SAMUEL GALVÃO ELIAS

CONTRIBUIÇÕES À SISTEMÁTICA DE *PHELLINOTUS* (BASIDIOMYCOTA, HYMENOCHAETALES) COM ÊNFASE NA DELIMITAÇÃO FILOGENÉTICA E FILOGEOGRAFIA PRELIMINAR DE *P. PIPTADENIAE*

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Esta Dissertação foi julgada adequada para obtenção do Título de "Mestre", e aprovada em sua forma final pelo Programa de Pós-graduação em Biologia de Fungos, Algas e Plantas.

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Florianópolis, 31 de Março de 2017

Dedico esse trabalho aos meus pais, Wânia e Evaldo.

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(Theodore Roosevelt)

RESUMO

Phellinotus, um gênero de Hymenochaetaceae representa um táxon restrito à região Neotropical. Atualmente, duas espécies são descritas para o gênero, *P. neoaridus*, com populações atualmente somente registradas no semi-árido brasileiro e associadas exclusivamente a espécies do gênero *Caesalpinia* (Fabaceae) e *P. piptadeniae*, com populações possívelmente distribuídas ao longo de diferentes grupos florísticos (GF) das florestas tropicais sazonalmente secas da América do Sul e associado a diferentes gêneros de leguminosas, como *Piptadenia*, *Senegalia*, *Mimosa*, *Pithecellobium*, *Libidibia* e *Pityrocarpa*. Até o momento, apenas as populações de *P. piptadeniae* ocorrentes no GF Caatinga foram testadas filogenéticamente. No presente trabalho, com o intuito de delimitar filogeneticamente *P. piptadeniae* considerando os diferentes hospedeiros e sua distribuição geográfica, foram realizadas reconstruções filogenéticas baseadas nos marcadores moleculares nucleares ITS e nLSU do rDNA, incluindo espécimes pertencentes a todas as populações conhecidas do táxon, assim como espécimes adicionais. Os resultados demonstram que *P. piptadeniae* possui duas populações ocorrentes de forma alopátrica no GF Caatinga e florestas estacional semidecidual e ombrófila densa na porção sul da Mata Atlântica. Os espécimes do GF Caatinga e GF Costa Central dos Andes e um único exemplar coletado no GF Missiones (nordeste da Argentina), previamente determinados como *P. piptadeniae*, ficaram agrupados em um clado distinto de *P. piptadeniae* e aqui é proposta como a espécie nova *P. teixeirae* sp. nov. Ad int. Adicionalmente, uma nova espécie de *Phellinotus* é descrita, *P. magnoporatu* sp. Noc. Ad Int., um táxon até o momento registrado somente no GF Costa Central dos Andes. Baseando-se nos resultados das análises filogenéticas, um estudo preliminar

da filogeografia de *P. piptadeniae* foi realizado, utilizando-se o marcador molecular ITS. Nossos resultados evidenciam que as populações de *P. piptadeniae* associadas à *P. gonoacantha*, que ocorrem na Mata Atlântica, juntamente com novos registros de populações localizadas na porção central do Cerrado, possuem alta diversidade genética em comparação com as populações associada aos demais hospedeiros conhecidos. Adicionalmente, sinais de recente expansão demográfica foram evidenciados através do marcador nuclear ITS.

Palavras-chave: Florestas tropicais sazonalmente secas, parasita, neotrópico, Fabaceae, diversidade genética, simpatria

ABSTRACT

The Hymenochaetoid genus *Phellinotus*, represents a restricted taxa to the Neotropical Region. Currentlly, two species are described from the genus, '*P. neoaridus*, with populations occurring on the brasilian semi-arid region and excluvelly associated to species of Caesalpinia (Fabceae) and *P. piptadeniae*, with populations possibly distributed througouth different floristic groups (FG) of the Seazonally Dry Tropical Forests of South America and associated to different genera of Fabeceae, as *Piptadenia, Senegalia, Mimosa, Pithecellobium, Libidibia* and *Pityrocarpa*. At this moment only the populations of P. piptadeniae that occur on the Caatinga FG were phylogenetucally tested. In this work, to address the phylogeny of P. piptadeniae considering different hosts and full geographical distribution, were performed phylogenetical reconstructions based on nuclear molecular markers ITS and LSU of rDNA, including specimes belong to all know populations to this taxon, and additional specimens. Our results show that P. piptadeniae has two populations that allopathically occur in the Caatinga FG and in the Semidecidious Forest of south portion of Atlantic Forest. Specimens collected on the Caatinga FG and Central Andes Coast FG and only one specimens collected on Missiones FG (northeast of Argentina), previously identified as P. piptadeniae, clustered on distinct clade of P. piptadeniae, and here we propose a new taxa *P. teixeirae* sp. nov. Ad int. Additionaly, a new species of *Phellinotus* are proposed, *P. magnoporatus* sp. Nov. Ad int., a species at this moment recorded only on Central Andes Coast FG. Based in our results of phylogenetic analisis, a preliminary phylogeographic study of *P. piptadeniae* was conducted based on the ITS marker. Ouer results indicate that populations of *P. piptadeniae* associated to *P. gonoacantha*, and that occur in Atlantic Forest, together the

populations located on the central portion of Cerrado, has high genetic diversity em comparison with populations associated to the other know hosts. Adictionally, signs of recent demographic expancion were evidenced trougthout the nuclear marker ITS.

Key-words: Seazonally dry tropical forests, parasitic, neotropic, Fabaceae, genetic diversity, simpatry.

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1 APRESENTAÇÃO

Neste trabalho com a delimitação filogenética de *Phellinotus piptadeniae* (Teixeira) Drechsler-Santos & Robledo (≡ *Phellinus piptadeniae* Teixeira), são descritas duas novas espécies e uma filogenia de *Phellinotus* é apresentada. Ainda, é apresentada a ampliação da distribuição geográfica e a filogeografia preliminar de *P. piptadeniae*. Este trabalho está dividido em Introdução (fundamentação teórica), Objetivos, Material & Métodos, Resultados & Discussão e Considerações Finais e Recomendações para Trabalhos Futuros. Os Resultados & Discussão estão subdivididos em dois capítulos:

Capítulo 1 – apresenta a reconstrução filogenética baseada em dois marcadores moleculares (ITS e LSU rDNA), com inclusão de espécimes anteriormente tratados como *P. piptadeniae* assim como alguns táxons adicionais. Esse capítulo é apresentado na forma de artigo a ser submetido para a revista *Plant Systematics and Evolution*.

Capítulo 2 – apresenta a ampliação da distribuição geográfica de *P. piptadeniae* baseada na distribuição de seu hospedeiro *Piptadenia gonoacantha*, bem como trata da filogeografia preliminar de *P. piptadeniae* com base no marcador molecular nuclear ITS. Esse capítulo é apresentado na forma de artigo a ser submetido para a revista *Molecular Ecology*.

Todas as referências bibliográficas citadas na Introdução e Material & Métodos são apresentadas após as Considerações Finais e Recomendações para Trabalhos Futuros, as referências dos demais capítulos são apresentadas no final de cada um deles.

2 INTRODUÇÃO

2.1 *PHELLINOTUS*: CONTEXTO FILOGENÉTICO E CLASSIFICAÇÃO

Macrofungos pertencentes à Hymenochaetales Oberw. (Agaricomycetes Doweld, Basidiomycota R.T. Moore), são caracterizados por produzirem basidiomas de formas variáveis incluindo ressupinados, pileados e clavarióides (Parmasto 2010, Hibbett 2014). Em termos ecológicos, podem ser degradadores de madeira totalmente generalistas [ex. *Fuscoporia gilva* (Schwein.) T. Wagner & M. Fisch. (Ryvarden, 2004)], parasitas de diversos gêneros de Angiospermas e Gimnospermas, ou apresenta diferentes graus de especialização com hospedeiros [ex. *Phellinotus piptadeniae* (Teixeira) Drechsler-Santos & Robledo (Salvador-Montoya et al. 2015, Drechsler-Santos et al. 2010, 2016)], ou ainda ser associados a diversas famílias de plantas como Fabaceae, Dipterocarpaceae e Myrtaceae em relações ectomicorrízicas [ex. *Coltricia* Gray (Tedersoo et al. 2007)]. Quando degradadores de madeira produzem a podridão branca (Hibbett 2014).

Originalmente proposta por Donk (19645), Hymenochaetaceae Donk possui cerca de 400 espécies descritas, representando a maior família da ordem (Larsson et al. 2006). Táxons pertencentes à Hymenochaetaceae são facilmente reconhecidos pela coloração amarelo-enxofre dos basidiomas, que quando em contato com KOH adquirem permanentemente a coloração negra (reação xantocróica, Fiasson 1982, Lee & Yun 2011, Hibbett 2014). Produzem basidiomas anuais a perenes, variando entre ressupinados e pileados, sésseis e estipetados, em sua maioria formadores de himenóforo poróide. Em

alugns casos o himenóforo liso é observado (ex. *Hymenochaete* Lév. e *Hymenochaetopsis* S.H.He & Jiao Yang.) (Larsson et al. 2006).

Microscopicamente são observados basicamente dois tipos de hifas, as generativas (comuns a todos os fungos) que possuem septação simples e regular, com ramificações frequentes, e as esqueletais, hifas de parede geralmente espessa, sem septação regular e raramente ramificadas. Espécies que apresentam apenas hifas generativas são tratadas como monomíticas, e dimíticas quando ambos os tipos hifais são observados na construção do basidioma (Wagner & Fischer, 2001; Larsson et al. 2006).

Em alguns casos, como nos gêneros recentemente descritos *Phellinotus* Drechsler-Santos, Robledo & Rajchenb., *Sanghuangporus* Sheng H. Wu, L.W. Zhou & Y.C. Dai e *Tropicoporus* L.W. Zhou, Y.C. Dai & Sheng H. Wu, observa-se um sistema hifal misto, com hifas esqueletais restritas a porção da trama himenoforal (dimítico), enquanto que no contexto são encontradas exclusivamente hifas generativas (monomítico) (Zhou et al. 2015, Drechsler-Santos et al. 2016). Por outro lado, nos gêneros *Hymenochaete* Lév. e *Hymenochaetopsis* S.H.He & Jiao Yang, observa-se um sistema hifal denominado pseudodimítico, no qual as hifas generativas não possuem septação regular porém apresentam paredes espessadas, semelhante às esqueletais, diferenciando-se apenas por apresentarem ramificações em diversos pontos (Parmasto 2001).

Inúmeros gêneros de Hymenochaetaceae possuem setas, estruturas caracterizadas como elementos terminais estéreis, pontiagudos e de paredes espessadas, que projetam-se a partir da trama do himenóforo ou da camada himenial, denominando-se setas tramais e

himeniais, respectivamente. Os basidiósporos de tamanho podem ser hialinos ou apresentarem coloração variando entre amarelo-claro e marrom-avermelhado em tons escuros além de apresentarem tamanho, forma e espessura de parede variáveis,. Alguns gêneros são caracterizados por apresentar a reação xantocróica nos esporos ou quando em contato com reagente de Melzer, adquirem coloração avermelhada (reação dextrinóide) (Wagner & Fischer 2001, Larsson et al. 2006, Hibbet 2014, Drechsler-Santos et al. 2016).

Em termos taxonômicos, gêneros poróides de Hymenochaetaceae foram historicamente combinados em grandes grupos como *Phellinus* Quél. e *Inonotus* P. Karst. (Larsen & Cobb-Poulle 1990, Ryvarden 2004). Entretanto, atualmente ambos os gêneros, no sentido amplo (*s.l.*), são reconhecidamente artificiais, e desse modo, gêneros anteriormente propostos com base em dados morfológicos [Fiasson & Niemelä 1984, ex. *Fomitiporia* Murrill, *Fulvifomes* Murrill, *Fuscoporia* Murrill, *Inonotus* Karst., *Inocutis* Fiasson & Niemelä, *Inonotopsis* Parm., *Phylloporia* Murrill, *Phellinidium* (Kotl.) Fiasson & Niemelä e *Porodaedalea* Murrill], foram resgatados (Fiasson & Niemelä 1984), e na sequência, corroborados através da inclusão de dados moleculares às reconstruções filogenéticas (Wagner & Fischer 2001, 2002).

Por sua vez, com o aumento da amostragem molecular dos inúmeros gêneros de Hymenochaetaceae, relações supragenéricas ganharam destaque, como é o caso do "clado Phellinotus" formado pelos gêneros *Arambarria* Rajchenb. & Pildain, *Fomitiporella* Murrill, *Fulvifomes*, *Inocutis*, *Phylloporia* e algumas linhagens ainda sem tratamento taxonômico, além do recém proposto *Phellinotus* (Drechsler-

Santos et al. 2016). A proximidade filogenética desses táxons, foi outrora confirmada por reconstruções filogenéticas publicadas por diferentes autores (Wagner & Fischer 2002, Larsson et al. 2006, Zhou 2014, Rajchenberg et al. 2015, Zhou et al. 2015). Espécies pertencentes ao clado Phellinotus caracterizam-se por produzirem basidiomas poróides, ausência de setas ou hifas setais, basidiósporos de paredes espessadas e coloração amarelo-pálido a marrom-avermelhada (Drechsler-Santos et al. 2016).

Phellinotus, representa uma linhagem com espécies descritas apenas para a região Neotropical até o momento, e é caracterizada por produzir basidiomas pileados, frequentemente com rimosidades na superfície superior e presença de uma linha negra resinosa que divide o contexto em dois extrados. Microscopicamente, possui sistema hifal intermediário (contexto monomítico e trama do himenóforo dimítico), os esporos ventralmente achatados possuem paredes espessadas, de coloração amarelo-claro tornando-se marrom-avermelhado em solução de KOH 3% (reação xantocróica). *Phellinotus* possui apenas duas espécies descritas, (i) *P. neoaridus* Drechsler-Santos & Robledo, a espécie tipo de *Phellinotus*, com populações amplamente distribuídas no domínio da Caatinga, crescendo em associação com espécies de *Caesalpinia* e (ii) *P. piptadeniae*, com populações possivelmente restritas às Florestas Secas da América do Sul e associadas a espécies de *Piptadenia*, *Senegalia* e *Mimosa* (Teixeira 1950, Drechsler-Santos et al. 2010, Salvador-Montoya et al. 2015, Drechsler-Santos et al. 2016).

