ADANS AGUSTIN COLMAN

ADIÇÕES À MICOBIOTA ASSOCIADA À PLANTA INVASORA *Dolichandra unguis-cati* NO BRASIL E NO PARAGUAI COM PARTICULAR REFERÊNCIA AOS FUNGOS FITOPATOGÊNICOS PARA O CONTROLE BIOLÓGICO

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

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APROVADA: 25 de fevereiro de 2014

_____	_____
Davi Mesquita de Macedo	Olinto Liparini Pereira

_____
Robert Weingart Barreto
(Orientador)

A Deus;

A minha mãe e avó, Elida e Vicenta;

As meus irmãos, Fabiola, Alba, Alexis, Dante e Juan;

A minha namorada Olga McLeod;

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BIOGRAFIA

ADANS AGUSTIN COLMAN, filho de Elida Rosa Colmán Medina, nasceu na cidade de Assunção, no dia 20 de janeiro de 1988, onde cursou o ensino fundamental e médio, concluindo-os em Dezembro de 2005.

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RESUMO

COLMAN, Adans Agustín, M.Sc., Universidade Federal de Viçosa, Fevereiro de 2014. **Adições à micobiota associada à planta invasora Dolichandra unguis-cati no Brasil e no Paraguai com particular referência aos fungos fitopatogênicos para o controle biológico.** Orientador: Robert Weingart Barreto.

Dolichandra unguis-cati (Bignoniaceae), comumente conhecida no Brasil e Paraguai como unha de gato, é uma planta trepadeira lenhosa perene, nativa da América, do México até a Argentina que tem uma ampla distribuição pelo Brasil e Paraguai. Depois de introduzida como uma planta ornamental em vários países naturalizou-se e tornou-se uma importante invasora causando sérios problemas em jardins, pomares, florestas cultivadas e nativas na Austrália, África do Sul, China, USA (Sul da Flórida) entre outros. A unha de gato representa uma ameaça significativa para a biodiversidade, em áreas densamente afetadas, pois cobre totalmente a vegetação, dominando-a e levando ao declínio de morte das plantas subjacentes. Os métodos convencionais de controle, como o químico e mecânico não podem ser aplicados no seu controle pois são inviáveis economicamente e danosos ao meio ambiente. Com isso, o controle biológico com fungos fitopatogénicos é considerado uma das medidas para o manejo desta invasora. Com o objetivo de complementar o trabalho feito na busca de agentes adicionais para o biocontrole de D. unguis-cati foi realizado um levantamento ampliado dos fungos associados a esta planta invasora no Brasil e Paraguai visando produzir uma descrição mais completa da micobiota fitopatogênica e orientar a seleção de potenciais agentes de controle biológico. Durante o levantamento foram obtidos 45 amostras, provenientes das regiões sul, sudeste do Brasil e da região oriental do Paraguai. Foram encontrados, descritos e fotografados quatorze fungos, dentre os quais três são aqui reconhecidos como novos taxa, dez são novas adições a micobiota do Brasil e doze são novos relatos para o Paraguai. Compreendem esta micobiota: sete hifomicetos- Alternaria alternata, Cercospora rodmanii, Cercospora appii, Passalora sp. nov. Passalora macfadyenae, Pseudocercospora unguis-cati, Ramularia sp. nov., e Myrothecium roridum; três celomicetos- Colletotrichum dematium, Colletotrichum karssi e Phoma sp. nov. e duas ferrugens- Uropyxis rickiana e Prospodium macfadyenae. Dentre as espécies encontradas, Uropyxis rickiana, Prospodium macfadyenae e Passalora macfadyenae

são considerados como tendo o maior potencial para uso em programas de controle biológico clássico.

ABSTRACT

COLMAN, Adans Agustín, M.Sc., Universidade Federal de Viçosa, February 2014. **Addition to mycobiota associated with the invasive plant Dolichandra unguis-cati in Brazil and Paraguai with particular reference to fungal pathogens for biological control**. Adviser: Robert Weingart Barreto

Dolichandra unguis-cati (Bignoniaceae), popularly known in Brazil and Paraguay as cat's claws, is a woody perennial climbing plant, native to America, being found from Mexico to Argentina and having a wide distribution in Brazil and Paraguay. Introduced as an ornamental plant in several countries it has became naturalized and it is causing serious problems as invasive in gardens, orchards, cultivated and natural forests of Australia, South Africa, China and USA (south Florida), among others. The cat's claws represent a significant threat to the biodiversity once its covers the vegetation, dominating it and leading to the decline and death to the adjacent plants. The conventional methods of control, as the chemical and mechanical controls, can not be used since they are uneconomical and damaging to the environment. Thereby the biological control is considered as the only viable measure to the management of this weed. In order to complement the search for biocontrol agents for cat's claws it was conducted a another survey of the fungi associated with this weed in Brazil and Paraguay aimed at the description of the plant pathogenic mycobiota associated with it and select potentials agents of biocontrol. This survey resulted in 45 samples, from the south, southeast of Brazil and oriental region of Paraguay. It was found, described and photographed fourteen fungi, among witch three are recognized as new taxa, ten as new reports to Brazil and twelve to Paraguay. Comprise this mycobiota: seven hyphomycetes - Alternaria alternata, Cercospora rodmanii, Cercospora appii, Passalora sp. nov., Passalora macfadyenae, Pseudocercospora unguis-cati, Ramularia sp. nov., Pseudocercosporella sp. nov. and Myrothecium roridum; three coelomycetes- Colletotrichum dematium, Colletotrichum karssi and Phoma sp. nov. and two rust- Prospodium macfadyenaeand Uropyxis rickiana. Among the species that were collected in the course of this work, Uropyxis rickiana, Prospodium macfadyenae and Passalora macfadyenae are consideredhere preliminarly to have the greatest potential for use in classical biological control programs.

INTRODUÇAO GERAL

As invasões biológicas são hoje a segunda maior ameaça a biodiversidade, perdendo apenas em importância para a destruição direta de habitats pela atividade humana. As primeiras transferências de espécies vegetais de uma região a outra do planeta foram intencionais e visavam, basicamente, suprir necessidades agrícolas, florestais e outras de uso direto. A introdução de plantas agrícolas, florestais ou ornamentais vindas de outras regiões pode produzir invasões de ecossistemas agrícolas e naturais provocando impactos desastrosos sobre o ambiente e afetando atividades econômicas ali realizadas (Ziller, 2001; Alves, 2008)

Trepadeiras e lianas estão entre as plantas invasoras mais destrutivas, impactando de forma significativa os ecossistemas que invadem (Harris & Gallager 2010). Como elas não são auto-sustentáveis, as trepadeiras podem destinar uma maior proporção de seus recursos no alongamento e produção de folhas em comparação com espécies arbóreas e arbustivas, permitindo-lhes, quando em situação de desequilíbrio, sufocar rapidamente a vegetação existente (Putz & Mooney, 1991). Devido a seu rápido crescimento, as trepadeiras são capazes de monopolizar a luz disponível no dossel, reduzindo a quantidade de radiação fotossinteticamente ativa que atinge tanto a planta que lhe serve de suporte quanto as que crescem no sub-bosque, reduzindo ainda mais o crescimento das árvores hospedeiras e suprimindo a regeneração das espécies nativas (Putz & Mooney, 1991; Harris et al. 2007)

Dolichandra unguis-cati (L.) Gentry, (syn. Macfadyena unguis-cati) Bignoniaceae, também conhecida como unha de gato ou unha de morcego, é uma trepadeira lenhosa perene que tem uma ampla distribuição na sua área nativa que vai desde o México e ao longo da América Central (incluindo Trinidad e Tobago) até o sul do Brasil e a Argentina (Everett, 1980; Rafter et al., 2008).

Escolhida como planta ornamental em função de sua bela floração amarela passou a ser cultivada em jardins e introduzida em muitas regiões tropicais e subtropicais escapando do cultivo, naturalizando-se e passando a invasora de ecossistemas naturais. Atualmente a sua erradicação de bosques, florestas, pomares e

plantações florestais tornou-se impossível (Henderson, 2001). Esta trepadeira se naturalizou em vários países da Ásia (China, Índia, Malasia, Nepal, Sri Lanka e Tailândia), Austrália e Oceania eilhas do Pacifico (Nova Zelândia, Indonésia, Micronésia, Nova Caledônia e Havaí), Europa, (Suíça, Servia e Montenegro, França e Grécia), África (Quênia, Ilhas Maurícias, África do Sul, Uganda e Zimbawe) e sul dosEUA (King & Dhileepan, 2009; Williams et al. 2008; Osunkoya et al. 2009; Dhileepan et al. 2010).

A unha de gato é uma trepadeira lenhosa alta, com hastes de até 6 cm de diâmetro e raízes que com a idade vão se intumescendo eformando tubérculos. Os ramos são verticais e horizontais e com o tempo podem desenvolver raízes adventícias. As folhas são opostas, compostas, com dois folíolos e três garras terminais bifurcadas que permitem que a planta se adira aos troncos de árvores, outros tipos de vegetação e estruturas artificiais, como cercas. As flores são amarelas e em forma de trombeta solitárias ou em pequenos grupos nas axilas das folhas. O fruto é uma cápsula linear plana, com 50 -100 cm de comprimento, oblongo com sementes aladas. Na Austrália, a planta geralmente tem um único pulso anual de floração no final da primavera ou no início do verão. A planta pode ser propagada a partir da semente, e vegetativamente a partir de tubérculos abaixo do solo. Hastes rasteiras ao longo do solo são capazes de produzir raízes nos nós. As sementes são dispersas pelo vento e pela água, as mesmas não permanecem viáveis por mais de um ano, o que sugere que, embora o mecanismo de propagação seja por meio de sementes, o mecanismo de persistência seja através do banco de tubérculos (Osunkoya et al. 2009; Vivian-Smith & Panetta 2004).

Na Austrália, a unha de gato é uma das principais plantas daninhas no ambiente de Queensland e Nova Gales do Sule tem o potencial de se espalhar por todo o leste da Austrália. Em Queensland e nordeste de Nova Gales do Sul , a trepadeira foi declarada oficialmente “erva daninha nociva”. A unha de gato representa uma ameaça significativa para a biodiversidade em áreas ribeirinhas, as comunidades da floresta, áreas não agrícolas e remanescentes de vegetação natural (Vivian-Smith & Panetta 2004; Downey & Turnbull 2007).

Em áreas densamente infestadas, esta trepadeira cobre totalmente a vegetação, incluindo arbustos e árvores de grande porte até 30 m de altura, causando finalmente colapso do dossel (Sparks, 1999). Em áreas sem vegetação em pé nem estruturas feitas

pelo homem, as trepadeiras crescem na superfície do solo da floresta formando tapetes densos o que suprime o desenvolvimento de plantas menores e a germinação das sementes de plantas nativas (Neser 1996, King & Dhileepan 2009).

O manejo desta trepadeira está focado na redução da taxa de crescimento da parte aérea para limitar a capacidade desta para subir e sufocar a vegetação nativa, bem como a redução da biomassa do tubérculo. A inacessibilidade dos tubérculos das raízes e sua capacidade para regenerar são um grande problema para o controle desta planta daninha. Opções de controle químico para o manejo desta trepadeira estão disponíveis, mas são de discutível viabilidade econômica e tem pouco sucesso por terem como alvo a parte aérea, tendo pouco impacto direto sobre o banco de tubérculos. A utilização e aplicação de produtos químicos são ainda mais complicadas pelo risco de efeitos sobre organismos não-alvos. Herbicidas de folhas largas só podem ser utilizados pontualmente pelo fato de que os ecossistemas ecologicamente mais sensíveis à invasão por D. unguis-cati e de maior importância econômica serem a vegetação ribeirinha e as florestas (Sparks, 1999; Dhileepan et al. 2005).

O controle mecânico de trepadeiras não é uma prática economicamente viável pois, os cortes de partes da planta localizadas acima do solo resultam em alivio apenas temporário para o problema (Pérez-Salicrup et al. 2001). A regeneração de plantas a partir dos tubérculos subterrâneos continua ao longo de muitos anos. Para alcançar o controle existe uma necessidade de tratar áreas infestadas com controle mecânico ou químico repetidamente, o que leva a efeitos não desejados. Portanto, o controle biológico é considerado como a única possibilidade para o manejo a longo prazo desta invasora em locais onde ela é generalizada (Raghu et al. 2007).

Os fungos representam o principal grupo de fitopatógenos utilizados para o controle biológico de plantas daninhas. Isso se deve à diversidade destes organismos associados a estas plantas, seu elevado potencial em produzir propágulos em grande quantidade, à capacidade de produzirem estruturas de resistência e ao fato de se dipersarem com facilidade (Barreto, 2009).

Há duas abordagens para o uso de fitopatógenos como agentes de controle biológico de plantas invasoras: o método clássico e o método bioherbicida. O método clássico ou inoculativo envolve a introdução de um patógeno inimigo natural de uma

planta-alvo desde seu centro de origem até a nova área de distribuição da planta onde ela, estando livre de seus inimigos naturais, tornou-se agressiva, visando restabelecer o equilíbrio. O método de bioherbicida ou inundativo, que tipicamente envolve o uso de fitopatógenos endêmicos, predominantemente fungos (denominados de mico-herbicidas) associados à planta alvo, que são produzidos em massa, formulados e aplicados de modo semelhante a um herbicida químico onde a população da invasora está estabelecida (Barreto, 2009; Pereira et al. 2003)

Em 1996 o controle biológico de D. unguis-cati foi iniciado na África do Sul pelo Plant Protection Research Institute (ARC-PPRI). Desde então, cinco insetos foram investigados e três foram aprovados para liberação no campo (King et al. 2011, Sparks, 1999).

Dolichandra unguis-cati pode exibir uma ampla diversidade genética ao longo de sua área nativa. Em contraste, a sua diversidade genética em áreas exóticas onde foi introduzida é muito baixa. Estudos genéticos utilizando microssatélites de cloroplastos para estimar a diversidade haplotípica demonstraram que mais de 90% das amostras das populações da unha de gato na maioria das áreas introduzidas, incluindo Austrália e África do Sul, pertenciam a um único haplotipo que tem afinidade com amostras provenientes do Paraguai e estão intimamente relacionadas geneticamente as populações da região da Bolívia e Argentina, representando a porção sul da faixa de distribuição em áreas nativas (Prentis et al. 2009).

Na Austrália, duas populações morfologicamente e geneticamente distintas ocorrem. (Sigg et al. 2006; Prentis et al. 2009). A variedade ‘short-pod’ é a mais invasiva e difundida em Queensland e Nova Gales do Sul, com uma segunda variedade ‘long-pod’ restrita a alguns locais no sudeste de Queensland. Ambas variedades tem uma flor amarela em forma de trombeta, mas a flor da variedade ‘long-pod’ tem um tom de amarelo mais profundo do que as flores da ‘short-pod’. As vagens da variedade ‘short-pod’ amadurecem no final do verão ou início do outono e as vagens da variedade ‘long-pod’’ amadurecem no final do inverno e início da primavera. A variedade ‘long pod’ tem folhas e vagens significativamente maiores, e mais sementes por vagens do que a variedade ‘short-pod’ (Shortus & Dhileepan 2011).

Rafter et al. (2008) realizaram estudos sobre a similaridade climática (utilizando o software CLIMEX) para priorizar áreas para a exploração de agentes de controle biológico onde M. ungüis-cati é nativa adequados para a liberação na Austrália e África do Sul e verificou-se que as áreas do centro e leste da Argentina, Sul do Brasil, Uruguai e partes de Bolívia e do Paraguai devem ser priorizadas para a exploração de novos agentes de controle biológico para esta planta.

O estudo de fungos associados a D. unguis-cati é recente. Levantamentos preliminares feitos no Brasil resultaram na descrição de cinco fungos associados a esta planta-alvo: Guignardia mangiferae, Meliola heteri, Passalora macfadyenae, Pseudocercospora ungüis-cati e Prospodium macfadyenae. Duas espécies descritas Passalora macfadyenae e Prospodium macfadyenae foramconsideradas pelos autores como tendo maior potencial para o uso no controle biológico clássico (Silva et al. 2012). No entanto, os autores reconheceram que o levantamento efetuado era apenas parciais e que o trabalho deveria ser continuado em busca de agentes adicionais para uso no controle biológico clássico desta importante invasora.

