded on *D. mollis*. In July 2009, a pioneering exploratory survey of the mycodiversity of *Dimorphandra* spp. was initiated. The first brief survey trip to the municipalities of Paraopeba and Caetanópolis (state of Minas Gerais, Brazil) has already yielded several fungi of interest. One interesting fungus was found colonizing trichomes of *D. mollis*. This fungus is described and discussed herein.

Material & methods

Samples of *D. mollis* foliage were collected, dried in a plant press and taken to the lab. After examination, selected leaves bearing fungus colonies were deposited in the local herbarium (Herbarium VIC). Isolations were attempted by direct transfer of spores onto plates containing VBA medium (Pereira et al. 2003) with the help of a fine needle. Fungal structures were removed from fresh leaves and mounted in lactophenol. Observations, measurements and illustrations were carried out with an OLYMPUS BX 51 light microscope fitted with a digital camera (EVOLT E330) and a drawing tube. Wherever possible, 30 measurements of each structure were taken.

Taxonomy

Alveariospora Meir. Silva, R.F. Castañeda, O.L. Pereira & R.W. Barreto,

anam. gen. nov.

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Fungus anamorphicus. Coloniae in substrato naturali effusae, pilosae, brunneae, olivaceae vel nigrae. Mycelium partim superficiale et partim in substrato immersum. Conidiophora macronematosa, mononematosa, simplicia, septata, brunnea vel olivacea, laevia. Cellulae conidiogenae integratae, terminales, primo monoblasticae, deinde polyblasticae, indeterminatae, cum cellulis conidiogenis rectis, successive sympodialiter sed rectilineare proliferantibus, cicatricibus primo apicalibus, deinde lateralibus, discoidibus, protuberantibus, nigris, incrassatis. Secessio conidiorum schizolytica. Conidia solitaria, alveariformia (ellipsoidea, ovalia usque ad navicularia, muriformia), distoseptata, verruculosa vel laevia, brunnea vel atrobrunnea, ad basim conspicue cicatricata et ad apicem cum appendicibus cellularibus, cylindricis sivel subulatis, brunneis praedita.

TYPE SPECIES: *Alveariospora distoseptata* Meir. Silva et al.

ETYMOLOGY: Latin, *alvearium*, meaning beehive, skep; and *spora*, meaning spores.

Anamorphic fungi. Colonies on the natural substrate effuse, hairy, brown, olivaceous or black. Mycelium partly superficial and immersed. Conidiophores distinct, single, unbranched, septate, brown or olivaceous, smooth. Conidiogenous cells integrated, terminal, at first with a single terminal conidiogenous locus, then indeterminate, polyblastic, with successive sympodial but rectilinear proliferation, rupturing the outer wall around each scar, resulting in a lateral displacement of scars, leaving conspicuous circumferential annular fringes of the torn wall, . Conidiogenous loci evident, lenticular, protuberant, thickened and black, conidial secession schizolytic. Conidia solitary, ellipsoidal, oval to broadly navicular, dictyoseptate, distoseptate, verruculose or smooth, brown or dark brown, conspicuously cicatrized at the base, with a cellular, cylindrical or subulate, brown apical appendage.

Alveariospora distoseptata Meir. Silva, R.F. Castañeda, O.L. Pereira & R.W. Barreto, *anam. sp. nov.* PLATES 1–4

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Fungus anamorphicus. Coloniae in substrato naturali effusae, pilosae, brunneae. Mycelium partim superficiale et partim in substrato immersum, ex hyphis septatis, ramosis, laevibus, brunneis, compositum. Conidiophora macronematosa, mononematosa, cylindracea, simplicia, erecta, recta, 3–6-septata, laevia, atrobrunnea sed pallide brunneae prope apicem, 120–160 × 7–10 µm. Cellulae conidiogenae integratae, terminales, 22–45 × 6–10 µm, primo monoblasticae, deinde polyblasticae, indeterminatae, cum cellulis conidiogenis rectis, successive sympodialiter sed rectilineare proliferantibus, cicatricibus primo apicalibus, deinde lateralibus, discoidibus, protuberantibus, nigris, incrassatis, 6–8 × 2–4 µm, 3–7 per conidiophorum., Secessio conidiorum schizolytica. Conidia solitaria, ellipsoidea vel late navicularia, dictyoseptata, distoseptata, verruculosa, brunnea, ad basim conspicue cicatricata, nigra, 62–85 × 26–45 µm et cum appendicibus cellularibus, cylindricis, vel usque leviter subulatis, laevibus, apice rotundato, brunneis, sed apicem versus pallidioribus vel hyalinis, 27–42 × 2.5–4.5 µm, interdum ad apicem cum velamento mucoso. Teleomorphosis ignota.

