

A new *Inocyclus* species (*Parmulariaceae*) on the neotropical fern *Pleopeltis astrolepis*

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Abstract: During a survey for fungal pathogens associated with ferns in Brazil, a tar spot-causing fungus was found on fronds of *Pleopeltis astrolepis*. This was recognised as belonging to *Inocyclus* (*Parmulariaceae*). After comparison with other species in the genus, it was concluded that the fungus on *P. astrolepis* is a new species, described here as *Inocyclus angularis* sp. nov.

Key words:
Ascomycota
Brazil
Neotropics
tropical ferns

Article info: 7 January 2014; Accepted: 29 April 2014; Published: 9 May 2014.

INTRODUCTION

The mycodiversity in Brazil is very rich, and numerous novel records of known and new fungal taxa have recently been published, as mycological activity appears to be gaining momentum in this country. Poorly exploited biomes, such as the semi-arid Caatinga (Isabel *et al.* 2013, Leão-Ferreira *et al.* 2013) and the savannah-like Cerrado, are having their mycobiota surveyed and described (Hernández-Gutiérrez & Dianese 2014, Soares & Dianese 2014), and host-plant focused fungal surveys (such as of native weeds and endangered plant species) have been conducted. Since 2009, a survey of fungi occurring on ferns (also a group of host-plants poorly studied by mycologists) is being conducted in southern and south-eastern Brazil. Numerous mycological findings have resulted and publications are in preparation to describe these. The first mycological novelty to be published as a result of this intensive study was a new genus of *Parmulariaceae*, *Rhagadolobiopsis* described on *Thelypteris serrata* (*Thelypteridaceae*) (Guatimosim *et al.* 2014). *Parmulariaceae* includes 59 genera of foliicolous biotrophic fungi, occurring mainly in the Neotropics and Paleotropics (Kirk *et al.* 2008). The family was recently reviewed (Inácio & Cannon 2008). Although numerous publications have covered this fungal family (Sivanesan 1970, Müller & von Arx 1973, von Arx & Müller 1975, Barr 1987, Sivanesan & Hsieh 1989, Sivanesan & Sinha 1989, Sivanesan *et al.* 1998, Inácio & Minter 2002a-i, Inácio 2003, Inácio & Cannon 2003a, b, Inácio 2005, Inácio *et al.* 2011a, b, Inácio *et al.* 2012) it is acknowledged to be still poorly known and many taxa in the family are still awaiting discovery. Delimitation of existing taxa is purely morphology-based, and a serious limitation to improving our understanding of *Parmulariaceae* is the absence of any molecular data for fungi in the family. Although a general

molecular-based reappraisal of the family is desirable, technical difficulties for dealing with such biotrophic parasites still frustrates progress. Nevertheless the description of novel taxa of *Parmulariaceae* should not be interrupted awaiting for adequate methodologies to become available for molecular studies. Herein, a new member of the family, found on a fern in Brazil during our ongoing surveys, is described based on its distinct morphology, as compared to related species. The host plant is *Pleopeltis astrolepis*, a member of a genus containing approximately 90 species and occurring primarily in the Americas, but also having species in Africa, India, and Sri Lanka (Mickel & Smith 2004, Otto *et al.* 2009, Smith & Tejero-Díez 2014). *Pleopeltis astrolepis* is a widespread fern occurring throughout the Neotropics and extending into Mexico and Florida in North America (Mickel & Smith 2004).

MATERIALS AND METHODS

Samples of leaves of the epiphytic fern *Pleopeltis astrolepis* (*Polypodiaceae*) bearing minute black (tar-spot-like) colonies were collected in a private garden and also on a fallen tree in an Atlantic forest area in the municipality of Nova Friburgo, state of Rio de Janeiro (Brazil), in 2013. These were dried in a plant press and later examined under a dissecting microscope. Freehand sections of fungal colonies on leaves were prepared and also fungal structures scraped from the plant surface were mounted in lactophenol, lactofuchsin, Lugol's solution, and Melzer's reagent. When necessary, sections were made using a Microm HM 520 freezing microtome. Fungal structures were observed, measured (at least 30 structures), and line drawings and photographs were prepared, with an Olympus BX51 light microscope fitted with a drawing tube and an Olympus E330 digital camera.