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Adition to mycobiota associated with the invasive plant Dolichandra unguis-cati in Brazil and Paraguay with particular reference to fungal pathogens for biological control

(Preparado de acordo com as normas da revista Mycologia)

Adition to mycobiota associated with the invasive plant Dolichandra unguis-cati in Brazil anda Paraguay with particular reference to fungal pathogens for biological control

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Abstract: A survey of fungi associated with the invasive plant Dolichandra unguis-cati was conducted in Brazil and Paraguay aiming at finding potential biological control agents for use in classical introductions in areas of the world where it has become a noxious invader. Fourteen fungal species were collected, identified, described and illustrated, including: two rust fungi (Uropyxis rickiana and Prospodium macfadyenae), three coelomycetes (Colletotrichum dematium, Colletotrichum karsii and Phoma sp.nov.), seven hyphomycetes (Alternaria alternata, Cercospora rodmanii, Cercospora appi, Pseudocercospora unguis-cati., Passalora sp. nov (Mycovelliosella-like)., Passalora unguis-cati., Ramulariopis sp. nov. and Myrothecium roridum). Four among these fungi represented new taxa which are described herein whereas ten are new adition to the mycobiota of D. unguis-cati in Brazil, and eleven are new reports for Paraguay. Observations of damage in the field and preliminary inoculation studies indicated that Prospodium macfadyenae, Uropyxis rickiana and Passalora sp.nov. have the greatestpotencial for use in classical biological control. Koch's postulates were performed with most culturable fungal species but typical disease symtoms were obtained only for inoculations involving Alternaria tenuissima, Colletotrichum dematium, Myrothecium roridum and Passalora sp. nov. Uropyxis rickiana was seen causing large gall symptoms on stems and, under controlled conditions caused rust symptoms on the two forms of cat's claws found in Australia.

Key words: Bignoniaceae, Macfadyena, fungal survey, taxonomy

General introduction

Biotic invaders are species that establish a new range in which they proliferate, spread, and persist to the detriment of the environment. They are the most important ecological outcomes from the unprecedented alterations in the distribution of the earth's biota brought about largely through human transport and commerce (Mack et al. 2000).

Cat's claw creeper, Dolichandraunguis-cati (L.) A.H.Gentry (Bignoniaceae), is a perennial woody climbing vine that is native from Mexico through Central America to tropical South America, including Trinidad e Tobago (Rafter et al. 2008; Dhileepan, 2013).Introduced as an ornamental vine, the exotic vine cat's claw creeper, has become a significant threat to the biodiversity of a variety of sensitive ecosystems in coastal and

subcoastal areas of subtropical eastern Australia and South Africa. (King and Dhileepan, 2012).

Two morphologically distinct cat's claw varieties have been identified ocurring in Australia, one of wich (short pod) is found throughout south eastern Australia, while the other (long pod) appears to be restricted to several sites in south-eastern Queensland (Shortus and Dhileepan 2010).Plant genotypic studies suggest that the invasive haplotypes in Australia and South Africa are similar to those in the southern parts of the native range of cat's claw creeper, Peru, Bolivia, Paraguay and Argentina (Sigg et al., 2006).

The seed of cat's claw creeper are capable of hydrochory and the plant can regenerate from under-ground tubers and broken stems. Costs, logistical constraints and the need reapplication of treatments associated with mechanical or chemical controls for this weed are such that biological control is regarded as the most practical and sustainable means of successfully managing the weed for long-term (Dhileepan, 2005; King et al. 2012)

The study of fungi associated with D. unguis- cati is recent. Preliminary studies in Brazil resulted in the description of five fungi associated with this plant: Guignardia mangiferae, Meliola heteri, Passalora macfadyenae, Pseudocercospora ungüis-cati and Prospodium macfadyenae. Two species described Passalora macfadyenae and Prospodium macfadyenaewere considered as having the greatest potential for use in classical biological control (da Silva et al. 2012). However, the authors acknowledged that their survey was only partial and that the work should be continued in search of additional agents to use for this purpose. During 2012 and 2013, the search was expanded to encompass novel areas in native situations in Brazil and Paraguay. This publication presents the results of this work including information on fungal taxonomy and the potential to use components of the mycobiota of D. unguis-cati in classical biological control.

Materials and methods

Survey and sampling

The samples included in this study were obtained during surveys conducted between the 2012 and 2013, covering the southern and southeastern Brazilian states of

Minas Gerais, Rio de Janeiro, São Paulo, Paraná, Santa Catarina and Rio Grande do Sul and the Central, Cordillera, Caazapa, Guaira, Paraguari and Caaguazudepartments of Paraguay. Sampling locations were selected arbitrarily during the trips and whenever target-plant populations were found representative parts bearing disease symptoms were taken, photographs were made and notes were taken. The collected samples were dried in a botanical press subsequently transferred to envelopes and deposited at the herbarium of the Universidade Federal de Viçosa (VIC). All samples were carefully examined under a dissecting microscope and whenever fungal structures seemed to be associated with the symptoms of the disease (and fungi were recognized as cultivable) attempts to obtain monosporic pure cultures were made by direct transfer of fungal structures onto plates containing vegetable broth-agar (VBA) as described by Pereira et al. (2003) with the help of sterile fine pointed needle. Pure cultures were preserved in tubes containing potato-carrot-agar (PCA), in silica-gel, as described in Dhingra and Sinclair (1996), and in cryotubes containing glycerol 10% and maintained at-80 ºC in a deep-freezer. Cultures were deposited in the culture collection Oswaldo Almeida Drummond of the Universidade Federal de Viçosa (COAD).

Culture descriptions

Culture description were based on observations of the colonies formed in plates containing potato dextrose-agar (PDA), PCA and oatmeal-agar (OA).These were incubated at 25^{o}C undera 12 h daily light regime (light provided by two fluorescent daylight lamps and oneNUV lamp placed 35 cm above the plates) for 10 days. An isolate of*Alternaria*was grown in*V8*-agar medium and plates were kept under the same conditions. Color terminology followed Rayner (1970).

Morphology studies

Observations of the fungus morphology were made in slides containing representative structures of the fungus mounted in lactophenol or lactofuchsin. Slides were performed by scraping externally produced structures with a scalpel or by freehand sectioning the plant tissue bearing the fungal structures or with afreezing microtome (Leitz, Kriomat) - sections having a 14–20 µm thickness. Observations of the morphology and illustrations were made under a Olympus BX 51 light microscope equipped with DIC, camera tube and Olympus E 330 camera.

Patogenicity and host range test

Demonstration of the pathogenicity of the fungus (Koch's Postulates) and preliminary evaluation of the possible impact of each fungus as a biocontrol agent was performed as follows: five D. ungüis-cati seeds (short pod and long pod varieties) from (Australia, South Africa, Brazil and Paraguay) were sown in plastic trays containing a commercial substrate (Bioplant) Thirty days after emergence the plants were transferred to plastic vases containing 1: 1 sand-cow manure and when they were well established Concentration of the inoculum used in each test was calibrated with a Neubauer chamber.

The fungus was seeded onto plates (60 mm diam) containing PDA and placed in a controlled temperature room adjusted to 25 ± 2º C under a light regime of 12 hours. After plates were fully colonized by the fungus (ten days for Colletotrichum spp., and thirty five days for cercosporoid fungi), 10 mL of sterile water were poured on each plate and the surface of the sporulating cultures was scraped with a rubber spatula. Two droplets of Tween 20 were added to the conidial suspension, and the concentration was adjusted to 1×10^6 conidia/mL (for Colletotrichum isolates). For cercosporoid fungi which did not sporulate in the culture inoculations were performed by the deposition of three 5mm diam culture discs (obtained from the margin of actively growing colonies) onto healthy young leaves.

Conidial suspensions were sprayed on testplants until runoff. Plants sprayed with a diluted Tween 20 solution served as controls. The plants were left in a moist chamber for 48 hours and taken to a greenhouse. Plants were examined at5-days intervals for the emergence of disease symptoms for45days after the inoculation.

A selected species – the rust fungus Uropyxis rickianaMagnus was preliminarly evaluated for its host-specificity to D. unguis-cati. A test plant list comprising 21 plant species (Table 1), similar to the one used for previously tested biological control agents for cat's claw creeper in Australia (Dhileeepan et al. 2005; 2007 a; b; 2013), was assembled. The test plant species were selected using the centrifugal phylogenetic method (Wapshere 1974; Briese 2003). The test, starting with the two forms of cat's claws and including the nearest relatives of the target within the family Bignoniaceae available and also including plants in other families in the order Lamiales (Dhillepan et al. 2013). For this test, plants were inoculated with a suspension

of 1×10^6 teliospores–uredinospores/mL, teliospores germinated on AA were also used in inoculations.

Results

Numerous samples of diseased D. unguis-cati were collected during the survey in Brazil and Paraguay. Fourteen different fungal pathogens were collected in association with cat's claws causing following disease: leaf spot, anthracnose and rust. Ten fungal species were isolate in culture pure. The mycobiota obtained in the present study is listed below and fungi that were newly collected on this work are described.

Alternaria alternata(Fr.) Keissler, Beih. Bot. Zentralbl. 29:434. 1912. Fig. 1

Lesions on living leaves starting as small necrotic spots becomingcircular to irregular dark brown, 5–10 mm diam and eventually coalescingand leading to necrosis of large portions of leaves. External mycelium absent. Internal mycelium indistinct. Stromata absent. Conidiophores amphigenous, single or in small groups of up to six, cylindrical, straight or slightly sinuous, geniculate, 25–102 × 2.5–4 μm, 2–11 septate, pale brown to brown, smooth. Conidiogenous cells terminal, integrated, proliferating simpodially, cylindrical 4–12 × 3.0–4.5 μm. Conidiogenous loci conspicuous 1–4 per cell, 2–3μm diam, darkened, thickened. Conidia single or catenulate pyriform to ovoid-obclavate, 22–64 × 6–10 μm, golden brown to brown, 3–7 tranversal septa, 1–5 longitudinal septa, smooth or slightly verruculose.

In culture: On PDA and V8, slow-growing (5.5 – 6 cm diam after 10 days), flat to slightly convex, entire edged, aerial mycelium cottony centrally to felty periphery, strong diurnal zonation alternatingly smoke grey and mouse grey, humid centrally, dark mouse grey reverse; abundant sporulation. In PCA, slow-growing (5–5.5 cm diam after 10 days), slightly convex, entire edged, of cottony to felty mycelium centrally becoming low at peryphery, pale olivaceous grey, with subtle diurnal zonation and humid centrally; smoke grey reverse, sporulation scarse.

Material examined:PARAGUAY, Central, San Lorenzo, on living leaves of D. unguis-cati 'long pod', 22 June 2013, A. A. Colman (VIC 39830).

Notes: No species of Alternariahave ever been reported in association with D. unguis – cati. Nevertheless three species of Alternaria have been found in asssociation

with other members of the Bignoniaceae: A. alternata , A. tenuissima (Nees) Wiltshire and A. catalpa (Ellis & Mont.) P.Joly (Farr & Rossman, 2014). Alternaria tenuissima is a species that is morphologically similar to A. alternata, but differs from the latter because of its longer conidial chains and darker conidia (Simmons 2007). The morphology of the fungus found on D. unguis-cati is equivalent to that describedby Simmons for A. alternata.

The genus Alternariais a ubiquitous fungal genus that includes saprophytic, endophytic and pathogenic species associated with a wide variety of substrates (Woudenberg et al. 2013). Alternaria alternata is a polyphagous and cosmopolitan species which has been recorded either as a saprophyte or as a pathogen on hundreds of hosts (Farr & Rossman, 2014). The fungus was observed only once in a single location causing necrotic lesions in D. unguis – catiplants. In the pathogenicity test, necrotic symptoms were observed 8 days after inoculation. Under controlled conditions, the disease caused by the fungus was less severe.This is the first report of A. alternata causing leaf spots on D. unguis – cati, in Paraguay and worldwide. (Farr & Rossman, 2014). The occurrence of A. alternata was observed only once in the field and the fungus was causing only a minor disease. Additionally it belongs to a polyphagouspathogenic species. Therefore it is regarded here as having no potential for classical biological of D. unguis-cati.

Cercospora apii Fresen. sensu lato, emend. Crous and Braun. Mycosphaerella and its anamorphs: 1. Names published in Cercospora and Passalora CBA Biodiversity Ser. 1: 33-36 (2003). Fig. 2

Lesions on living leaves, necrotic, initially circular to ellipsoid, later coalescing to form large spots, with indistinct margins, brow, 5–30 mm. Internal mycelium indistinct. External mycelium absent. Stromata reduced to few cells on substomatal cavity. Conidiophores hypophyllous arising through stomata, fasciculate, erect, straight, subcylindrical, 137– 475 × 3–4.5 μm, 4–14 septate, unbranched, brown paler at apices, thin-walled, smooth. Conidiogenous cells terminal, integrated, proliferating simpodially, 25 – 40 × 2.5– 4μm, light brown. Conidiogenous loci conspicuous, 1–3 per cell, 2.5–3.5 μm diam, thickened, darkened. Conidia dry, solitary, ranging from oblcavate-cylindrical to (mostly) acicular-filiform, straight to curved, 87.5–300 × 3–4

μm, apex subacute, base truncate, 7–22 septate, guttulate, hyaline, thin walled, smooth, hila thickened and darkened.

In culture: on PDA, slow-growing (3.6–4.5 cm diam after 20 days), flat to slightly convex, lobate, aerial mycelium scarce, cottony centrally,pale olivaceous grey, peripheral region of immersed mycelium, subtle diurnal zonation, greenish black in the reverse; no sporulation. On PCA, slow-growing (2–2.5 cm diam after 20 days), colony edges entire, flat to slightly convex white to pale olivaceous grey, centrally, periphery immersed, diurnal zonation either pronounced or subtle, reverse olivaceous black; no sporulation.

Material examined: BRAZIL, Minas Gerais, Brumadinho, on living leaves of D. unguis-cati 'short pod variety', 13 June 2013, A. A. Colmán (VIC 39819).BRAZIL, Minas Gerais, Viçosa, on living leaves of D. unguis-cati 'short pod variety', 13 June 2013, A. A. Colmán (VIC 39821). BRAZIL, Minas Gerais, Paraopeba, on living leaves of D. unguis-cati 'short pod variety', 23 July 2013, A. A. Colmán (VIC 39826).

Notes: The genus Cercospora (Fresen) was first described by Fresenius in 1863 (in Fuckel) and currently is one of the largest and most heterogeneous genera of hyphomycetes (Crous & Braun, 2003). Johnson & Valleau (1949) stated that most of the morphologically uniform Cercospora belong to a single species of Cercospora which occur in a wide host range and is morphologically indistinguishable from C. apii. Cercospora apii is the oldest available name for this large complex of morphologically indistinguishable species (Groenewald et al. 2006). Eleven species of Cercospora have been reported occurring on members of the Bignoniaceae, and C. apii was one such species. It was reported in association with Tabebuia serrattifolia (Farr & Rossman, 2014). The fungus found on D. unguis-cati in our surveys fits well within the morphological delimitation of Cercospora apii sensu lato as described in Crous and Braun (2003).This fungus was found in samples collected in Brazil and Paraguay causing necrotic spots the plant D.unguis-cati.

Pathogenicity tests were performed and typical symptoms were observed 9 days after inoculation. Recent studies have indicated that Cercospora apii is a species complex including forms having a broad host range and others that are phylogenetically distinct and are host-specific, deserving recognition as separate species (Groenewald et al., 2006). This may be the case of the Cercospora on cat's claws but this remains to be

clarified. Inoculation studies indicated a weak impact of this fungus on the host and other fungi in the mycobiota of D. unguis-cati deserve more attention for seemingly having better potential for use in biocontrol of this weed.

Cercospora rodmanii Conway,Can. J. Bot.54(10): 1082 (1976).Fig. 3

Lesions on living leaves, similar to those caused by C. apii(see above), necrotic, initially circular to ellipsoid, later coalescing to form large spots, with indistinct margins, dark brow 20 – 50 mm.External mycelia and stromata lacking**.** Internal mycelium indistinct. Conidiophores arising through the stomata, predominantly hypophyllous, solitary, or forming fascicles of up to 5, sub-cylindrical, straight or slightly curved or sinuose and geniculate, 44–107.5 × 3.5–4.5 μm, unbranched, 3–4 septate, pale brown smooth. Conidiogenous cells integrated, holoblastic, sub-cylindrical, terminal, sympodial, 15–30 × 3–4.5 μm, pale brown. Conidiogenous loci 1–4 per cell, 2.5–3 μm, thickened, darkened. Conidia obclavate, straight to somewhat curved or slightly sinuous, 31–175 × 2.5– 3.5 μm, apex rounded, base subtruncate, 2–8 septate, hyaline, smooth.