TYPE: BRAZIL, Minas Gerais, Paraopeba, Floresta Nacional de Paraopeba, on trichomes of *Dimorphandra mollis*, 10 Jul. 2009, M. Silva & O.L. Pereira (**Holotype**, VIC 31399).

ETYMOLOGY: Latin, *distoseptata*, meaning distoseptate, having the individual cells each surrounded by a sac-like wall distinct from the outer wall.

Anamorphic fungus. Colonies on the natural substrate effuse, hairy, brown. Mycelium partly superficial and partly immersed, composed of septate, branched, smooth, brown hyphae. Conidiophores distinct, single, unbranched, erect, straight, 3–6-septate, dark brown, pale brown near the apex, smooth, 120–160 × 7–10 µm. Conidiogenous cells integrated, terminal, 22–45 × 6–10 µm, at first with a single terminal conidiogenous locus, then indeterminate, polyblastic, with successive sympodial but rectilinear proliferation, rupturing the outer wall around each scar, resulting in a lateral displacement of scars, leaving conspicuous circumferential annular fringes of the torn wall. Conidiogenous loci evident, lenticular, black, 6–8 µm diam., 2–4 µm thick, 3–7 per conidiophore. Conidial secession schizolytic. Conidia solitary, ellipsoidal to broadly navicular, dictyoseptate, distoseptate, verruculose, brown, 62–85 × 26–45 µm, with a conspicuous protuberant, melanized, lenticular hilum at the base, 7–8 µm diam., 3–4 µm thick, and with a cellular, cylindrical or slightly subulate, smooth terminal appendages, 27–42 × 2.5–4.5 µm, brown and pale brown to subhyaline at the end, sometimes surrounded by a hyaline, mucilaginous sheath. Teleomorph unknown. In culture, no conidial germination was observed, even after a period of four weeks.

COMMENTS — The pattern of ontogeny in *Alveariospora* can be classified as conidial development type 17 (Kirk et al. 2008) (holoblastic, delimitation by 1 septum, schizolytic secession, maturation by diffuse wall-building, percurrent enteroblastic conidiogenous cell extension, followed by conidial ontogeny by replacement apical wall-building; strongly melanized, each successive conidium seceding before the next percurrent elongation of the conidiogenous cell) but sometimes also holoblastic, sympodial proliferation occurring and two or more conidia are produced, a pattern of ontogeny classified as conidial development type 10 (holoblastic, regularly alternating with sympodial proliferation, maturation by diffuse wall-building and secession schizolytic). The mode of rectilinear proliferation and the combination of conspicuous annular structures and thickened, darkened conidiogenous loci in *Alveariospora distoseptata* is very unusual and only comparable with *Annellophragmia* Subram. (Ellis 1971), *Annellosympodia* McTaggart et al. (McTaggart et al. 2007) and *Spiropes dictyosporus* Seifert & S. Hughes (Seifert & Hughes 2000). However, the latter species, inhabiting a sooty mould in New Zealand, is characterized by having synnematosous conidiomata and euseptate conidia. *Annellophragmia* differs in having synnematosous conidiomata and phragmosporous (but also distoseptate) conidia. *Annellosympodia* is characterized by its sporodochial conidiomata with ampulliform, doliiform to obovoid conidiogenous cells, 0–1-euseptate conidia and rhexolytic conidial secession. Among other