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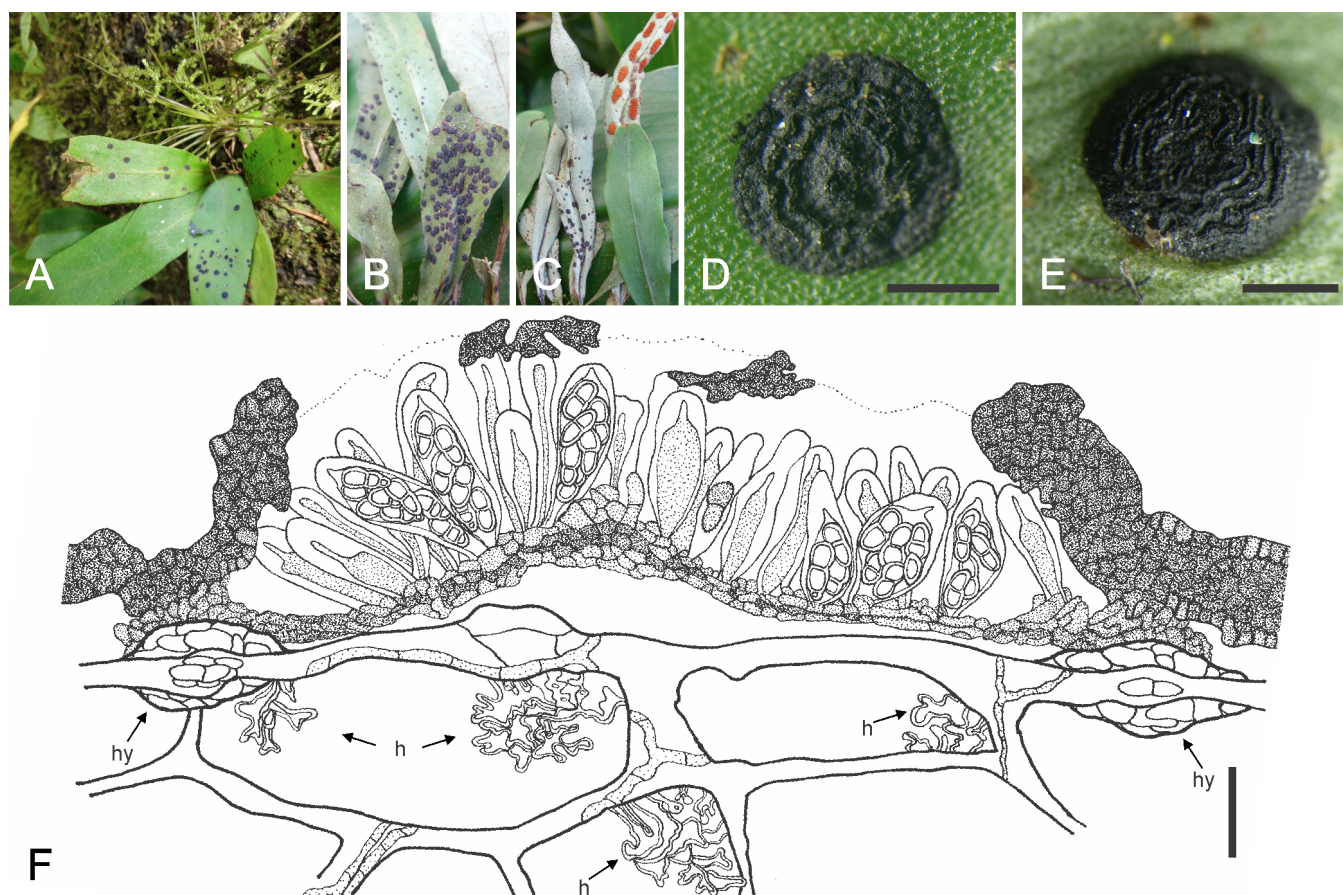


Fig. 1. *Inocyclus angularis* (VIC 39747). **A–E.** Colonies on *P. astrolepis*. **F.** Ascoma with asci and ascospores. h = coraloid haustoria, hy = hypostroma that connects the ascoma with the host tissue. Bars: D–E = 4 mm, F = 20 μ m.