In culture:on PDA, slow-growing (1.5–2.5 cm diam after 20 days), edge entire, flat to slightly convex, aerial mycelium scarce, cottony, dark olivaceous greycentrally, periphery of immersed mycelium, pigmenting the medium in pink,humid centrally, diurnal zonation subtle, greenish black reverse; no sporulation. On PCA, slow-growing (3.5–4.5 cm diam after 20 days), entire edge, flat to slightly convex, aerial mycelia organza-like to powdery, olivaceous grey, peripheral region of immersed mycelium, diurnal zonation absent; olivaceous black to dark olivaceous grey reverse; no sporulation.

Material examined: PARAGUAY, Central, Capiatá, on living leaves of D. unguis-cati ‘short and long pod variety’, 22 May 2013, A. A. Colmán (VIC 39806). PARAGUAY, Guaira, Tebicuary, on living leaves of D. unguis-cati ‘short pod variety’, 27 May 2013, A. A. Colmán (VIC 39814). BRAZIL, Rio Grande do Sul, Itaimbezinho, on living leaves of D. unguis-cati ‘short pod variety’, 13 June 2013, A. A. Colmán (VIC 39820). BRAZIL, Bom Retiro, on living leaves of D. unguis-cati ‘short pot variety’, 15 May 2013, A. A. Colmán (VIC 39797). PARAGUAY, Central, Capiatá, on living leaves of D. unguis-cati ‘long pod variety’, 23 December 2013, A. A. Colmán (VIC 39833).

Notes: The genus Cercospora includes numerous important pathogenic fungi, which affect a wide range of hosts; information on most species is restricted to in vivo

morphological characters (Groenewald et al. 2012).Morphological features of conidiophores, conidiogenous cells and conidia have been traditionally used to divide morphologically similar genera within cercosporoid fungi (Crous & Braun 2003).

Within the family Bignoniaceae only one species of Cercospora has been reported to occur in D. unguis-cati namely Cercospora duplicata Ellis & Everh (1889). This was described on basis of a specimen collected in Barbados (Farr & Rossman 2014). Our sample was compared with this species and also with Cercospora species occurring in the Bignoniaceae and it was found that its morphology fits well within that described for Cercospora rodmanii.

After 20 days, plants inoculated with C. rodmanii showed characteristic foliage necrosis as seen in the field. This fungus has been originally described in association with with Eichhornia crassipes (Mart.), Pontederiaceae (Farr & Rosmann, 2014). To our knowledge this is the first report of Cercospora rodmanii causing leaf spotting on D. unguis-cati found in Brazil and Paraguay. Unlike C. apii, C. rodmanii was observed in the field causing a more severe disease. Atlthough, at this stage, this species doesn't deserve priority as a candidate for use in cat's claws biocontrol, because of the higher potential of other species it would deserve further evaluation at later stages if other biocontrol agents fail to deliver an adequate level of control.

Psedocercospora unguis-cati (Speg.) U. Braun, Mycotaxon 51:49. 1994. Fig. 4

Description see in: Da Silva M, Barreto RW, Pereira OL. 2012. Fungal pathogens of 'cat's claws' from Brazil for biocontrol of Macfadyena unguis-cati. Mycotaxom. 119, 181-195

In culture:On PDA, slow-growing (1.5–2 cm diam after 38 days), colonies umbonate, undulate, aerial mycelium scarce, slightly raised centrally, cottony to lavender grey, periphery immersed, no diurnal zonation; greenish grey reverse; no sporulation. On PCA, slow- growing (2–3 cm diam after 38 days), colonies with slightly lobate edges, aerial mycelium cottony to pale olivaceous grey centrally, periphery composed of immersed mycelium,slightly humid centrally, diurnal zonation absent ; dark honey to olivaceous black reverse; no sporulation.

Material examined:BRAZIL, São Paulo, Bragança Paulista, on living leaves of M. unguis-cati 'long pot variety', 2 May 2013, A. A. Colmán. (VIC 39792). BRAZIL, São Paulo, Barra do Turvo, on

living leaves and stems of M. unguis-*cati* 'short pot variety', 13 May 2013, A. A. Colmán (VIC 39793). BRAZIL, São Paulo, Iporanga, on living leaves of M. unguis-*cati* 'long pot variety', 13 May 2013, A. A. Colmán (VIC 39795). BRAZIL, Santa Catarina, Alfredo Wagner, on living leaves of M. unguis-*cati* 'long pot variety', 15 May 2013, A. A. Colmán, (VIC 39796). BRAZIL, Santa Catarina, Alfredo Wagner, Bom Retiro, on living leaves of M. unguis-*cati* 'short pot variety', 15 May 2013, A. A. Colmán (VIC 39797). BRAZIL, Santa Catarina, Urubici, on living leaves of M. unguis-*cati* 'long pot variety', 15 May 2013, A. A. Colmán (VIC 39798). BRAZIL, Paraná, Jaguanaúva, Itararé, on living leaves of M. unguis-cati 'short and long pod variety', 19 April 2013, A. A. Colmán (VIC 39799). BRAZIL, Paraná, Sengés, 19 May 2013, A. A. Colmán, (VIC 39800). BRAZIL, Paraná, Jaguanaúva, Itararé, on living leaves of M. unguis-cati 'short pod variety', 19 April 2013, A. A. Colmán (VIC 39799). BRAZIL, Paranáá, Itararé, on living leaves of M. unguis-cati 'long pot variety', 19, April 2013, A. A. Colmán (VIC 39801). BRAZIL, São Paulo, Cerquilho, on living leaves of M. unguis-cati 'long pot variety',19 April 2013, A. A. Colmán (VIC 39803). BRAZIL, Minas Gerais, Viçosa, on living leaves of M. unguis-cati 'long pot variety', 8 May 2013, A. A. Colmán (VIC 39804). BRAZIL, Rio de Janeiro, Nova Petrópolis, on living leaves of M. unguis-cati 'short pot variety', 13 January 2013, R. W. Barreto (VIC39836). BRAZIL, Minas Gerais, Paraopeba, on living leaves of M. unguis-cati 'long pot variety', 31 March 2013, M. Silva (VIC 39838). PARAGUAY, Central, San Lorenzo, on living leaves of M. unguis-cati 'short pod variety', 20 May 2013, A. A. Colmán (VIC 39805). PARAGUAY, Cordillera, Piribebuy, on living leaves of M. unguis-cati 'long pod variety', 23 May 2013, A. A. Colmán (VIC 39808). PARAGUAY, Cordillera, Caraguatay, on living leaves of M. unguis-cati 'short pod variety', 25 May 2013, A. A. Colmán (VIC 39809). PARAGUAY, Caazapa, General Morinigo, on living leaves of M. unguis-cati 'long pod variety', 23 May 2013, A. A. Colmán (VIC 39810). PARAGUAY, Caazapa, General Morinigo, on living leaves of M. unguis-cati 'short pod variety', 27 May 2013, A. A. Colmán (VIC 39811). PARAGUAY, Guaira, Ñumi, on living leaves of M. unguis-cati 'short pod variety', 27 May 2013, A. A. Colmán (VIC 39812). PARAGUAY, Guaira, Coronel Martinez, on living leaves of M. unguis-cati 'short pod variety', 27 May 2013, A. A. Colmán (VIC 39813). PARAGUAY, Guaira, Tebicuary, on living leaves of M. unguis-cati 'long pod variety', 27 May 2013, A. A. Colmán (VIC 39814). PARAGUAY, Paraguari, on living leaves of M. unguis-cati 'long pod variety', 27, May 2013, A. A. Colmán (VIC 39815). BRAZIL, Minas Gerais, Juatuba, on living leaves of M. unguis-cati 'short pod variety', 13 June 2013, A. A. Colmán (VIC 39817). BRAZIL, Minas Gerais, Florestal, on living leaves of M. unguis-cati 'long pod variety', 13 June 2013, A. A. Colmán (VIC 39818). BRAZIL, Minas Gerais, Brumadinho, on living leaves of M. unguis-cati 'long pod variety', 13 June 2013, A. A. Colmán (VIC 39819). BRAZIL, Minas Gerais, Viçosa, on living leaves of M. unguis-cati 'short pod variety', 13 June 2013, A. A. Colmán (VIC 39821). BRAZIL, Minas Gerais, Bocaiuva, on living leaves of M. unguis-cati 'short pod variety', 22 July 2013, A. A. Colmán (VIC 39823). BRAZIL, Minas Gerais, Mirabela, on living leaves of M. unguis-cati 'short pod variety', 22 July 2013, A. A. Colmán (VIC 39824). BRAZIL, Minas Gerais, Paraopeba, on living leaves of M. unguis-cati 'short pod variety', 23 July 2013, A. A. Colmán (VIC 39825) BRAZIL, Minas Gerais, Paraopeba, on living leaves of M. unguis-cati 'long pod variety', 23 July 2013, A. A. Colmán (VIC 39826). BRAZIL, São Paulo, on living leaves of M. unguis-cati 'long pod variety', 22 July 2013, A. A. Colmán (VIC 39828)

Notes:Pseudocercospora is a large genus of hyphomycete fungi, including more than 1200 species (Kirk et al. 2008). A fungus originally collected in Argentina on D.unguis-cati was described by Spegazzini as - Cercosporella unguis- cati Speg. Later Braun (1994) recombined that species to Pseudocercospora unguis-cati by Braun (1994). The latter author observed inconspicuous, unthickened conidial scars and faintly coloured stromata and conidiophores. Several species of Pseudocercospora have been reported on members of the Bignoniaceae (Farr & Rossmam, 2014). Specimen collected in Brazil and Paraguay, showed morphological similarities to P. unguis-cati. This is the first report of P. unguis-cati, originally described from Argentina and Brazil, on D.unguis-cati in Paraguay. (Farr & Rossman, 2014). Disease caused by P. unguis-cati is rather severe. Inoculations with our isolates reproduced the disease forty days after inoculation, plants inoculated showed severe defoliation. It appears to have good potential for use in classical biocontrol of D. unguis-cati.

Passalora sp. nov. Fig. 6

Lesions on living leaves, amphigenous, subcircular, well delimited, infected tissue initially dark brown surrounded by a pale brown halo, becoming grayish centrally with a narrow dark brown outer rim at periphery, 3–3.5 mm diam, coalescending and leading to blight of extensive areas on leavesand leaf drop. Internal mycelium intercellular 1–2 μm. External mycelium present 2.5–3 μm, septate, branched, pale brown. Stromata absent or small and composed of only a few subtomatal swollen cells, pale brown. Conidiophores hypophyllous, either primary or secondary - primary emerging through stomata, solitary or forming loose fascicles of few conidiophores, straight to slightly curved, cylindrical, 22–175 × 3–4 μm, 2–24 septate, branched – secondary light brown 20–45 × 2.5–3.0 μm, pale brown, smooth, with inconspicuous scars. Conidiogenous cells terminal, integrated, proliferating simpodially, holoblastic, cylindrical, 12.5–22.5 × 2.5–3.5, brown. Conidiogenous loci inconspicuous. Conidia dry, solitary, acicular-obclavate, straight to slightly curved, 25–150 × 2.5–3.5μm, base slightly subtruncate to obconic, apex rounded, 3-16 septata, hilum unthickened, not darkened, pale brown, smooth.

In culture:on PDA, slow-growing (3–3.5 cm diam after 20 days) slightly convex, lobate margins, aerial mycelium abundant velvety, pale mouse grey to mouse

grey, periphery composed of superficial mycelium; diurnal zonation absent; slightly humid centrally; reverse olivaceous black; no sporulation. On PCA, slow-growing (1–2 cm diam after days), slightly convex, lobate margins, cottony centrally, pale olivaceous grey, periphery of immersed mycelium. Reverse greenish-black, diurnal zonation absent; no sporulation.

Material examined: BRAZIL, Minas Gerais, Viçosa, on living leaves of D. unguis-cati 'long pot variety', 26 August 2013, A. A. Colmán. (VIC 39829)

Notes: Only one species of Passalora has been described in association with D. unguis-cati, namely Passalora macfadyenae (Silva et al. 2012). Passalora sp. nov. was found to be significantly different from the other species in this genus described on the Bignoniaceae (Table 2). Various morphological features, such as conidial size, are used for differentiating Passalora species. Passalora sp.nov. is clearly different from P. macfadyenae. Differences are in conidial size (31.5–114 × 3–4.5 µm in P. macfadyenae) the lack of stromata and presence of external mycelium in the new species, among others . P. markhamiae (X.J Y.L. Liu & Guo), P. tabebuiae (JJ & Muchovej F.AFerreira), P. tabebuiae-ochraceae (Ignácio & Dianese) and P. tecomariae (Crous & Sutton, 1997) are all different from the the new species, because because of having shorter conidia. Additionally P. catalapae (Chupp) Braun & Crous, P. pyrostegiae (Viégas) U. Braun & Crous, P. leprous (Speg) U. Braunhave well developed stromata. Passalora adenocalymmatis (Chupp) Crous & Braun, differs from the new species on having stromata and having conidiophores of different length. P. arrabidaeae (Chupp & Viégas) has shorter conidiophores and produces no superficial mycelium. Finally P. catalparum (Chupp) Crous & Braun, has conidia and conidiophores of size differing from that of the new species.

Inoculations with our isolates did not reproduce the disease. Passalora sp. nov. causes a more severe disease than P. macfadyeane and appears to have more potential for the biologically control of D. unguis-cati.

Passalora macfadyenae Meir. Silva, O.L. Pereira & R.W. Barreto .Mycotaxon. 119:15 (2012). Fig. 7

Description: See in: Silva M, Barreto RW, Pereira OL. 2012. Fungal pathogens of 'cat's claws' from Brazil for biocontrol of Macfadyena unguis-cati. Mycotaxom. 119, 181-195

In culture: on PDA, slow- growing (2.5–3.5 cm diam after 20 days), pronouncedly lobateedges, flat convex, aerial mycelium velvety, dark olivaceous grey cerebriform centrally, periphery of immersed mycelium, diurnal zonation absent, humid centrally; reverse olivaceous black; no sporulation. On PCA, slow-growing (2–2.5 cm diam after 20 days), silghtly convex, lobate margins, irregular cottony centrally, dark olivaceous grey, diurnal zonation absent; reverse greenish black; no sporulation.

Material examined: PARAGUAY, Caazapa, General Morinigo, on living leaves of D. unguis-cati 'short pod variety', 23 May 2013, A. A. Colmán (VIC 39810). BRAZIL, Minas Gerais, Florestal, on living leaves of D. unguis-cati 'long pod variety', 13 June 2013, A. A. Colmán (VIC 39818). BRAZIL, Santa Catarina, Urubici, on living leaves of D. unguis-cati 'long pod variety', 22 July 2013, A. A. Colmán (VIC 39822). BRAZIL, Minas Gerais, Bocaiuva, on living leaves of D. unguis-cati 'short pod variety', 22 July 2013, A. A. Colmán (VIC 39823). BRAZIL, Minas Gerais, Mirabela, on living leaves of D. unguis-cati 'long pod variety', 22 July 2013, A. A. Colmán (VIC 39824). BRAZIL, Minas Gerais, Juatuba, on living leaves of D. unguis-cati 'short pod variety', 23 July 2013, A. A. Colmán (VIC 39831).

Notes:Passalora macfadyenaewas widely collected in Brazil and Paraguayan causing necrotic spots on D. unguis-cati. This fungus was first reported in Brazil, but this is the first report of P. macfadyenae in Paraguay. Pathogenicity tests were done but typical disease symptoms did not result. Again, here this failure on reproducing the disease may have resulted from the use of culture disks instead of spore as inoculums. This fungus was already considered as a potential candidate for further evaluation as a biocontrol agent of cat's claw by Silva et al. (2012) and our observations here confirmed those views.Inoculations with our isolates did not reproduce the disease.

Colletotrichum dematium (Pers.) Grove, J. Bot., Lond. 56: 341 (1918). Fig. 8

Lesion on living leaves, amphigenous, starting as small necrotic spots which later coalesced resulting in irregularly shaped necrotic areas 5–50 mm. Internal mycelium indistinct. External mycelium absent. Conidiomata acervular, amphigenous, subcuticular, 30–80 µm diam. Setae abundant, mostly uniformly medium to dark brown but lighter apically when larger, cylindrical, slightly swollen at base, tapering apically to subacute apex, straight to slightly curved, (1–3 septate, 55–150 × 4–6 µm). Conidiophores mostly reduced to the conidiogenous cells, cylindrical, phialidic, 6–19 × 3 – 4.5, 1–2 septate, unbranched, hyaline, smooth. Conidia falcate with acute ends, 15–

21 × 2.5–3.5 µm, aseptate, gutulate, hyaline to sub-hyaline, smooth. Apressoria (observed in slide cultures) borne on hyaline thin-walled supporting hyphae, globose to ellipsoid, sometimes irregular to lobate, solitary or in groups (6–28 × 5–10), medium brown to dark brown, smooth.