genera of anamorphic fungi hitherto described, *Alveariospora* appears superficially similar to, *Briansuttonia* R.F. Castañeda et al. (Castañeda et al. 2004), *Dictyospiropes* M.B. Ellis (Ellis 1976) and *Veracruzomyces* Mercado et al. (Mercado-Sierra et al. 2002). *Briansuttonia* is characterized by monotretic, terminal, determinate or indeterminate with enteroblastic percurrent conidiogenous cells. *Dictyospiropes* is quite distinct from *Alveariospora* by having polyblastic, sympodial conidiogenous cells with strongly melanized, lenticular conidiogenous loci, lacking percurrent extensions, and the conidia are euseptate-dictyoseptate without strongly thickened and melanized basal hilum. The genus *Veracruzomyces* is clearly distinguished from *Alveariospora* by its monoblastic, percurrently proliferating conidiogenous cells without cicatrized conidiogenous loci.

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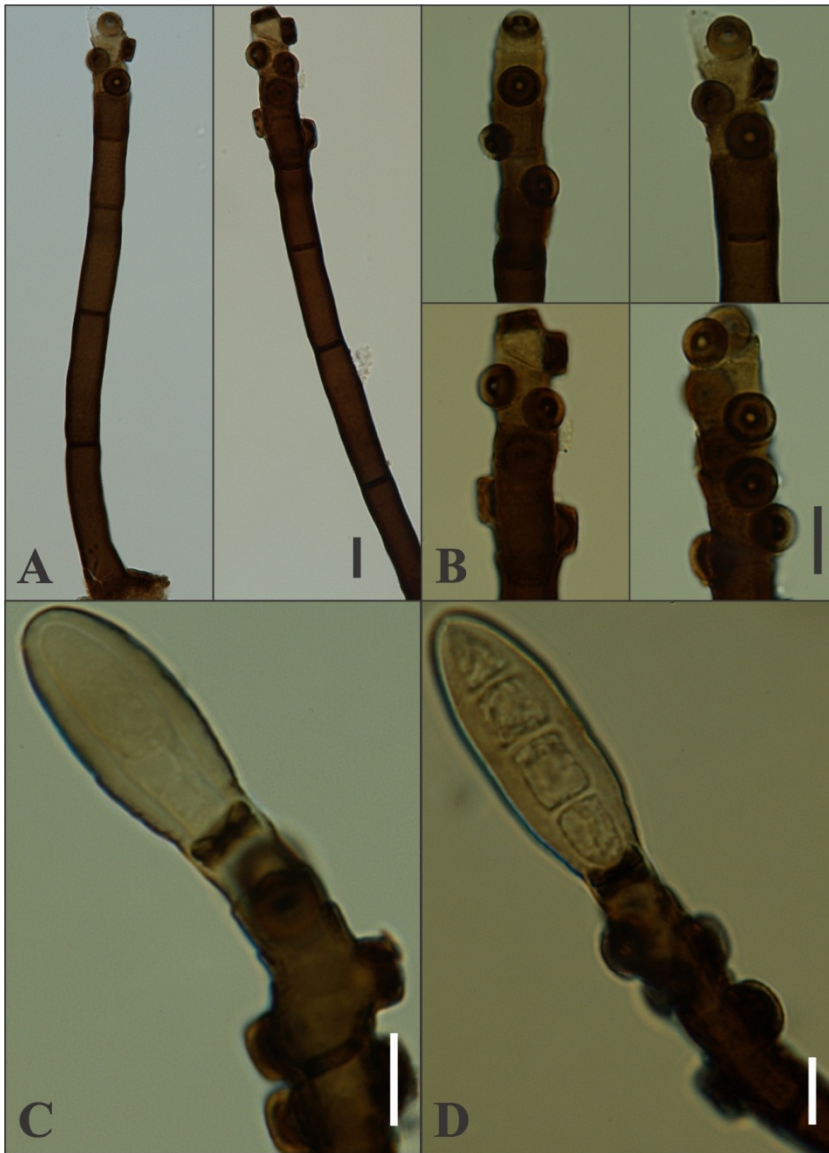


PLATE 1. *Alveariospora distoseptata*. Macronematous conidiophores with polyblastic conidiogenous cells (A); detail of protuberant lenticular loci on enteroblastic percurrent proliferations (B); initial conidial formation (C) and initial conidial septation (D). Bars: 10 μ m.