Representative specimens were deposited at the herbarium of the Universidade Federal de Viçosa (VIC).

In order to observe details of ascospore germination and to investigate the possibility of obtaining pure cultures of the fungus, ascospores were ejected onto the surface of PDA agar in Petri plates (Crous et al. 2009). This was done by attaching 1cm² frond pieces bearing fertile ascomata to the inside of the upper lids of Petri dishes, using vaseline, with the ascomata facing the medium. Plates were left in a growth chamber adjusted to 25 ± 2 °C under a light regime of 12 h for 2 d. Additionally, ascospores were also directly ejected onto sterile microscope slides under similar conditions with an equivalent apparatus, but using a Petri dish lined with sterile filter paper soaked with sterile water over which a sterile microscope slide was kept suspended on sterile glass rods.

TAXONOMY

Inocyclus angularis Guatimosim & R.W. Barreto, **sp. nov.**

Mycobank MB805976
(Figs 1–2)

Etymology: *angularis*, derived from the angle formed by the germ-tube during ascospore germination.

Diagnosis: Differs from *Inocyclus discoideus* by having amphigenous ascomata and roughened versicoloured ascospores (i.e. with one brown and one subhyaline cell).

Type: **Brazil:** *Rio de Janeiro:* Nova Friburgo, Mury, Sítio Colonial, on living leaves of *Pleopeltis astrolepis*, 30 Mar. 2013, R.W. Barreto (VIC 39747 – **holotype**).

Description: Symptoms visible as superficial amphigenous black tar-spot-like colonies, numerous and scattered over leaves, not associated with necrosis, occasionally confluent, mostly ellipsoid to discoid, $3\text{--}10 \times 7\text{--}8$ mm. **External mycelium** absent. **Internal mycelium** intra- and intercellular, deeply penetrating the mesophyll, branched, $2\text{--}3$ μ m, sub-hyaline, smooth. **Haustroria** coralloid, several per host cell, hyaline. **Internal stromata** absent. **External stromata** superficial with radiating cells, amphigenous, ellipsoid to discoid, opening in circumferentially arranged locules connected to the host mesophyll at multiple points by discrete pulley wheel-like (in section) pads composed of internal and external aggregations of pale brown hyphae connected by a peg emerging through the cuticle. **Ascomata** black, initially circular, becoming ellipsoidal, producing locules arranged in one-two rings with undulated surface, $800\text{--}980 \times 650\text{--}670$ μ m, composed of dark brown *textura prismatica* (cells 9×4 μ m). In vertical section: stromata entirely superficial, strongly connected to the leaf, delimited

