UNIVERSIDADE FEDERAL DO PAMPA

EHIDY ROCIO PEÑA CAÑÓN

FUNGOS ENDOLIQUÊNICOS ASSOCIADOS À *Cladonia curta* Ahti & Marcelli NO PAMPA GAUCHO - BRASIL

São Gabriel

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Dissertação apresentada ao programa de Pós-graduação *Stricto Sensu* em Ciências Biológicas da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Mestre em Ciências Biológicas.

Orientador: Dr. Antônio Batista Pereira

Co-Orientador: Dr. Filipe de Carvalho Victoria

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A ti meu anjo, que desde sempre e em qualquer lugar cuidas de mim, mãe tu é o meu maior exemplo a seguir, te amo!

AGRADECIMENTOS

"La gente piensa que las historias son moldeadas por la gente.

De hecho, es al revés."

Terry Pratchett

RESUMO

Os liquens são considerados uma associação simbiótica composta por um micobionte, um ou mais fotobiontes e inclusive um terceiro simbionte *Basidiomycota*, representam um dos estilos de vida de maior sucesso para os fungos e uma das formas mais bem sucedidas da simbiose na natureza. Liquens exploram todos os ambientes naturais e se adaptam a condições extremas como as causadas pelas perturbações humanas, tornando-se importantes bioindicadores da qualidade ambiental. *Cladonia curta* Ahti & Marcelli é uma espécie de líquen escamoso da família *Cladoniaceae* distribuído em três locais ao sudeste do Brasil, em florestas mesófilas e no Cerrado e coletado no bioma Pampa durante o presente estudo, tornando-se um registro de nova ocorrência. Por outro lado, existem estudos que demonstram a presença de fungos que ocorrem dentro dos talos aparentemente saudáveis dos liquens, conhecidos como endoliquênicos, associados principalmente ao fotobionte. Durante os últimos 30 anos, fungos endoliquênicos têm sido estudados com base em métodos dependentes do cultivo *in vitro* e o uso de ferramentas moleculares empregadas principalmente na sua detecção e identificação. Assim o objetivo do presente trabalho foi conhecer a diversidade de fungos endoliquênicos abrigados no talo de *Cladonia curta* e suas relações filogenéticas com outros fungos endófitos. As análises filogenéticas baseadas na amplificação das regiões ITS do rDNA e do gene da β-Tubulina infere as linhagens de fungos endoliquênicos no gênero *Xylaria*. Do mesmo modo, as características morfológicas das colônias e dos estromas anamórficos obtidos em cultura confirmam esta classificação. Nossos resultados baseados em marcadores moleculares fornecem evidências de que fungos endoliquênicos isolados de *Cladonia curta* estão intimamente relacionados com fungos endófitos e saprofíticos; no entanto, os isolados se mostram mais estreitamente relacionados com fungos endófitos, sugerindo que a sua associação ao talo não é puramente acidental e respaldando a relação filogenética entre estes três estilos de vida, como previamente relatado por outros autores. Estudos adicionais são necessários para demostrar que os isolados *Xylaria* spp. e *Xylaria berteri* sejam conspecíficos. Além disso, nós sugerimos o uso do meio de cultura MS como uma alternativa nos esforços para conhecer a grande diversidade de fungos endófitos que habitam tecidos vivos de liquens. Finalmente, a diversidade e a prevalência dos fungos endoliquênicos continua sendo estudada sendo este o primeiro relato de isolamento e identificação de fungos endoliquênicos no Brasil.

Palavras-chave: *Xylaria*, endosimbiontes, *Cladoniaceae*, liquens.

ABSTRACT

Lichens considered as a symbiotic association between a mycobiont, one or more photobiont and even a third *Basidiomycota* symbiont represent one of the most successful lifestyles for fungi and one of the most successful forms of symbiosis in nature. Lichens explore all natural environments and adapt to extreme conditions such as those caused by human disturbances, becoming important bioindicators of environmental quality. *Cladonia curta* Ahti & Marcelli is squamulose lichen of *Cladoniaceae* family distributed in three sites in southeastern Brazil, in mesophyll forests and in the Cerrado and collected in the Pampa biome during the present study, becoming a record of new occurrence. On the other hand, there are studies demonstrating the presence of fungi that occur within apparently healthy lichen thalli, known as endolichenic, associated mainly with the photobiont. During the last thirty years, endolichenic fungi have been studied based on in vitro culture-dependent methods and the use of molecular tools mainly used in their detection and identification. Thus, the aim of the present work was to know the diversity of endolichenic fungi harbored in *Cladonia curta* thallus and their phylogenetic relationships with other endophytic fungi. Phylogenetic analyzes based on the amplification of the ITS regions of the rDNA and the β-Tubulin gene infers the endolichenic fungi lineages in the genus *Xylaria*. Likewise, the morphological characteristics of the colonies and the anamorphic stromatas obtained in culture confirm this classification. Our results based on molecular markers provide evidence that endolichenic fungi of *C. curta* are closely related to endophytic and saprophytic fungi; however, they are closely related to endophytic fungi, suggesting that their association with thallus is not purely accidental and supporting the phylogenetic relationship between these three lifestyles, as previously reported by other authors. Additional studies are needed to demonstrate that isolates of *Xylaria* spp. and *Xylaria berteri* are conspecific. In addition, we suggest the use of the MS culture medium as an alternative in the efforts to know the great diversity of endophytic fungi that inhabit living lichen tissues. Finally, the diversity and prevalence of endolichenic fungi continues to be studied with this, the first report of isolation and identification of endolichenic fungi in Brazil.

Key Word: *Xylaria*, Endosymbionts, *Cladoniaceae*, lichens.

LISTA DE FIGURAS

LISTA DE TABELAS

Manuscrito

Material Suplementar

SUMARIO

1. INTRODUÇÃO

Os liquens são considerados como uma associação simbiótica entre um micobionte (fungo filamentoso) e um ou mais fotobiontes (alga ou cianobactéria), representando um dos estilos de vida de maior sucesso para os fungos (Ahmadjian, 1966; Honegger, 1998; De Priestt, 2004). Inclusive, um terceiro simbionte do filo *Basidiomycota* formando parte do córtex de *Bryoria tortuosa* (G. Merr.) Brodo & D. Hawksw., foi recentemente descoberto (Spribille et al., 2016). Sem dúvida, os liquens representam uma das formas mais bem sucedidas da simbiose na natureza, encontrando-se distribuídos em todo o globo, explorando não só os ambientes naturais, mas em muitos casos adaptando-se a condições extremas, incluindo algumas provocadas por perturbações humanas (Seaward, 2008). Estes organismos são reconhecidos por mostrar alta sensibilidade a poluentes já que absorvem metais pesados do ar e da água (Brodo et al., 2001); moléculas prejudiciais são absorvidas com os nutrientes, e os liquens são frequentemente incapazes metabolicamente de processar ou de isolar estas rapidamente, causando não somente a diminuição da sua vitalidade, como também a alteração do balanço simbiótico entre o fotobionte e o micobionte e sintomas externos característicos (Valencia e Ceballos, 2002; Martins-Mazzitelli et al., 2006). A relação demostrada anteriormente torna os liquens importantes bioindicadores da qualidade ambiental (Hawksworth et al., 2005; O-H NG et al., 2005; Martins et al., 2008; Lijteroff et al., 2009).

Existem três formas principais de crescimento ou de configuração geral dos talos liquênicos sendo estes: foliáceos, fruticosos e crostosos. Porem, um quarto tipo, os liquens escamosos também podem ser reconhecidos (Fleig et al., 2008). O talo dos liquens foliosos é achatado e prostrado com diferenças na cor e nas características das superfícies superior e inferior; geralmente o talo esta dividido em numerosos ramos chamados lóbulos e ligado ao substrato por rizinas. Os liquens fruticosos consistem em ramos simples ou divididos, redondos a aplanados, com pouca diferença entre as superfícies superior e inferior, desprovidos de rizinas. Nos talos crostosos, a parte central do talo é areolada, apresentando margens inteiras que às vezes desaparecem no substrato tornam-se indistintas. O talo de liquens escamosos constitui-se de lóbulos pequenos e separados, sem córtex inferior nem rizinas (Hale, 1979).

Cladonia Curta Ahti & Marcelli é um líquen escamoso de talo primário persistente que consiste em escamas esverdeadas, macias e muito pequenas com margens ligeiramente sorediadas; apresenta podécios muito curtos, espessos, sem ramificações e com apotécios

vermelhos cónicos; cresce sobre troncos em decomposição na borda da floresta e é considerada uma espécie muito rara (Fig. 1). No Brasil, *C. curta* é distribuída em três locais ao sudeste do país, em florestas mesófilas e no Cerrado nos estados de Minas Gerais (Município Catas Altas) e São Paulo (Município São Bernardo do Campo, 1970), além do Distrito Federal (Jardim botânico de Brasília, 0,5 km NW da escola Fazendária e Reserva ecológica de IBGE), entre 800-1200 metros acima do nível do mar (a.n.m.) (Ahti, 2000). Recentemente a espécie foi coletada no estado Rio Grande do Sul inserido no bioma Pampa tornando-se um registro de nova ocorrência (Artigo 2).

Figura 1 - Detalhe dos exemplares de *Cladonia Curta* Ahti & Marcelli. (A) Habito da espécie. (B) Aparência do talo.

Fonte: fotos Geferson Metz e Rocio Peña.

No Brasil o bioma Pampa é caracterizado por apresentar uma vegetação típica de pastagem nativa, com arbustos escassos e formações arbóreas (Overbeck et al., 2007). Porém, a região principal de formação de pastagens esta principalmente distribuída na região sudoeste do bioma e é chamada "Campanha Gaúcha". O bioma Pampa esta localizado na região sul do país, no estado Rio Grande do Sul, dentro da Zona Temperada, apresentando climas subtropicais e temperados. Embora seja frequentemente interpretado como um ambiente de pastagem pura é dividido em cinco ambientes principais: costeiro, cerrado, estepe, estepe-savana e parque do espinilho (Roesch et al., 2009). Representando cerca do 2,07% do território nacional é um dos menores, ocupando uma área de 176.496 km, e foi oficialmente reconhecido em 2004 pelo Instituto Brasileiro de Geografia e Estatística (IBGE, 2004). Com tais características e o tipo de vegetação que apresenta, este bioma possui um grande valor ecológico, não obstante, encontra-se em estado de vulnerabilidade (Overbeck et al., 2007).

O ramo da taxonomia de fungos vem demostrando a presença de espécies do filo *Ascomycota*, diferentes ao micobionte, ocorrendo em simbiose nos talos liquênicos (Petrini et al., 1990; Girlanda et al., 1997; Suryanarayanan et al., 2005; Li et al., 2007). Assim, o talo dos liquens é considerado um dos nichos ecológicos para fungos (Nash, 2008), abrigando três tipos de fungos ecologicamente diferentes: liquenícolas, incidentes e endoliquênicos (Lutzoni et al., 2001; Lawrey e Diederich, 2003; Arnold et al., 2009). Os fungos liquenícolas são um grupo altamente especializado e bem sucedido de organismos que se desenvolvem e esporulam sobre liquens (Lawrey e Diederich, 2003), entrando numa relação estável que pode causar perda de cor, formação de galhas e inclusive a morte dos liquens. Estes, bem como fungos saprófitos ocorrem em talos em decomposição (Petrini et al., 1990). Assim mesmo, aqueles fungos que habitam nas superfícies dos talos liquênicos são conhecidos como fungos incidentes (Lutzoni et al., 2001).

Por outro lado, os fungos endoliquênicos são um grupo de endosimbiontes que ocorrem dentro dos talos de liquens aparentemente saudáveis, preferencialmente associados á camada do fotobionte (Suryanarayanan et al., 2005; Arnold et al., 2009; U´Ren et al., 2010; Tripathi e Joshi, 2015; Suryanarayanan et al., 2017). Estes fungos habitam os espaços intercelulares dos simbiontes liquênicos (Wu et al., 2011), sua colonização é assintomática, são hiperdiversos e muitos são transmitidos horizontalmente como análogos aos endófitos das plantas (Arnold et al., 2009; Kannangara et al., 2009; U´Ren et al., 2012). Nos últimos trinta anos, os fungos endoliquênicos foram conhecidos de todas as espécies de liquens amostradas para ecossistemas que vão desde desertos quentes até florestas úmidas e tundras árticas (U´Ren et al., 2010; Tripathi et al., 2014; Padhi e Tayung, 2015; U´Ren et al., 2016; Suryanarayanan et al., 2017).

Existem diferentes estudos para estimar a diversidade de fungos endoliquênicos. Suryanarayanan et al., (2005) e Suryanarayanan et al., (2017) recuperaram um total de 24 espécies e 33 morfoespécies a partir de espécies de liquens foliáceos e fruticosos de regiões tropicais na Índia pertencentes a cinco famílias (*Caliciaceae*, *Collemataceae*, *Lobariaceae*, *Parmeliaceae*, *Physiaceae*, *Ramalinaceae* e *Roccellaceae*). Em contraste, Tripathi e Joshi (2015) e Tripathi et al. (2014) isolaram 10 espécies a partir de liquens coletados em diferentes regiões temperadas de Kumaun Himalaya. Na China, foram investigados um total de 32 fungos endoliquênicos isolados desde sete espécies de liquens pertencentes às famílias *Cladoniaceae*, *Parmeliaceae*, *Ramalinaceae*, *Teloschistaceae* e *Verrucariaceae* (Li et al,

2007). No Sri Lanka, 29 morfoespécies foram isoladas de *Parmotrema* sp., *Usnea* sp. e *Pseudocyphellaria* sp. No entanto, este número é pequeno quando comparado com a diversidade de fungos endoliquênicos relatados nos Estados Unidos (Arnold et al., 2009; U´Ren et al., 2010; U´Ren et al., 2012; U´Ren et al., 2016). Da mesma forma, foram feitas pesquisas sobre a produção de metabólitos (Wu et al., 2011) e a avaliação dos potenciais antimicrobianos das sustâncias produzidas pelos fungos endoliquênicos (Padhi e Tayung, 2015). Recentemente foi apresentada uma revisão das relações filogenéticas moleculares dos fungos endófitos e endoliquênicos da família *Xylariaceae*, mostrando que a grande maioria dos isolados foi incluída dentro das subfamílias *Hypoxyloideae* e *Xylarioideae* (U´Ren et al., 2016). Após revisão bibliográfica, foi evidenciado que o presente trabalho é o primeiro estudo na América do Sul sobre a diversidade de fungos endoliquênicos abrigados nos talos de liquens.

O gênero *Xylaria* Hill ex Schrank é um dos mais diversos dentro da família *Xylariaceae* com cerca de 600 espécies a maioria tropical, tem mostrado como um grupo parafilético (Hsieh et al., 2010; Rogers e Ju, 2012); junto a outros 85 gêneros da família é classificado dentro da classe *Sordariomycetes* (Stadler et al., 2013). Os membros deste gênero são considerados saprófitos importantes, por vezes desde ligeiramente até fortes parasitas em madeira de árvores, encontrados em folhas e raramente observados em frutos (Lee et al., 2000; Rogers 2000; Rogers e Ju, 2012). *Xylaria* são visíveis durante a esporulação sexual, formando estromas macroscópicos relativamente grandes (Davis e Shaw, 2008). Apesar da sua condição saprófita, espécies de *Xylaria* são típicos fungos endófitos isolados a partir de fragmentos de plantas e liquens aparentemente saudáveis (Guo et al., 2000; Shibuya et al., 2003; Arnold et al., 2009; Thomas et al. 2016; U´Ren et al., 2016) e predominantes em estudos de diversidade tanto de fungos endoliquênicos quanto de endófitos (Bayman et al., 1998; Vaz et al., 2014; Suryanarayanan et al., 2017).

Fungos endófitos em plantas e liquens têm sido estudados e descritos com base em métodos dependentes do cultivo *in vitro*. No entanto, muitas *Xylariaceae* permanecem frequentemente estéreis em cultura ou reproduzem-se só assexuadamente sobre meios de cultura padrão (Stadler et al., 2013). Assim, na ausência de características do teleomorfo, as culturas podem ser classificadas, em alguns casos, com base em características coloniais e anamórficas observadas, tais como taxas de crescimento, cor, morfologia superficial das colônias, bem como na ramificação de conidióforos e a natureza da proliferação celular

conidiogênica (Petrini e Petrini, 1985; Callan e Rogers, 1993; Stadler et al., 2013). Porém, várias ferramentas moleculares foram adicionadas a esta metodologia utilizada para caracteriza-los. Regiões ITS do rDNA e o gene da β-Tubulina são atualmente empregados na detecção e identificação de fungos endoliquênicos para analisar posições ou relações filogenéticas ao nível de espécie ou interespécies (Lee et al., 2000; Arnold et al., 2009; Hsieh et al., 2010; U´Ren et al., 2016). Tendo em conta a vasta diversidade ainda pouco explorada dos fungos endoliquênicos, que ainda há muito a ser entendido e explicado sobre as suas características, particularidades e as condições da sua associação com os simbiontes dentro dos talos liquênicos, um esforço imenso e estudos adicionais ainda são necessários para aumentar seus conhecimentos e compreender sua relação filogenética com outros fungos endófitos de plantas e líquenes.

2. OBJETIVOS

2.1 Objetivo geral

Conhecer a diversidade de fungos endoliquênicos abrigados no talo de *Cladonia curta* Ahti & Marcelli, espécie de líquen registrada no bioma Pampa, RS − Brasil, e suas relações filogenéticas com fungos endófitos isolados de plantas e liquens.

2.2 Objetivos específicos

- Caracterizar morfologicamente as linhagens de fungos recuperados em cultura desde podécios do líquen *Cladonia curta.*
- Identificar o posicionamento taxonômico dos fungos endoliquênicos isolados.
- Inferir relações filogenéticas para os isolados obtidos *in vitro* a partir de *Cladonia curta* usando regiões ITS do rDNA e do gene da β-Tubulina.

3. ARTIGO 1: Caracterização morfológica e molecular de três isolados de *Xylaria* (*Xylariaceae*), endoliquênicos em *Cladonia curta* Ahti & Marcelli (*Cladoniaceae*)

(Artigo a ser submetido para a revista Fungal Biology, conforme normas da revista).

Morphological and molecular characterization of three isolates of *Xylaria* (*Xylariaceae*), endolichenic in *Cladonia curta* Ahti & Marcelli (*Cladoniaceae*)

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Abstract

The biology of endophytes is a branch of science that is contributing to understand the diversity and ecology of fungi that live inside plants, fungi and lichen. Considering that this diversity of endolichenic fungi is little explored and there is still much more to be understood and explained about its phylogenetic relationship with other lifestyles (endophyte and saprotrophism), this paper presents data on axenic growing and phylogenetic relationships of three endolichenic fungi, isolated in MS medium. *Cladonia curta* Ahti & Marcelli a species of lichen described in Brazil is distributed in three sites in the southeast of the country, in mesophilous forests and Cerrado. The initial growth of hyphae of *Xylaria* spp. on *C. curta* podetia started four days after inoculation and continued up to the next 13 days until the hyphae completely covered the podetia. Stromata formation and differentiation were observed, occurring approximately one year after of isolation and consecutive subculture of

lineages. The phylogenetic analyses based on ITS rDNA and β-tubulin infers lineages of endolichenic fungi in the genus *Xylaria,* even as the morphological characteristics of colonies and anamorphous confirm this classification. Our preliminary result based in molecular marker provides evidences that this endolichenic fungi are closely related to both endophytic and saprophytic fungi, nevertheless, they are more closely related to endophytic fungi suggesting that associations are not purely incidental. Further studies are needed for accept or reject the hypothesis that endolichenic fungi isolated *Xylaria* spp. and *X. berteri* are conspecific, specially phylogenetic analyses using robust multi-locus dataset.