2.2 EVOLUÇÃO DO CONCEITO DE *PHELLINOTUS PIPTADENIAE*

Em um contexto histórico, na descrição original de Teixeira (1950), *P. piptadeniae* é caracterizado como uma espécie possivelmente parasita, especialista de Pau-Jacaré [*Piptadenia communis* Benth. = *P. gonoacantha* (Mart.) J. F. Macbr., Fabaceae]. A partir das coleções de diferentes localidades do estado de São Paulo, Teixeira estabeleceu a hipótese primária acerca da distribuição geográfica do táxon, através da afirmação:

> "*Tratando-se de um fungo aparentemente especializado sobre "pau-jacaré", provavelmente a sua distribuição geográfica se estenda por onde quer que haja essa essência florestal*" (Teixeira 1950).

Considerando as premissas de Teixeira, a possível distribuição geográfica de *P. piptadeniae* seria equivalente à de *P. gonoacantha*, ou seja, suas populações seriam encontradas ao longo das florestas ombrófilas e semideciduais na porção sul e sudeste da Mata Atlântica e regiões sudeste e central do domínio do Cerrado (Morim, 2013). Ao mesmo tempo que esta hipótese fornece um importante indício acerca da provável distribuição do fungo, essa possui baixo poder explicativo, considerando que as coletas apresentadas por Teixeira são geograficamente restritas à floresta estacional semidecidual do estado de São Paulo (municípios de Campinas, Torrinha, Piracicaba, Monte Alegre do Sul e SãoPaulo).

Seis décadas mais tarde, com a publicação de Drechsler-Santos et al (2010), novas ocorrências de *P. piptadeniae* foram registrados para o Semi-árido Brasileiro. Na obra, juntamente com a publicação da ampliação da distribuição geográfica, os autores demonstram que *P. piptadeniae* não representa um táxon exclusivamente associado a *P.*

gonoacantha, já que no domínio da Caatinga, possui a capacidade de crescer em outras espécies de *Piptadenia* [*P. stipulacea* (Benth.) Ducke] e adicionalmente em outros gêneros de leguminosas como *Pityrocarpa* (Benth.) Britton & Rose, *Senegalia* Raf. e *Mimosa* R.Br.

Recentemente, novos registros do táxon foram apresentados para a região sul da Mata Atlântica (estados de São Paulo e Santa Catarina) e Florestas Secas à noroeste do Peru (Salvador-Montoya et al. 2015). Os espécimes da Mata Atlântica foram novamente amostrados crescendo em *P. gonoacantha* – conforme reconhecido por Teixeira (1950) – enqanto os espécimes pertencentes às florestas secas do Peru foram amostrados em associação com *Pithecellobium excelsum* (Kunth) Mart. e *Libidibia glabrata* Kunth, ambas leguminosas. Apesar da distribuição geográfica ampla e das populações de *P. piptadenaie*, a minunciosa análise de atributos morfológicos apresentada pelos autores identificou alta sobreposição fenotípica entre as populações. Com exceção das dimensões dos basidiósporos, os demais atributos macro e microscópicos não apresentaram diferenças relacionadas à distribuição geográfica ou mesmo à diferenciação ecológica (capacidade de crescer em diferentes hospedeiros) observada entre as populações de *P. piptadeniae* (Salvador-Montoya et al. 2015).

Com base nesse padrão, Salvador-Montoya et al (2015) estabelecem duas hipóteses acerca do histórico biogeográfico do táxon: (i) *P. piptadeniae* corresponderia a um complexo taxonômico composto por três espécies distintas e especializadas a distintos hospedeiros, ou alternativamente, (ii) *P. piptadeniae* seria uma única espécie com populações disjuntas, amplamente distribuídas e associadas às Florestas Secas da América do Sul.

Considerando casos semelhantes registrados na literatura micológica – ausência de diferenciação morfológica acompanhada por especiação ecológica e ampla distribuição geográfica (Geml et al. 2006, Kauserud et al. 2007, Geml et al. 2008, Carlsen et al. 2011, Seierstad et al. 2013) – torna-se indispensável a utilização de dados moleculares para testar as hipóteses de Salvador-Montoya et al (2015), bem como, delimitar filogenéticamente *P. piptadeniae*. Entretanto, até o momento, apenas as populações de *P. piptadeniae* localizadas no domínio da Caatinga possuem amostragem de sequências nucleotídicas (Drechsler-Santos et al. 2016). Desse modo, a ampliação da amostragem molecular, com a inclusão de espécimes, assim como a amostragem de espécimes associados a diferentes hospedeiros, torna-se necessária para elucidação do provável histórico evolutivo da linhagem.

2.3 ESTUDOS FILOGEOGRÁFICOS EM FUNGOS

A inclusão de caracteres microscópicos na taxonomia dos fungos trouxe consideráveis avanços, não só por aumentar o número de caracteres, mas também na compreensão das relações evolutivas do grupo (e.g. Patouilard 1900). Apesar disso, a taxonomia clássica baseada apenas em caracteres morfológicos (macro e microscópicos) é frequentemente divergente quando confrontada a outros conceitos de espécie, como biológico ou filogenético (Petersen & Hughes 1999; Taylor et al. 2000). Muitos grupos possuem variados e complexos ciclos de vida e a cada etapa sofrem diferenciadas pressões evolutivas, representando um desafio para o uso da morfologia única e exclusivamente, a qual nos fungos, na grande maioria dos casos, é ainda restrita somente ao seu estágio reprodutivo (e.g. Petersen & Hughes

1999). Sob tais circunstâncias – informação morfológica limitada e observação ecológica difícil – a utilização de técnicas moleculares baseadas em polimorfismos de DNA, tornaram-se indispensáveis na compreensão dos grupos, quanto às relações e histórico evolutivo das linhagens.

Fenômenos ambientais complexos como flutuações climáticas e acidentes geográficos, influenciam direta e/ou indiretamente a evolução das linhagens, afetando sob diferentes taxas, a diferenciação genética das espécies pela finxação das mutações nas populações (Bromham 2009, Bromham et al. 2013, Bromham et al. 2015). Logo, o estudo da relação causal entre eventos geográficos e climáticos e a distribuição espacial das mutações genéticas representa o cerne da filogeografia (Avise 2000), a área da ciência que une definitivamente as disciplinas de filogenética e a genética de populações (Hickerson et al. 2010).

A partir do seu estabelecimento, inúmeros trabalhos filogeográficos foram realizados com grupos taxonômicos bem estudados, como animais vertebrados e invertebrados e plantas, permitindo assim, a compreensão de grandes processos evolutivos ocorridos em diversos continentes, como Europa (Feliner 2011), América do Norte (Shafer et al. 2010), Nova Zelândia (Wallis & Trewick 2009), Austrália (Byrne 2008), África (Lorenzen et al. 2012) e América do Sul (Turchetto-Zolet et al. 2013).

Entretanto, essa não é uma realidade observada em estudos micológicos. Dentre os poucos trabalhos filogeográficos existentes (Kasuga et al. 2003, Geml et al. 2006, Kauserud et al. 2007, Geml et al. 2008, Moncalvo & Buchanan 2008, Linzer et al. 2008, Seierstad et al. 2013), poucos incluem táxons que ocorrem no hemisfério sul,

destacando-se Kasuga et al (2003) e Moncalvo & Buchanan (2008). Entretanto, apesar de ocorrentes no Hemisfério Sul, os táxons abordados nesses estudos (*Histoplasma capsulatum* Darling e o complexo *Ganoderma australe-applanatum*, respectivamente) representam linhagens mundialmente distribuídas, sendo desse modo, pouco informativos para o Hemisfério Sul especificamente.

Nesse contexto, o desenvolvimento de estudos filogeográficos baseados em linhagens exclusivamente Neotropicais – o qual é o caso de *Phellinotus piptadeniae* (Salvador-Montoya et al. 2015, Drechsler-Santos et al. 2016) – possui grande relevância científica, fornecendo importantes informações acerca dos processos evolutivos atuantes sobre a micota dessa grande e complexa área continental que é a América do Sul. Além disso, considerando a distribuição geográfica ampla e disjunta de *P. piptadeniae*, assim como sua capacidade de se associar a diferentes hospedeiros, a utilização da técnicas filogeográficas possui grande valor na compreensão da história evolutivo deste táxon.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Atualizar o status taxonômico e filogenético do gênero *Phellinotus*, bem como apresentar um estudo preliminar da filogeografia de *P. piptadeniae*.

3.2 OBJETIVO ESPECÍFICOS

- Testar filogeneticamente espécimes previamente tratados como *P. piptadeniae* com a utilização de marcadores moleculares;
- Apresentar uma sinopse das linhagens filogenéticas de *Phellinotus* a partir de dados morfológicos, distribuição geográfica e asssociação com hospedeiros;
- Identificar e confirmar a distribuição geográfica das populações de *P. piptadeniae* associadas à *P. gonoacantha*;
- Realizar um estudo preliminar da filogeografia de *P. piptadeniae* com base no marcador ITS

4 MATERIAL & MÉTODOS

4.1 ESPÉCIMES EXAMINADOS E PROCESSAMENTO DO MATERIAL

Para o primeiro capítulo foram examinados espécimes de localidades do Peru, Argentina e Brasil (para maiores detalhes nas seções "*Material & Methods*" e "*Taxonomic treatment*" do respectivo capítulo), coletados no âmbito de pesquisas realizadas por pesquisadores colaboradores.

Na produção do segundo capítulo, foram realizadas expedições de campo baseadas na distribuição geográfica do principal hospedeiro de *P. piptadeniae* na Mata Atlântica, *P. gonoacantha* (maiores detalhes seção "*Potential Distribution of P. piptadeniae*" do respectivo capítulo).

Em campo os basidiomas foram coletados e cada espécime georeferenciado utilizando-se aparelho de sistema de posicionamento global (GPS). Informações referentes à localização geográfica, condições do substrato/hospedeiro assim como demais informações pertinentes foram tomadas em nota. Em laboratório os espécimes coletados foram mantidos em estufa de ventilação forçada (35º - 40º) até total desidratação dos basidiomas. Anteriormente à desidratação, segmentos dos basidiomas foram envoltos em papel manteiga e preservados em sacos plásticos (tipo ziplock) contento sílica para posterior extração de DNA e demais análises moleculares.

4.2 ANÁLISES MORFOLÓGICAS

Macroscopicamente foram descritas forma, dimensões do basidioma (contexto e tubos), características do abhimênio (formação de

sulcos, lóbulos, rimosidade, etc), característica do himenóforo (número de poros por milímetro linear, formato), hospedeiro (vivo, morto, identificação botânica) e a determinação das cores de acordo com o catálogo de Munsell (1975).

Para observação de características micromorfológicas, foram realizados cortes a mão livre de diferentes partes do contexto e himenóforo e estes montados sob lâmina e lamínula. As observações do sistema hifal e elementos reprodutivos (basídios e basidiósporos) foram realizadas em KOH 2-3% (hidratante e reagente para observação da reação xantocróica nos basidiósporos), Floxina 1% (corante citoplasmático para observação principalmente de hifas generativas e basídios) e reagente de Melzer (para verificar possível reação amilóide e/ou dextrinóide em hifas e basidiósporos). Fragmentos do contexto e tubos imersos em solução de NaOH 3%, foram incubados por 24-48h para posterior dissecação em estereomicroscópio e descrição do sistema hifal, conforme Teixeira (1995). As observações foram tomadas em microscópio óptico com ocular milimetrada.

4.3 ANÁLISES MOLECULARES

4.3.1 Extração, Amplificação e Sequenciamento

A extração do DNA genômico total seguiu protocolo de Doyle & Doyle (1987) adaptado por Góes-Neto et al (2005). Para amplificação a partir do produto de extração (PCR1), foram utilizados os pares de primers ITS1-F/ITS4-R, LR0R/LR5 e EF1-983F/EF1-2212R para as regiões nucleares ITS, LSU e tef1-α, respectivamente e ATP6-2/ATP6-3 para a região ATP6 do DNA mitocondrial. A descrição dos ciclos utilizados na reação de PCR1 estão descritos na Tabela 1. Todos os

produtos de amplificação foram purificados de conforme protocolo de PEG 20% [Poly(ethylene glycol) 8,0 plus NaCl 2.5M] e enviados para reação de sequenciamento (PCR2) realizada na Plataforma de Tecnologias (PDTIS), Fiocruz – Belo Horizonte, no âmbito do projeto BrBol - "Identificação molecular de fungos do Brasil". As reações de sequenciamento foram preparadas com 1 μL de BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA), 1 μL 5x Buffer, 1 μL primer, 5 μL H2O q.s.p. e 2-2,5 μL do produto da PCR. Nesta etapa foram utilizados os mesmos pares de primer citados anteriormente, com exceção da região tef1-α que devido ao elevado comprimento, foram adicionados os primer EF1-1567R e EF1-1577F para obtenção de melhor cobertura nos cromatogramas.

4.3.2 Processamento dos Dados Moleculares

Todos os cromatogramas foram vistoriados e editados manualmente no software Geneious versão 9.0.5 (Kearse et al. 2012). A presença de ambiguidades indicando posições heterozigotas, foram notadas de acordo com o código do International Union of Pure and Applied Chemistry (IUPAC). Todas as sequencias geradas nesse trabalho serão posteriormente disponibilizadas no banco de dados Genbank (www.ncbi.nlm.nih.gov).

O alinhamento das sequências resultantes, foi realizado no software MAFFT versão 7.305 (Katoh and Standley 2013), utilizando-se diferentes estratégias de acordo com as características de cada marcador molecular. Para reconstrução da árvore filogenética do gênero *Phellinotus* apresentada no primeiro capítulo, foram utilizados dois métodos de busca, a busca por Máxima Verossimilhança e por

Inferência Bayesiana (maiores detalhes ítem Material & Methods capítulo 1). Para análise da estrutura filogeográfica de *P. piptadeniae*, foram implementados métodos baseados tanto em análises Bayesianas como frequentistas (maiores detalhes item "*Material & Methods*" capítulo 2).

Tabela 1. Configurações utilizadas na amplificação dos cinco marcadores moleculares via reação de PCR. D = desnaturação, AN = anelamento, E = extensão, ^{oo} = tempo indeterminado, - etapa não aplicável, [1]genôma nuclear, [2]gênoma mitocondrial.