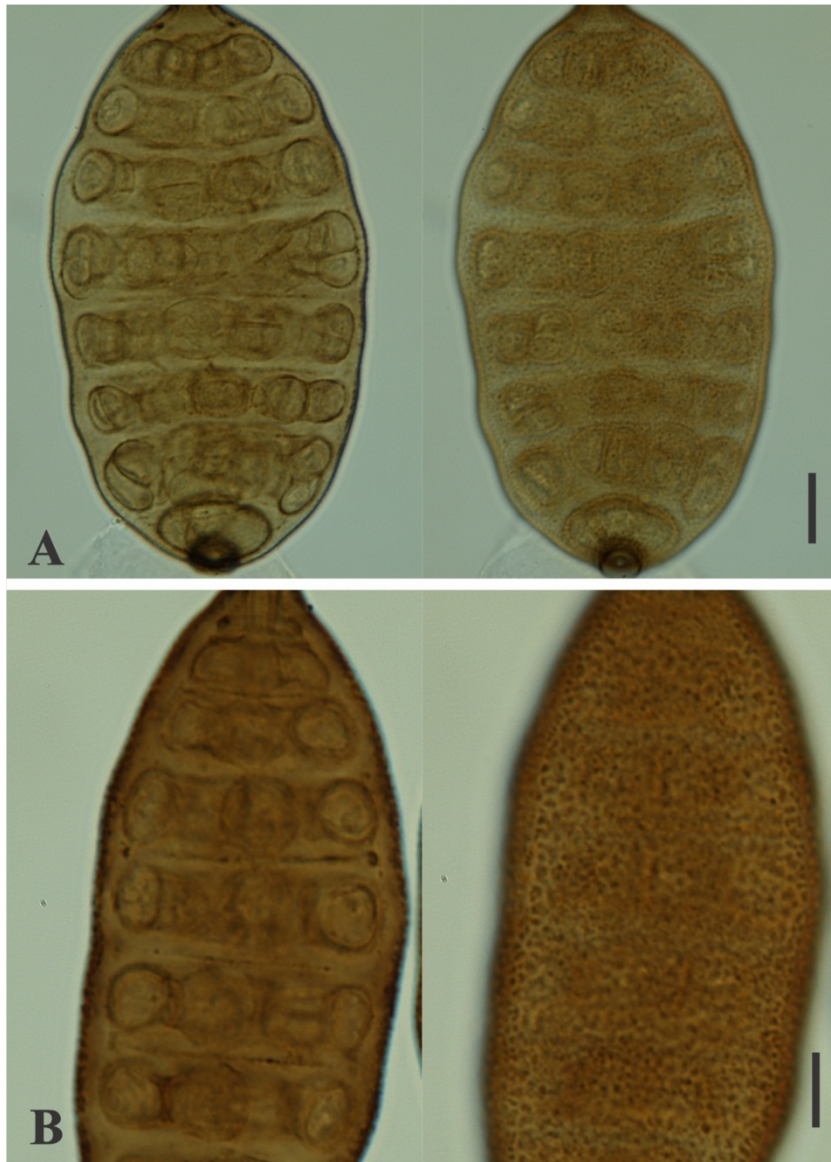


PLATE 2. *Alveariospora distoseptata*. Detail view of muriform, distoseptate conidia (left) with verruculose wall (right) on young yellowish conidia (A) and reddish-brown mature conidia (B). Bars: 10 μ m.