Key Word: Anamorph, Fungi, Phylogeny, Lichen, Brazil, *Xylaria berteri, Xylaroideae.*

1. Introduction

The biology of endophytes is a recent science that is starting to known the diversity and ecology of endolichenic fungi, a group of endosymbionts which occur inside of symptomless lichen thalli preferentially associated with the green algal photobiont (Arnold et al., 2009; Suryanarayanan et al., 2005; Suryanarayanan et al., 2017; Tripathi and Joshi, 2015; U´Ren et al., 2010). They inhabit the intercellular spaces of the hosts (Wu et al., 2011), their colonization is asymptomatic, are hyperdiverse and many are transmitted horizontally as analogous to the plant endophytes (Arnold et al., 2000; Arnold et al., 2009; Kannangara et al., 2009; U´Ren et al., 2012). In the last thirty years, endolichenic fungi are known from every lichen species sampled to date in ecosystems ranging from hot deserts to moist forests and arctic tundra (Arnold et al., 2009; Li et al., 2007; Padhi and Tayung, 2015; Suryanarayanan et al., 2005; Suryanarayanan et al., 2017; Tripathi et al., 2014; U´Ren et al., 2010; U´Ren et al., 2012; U´Ren et al., 2016; Wang et al., 2016).

The efforts have been made to compare the different fungi that inhabit the tissues of lichens and their host tissues (Suryanarayanan et al., 2005; U´Ren et al., 2010) establishing

effective methods for surface sterilization in order to estimate their diversity (Li et al., 2007; Suryanarayanan et al., 2017; Tripathi et al., 2014). In the same way, some studies try to explain both the evolution of endophism and the diversification of the Ascomycota endolichenic (Arnold et al., 2009) and evaluate the biotic, biogeographic and abiotic factors that structure their communities (U´Ren et al., 2012;Wang et al., 2016). Likewise, research has been carried out on the metabolites produced (Wu et al., 2011) and the evaluation of the antimicrobial potentials of endolichenic fungi (Padhi and Tayung, 2015). Recently, a review of the molecular phylogenetic relationships of the endophytic and endolichenic fungi of the *Xylariaceae* family was presented, showing that the great majority of the isolates were included within the *Hypoxyloideae* and *Xylarioideae* subfamilies (U'Ren et al., 2016).

To date, studies on endolichenic fungi in India from foliose and fruticose lichen species belonging to five families (*Caliciaceae*, *Collemataceae*, *Lobariaceae*, *Parmeliaceae*, *Physiaceae*, *Ramalinaceae* and *Roccellaceae*) have recovered a total of 24 species (Suryanarayanan et al. , 2005) and 33 morphospecies (Suryanarayanan et al., 2017) from tropical regions. In contrast, Tripathi and Joshi (2015) and Tripathi et al. (2014) worked on endolichenic fungi of temperate regions of Kumaun Himalaya and isolated a total of 10 species that are reported as true endolichenic fungi. In China endophytic fungi of seven lichen species belonging to five families (*Cladoniaceae*, *Parmeliaceae*, *Ramalinaceae*, *Teloschistaceae* and *Verrucariaceae*) were investigated, with a total of 32 taxa (Li et al, 2007). In Sri Lanka, 29 endolichenic fungal strains were isolated from the lichens *Parmotrema* sp., *Usnea* sp. and *Pseudocyphellaria* sp. However, this number is small when compared with those of some more studied in North American states (Arnold et al., 2009; Chagnon et al., 2016; U'Ren et al. 2010; U'Ren et al. 2012; U'Ren et al. 2016). After a bibliographic review, it was evidenced that the present work is the first study in South America on the diversity of endolichenic fungi harbored in lichen thallus.

The genus *Xylaria* Hill ex Schrank is one of the most diverse within the family *Xylariaceae*, with about 600 species, mostly tropical has been shown to be paraphyletic (Hsieh et al., 2010; Rogers and Ju, 2012) and along with 85 other genera of family is classified within the class *Sordariomycetes* (Stadler et al., 2013). Members of this genus are considered important saprophytes, sometimes from slightly to strongly parasites in wood of trees and even found on leaves and rarely on fruits (Lee et al., 2000; Rogers 2000; Rogers and Ju, 2012). *Xylaria* are visible during sexual sporulation forming relatively large, macroscopic stromata (Davis and Shaw, 2008). Despite their condition saprophytic, *Xylaria* species are an example of typical endophytic fungi that have been isolated from fragments of apparently healthy plants and lichens (Arnold et al., 2009; Guo et al., 2000; Shibuya et al., 2003; Thomas et al., 2016; U´Ren et al., 2016) and predominant in studies of diversity of both endolichenic and endophytic fungi (Bayman et al., 1998; Suryanarayanan et al., 2017; Vaz et al., 2014).

Xylariaceae are often remaining sterile in culture or reproduce only asexually (Stadler et al., 2013), therefore, in the absence of teleomorph features, cultures can be classified, in the some cases, based on colonial and anamorphic features observed, such as growth rates, color, colony surface morphology, as well as conidiophore branching and the nature of conidiogenous cell proliferation (Callan and Rogers, 1993; Petrini and Petrini, 1985; Petrini and Petrini, 1990; Stadler et al., 2013). Additionally, molecular techniques have been employed in the detection and identification of endolichenic fungi using principally ITS regions and β-tubulin gene to analyze phylogenetic positions or relationships at a species or interspecies level (Arnold et al., 2009; Hsieh et al., 2010; Lee et al., 2000; U´Ren et al., 2016).

Cladonia curta Ahti & Marcelli is squamulose lichen that grows on decomposing logs at the edge of the forest and is considered a very rare or ignored species. In Brazil, this species is distributed in three sites in the southeast of the country, in mesophilous forests and Cerrado

in the states of Minas Gerais and São Paulo and in the Federal District of Brasília, between 800-1200 msl. (Ahti, 2000). Considering the vast diversity still little explored of endolichenic fungi, that there is still much to be understood and explained about their features, particularity and conditions of their association with symbiont within lichen thallus, an immense amount of effort and additional studies is still required to increase their knowledge and understand the phylogenetic relationship with other endophytic fungi isolated of plant and lichens. Thus, the aim of the present work was to know the diversity of endolichenic fungi harbored in *Cladonia curta* thallus, collected in the south of Brazil and their phylogenetic relationships with other endophytic fungi.

2.   Materials and Methods

2.1. Collection

The samples of lichen *Cladonia curta* were identified and collected in the municipality of São Gabriel, state of Rio Grande do Sul, Brazil at Federal University of Pampa (UNIPAMPA) campus (30° 20' 06.3" S, 054° 21' 46.5" W) at 124 m asl., on dead wood of an *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) trunck. The local of collection lies within the Pampa biome located in the southern region of Brazil. The lichen sample was deposited in the Bruno Edgar Irgang herbarium (HBEI- Federal University of Pampa) under the voucher number HBEI 023.

2.2. Fungal isolation and culturing

The endolichenic fungi were isolated from podetia of *Cladonia curta* apparently healthy without symptoms of colonization of other fungi, one day after collected the lichen. The isolation of endolichenic was achieved following the protocol of the spore-shot method for mycobionts (McDonald et al.*,* 2013) in which under sterile conditions, the podetia of lichen

were removed, rinsed, disinfested and affixed to the lid of an inverted petri dish with sterile solid Vaseline (Synth®). Surface sterilization of the fragments was performed by consecutive immersion in 25% bleach solution for 3 min, ethanol (70%) and rinsed three times for 2 min, with ultra-pure water and subsequently dried on sterile filter paper, followed a modified version of the procedure from Arnold *et al*. (2000). We use the medium Murashige and Skoog (MS) (Murashige and Skoog, 1962) modified, for the growth and maintained of endolichenic fungi. The MS medium modified (pH 5.5) contains: 1.650 g. L^{-1} NH_4NO_3; 1.9 g. L^{-1} KNO_3; 370 mg. L^{-1} $MgSO_4$ x H_2O; 16.9 mg. L^{-1} $MnSO_4$ x H_2O; 8.6 mg. L^{-1} $ZnSO_4$ x H_2O; 0.025 mg. L^{-1} de $CuSO_4$ x H_2O; 333 mg. L^{-1} de $CaCl_2$; 6.2 mg. L^{-1} H_3BO_3; 170 mg. L^{-1} KH_2PO_4; 0.83 mg. L^{-1} KI; 0.25 mg. $L^{-1}NaMoO_4$ x $2H_2O$; 0.025 mg. L^{-1} $CoCl_2$ x $6H_2O$; 37.25 mg. L^{-1} Na_2EDTA; 27.85 mg. L^{-1} $FeSO_4$ x $7H_2O$; 1 mg. L^{-1} thiamine; 0.5 mg. L^{-1}de Pyridoxine; 0.5 mg. L^{-1} nicotinic acid; 2 mg. L^{-1} glycine; 30 g. L^{-1} sucrose; 9 g. L^{-1} Agar and without myo-inositol content. After 18 days the hyphae from the growth on the podetia were transferred to new MS plates. The plates were maintained in culture chamber at 20 ± 1°C without light incidence. A month later the endolichenic fungi isolated were subculture in MS and maintained at 20 ± 1°C and photoperiod of 16h light and 8h dark until the stromata formation. For stimulate the abundant formation of stromata in the mycelium, endolichenic fungi were sub-cultured in 2% malt extract agar (2% MEA) (Persŏh et al., 2009). The cultures were preserved in sterile water by Castellani method (Hartung et al., 1989) and deposited at HBEI under the vouchers numbers 001, 002 and 003, together with dry stromata of isolated *Xylaria* sp. 1 and *Xylaria* sp. 2, vouchers HBEI 021 and HBEI 022 respectively.

2.3. Morphological characterization

We described texture, topography, margin, exudates, coloration verse and reverse, amount of aerial mycelium and stromata production from the colonies formed by the

endolichenic fungi isolated. Stromata size, shape, surface and color were observed and measurements were based on available stromata in culture. To describe the color nomenclature was used the dictionary Maerz and Paul (1930). For measuring and characterizing microscopic reproductive structures, cross-sections were made by hand of the stromata and the material was mounted in water, Melzer iodine reagent and 5% KOH. The mean spore width and height was measurement taken from 20 randomly selected spores in water mounts. Microscopic features were measured (increasing 100x) and examined by differential interface contrast (DIC) and bright-field microscopy with optical microscope Axio Imager A2 (ZEISS®) equipped with Axiocam ERc5s (ZEISS®) and software ZEN 2 v 4.0.

2.4. DNA Extraction, Amplification, PCR Purification and Sequencing

Total DNA from mycelia were extracted using DNeasy Plant Mini Kit (Quiagen®) following the manufacturer's instructions. PCR amplification of the nuclear ribosomal internal transcribed spacers and the region 5.8S (ITS rDNA), and β-tubulin gene were performed using primers ITS1_F with ITS4_R (White et al., 1990) and Bt2b_F with Bt2a_R (Glass and Donaldson, 1995). The following cycle parameters were used for ITS1 and ITS4: initial denaturation at 94°C for 2 min, 30 cycles of 45 s at 94°C, 30 s at 55°C and 35 s at 72°C and a final elongation for 7 min at 72°C. For the Bt2b and Bt2a the amplification conditions were: 2 min at 94°C (denaturing), 29 cycles of 10 s at 94°C, 10 s at 58°C (annealing), 20 s at 72°C and 5 min at 72°C (extension). For the PCR reaction were used 4 μl DNA sample, 0.75 μl Milli-Q® H_2O, 1.25 μl solution of each primer and 1.2 μl of GoTaq® PCR Master Mix (Taq DNA polymerase, dNTPs and $MgCl_2$). PCR products were purified with the Wizard PCR Preps DNA Purification System (Promega®) kit according to the manufacturer's protocol and quantified using NanoVue™ Plus spectrophotometer. All products were sequenced in ABI-Prism 3500 Genetic Analyzer (Applied Biosystems). The sequences obtained were manually

adjusted using the software Bioedit v. 7.2.5 (Hall, 1999) and a consensus sequence was obtained using SeqMan package of Lasergene software v 14.0.0.86 (DNASTAR/Inc.). New sequences were deposited in Genbank under the accession numbers KY962975, KY962976, KY962977.

2.5. Phylogenetic analyses

ITS regions of rDNA and β-tubulin consensus sequences were compared to the Genbank database (www.ncbi.nlm.nih.gov) using the BLASTn to estimate the taxonomic placement of each isolate. The closest matches sequences with query cover and maximum identity ≥ 98% and ≥ 97% for sequences ITS and β-tubulin and e-value ≥ 0, were included in the phylogenetic analysis (Nilsson et al., 2008). Available sequences (November 2016) of endolichenic and endophytic fungi for ITS regions of rDNA and sequences of endolichenic fungi provided by U´Rent et al. (2016) for β-tubulin were downloaded from NCBI. In order to provide a phylogenetic context based in ITS rDNA for the isolated fungi from *C. curta*, four clusters of sequences were established: a) BLASTn search; b) endophytic and endolichenic fungi obtained by Arnold et al. (2009); c) isolated fungi from the interior of *Cladonia* and other lichen species (U'Ren et al., 2016) and d) liverwort endophytes fungi cultured by David et al. (2003). For β-Tubulin, one sequence group was formed including the sequences resulting from the use of the BLASTn algorithm and sequences of endolichenic isolated by U'Ren et al. (2016). The Genbank accession numbers for sequences of taxa included in phylogenetic analyses in this study are showed in Table 2 and Table 3 of supplementary material. For each data group, the pairs and multiples alignment were performed by using CulstalW as implemented in MEGA v. 6.06 (Tamura et al., 2013). Models for nucleotide substitution were estimated and these with the lowest BIC scores (Bayesian Information Criterion) were considered to describe the best substitution pattern (Supplementary material –

Table 4). Phylogenetic trees for each dataset of sequences were constructed with MEGA using maximum likelihood with Neighbor-Joining method and bootstrap values calculated from 1000 replicate (using all sites).

From each phylogenetic tree obtainded, based in ITS regions, the sequences of endophytes and endolichenic fungi most closely related to the species isolated during this work were selected (Supplementary material – Fig 8 -11) and the phylogenetic relationships between these were analyzed using Maximum Likelihood (ML) and Bayesian Inference (BI). Maximum likelihood as implemented in MEGA was used with bootstrap values calculated from 1000 replicate, Neighbor-Joining method and the Kimura 2-parameter substitution model. Bayesian analyses were conducted on the aligned data set using BEAST v. 1.8.3 (Drummond et al., 2012) using the Tamura-Nei model of equal base frequencies and gamma distribution with five categories. In order to identify the posterior probability tree set 10,000,000 million Markov chains Monte Carlo (MCMC) were run and trees were sampled every 1000 generations. Tracer v1.6 (Rambaut et al., 2014) was used to evaluate the effective population size (ESS >100) and TreeAnnotator v1.8.3 (of package BEAST) for condensed the information of the trees sampled by MCMC. The phylogenies produced from ML and BI based in ITS region of rDNA were rooted with sequences of *Ophiostoma valdivianum* (Butin) Rulamort (NR145317), *Ophiostoma eucalyptigena* Barber & Crous (NR137979), *Ophiostoma tetropii* Math.-Käärik (NR145271) and *Peziza fascicularis* (LT158418). The phylogenetic trees of β-tubulin were constructed as describe above for analyses of ML and BI. Maximum likelihood were estimated using Tamura-Nei substitution model and Tamura-Nei model of equal base frequencies and gamma distribution was used for Bayesian analyses. *Ophiostoma tetropii* (AY305701), *Ophiostoma grandicarpum* (Kowalski & Butin) Rulamort (KX590762) and *Ophiostoma microsporum* Arx (KX590764) were used to rooted. Alignments and trees are deposited in TreeBase.

3.   Results

3.1.   Endolichenic fungi isolated

We characterize both for molecular and morphological features three endolichenic fungi isolated from *Cladonia curta.* The initial growth of hyphae of isolated *Xylaria* spp. on *C. curta* podetia started four days after inoculation and continued for the next 13 days until the hyphae completely covered the podetia (Fig 1A-B). The podetia transferred to petri dishes with fresh culture medium continued the growth and developed mycelium until colony formation (Fig 1C). The appearance of colonies of isolates *Xylaria* spp. on MS in photoperiod and in darkness are present in Material supplementary (Fig 13 – 15). The stromata formation and differentiation occurred ten months (isolates MS1 and LB1) and one year (isolated MS2) after of isolation and consecutive subculture of lineages.

3.2. Phylogenetic analyses

The sequences obtained of axenic cultures of the endolichenic fungi from *Cladonia curta* resulted in BLASTn hits close for xylariaceous fungi. The isolates were considered as belonging to the *Xylaria* genus after a comparison of their nucleotide sequences that revealed an identity above 98% and 97% for regions ITS of rDNA and β-tubulin, respectively. The compatibility of species of endolichenic fungi mainly with sequences deposited as *Xylaria berteri* and reported as endophytic fungi of angiosperm (Douanla-Meli and Langer, 2012; Gazis and Chaverri, 2010; Hsieh et al., 2005; Hsieh et al., 2010; De Souza Leite et al., 2013; Rojas-Jiménez et al., 2016; Shibuya et al., 2003; Smith et al., 2008; Vaz et al., 2014; Vega et al., 2010), liverworts (Davis and Shaw, 2008) and ferns (Fu et al., 2013) (Table 1).

The total number of sequences of ITS regions of rDNA that were compared with sequences of endolichenic fungi of *Cladonia curta* was 52. The maximum likelihood (Fig 2) and Bayesian Inference (Fig 3) trees based in this dataset were congruent and differ slightly in their topology. Based on our results four distinct clade are supported (A, B, C and D). The clade A be comprised of a total of 14 endolichenic fungi sequences including *Xylaria arbuscula* Sacc., *Xylaria venustula* Sacc., 7 species of *Xylaria* isolated from six species of lichens (*Cladonia evansii*, *Usnea subscabrosa*, *Cladonia subradiata*, *Flavoparmelia caperata* and *Cladonia didyma*) (U´Rent et al., 2016) and four species sequences of endolichenic fungi isolated from *Peltigera neopolydactyla* s.l. lichen by Arnold et al. (2009). The formation of this clade is few supported using both the maximum likelihood and the Bayesian analyses with bootstrap values of 1% and posterior probability of 0.25. An endophytic fungus of *Faramea occidentalis* (*Rubiaceae*) (KT289626) was included in this clade using BI and inferred as sister group of two species sequences reported as endolichenic fungi (PP = 0.21).

The comparison of the phylogenetic trees obtained from analyzes of maximum likelihood and Bayesian Inference, showed that the clade B formation presents slight differences in composition and in grouping of the species. Within this clade were grouped new sequences from this study and sequences of fungi principally reported as endophytic and some endolichenic. Of these taxa, 9 are identified as *Xylaria berteri* in Genbank and were isolated from angiosperms in different studies: 6 sequences were obtained of fungi isolated from soybean cultivars *Glycine max* (*Fabaceae*) by De Souza Leite et al. (2013), two from dicotyledons by Hsieh et al. (2010) and one from *Myrceugenia ovata* var. nanophylla (Myrtaceae) by Vaz et al. (2014). Among the remaining species that comprise the clade B were included three species of endophytic fungi from liverworts that correspond to two species of *Xylaria* isolated from *Bazzania* sp. (*Lepidoziaceae*) and *Trichocolea tomentella* (Ehrhart) Dumortier (*Trichocoleaceae*) and an unidentified isolate from *Plagiochila*

(*Plagiochilaceae*) (EU686041), as well as, one sequence of endophytic *Xylaria* in *Cinchona pubescens* (*Rubiaceae*), two endophytic fungi of the *Xylariaceae* family, grown from the interior of healthy leaves of *Coffea arabica* (*Rubiaceae*), two isolated endophytic fungi from *F. occidentalis* and *Arundinaria gigantea* (*Poaceae*) respectively and three endolichenic fungi isolated from *Lecanora oreinoides* (JQ761659), *Usnea mutabilis* (JQ760593) and *Umbilicaria mammulata* (KT289568) lichens. This clade showed low ML support (1%) and was moderately supported on BI (PP= 0.49). The Bayesian analysis resulted in a modification of the clade B showed by ML, excluding *F. occidentalis* and an endolichenic of *P. neopolydactyla* s.l. (KT289550) were grouped with hepatic endophytic species that comprise the clade C.