MARCADOR - Referência / Primers	D1	D2	AN1	AN2	E1	D3	AN3	E2	E3	Final
[1]ITS2 - Gardes & Bruns (1993)			35x							
ITS1F - 5' CTTGGTCATTTAGAGGAAGTAA 3' ITS4R - 5' CAGGAGACTTGTACACGGTCCAG 3'	94º / 5'	94º / 3'	61º / 30"	-	72º / 1'	-	-	-	72º / 10'	14º / ^{oo}
[1]ITS2 - Dentinger et al (2010)			5x				25x			
ITS8F - 5' AGTCGTAACAAGGTTTCCGTAGGTG 3' ITS6R - 5' TTCCCGCTTCACTCGCAGT 3'	94º / 2'	94º / 30"	60º / 30"	-	72º / 1'	94º / 30"	55º / 30"	72º / 1'	72º / 10'	14º / ^{oo}
[1]LSU - Vilgalis Laboratory			30x							
LR0R - 5' ACCCGCTGAACTTAAGC 3' LR5 - 5' TCCTGAGGGAAACTTCG 3'	72º / 1'	94º / 1'	50º / 45"	-	72º / 1'	-	-	-	72º / 7'	14º / ^{oo}
[1]TEF1-α - Morehouse et al (2003)			10x				36x			
EF1-983F - 5' GCYCCYGGHCAYCGTGAYTTYAT 3' EF1-1567R - 5' ACHGTRCCRATACCACCRATCTT 3' EF1-1577F - 5' CARGAYGTBTACAAGATYGGTGG 3' EF1-2212R - 5' CCRACRGCRACRGTYYGTCTCAT 3'	94º / 2'	95º / 30"	66º / 30"	57º / 30"	72º / 1'	94º / 30"	56º / 30"	72º / 1'	72º / 10'	14º / ^{oo}
[2]ATP6 - Kretzer & Bruns (1999)			5x				30x			
ATP6-2 - 5' TAATTCTANWGCATCTTTAATRTA 3' ATP6-3 - 5' TCTCCTTTAGAACAATTTGA 3'	-	94º / 35"	37º / 55"	-	72º / 1'	94º / 35"	45º / 55"	72º / 1'	72º / 10'	14º / ^{oo}

5 RESULTADOS & DISCUSSÃO

Ao todo foram realizadas seis expedições de campo para coleta nas regiões previamente definidas como de potencial ocorrência de *P. piptadeniae*, abrangendo os estados de Santa Catarina (2), Paraná (1), São Paulo (1), Rio de Janeiro (1) e Distrito Federal (1). Foram coletados 46 espécimes de *P. piptadeniae*, distribuídos entre as seis localidades acima citadas. Adicionalmente, foram incluídos nas análses do primeiro e do segundo capítulo, espécimes de *P. piptadeniae* publicados em trabalhos anteriores (Drechsler-Santos et al. 2010, Salvador-Montoya et al. 2015, Drechsler-Santos et al. 2016), assim como de coletas realizadas por outros pesquisadores colaboradores. A lista completa contendo detalhes de todos os espécimes utilizados no desenvolvimento desse trabalho, assim como os marcadores sequenciados para cada espécime, é apresentada na Tabela 2.

Os marcadores moleculares com o maior número de espécimes sequenciados foram ITS e ATP6. Apesar de apresentar cromatogramas de alta qualidade (dados não mostrados) e, por consequência, sequências fiéis, o marcador ATP6 não apresentou variação genética, sendo portanto, excluído das posteriores análises moleculares. Em relação ao marcador molecular tef1-α, não foram obtidas sequências suficientes para representação de todas as populações amostradas neste estudo, e portanto, também foram excluídas das análises.

Os marcadores ITS e LSU foram incluídos nas reconstruções filogenéticas do primeiro capítulo. Apenas o marcador ITS foi utilizado na filogeografia preliminar de *P. piptadeniae*, devido a sua maior representatividade nas populações amostradas. Os demais marcadores deverão ser re-sequenciados para posterior publicação dos resultados da

presente dissertação. Os resultados e suas respectivas discussões são apresentados de forma separada em dois capítulos:

O Capítulo 1 apresenta a proposta de filogenia para o gênero *Phellinotus*, com ênfase na delimitação filogenética de *P. piptadeniae*. Ainda, para o tratamento taxonômico foi realizada a caracterização morfológica, ecológica e filogenética de duas linhagens correspondentes a novas espécies de *Phellinotus*, *P. teixeirae* sp. nov. Ad int e *P. magnoporatus* sp. nov. Ad int.

O Capítulo 2 propõe a ampliação da distribuição geográfica de *P. piptadeniae* com base em novas coletas feitas em regiões marginais da distribuição geográfica da leguminosa *P. gonoacantha*, o único hospedeiro conhecido para *P. piptadeniae* na Mata Atlântica. Adicionalmente, é apresentado um estudo preliminar da filogeografia de *P. piptadeniae* com base no marcador molecular nuclear ITS.

Tabela 2. Lista de espécimes utilizados. Coordenadas geográficas expressas em graus-decimais. [1]espécimes coletados durante a realização desse trabalho, porém não necessáriamente utilizados nas análises, [2]novos registros para a respectiva localidade.

Espécie / População (Localidade) / Voucher	Longitude	Latitude	Hospedeiro	ITS	LSU	tef	ATP6
Phellinotus piptadeniae (Teixeira) Drechsler-Santos & Robledo							
SC1 (Brazil, SC, Criciúma)							
[1,2]SGE_111	-49,3587	-28,6885	*Piptadenia gonoacantha*	1	1		1
[1,2]SGE_113	-49,3586	-28,6889	*Piptadenia gonoacantha*	1	1	1	1
[1,2]SGE_114	-49,3590	-28,6890	*Piptadenia gonoacantha*				
[1,2]SGE_115	-49,3596	-28,6891	*Piptadenia gonoacantha*	1	1	1	1
[1,2]SGE_117	-49,3596	-28,6893	*Piptadenia gonoacantha*	1	1	1	1
[1,2]SGE_87	-49,4319	-28,6897	*Piptadenia gonoacantha*				
[1,2]SGE_93	-49,4309	-28,6904	*Piptadenia gonoacantha*				
SC2 (Brazil, SC, Tubarão)							
AGS_48 (FLOR 51451)	-49,0323	-28,4854	*Piptadenia gonoacantha*				
SC3 (Brazil, SC, Florianopolis)							
[1]SGE_120	-48,4759	-27,5911	*Piptadenia gonoacantha*				
[1]SGE_127	-48,4793	-27,5954	*Piptadenia gonoacantha*	1	1	1	1
[1]SGE_126	-48,4792	-27,5953	*Piptadenia gonoacantha*	1	1		1
[1]SGE_124	-48,4746	-27,5903	*Piptadenia gonoacantha*	1	1		1
[1]SGE_125	-48,4781	-27,5934	*Piptadenia gonoacantha*	1	1		1
[1]SGE_123	-48,4759	-27,5911	*Piptadenia gonoacantha*	1			1
MABS_136 (FLOR 39574)	-48,5492	-27,5967	*Piptadenia gonoacantha*	1			
MABS_135 (FLOR 39573)	-48,5492	-27,5967	*Piptadenia gonoacantha*	1			
MABS_106 (FLOR 39571)	-48,5492	-27,5967	*Piptadenia gonoacantha*	1			
PR (Brazil, PR, Maringá)							
[1,2]SGE406	-51,9387	-23,4046	*Piptadenia gonoacantha*	1			1
[1,2]SGE410	-51,9320	-23,4264	*Piptadenia gonoacantha*				
[1,2]SGE417	-51,9320	-23,4264	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE420	-51,9320	-23,4264	*Piptadenia gonoacantha*				
[1,2]SGE432	-51,9320	-23,4264	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE444	-51,9320	-23,4264	*Piptadenia gonoacantha*	1			1
SP1 (Brazil, SP, Caraguatatuba)							
[1,2]MAR_1178_16	-45,4296	-23,5936	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE_220	-45,4286	-23,5928	*Piptadenia gonoacantha*	1		1	
[1,2]SGE_221	-45,4292	-23,5959	*Piptadenia gonoacantha*	1		1	
[1,2]SGE_222	-45,4292	-23,5959	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE_223	-45,4297	-23,5967	*Piptadenia gonoacantha*				
[1,2]SGE_224	-45,4297	-23,5967	*Piptadenia gonoacantha*	1			1
[1,2]SGE_225	-45,4298	-23,5966	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE_226	-45,4298	-23,5969	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE_242	-45,4296	-23,5960	*Piptadenia gonoacantha*	1			1
[1,2]SGE_243	-45,4298	-23,5958	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE_244	-45,4298	-23,5958	*Piptadenia gonoacantha*				
[1,2]SGE_245	-45,4297	-23,5959	*Piptadenia gonoacantha*	1			1
SP2 (Brazil, SP, Botucatu)							
MF_043	-48,4299	-22,8364	*Piptadenia gonoacantha*	1	1		
MF_032a	-48,4263	-22,8364	*Piptadenia gonoacantha*	1	1		
MF_046	-48,4263	-22,8364	*Piptadenia gonoacantha*	1	1		

MF_031	-48,4263	-22,8364	*Piptadenia gonoacantha*	1	1		
MF_044	-48,4249	-22,8371	*Piptadenia gonoacantha*	1	1		
MF_040	-48,4250	-22,8374	*Piptadenia gonoacantha*	1	1		
MF_032b	-48,4263	-22,8364	*Piptadenia gonoacantha*		1		
MF_035	-48,4293	-22,8370	*Piptadenia gonoacantha*	1			
MF_037	-48,4301	-22,8369	*Piptadenia gonoacantha*	1			
RJ (Brazil, RJ, Rio de Janeiro)							
[1,2]MAR_836/14	-43,2525	-22,9570	*Piptadenia gonoacantha*	1			1
[1,2]MAR_920/14	-43,2525	-22,9570	*Piptadenia gonoacantha*				
[1,2]SGE253	-43,2501	-22,9730	*Piptadenia gonoacantha*	1			1
[1,2]SGE348	-43,2511	-22,9727	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE349	-43,2529	-22,9722	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE350	-43,2497	-22,9733	*Piptadenia gonoacantha*	1			1
[1,2]SGE352	-43,2497	-22,9733	*Piptadenia gonoacantha*				
[1,2]SGE353	-43,2506	-22,9728	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE354	-43,2527	-22,9724	*Piptadenia gonoacantha*	1		1	1
DF (Brazil, DF, Brasilia)							
[1,2]SGE385	-47,8843	-15,7408	*Piptadenia gonoacantha*	1		1	1
[1,2]SGE386	-47,8861	-15,7410	*Piptadenia gonoacantha*	1			1
[1,2]SGE387	-47,8860	-15,7410	*Piptadenia gonoacantha*	1			1
[1,2]SGE389	-47,8860	-15,7410	*Piptadenia gonoacantha*	1			1
[1,2]SGE388	-47,8853	-15,7407	*Piptadenia gonoacantha*	1			1
[1,2]SGE390	-47,8868	-15,7416	*Piptadenia gonoacantha*	1			
PE1 (Brazil, PE, Serra Talhada)							
DS139PE (URM 80768)	-38,3014	-7,9003	*Piptadenia sp.*	1	1		
DS97PE (URM 80360)	-38,3047	-7,8914	*Mimosa sp.*	1	1		
PE2 (Brazil, PE, Barro Branco)							
DS300 (URM 80595)	-37,2472	-8,5924	*Piptadenia stipulacea*	1			
PE3 (Brazil, PE, Caruaru)							
DS110PE (URM 80345)	-35,9200	-8,2314	*Senegalia sp.*	1	1		
DS109PE (URM 80322)	-35,9200	-8,2314	*Mimosa sp.*	1	1		
DS163PE (URM 80766)	-35,9206	-8,2306	*Mimosa sp.*	1	1		
DS128PE (URM 80361)	-35,9203	-8,2308	*Senegalia sp.*	1	1		
Phellinotus teixeirae Salvador-Montoya, Galvão-Elias & Drechsler-Santos							
AR (Argentina, Corrientes, Corrientes)							
[2]Popoff_Dichtiar_34J	-58,8296	-27,4731		1			
PERU (Perú, Piura, Piura)							
[1]CS 457b (FLOR 16945)	-80,6642	-5,1849	*Libidibia glabrata*	1			
[1]CS_377 (FLOR 7554)	-80,6642	-5,1849	*Pithecellobium excelsum*	1			
[1]CS 454b (FLOR 16944)	-80,6642	-5,1849	*Libidibia glabrata*	1			
SE (Brazil, SE, Niterói)							
[1]DS44PE (URM 80403)	-37,4625	-9,7550		1			
PE (Brazil, PE, Buíque)							
[1]DS257 (URM 80889)	-37,2472	-8,5924	*Pityrocarpa moniliformis*	1			
[1]DS108 (URM 80636)	-37,2472	-8,5924	*Piptadenia* sp.	1			

5.1 CAPÍTULO 1 - THE GENUS *PHELLINOTUS* (HYMENOCHAETACEAE, BASIDIOMYCOTA): A MORPHOLOGICAL AND MOLECULAR STUDIES REVEAL NEW SPECIES FROM NEOTROPICS

The genus *Phellinotus* (Hymenochaetaceae, Basidiomycota): a morphological and molecular studies reveal new species from neotropics

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Abstract

The hymenochaetoid genus Phellinotus is a legume-associated polypore with two recognized species. Here, we investigate based on the internal transcribed spacer (ITS) and nuclear large subunit (nLSU) of robossomal DNA/RNA regions, in order to provide a phylogenetic delimitation for this species. Additionally, two new species are descrubed, '*P. teixeirae* sp. nov. Ad int. and *P. magnoporatus* sp. nov. Ad int. Overal morphology of *P. piptadeniae* and *P. teixeirae* overlap, but the polypore are associated to different legume hosts. *P. magnoporatus* has large pores and the distinctive black line that

characterize the genus *Phellinotus* is missing in this taxa. *P. magnoporatus* grows in *Ocotea aurantiodora*, representing the first record in Lauraceae host.

Key words

Hymenochaetaceae, Seazonally Dry Tropical Forests, South America, phylogeny and taxonomy.

Introduction

The newly described Hymenochaetoid genus *Phellinotus* Drechsler-Santos, Robledo & Rajchenb. is mainly characterized by a dimitic hyphal system with skeletal hyphae restricted to the tube layers, distinct black line across the context and pale yellow basidiospores that turn brown in KOH solution (Drechsler-Santos et al. 2016). Two species are recognized for the genus, the type species *P. neoaridus* Drechsler-Santos & Robledo with populations widely distributed in the Brazilian semiarid region (Caatinga dry woodlands) and *P. piptadeniae* (Teixeira) Drechsler-Santos & Robledo, a species with disjunct populations potentially distributed along to the South America Seasonally Dry Tropical Forests (SDTF) biome (Salvador-Montoya et al. 2015, Drechsler-Santos et al. 2016). Both species are found growing on living members of the family Fabaceae (Teixeira 1950, Drechsler-Santos et al. 2010, as *Phellinus rimosus* and *P. piptadeniae*, Drechsler-Santos et al. 2016).