In culture: On PDA and PCA, fast-growing (5.5–7.5 mm diam after 7 days), edge entire, slightly convex, aerial mycelium cottony centrally, followed by an area of sparse mycelia, centrally pale greenish grey alternate with dark mouse grey, periphery composed of immersed mycelium, diurnal zonation present; slightly humid centrally, olivaceous black reverse; no sporulation.

Material examined: BRAZIL, Minas Gerais, Viçosa, on living leaves of D. unguis-cati 'short pod variety', 13 June 2013, A. A. Colmán (VIC 39821). BRAZIL, Minas Gerais, Brumadinho, on living leaves of D. unguis-cati 'long pod variety', 13 June 2013, A. A. Colmán (VIC 39816). BRAZIL, Minas Gerais, Juatuba, on living leaves of D. unguis-cati 'short pod variety', 13 June 2013, A. A. Colmán (VIC 39817). BRAZIL, Minas Gerais, Florestal, on living leaves of D. unguis-cati 'long pod variety', 13 June 2013, A. A. Colmán (VIC 39818). BRAZIL, Rio Grande do Sul, Itaimbezinho, on living leaves of D. unguis-cati 'long pod variety', 13 June 2013, A. A. Colmán (VIC 39820). BRAZIL, Minas Gerais, Viçosa, on living leaves of D. unguis-cati 'short pod variety', 13 June 2013, A. A. Colmán (VIC 39821). BRAZIL, Minas Gerais, Paraopeba, on living leaves of D. unguis-cati 'short pod variety', 23 July 2013, A. A. Colmán (VIC 39826). BRAZIL, Minas Gerais, Paraopeba, on living leaves of D. unguis-cati'long pod variety', 23 July 2013, A. A. Colmán (VIC 39827). BRAZIL, São Paulo, on living leaves of D. unguis-cati 'short pod variety', 22 July 2013, A. A. Colmán (VIC 39828)

Notes: Colletotrichum is a causal agent of anthracnose and other diseases on leaves, stems and fruits of many plants species, including many crops of importance (Cai et al. 2009). Five species of Colletotrichumare know to occur on members ofthe Bignoniaceae. Colletotrichum dematium has already been reported causin leaf spots in D. unguis-cati in India (Farr & Rossman, 2014). Discriminating members of Colletotrichum on morphology alone has always been problematic. These have few reliable morphological features and many of them are influenced by environmental and cultural conditions. The samples collected in Brazil and Paraguay had a morphology similar to that described for C. dematium . Molecular information gathered for isolates obtained in this survey confirmed its placement in C. dematium (data not presented). This is the first report of C. demaitum causing anthracnose in plants of D. unguis-cati in Brazil and Paraguay. (Farr & Rossman, 2014).

After 8 days, plants inoculated with C. dematium showed characteristic foliage necrosis as seen in the field. A significant contrast between disease severity in the field and under controlled conditions was observed. In the greenhouse damage was weaker than in the field. We suspect that this may be because of interaction of the fungus with insects in the field but this requires further investigation.

Colletotrichum species have been used in several instances in weed biocontrol (Meyer et al 2008; Killgore et al. 1999; Peng et al 2005; Trujillo et al 1986). The fungus isolated from D. unguis-catimay have potentialfor use in classical biological control of D. unguis-cati but additional studies are needed in order to confirme that.

Colletotrichum karstti Y.LYang, Zuo Y. Liu, K.D. Hyde & L. Cai, Cryptogamie Mycologie 32: 241. 2011. Fig. 9

Lesions on leaves, initially as small circular spots, brown, surrounded by a chlorotic halo, later becoming large circular, elliptic to irregular spots, leading to necroses 10–60 mm, sometimes with dark brown margin. Internal mycelium intercellular 1–2 µm, septate, branched, pale brown. External mycelium absent. Conidiomata acervular, subepidermal, 25–100 µm wide, olivaceous brown, disrupting outer epidermal cell wall of host. Setae scarce or absent, pale brown, cylindrical, slightly swollen at base,tapering apically to subacute apex (40–80 × 2.5–5,.5), 1–3 septate. Conidiogenous cells terminal, cylindrical to. rarely ampuliform, with acute apices, 9.5–26 × 2–5 µm, hyaline. Conidia in yellow to orange mucilaginous masses, cylindrical, straight to slightly allantoid, apices rounded, 10–15 × 4.5–5 µm, aseptate, guttulate hyaline, smooth. Appressoria globose to ellipsoid, sometimes irregular, 7–10 × 5–8 µm, dark brown to olivaceous brown, smooth.

In culture: On PDA, fast-growing (6.4–7 mm diam after 7 days), flat or effuse, edges entire, aerial mycelium cottony centrally followed by periphery of powdery mycelium, white centrally to rosy at periphery, periphery of immersed mycelium, diurnal zonation present, humid centrally; apricot to ochraceous reverse. Small stromatic aggregates bearing abundant sporulation produced on surface. On PCA, fast-growing (5.5 – 6 mm diam after 7 days), flat or effuse, edge entire, mycelium cottony

centrally to scarce in the periphery, white to light rosy buff; diurnal zonation present; light apricot centrally reverse; sporulation abundant.

Material examined: BRAZIL, São Paulo, Bragança Paulista, on living leaves of D. unguis-cati 'long pot variety', 2 May 2013, A. A. Colmán. (VIC 39792). BRAZIL, Santa Catarina, Urubici, on living leaves of D. unguis-*cati* 'long pot variety', 15 May 2013, A. A. Colmán (VIC 39798). BRAZIL, Sao Paulo, Itapevá, on living leaves and stems of D. unguis-*cati* 'long pot variety', 19 April 2013, A. A. Colmán (VIC 39802). BRAZIL, Sentido Cerquillo, on living leaves of D. unguis-cati 'long pot variety',19 April 2013, A. A. Colmán (VIC 39803). BRAZIL, Minas Gerais, Viçosa, on living leaves of D. unguis-cati 'long pot variety', 8 May 2013, A. A. Colmán (VIC 39804).

Notes:Only one species of Colletotrichum was previously known to occur on D. unguis-cati-C. dematium (Farr & Rossman, 2014). Colletotrichum karstii was recently described from Vanda sp. (Orchidaceae) in China, and was also reported on several other orchidsas a pathogen causing dark brown to black, ellipsoid lesions on leaves and was also isolated as an endophyte from roots (Yang et al. 2011).

The fungus on D. unguis-cati clearly fits the morphological delimitation of C. karstii.Thispathogen occurs on many host plants and is the most common and geographically diverse species in the C. boninense complex (Damm et al. 2012). In addition, C. karstii was isolated from grape (Vitis vinifera), chili (Capsicum spp.) and tomato (Lycopersicon esculentum) associated with anthracnose in China (unpublished data), and this suggested this taxon has a wide range of hosts (Yang et al. 2011).

This species was frequently found in all Brazil and Paraguay during the survey. This is the first report of Colletotrichum karstii causing leaf spots on M. ungui-cati worldwide. (Farr & Rossman, 2014).

Eight days after inoculation, the first symptoms observed were small spots that quickly led to the formation of necrotic area. In the field conditions, it caused large necrotic lesions on leaves of D. unguis-cati and was particularly damaging to the host. The broad host range (including species of agricultural importance) would prevent further considerations about the use of this fungus for biological control. Nevertheless, the possibility of host-specificity within C. karstii at the forma specialis level must be evaluated before a final decision.

Myrothecium roridum Tode, Fung. macklenb. sel. (Lunerburg) 1: 25 (1790). Fig. 10

Lesions on living leaves and other parts of the plant, pale brown necrotic spots which may eventually lead to infected tissue being shed causing shot-hole symptoms. External mycelium absent. Internal mycelium indistinct. Stromata absent. Conidiophores, cylindrical, 1–2 μm diam. Sporodochia sessile, up to 1,5 mm diamenter, at first green, later black with a white margin. Setae present unbranched (80 –105 × 1–1.5 μm). Conidiogenous cells monophialidic, discrete, cylindrical, 14–34 × 1.5–2 μm. Conidia aggregated in slimy masses, cylindrical with rounded ends, hyaline topale olive, green to black in mass, mostly 5.5–7.5 × 1 –2 μm.

In culture: on PDA, slow- growing (6 – 6.5 cm diam after 10 days), slightly convex, edge entire, aerial mycelium cottony to felty, white to light rosy vinaceous, centrally with olivaceous blackdroplets, corresponding to sporulation, periphery of immersedmycelium; humidity centrally, pronounced diurnal zonation; reverse saffron; sporulation abundant. On PCA, (5.5 – 6 cm diam after 10 days), slightly convex, edge entire, aerial mycelium cottony to felty, white to rosy buff, centrally with small olivaceous blackdropscorresponding to sporulation, periphery immersed, humid centrally, with subtle diurnal zonation; reverse light saffron; sporulation abundant.

Material examined:BRAZIL, Minas Gerais, Viçosa, on living leaves of D. unguis-cati 'short pod variety', 23 February 2013, A. A. Colmán (VIC 39801). BRAZIL, Minas Gerais, Paraopeba, on living leaves of D. unguis-cati 'short pod variety', 23 July 2013, A. A. Colmán (VIC 39800)

Notes: The genus Myrothecium Tode (Sordariomycetes) was originally proposed by Tode in 1790 having as type species Myrothecium inundatum. Sixteen species are currently accepted for the genus Myrothecium (Kirk et al. 2008). Several of the most common species are polyphagous causing spots on leaves or other parts of different living plants or grow on decaying plant tissue (Ellis, 1971; Sutton 1985).

Biometric data showed that our isolated belong to the species M. roridum, which has been reported causing leaf spots in Campsis radicans(L) Seem (Bignoniaceae) in Texas (Anonymus, 1960). This is the first report of M. roridum causing leaf spots on D. unguis – cati, in Brazil and Paraguay (Farr & Rossman, 2014). Although other species of Myrothecium (as M. verrucaria) have been investigated as potential mycoherbicide against several weed species with very satisfactory results (Anderson & Hallett, 2004),

this is not the case for M. roridum because it is a highly polyphagous fungus which attacks crops of agricultural importance.

Phoma sp. nov. Fig. 10

Lesions on living leaves, amphigenous, subcircular, well delimited, infected tissue initially dark brown surrounded by a pale brown halo, centrally with dots that correspond to areas where picnidia accumulates, 10 – 40 mm coalescing and leading to blight of extensive areas on leaves. External mycelium absent. Internal mycelium indistinct. Conidiomata pycnidial, amphigenous, subcuticular, group scattered in necrotic tissues, subglobose, , 60–170 × 65.5–92 µm, walls of brown textura angularis; dehiscence ostiolate. Conidiophores reduced to the conidiogenous cell, enteroblastic, lageniform or ampulliform, 5 – 10 × 1.5 – 3 µm, hyaline, smooth. Conidia ellipsoid to sub cylindrical, straight to slightly curved, 5–7 × 2–3µm, aseptate, ends rounded, with 2 small polar guttules, subhyaline, smooth.

Material examined:PARAGUAY, Cordillera, Caacupe, on living leaves of M. unguis-cati 'short pod variety', 23, May 2013, A. A. Colmán (VIC 39807). PARAGUAY, Cordillera, Piribebuy, on living leaves of M. unguis-cati 'long pod variety', 23, May 2013, A. A. Colmán (VIC 39808). BRAZIL, Minas Gerais, Paraopeba, on living leaves of M. unguis-cati 'long pod variety', 23, July 2013, A. A. Colmán (VIC 39825).

Notes:Phoma is a coelomycete genus characterized by having hyaline, unicellular conidia that may become septate due to secondary septation, phialidic, ampulliform to dolliform conidiogenous cells and (sub) globose, glabrous to pilose or setose, pseudoparenchymatous or scleroplectenchymatous pycnidia. (Gruyter, 2010).

The generic name Phoma was originally reserved for plant pathogenson stem, but nowadays the genus comprises pathogens, opportunistic as well as saprophytic species on a much wider range of substrates (Aveskamp, 2008). The nearly 220 Phoma species that are currently recognized were classified in nine sections according to pycnidial, conidial and cultural characters (Boerema et al. 2004).

Only one specie of Phoma has been recorded on D. unguis-cati. This was described based on specimens collected on D. unguis-cati in Cuba and West Indies (Farr & Rossman, 2014). Five Phoma species are known to attack Bignoniaceae representatives, but all are dissimilar to the fungus collected on D. unguis-cati in Brazil

and Paraguay. Conidial sizes was used to distinguish this species from other, Phoma sp. nov. have larger conidia,while Phoma botryoidea Gz. Frag. (3 –6 × 3 – 2 µm) and Phoma anemopaegmae Gz. Frag (3 –4 × 1 µm) have lower conidia. This is recognized here as a taxonomic novelty for the genus whichwill be published as new in the future.

This fungus only found at three occasions on cat's claws during this survey (in Brazil and Paraguay). Inoculation performed under controlled conditions did not result in any symptom development. Further studies are necessary to clarify the potential of this fungus as a biocontrol agent.

Ramulariopsis sp. nov. Fig. 11

Leaf spots amphigenous, subcircular to irregular, 1-10 mm wide, medium to dark brown, ocacionally somewhat zonate, margin indefinite or darker. Internal mycellium indistinc. External mycelium absent. Stromata absent or small and composed of only a few substomatal sub-hyaline swollen cells. Conidiophores hyphophyllous emerging through stomata, solitary or forming loose fascicles of few conidiophores, usually dense, subcylindrical, straight to slightly curved or sinuose, sometimes restricted to the conidiogenous cell, 10–60 × 2.5–4 µm, 0–5 septate, branched, sub-hyaline, smooth. Conidiogenous cell integrated terminal, 13–16 × 2.5–3,0 µm, sub-hyaline. Conidiogenous loci conspicuous, 1-3 per conidiogenous cell, 1–1.5 µm wide, slightly thickened. Conidia dry, in simple chains, narrowly ellipsoid-ovoid, fusiform to cylindrical, 8–40 × 2.5–4 µm, 1–6 septate, smooth, ends obtuse to subacute.

In culture: on PDA, slow-growing (1–1.5 cm diam after38 days), edge entire, slightly convex to flat, aerial mycelium scarce felty, centrally white to vinaceous buff, periphery of superficial mycelium; slightly humid centrally; hazel to honeyreverse; no sporulation. On PCA, slow growing (1.5 cm diam after 38 days), edge entire to lobate, white to lavender grey centrally, felty, periphery composed of superficial mycelium. Honey reverse; no sporulation.

Material examined: PARAGUAY, Central, San Lorenzo, on living leaves of D. unguis-cati 'long pod variety', 20, May 2013, A. A. Colmán (VIC 39805). PARAGUAY, Central, Capiatá, on living leaves of D. unguis-cati 'long pod variety', 23, December 2013, A. A. Colmán (VIC 39833).

Notes:The genus Ramulariopsis Speg.(Mycosphaerellaceae, Ascomycota) comprises moniliaceous hyphomycetes, most of which are plant pathogens causing leaf spots, but occasionally also saprobic. The conidiophores of Ramulariopsisare mostly fasciculate, arising from internal hyphae or stromata, through stomata or erumpent, hyaline, septate smooth, simple or branched and the conidiogenous cells are integrated, terminal, intercalary, subcylindric, poliblastic, sympodial, cicatrized. The conidia catenate, formed singly or in chains, ellipsoid-ovoid, 0–1, to pluriseptate. (Braun, 1998). No Ramulariopsis species are known in association with D. unguis-cati. This species is dissimilar to the fungus collected on D. unguis-cati

Ramulariopsis sp. was found only twice during this survey and only in Paraguay. Symptom observed on leaves were not very conspicuous and damage caused by the fungus do not appear significant. Inoculations performed under controlled conditions did not result in any symptom development. Such a failure may have been caused by the use of mycelium as inoculum (which might be not infective), the fungus having lost pathogenicity in culture, incompatible host biotype/fungus strain association among others. At this stage, Ramulariopsissp. appears inadequate for use in biological control of D. unguis-cati. This is a new species of Ramulariopsis sp., which will be published later.