The isolated *Xylaria* sp.1 (LB1) was inferred together with an endophytic fungus identified as *Xylaria berteri* cultivated from soybean leaves of the "Conquista" cultivar in a clade poorly supported by maximum likelihood (7%). In the same way, the posterior probability that *Xylaria* sp.1 is closest to soybean endophytic was low (0.08). The culturing *Xylaria* sp.2 (MS1) was showed forming a clade with one endophytic fungus of *F. occidentalis* (KT289626) and a endolichenic fungi cultured from *P. neopolydactyla* s.l. (KT289550) (ML = 3%). Using BI, this isolated shows close relationship with seven sequences of endophytic fungi from angiosperms identified as *X. berteri*. The isolated *Xylaria* sp.3 (MS2) was inferred as sister of the others lineages (PP= 0.38). Sequence similarities by ML between isolated *Xylaria* sp.3 (MS2) and the taxon identified as *X. berteri* (GU324750) isolated from bark of a dicotyledonous was 6%. Similarly, this isolate was inferred within the same clade with the endophytic *X. berteri* of *M. ovata* var. *nanophylla*, supported with posterior probability value of 0.07. Lower sequences similarities of ML analysis between *Xylaria* sp.3 (MS2) and the taxon identified as *X. berteri* (GU324750) isolated from bark of a dicotyledonous was found, up to 6% from both homologues. Likewise, this isolate was

inferred within the same clade with the endophytic *X. berteri* of *M. ovata* var. *nanophylla*, supported with posterior probability value of 0.07.

Seven species of *Xylaria* isolated by Davis et al. (2003) from liverworts rhizoids of *Bazzania* species and three species of endolichenic fungi of *P. neopolydactyla* s.l. comprises the clade C. This clade has low support with bootstrap value of 28% and a posterior probability of 0.18. The clade D it grouped together four endolichenic fungi isolated from *P. neopolydactyla* s.l. and a *Xylaria* isolated from *Bazzania* sp. with weak bootstrap support (18%) while that by posterior probability was strongly supported (0.99), however, no include the sequence of *Xylaria* sp. (AY315388) in this clade.

A total of 43 sequences from the β-tubulin gene were compared to the sequences obtained in this study (LB1 and MS1). Of these, two sequences were the result of the BLASTn search, one sequence of *Xylaria berteri* cultivated by Hsieh et al. (2010) and the rest of the sequences correspond to species of endolichenic fungi isolated and cultivated by U'Ren et al. (2016). The fungal isolate MS2 was not included in the phylogenetic analysis because it sequence presented a size smaller, low quality and e-value > 0 in the BLASTn query that generated conflict during the alignment with other sequences of endophytic and endolichenic fungi. Taking into account the results generated by the ITS regions of rDNA phylogeny, we consider pertinent to include the sequence of *X. berteri* (GQ502698) grown in culture from ascospores. The trees generated by the ML and BI analyses based on β-tubulin dataset were highly similar in topology. Since these trees are congruent only the tree obtained by Bayesian inference (Fig 4) is present and described in this document. The tree generated by ML is provided in the Fig 12 of supplementary material.

The phylogenetic tree inferred showed clearly the formation of two large clades and the sequence of endolichenic fungus from *Pseudevernia consocians* outside the groups. One of

them, the clade with high support (PP= 0.83) included the species of endolichenic fungi isolated from *Cladonia curta* (LB1 and MS1), three sequences of *X. berteri* and sequences of endophytic fungi isolated mainly from lichen species of the genus *Cladonia* (*C. evansii, C. didyma, C. subradiata, C. subtenius*) and other lichens (*Usnea subscrabosa* and *Flavoparmelia caperata*). The species remaining of endolichenic fungi formed a branch with low posterior probability support (0.35). The sequences of fungal isolates (MS1 and LB1) were close related to three sequence of *X. berteri*, two saprophytic and one endophytic (PP= 1) and sequences of *Xylaria cubensis* (Mont.) Fr. and *Xylaria* cf. *heliscus*, endolichenic fungi in *Lecanora oreinoides* and *Usnea mutabilis* respectively (PP= 1). This clade was strong supported with posterior probability value of 1.

4. Taxonomy

Isolated: *Xylaria* sp. 1 (LB1 – Fig 5)

Culture: colonies on MS medium cover a 9 cm plate in 18 days. In photoperiod of 16 h light and 8h dark, at first white, cottony, zoned in the center and irregularly radial toward the edge; abundant aerial mycelium forming prominent protrusions with flattened topography and finally forming irregular beige dark spots (M&P K1–Plate 9). Reverse mostly remaining white for up to four weeks, finally turning yellowish white (M&P G1–Plate 11); radial with black scores up to one millimeter in diameter forming concentric zones. In the center of the colonies was observed a primordium of stroma, pyramidal-acute, black with cylindrical stipe up to 0.8 mm of height with irregular flattened branches. In dark, the colonies were uniformly white, cottony, zoned in the center, radial towards the margin with elevated topography. Reverse radial, uniformly yellowish white. Clear droplets were exudate on surface of young colonies and (M&P B9–Plate 13) droplets were found in mature colonies. On 2% MEA the primordia grew and the mycelium formed more stromata.

Stromata: 10 stromata solitaries or gregarious were formed; stipe initially cylindrical to clavate later bifurcate to cerebriform with several small digitate projections, 1.5 – 3 mm (base) and 2 – 6 mm (apex) wide × 11 – 16 mm high, verruculose to dendroid surface; apex white and pale orange (M&P B9–Plate 4) at base during the formation of conidia to finally gray ocher (M&P E1–Plate 8) (Fig 5A-C). Hyphae with thick wall, dark brown, regularly septated with branches forming 90° angles, without reaction to the Melzer reagent (Fig 5E). The central axis of the stroma presents thin hyphae immersed in a gelatinous matrix.

Conidiospore: ovoid to clavate, 1.72 – 2.96 (3.47) × 4.30 – 6.35 (8.22) μm (M = 5.66 – 2.41 μm) of thin wall, smooth ornamentation, hyaline with flattened basal scar (Fig 5G-H). Conidiophores laterally compressed into tight layer or palisade.

Perithecia: the initial formation of primordium perithecium-like subglobose structures, that reaction with KOH 5% was observed (Fig 5D).

Ascospores: at least two ascospores, reniform, 2.27 – 2.20 × 5.01 – 5.10 μm, dark brown to blackish brown of thin wall with smooth ornamentation and longitudinal germ slit, straight were observed (Fig 5F).

Specimens examined: BRAZIL, Rio Grande do Sul – São Gabriel. Endolichenic fungi isolated from *C. curta*. February 11th, 2016. Isolator: Peña-Cañón, R. HBEI 001 and HBEI 021.

Commentary: based on synoptic key to *Xylaria* species of Callan and Rogers (1993) the isolated presents colonial and anamorphic features observed in cultures that are shared with *Xylaria multiplex* (Kunze) Fr. and *Xylaria longiana* Rehm.

Isolated: *Xylaria* sp. 2 (MS1 – Fig 6)

Culture: colonies on MS medium cover a 9 cm plate in 21 days. In photoperiod of 16 h light and 8h dark, the mycelium was uniformly white, cottony, radial non-zoned with flat

topography. Reverse radial and uniformly yellowish white (M&P G1–Plate 11). In the colonies was observed a primordium of stroma not-central, pyramidal-obtuse, black and irregular in appearance and shape on the reverse. In dark, colonies were white with center greyish white (M&P A1–Plate 39), cottony, radial, margin forming wide lobes and irregular topography. Reverse yellowish white (M&P G1–Plate 11) becoming more pale colored towards the margin, except in an area where developing partridge brown (M&P L12–Plate 15) to black patches. Clear droplets were exudate on surface of young colonies, orange (M&P B12–Plate 13) droplets in mature colonies. Present black immature stromata with cylindrical stipe up to 0.8 mm high, apex branched and irregularly flattened. On 2% MEA the primordia of stromata grew and the mycelium formed more stromata.

Stromata: on MS medium, cylindrical stipe of 12 mm high, central and irregularly branched in the apex; initially orange (M&P E8–Plate 13) later black at base with white apex (Fig 6A). On MEA 2%, 17 irregular to irregularly clavate stromata, $1 - 4$ mm (base) and $1 - 7$ mm (apex) wide $\times$ $9 - 16$ mm high were formed, verrucose to dendroid surface with distal lobes, irregularly verrucose extended up to 5mm from a base with two to three millimeters of high. Base of stipe white passing of willow brown color (M&P L7–Plate 15) to smoke brown (M&P A2–Plate 16) almost black and apex pale orange (M&P B9–Plate 4) (Fig 6B-C). Hypothecium of ectostroma formed by pseudo-catenulate, erect and varied bifurcated hyphae of thin wall, probably being the beginning of hymen formation; some hyphae presented anastomoses (Fig 6E).

Conidiospore: obovoid to clavate, $4.38 - 5.86 \times 9.92 - 10.99$ (13.35) μm (M = $5.21 - 10.60$ μm, n = 20), of thin wall, smooth ornamentation, hyaline with flattened basal scar (Fig 6F-H).

Perithecia: initials formation of perithecium were observed but without ascospores development (Fig 6D).

Specimens examined: BRAZIL, Rio Grande do Sul − São Gabriel. Endolichenic fungi isolated from *C. curta.* February 11th, 2016. Isolator: Peña-Cañón, R. HBEI 002 and HBEI 022.

Commentary: based on synoptic key to *Xylaria* species of Callan and Rogers (1993) the isolated shares a greatest number of colonial and anamorphic features with *Xylaria longipes* Nitschke, *Xylaria polymorpha* (Pers.) Grev. and *Xylaria schweinitzii* Berk. & M.A. Curtis.

Isolated: *Xylaria* sp. 3 (MS2 − Fig 7)

Culture: colonies on MS medium cover a 9 cm plate in 28 days. In photoperiod of 16 h light and 8h dark, at first white, cottony, non-zoned, margin forming wide lobes and elevated topography. The aerial mycelium later varied to pale pink (M&P D1−Pate 1) towards the margin. Reverse yellowish white with irregular zonation of semicircular brown spots (M&P H5−Plate 7). The formation of stromata primordia were observed at prolonged incubation times. Towards the edge of the colony was observed some primordium of stroma with apex pyramidal-acute, varying of pale orange (M&P B9−Plate 4) to white and cylindrical stipe, orange (M&P E8−Plate 13), between 0.3 − 0.9 mm in height. In dark, colonies white, cottony, with finely lobed margins and flat topography. Reverse yellowish white (M&P G1−Plate 11) and with black spots arranged radially.

Stromata: two stromata of 0.6 − 0.9 mm of height with 1 mm in diameter, smooth and orange (M&P A12−Plate 4) basal region and apex bifurcated, cottony and whitish covered with conidiophores (Fig 7A).

Conidiospore: obovoid to clavate, 1.67 − 2.41 (3.65) × (4.10) 4.81 − 6.26 (6.66) μm (M = 2.19 − 5.45 μm, n = 20), of thin wall, smooth ornamentation, hyaline with flattened basal scar (Fig 7B). Conidiophores laterally compressed into tight layer or palisade (Fig 7C).

Specimens examined: BRAZIL, Rio Grande do Sul - São Gabriel. Endolichenic fungi isolated from *C. curta*. February 11th, 2016. Isolator: Peña-Cañón, R. HBEI 003.

Commentary: based on synoptic key to *Xylaria* species of Callan and Rogers (1993) the isolated shares the greatest number of colonial and anamorphic features with *Xylaria polymorpha* (Pers.) Grev. and *Xylaria longiana* Rehm.

5. Discussion

The phylogenetic analyses of ML and BI based on the ITS regions of rDNA and β-tubulin dataset, infer and support the placement of the three fungal isolated from disinfected surface of podetia of *Cladonia curta* within the genus *Xylaria*. Unifying morphological characters for the *Xylaria* genus include conidiophores usually compressed laterally into tight layer or palisade covering all or part of the stromata surface, conidia hyaline, ovoid to ellipsoid and a flattened basal scar indicating the former point of attachment to the conidiogenous cell (Callan and Rogers, 1993). Thus, the morphological characteristics observed in anamorphic state of endolichenic fungi obtained in culture confirm their classification into this genus. The presence of *Xylaria* representatives as endolichenic in *C. curta* agreed with previous studies that revealed a particular diversity and richness of xylariaceous fungi into lichens of the diverse growth form and substrates, in subtropical, temperate and boreal environments (Arnold et al., 2009; Suryanarayanan et al., 2005; Suryanarayanan et al., 2017; U´Ren et al., 2016; Wu et al., 2011).

Taking into account that contemporaneously endophytic and endolichenic associations are two ecologically similar types of interactions that have influence on ecological network structure (Chagnon et al., 2016), both live within apparently healthy hosts and often represent the same phylogenetic lineages (Arnold et al. 2009; U'Ren et al. 2012), the molecular

phylogeny based in ITS region of rDNA was congruent inferring sequences of endolichenic fungi *Xylaria* spp., recovered in this work, as close to sequences of the endophytic fungi cultivated from angiosperms. Likewise, these sequences were grouped together in the major clades with sequences obtained from other endolichenic fungi showing topologies consistent with its reported by Arnold et al. (2009) and U´Rent et al. (2016), where endolichenic and endophytic fungi of order *Xylariales* were grouped in the same clades. This fact, confirmed the endolichenic lifestyle of these species as shown by previous work (Arnold et al., 2009; Petrini and Petrini, 1985; Suryanarayanan et al., 2005; Suryanarayanan et al., 2017; U´Ren et al., 2016; Wu et al., 2011). Nevertheless, we obtained reduced bootstrap support, as well as medium supported from posterior probability.

In the phylogenetic trees ITS region of rDNA, isolated *Xylaria* spp. were clustered with sequences of *Xylaria berteri* (Mont.) Cooke (in Index Fungorum (www.indexfungorum.org) the current name is X*ylaria berteroi* (Mont.) Cook ex J.D. Rogers & Y.M. Ju. [as *'berteri'*]), a species of saprophytic fungus reported as endophytic of angiosperms but which before had not been registered as endolichenic. However, sequences from type material of *X. berteri* are no available in Genbank database and in consequence some authors have assigned an endophytic lifestyle for *X. berteri* based only in query BLASTn (De Souza Leite et al.*,* 2013; Vaz et al.*,* 2014; Vega et al.*,* 2010); in Genbank database, 38 sequences of *X. berteri* were available: 27 correspond to sequences of 18S, 5.8S and 28S genes and internal transcribed spacer (ITS 1 and ITS 2) of rDNA, 6 sequences of the β-tubulin gene and four sequence of genes, Calmodulin, 1-alpha, alpha-actin and DNA-dependent RNA polymerase II (as of November 2016). In addition, it is important to note that in the case of the endophytic fungi is recommended that comparisons of sequence data be made using sequences from material type of species, and if such sequences are no available, then the data must be treated with caution (Ko Ko et al., 2011).

In accordance with U'Ren et al. (2010), our analyses based on β-tubulin data set revealed that endolichenic *Xylaria* spp. could be phylogenetically related with the saprophytic *X. berteri,* nevertheless they are more closely related to an endophytic symbiont isolated from bark of *Cyathea lepifera* (*Cyatheaceae*) (Fu et al., 2013), suggesting that associations of endolichenic fungi with lichen thalli are not purely incidental. Although endolichenic fungi of *Xylaria* are often host-generalists, Thomas et al., (2016) specify that they are not obligated and suggest that Foraging *Ascomycota* strategy to be a specialized survival or dispersal mechanism utilized by a subset of fungi as *Xylaria* and that the variation in niche or preferred habitat would modulate the selective advantage of endophytism.

On the other hand, there are a number of indications that support relationship between isolated *Xylaria* spp. endolichenic in *C. curta* and *X. berteri* with saprophytic lifestyle. First, the endolichenic fungi identified as *Xylaria cubensis* and *Xylaria* cf. *heliscus*, recovered from *L. oreinoides* and *U. mutabilis* (U'Ren et al., 2016) were nested within the same in-group where endolichenic of *C. curta* were located. Second, the results of the search using BLASTn algorithm to find sequences highly similar to ours included two sequences of *Xylaria allantoidea* (Berk.) Fr. (KR534643 and KR534722) with similarity and coverage values ≥ 98%; this species is considered part of the *X. cubensis* aggregate (Hsieh et al., 2010; Ju et al., 2012) and were isolated from healthy leaves of *Nertera granadensis* and *Leandra longicoma* (Rojas-Jiménez et al., 2016). In third place, *X. allantoidea*, *X.* cf. *heliscus* and *X. cubensis,* all are located in the clade "PO" (the clade containing *X. polymorpha* and closely related species) together with *X. berteri* in the currently phylogenetic status of the subfamily Xylarioideae (Hsieh et al., 2010; U´Ren et al., 2016). Finally, in the same way also contribute the similarity in the β-tubulin alignment of our sequences of endolichenic fungi with sequences obtained of *X. berteri*, from two saprophytic fungi that grow on wood of *C. carlesii* var. *sessilis* (Hsieh et al., 2005) and on dicot bark (Hsieh et al., 2010), both cultured from ascospores. In

concordance, U´Ren et al. (2016) suggest that in general, temperate and boreal xylariaceous endophytes, both plant and lichens, have endophytic and saprotrophic life stages. Also, *Xylaria* displaying both life stages were found in the endophytic and saprotrophic phases (Thomas et al., 2016).

The use of anamorphic characters of the cultures often lack sufficient information for taxonomic identification (Arnold et al., 2007; Petrini and Pertrini, 1985). Furthermore, the colonial and anamorphic features described in the key of Callan and Rogers (1993) for *Xylaria* species of continental United States and Canada can represent an alternative to approach to identification of lineages, however, in the same way delimits the identification to the species included. Thus, the endolichenic fungi recovered in this study exhibit similar morphological appearance of state anamorphic of five species, *X. multiplex*, *X. longiana*, *X. longipes*, *X. polymorpha* and *X. schweinitzii*. Nevertheless, is not possible to corroborate this information with other authors, since descriptions of *Xylaria* usually include descriptions of teleomorphic state (Da Silva and Cortez, 2015; Hladki and Romero, 2010; Medel et al., 2008; Rogers, 1983; Rogers and Ju, 2012) and this feature has not been obtained *in vitro* for endolichenic *Xylaria* spp.

The morphological features that characterize the teleomorph of *X. berteri*, including stromata ± pulvinate to discoid, with stipe or connective narrow and margins usually drooping (parasol- shaped) (Rogers and Ju, 2012), differs from the features observed in the anamorphous stromata of endolichenic fungi obtained in culture. Besides, none of taxa of endolichenic isolated present stromata sessile, a defining feature of all known taxa in the *Xylaria cubensis* (Mont.) Fr. aggregate, that is the group that harbors penzigioid species in which *X. berteri* is probably the most frequently observed (Ju et al., 2012). This species is reported throughout the tropics of both hemispheres growing on *Acacia koa*, *Albizia* sp.,

Alnus nepalensis, *Eucalyptus robusta*, *Fraxinus uhdei*, *Macadamia* sp., *Metrosideros polymorpha*, *Psidium* sp., *Sapindus saponari*, *Spathodea campanulata* (Rogers and Ju, 2012; Rogers and Ju, 2015). We also encountered that the size (8)12-13.5 x 6-7.5(8) of ascospores of *X. berteri* (Rogers and Ju, 2012) was greater than to present by isolated *Xylaria* sp. 1 (2.27 − 2.20 × 5.01 − 5.10), the unique specie of that only spores were observed. However, this observation is not conclusive considering the small number of spores found.