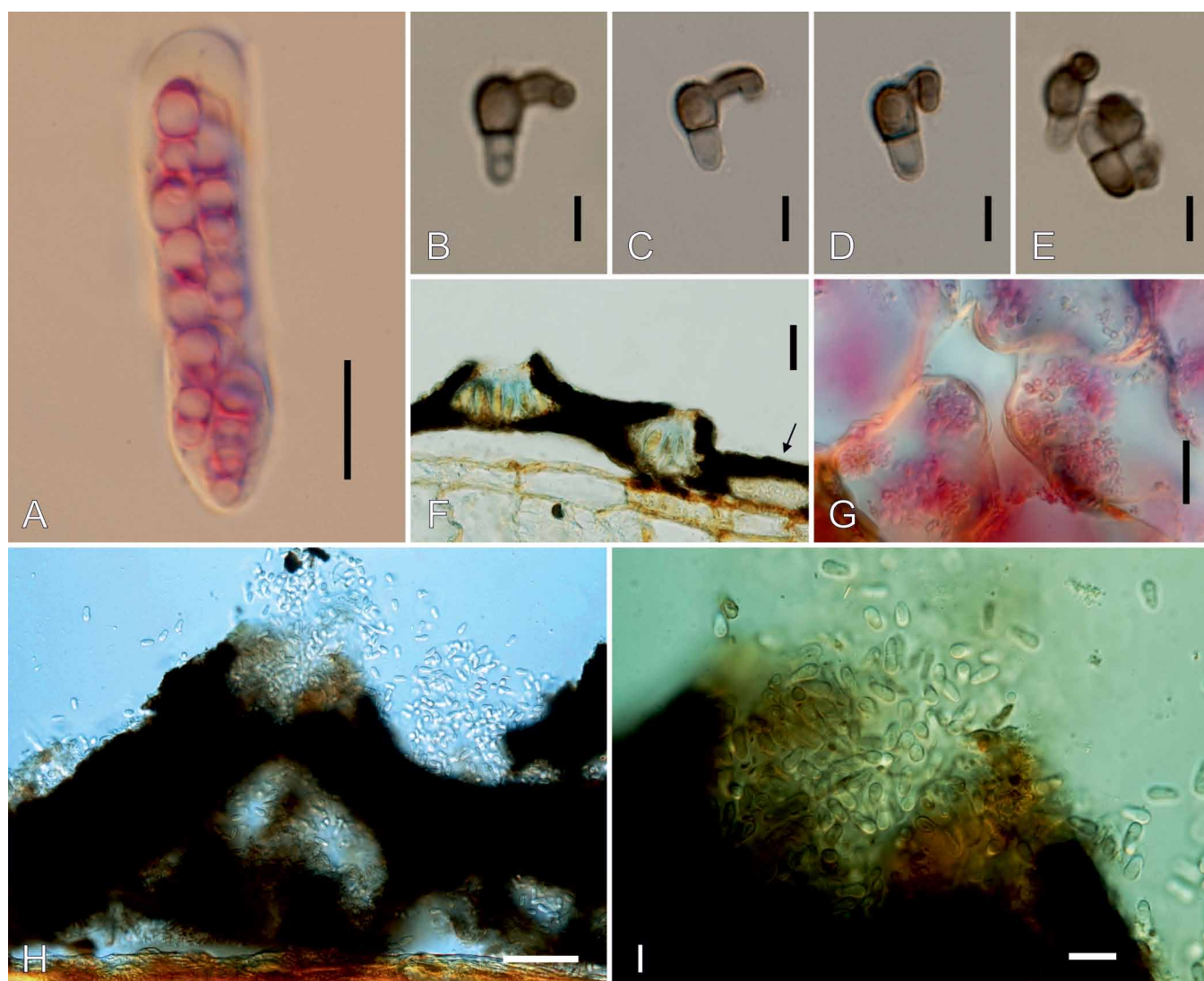


Fig. 2. *Inocyclus angularis* (VIC 39747). **A.** Ascus with hyaline ascospores (mounted on lactofuchsin). **B–E.** Germinated conidia, showing the nearly right angle formed by the germ tube. **F.** Amyloid reaction of the asci matrix on IKI. **G.** Detail of coraloid haustoria. Note the presence of the asexual morph (arrowed) intermixed with the sexual morph. **H–I.** Asexual morph with conidia. Bars: A = 10 µm, B–E = 5 µm, F = 100 µm, G, I = 20 µm, H = 50 µm.