Prospodium macfadyenae Meir. Silva, O.L. Pereira & R.W. Barreto. Mycotaxon. 119:15 (2012). Fig. 13

Description: See in: Silva M, Barreto RW, Pereira OL. 2012. Fungal pathogens of 'cat's claws' from Brazil for biocontrol of Macfadyena unguis-cati. Mycotaxom. 119, 181-195

Material examined:BRAZIL, Paraná, Jaguanaúva, Itarare, on living leaves of M. unguis-cati 'short pod variety', 19 April 2013, A. A. Colmán (VIC 39799)

This rust species was only collected once during this survey and was not found to be abundant in the field. Its presence was only noticed during the screening of samples collected at the locality because of attack by other fungi. Although Silva et al. (2012) considered the potential of this species for biocontrol as high in the context of the present paper this do not appear to be the case.

Uropyxis rickiana Magnus, Hedwigia 45: 176. 1906(= **Uropyxis reticulata** Cummins, Mycologia 31.171. 1939). Fig. 14

Galls up to 10 cm diam 25 – 30 cm long formed on stems, smaller on pods and leaves, interfering with seed formation; on leaves producing small amphigenous galls associated to the formation of slight depression on the reverse.Spermogonia amphigenous, type 7, subepidermal, erumpent, in groups, forming on small distorted convex portion of leaves. Aecia developing around spermogonia, dark brown, without paraphyses**;** aeciospores borne singly on pedicels, variable in size and shape, mostly obovoid, 25 –30 × 20 –28 μm , wall 1.5 –3 μm thick, cinnamon to chestnut brown, reticulate with meshes 2–3 μm diam, germ pores,two, equatorial on slightly flattened sides. Uredinia hypophyllous, small, scattered, not associated to hypertrophic tissues, dark brown, without paraphyses; urediniospores mostly obovoid, 26 –37.5 × 20 – 34μm, wall 2–2.5μm thick, cinnamonbrown, reticulate as for aeciospores, germ pores,two, equatorial.Telia associated with spermogonia and aecia, brown, formed in fissures of the galls and on leaves with uredinia; teliospores mostly broadly oblong-ellipsoid, 81– 175× 25-37.5μm, wall 2.5– 4μm thick, chestnut brown, inconspicuously bilaminate in lactophenol mounts, verrucose,germ pores two on each probasidial cell, equatorial; pedicel subcylindrical thick-walled, rugose totowards the base with spiral ornament, 50 – 112. 5 – 5-7 μm.

Material examined: BRAZIL, Rio Grande do Sul, Nova Petropolis, on living leaves and stems of D. unguis-c*ati* 'long pot variety', 11 November 2012, R. W. Barreto (VIC 39837).BRAZIL, Paraná, Itararé, on living leaves, pod and stems of D. unguis-cati 'long pot variety', 19 April 2013, A. A. Colmán (VIC 39801).BRAZIL, Paraná, Itapevá, on living leaves and stems of D. unguis-cati 'long pod variety', 19 April 2013, A. A. Colmán (VIC 39802). PARAGUAY, Caazapa, General Morinigo, on living leaves and stems of D. unguis-cati'long and short pot variety', 27 June 2013, A. A. Colmán (VIC 39811). BRAZIL, Rio Gande do Sul, Nova Petrópolis, on living leaves and stems of D. unguis-cati 'long pot variety', 13 January 2013, R. W. Barreto (VIC 39837)

Notes: Two species of Uropyxis have been described on D. unguis-cati: U. rickiana Magnus and U. reticulata Cummins but these are now considered to be synonyms. Uropyxis rickiana is an autoecious macrocyclic rust which causes infections through basidiospores formed on metabasidia originating from teliospores, but also through uredinospores and aeciospores. Such infections lead to the formation of galls on

stems, leaves and pods. This species is distinguishable from other species by have uredinosporos wall reticulate and by the presence of two germinal pore probasidial in each cell.

Uropyxis rickiana causes conspicuous galls that are easily spotted where the rust occurs. The galls can be rather large on older stems. On the galls, telia and uredinia can be found, whereas spermogonia are found on the leaves.

Hernandez & Hennen (2003) reported that all other species of Uropyxis are know for attack members of the Fabaceae and none have reticulate uredinispore walls and the discretely layered teliospore walls as in U. rickinana. Such features onlyoccuring on rusts on the Bignonicaceae. These authors suggest that U. rickianamay be misplaced in Uropyxis being kept in this genus only because of the presence of two germ pores in each probasidial cell. It is possible that U. rickiana in fact represents an independent monotypic genus separate from Uropyxis. We are in agreement with Hernandez and Hennen's views. Nevertheless it is regarded here as better to await for molecular evidence separating U. rickiana from related taxa before a new genus is proposed. Uropyxis rickiana has been reported on D. unguis-cati in Brazil and Argentina, (Hernandez & Hennen 2003; Hennen et al. 2005). However this is the first report of this fungal species in Paraguay.

Inoculations of U. rickinana have been made on both varieties ofD. unguis-cati (short and long pod), and after 20 days small necrotic lesions appeared on leaves and 30 days after inoculation teliospores and uredinospores were observed on inoculated plants of both varieties. The preliminary host range test indicated that U. rickiana is specific toD. unguis-cati. None of the other 20 plant species, belonging to ten different families, involved in the host range test became diseased after inoculation with U. rickiana. At this stage U. rickiana appears to have an excellent potential for use in classical biocontrol of cat's claws.

Discussion

A fundamental step in any classical or inundative biological control project involves the search and selection of potential biological control agents for potential use. Brazil is part of the origin of many important invasive plant worldwide, and many studies on the micobiota of selected invasive plants has been made (Alves, et al., 2008;

Barreto & Evans 1994, 1995; Silva et al. 2014, Pereira & Barreto 2000; Pereira et al., 2007; da Silva et al., 2012). Such searches normally start in literature reviews. A list of fifteen fungi likely to be pathogenic to D. unguis-cati have been reported in the the literature (Table 3).Here this list is expanded to twenty-two species. It is worthy of notice that many of the fungi included in Table 3 are of no interest for classical biocontrol either because of being weak pathogens (such as the three species of Meliola which cause black mildew – usually a minor/benign disease) or polyphagous pathogens that attack important crops such as Phymatotrichum omnivorum.Interestingly, promising agents found among the existing list and the novel taxa found on D. unguis-cati during our surveys are either cercosporoid fungi, anthracnose-causing fungi or rust fungi.

The cercosporoids Passalora macfadyenae and Passalorasp.nov. appear promising biological control agent although initially P. unguis-cati not having been considered useful (Silva et al., 2012). During this new round of survey it was observed that this pathogen can cause severe lesions on young plants, unlike P. macfadyenae and Passalora sp. nov. which were observed only attacking adult plants. Preventing the establishment of young plants is particularly useful in weed biocontrol. Complementary studies are being conducted to evaluate the potential of these fungi.

Rust fungi have a long and favorable history in weed biocontrol with some spectacular results obtained from their introduction against major invasive weeds including the first examples of the use of plant pathogens in classical biocontrol of weeds Puccinia chondrillina Bubak & Sidenham against Chondrilla juncea L. (Cullen et al. 1973) and Phragmidium violaceum against Rubus constrictus (Oehrens, 1977) and also Maravalia cryptostegiae (Cummins) Ono used in Australia to control rubbervine Cryptostegia grandiflora Roxb. Ex R. Br (Evans et al. 2001) and Puccinia myrsiphylli G.Winterwhich was introduced in Australia with great success for the control of Asparagus asparagoides (L.) W. Wight (Morin & Edwards, 2006). These fungi are often considered to be the first choice fungal pathogens to be deployed against invasive weeds. Gall causing-rusts have also demonstrated their value in the spectatular example of the classical introduction of Uromycladium tepperianum (Sacc.) McAlpine that led to the successful control of a “difficult” target Acacia saligna (Labill.) Wendl. an invasive alien shrub from Australia that was widely established in South Africa causing great

environmental damage (Morris, 1987, Wood & Morris, 2007; Wood 2012). Uropyxis rickiana is therefore the natural choice as a priority agent to concentrate studies upon for possible later introductions into Australia, South Africa and other regions where cat's claw became a noxious invader.

Little is known about the biology this fungus but circumstantial and experimental evidence indicate that this rust species is both autoecious and host-specific, which are critical requirements for a rust to be used in classical biocontrol. The mycobiota described herein may represent only a fraction of the existing diversity of fungal pathogens on this host as only part of the distribution range of this plant was covered during the two survey rounds (da Silva et al. 2012 and this work). It is likely that an expansion of the survey, in Brazil, Southern Paraguay and Argentina will significantly increase the list of fungi associated to D. unguis-cati and maybe uncover additional fungi with biocontrol potential.

Acknowledgements

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Tables

Table 1. List of plants used in host specificity tests

Order	Family	Genus	Results
	Achantaceae	Pachystachys sp.	-
		Thumbergia sp.	-
		Adenocalymmba sp.	-
		Cuspidaria sp	-
		Friedericia sp.	-
		Jacaranda sp.	-
	Bignoniaceae	Dolichandra unguis-cati "shor pod"	+
		Dolichandra unguis-cati "long pod"	+
		Pandorea sp.	-
Lamiales		Spathodea campanulata	-
		Pyrostegia sp.	-
		Tabebuia sp.	-
		Tecoma sp.	-
		Episcia sp.	-
	Lamiaceae	Origanum sp.	-
	Oleaceae	Jasminum sp.	-
	Pedaliaceae	Sesamum sp.	-
	Plantaginaceae	Plantago sp.	-
	Scrophulariaceae	Nemesia sp.	-
Solanales	Solanaceae	Solanum sp.	-
Ericales	Ericaceae	Azalea sp.	-

+ (Positive)
− (Negative)

Table 2. Biometric data for Passalora species recorded on members of the Bignoniaceae.

Species	Conidia (µm)	Stromata(µm)	Conidiophores (µm)
P. adenocalymmatis [a,b]	351–50 × 03–4,5	40–60	4–5.5 × 20–65
P. arrabidaeae [a,b]	35–150 × 3.5 –5	Absent	4–6 × 10–75
P. catalpae [a,b]	40–120 × 2.5 –4,5	0–50	3–5.5 × 10–125
P. catalparum [a,b]	35 –125 × 3.5– 6	absent	4–7.5 × 15–70
P. leprosa [a,b]	30–80 × 3.5 –6	300–500	5–8 × 40–150
P. macfadyenae [c]	31.5–114 × 3– 4.5	63–142 ×35–95	13–6 × 16–114
P. markhamiae [d]	16–92.5 × 2.5–55.5	15–60	16–92.5 × 2.5–55.6
P. pyrostegiae [a,b]	25–170 × 3 – 4.5	40–80	4–6 × 10–45
P. tabebuiae [e]	(6–) 8–30 (34) × 2.5– 3	absent	2.5-3.2 × 0–50
P.tabebuiae-ochraceae [f]	31–75 × 5–8	24–130	5–8 × 31–75
Passalora sp. nov. [g]	25–150 × 2.5–35	absent	3–4 × 22–175
P. tecomariae [b]	35–90 × 5–6	absent	5–6 × 35–90

a. Chupp (1954)
b. Crous and Braun (2003)
c. Da Silva et al. (2012)
d. Liu & Guo (1982)
e. Muchovej & Ferreira (1981)
f. Inacio & Dianese (2006)
g. This paper

Table 3. Fungi recorded on Dolichandra unguis-cati worldwide*

Fungi	Distribution
Cercospora duplicata Ellis & Everh (1989)	Barbados
Cercosporella unguis-cati Speg. (1911)	Argentina
Colletotrichum dematium Pers. Grove. (1918)	India
Diplodia catalpae Speg.. (1879)	Portugal
Glomerella cingulata (Stoneman) Spauld. & H. Scherenk. (1903)	Barbados
Meliola bidentata Cooke. (1882)	Puerto Rico, Virgin Islands, West Indies
Meliola stevensiana Cif. (1954)	Trinidad & Tobago
Meliola thaxteri Hansf. (1961)	Ecuador, Trinidad & Tobago, West Indies
Phoma sp.	Cuba, West Indies
Phymatotrichum omnivorum Duggar. (1916)	Texas
Pseudocercospora unguis-cati (Speg.) Braun (1994)	Argentina, Brazil
Mycena citricolor (Berk.& M.A. Curtis) Sacc. (1887)	Venezuela
Passalora macfadyenae Meir. Silva, O. L. Pereira & R.W. Barreto (2012)	Brazil
Prospodium macfadyenae Meir. Silva, O. L. Pereira & R.W. Barreto (2012)	Brazil
Uropyxis rickiana Magnus (1906)	Argentina, Brazil

*Adapted from Farr & Rossman, 2014

Figures

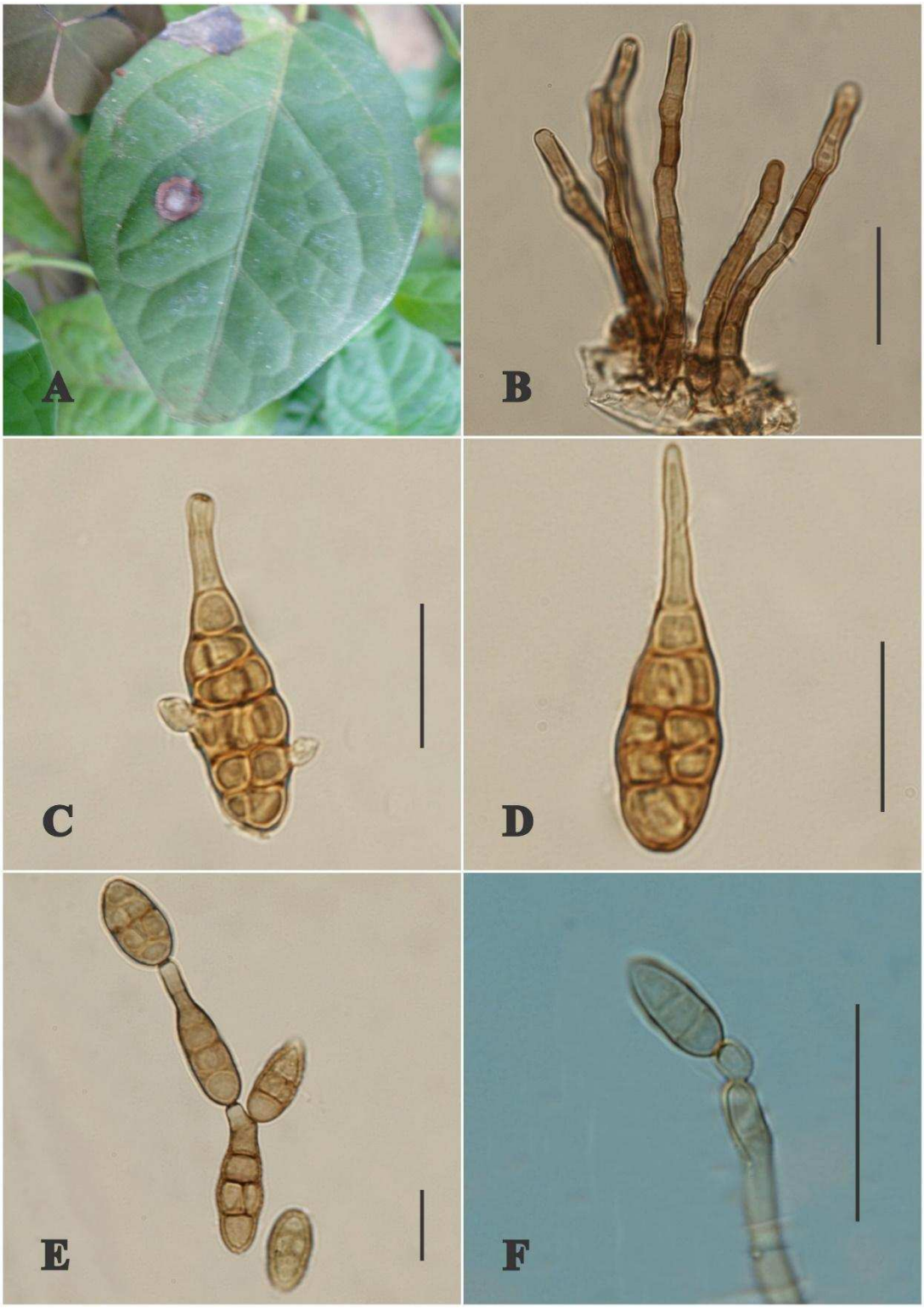

Fig.1: Alternaria alternata on Dolichandra unguis-cati.**A.** Leaf spot symptons. **B.** Conidiphores dark **C.** Germinating conidium - **D.** Obclavate muriform conidium. **E.** Chain of conidia. **F.** Conidium still attached to the conidiophore. Bars= 20 µm.