In vitro isolates of *Xylariaceae* specimens can be identified only after comparison with cultures that was originated from identified teleomorphic material (Callan and Rogers, 1990). Consequently, is not possible to determine with certainty the identification to specie-level of the tree isolated and culturing from lichen *C. curta* from cultural or anamorphic features. As recommended by Stlader et al. (2013) the teleomorphic material is needed to establish more teleomorph-anamorphism relations and finally apply a unified nomenclature to the *Xylariaceae* in general using a polyphasic taxonomic approach, especially in the tropical regions of the world and in the southern hemisphere. In the case of a conspecific relationship between isolated *Xylaria* spp and *X. berteri*, the group of taxa reported as endophytic fungi of angiosperms and species isolated in this work, at first time recorded as endolichenic, could be a species complex. Considering that the characterization on the anamorph of *Xylaria* is required to separate closely related taxa in certain species complexes (Callan and Rogers, 1990) this work provide primordial information for answer this question.

According to our results sequences of endophytic fungi in liverworts (Davis et al., 2003) that were observed growing inside the rhizoids without penetrating the thalli of their hosts were grouped in a separate clade of the sequences of endophytic fungi of angiosperms and lichens used in the present study. At the same time two endophytes in which hyphal growth

was not visible in or near the rhizoids using optical microscopy are shown integrating the clade that includes the sequences of endolichenic in *Cladonia curta*.

The most favorable medium for stromatal and conidial production by most *Xylaria* is 2% oatmeal agar (Callan and Rogers, 1993), 2% MEA (Persŏh et al., 2009) or PDA (Tripathi and Joshi, 2015). Nevertheless, based on experimental observations during the progress of this work, we recommend the use of culture medium MS as an alternative for the colony development of endolichenic fungi, taking into account that it could favors growth. Despite this, it is necessary using culture medium established (i.e. MEA 2%) for differentiation and development of structures of the anamorphic phase these fungi in culture.

6. Conclusion

Our work highlight the less explored diversity of endolichenic fungi reported at present, including the characterization morphological and molecular of three lineages of fungi that inhabit inside of symptomless lichen thallus of *Cladonia curta*. The phylogenetic analyses based on ITS region of rDNA and β-tubulin infers these endolichenic fungi close to the genus *Xylaria*. The morphological characteristics of colonies and anamorphous also confirm this classification. The phylogenetic relationships among species of endophytic fungi identified as *X. berteri* isolated of angiosperms and endolichenic *Xylaria* spp. is supported. However, the morphological features of anamorphic stromata obtained in culture provide arguments that discuss the identification as *X. berteri* of endolichenic fungi isolated in this work. Given the current situation, it is premature assign taxonomic names to specie-level for isolated endolichenic *Xylaria* spp. Our preliminary data based in molecular marker ITS rDNA and β–tubulin provides information complementary for evidence that endolichenic fungi are closely related to endophytic fungi and saprophytic fungi. Further studies are needed for accept or reject the hypothesis that endolichenic fungi isolated *Xylaria* spp. and *X. berteri* are

conspecific, specially phylogenetic analyses using robust multi-locus dataset. Besides, the use of culture medium MS constitute an alternative in the efforts to know the great diversity of endophytic fungi that inhabiting living tissues of lichens. Finally, the diversity and prevalence of the endolichenic fungi have not been studied extensively and this is the first report of isolation and identification of endolichenic fungi from a species of lichen collected in the south of Brazil.

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Disclosure Declaration

The authors declare that there is no conflict of interest.

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Table 1 Identification of fungal endolichenic isolated from *Cladonia curta* based on ITS region of rDNA and β-Tubulin gene sequences data comparison with sequences available in the Genbank database using BLASTn algorithm. Sequences of fungi of accession numbers JF773597, KP133344, JN418792, KC771483 and HQ117853 were not included; although they met the statistical significance the studies reporting these sequences have not yet been published.

ID Isolate (Size)	Region	Sequences of Genbank						
		Nearest match	Accession	Query cover	Max Identity	Substrate types	Host species	Reference
LB1 (565bp)	ITS rDNA	*Xylaria berteri*	GU324749	99%	98%	Bark	Dicotyledons	Hsieh et al., 2010
		Xylaria berteri	JQ936299	98%	99%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylariaceae sp.	EU009985	98%	98%	Leaves	*Coffea arabica*	Vega et al., 2010
		Xylaria berteri	JQ327861	99%	98%	Leaves	*Myrceugenia ovata* var. *nanophylla*	Vaz et al., 2014
		Xylaria berteri	JQ936295	99%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylariaceae sp.	EU009986	98%	98%	Leaves	*Coffea arabica*	Vega et al., 2010
		Xylaria berteri	JQ936294	99%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria berteri	JQ936291	98%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria sp.	AB743839	99%	98%	Stems	*Cinchona pubescens*	Shibuya et al., 2003
		Xylaria berteri	GU324750	99%	98%	Bark	Dicotyledons	Hsieh et al., 2010
MS1 (566bp)	ITS rDNA	*Xylaria berteri*	GU324749	100%	99%	Bark	Dicotyledons	Hsieh et al., 2010
		Xylaria berteri	JQ327861	100%	98%	Leaves	*Myrceugenia ovata* var. *nanophylla*	Vaz et al., 2014
		Xylariaceae sp.	EU009985	98%	99%	Leaves	*Coffea arabica*	Vega et al., 2010
		Xylaria berteri	JQ936295	100%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylariaceae sp.	EU009986	98%	98%	Leaves	*Coffea arabica*	Vega et al., 2010
		Xylaria berteri	JQ936294	100%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria berteri	JQ936291	99%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria allantoidea	KR534643	98%	98%	Leaves	*Nertera granadensis*	Rojas-Jiménez et al., 2016
		Xylaria berteri	GU324750	100%	98%	Bark	Dicotyledons	Hsieh et al., 2010
MS2 (515bp)	ITS rDNA	Fungal endophyte	EU686041	99%	99%	leaves	*Plagiochila* sp.	Davis and Shaw, 2008
		Xylaria berteri	GU324749	100%	99%	Bark	Dicotyledons	Hsieh et al., 2010

Table 1 Continued

| ID Isolate (Size)	Region	Sequences of Genbank						
		Nearest match	Accession	Query cover	Max Identity	Substrate types	Host species	Reference
		Xylaria berteri	JQ936300	99%	99%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria berteri	JQ936295	100%	99%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria berteri	JQ327861	100%	99%	Leaves	*Myrceugenia ovata* var. *nanophylla*	Vaz et al., 2014
		Xylaria berteri	JQ936294	100%	99%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria berteri	JQ936291	100%	99%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria allantoidea	KR534643	100%	98%	Leaves	*Nertera granadensis*	Rojas-Jiménez et al., 2016
		Xylaria berteri	JQ936297	100%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria sp.	AB743839	100%	99%	Stems	*Cinchona pubescens*	Shibuya et al., 2003
		Xylaria berteri	JQ936292	100%	99%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria berteri	JQ936301	98%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria berteri	JQ936298	98%	98%	Leaves	*Glycine max*	De Souza Leite et al., 2013
		Xylaria allantoidea	KR534722	100%	98%	Leaves	*Leandra longicoma*	Rojas-Jiménez et al., 2016
		Xylaria sp.	JQ341065	100%	98%	Leaves	*Diospyros crassiflora*	Douanla-Meli and Langer, 2012
		Xylaria allantoidea	FJ884194	99%	98%	Leaves	*Hevea brasiliensis*	Gazis and Chaverri, 2010
		Xylaria berteri	GU324750	98%	99%	Bark	Dicotyledons	Hsieh et al., 2010
		Fungal endophyte	EU977315	99%	98%	Twigs	Angiosperm	Smith *et al.*, 2008
LB1 (404bp)	β - tubulin	*Xylaria berteri*	AY951763	100%	98%	On wood	*Castanopsis carlesii* var. *sessilis*	Hsieh et al., 2005
		Xylaria berteri	KC473561	100%	98%	Bark	*Cyathea lepifera*	Fu et al., 2013
MS1 (409bp)	β - tubulin	*Xylaria berteri*	AY951763	100%	97%	On wood	*Castanopsis carlesii* var. *sessilis*	Hsieh et al., 2005
		Xylaria berteri	KC473561	100%	97%	Bark	*Cyathea lepifera*	Fu et al., 2013
MS2 (242bp)	β - tubulin	*Xylaria berteri*	FJ904911	90%	77% 1e-21	Leaves	*Grevillea robusta*	Unpublished
		Xylaria berteri	AY951763	90%	77% 1e-21	On wood	*Castanopsis carlesii* var. *sessilis*	Hsieh et al., 2005
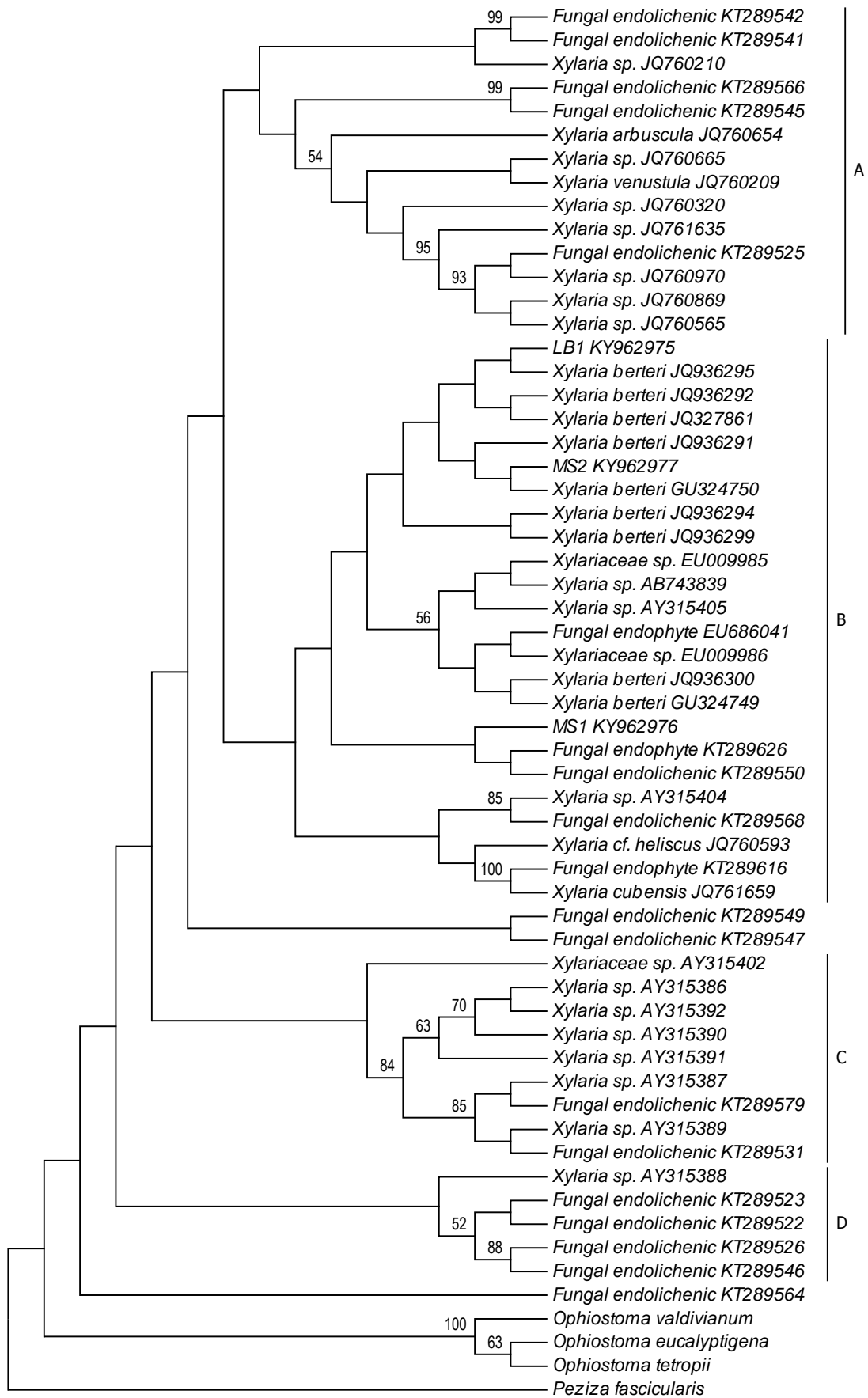
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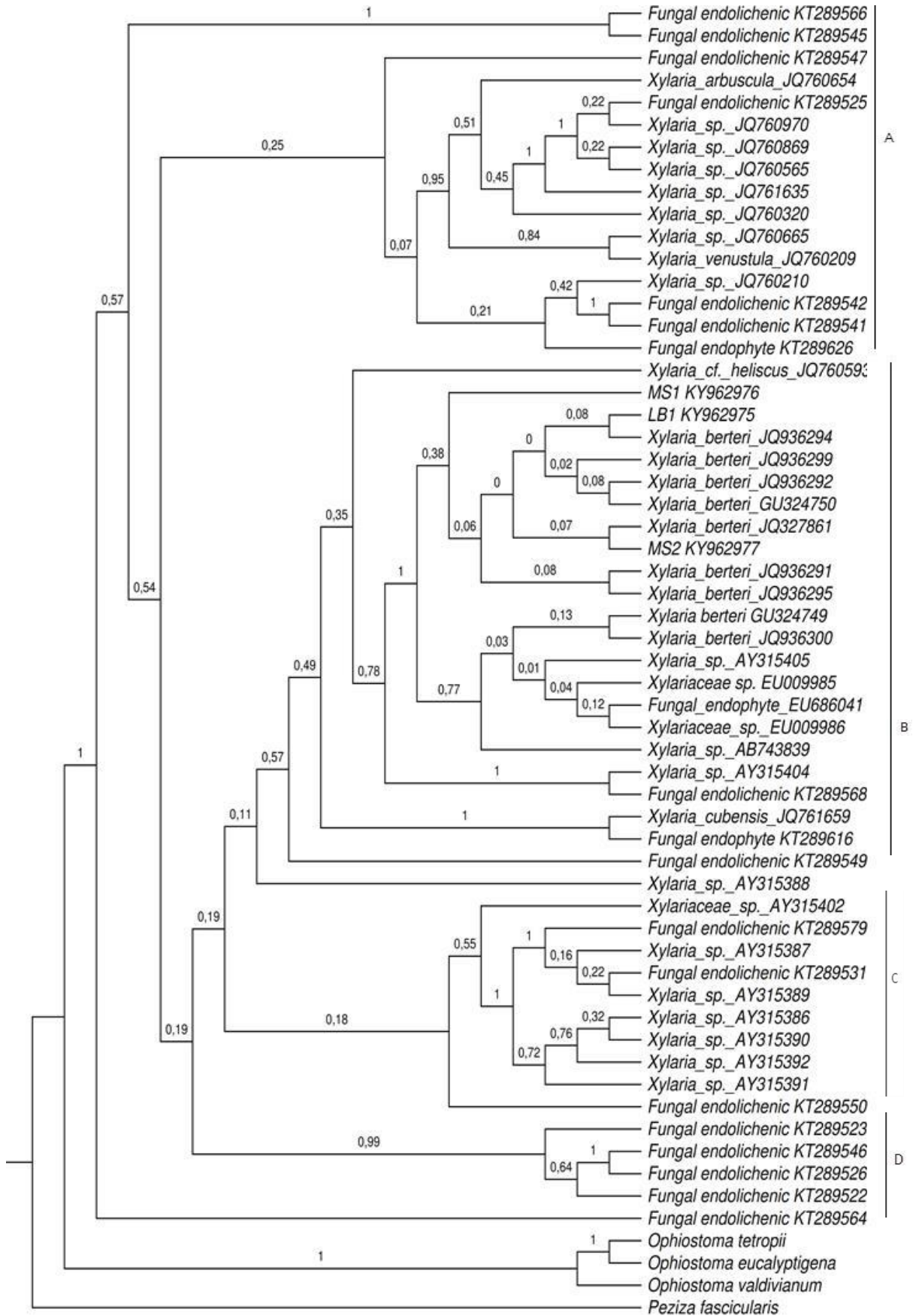
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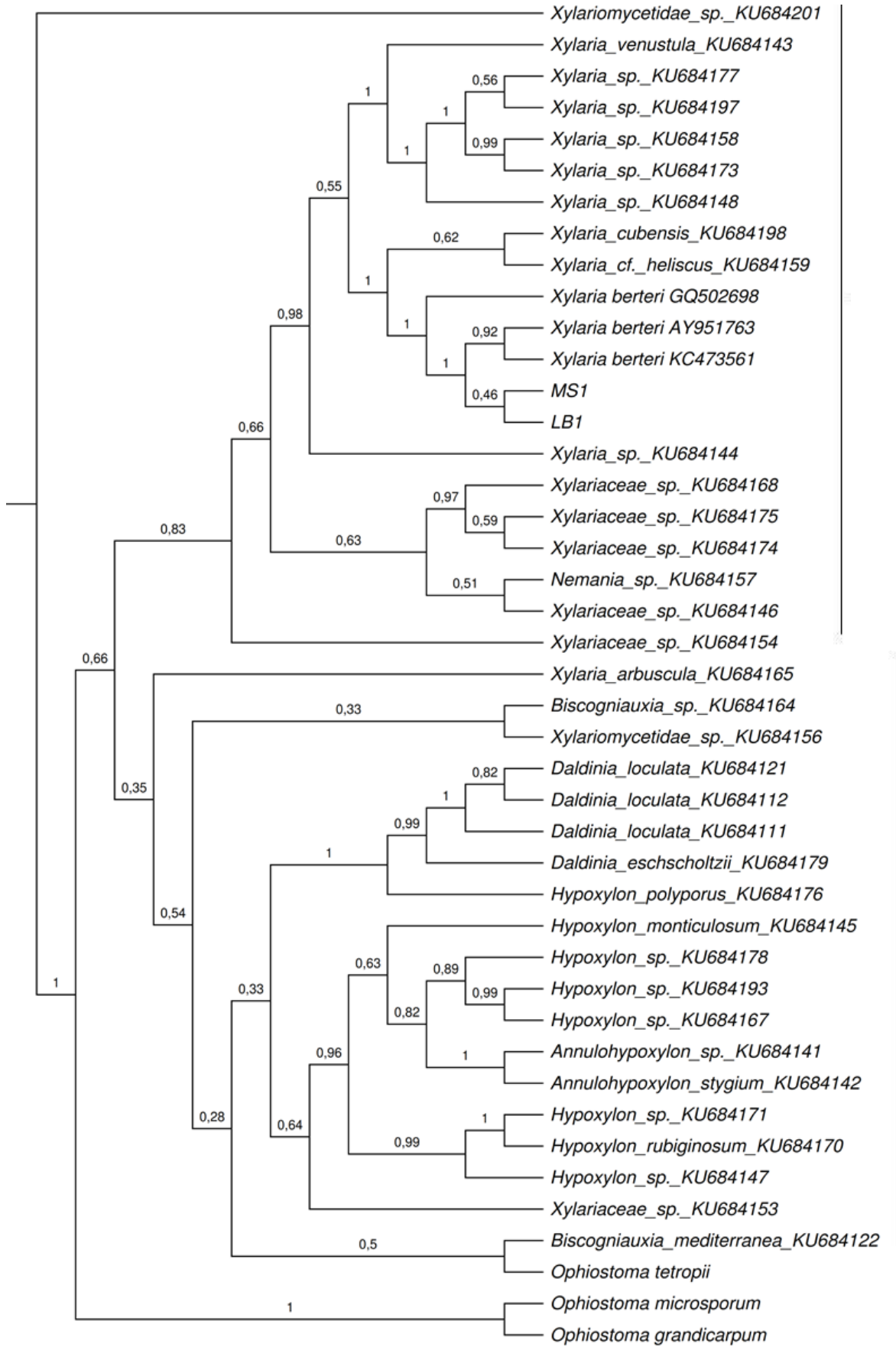
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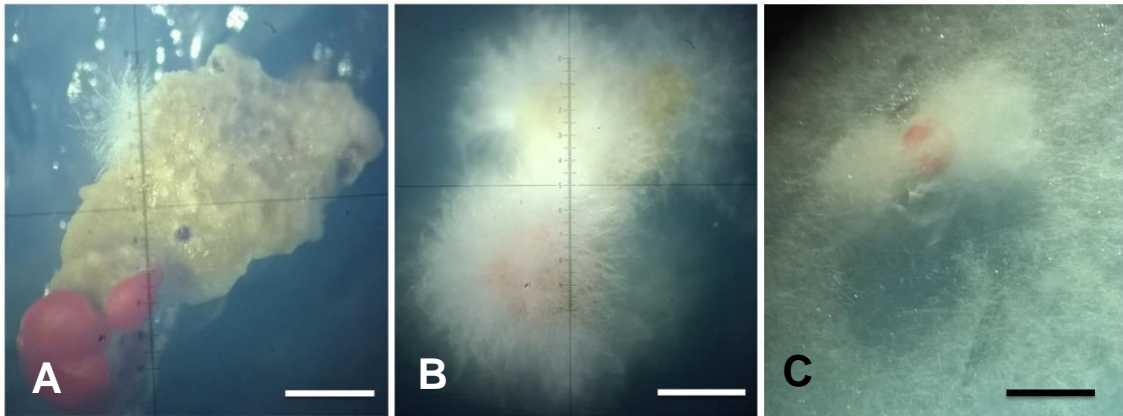
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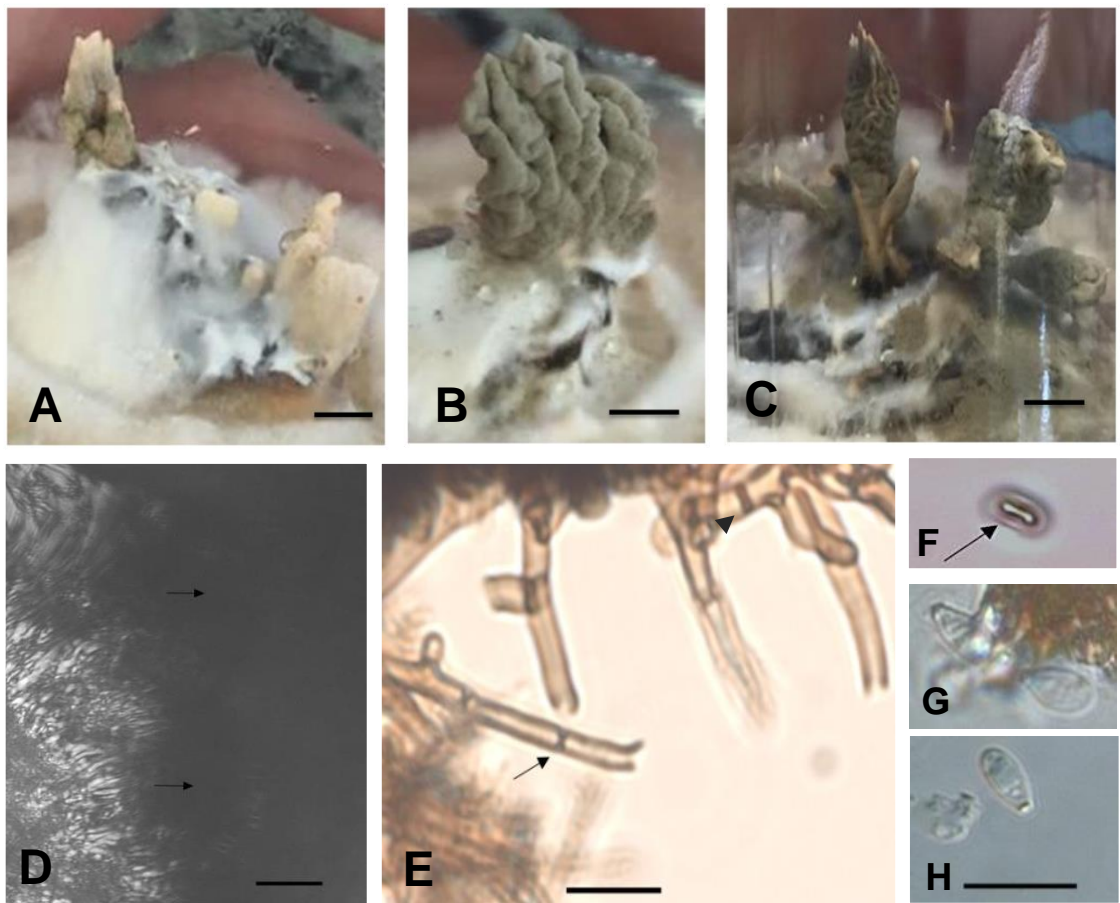
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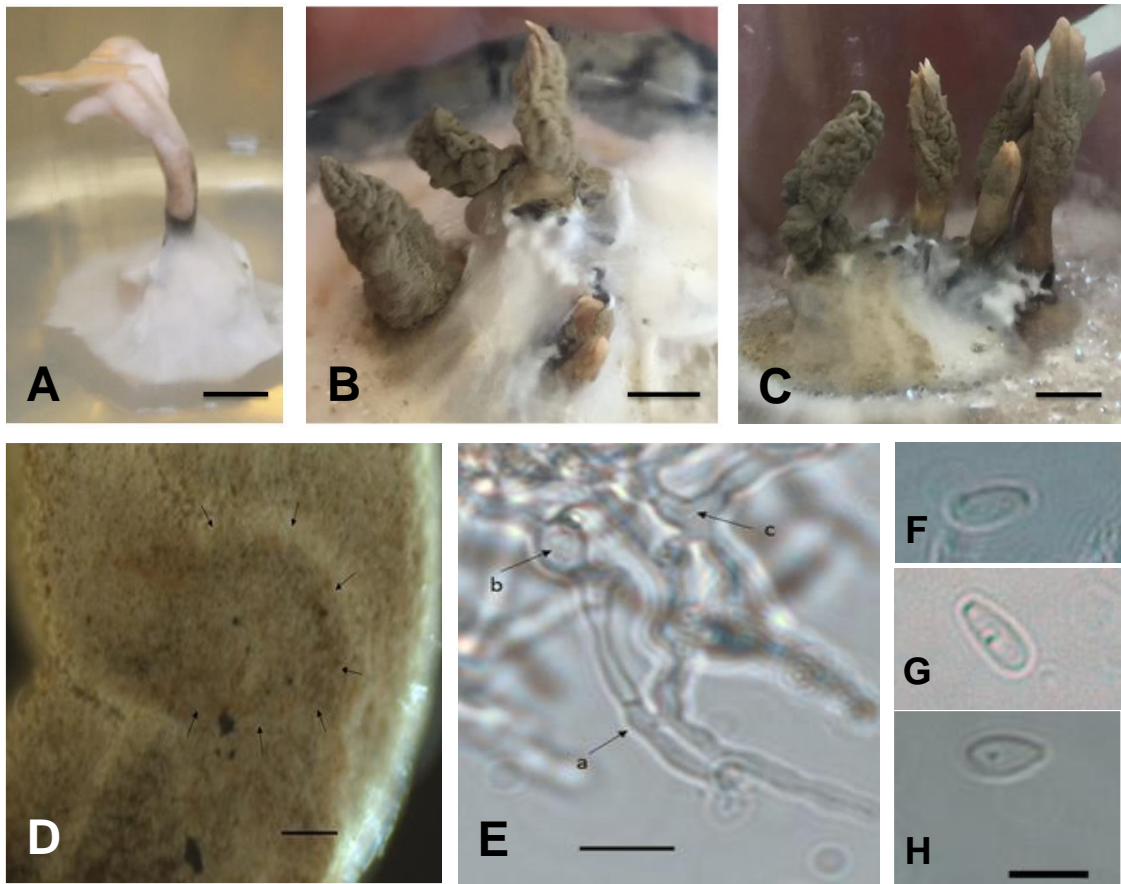
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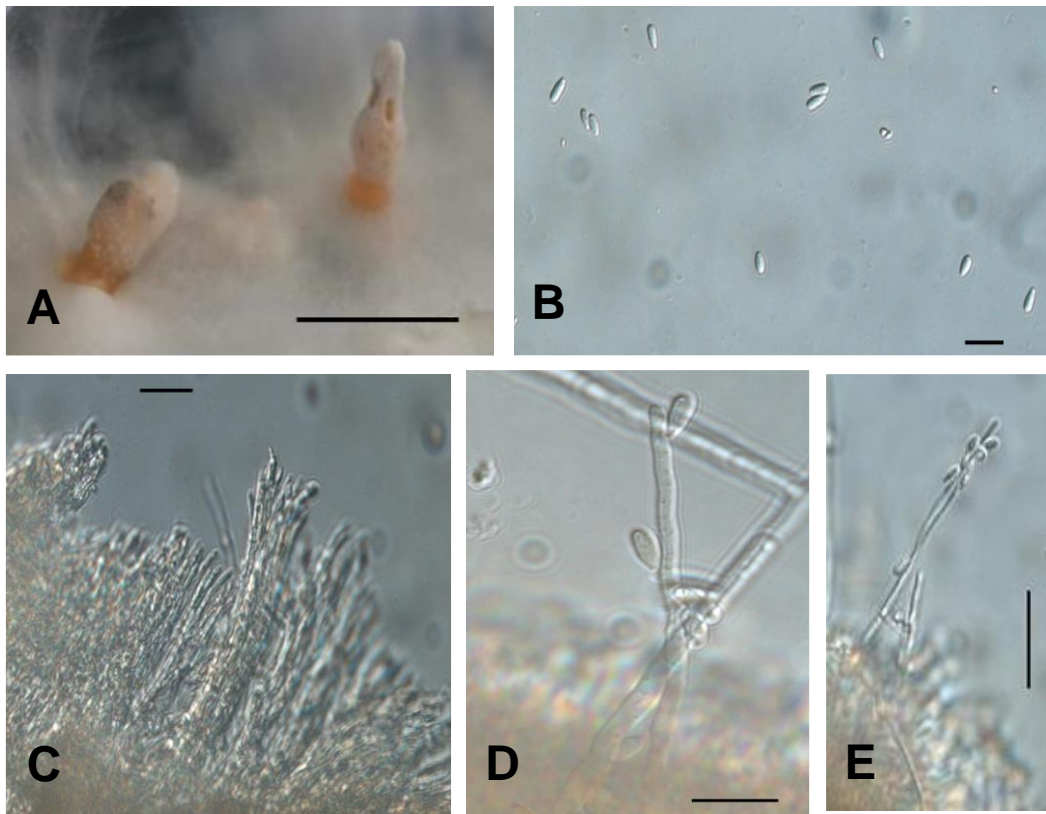
Fig. 7