The original description of *P. piptadeniae* (as *Phellinus piptadeniae* Teixeira) was based on collections of São Paulo state located in the Atlantic Forest domain (Teixeira 1950) (Figure 1). However, with

additional recent sampling effort in the northwestern of Brazil (Drechsler-Santos et al. 2010), the geographic range of the species has been extended to the Caatinga domain (Figure 1). Together to the geographical range, evidences of host-shift are provided by the authors. While the Atlantic Forest specimens grown exclusively on *Piptadeniae gonoacantha* (Mart.) J.F. Macbr. (as *Piptadenia communis* in Teixeiras 1950) a legume widely distributed throughout the semideciduous and ombrophilous formations of the Atlantic Forest and Cerrado domains (Morim 2013). Alternatively, Caatinga specimens grow on other Mimosoideae hosts, as other species of the genus *Piptadenia* Benth., besides the genera *Senegalia* Raf. and *Mimosa* R.Br (Drechsler-Santos et al. 2010). More recently, after new surveys in Peruvian dry woodlands and Brazilian Atlantic Forest, new hosts were recorded for the *P. piptadeniae* (Salvador-Montoya et al. 2015), and an interesting new biogeographical picture reveal the possible first case of fungal affinities to SDTF biome of the Neotropics. However, comprehensive molecular samplings including the disjunct populations know for the species remain to be done, in order to test the phylogenetic status of *P. piptadeniae*.

In this work, during the investigation of *Phellinotus* genus, we present a phylogenetic delimitation of *P. piptadeniae*, and based on morphological, ecological and molecular data, two new species are described. A key to *Phellinotus* species is provided.

Materials and methods

Study area and collections – The specimens included in this study were sampled in two different Floristic Groups (FG) of the South America

SDTFs (sensu DRYFLOR 2016), (i) Caatinga FG (dry woodlands of the Brazilian northeast) and (ii) Central Andes Coast FG (lowland on the northwest of Peru); and specimens from semidecidious formations of south Atlantic Forest. These specimens have been previously treated as *P. piptadeniae* in a wide sense (Salvador-Montoya et al. 2015, Drechsler-Santos et al. 2016). New specimens were collected on the Caatinga FG (DS44CE, DS108 e DS257) and Missiones FG (Popoff y Dichtiar 34J). Addictionally, one single specimen that partially fits in the *Phellinotus* concept (CS372), also collected in the Central Andes Coast FG, was included in phylogenetical and morphological analysis.

Morphological analysis – Macro and microscopical analysis of basidioma follows Teixeira (1995) and Ryvarden (2004). Size, shape, and color (following Munsell Color Company 1975) of basidiomata, as well as the pore surface (number of tubes strata and of pores per linear millimeter) were observed to describe seasonality. Microscopical examination and measurements were done in lactophenol (non-reaction), 3 % (v/w) KOH solution (xanthochroic), Melzer (dextrinoid or amyloid) and Cotton Blue (cyanophilia) reagents to determine the presence or absence of reactions. All microscopic measurements (n = 40) and drawings were made in 3 % (v/w) KOH solution. Size ranges of the microscopic measurements are reported after exclusion of 5 % of the smalles and bigger measurements and are given in parentheses.

DNA extraction, amplification and sequencing - Extraction of total genomic DNA followed the protocol of Doyle and Doyle (1987) adapted by Góes-Neto et al. (2005). We used the primer pairs ITS1-F/ITS4-R and LR0R/LR5 to amplify the nuclear ITS (ITS1-5.8S-ITS2) and nLSU (28S) regions respectively. Polymerase chain reaction were

implemented according to the parameters described in Dentinger et al (2010) for ITS and Vilgalys and Hester (1990) for LSU. We purified all PCR products with PEG 20 % [Poly(ethylene glycol) 8,000 plus NaCl 2.5M], and performed sequencing of the PCR products with addition of a mix cimposed by 1 μL BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) following manufacturer instructions and using the same primers as above.

Phylogenetic analysis – Taxa included in the phylogenetic analysis and their corresponding GenBank accession number are listed in Table 1. For the ITS analysis our newly generated sequences were aligned with previous published sequences of *P. piptadeniae* and *P. neoaridus* and *Fulvifomes fastuosus* (Lév.) Bondartseva & S. Herrera and *F. robiniae* (Murrill) Murrill were used as outgroup. In the concatenate analysis of nLSU and ITS, 19 sequences of 12 taxa of Hymenochaetaceae were aligned to our data, including the genera *Phylloporia* Murrill, *Fulvifomes* Murrill, *Inocutis* Fiasson & Niemelä and residual taxa of "phellinotus Clade" (Drechsler-Santos et al. 2016). In this analysis *Phellinus alni* (Bondartsev) Parmasto and *P. tremulae* (Bondartsev) Bondartsev & P.N. Borisov were selected as outgroup. The newly dataset was aligned using MAFFT version 7.305 (Katoh and Standley 2013) using X-INS-i strategy in which secondary structure information of RNA/DNA is considered (Katoh and Toh 2008). Maximum Likelihood (ML) and Bayesian Inference (BI) were used to conduct the phylogenetic analysis. The ITS region was divided in ITS1, 5.8s and ITS2. To obtain the best fit model of nucleotide evolution we consider the BIC criterion implemented in jModelTest version 2.1.6 (Guindon and Gascuel 2003, Darriba et al. 2012). A ML analysis was carried out

in RaxML version 8.2.9 (Stamatakis 2014), available in the CIPRES science gateway (Miller et al. 2010, http://www.phylo.org/). The analysis first involved 100 ML searches, each one starting from one randomized stepwise addition parsimony tree under a GTRGAMMA model. To access the branch support 500 bootstrapping (BS) replicates under the same model were computed. Bayesian Inference was carried in the software Mr. Bayes 3.2.6 (Ronquist and Huelsenbeck 2003) implemented on the CIPRES Science Gateway 3.1 (Miller et al. 2010). Two independent runs were used, each one starting from random trees and four simultaneous independent chains at $2x10^6$ generations, trees were sampling every 1000th generation. Four rate categories were used to approximate the gamma distribution. Estimate of nucleotide substitution rate performed in jModelTest were used under Dirichlet distribution as prior. Of all sampled trees 15% were discarded as burn-in and checked by the convergence criterion (frequencies of average standard deviation of split <0.01) in Tracer v.1.6 (Rambaut et al. 2014), while the remaining ones were used to estimate Bayesian posterior probabilities (BPP) of the branches.

Results

Molecular phylogeny – We generated a total of 20 new sequences of ITS and 15 of nLSU in this study. The ITS alignmed matrix containe 745 characters including gaps. The best-fit evolutionary models selected for each partition of this matrix were TPM3uf+I+G (ITS1), K80+G (5.8S) and TPM1uf+G (ITS2). The final concatenate alignmed matrix contained 1722 characters including gaps. The best-fit evolutionary

model selected to LSU was GTR+I+G. Informations of the priors used in BI of ITS and concatenate datasets are summarized in Table 2.

On the ITS-based and concatenate-based phylogeny (Figure 4 and 5), specimens previously treated as *Phellinus piptadeniae* by Salvador-Montoya et al. (2015) form two highly supported clades in the BI and ML analysis (Figure 4 and 5), here treated as *Phellinotus piptadeniae* and *Phellinotus teixeirae* sp. nov. *Ad. int*. The *P. piptadeniae* clade (ITS: ML=88/BI=0.96; concatenate: ML=98/BI=1) includes specimens from the semideciduous forest and lowlands of the Atlantic Forest domain, growing exclusively on *P. gonoacantha*, some of them are from an area near to the type locality (Table 1, Botucatu, Salvador-Montoya et al. 2015). In addiction, those Caatinga specimens that grow on *Mimosa* (DS109), *Senegalia* (DS110) and *Piptadenia* (DS139) grouped in the *P. piptadeniae* clade. In this case, we consider *P. piptadeniae* as a phylogenetic lineage represented by specimens from Atlantic Forest and Caatinga domains. Those specimens from Caatinga FG (DS108 and DS257), Missiones FG (Popoff & Dichtiar 34J) and Central Andes Coast FG (CS377, CS454b, CS457b and CS461b), grouped in a sister clade (ITS: ML=100/BI=1; concatenate: ML=100/BI=1) of *P. piptadeniae*, representing a new species, taxonomic treated here as *P. teixeirae* sp. nov. *Ad int*. Additionally, besides its disjunctive populations (Argentina, Brazil and Peru, Figure 1), the species were found growing on different legume hosts, *Pithecellobium*, *Libidibia*, *Pityrocarpa* and *Acacia*, without a host-specialization pattern.

Phellinotus neoaridus was recovered as a monophylitec species (ITS: ML=100/BI=1; concatenate: ML=100/BI=1), as presented by Drechsler-Santos et al. (2016). The Peruvian collection, founded on *Ocotea*

aurantiodora (Ruiz & Pav.) Mez, represent another new species of *Phellinotus*, taxonomic treated here as *P. magnoporatus* sp. nov. *Ad int*.

Taxonomic treatment

Phellinotus piptadeniae (Teixeira) Drechsler-Santos & Robledo, *Phytotaxa* 261(3): 218-239. 2016. (Fig. 2).

=*Phellinus piptadeniae* Teixeira, Bragantia 10(4): 118. 1950.

=*Fomitiporella piptadeniae* (Teixeira) Teixeira, Revista Brasileira de Botânica 15(2): 126. 1992.

Description in Drechsler-Santos et al. 2016.

Remarks — According to Salvador-Montoya et al. (2015) and Drechsler-Santos et al. (2016), *Phellinotus piptadeniae* is characterized by its aplannate to triquetous basidiomes with reddish yellow to olive or black grayish, concentrically sulcate and cracked to rimose pilear surface, a contextual distinct black line, 4-6 pores/mm, distinctly stratified tubes with skeletal hyphae restricted to the trama and broadly ellipsoid to ellipsoidwith a flattened side and yellowish basidiospores, becoming chestnut brown in KOH. Several specimens from seasonally dry tropical forests (SDTFs) were studied by the author (Salvador-Montoya et al. 2015, Drechsler-Santos et al. 2016), considering that *P. piptadeniae* is widely variable. However, the carefully revision of some specimens from Caatinga domain and Atlantic Forest of Brazil (including the type) help us to realized that concentrically wavy basidiomata with dark brown pilear surface of young specimens, turning lobulate with concentrically deep furrows delimiting wide lobes (frequently at the margin), coarsely deeply cracked and olive gray in well-developed and mature specimens should be considered as a

morphological pattern. In addition, the deterioration caused by insects showed a rimose appearance (Table 1, Fig. 2). On the other hand, specimens from Peru, Argentina and some others from Brazilian Caatinga present a morphological pattern characterized by its radially concentric rimose and dark grayish brown pilear surface (Table 1, Fig. 2) in mature specimens, besides its concentrically wavy and dark brown pilear surface in young specimens. Additionally, Brazilian specimens are recurrently collected on living tree of *P. gonoacantha*, while specimens from Peru, Argentina and others from Brazilian Caatinga domain (treated as new species) were founded on living tree of other leguminoses (Table 1). In this context, the specimens from Brazil represent the concept of *P. piptadeniae*.

Specimens examined — BRAZIL. São Paulo, Campinas, Bosque dos Jequitibás, on *P. communis*, 12 October 1943, A. R. Teixeira & P. R. Santos s.n. (IAC 4365, paratype), ibid Município de Botucatu, trilha Ecológica Casa da Natureza, Fazenda Experimental do Lajeado, on live trunk of *P. gonoacantha*, 30 January 2013, M. Fernandes MF7 (FLOR 19926), M. Fernandes MF8 (FLOR 30457), ibid on live trunk of *P. gonoacantha*, 4 July 2013, M. Fernandes MF26 (FLOR 39430), M. Fernandes MF27 (FLOR 51449), ibid on log trunk of *P. gonoacantha*, 4 July 2013, M. Fernandes MF29 (FLOR 51450); Santa Catarina, Florianópolis, Campus Universitário/UFSC, on live trunk of *P. gonoacantha*, 25 January 2011, M. A. Borba-Silva MABS106 (FLOR 39571), ibid on live trunk of *P. gonoacantha*, 14 April 2011, M. A. Borba-Silva MABS135 (FLOR 39572), M. A. Borba-Silva MABS136 (FLOR 39573), ibid. Tubarão, Fazenda Lunard, trilha do rio, on log trunk of *P. gonoacantha*, 14 November 2012, A. G. Silva-Filho AGS48

(FLOR 51451); Pernambuco, Estação Experimental do IPA, Caruarú, on *Mimosa* sp., 10 December 2008, E. R. Drechsler-Santos 109PE (URM 80322), ibid on *Senegalia* sp., 10 December 2008, E. R. Drechsler-Santos 110PE (URM 80345); ibid. Serra Talhada, on *Piptadenia* sp., 5 Mar 2009, E. R. Drechsler-Santos 139PE (URM 80768).

Phellinotus teixeirae Salvador-Montoya, Galvão-Elias & Drechsler-Santos, sp. nov. Ad int. (Fig. 2).

Typus — PERU, Piura, Las Lomas, Parque Nacional Cerros de Amotape, (4.194472S × 80.461320W, 502m), on living tree of *Pithecellobium excelsum* (Kunth) Mart., 7 December 2011, C. A. Salvador-Montoya 377 (USM 250528 holotype, FLOR 7554).

Diagnosis — Basidiomata perennial, triquetrous, pilear surface rimose, margin round, hymenophore poroid (4–6/mm). Context with a distinct sinuous black line that runs from the base to the margin and thin to slightly thick dark brown crust on pilear suface. Hyphal system monomitic in the context and dimitic in the tubes. Basidiospores broadly ellipsoid to ellipsoid (4.5–6.5 × 3.5–5.5 µm), adaxially flattened, thick-walled, yellowish, chesnut to ferruginous in KOH, on living tree of leguminose in seasonally dry tropical forests (SDTFs).

Etymology — Phellinotus teixeirae, in honor to the Brazilian mycologist Alcides Ribeiro Teixeira, who significantly contributed for the taxonomy of polypores.