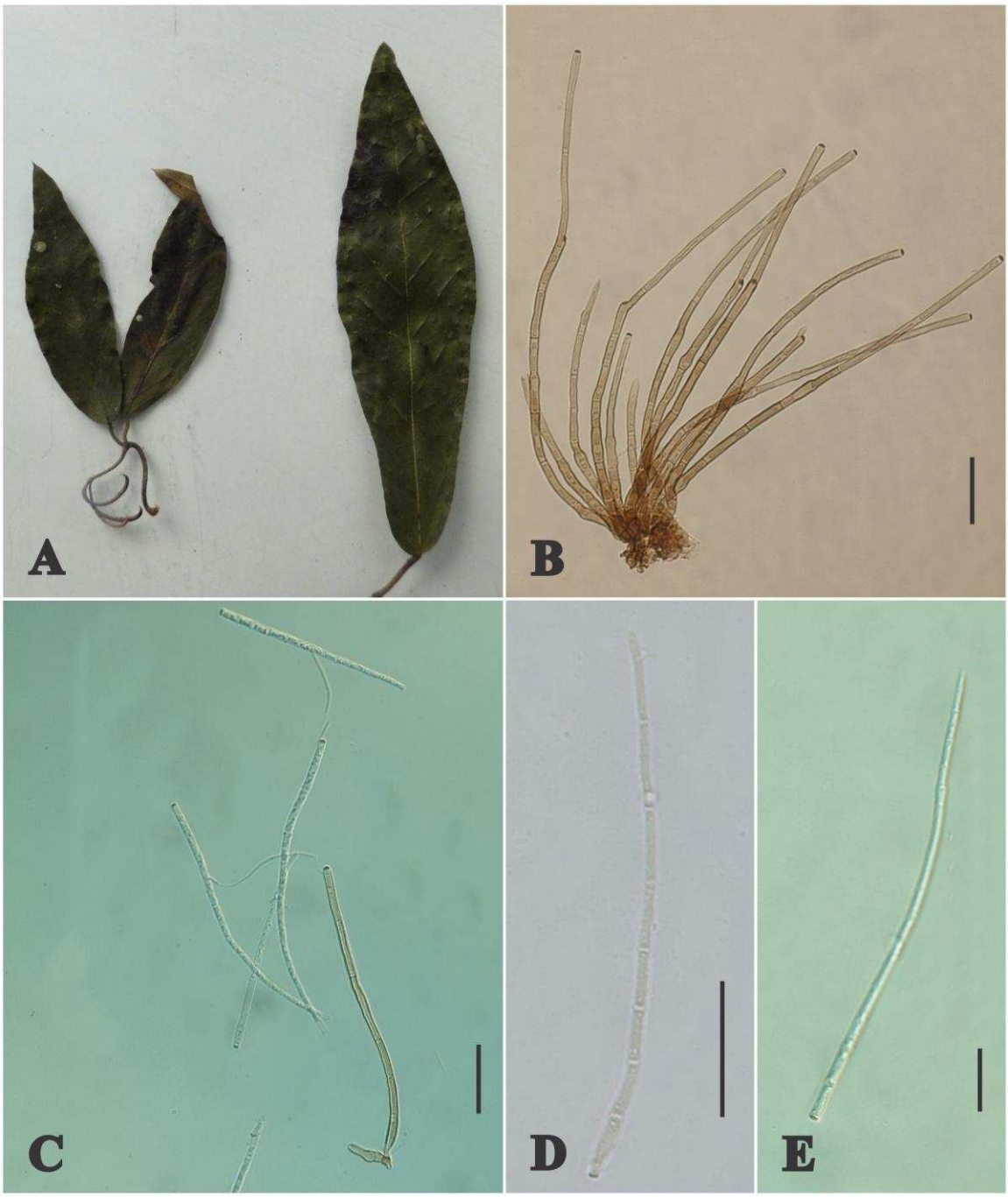

Fig. 2: Cercospora apii on Dolichandra unguis-cati.**A.** Symptons of leaf spots **B.** Conidiophores fasciculate. **C.** Conidiophore and conidia. **D-E.** Filiform conidia. Bars= 20 µm

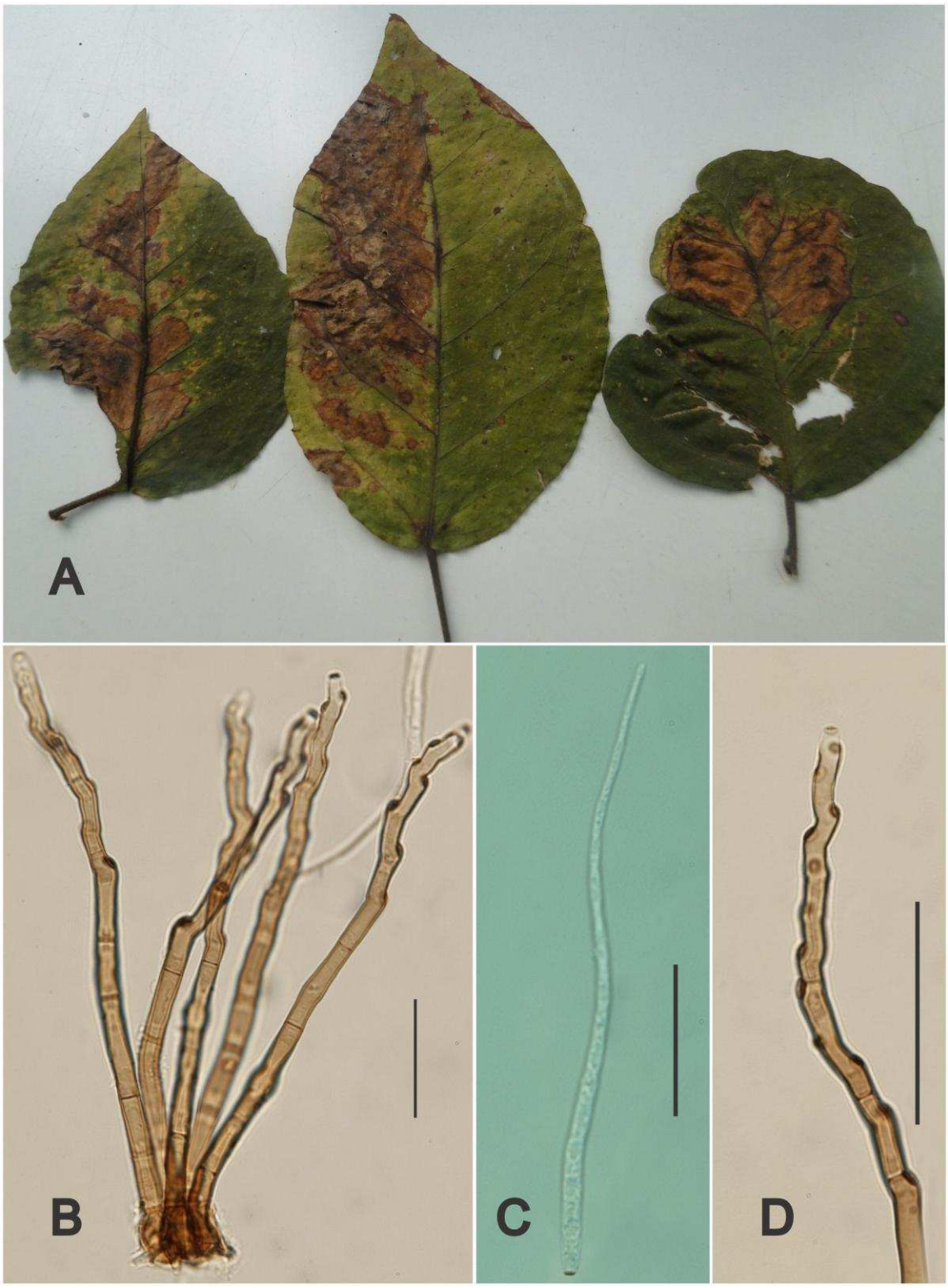

Fig.3: Cercospora rodmanii on Dolichandra unguis-cati. **A.**Leaf spot symptons **B.** Conidiophores fascicle **C.** Filiform conidia. **F**. Conidiogenous cell and loci. Barrs= 20 µm

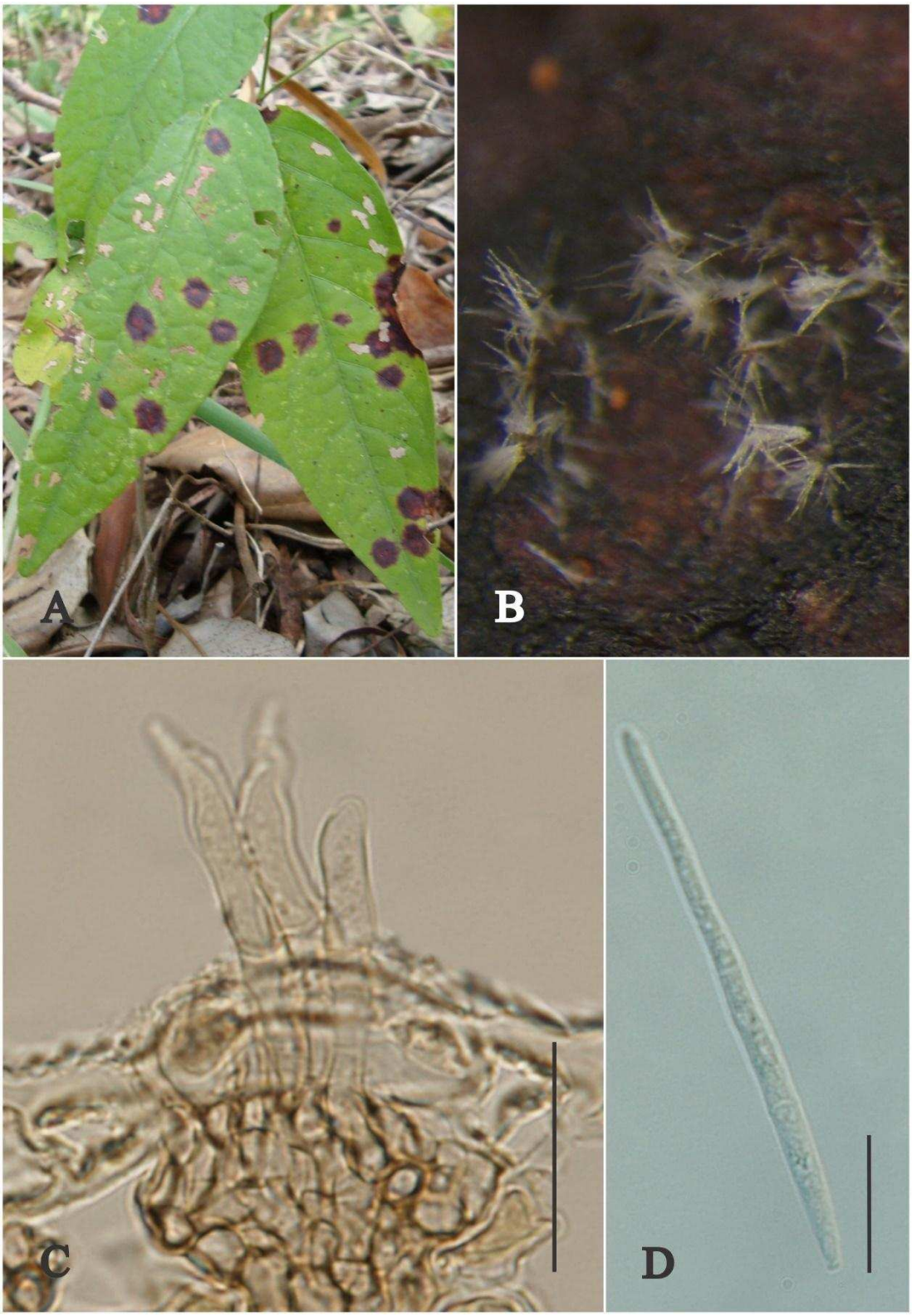

Fig. 4: Pseudocercospora unguis-cati on Dolichandra unguis-cati.**A.** Symptons of leaf spots on young plant. **B.** White tufts of conidia on foliar surface**. C.** Conidiophores arising through stoma. **D.** Filiform conidia. Bars= 20 µm

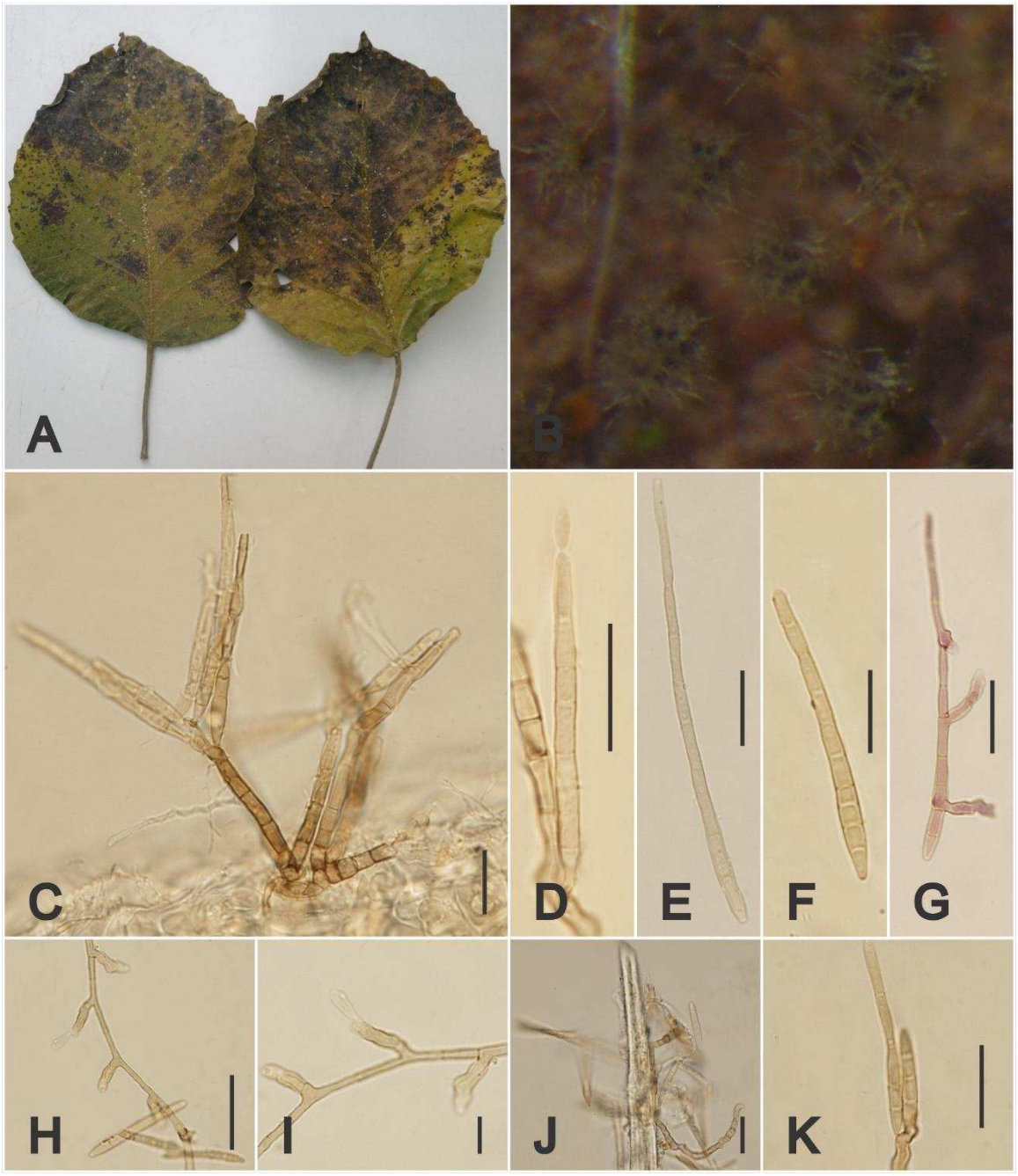

Fig. 5: Passalora sp. nov. on Dolichandra unguis-cati**. A.** Leaf spot symptons .**B.**Tufts of mature and immature conidia on foliar surface. **C.**Conidiophores arising through stoma. **D.** Conidial chain. **E-F**. Filiform conidia. **G.** Germinating conidia forming secondary conidiophores. **H-I.** Superficial mycelium and conidia. **J.** Conidiophores formed on trichomes. **K.** Conidiogenous cell bearing conidia. Bars= 20 µm