Figure legends

Fig 1 – Endolichenic *Xylaria* growing on podetia of *Cladonia curta*. (A – B). Stereoscope (increasing 1.6); (C). Stereoscope (increasing 3.2); (A). Initial growth of hyphae on podetium after four days of inoculation; (B). Appearance of hyphae 18 days after isolation; (C). Formation of the mycelium in culture medium MS. – Scale bars (A – C) = 500 μm.

Fig 2 - Phylogenetic relationships among endolichenic fungi isolated from *Cladonia curta* and sequences of endophytic fungi from plants (BLASTn; Arnold et al.,2009), liverworts (Davis et al., 2003) and lichens (Arnold et al.,2009; UˊRen et al., 2016). MS1, MS2 and LB1 corresponding to sequences obtained in the present study. The phylogenetic tree inferred by maximum likelihood based on one-locus ITS region of rDNA dataset. The numbers at each internode indicates bootstrap support values. Three species of *Ophiostoma* (*O. valdivianum*, *O. eucalyptigena* and *O. tetropii*) and *Peziza fascicularis* were used as outgroup.

Fig 3 - Phylogenetic relationships among endolichenic fungi isolated from *Cladonia curta* and sequences of endophytic fungi from plants (BLASTn; Arnold et al.,2009), liverworts (Davis et al., 2003) and lichens (Arnold et al.,2009; UˊRen et al., 2016). MS1, MS2 and LB1 corresponding to sequences obtained in the present study. A phylognetic tree generated by using BI analysis from the ITS region of rDNA dataset. Number at internodes represent posterior probability values of a 95% majoritty rule consensus tree from 10,000,000 million generation Markov Chain Monte Carlo analysis. Three species of *Ophiostoma* (*O. valdivianum*, *O. eucalyptigena* and *O. tetropii*) and *Peziza fascicularis* were used as outgroup.

Fig 4 - Phylogenetic relationships among endolichenic fungi isolated from *Cladonia curta*, sequences of endophytic fungi from other lichens (UˊRen et al., 2016) and closest match BLASTn. MS1 and LB1 corresponding to sequences of two fungi obtained in the present study. The phylogenetic tree created by using Bayesian statistical analysis with 10,000,000 million generation Markov Chain Monte Carlo based on one-locus dataset β-tubulin. The numbers at each internode indicates posterior probability

values of a 95% majoritty rule consensus tree. *Ophiostoma tetropii*, *Ophiostoma grandicarpum* and *Ophiostoma microsporum* were used to rooted the trees.

Fig 5 – Isolated *Xylaria* sp. 1. (HBEI 021) (A – C). Stromata obtained in culture on MEA 2% medium; (D – F). Bright - field microscopy; (D). Initial formation perithecia (arrows); (E). Hyphae regularly septated (arrow) with branches forming 90° angles (arrowhead); (F). Ascospores with longitudinal germ slit (arrow); (G – H) DIC microscopy, Conidiospore. – Scale bars (A – C) = 4 mm, (D – H) = 10 µm.

Fig 6 - Isolated *Xylaria* sp. 2. (HBEI 022) (A).Stroma obtained in culture on MS medium; (B – C). Stromata on MEA 2% medium; (D). Initial formation of the perithecium (arrows); (E). Bright - field microscopy. Hyphae of hypothecium with anastomose (arrow a) and basal (arrow b) and irregular hyphae (arrow c). (D, F – H). DIC microscopy; (F – H). Conidiospore. – Scale bars (A – C) = 4 mm, (D – H) = 10 µm.

Fig 7 - Isolated *Xylaria* sp.3. (A). Stromata obtained in culture on MS medium; (B – E). DIC microscopy (40x); (B). Conidiospore; (C). Palisade of mature conidiophores; (D). Mature conidiophores; (E). Conidia produced at the tip of a conidiophore. – Scale bars (A) = 1 mm, (B – E) = 10 µm.

4. ARTIGO 2: Occurrence of the *Cladonia curta* Ahti & Marcelli (*Cladoniaceae*, lichenized *Ascomycota*) in the Pampa biome

(Artigo a ser submetido como Nota Científica para a Acta Botânica Brasílica).

Occurrence of the *Cladonia curta* Ahti & Marcelli (*Cladoniaceae*, lichenized *Ascomycota*) in the Pampa biome

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Abstract. *Cladoniaceae* is a family with more than 450 species registered and distributed in the most varied environments. It presents species with large thalli, as well as species with diminutive thalli, which often are neglected even by lichenologists. The aim of this note is to record a occurrence of *Cladonia curta* in the Pampa biome, a species previously known only from Cerrado and mesophyll forest in the states of Minas Gerais, São Paulo and the Federal District, considered a very rare or ignored species.

Introduction

The Brazilian portion of the Pampa biome is located in the Southern region of the country, in the state of Rio Grande do Sul, within the Temperate Zone showing both subtropical and temperate climates. The biome represents about 2.07% of the national territory, occupying an area of 176,496 km². Although is frequently interpreted as a pure grassland environment, it comprises at least seven different physiographic formations: savanna, steppe, steppe-savanna,

coast, transition areas and patches of seasonal deciduous and semideciduous forests (Roesch *et al.*, 2009).

The state of Rio Grande do Sul has a well-documented lichen biota (although probably underestimated). Spielmann (2006) listed 912 species of lichenized fungi for the state, distributed among the most diverse environments, among which, the Pampa biome was an important collection area, with researches carried out mainly by the lichenologists: Gustaf O. An. Malme, Mariana Fleig and Hector S. Osorio. Fleig *et al.* (1995) recorded 40 species of Cladoniaceae for the state, distributed in four different genera: *Cladia* Nyl., *Cladina* (Nyl.) Nyl. (=*Cladonia*), *Cladonia* P. Browne and *Pycnothelia* (Ach.) Dufour. Three species of *Cladonia* were described as new (*C. maculate*, *C. paranaensis* and *C. quiririensis*) and two new records for Brazil, as well as several new records for the state (Charnei et al., 2014). However, the last initiative to inventoried lichenized fungi species for the Pampa Biome was carried out punctually in a conservation area, where 254 taxa were recorded, including three *Cladonia* species, such *Cladonia ceratophylla* (Sw.) Spreng., *Cladonia chlorophaea* (Flörke *ex* Sommerf.) Spreng. and *Cladonia* cf. *sphacelata* Vain (Käffer *et al.,* 2015).

Cladoniaceae has species very variable morphologically (Fleig *et al.* 1995; Ahti 2000), from species with very prominent thalli occupying large areas (e.g., *Cladonia crispatula* (Nyl.) Ahti and *C. confusa* R. Sant.), to very discrete species, with reduced podetia and/or primary squamules (*C. cartilaginea* Müll. Arg. and *C. nana* Vain.), these usually overlooked in field.

The present article aim to contribute to the knowledge about the distribution of the family *Cladoniaceae* in Brazil, reporting the occurrence of the specie *Cladonia curta* Ahti & Marcelli in Pampa biome. Until then, according to Ahti (2000), the species endemic from Brazil, was known in three localities to the Southeast region, from Federal District, state of

Minas Gerais, and state of São Paulo, in mesophilic forests and Cerrado, between 800 and 1200 alt.

Materials and Methods

The samples of lichen were collected in the municipality of São Gabriel, state of Rio Grande do Sul – Brazil at Federal University of Pampa (UNIPAMPA) campus (30° 20' 06.3" S, 054° 21' 46.5" W) at 124 m asl., on dead wood of an *Eucalyptus camaldulensis* Dehnh (Myrtaceae) trunck, in edge of vegetable spot with *Eucalyptus* understory. Predominant native species of the generational state include *Schinus polygamus* (Cav.) Cabrera, *Lithraea molleoides* (Vell.) Engl.*, Saccharum angustifolium* (Nees) Trin.*, and representative of genera *Baccharis*, *Eugenia*, *Vachellia* and *Eryngium*. Four samples of material vegetal were collected from October 2015 to February 2016; rainfall average 196 ±1 mm, humidity was typically medium (~57 %), and daily temperatures at the site were range from 18°C to 27°C (INMET, 2017).

The lichen samples were deposited in the Bruno Edgar Irgang Herbarium (HBEI-Federal University of Pampa) under the voucher number HBEI 023 and Joinvillea herbarium (JOI- University of Joinville Region). The specimens were examined using standard stereoscopic and light microscopic techniques according to Brodo *et al.* (2001). Sections of primary thallus, podetia and ascomata were mounted in water. Chemical constituents were identified by spot tests, ultraviolet light exposure and thin layer chromatography using solvent system C (Orange *et al.,* 2001; Elix, 2014).

Results and discussion

Cladonia curta Ahti & Marcelli, Flora Neotropica 78: 192. 2000.

Type. Brazil. Minas Gerais: Mun. Catas Altas, 0.5 km S of Santuario do Caraca, 1100-1200 m, 1993, *Marcelli, Ahti, et al. 25916* (holotype, SP; isotype, H).

Description. Primary thallus persistent, irregular, squamules 0.8–3.5 mm wide, 0.5–4.5 mm long, slightly to clearly arachnoid above, whitish to beige, in part dissolved into soredia, margins irregularly crenate, slightly sorediate, soredia 200–800μm in diam.; upper surface greenish, slightly maculate, smooth, epruinose, cortex 60–120 μm thick, algal layer continuous, 15–25 μm thick, medulla 100–500 μm thick. Pycnidia not seen. Podetia common, inconspicuous, 0.5–0.8 mm tall, 0.2–1.0 mm thick, greenish, submarginal to marginal, unbranched, ascyphose; surface verruculose-corticate, in part denuded mainly near the hymenial discs, esorediate, usually esquamose, not melanotic; cortex 0–50 μm thick, medulla loose, not clearly differentiated between stereome and central cavity. Pycnidia not seen. Hymenial disks red, 1.0–1.8 mm diam., hymenium hyaline, 25–30 μm thick, hipohymenium 25–38 μm thick, ascospores not seen (Figure 1).