Basidiome perennial, sessile, applanate to triquetrous, usually solitary or gregarious, up to 135 mm long, 65 mm wide and 58 mm thick, woody hard; pilear surface first pubescent and dark brown (HUE 7.5YR, 4/6), soon glabrous and dark grayish brown (HUE 2.5Y, 4/2); concentrically

wavy with shallow fisures when young, latter turning concentrically sulcate and radailly coarsely deeply cracked, when well developed and mature radially concentric rimose; margin entire, thin to thick, round, pubescent and dark yellowish brown (HUE 10YR, 4/6) in the young or in active growth, when drying dark grayish brown (HUE 2.5Y, 4/2); pore surface dark brown (HUE 7.5YR, 3/4); pores round, regular, (3–)4–6(–7) per mm, (110–)130–350(–390) µm diam., dissepiments entire, (30–)40–130(–140) µm thick; context up to 20 mm thick at the base in young, 8 mm thick in well-developed specimens, zonate, with a distinct sinuous black line that runs from the base to the margin, a thin to slightly thick dark brown crust present above context, dark yellowish brown (HUE 10YR, 4/6); tubes indistinctly to distinctly stratified with a thin context layer, up to 40 mm long, dark brown (HUE 7.5YR, 3/4).

Hyphal system, monomitic in the context and dimitic in the trama of tubes; context dominated by generative hyphae, (1.5–)2–8(–9) µm diam, regularly septate, branched, thin-walled, gradually thick-walled, occasionally portions with few septa are observed; trama of tubes dimitic with thin- to slightly thickwalled generative hyphae, simple septate, branched, and unbranched skeletal hyphae, thick-walled with a visible lumen to almost solid, (135–)138–630(–675) µm long × (3–)3.5–6.5(–7) µm diam. (L avg. = 384.5 µm, W avg. = 4.73 µm), tapering to the apex where the wall is almost thin and three to four adventitious septa are present; setae absent; basidia not observed; basidiospores broadly ellipsoid to ellipsoid, with the ventral side flattened, (4–)4.5–6.5(–7) × (3–)3.5–5.5(–6) µm (L avg. = 5.59 µm, W avg. = 4.20 µm), Q = 1.10–1.57 (Q avg. = 1.33), thick-walled, smooth, pale yellow in

lactophenol, showing a xantochroic reaction in KOH and basidiospores turning chestnut to ferruginous brown in KOH (KOH+), CB-, IKI-.

Habit and distribution — Basidiomes found in the base trunk of the living tree of *Pithecellobium excelsum* (Kunth) Mart., *Libidibia glabrata* (Kunth) Castellanos & G.P.Lewis, *Pityrocarpa moniliformis* Benth. and *Acacia* sp., in Caatinga, Misiones, Piedmont and Central Andes Coast floristic groups of SDTFs (Särkinen et al. 2011, DRYFLOR 2016).

Remarks — *Phellinotus teixeirae* is characterized by presenting triquetrous basidiomes with a rimose and dark grayish brown pilear surface, 4-6 pores/mm and context with a distinct black line. When the basidiomes are young, it resemble *P. piptadeniae*, by presenting concentrically wavy and dark brown pilear surface, besides the same pores per milimeter and context duplex. However, when mature, *P. piptadeniae* presents a lobulate with concentrically deep furrows delimiting wide lobes, coarsely deeply cracked, and olive gray pilear surface (Table 1, Figs. 2 and 3). In addition, *P. piptadeniae* is a exclusive parasitic of *P. gonoacantha* in Atlantic Forest domain and associated to several legume hosts in the Caatinga domain, while *P. teixeirae* is a parasitic polypore of different species of leguminose and distributed in different floristic groups of SDTFs in South America. The new species could also be compared to *Phellinotus neoaridus* Drechsler-Santos & Robledo, but this later present rimose and black upper surface, besides one or more indistinct black lines and distinct granular core in the context. In addition, *P. neoaridus* being parasitic of living trees of *Caesalpinea* spp., found in the Caatinga domain in Brazil (Drechsler-Santos et al. 2016).

Specimens examined — ARGENTINA, Corrientes, Itatí, Scorza Cué, (27.278150S × 58.246331W, 67m), 11 January 1988, O. Popoff et Dichtiar OP345 (CTES 515266); Anta, Salta, Parque Nacional El Rey, (24.694444S × 64.611056W, 980m), on *Acacia* sp., 24 March 2007, O. Popoff et al.OP4566 (CTES 569014). BRAZIL, Pernambuco, Buíque, Parque Nacional do Catimbau, Quixadeira/Morro do cachorro, (8.409722S × 37.248333W, 744m), on living tree of *Pityrocarpa moniliformis* , 30 October 2007, E. R. Drechsler-Santos et al. DS257 (URM80889), ibid, Trilha das Torres/Igrejinha, (8.571389S × 37.24611W, 765m), on living tree of *Pityrocarpa moniliformis*, 08 December 2006, E. R. Drechsler-Santos et al. DS108 (URM 80636); Sergipe, Niterói, 16 June 2008, (9.755S × 37.4625W, 33m), E. R. Drechsler-Santos et al. DS44SE (URM 80403). PERU. Piura, Las Lomas, Parque Nacional Cerros de Amotape, (4.312185S × 80.546895W, 329m), on living tree of *Libidibia glabrata*, 28 August 2012, C. A. Salvador-Montoya 454b (USM 278225, FLOR 16944), ibid (4.281991S × 80.536396W, 312m), on living tree of *Libidibia glabrata*, 29 August 2012, C. A. Salvador-Montoya 457b (USM 258362, FLOR 16945), ibid (4.287833S × 80.534302W, 293m), on living tree of *Libidibia glabrata*, 29 August 2012, C. A. Salvador-Montoya 461b (USM258366, FLOR 16946).BRAZIL, Pernambuco, Buíque, Parque Nacional do Catimbau, Quixadeira/Morro do cachorro, (8.409722S × 37.248333W, 744m), on living tree of *Pityrocarpa moniliformis*, 30 October 2007, E. R. Drechsler-Santos et al. DS257 (URM80889), ibid, Trilha das Torres/Igrejinha, (8.571389S × 37.24611W, 765m), on living tree of *Pityrocarpa moniliformis*, 08 December 2006, E. R. Drechsler-Santos et al. DS108 (URM 80636); Sergipe, Niterói, 16 June 2008,

(9.755S × 37.4625W, 33m), E. R. Drechsler-Santos et al. DS44SE (URM 80403). ARGENTINA, Corrientes, Itatí, Scorza Cué, (27.278150S × 58.246331W, 67m), 11 January 1988, O. Popoff et Dichtiar OP345 (CTES 515266); Anta, Salta, Parque Nacional El Rey, (24.694444S × 64.611056W, 980m), on Acacia sp., 24 March 2007, O. Popoff et al.OP4566 (CTES 569014).

Additional specimens examined — *Phellinotus neoaridus*: BRAZIL, Pernambuco: Serra Talhada, Estação Experimental do IPA, (7.891389S × 38.304722W, 490m), on living caatingueira tree (*Caesalpinia* sp.), 09 December 2008, Drechsler-Santos DS105PE (FLOR 53152 isotype), ibid Barra da Jangada, Jobatão dos Guararapes, (9.546944S × 37.557500W, 224m), on living tree of leguminose, September 2003, Silva GT s/n (URM 77673); Bahia, Curaçá, 21 February 2011, Lira CRS141 (URM83203).

Phellinotus magnoporatus Salvador-Montoya, Galvão-Elias & Drechsler-Santos, sp. nov. (Fig. 2)

Typus — PERU, Piura, Las Lomas, Parque Nacional Cerros de Amotape, (4.194472S × 80.461320W, 502m), on living tree of *Ocotea aurantiodora* (Ruiz & Pav.) Mez., 6 December 2011, C. A. Salvador-Montoya 372 (USM 250523 holotype, FLOR 51897).

Diagnosis — Basidiomes perennial, ungulate, pilear surface shallow coarsely cracked at the base, margin round, hymenophore poroid (1-2/mm). Context with a thin dark brown line (crust) on pilear suface and granular core in the base of basidiomes. Hyphal system monomitic in the context and dimitic in the tubes. Basidiospores broadly ellipsoid to ellipsoid (4.5–5.5 × 4–4.4 µm), adaxially flattened, thick-walled,

yellowish, chesnut to ferruginous in KOH, on living tree of *Ocotea aurantiodora*.

Etymology — P. magnoporatus, in reference to the large pores visible to the naked eyes.

Basidiome perenial, sessile, triquetrous to ungulate, solitary, up to 53 mm long, 80 mm wide and 45 mm thick, woody hard; pilear surface glabrous and brown to very dark grayish brown (HUE 10YR, 3/2), concentrically wavy, rough with scarce shallow fissures when young and when well developed and mature concentrically zonate and shallow coarsely cracked at the base of basidiome, with a thin crust in cross section; margin round, pubescent and dark yellowish brown (HUE 10YR, 4/6); pore surface dark brown (HUE 7,5YR, 3/4), pores round, some elongated, 1–2(–3) per mm, (300–)350–600(–650) µm diam, dissepiment entire, (100–)110–250(–270) µm thick; context up to 37 mm thick at the base in well-developed specimens, azonate, with a indistinct and thin dark brown line (crust) on pilear suface and granular core in the base of basidiome; tubes indistinctly stratified, up to 15 mm long, with whitish mycelia strands usually filling the old tubes, yellowish brown (HUE 10YR, 5/8).

Hyphal system, monomitic in the context and dimitic in the trama of tubes; context dominated by generative hyphae, (2–)3–5.5(–6) µm diam, regularly septate, branched, thin-walled, gradually thick-walled, occasionally portions with few septa (skeletal-like hyphae) are observed; trama of tubes dimitic with thin- to slightly thickwalled generative hyphae, simple septate, branched, and unbranched skeletal hyphae, thick-walled with a visible lumen to almost solid, (130–)193–448(–497) µm long × (3.5–)3–4(–4.5) µm diam. (L avg. = 293.2 µm, W

avg. = 3.7 µm), tapering to the apex where the wall is almost thin and three to four adventitious septa are present; setae absent; basidia not observed; basidiospores broadly ellipsoid to ellipsoid, with the ventral side flattened, (4–)4.5–5.5(–6) × (3–)4–4.5 µm (L avg. = 5.0 µm, W avg. = 4.0 µm), Q = 1.1–1.4 (Q avg. = 1.24), thick-walled, smooth, pale yellow in lactophenol, showing a xantochroic reaction in KOH and basidiospores turning chestnut to ferruginous brown in KOH (KOH+), CB-, IKI-.

Habit and distribution — Found on the base trunk of the living tree of *Ocotea aurantiodora*, in lowland seasonally dry tropical forest of Pacific in Peru corresponding to Central Andes Coast floristic group of SDTFs (Linares-Palomino 2006, Särkinen et al. 2011, DRYFLOR 2016).

Remarks — *Phellinotus magnoporatus* is characterized by presenting ungulate basidiome with a zonate, shallow coarsely cracked and very dark grayish brown pilear surface, 1–2 pores/mm and context with a granular core in the base of basidiome, besides a indistinct and thin dark line (crust) on pilear suface (Tabela 1, Figs. XXX). The new species could be compared to *Phellinotus neoaridus* by presenting a distinct granular core. However, *P. neoaridus* presents a black and rimose basidiome, 3–6 pores/mm and context with one or more dark lines below pilear surface, besides being parasitic of living trees of *Caesalpinea* spp. (a leguminose) in the Brazilian semiarid region (Caatinga dry woodlands) (Drechsler-Santos et al. 2016). While *P. magnoporatus* collected on a living tree of Lauraceae Juss. species (*O. aurantiodora*) in lowland dry tropical forest of Pacific in Peru.

Discussion

In this work, based on morphological, ecological and molecular data, we show that *P. piptadeniae*, as treated by Salvador-Montoya et al. (2015) do not is monophyletic and we propose a new species, *P. teixeirae*, for the sister lineage of *P. piptadeniae*. Additionally, we illustrate the new interesting taxa *P. magnoporatus*. The current concept of the *P. piptadeniae*, include specimens that produce perennial basidiomes with triquetrous to ungulate shapes, and the upper surfaces becoming rimose whit age (more details in taxonomic treatment). Thus, previous studies indicate that populations of *P. piptadeniae* are widely distributed throughout the Seasonally Dry Tropical Forests of the South America (Salvador-Montoya et al. 2015).

Based on two nuclear rDNA markers of a representative sampling of *P. piptadeniae* populations, we show that some specimens collected in the Caatinga, Missiones and Central Andean Coast FG's form a highly supported sister clade here treated as *P. teixeirae*. In a wide sense (Salvador-Montoya et al. 2015) *P. piptadeniae* are recognized to produce variable basidiomes with a rimose upper surface, however, our molecular evidences reveal that this character is restricted to *P. teixeirae* (Figure 3, Table 3). On *P. piptadeniae*, pilear surface of the younger specimens are similar to *P. teixeirae*, but turn lobulate on well developed basidiomes (Figure 3, Table 3). In general, microscopic features that distinguish the two species are subtle. Salvador-Montoya et al (2015) pointed slight differences in the basidiospore size among Peruvian and Brazilian specimens, based in our phylogenetic reconstruction, we show that despite overlapped, basidiospores are larger in some collections of *P. teixeirae* (Table 3).

Given the morphological overlap between the lineages, ecological features (different legume hosts) should be considered to recognize each species. In the original description of the *P. piptadeniae*, Teixeira (1950) postulate that the fungal species should be a parasitic species, growing exclusively on *P. gonoacantha*, a legume widely distributed throughout the south Atlantic Forest and southwestern Cerrado (Carvalho 2004, Morim 2013). Currently we recognize that in the Atlantic Forest, *P. gonoacantha* is the only host of the *P. piptadeniae*. However the geographic distribution of this taxon extend from Caatinga FG (Drechsler-Santos et al. 2010, Drechsler-Santos et al. 2016). Under semiarid environmental conditions, specimens of *P. piptadeniae* grows on other legume species of the genus *Senegalia*, *Mimosa* and *Piptadenia*. The closest related species *P. teixeirae* probably has an ecological predilection for SDTF´s (Figure 1). This polypore species grows in association with different hosts from those previously recorded for *P. piptadeniae*. In the Caatinga FG *P. piptadeniae* is collected on *P. monoliformis*, in the Piedmont FG (not included in phylogenetic analysis but fits morphologically in the *P. teixeirae* concept) on *Acacia* sp. and in the Central Andean Coast FG is collected on *L. glabrata* and *P. excelsum*.