by a covering layer (above the fertile locules) and a lower layer. Covering layer 12–16.5 µm thick, black, consisting of dense dark brown-pigmented radiating cells of *textura angularis* (cells 4 × 5 µm). Lower layer underneath the hymenium adjacent to the host cuticle, 5–12 µm thick, composed of brown to light brown *textura angularis* (cells 2–3 × 5 µm). Locule composed of a thin basal cushion above the lower layer, with asci, immersed in amyloid gelatinous stratum, 35–192 × 45–65 µm. *Hamathecium* not seen, possibly evanescent. *Asci* maturing sequentially, with young and mature asci in the same locule; young asci variable in shape before spores can be distinguished, truncated at the base, subcylindrical; mature asci bitunicate in structure, dehiscence not observed, subcylindrical, 31–45 × 10–12 µm, non-amyloid, 8-spored, biseriate or inordinate becoming uniseriate at maturity. *Ascospores* ellipsoidal to clavate, initially hyaline, becoming versicoloured, 1-septate, constricted at the septum, with unequal cells (apiospores), the upper cell larger, darker and rounded and the lower

cell smaller and acute, 10–13 × 3–4 µm, roughened; only versicoloured ascospores were ejected; germination through the upper cell only, germ tubes readily folding at approximate right angles to the main ascospore axis. *Asexual morph* intermixed with the ascomata, occupying the same stromata located in the central region of the colonies. *Conidia* hyaline, aseptate, smooth, fusiform to clavate, with one large guttule at the rounded side, 7–10 × 3–4 µm.

Host: *Pleopeltis astrolepis* (Polypodiaceae), an epiphytic fern from the tropical and subtropical Americas (Florida to Southern Brazil).

Additional specimens examined: **Brazil:** Rio de Janeiro: Nova Friburgo, Mury, Sítio Colonial, on living leaves of *P. astrolepis*, 8 June 2013, R.W. Barreto (VIC 39748); Nova Friburgo, Riograndina, Fazenda Barreto, on living leaves of *P. astrolepis*, 9 June 2013, R.W. Barreto (VIC 39749).

DISCUSSION

Inocyclus angularis, as other *Parmulariaceae*, seems to be unculturable on artificial media. When ascospores were ejected onto culture medium, they readily germinated but, shortly afterwards ceased to grow.

In *Parmulariaceae*, five genera (besides *Inocyclus*) are known on ferns: *Pachypatella*, *Polycyclus*, *Polycyclina*, *Rhagadolobium*, and *Rhagadolobiopsis* (Inácio & Cannon 2008, Guatimosim et al. 2014). Except for *Polycyclus*, all are easily separated from *Inocyclus* through observation of morphological features, as indicated in the dichotomous key for the identification of genera of *Parmulariaceae* on ferns provided by Guatimosim et al. (2014).

Separation between *Polycyclus* and *Inocyclus* is somewhat tenuous. The most relevant differences between these two genera, according to Inácio & Cannon (2008), are as follows. In *Inocyclus* the ascogelatinous layer has a strong amyloid reaction and the locules are irregularly or radially arranged, whereas in *Polycyclus* no amyloid reaction is observed and the locules are circumferentially arranged.

The current generic delimitations in *Parmulariaceae* are highly artificial and this status will remain unchanged until molecular information becomes available for fungi in this family. The new species has circumferentially arranged locules as in *Polycyclus*. However we preferred to place it in *Inocyclus* because of the highly intense amyloid reaction observed in its hymenial gel.

The genus *Inocyclus* includes seven accepted species, namely the type species *I. psychotriae*, and *I. blechni*, *I. calotheus*, *I. myrtacearum*, *I. discoideus*, *I. australiensis*, and *I. dovyalidis* (<http://nt.ars-grin.gov/fungaldatabases>).

Among all *Inocyclus* species, only *I. discoideus* is known from the host family *Polypodiaceae*. It has been recorded on different species of *Polypodium* from Indonesia and the Philippines, and differs from *I. angularis* in having hypophyllous ascomata (while *I. angularis* has amphigenous ascomata) and smooth, pigmented ascospores (roughened and versicoloured in *I. angularis*).

Inocyclus angularis is the first pathogenic fungus recorded on a species of *Pleopeltis* worldwide.

ACKNOWLEDGEMENTS

We thank Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional do Desenvolvimento Científico e Tecnológico (CNPq) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for financial support.

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