Chemistry. Spot tests: [primary squamules and podetia] K-, C-, KC-, P-, UV-. TLC: barbatic and didymic acids, traces of condidymic. Hymenia with rhodocladonic (K+ purple).

Specimens examined: Brazil. State of Rio Grande do Sul, Municipality of São Gabriel, 30°20'06.3"S, 54°21'46.5"W, on dead wood, Pena-Cañón, R. s.n. (HBEI 023; JOI).

Comments. *Cladonia curta* is characterized by the discrete primary squamules, and by the short podetia with red hymenial disc. The primary squamules are arachnoid above and slightly sorediated at margins. The podetia are mainly corticated without symbiotic propagules.

The species is similar to *C. pumila* Ahti by the tiny podetia, but differs by the chemistry (sekikaic acid) and byssoid primary squamules in the last (Ahti, 2000). The constant presence of the red hymenial discs could remind *C. didyma* (Fée) Vain. or even a tiny podetia of *C. macilenta* Hoffm., however, both species have a ecorticate podetia, added *C. didyma* present squamules on podetia, and *C. macilenta* are dense sorediate (Gumboski & Eliasaro 2012).

Specimens of *Cladonia ahtii* S. Stenroos and *C. miniata* G. Mey grows on wood and could remind *C. curta*. However, *C. ahtii* and *C. miniata* have a well-developed primary squamules, *C. ahtii* present a clearly sorediate under surface and margins, while *C. miniata* have a reddish medulla on primary thallus (Stenroos 1989; Fleig *et al.* 1995; Ahti 2000).
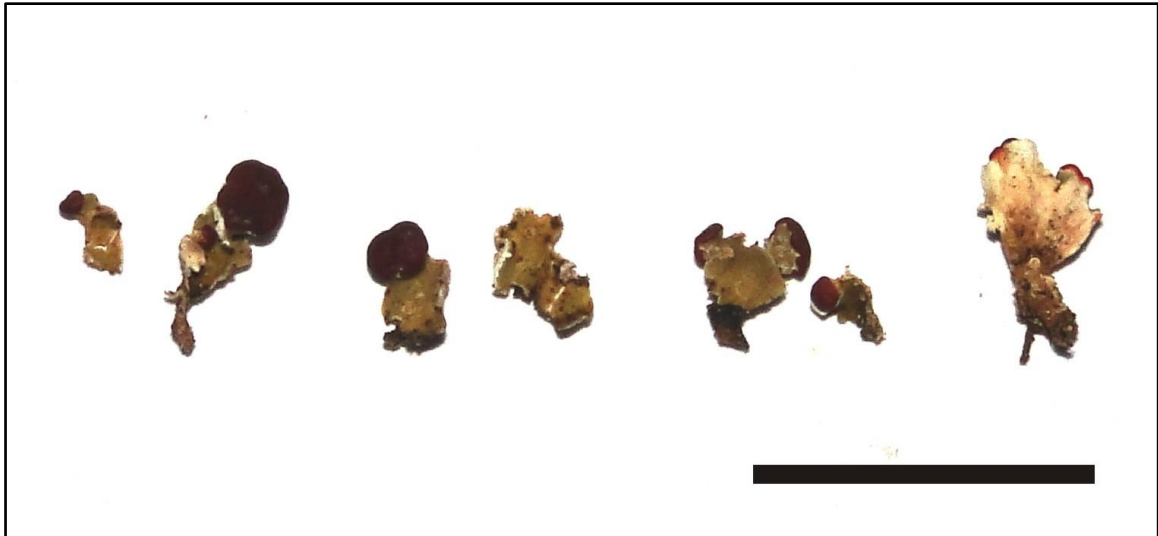
Figure 1 - Podetia of *Cladonia curta* (specimens on JOI). Scale bar = 1.0 cm.

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5.   CONSIDERAÇÕES FINAIS

Com base nos dados obtidos no presente trabalho pode-se concluir que a caracterização morfológica e molecular de três linhagens de fungos que habitam dentro do talo assintomático do líquen *Cladonia curta* Ahti & Marcelli, fornece informações complementares para evidenciar que fungos endoliquênicos estão intimamente relacionados com fungos endófitos e fungos saprófitos. Porém a relação parece ser mais estreita com fungos endófitos em angiospermas. As análises filogenéticas baseadas na amplificação das sequencias da região ITS do rDNA e do gene β- Tubulina, infere os fungos endoliquênicos recuperados durante o presente estudo como próximos ao gênero *Xylaria*. Da mesma maneira, as características morfológicas das colônias e dos estromas obtidos *in vitro* também confirmam esta classificação.

Por sua parte, a filogenia molecular baseada em sequencias da região ITS do rDNA, suporta a relação filogenética entre as três linhagens de fungos endoliquênicos isolados com fungos endófitos de angiospermas identificados na base de dados do Genbank como *Xylaria berteri*, uma espécie saprófita antes não citada como endoliquênica. No entanto, o formato cilíndrico a clavado dos estromas da fase assexuada obtidos em cultura é dissimilar ao estroma séssil, tipo característico observado nesta espécie penzigioide. Assim, se estabelecem argumentos que discutem a identificação dos fungos endoliquênicos isolados neste trabalho como *Xylaria berteri* (Mont.) Cooke. Com tudo, é prematuro atribuir nomes taxonômicos ao nível de espécie para os isolados *Xylaria* spp. Estudos adicionais são necessários para confirmar ou contradizer que estes fungos sejam conspecíficos com *X. berteri*.

Considerando que a espécie de líquen objeto de estudo corresponde a um novo registro de ocorrência para o sul do Brasil, região do país incluída no bioma Pampa, o presente trabalho contribui a ressaltar a grande importância desde bioma como fonte de biodiversidade que requer ser explorada. Ao mesmo tempo, a diversidade e a prevalência dos fungos endoliquênicos continua sendo estudada com este, o primeiro relato de isolamento e identificação de fungos endoliquênicos no Brasil.

Embora, os meios de cultura previamente estabelecidos para o isolamento de fungos endoliquênicos não foram usados nas praticas experimentais, o meio Murashige e Skoog (MS) resultou ser útil na recuperação de fungos endoliquênicos. Sugere-se seu uso como uma alternativa para o isolamento, crescimento e desenvolvimento deste tipo de fungos, numa temperatura de 20°C. Do mesmo modo se ressalta a necessidade e importância de usar meios tradicionais, no caso MEA 2%, para a formação de estromas anamórficos em cultivo.

6.   PERSPECTIVAS FUTURAS

- Tendo em conta que fungos endoliquênicos residem dentro do talo dos liquens numa estreita associação com o fotobionte, se espera utilizar o cultivo dos fungos endoliquênicos *Xylaria* spp. e o fotobionte de *Cladonia curta* Ahti & Marcelli (Fig. 2), isolados durante o presente estudo, em processos de ressíntese, tentando assim ter uma melhor compreensão dos processos de reconhecimento e de interação existentes entre eles e identificar os níveis de expressão de genes relacionados com a simbiose.

Figura 2 – Fotobionte de *Cladonia curta* obtido em cultura. (A). Aspecto macroscopico da colonia crescendo em meio trebouxia a 20°C em fotoperiodo 16h luz / 8h osuridad. (B). Aspecto microscopico do fotobionte em aumento 40x.
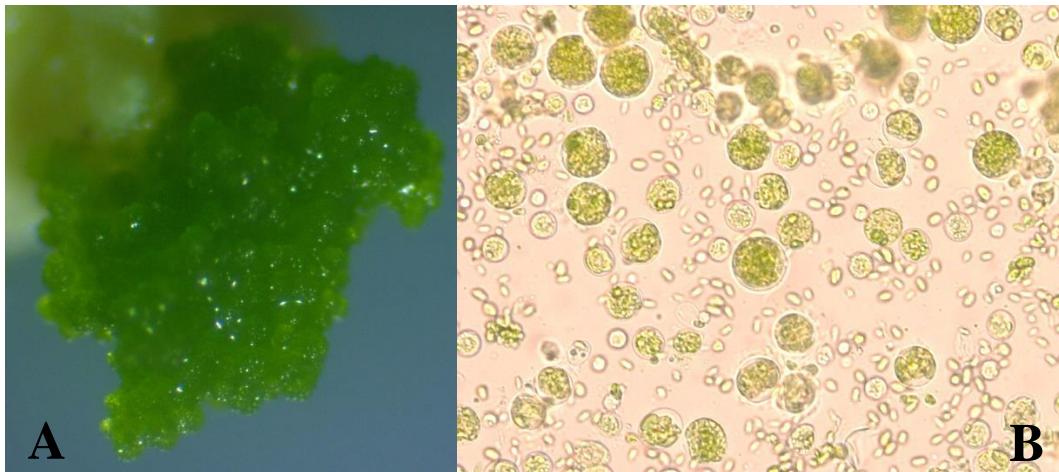
Fonte: Autora

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8. ANEXOS

Supplementary Material

Table 2 Genbank accession numbers and references for ITS region of rDNA sequences of taxa included in phylogenetic analyses in this study.

Accession	Reference	Accession	Reference
AY315386	Davis et al., 2003	KT289545	Arnold et al., 2009
AY315387	Davis et al., 2003	KT289546	Arnold et al., 2009
AY315388	Davis et al., 2003	KT289547	Arnold et al., 2009
AY315389	Davis et al., 2003	KT289548	Arnold et al., 2009
AY315390	Davis et al., 2003	KT289549	Arnold et al., 2009
AY315391	Davis et al., 2003	KT289550	Arnold et al., 2009
AY315393	Davis et al., 2003	KT289551	Arnold et al., 2009
AY315394	Davis et al., 2003	KT289552	Arnold et al., 2009
AY315395	Davis et al., 2003	KT289553	Arnold et al., 2009
AY315396	Davis et al., 2003	KT289554	Arnold et al., 2009
AY315400	Davis et al., 2003	KT289555	Arnold et al., 2009
AY315401	Davis et al., 2003	KT289556	Arnold et al., 2009
AY315402	Davis et al., 2003	KT289557	Arnold et al., 2009
AY315404	Davis et al., 2003	KT289558	Arnold et al., 2009
AY315405	Davis et al., 2003	KT289559	Arnold et al., 2009
AY315406	Davis et al., 2003	KT289560	Arnold et al., 2009
AY315407	Davis et al., 2003	KT289561	Arnold et al., 2009
KT289521	Arnold et al., 2009	KT289562	Arnold et al., 2009
KT289522	Arnold et al., 2009	KT289563	Arnold et al., 2009
KT289523	Arnold et al., 2009	KT289564	Arnold et al., 2009
KT289524	Arnold et al., 2009	KT289565	Arnold et al., 2009
KT289525	Arnold et al., 2009	KT289566	Arnold et al., 2009
KT289526	Arnold et al., 2009	KT289567	Arnold et al., 2009
KT289527	Arnold et al., 2009	KT289568	Arnold et al., 2009
KT289528	Arnold et al., 2009	KT289569	Arnold et al., 2009
KT289529	Arnold et al., 2009	KT289570	Arnold et al., 2009
KT289530	Arnold et al., 2009	KT289571	Arnold et al., 2009
KT289531	Arnold et al., 2009	KT289572	Arnold et al., 2009
KT289532	Arnold et al., 2009	KT289573	Arnold et al., 2009
KT289533	Arnold et al., 2009	KT289574	Arnold et al., 2009
KT289534	Arnold et al., 2009	KT289575	Arnold et al., 2009
KT289535	Arnold et al., 2009	KT289576	Arnold et al., 2009
KT289536	Arnold et al., 2009	KT289577	Arnold et al., 2009
KT289537	Arnold et al., 2009	KT289578	Arnold et al., 2009
KT289538	Arnold et al., 2009	KT289579	Arnold et al., 2009
KT289539	Arnold et al., 2009	KT289580	Arnold et al., 2009
KT289540	Arnold et al., 2009	KT289581	Arnold et al., 2009
KT289541	Arnold et al., 2009	KT289582	Arnold et al., 2009
KT289542	Arnold et al., 2009	KT289583	Arnold et al., 2009
KT289543	Arnold et al., 2009	KT289584	Arnold et al., 2009

Table 2 Continued

Accession	Reference	Accession	Reference
KT289544	Arnold et al., 2009	KT289585	Arnold et al., 2009
KT289586	Arnold et al., 2009	KT289634	Arnold et al., 2009
KT289587	Arnold et al., 2009	KT289635	Arnold et al., 2009
KT289588	Arnold et al., 2009	JQ759362	U´Ren et al.,2016
KT289589	Arnold et al., 2009	JQ759383	U´Ren et al.,2016
KT289590	Arnold et al., 2009	JQ760192	U´Ren et al.,2016
KT289591	Arnold et al., 2009	JQ760209	U´Ren et al.,2016
KT289592	Arnold et al., 2009	JQ760257	U´Ren et al.,2016
KT289593	Arnold et al., 2009	JQ760320	U´Ren et al.,2016
KT289594	Arnold et al., 2009	JQ760565	U´Ren et al.,2016
KT289595	Arnold et al., 2009	JQ760593	U´Ren et al.,2016
KT289596	Arnold et al., 2009	JQ760665	U´Ren et al.,2016
KT289597	Arnold et al., 2009	JQ760666	U´Ren et al.,2016
KT289598	Arnold et al., 2009	JQ761035	U´Ren et al.,2016
KT289599	Arnold et al., 2009	JQ761586	U´Ren et al.,2016
KT289600	Arnold et al., 2009	JQ761635	U´Ren et al.,2016
KT289601	Arnold et al., 2009	JQ761899	U´Ren et al.,2016
KT289602	Arnold et al., 2009	JQ761992	U´Ren et al.,2016
KT289603	Arnold et al., 2009	HM122805	U´Ren et al.,2016
KT289604	Arnold et al., 2009	HM123248	U´Ren et al.,2016
KT289605	Arnold et al., 2009	HM123416	U´Ren et al.,2016
KT289606	Arnold et al., 2009	JQ760181	U´Ren et al.,2016
KT289607	Arnold et al., 2009	JQ760182	U´Ren et al.,2016
KT289608	Arnold et al., 2009	JQ760210	U´Ren et al.,2016
KT289609	Arnold et al., 2009	JQ760306	U´Ren et al.,2016
KT289610	Arnold et al., 2009	JQ760314	U´Ren et al.,2016
KT289611	Arnold et al., 2009	JQ760457	U´Ren et al.,2016
KT289612	Arnold et al., 2009	JQ760469	U´Ren et al.,2016
KT289613	Arnold et al., 2009	JQ760489	U´Ren et al.,2016
KT289614	Arnold et al., 2009	JQ760548	U´Ren et al.,2016
KT289615	Arnold et al., 2009	JQ760549	U´Ren et al.,2016
KT289616	Arnold et al., 2009	JQ760650	U´Ren et al.,2016
KT289617	Arnold et al., 2009	JQ760654	U´Ren et al.,2016
KT289618	Arnold et al., 2009	JQ760728	U´Ren et al.,2016
KT289619	Arnold et al., 2009	JQ760786	U´Ren et al.,2016
KT289620	Arnold et al., 2009	JQ760795	U´Ren et al.,2016
KT289621	Arnold et al., 2009	JQ760869	U´Ren et al.,2016
KT289622	Arnold et al., 2009	JQ760904	U´Ren et al.,2016
KT289623	Arnold et al., 2009	JQ760970	U´Ren et al.,2016
KT289624	Arnold et al., 2009	JQ760995	U´Ren et al.,2016
KT289625	Arnold et al., 2009	JQ761025	U´Ren et al.,2016
KT289626	Arnold et al., 2009	KT289630	Arnold et al., 2009
KT289627	Arnold et al., 2009	KT289631	Arnold et al., 2009
KT289628	Arnold et al., 2009	KT289632	Arnold et al., 2009
KT289629	Arnold et al., 2009	KT289633	Arnold et al., 2009

Table 3 Genbank accession numbers and references for β-Tubulin sequences of taxa included in phylogenetic analyses in this study.

| Accession	Reference
GQ502698	Hsieh et al., 2010
KU684111	U´Ren et al.,2016
KU684112	U´Ren et al.,2016
KU684121	U´Ren et al.,2016
KU684122	U´Ren et al.,2016
KU684141	U´Ren et al.,2016
KU684142	U´Ren et al.,2016
KU684143	U´Ren et al.,2016
KU684144	U´Ren et al.,2016
KU684145	U´Ren et al.,2016
KU684146	U´Ren et al.,2016
KU684147	U´Ren et al.,2016
KU684148	U´Ren et al.,2016
KU684153	U´Ren et al.,2016
KU684154	U´Ren et al.,2016
KU684156	U´Ren et al.,2016
KU684157	U´Ren et al.,2016
KU684158	U´Ren et al.,2016
KU684159	U´Ren et al.,2016
KU684164	U´Ren et al.,2016
KU684165	U´Ren et al.,2016
KU684167	U´Ren et al.,2016
KU684168	U´Ren et al.,2016
KU684170	U´Ren et al.,2016
KU684171	U´Ren et al.,2016
KU684173	U´Ren et al.,2016
KU684174	U´Ren et al.,2016
KU684175	U´Ren et al.,2016
KU684176	U´Ren et al.,2016
KU684177	U´Ren et al.,2016
KU684178	U´Ren et al.,2016
KU684179	U´Ren et al.,2016
KU684193	U´Ren et al.,2016
KU684197	U´Ren et al.,2016
KU684198	U´Ren et al.,2016
KU684201	U´Ren et al.,2016

Table 4 Nucleotide substitution models of group of sequences established in order to provide a phylogenetic context for the isolated endolichenic fungi from *Cladonia curta* based in ITS region of rDNA. The groups of sequences (G) were: 1) BLASTn search; 2) Arnold et al. (2009); 3) U'Ren et al., 2016 and 4) David et al. (2003). BIC: Bayesian Information Criterion; AICc: Akaike Information Criterion, corrected; *lnL*: Maximum Likelihood value; P: number of parameters including branch lengths. *R*: Values of transition/transversion bias; *f:* nucleotide frequencies; *r:* rates of base substitutions; +*G*: Gamma distribution; +*I*: Invariant sites; HKY: Hasegawa-Kishino-Yano; TN93: Tamura-Nei; T92: Tamura 3-parameter; K2: Kimura 2-parameter.