Despite the previous biogeographic hypothesis of *P. piptadeniae* association with SDTF regions (Salvador-Montoya et al. 2015), the new current scenario indicate that the SDTF-associated taxa is actually *P. teixeirae*, while *P. piptadeniae* distribution is possibly host-driven. The association of *P. teixeirae* with several hosts in different FG of the SDTF's, and the presence of the common host (*Piptadenia*) between the

Atlantic Forest and Caatinga disjunct populations of *P. piptadeniae*, support this assumption.

Examples of associated species to SDTF and 'open-diagonal' of South America are widely available to plants and animals (Dirzo et al. 2011; Werneck et al. 2012; Vieira et al. 2015; Banda et al. 2016), however, examples of fungi are unavailable. In this way, *P. teixeirae* is the first case of fungal affinities to SDTF formations of the Neotropical region.

Additional insights on the biogeographic history of this lineage is added when considering geographical range of the congeneric species *P. neoaridus*. This lineage is widely distributed throughout the dry woodlands of Caatinga, up to now knowing as endemic (Drechsler-Santos et al. 2016). Considering the three species that living sympatrically in this region, we postulate the hypothesis that the Caatinga can be a center of diversification of the *Phellinotus* lineage, which remain to be tested.

The new species *P. magnoporatus* represents a morphological exception of the *Phellinotus* concept. The distinctive black line that characterize the genus *Phellinotus* is missing in this taxa, in addiction, the pore size is considerably much larger in comparison whit the remains species of the genus. However, microscopic features as hyphal system (monomitic in the context and dimitic in the tubes layers), basidiospores size and granular core in the base of basidiomes and phyogenetic placement of the specimens, support the hypothesis that this species belong to the taxa *Phellinotus*.

Key to *Phellinotus* species

1. Pilear surface rimose …..……..……2

1’. Pilear surface fissured to cracked or lobulate with concentrically deep furrows delimiting wide lobes……..……...……3

2. Context with one or more indistinct dark lines and distinct granular core……...…….…… *P. neoaridus*

2’. Context with a distinct dark line without granular core……...…….…… *P. teixeirae* sp. nov.

3. Pores 4-6 per mm, context duplex……..…….... *P. piptadeniae*

3’. Pores 1-3 per mm, context with a granular core……... *P. magnoporatus* sp. nov.

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Table 1. List of species, collection and sequences (when applicable) used in the phylogenetic analysis. Vouchers superscribed by * representing new sequences generated in this study.

Species/voucher	Host	Locality	GenBank accession	
			nLSU	ITS
Phellinotus piptadeniae (Teixeira) Drechsler-Santos & Robledo				
*MF026	*Piptadenia gonoacantha*	Brazil, SP, Botucatu	KP412270	KP412290
*MF034	*P. gonoacantha*	Brazil, SP, Botucatu	KP412276	KP412295
*MF038	*P. gonoacantha*	Brazil, SP, Botucatu	KP412278	KP412299
*MF031	*P. gonoacantha*	Brazil, SP, Botucatu	KP412273	KP412293
*MF032	*P. gonoacantha*	Brazil, SP, Botucatu	KP412274	KP412294
*MF043	*P. gonoacantha*	Brazil, SP, Botucatu	KP412281	KP412304
*MF044	*P. gonoacantha*	Brazil, SP, Botucatu	KP412282	KP412305
*AGS48	*P. gonoacantha*	Brazil, SC, Tubarão	-	-
*SGE124	*P. gonoacantha*	Brazil, SC, Florianopolis	-	-
*SGE126	*P. gonoacantha*	Brazil, SC, Florianopolis	-	-
*SGE127	*P. gonoacantha*	Brazil, SC, Florianopolis	-	-
*MABS106	*P. gonoacantha*	Brazil, SC, Florianopolis		-
*SGE125	*P. gonoacantha*	Brazil, SC, Florianopolis		-
DS163PE	Mimosa sp.	Brazil, PE, Caruarú		-
DS110PE	Senegalia sp.	Brazil, PE, Caruarú	-	-
DS109PE	Mimosa sp.	Brazil, PE, Caruarú	-	-
DS128PE	Senegalia sp.	Brazil, PE, Caruarú		-
DS139PE	*Piptadenia sp.*	Brazil, PE, Serra Talhada	-	-
Phellinotus teixeirae Salvador-Montoya, Galvão-Elias & Drechsler-Santos, sp. nov.				
*CS377	*Pithecellobium excelsum*	Perú, Las Lomas, Piura	-	-
*CS454b	*Libidibia glabrata*	Perú, Las Lomas, Piura	-	-
*CS457b	*Libidibia glabrata*	Perú, Las Lomas, Piura	-	-
*CS461b	*Libidibia glabrata*	Perú, Las Lomas, Piura	-	-
*Popoff y Dichtiar 34J		Argentina, Corrientes, Corrientes		-
*DS44CE		Brazil, CE,		-
*DS108	*Piptadenia sp.*	Brazil, PE,		-
*DS257	*Piptadenia moniliformis*	Brazil, PE,		-
Phellinotus neoaridus Drechsler-Santos & Robledo				
URM 77673	-	Brazil, PE,	-	-
URM 83203	-	Brazil, PE,	-	-
URM 80362	-	Brazil, PE,	-	-
Phellinotus magnoporatus Salvador-Montoya & Drechsler-Santos, sp. nov.				
*CS372	-	Perú, Las Lomas, Piura	-	-
Fulvifomes fastuosus (Lév.) Bondartseva & S. Herrera				
CBS213/36	-	-	AY059057	AY558615
Fulvifomes robiniae (Murrill) Murrill				
CBS211/36	-	-	AY0590381	AY558646
Fulvifomes nilgheriensis (Mont.) Bondartseva & S. Herrera				
CBS209/36	-	-	AY0590231	AY558633
Hymenochaetaceae_sp				
CIEFAPcc107	-	-	KP347525	KP347537
CIEFAPcc88	-	-	KP347524	KP347536
Arambarria destruens Rajchenb. & Pildain				
CIEFAPcc347	-	-	KP347523	KP347538
Inonotus tenuissimus H.Y. Yu, C.L. Zhao & Y.C. Dai				
Dai_12245	-	-	KC9999021	KF6951211
Dai_12255	-	-	KC9999031	KC456243
Phylloporia sp				
FLOR51153	-	-	KJ631414	KJ639057
FLOR51173	-	-	KJ631412	KJ639055

Phylloporia aff *crhysites*				
FLOR51239	-	-	KJ631407	KJ639052
Inocutis dryophila (Berk.) Fiasson & Niemelä				
L61520A	-	-	AM2698461	AM269782
SP25	-	-	AM2698451	AM269782
Fulvifomes chinensis (Pilát) Y.C. Dai				
LWZ_201307137	-	-	KJ7878081	KJ7878171
LWZ_201309163	-	-	KJ7878091	KJ787818
Fulvifomes inermis (Ellis & Everh.) Y.C. Dai				
LWZ_201308095	-	-	KJ7878101	KJ7878211
LWZ_201308098	-	-	KJ787811	KJ7878211
Fomitiporella sp				
Oe5	-	-	JQ9109081	JF8954661
Oe6	-	-	JQ9109091	JF895467
Phellinus alni (Bondartsev) Parmasto				
TW162	-	-	KU139211	KU139159
Phellinus tremulae (Bondartsev) Bondartsev & P.N. Borisov				
243	-	-	KU139206	KU139136

Table 2. Summary of priors used in Bayesian analysis.

Proprieties	Partition			
	ITS1	5.8S	ITS2	nLSU
Evol. Model	TPM3uf+I+G	K80+G	TPM1uf+G	GTR+I+G
Likelihood score	3930,0713	551,1919	2249,3497	3741,841
Base frequencies	-	equal	-	-
Freq. A	0,1979	-	0,1979	0,2350
Freq. C	0,2142	-	0,2210	0,2236
Freq. G	0,2738	-	0,2602	0,3262
Freq. T	0,3141	-	0,3208	0,2153
Transition Rate	-	equal	-	-
R (AC)	1,4683	-	1,0000	0,7614
R (AG)	3,9709	-	2,9723	3,8639
R (AT)	1,0000	-	0,5559	0,1999
R (CG)	1,4683	-	0,5559	0,8734
R (CT)	3,9709	-	2,9723	8,4543
R (GT)	1,0000	-	1,0000	1,0000
Proportion of invariable sites	0,0880	-	-	0,4260
Gamma shape	1,7090	0,1760	0,4690	0,5340

Table 3. Comparison of morphological and ecological features of *Phellinotus* species. [a]Type; A = Aplannate; U = Ungulate; Lb = Lobulate; W = Concentrically wavy; F = Fisured; C = Cracked; L = Dark Line; G = Granular core M = Monomitic; D = Dimitic; AF = semideciduous forest of the Brazilian Atlantic Forest domain; CAA = Brazilian Caatinga dry woodland; CAC = Central Andes Coast floristic group; MIS = Misiones floristic group; PIED = Piedmonte floristic group; PER = Peru; AR = Argentina, SP = São Paulo states; SC = Santa Catarina, Pip. = Piptadenia, Pit. = Pithecellobium; Oco. = Ocotea.

Specimens	Basdioma	Upper surface	Pore/mm	Context	Tubes	Basidiospores	Substrata	Dsitribution
Phellinotus piptadeniae								
IAC 4365[a]	A	Lb/C	(3–) 4–5 (–6)	L/M	D	4.5–5.5 × 3.5–4.5	*Pip. gonoacantha*	AF-SP
FLOR 19926	A	W	3–4	L/M	D	4.5–5 × 3.5–4	*Pip. gonoacantha*	AF-SP
FLOR 30457	A	W	4–5	L/M	D	4.5–5 × 3.5–4	*Pip. gonoacantha*	AF-SP
FLOR 39430	A	W	4–5 (–6)	L/M	D	4.5–5 × 3.5–4	*Pip. gonoacantha*	AF-SP
FLOR 51449	T	W	4–5	L/M	D	5–5.5 × 3.5–4	*Pip. gonoacantha*	AF-SP
FLOR 51450	T	W	(3–) 4–5	L/M	D	5–5.5 × 3.5–4	Log or dead trunk	AF-SP
FLOR 51451	U	W	5–6	L/M	D	4.5–5 × 3–3.5	Log or dead trunk	AF-SC
FLOR 39571	U	W	5–6 (–7)	L/M	D	4–5 × 3–4.5	*Pip. gonoacantha*	AF-SC
FLOR 39572	A	W/F	5–6 (–7)	L/M	D	5–5.5 × 4–4.5	*Pip. gonoacantha*	AF-SC
FLOR 39573	U	Lb/C	5–6 (–7)	L/M	D	5–6 × 4–4.5	*Pip. gonoacantha*	AF-SC
URM 80322	A	W	4–5 (–6)	L/M	D	5–6 × 3.5–4.5	*Mimosa* sp.	CAA
URM 80345	A	W	(4–) 5–6 (–7)	L/M	D	5–5.5 × 3.5–4	*Senegalia* sp.	CAA
URM 80768	A	W	(4–) 5–6	L/M	D	4.5–5.5 × 3.5–4	*Piptadenia* sp.	CAA
Phellinotus teiceirae								
[a]USM 250528	T	C	(4–) 5–6 (–7)	L/M	D	(4–) 4.5–6 (–7) × (3–) 3.5–4.5 (–5)	*Pit. excelsum*	CAC-PER

USM 278225	A	W	4–5 (–6)	L/M	D	5–6 × 4–5	*Libidibia glabrata*	CAC-PER
USM 258362	A	W/F	4–5 (–6)	L/M	D	5–6 (–7) × 4–5	*Libidibia glabrata*	CAC-PER
USM 258366	T	W	4–5 (–6)	L/M	D	5–6 (–6.5) × 4–4.5 (–5)	*Libidibia glabrata*	CAC-PER
URM 80889	A	W	5–6	L/M	D	(4–) 5–5.5 × (3–) 3.5–4 (–4.5)	*Pip. moniliformis*	CAA
URM 80636	A	W	5–6 (–7)	L/M	D	5–5.5 × 3.5–4	*Pip. moniliformis*	CAA
URM 80403	A	W/F	(4–) 5–6	L/M	D	(4.5–) 5–5.5 × (3–) 3.5–4 (–4.5)	*Piptadenia* sp.	CAA
CTES 569014	T	W	5–6 (–7)	L/M	D	5.5–6 × 4.5–5	*Acacia* sp.	PIED-AR
CTES 515266	T	R	(3–) 4–5	L/M	D	(5.5–) 6–6.5 (–7) × (4–) 4.5–5.5 (–6)	Unknwon	MIS-AR
Phellinotus magnoporatus								
USM 250523	U	C	1–2 (–3)	G/M	D	4.5–5.5 × 4–4.5	*Oco. aurantiodora*	CAC-PER

Figure 1. Distribution map of *Phellinotus* species. *Phellinotus piptadeniae* = green rhombus (specimens of Salvador-Montoya et al. 2015) and yellow stars (type collections of the Teixeira 1950), *Phellinotus teixeirae* = blue triangle, *P. magnoporatus* = purple circle, and *P. neoaridus* = orange circles. Hatched area indicate SDTF potential distribution *sensu* Särkinen et al. (2011).