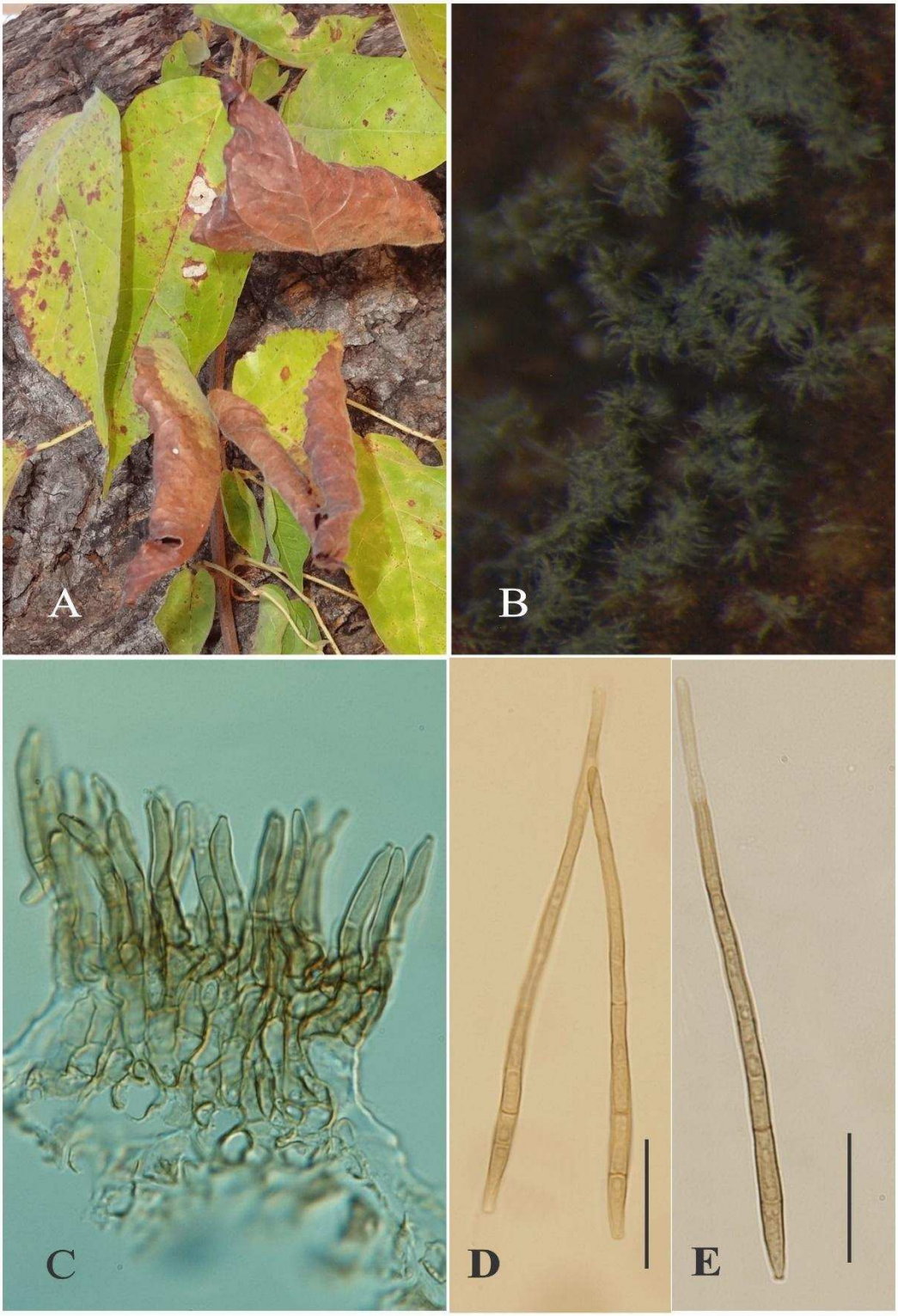

Fig. 6: Passalora macfadyenae on Dolichandra unguis-cati.**A.** Leaf blight.**B.**Tufts of mature and immature conidia on foliar surface. **C.** stroma and conidiophores. **D-E.** Filiform conidia. Bars= 20 µm

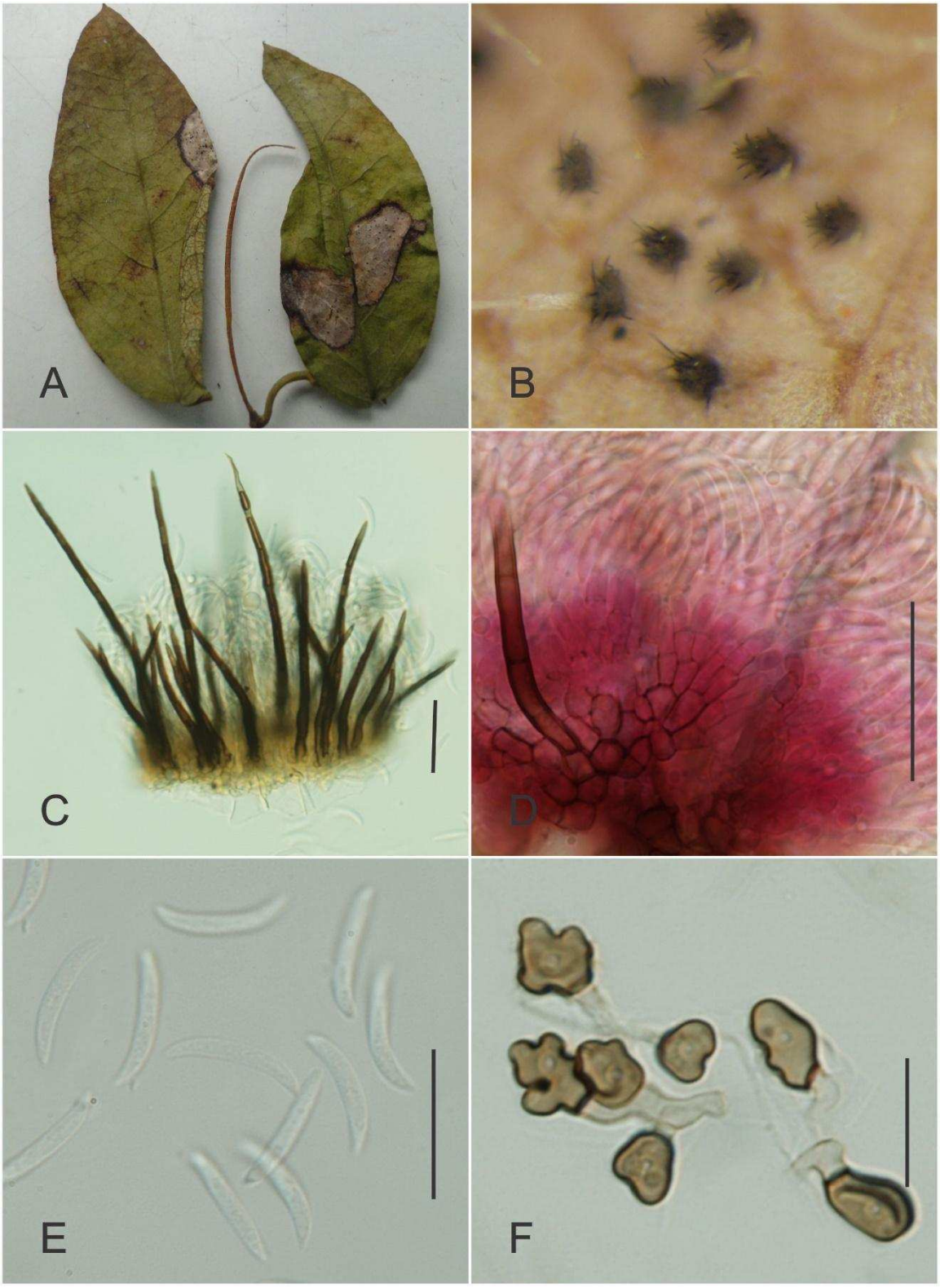

Fig. 7: Colletotrichum dematium on Dolichandra unguis-cati.**A.** Antracnosesymptons. **B.** Acervuli on foliar surface. **C.** Section through acervulus. **D.** Conidiogenous cell and conidia. **E.** Falcate conidia. **E.** Apressoria. Bars= 20 µm

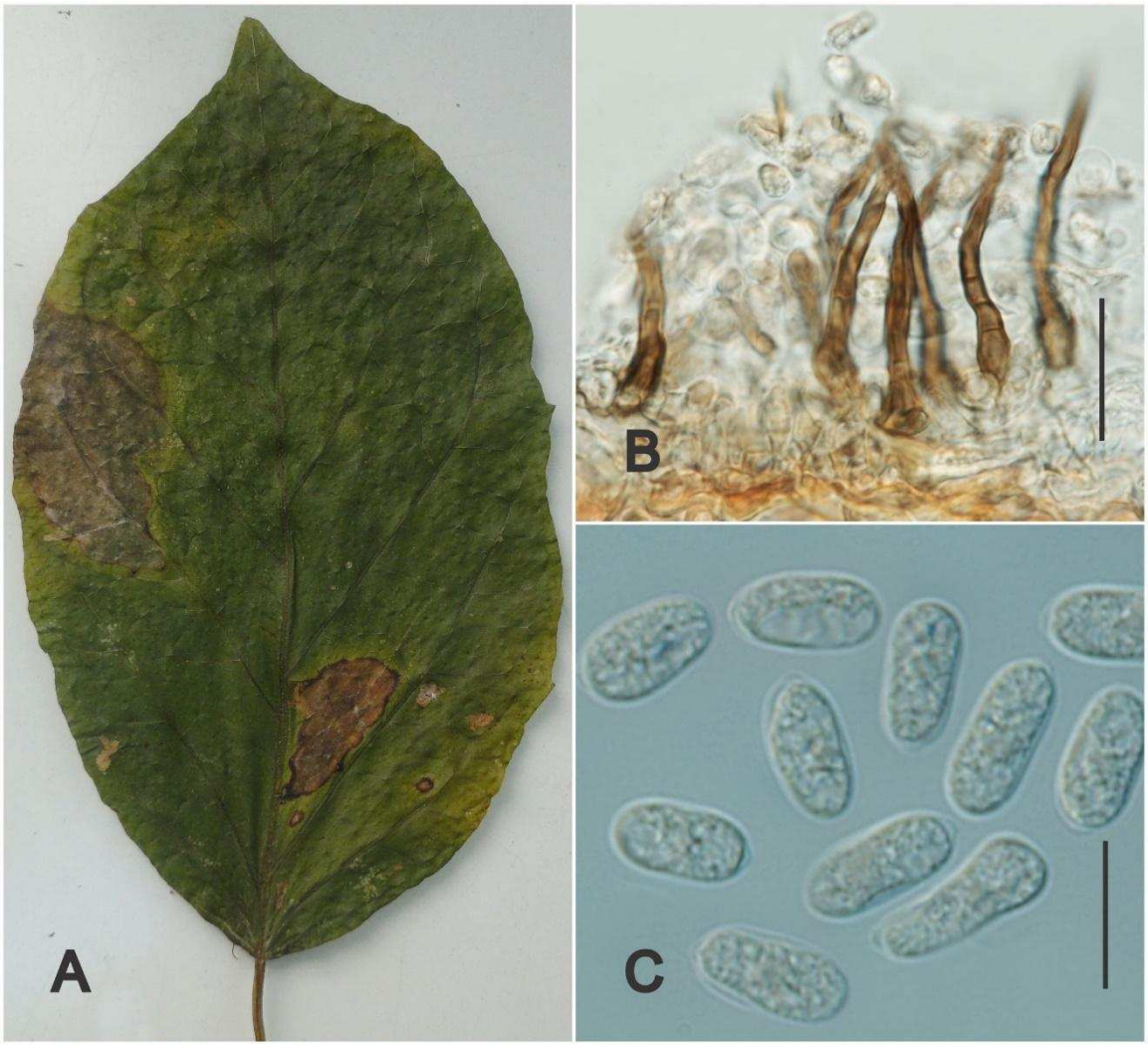

Fig. 8: Colletotrichum karstii on Dolichandra unguis cati.**A.** Anthracnose symptons on leaf. **B.** Section through acervulus**. C.** Oblong conidia**.** Bars= 20 µm

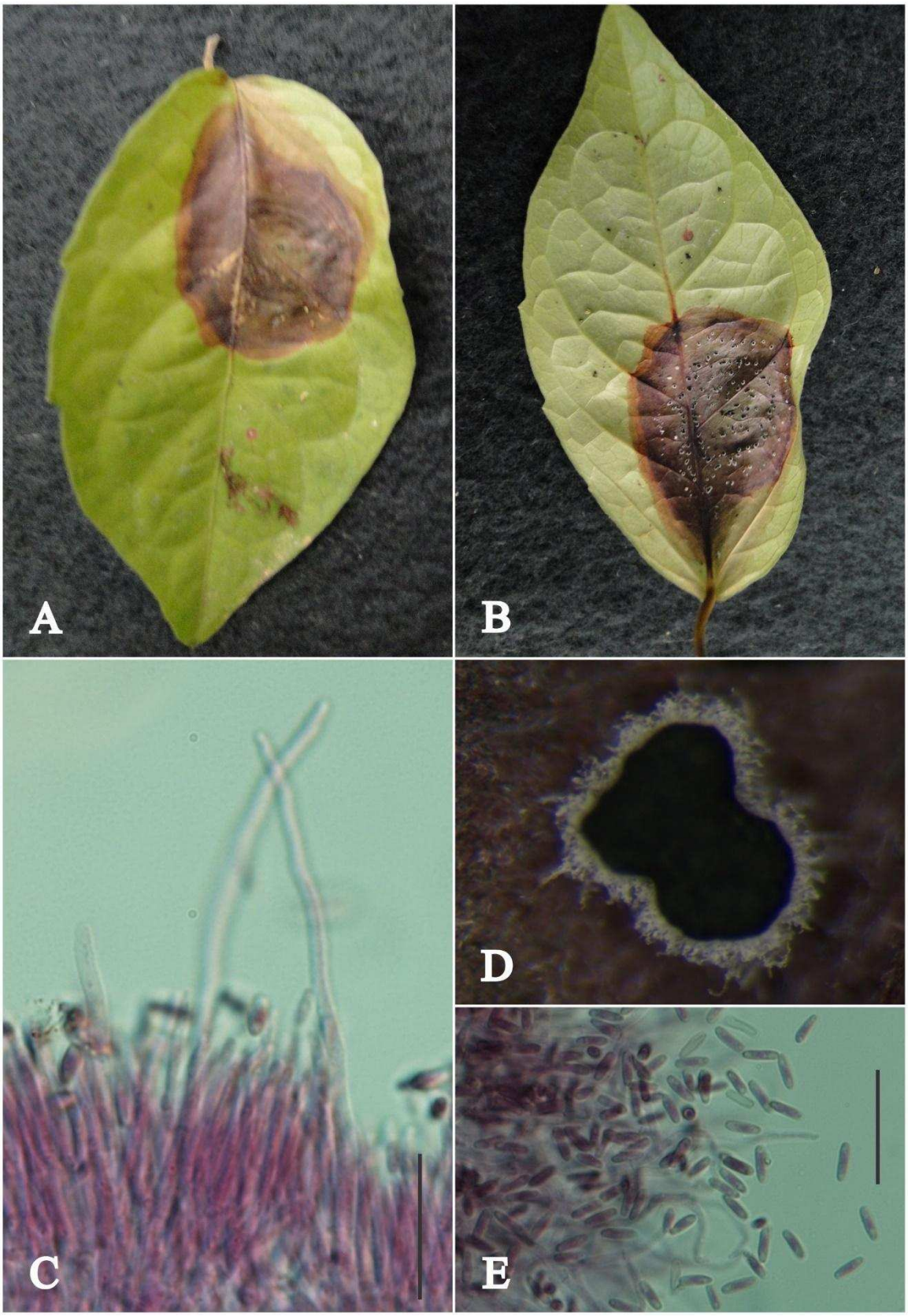

Fig. 9: Myrothecium roridum on Dolichandra unguis-cati.**A-B.** Leaf spot symptons. **C**. Conidiophores and setae. **D**. Sporodochium on leaf surface. **E.** Conidia . Bars= 20 µm