| G	Model	P	BIC	AICc	*lnL*	(+I)	(+G)	*R*	*f*(A)	*f*(T)	*f*(C)	*f*(G)	*r*(AT)	*r*(AC)	*r*(AG)	*r*(TA)	*r*(TC)	*r*(TG)	*r*(CA)	*r*(CT)	*r*(CG)	*r*(GA)	*r*(GT)	*r*(GC)
1	K2+G+I	58	5708.971	5271.012	-2577.263	0.25	1.21	1.56	0.250	0.250	0.250	0.250	0.049	0.049	0.152	0.049	0.152	0.049	0.049	0.152	0.049	0.152	0.049	0.049
	K2+I	57	5709.381	5278.965	-2582.248	0.38	n/a	1.47	0.250	0.250	0.250	0.250	0.051	0.051	0.149	0.051	0.149	0.051	0.051	0.149	0.051	0.149	0.051	0.051
	K2+G	57	5723.389	5292.973	-2589.252	n/a	1.21	1.34	0.250	0.250	0.250	0.250	0.053	0.053	0.143	0.053	0.143	0.053	0.053	0.143	0.053	0.143	0.053	0.053
	T92+G+I	59	5723.673	5278.171	-2579.835	0.25	1.18	1.55	0.256	0.256	0.244	0.244	0.050	0.048	0.148	0.050	0.148	0.048	0.050	0.156	0.048	0.156	0.050	0.048
2	TN93+G+I	242	13900.845	11895.883	-5703.950	0.33	0.50	1.79	0.242	0.256	0.248	0.254	0.046	0.044	0.112	0.043	0.208	0.046	0.043	0.215	0.046	0.106	0.046	0.044
	K2+G+I	238	13903.158	11931.270	-5725.709	0.34	0.52	1.80	0.250	0.250	0.250	0.250	0.045	0.045	0.161	0.045	0.161	0.045	0.045	0.161	0.045	0.161	0.045	0.045
	T92+G+I	239	13911.795	11931.639	-5724.877	0.34	0.52	1.80	0.249	0.249	0.251	0.251	0.044	0.045	0.161	0.044	0.161	0.045	0.044	0.160	0.045	0.160	0.044	0.045
	HKY+G+I	241	13926.847	11930.153	-5722.102	0.34	0.52	1.80	0.242	0.256	0.248	0.254	0.046	0.044	0.163	0.043	0.159	0.045	0.043	0.165	0.045	0.156	0.046	0.044
3	K2+G	93	9811.489	9103.878	-4458.356	n/a	0.26	2.09	0.250	0.250	0.250	0.250	0.040	0.040	0.169	0.040	0.169	0.040	0.040	0.169	0.040	0.169	0.040	0.040
	K2+G+I	94	9814.955	9099.748	-4455.278	0.37	0.74	2.11	0.250	0.250	0.250	0.250	0.040	0.040	0.170	0.040	0.170	0.040	0.040	0.170	0.040	0.170	0.040	0.040
	T92+G	94	9823.774	9108.568	-4459.688	n/a	0.26	2.09	0.259	0.259	0.241	0.241	0.042	0.039	0.163	0.042	0.163	0.039	0.042	0.175	0.039	0.175	0.042	0.039
	T92+G+I	95	9826.986	9104.183	-4456.483	0.37	0.73	2.12	0.259	0.259	0.241	0.241	0.041	0.039	0.164	0.041	0.164	0.039	0.041	0.176	0.039	0.176	0.041	0.039
4	K2+G+I	56	5722.084	5323.500	-2605.402	0.39	0.87	2.00	0.250	0.250	0.250	0.250	0.042	0.042	0.167	0.042	0.167	0.042	0.042	0.167	0.042	0.167	0.042	0.042
	K2+G	55	5722.243	5330.764	-2610.046	n/a	0.29	2.02	0.250	0.250	0.250	0.250	0.041	0.041	0.167	0.041	0.167	0.041	0.041	0.167	0.041	0.167	0.041	0.041
	T92+G	56	5723.685	5325.102	-2606.203	n/a	0.29	2.05	0.268	0.268	0.232	0.232	0.044	0.038	0.156	0.044	0.156	0.038	0.044	0.181	0.038	0.181	0.044	0.038
	T92+G+I	57	5724.678	5318.989	-2602.134	0.39	0.82	2.04	0.268	0.268	0.232	0.232	0.044	0.038	0.156	0.044	0.156	0.038	0.044	0.180	0.038	0.180	0.044	0.038
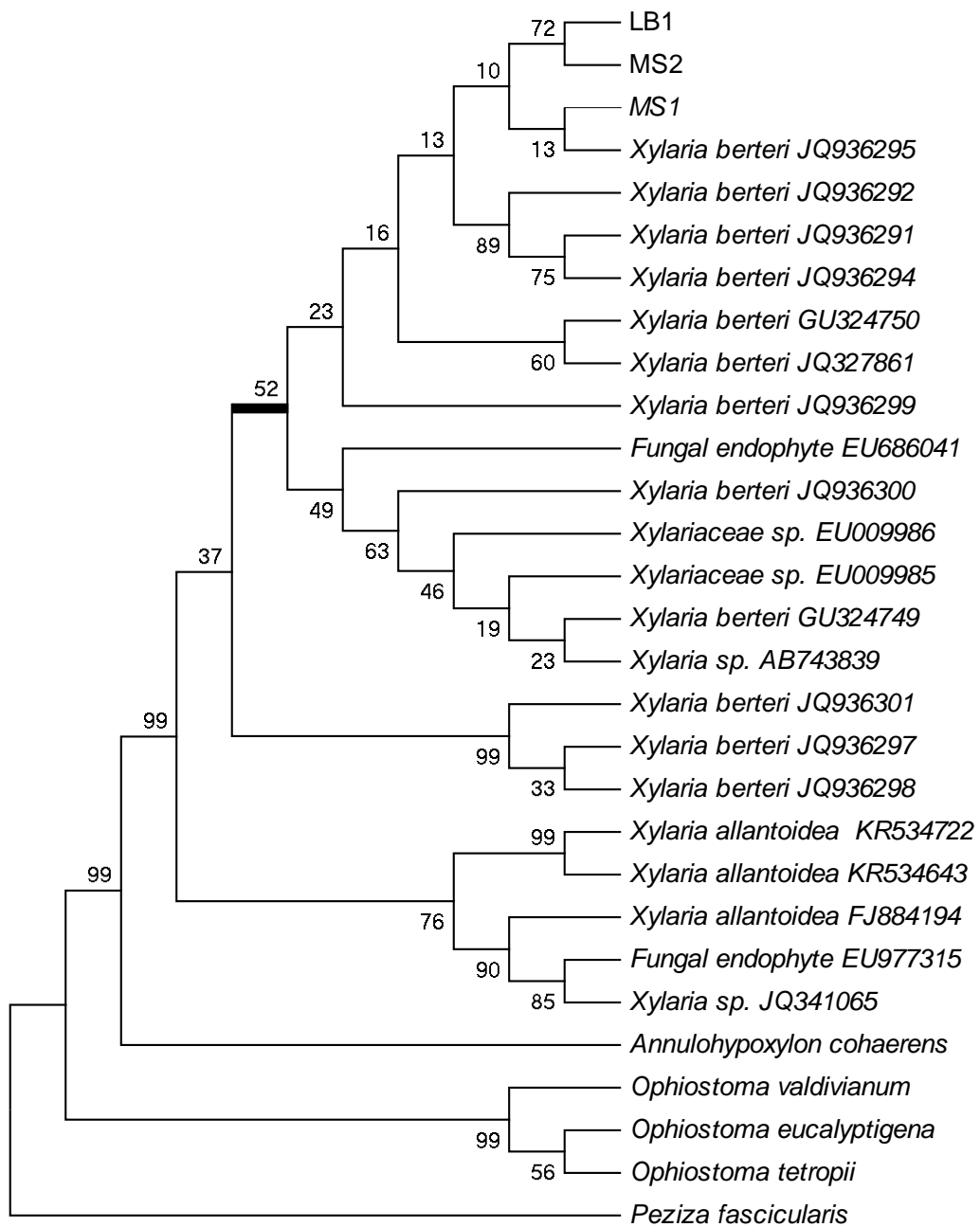
Fig 8 - Phylogenetic relationships among endolichenic fungi isolated from *Cladonia curta* and sequences of endophytic fungi with máximum similarity using BLASTn in GenBank database. MS1, MS2 and LB1 corresponding to sequences obtained in the present study. The tree was inferred by Maximum Likelihood based on one-locus dataset ITS region of rDNA. The numbers at each internode indicates bootstrap support values. Three species of *Ophiostoma*, *Annulohypoxylon cohaerens* and *Peziza fascicularis* were used as outgroup. Branch leading to clade with sequences of endophytes most closely related to the isolated fungi during this work are in bold.
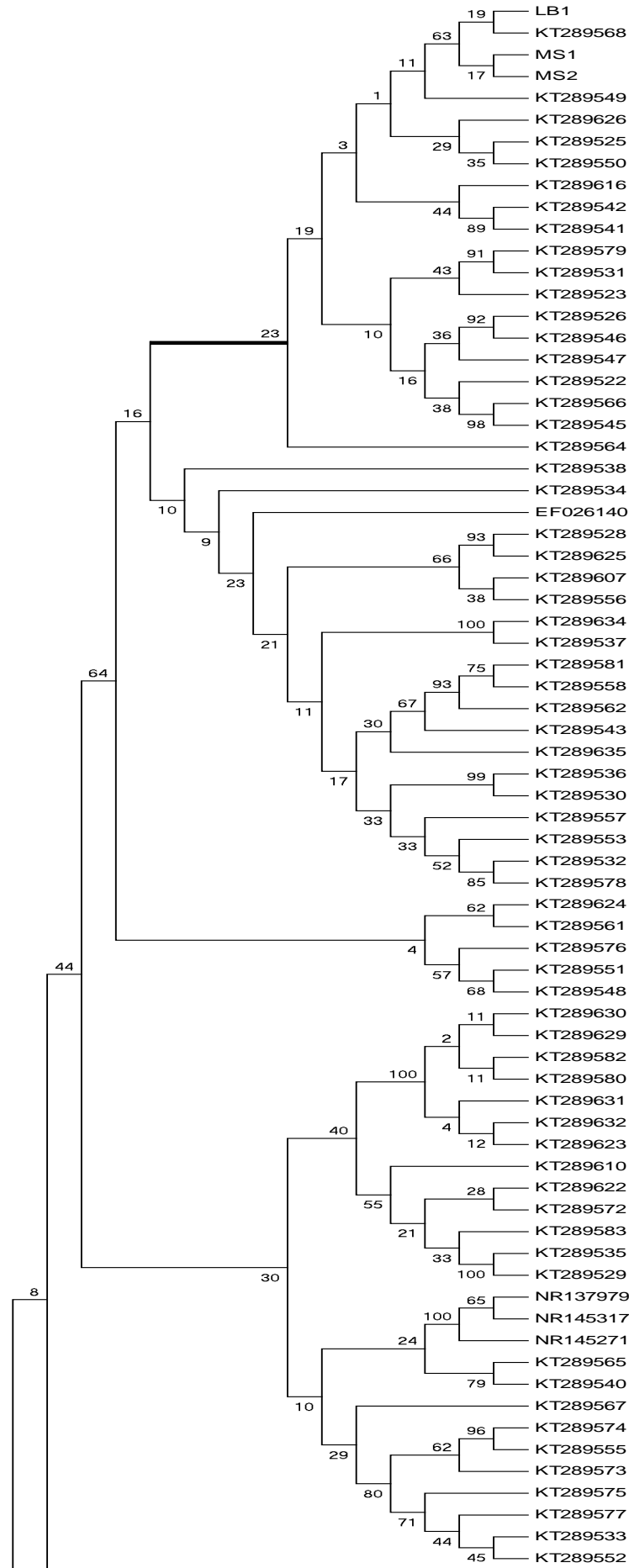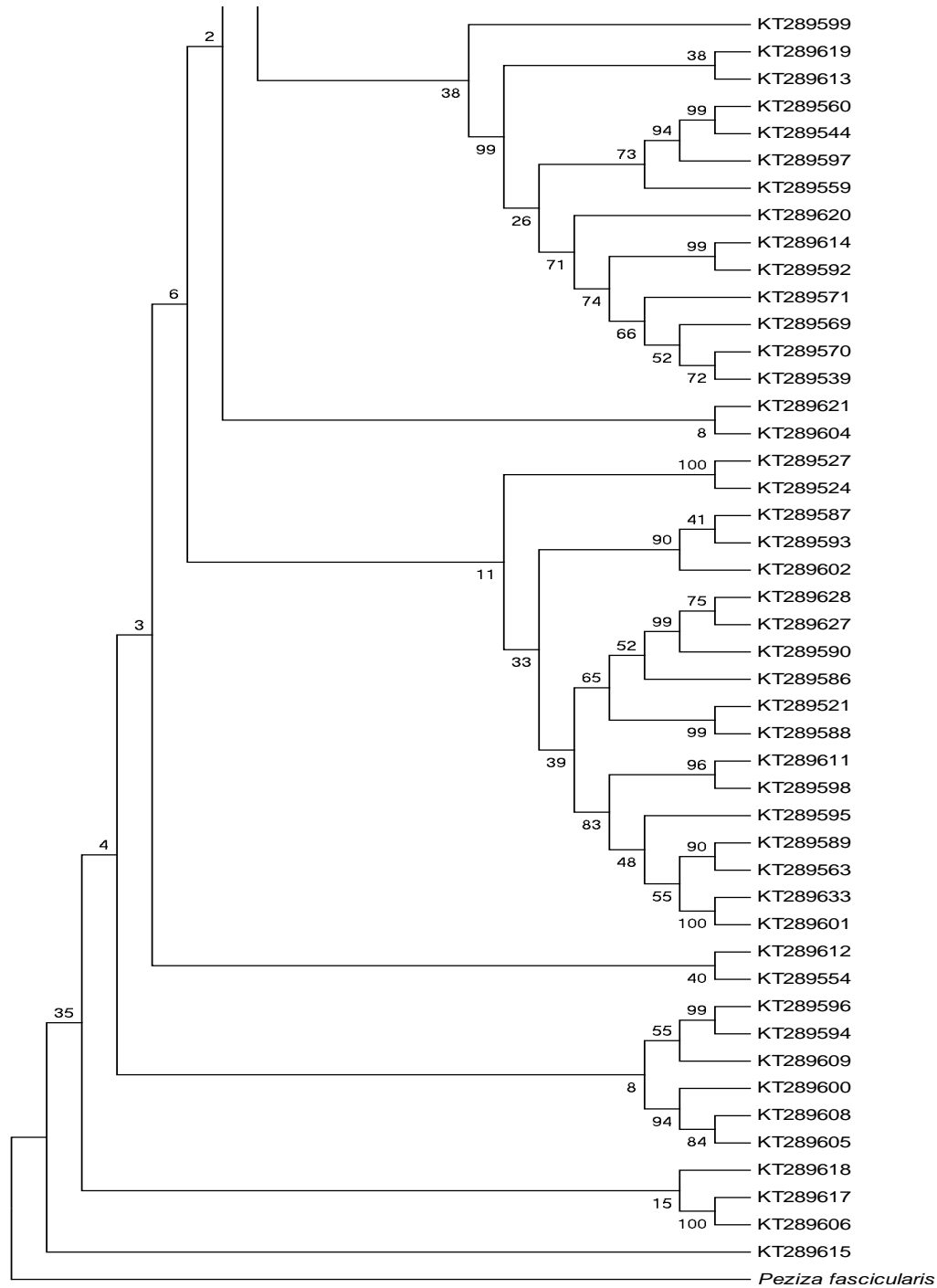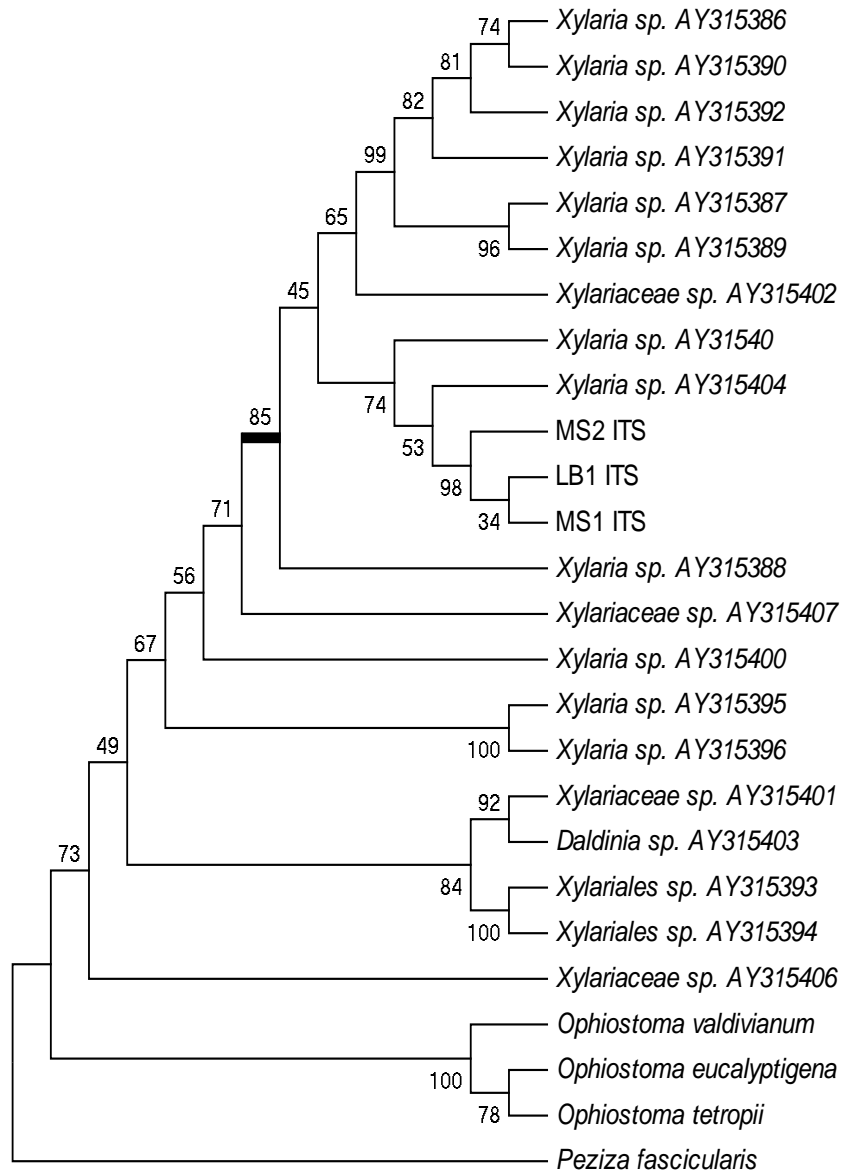
Fig 9 - Phylogenetic relationships of endolichenic fungi isolated from *Cladonia curta* (MS1, MS2 and LB1) and endophytic fungi isolated from plant and lichens obtained by Arnold et al. (2009) based on analysis of ITS region of rDNA sequences. The tree was inferred by Maximum Likelihood and numbers above branches indicates bootstrap support values. Three species of *Ophiostoma* (NR145317, NR137979 AND NR145271), *Annulohypoxylon cohaerens* (EF026140) and *Peziza fascicularis* (LT1558418) were used to root the tree. Branch leading to clade with sequences of endophytes most closely related to the isolated fungi in this work are in bold.
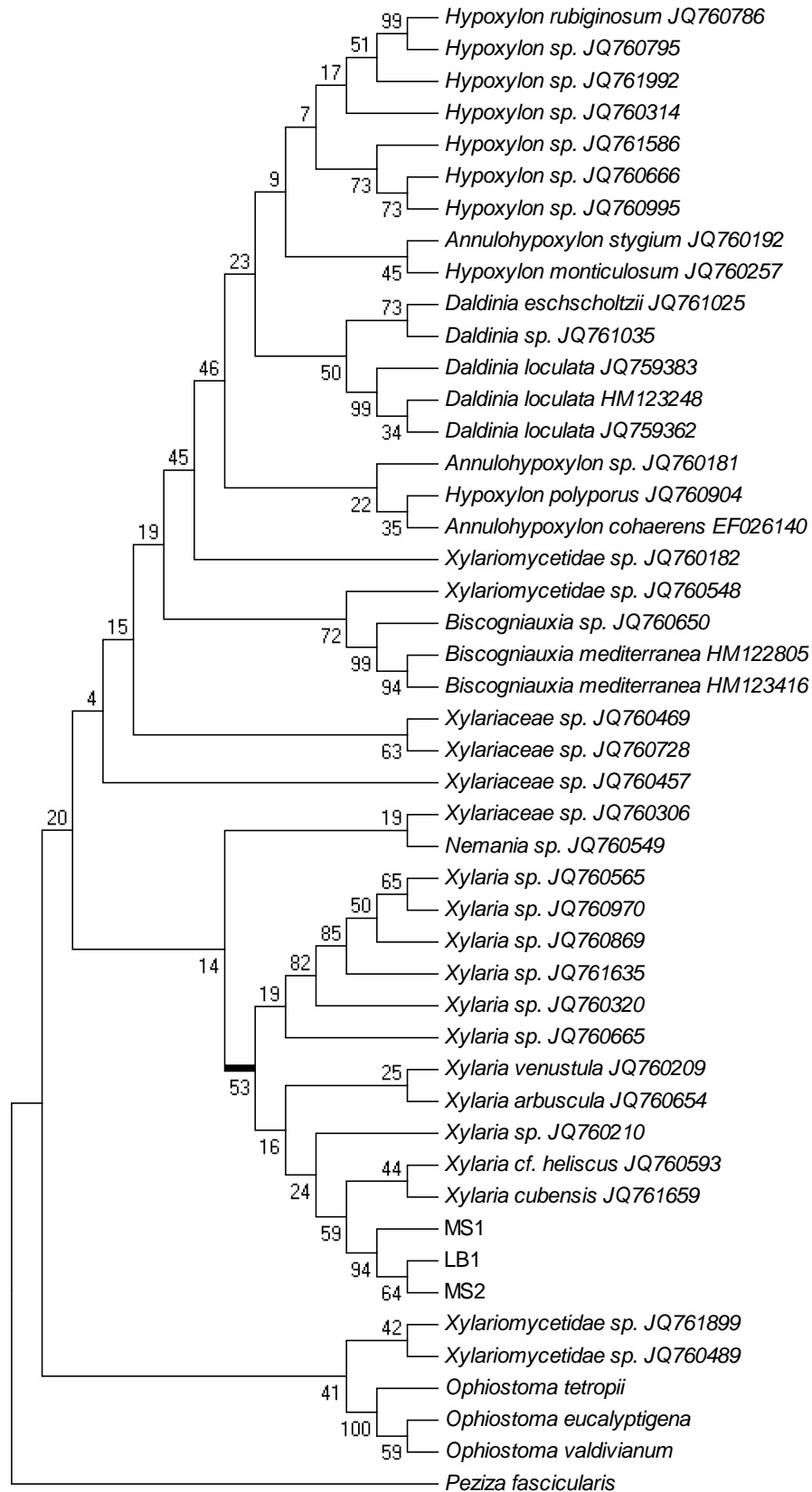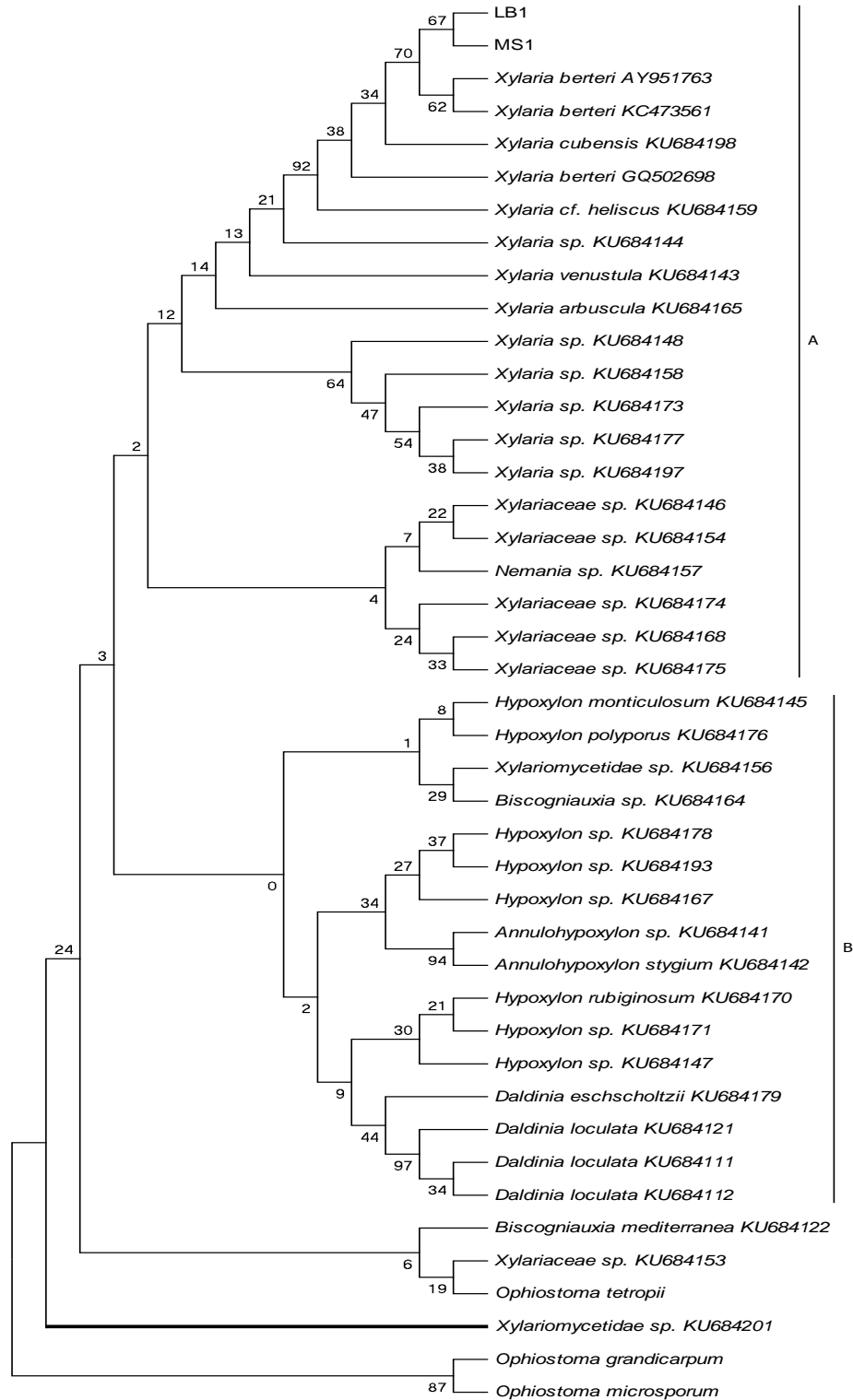
Fig 10 - Phylogenetic relationships among endolichenic fungi isolated from *Cladonia curta* and fungi isolated from the interior of *Cladonia* species and other lichens reported by U´Ren et al. (2016). MS1, MS2 and LB1 corresponding to sequences obtained in the present study. The tree was inferred by