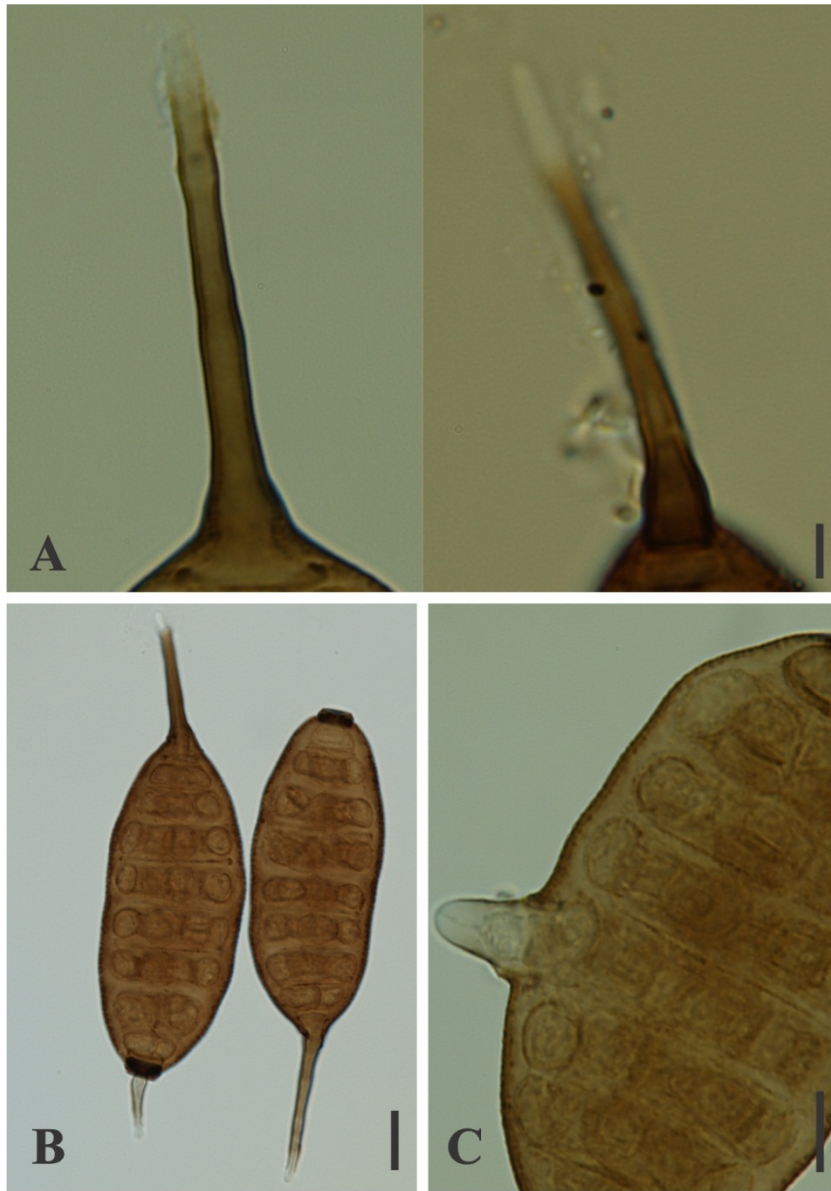


PLATE 3. *Alveariospora distoseptata*. Conidial appendage ends, sometimes surrounded by a hyaline, mucilaginous tunica (A); germination of the conidia, through hilum (B) and laterally through wall (C). Bars: A = 5 μ m; B = 20 μ m; C = 10 μ m.