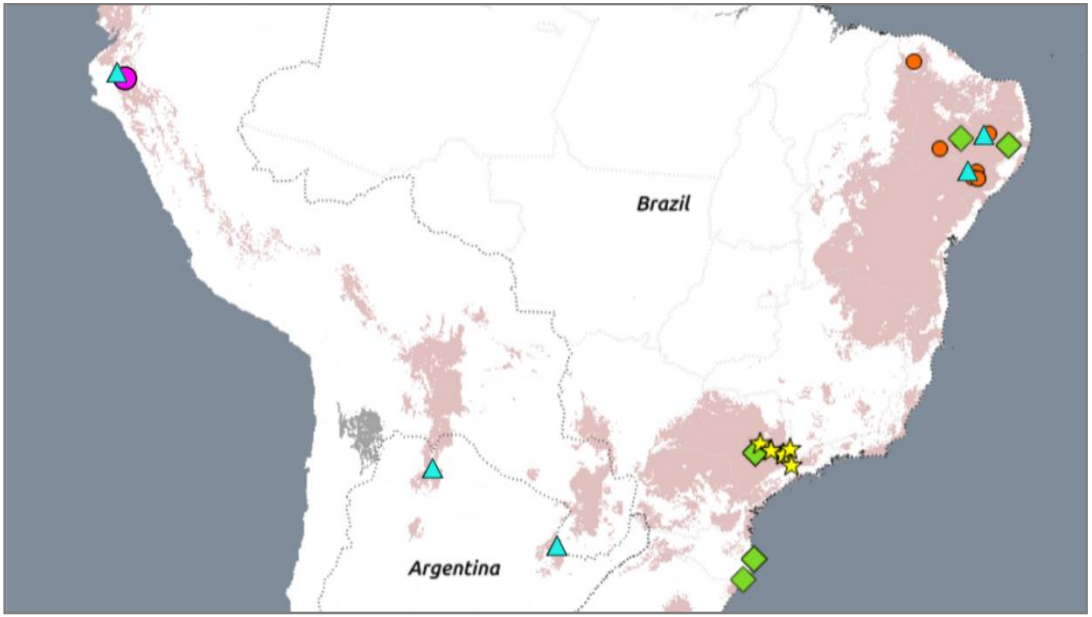


Figure 2. Morphological features of *Phellinotus* species: *Phellinotus piptadeniae versus P. teixeirae* (A-H): A. Paratype of *P. piptadeniae* (IAC 4365), B. Holotype of *P. teixeirae* in natura (USM 250528), C. Concentrically wavy pilear surface of young specimen of *P. piptadeniae* (URM80768), D. Concentrically wavy pilear surface of young specimen of *P. teixeirae* (URM80636), E. Lobulate and coarsely deeply cracked, beside deteriored by insects, pilear surface in well and developed specimen of *P. piptadeniae* (FLOR 39572), F. Rimose pilear surface in well and developed specimen of *P. teixeirae* (CTES 515266), G. Basidiome with deep furrow delimiting wide lobes and a dark line in the context in *P. piptadeniae* (IAC 4365, paratype), H. Basidiome with (H*) a dark line in the context in *P. teixeirae* (CTES 515266); *Phellinotus magnoporatus* (I-K): I. Basidiome in natura with shallow coarsely cracked pilear surface (USM 250523, holotype), J. Context with a granular core at the base of basidiome, in detail (J*) the indistinct and thin dark brown line (crust) on pilear suface, K. Pore surface.

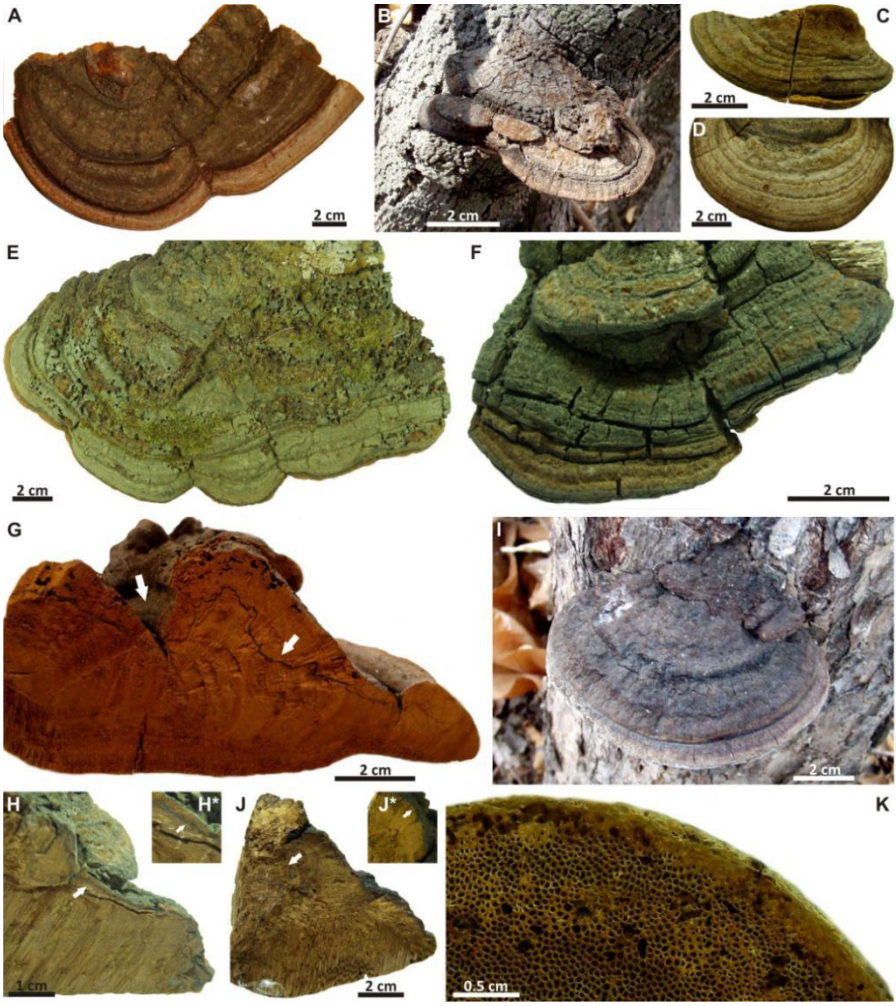

A
2 cm
B
2 cm
C
2 cm
D
2 cm
E
2 cm
F
2 cm
G
2 cm
I
2 cm
H
H*
1 cm
J
J*
2 cm
K
0.5 cm

Figure 3. Micromorphological features of *Phellinotus teixeirae* and *P. magnoporatus*: *P. teixeirae* (A-C): A. Generative hyphae of the context, B. Skeletal hyphae of the trama of tubes, C. Broadly ellipsoid to ellipsoid basidiospores with flattened side; *Phellinotus magnoporatus* (D-F): D. Generative hyphae of the context, E. Skeletal hyphae of the trama of tubes, F. Broadly ellipsoid to ellipsoid basidiospores with flattened side.

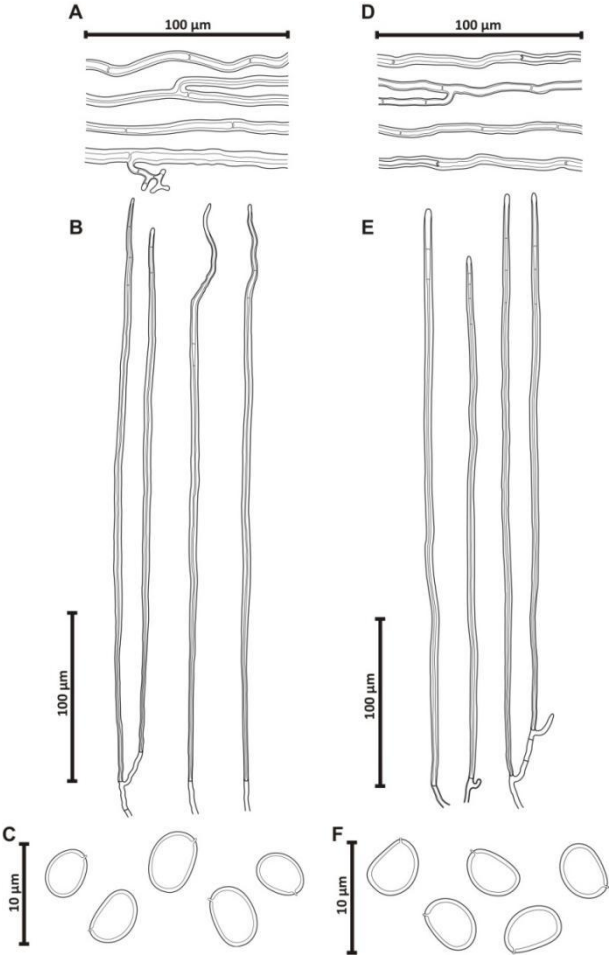


Figure 4. Phylogram of *Phellinotus* genus recovered from ITS (ITS1-5.8S-ITS2), inferred by Bayesian Inference (BI) and Maximum Likelihood (ML). Support values along branches are BS from ML and BPP from BI. Sequences of *Fulvifomes* species (*F. fastuosus* and *F. robinae*) were used to root the tree.

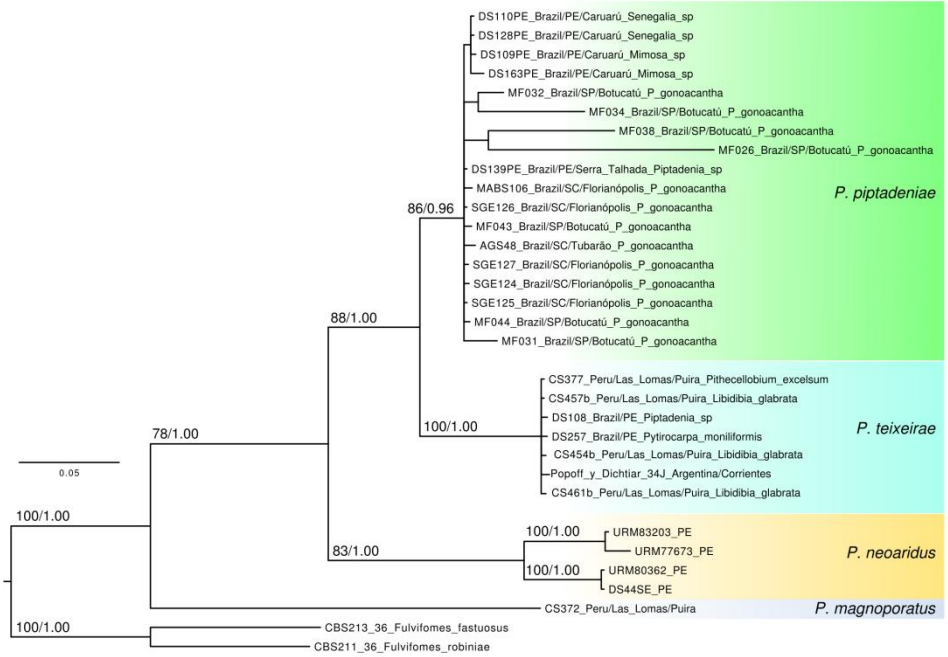
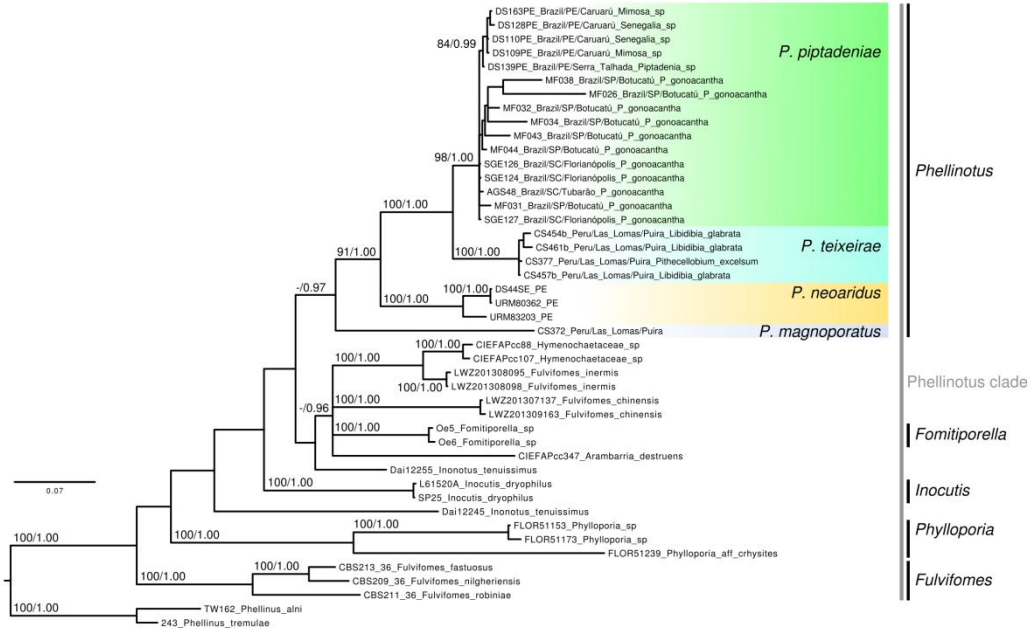


Figure 5. Phylogram of "phellinotus clade" recovered from ITS (ITS1-5.8S-ITS2) and nLSU regions of rDNA, inferred by Bayesian Inference (BI) and Maximum Likelihood (ML). Support values along branches are BS from ML and BPP from BI. Sequences of *Phellinus* s.s. species (*P. alni* and *P. tremulae*) were used to root the tree.



5.2 CAPÍTULO 2 - PRELIMINARY INSIGHTS INTO THE PHYLOGEOGRAPHY OF *PHELLINOTUS PIPTADENIAE* (HYMENOCHAETACEAE, BASIDIOMYCOTA)

Preliminary insights into the phylogeography of *Phellinotus piptadeniae* (Hymenochaetaceae, Basidiomycota)

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Abstract

Phellinotus piptadeniae is a legume-assocoated polypore with disjunt geographical distribution, with populations occurring on the Atlantic Forest and Caatinga domains. We use rDNA sequences of nuclear Internal Transcribed Spacer (ITS) in order to identify the preliminary phylogeographical structure of the populations. We identify the highest genetic diversity in populations associated associated to *Piptadenia gonoacantha*, 'the only host in the Atlantic Forest, and additionally we demonstrate that this polypore also occurr on the Brasilian Cerrado, following the *P. gonoacantha* distributons. Exclusive hapotypes associated to this populations iindicate recent demographic rxpansions.

Our results indicate that host association can drives the population structure of *P. piptadeniae*.

Key words

Host-driven distribution, sympatric species, genetic diversity, Structure analysis.

Introduction

South America is a large continent extending over a wide latitudinal range, including equatorial and temperate climatic regimes, complex topography, along the Andean uplift in pacific side, and Serra do Mar and Mantiqueira mountain uplifts in the atlantic side (Turchetto-Zolet et al. 2013). Across these environmental conditions, it is enevitable the divergence of biological lineages. Several examples of animal and plant speciation studies in the South America are available (Grazziotin et al. 2006, Cabanne et al. 2007, Fitzpatrick et al. 2009, Martins et al. 2009, D'Horta et al. 2011, Batalha-Filho et al. 2012, Werneck et al. 2012, Werneck et al. 2015), however, the Neotropical mycota remain unexplored in terms of its phylogeography.