Maximum Likelihood based on one-locus dataset (ITS region of rDNA) and numbers at each internode indicates bootstrap support values. Three species of *Ophiostoma* and *Peziza fascicularis* were used as outgroup. Branch leading to clade with sequences of endophytes most closely related to the isolated fungi in this work are in bold.


Fig 11 - Phylogenetic relationships of endolichenic fungi isolated from *Cladonia curta* and endophytes of liverworts (*Bazzania, Odontoschisma, Tricholea, Plagiochila* and *Metzgeria*) obtained by Davis et al. (2003) based on analysis of ITS region of rDNA sequences. MS1, MS2 and LB1 corresponding to sequences obtained in the present study. The tree was inferred by maximum likelihood and numbers above branches indicates bootstrap support values. Three species of *Ophiostoma*. Branch leading to clade with sequences of endophytes most closely related to the isolated fungi during this work are in bold.

Fig 12 - Phylogenetic relationships among endolichenic fungi isolated from *Cladonia curta*, BLASTn similarity sequences and sequences of endophytic fungi isolated from other lichens (U´Ren et al., 2016). MS1 and LB1 corresponding to sequences of two fungi recovered in the present study. The tree was inferred by maximum likelihood based on β-tubulin gene. The numbers at each internode indicates bootstrap support values. *Ophiostoma tetropii*, *Ophiostoma grandicarpum* and *Ophiostoma microsporum* were used to rooted.
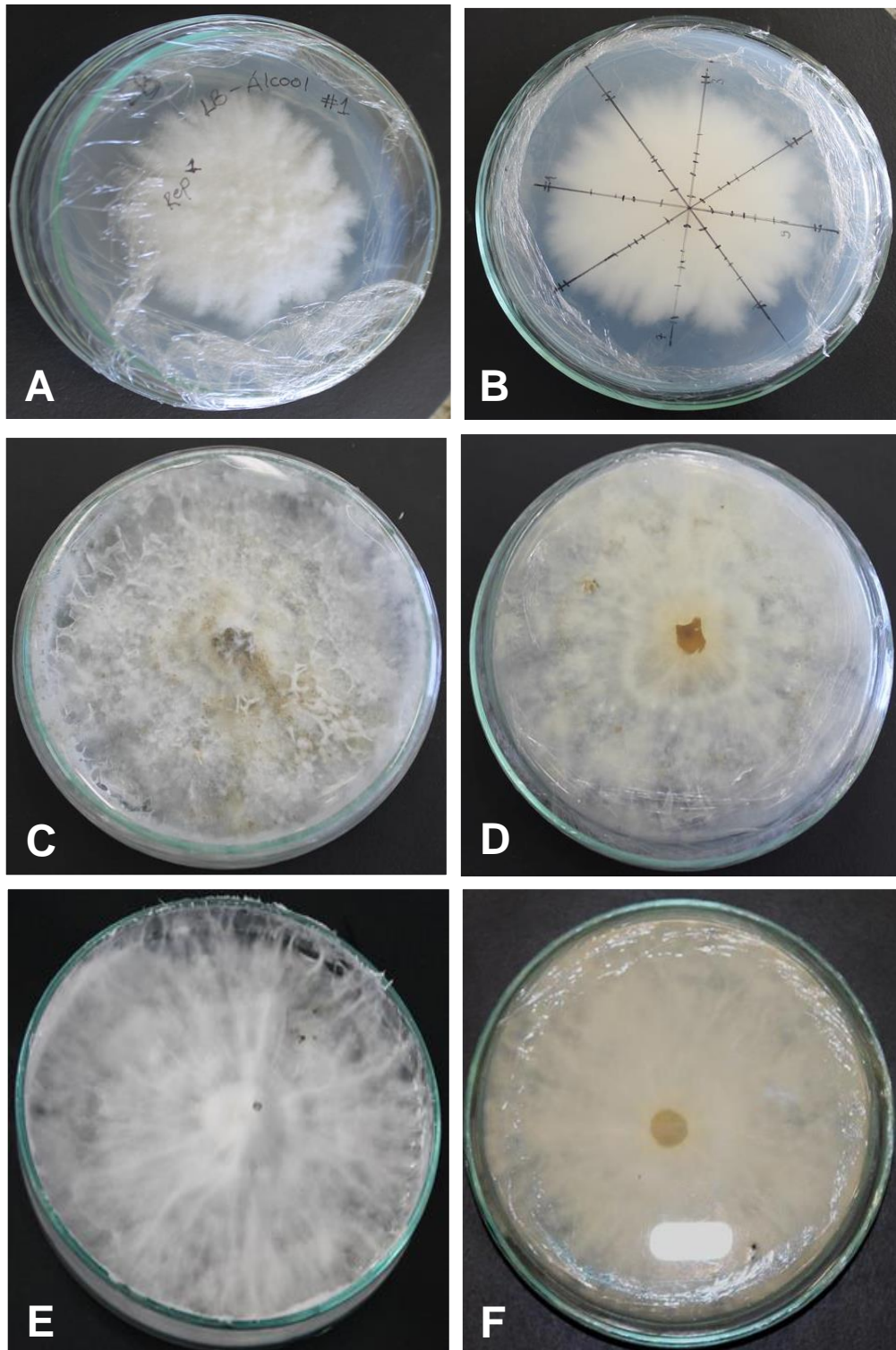
Fig 13 - Appearance of isolated *Xylaria* sp. 1 (HBEI 001), colonies on MS medium. A **-**B. Pure culture of the isolated fungi. C – D. Appearance of endolichenic fungi in photoperiod of 16h light / 8h dark. E **-** F. Appearance of endolichenic fungi in darkness.
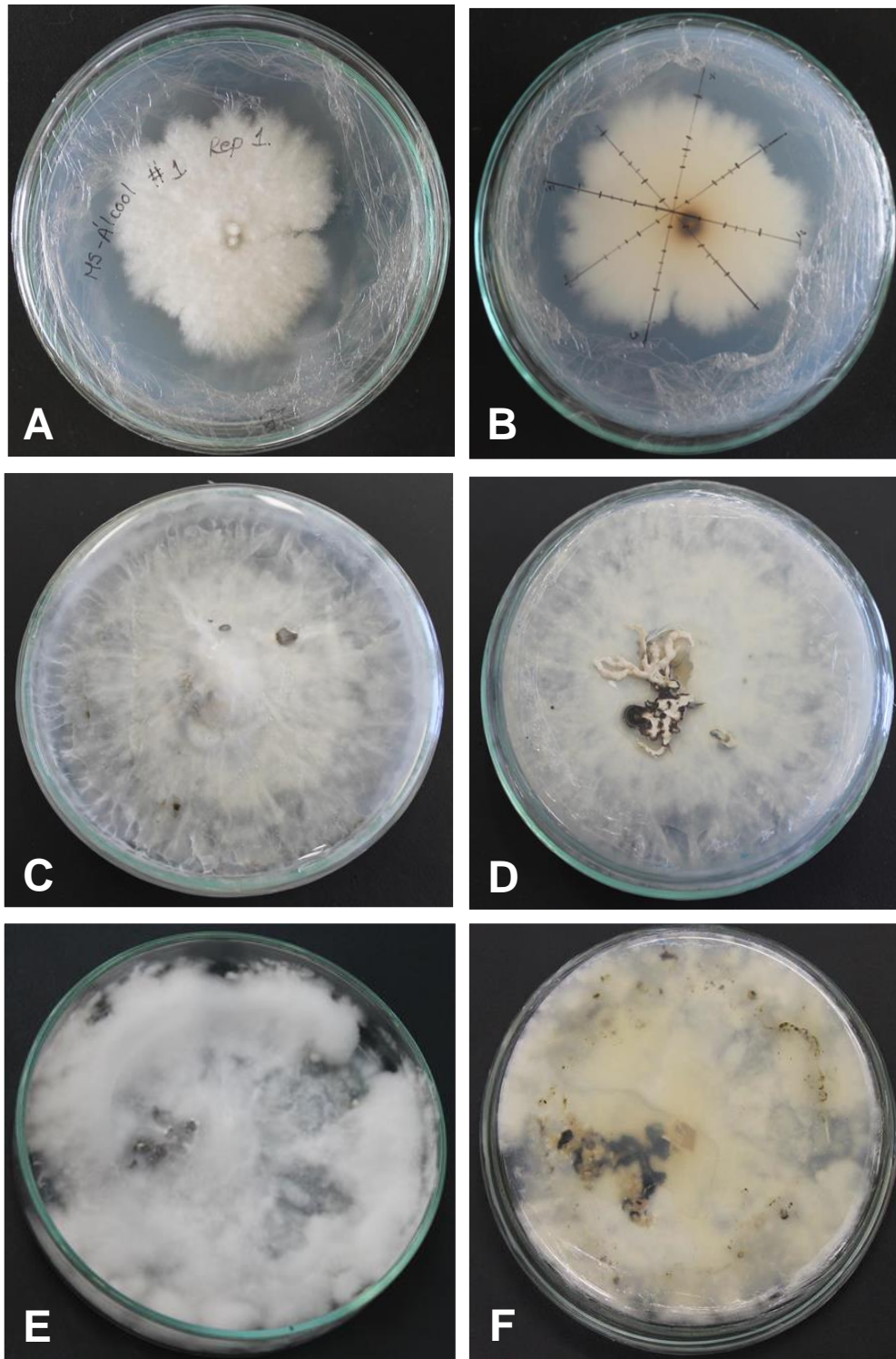
Fig 14 - Appearance of isolated *Xylaria* sp. 2 (HBEI 002), colonies on MS medium. A **-**B. Pure culture of the isolated fungi. C – D. Appearance of endolichenic fungi in photoperiod of 16h light / 8h dark. E **-** F. Appearance of endolichenic fungi in darkness.
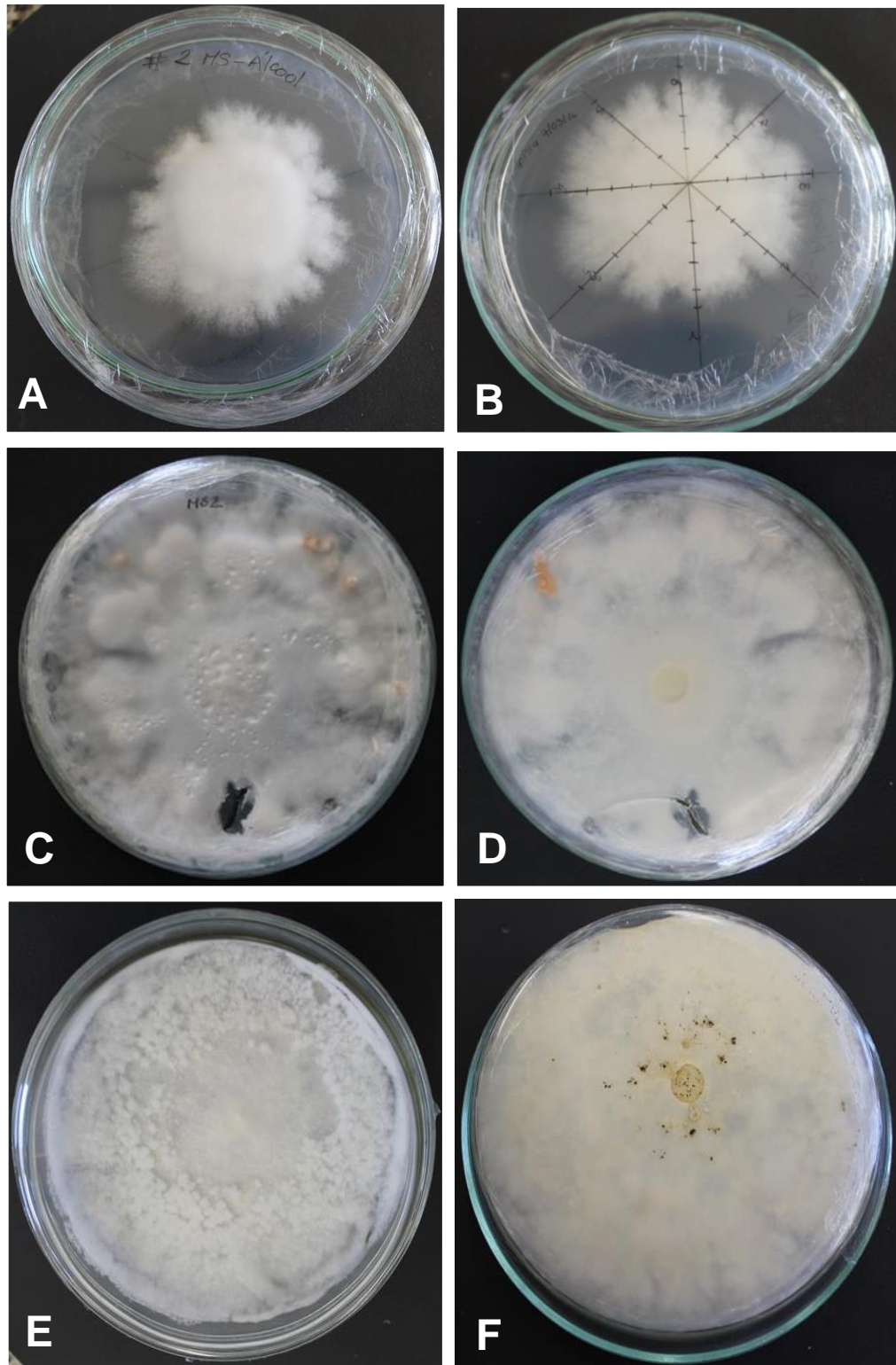
Fig 15 - Appearance of isolated *Xylaria* sp. 3 (HBEI 003), colonies on MS medium. A **-**B. Pure culture of the isolated fungi. C – D. Appearance of endolichenic fungi in photoperiod of 16h light / 8h dark. E **-** F. Appearance of endolichenic fungi in darkness.