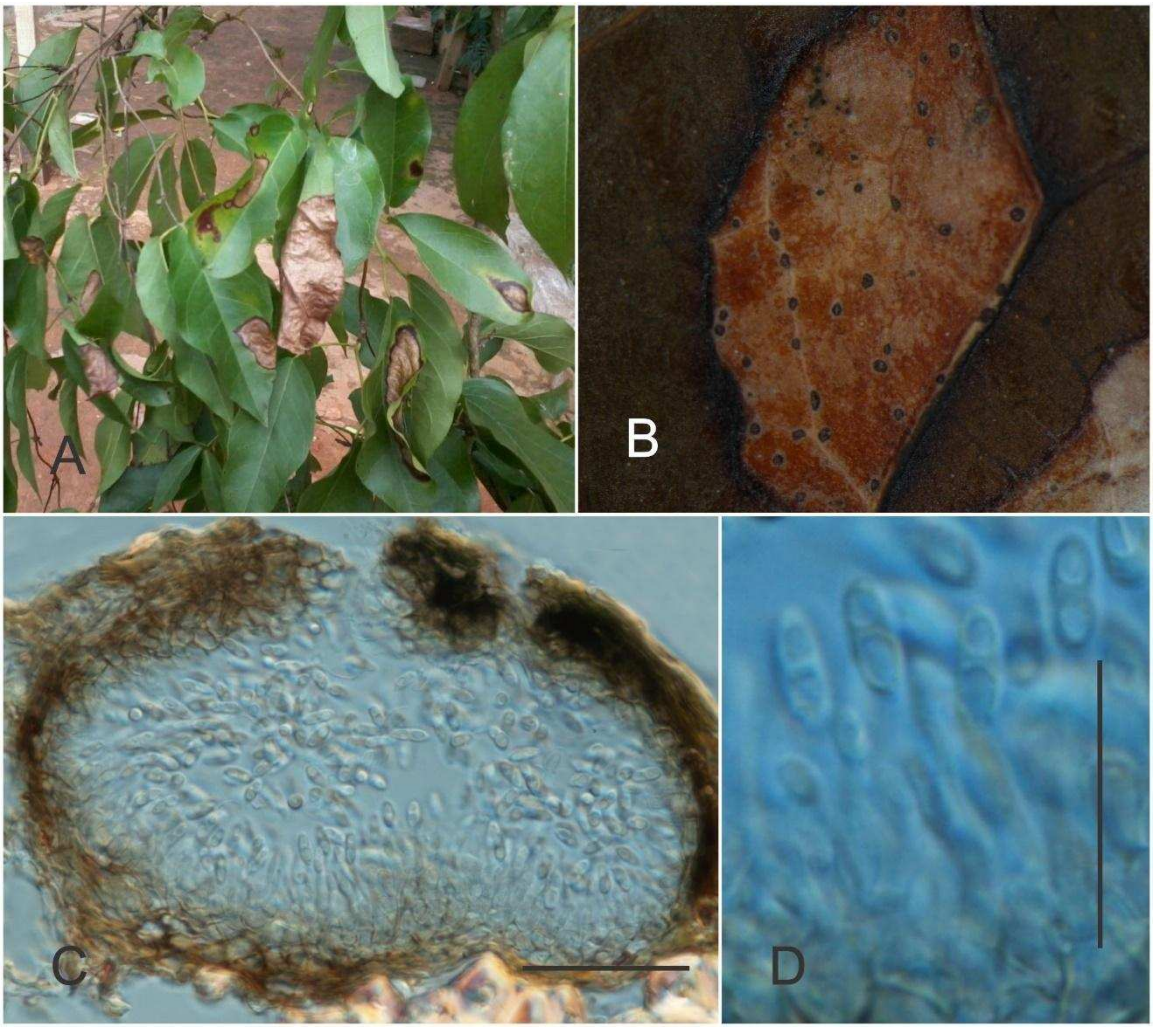

Fig. 10: Phoma sp. nov. on Dolichandra unguis-cati. **A.** Blight symptons of leaves. **B**. Leaf spot bearing several pycnidia. **C.** Section through pycnidium. **D**. Conidiogenous cells and conidia. Bars= 20 µm

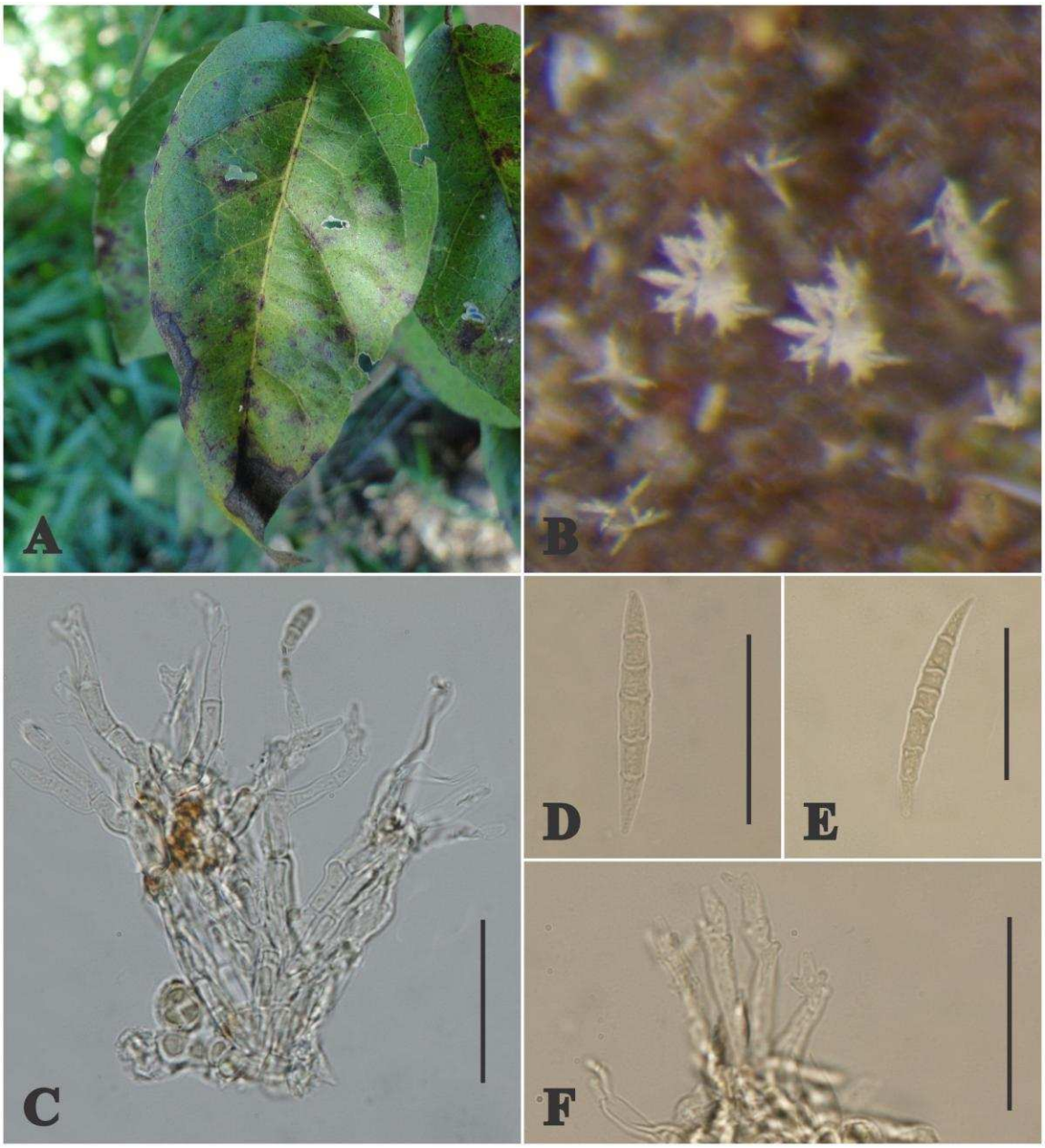

Fig. 11: Ramularia sp. nov. on Dolichandra unguis-cati. **A.** Infected leaf. **B**. White tufts of conidia on leaf surface. **C.-F**. Unbrached and branched conidiophores. **D-E.** Conidia dry. Bars = 20 µm

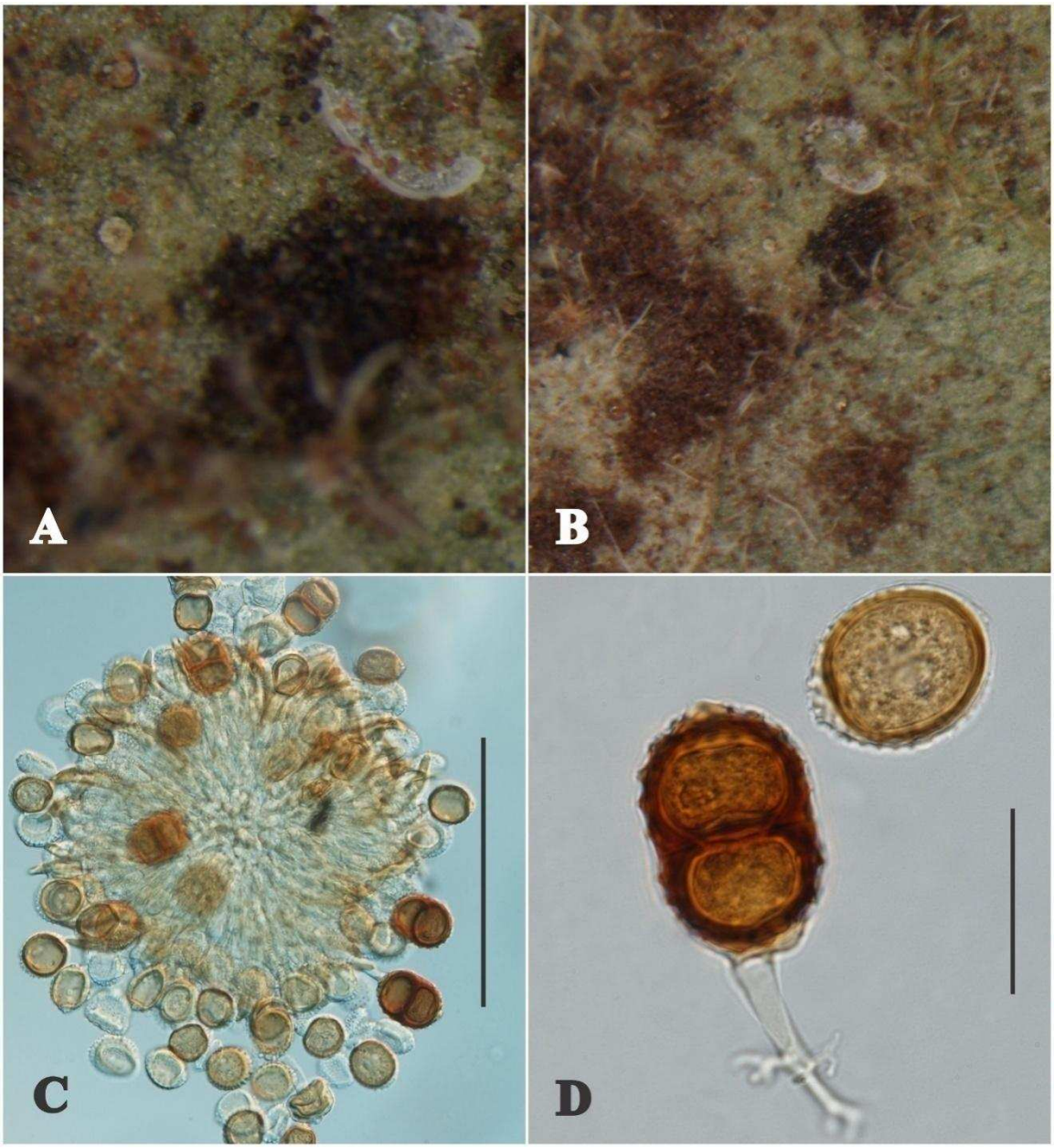

Fig. 13: Prospodium macfadyenae on Dolichandra unguis-cati.**A-B.** Telia and uredinia on infected plant. **C**. Basket-like sori (squash-mount). **D**. Echinulate uredinospore and pedicellate 2-celled teliospore. Bars = 20 µm.

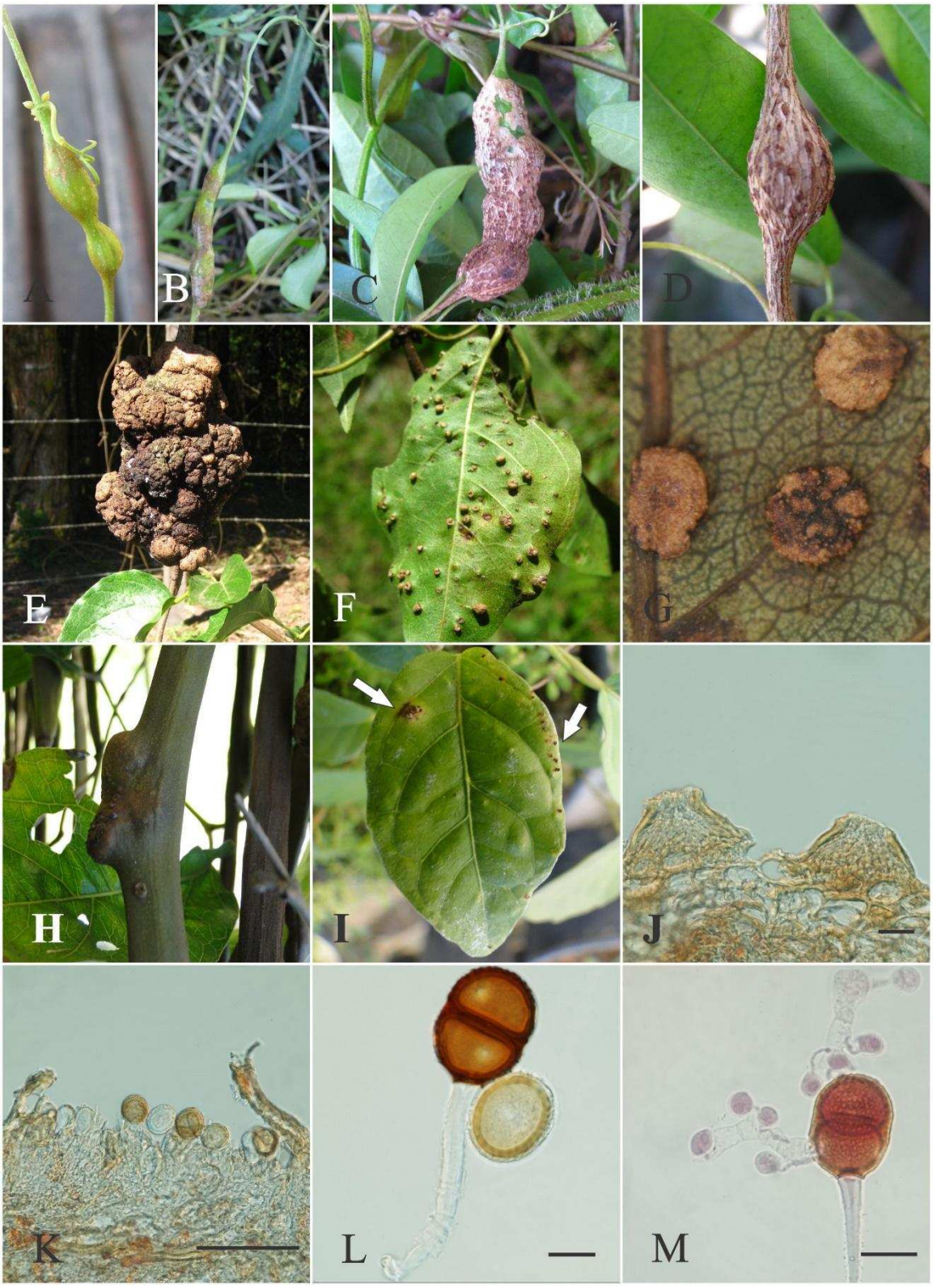

Fig. 12: Uropyxis rickiana on Dolichandra unguis cati. **A-E.** Galls on stem – progressive stages of development. **F-G**. Distortions on leaves to which aecia and spermogonia are associated**. H** Gall on pod. **I.** Symptons observed in greenhouse after

30 days of inoculation. **J**. Type 7 spermogonium. **K.** Aecia and aeciospores. **L**. Uredinospore and teliospore with spirall in the base. **M.** Germinated with metabasidia and basidiospores. Bars = 20µm