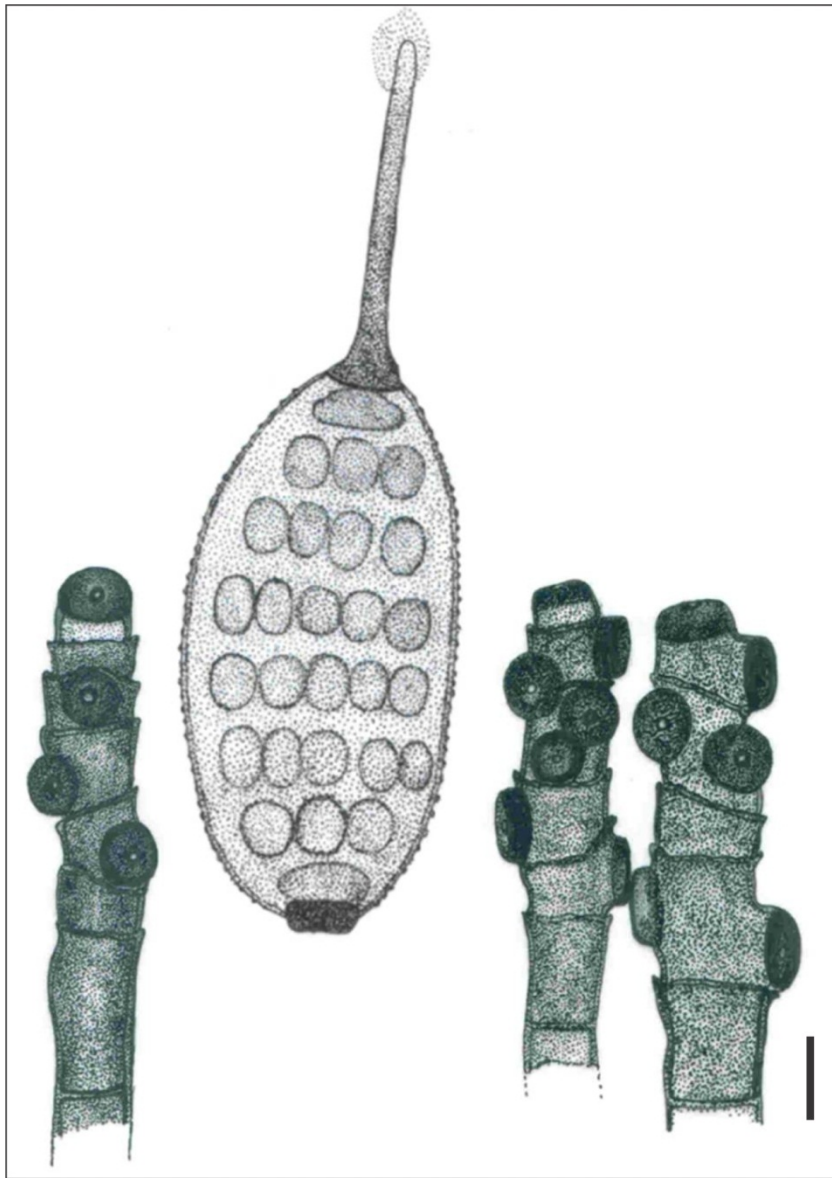


PLATE 4. Drawing of *Alveariospora distoseptata* conidia and conidiogenous cells. Bar: 10 μ m

CONCLUSÕES GERAIS

Treze espécies fúngicas foram encontradas associadas a *D. wilsonii*: *Vesiculohyphomyces cerradensis*, *Johansonia chapadiensis*, *Trichatomyces byrsonimae*, *Piricauda paraguayensis*, *Geastrumia polystigmatis*, *Phillipsiella atra*, *Stomiopeltis suttoniae*, *Microcalliopsis dipterygis*, *Janetia* sp. 1 (provável espécie nova), *Byssogene* sp. (provável espécie nova), *Pseudocercospora* sp. (provável espécie nova), *Pseudocercospora* sp. (provável espécie nova), cf. *Radulidium* sp. (provável gênero novo).

Seis espécies foram encontradas associadas a *D. mollis*: *Trichatomyces byrsonimae*, *Piricauda paraguayensis*, *Pseudocercospora* sp. (provável espécie nova), *Pseudocercospora* sp. (provável espécie nova), *Janetia* sp. 2 (provável espécie nova) e *Alveariospora distoseptata* (proposto como novo gênero e espécie).

Quatro fungos ocorreram em ambas às espécies de *Dimorphandra*: *Pseudocercospora* sp. (provável espécie nova), *Pseudocercospora* sp. (provável espécie nova), *Piricauda paraguayensis* e *Trichatomyces byrsonimae*.

Conclui-se que há possibilidade de que os três novos taxa associados unicamente a *D. wilsonii*: *Janetia* sp., *Byssogene* sp. e cf. *Radulidium* sp., mereçam o status de espécies possivelmente ameaçadas de extinção.