The Hymenochaetoid genus *Phellinotus* Drechsler-Santos, Robledo & Rajchenb. is an interesting example of neotropical polypore lineage (Drehsler-Santos et al. 2016) associated with legumes' hosts and widely distributed in South America. Currently, this lineage includes four species, '*P. magnoporatus* sp. nov. Ad int, *P. neoaridus*, *P. piptadeniae* and *P. teixeirae* sp. nov. Ad int. Exceptionally by *P. magnoporatus*, that occur only Central Andean Coast, species are sympatric in the Brazilian Caatinga. Up to now, the species *P. neoaridus* is considered endemic to

this semiarid regions, instead *P. piptadeniae* and *P. teixeirae*, that show populations widely distributed and disjunct throughout the Seazonally Dry Tropical Forests (SDTF) and Atlantic Forest of South America (Salvador-Montoya et al. 2015, Elias et al. 2017, unpublished data).

Phellinotus piptadeniae, first described as *Phellinus piptadeniae* Teixeira, has a complex history of attributed geographic distribution and host-association. In the original description of the species, Teixeira (1950) postulated that should be a fungus specific of *Piptadenia gonoacantha* (Mart.) J.F. Macbr., and point out that this fungus probably follows the geographical distribution of the host. Despite this observation, collections performed by Teixeira are geographically restricted to semideciduous forests of São Paulo state (Figure 1). Considering the widely distribution of *P. gonoacantha* 'throughout the semideciduous and ombrophilous formations of the Atlantic Forest and Cerrado domains (Morim 2013), the Teixeira's hypothesis remain to be tested.

Currently, the geographical range of *P. piptadeniae* is recognized to exceeds the *P. gonoacantha* distribution, and the *P. piptadeniae* range extends to the Brazilian semi-arid regions and the fungus also grows different legume hosts from the genus *Senegalia* Raf., *Mimosa* L. and *Piptadenia.* However, in the Atlantic Forest domain, the fungus grows exclusively on *P. gonoacantha* (Figure 1, Drechsler-Santos et al. 2010; Salvador-Montoya et al. 2015, Elias et al. 2017, unpublished data).

The phenomenon of host-shift observed in *P. piptadeniae* are recurrently observed in some cases of wood-decay fungi, in this cases, host-shift is associated to exclusive mutations that allow reconstruct the

natural history of the lineages (Geml et al. 2006, Geml et al. 2008, Seierstad et al. 2013).

In this way, we presented two main question: (i) the geographical distribution of *P. piptadeniae* is predicted by the range of *P. gonoacantha* (ii) the association with different hosts are associated to exclusive mutations.

Material & Methods

Potential Distribution of P. piptadeniae – In order to predict the host-based most probable distribution of *P. piptadeniae*, we compiled georeferenced herbarium data, fieldwork data and literature records to construct the Kermel density map of the main host *P. gonoacantha* populations, as cited following. We downloaded herbarium data for the host from CRIA speciesLink (splink.cria.org.br, May 2015) and manually cleaned it by exclusion of poor records, obvious outliers and duplicate specimens when necessary. The final cleaned dataset include 1137 records. To access regions with high population density, we use the Kernel Density Estimator considering 5km of influence radius. All analysis were performed in QGIS version 2.18.2 under Linux platform. We performed field expeditions in order to validate the selected area identified by this analysis, confirming the presence of *P. piptadeniae*, and collecting specimens for molecular analyses.

DNA extraction, amplification and sequencing – Extraction of total genomic DNA followed the protocol of Doyle and Doyle (1987) adapted by Góes-Neto et al. (2005). The primer pairs ITS1-F/ITS4-R were used to amplify the ITS (ITS1-5.8S-ITS2) region through polymerase chain reaction, according to the cycle parameters described

by Dentinger et al (2010). Sequencing was performed with BigDye Terminator 3.1 Cycle Sequencing Kit following manufacturer procedures (Applied Biosystems, Foster City, CA, USA) using the same primers above cited. Sequencing resources were awarded through the FungiBrBOL project in FIOCRUZ-BH (Brazil) sequencing platform (www.brbol.org).

Sequence edition and haplotype reconstruction - Electropherograms were manually checked and edited in Geneious 9.0.5 (Kearse et al. 2012). Generated sequences were aligned using MAFFT version 7.305 (Katoh and Standley 2013) using FFT-NS-i strategy (Katoh and Toh 2008). When present, double peaks in both strands were coded according to the IUPAC code. We used the PHASE algorithm to estimate the gametic phase, with 0.9 as minimum probability threshold (Stephens 2001, 2003). Haplotype reconstruction was performed in DnaSP version 5 (Librado and Rozas, 2009).

Populations structure – To graphical explore the haplotype relationship we construct a Median-Joining network (Bandelt et al. 1999) using SplitsTree software (Huson and Bryant 2006). To access the most probable number of genetic clusters (k) given the haplotype data, we use the Bayesian approach implemented on software Structure version 2.3.4 (Pritchard et al. 2000), using the population information as prior. Considering the total number of localities sampled in this study (11), we assume two to eleven groups (k=2-11) to be estimated, including specimens of *Phellinotus teixeirae* as outgroup. For each k we run 10 independent Markov chains with burn-in of 250.000 iterations and run length after this of $1x10^6$ iterations. The best number of k was determined according the DeltaK statistic (Earl & Holdt 2012). Analysis

of Molecular Variance (AMOVA, Excoffier et al. 1992) with three hierarchical levels were performed to access the population genetic structure at the clusters indicated by Structure analysis. Additionally, we test an Alternative design (Table 4), assuming two clusters that include Caatinga populations (PE1, PE2 and PE3) vs Atlantic Forest and Cerrado populations (SC1, SC2, SC3, SP1, SP2, RJ, PR and DF). Significance of F statistics were accessed by 10000 bootstrap pseudoreplications.

Demographic dynamics – Number of haplotypes (h), nucleotide diversity (Pi) and haplotype diversity (Hd) were calculated in DnaSP. Evidences of demographic expansion were tested with Tajima's D (Tajima 1989), Fu's FS (Fu 1997) and the population size change test R2 (Ramos-Onsins and Rozas, 2002). Significance of the tests were obtained by 1000 coalescence simulations in the same above cited software.

Results

Potential distribution of P. piptadeniae – Final map containing regions of high density of *P. gonoacatha* populations is presented in Figure 1. Regions of high Kernek density were identified for five states: PR, SP, RJ, MG and DF. In the São Paulo state two regions of high host density were identified in Campinas and São Paulo countries and middle density in Piracicaba country, the three countries are type localities of *P. piptadeniae* (Figure 1, yellow stars, Teixeira 1950). Marginal regions located in four of the five above cited states were visited to perform field collections. In all localities the presence *P. piptadeniae* was confirmed (Table 1).

Genetic analysis – We obtained 369bp consensus sequences for ITS marker that were aligned to previous published sequences of *P. piptadeniae* (Drechsler-Santos et al. 2016). Final alignment with 56 individuals containing 13 segregating sites (including gaps) were used to estimate the haplotypes used in the further analysis. PHASE reconstruction result in 112 sampled chromosomes with high probability and collapsed haplotypes is presented in Table 2.

Population structure – Geographic distribution of haplotypes and this relationship is presented in Figure 2. Analysis performed by Structure identify three (K = 3, excluding the outgroup cluster K1) most likely distinct genetic clusters at the 56 specimens included in this analysis (Figure 3, Table 3). Specimens of Atlantic Forest (SC1, SC2, PR, SP1 and RJ) and Cerrado domains (DF) cluster in K3 (Figure 3, Table 3), already, insular population SC3 show intermediary affinities with K3 and simultaneously with K2 (composed by SP2 population). In order to test this incoherence, two models of AMOVA were generated including SC3 in K2 and K3, respectively, to search the best fit model to genetic variation (Table 4, Models 1 and 2). Caatinga populations form a well-defined genetic cluster (K4, Figure 3, Table 3).

All levels of the genetic variation (among genetic cluster, F_{CT}; among populations within clusters, F_{SC}; and within populations, F_{ST}), show statistical significance at the bootstrap test to non-random distribution of haplotypes (Table 4). However, the Alternative model show higher values to all fixation index (F_{CT}= 0.39; F_{SC}= 0.17; F_{ST}=0.49), indicating lack or low genetic structure in populations associated to *P. gonoacantha*, given the single cluster assumed by the Alternative model from Atlantic Forest and Cerrado populations (Table 4).

Historical demography – Neutrality tests (except Tajima's D) show statistical significance to reject the null hypothesis of constant population size (Table 5), indicating sign of recent demographic expansion of the *P. piptadeniae* populations. R_2 is a singleton-sensitive test appropriate to small sample sizes (Ramos-Onsins and Rozas, 2002). Six of the thirteen haplotypes observed in our study are singletons, and those belong to the populations of SC3, SP1, SP2 and RJ (Figure 2). The last two (SP2 and RJ) show highest haplotypes number but only SP2 present high nucleotide diversity (Pi, Table 3). Is expected that higher values of Pi is observed in larger populations associated to historically stable areas (Avise 2000).

Discussion

Geographical Distribution of P. piptadeniae – The initial hypothesis of geographic and host range of *P. piptadeniae* was postulated by Teixeira (1950). Under the Teixeira's assumptions, *P. piptadeniae* probably follows the geographical distribution of its highly specific host *P. communis* (= *P. gonoacantha*). Previous evidences presented by Drechsler-Santos et al (2010), Salvador-Montoya et al (2015), Drechsler-Santos et al (2016) and Elias et al (2017, unpublished data) showed that *P. piptadeniae* has association with other legume hosts. However, populations of Atlantic Forest domain are exclusively associated to *P. gonoacantha.*

In our study, distribution of the *P. gonoacantha* was used to predict the host-based most probable distribution of *P. piptadeniae*. After new field samplings, we demonstrate that the geographic range of *P. gonoacantha* can be an important predictor of the *P. piptadeniae* distribution. The

new distribution here presented, extend significantly the current geographic range of *P. piptadeniae*. New records include nuclear region of Cerrado domain, semideciduous forests of Paraná, ombrophilous forest of São Paulo and Rio de Janeiro and expansion of the meridional limit of the species, with collections performed in lowlands of the southwestern of Santa Catarina state (Criciúma populations, Table 1, Figure 2).

Our results have important implications to understand the natural history of *P. piptadeniae*, and simultaneously of the highly specific host *P. gonoacantha*. Understand the environmental features that determine the geographical distribution of the species, is the first step to establish conservation strategies. However, the perfect estimation of the species distribution depends intensively on the precise detection of the environmental characteristics supported by each taxa (Guillera-Arroita 2017). In this way, several factors can be determine the suitability of the species to particular region, thus, when it come to parasitic species, the identification of the host-relationship has great value to trace current and future distribution of the species (Pickles et al. 2013).

Genetic structure and historical demography – Results obtained by Structure analysis, genetic structure explained by AMOVA and evidences of recent demographic expansion, reveal an interesting scenario. Genetic diversity of *P. piptadeniae* was not randomly distributed in the space and two main genetic clusters were observed in nuclear ITS rDNA marker: Caatinga populations (CP) associated to different hosts and (ii) Atlantic Forest and Cerrado populations (AFCP) associated exclusively to *P. gonoacantha*.

Specimens of CP show low genetic diversity, with only one exclusive haplotype, and another shared with all specimens associated to *P. gonoacantha* (Figure 2). The specimen that holds this haplotype (voucher DS139 in Table 1, Haplotype 2 in Figure 2) was collected in unidentified species of *Piptadenia*, indicating the possible link between the CP and AFCP populations.

Specimens associated to *P. gonoacantha* (AFCP) compose populations with high genetic diversity and many divergent haplotypes (Figure 2). Several singletons were identified in populations SP1, SP2, RJ and insular population SC3. Many divergent haplotypes, that leading to high nucleotide diversity were observed in population SP2, in this way, based on variation of partial ITS region, this is a possible center of genetic diversity of *P. piptadeniae*. This genetic center can be associated to historically stable regions, considering that is expected to observe higher values of genetic variation in historically stable populations (Avise 2000).

The genetic profile observed on ITS nuclear marker, reveal a interesting scenario, in which, the previous hypothesis of ecological predilection to dry woodlands postulated by Salvador-Montoya et al (2015) do not is supported. Predilection to environmental conditions imposed by SDTF formations, would imply in populations with high genetic diversity associated to historically stable region of these formation. However, paloedistribution models of SDTF stable regions (Werneck et al. 2011), indicate that region of historical climatic stability are located mainly on the Caatinga domain. The genetic profile of populations associated to Caatinga domain (PE1, PE2 and PE3), indicate poor genetic variation, thereby contradicting the SDTF predilection hypothesis.

Additionally, the pattern of genetic variation observed in this study, has been founded on several Atlantic Forest associated species of animals (Grazziotin et al. 2006; Cabanne et al. 2007; Fitzpatrick et al. 2009; Martins et al. 2009; D'Horta et al. 2011; Batalha-Filho et al. 2012). In this way, the hypothetical association of *P. piptadeniae* to Atlantic Forest domain, represent an alternative scenario to previous postulated by Salvador-Montoya et al (2015). Atlantic Forest climatic stable regions under Carnaval and Moritz (2008) paloedistribution model, are possibly appropriate to explain about the natural history of *P. piptadeniae*.

On the other hand, the common host of the genus *Piptadenia* shared between CP and AFCP, and additionally the three species of *Phellinotus* that sympatrically living on Caatinga domain (Drechsler-Santos et al. 2016; Elias et al. 2017, unpublished data), points out that the Caatinga domain represents a diversification center of the *Phellinotus* lineage, with posterior diversification of *P. piptadeniae* driven by the host association.

A similar case was registered in the fly-agaric *Amanita muscaria* (L.) Lam. (Gem et al. 2006). The historical dispersal pattern of the fly-agaric involves an initial speciation of three lineages, which sympatrically occur in Siberian–Beringian region, follows by worldwide dispersal and the development of ecoregional endemic lineages (Gem et al. 2006). Additionally, examples of host-driven speciation can be observed in some cases of wood-decay polypores (*Gloeoporus taxicola*, Seierstad et al. 2013) and human associated fungi (the genus *Paracoccidioides*, Theodoro et al. 2012), and entomopathogenic fungus (*Metarhizium flavoviride*; Keyser et al. 2015).

In conclusion, our findings provide evidences that host association can partially drives population dynamics of the studied associated fungus. However, since this is a preliminary results based on partial ITS sequences, more detailed studies including another molecular markers of nuclear and mitochondrial genomes, and an extensive field sampling, should be done to test this hypothesis.

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Table 1. Specimens used in this study. Longitude and Latitude coordinates are presented in decimal degrees. * before the voucher identification indicate a new record to respective locality.

Pop (Locality)/Voucher	Long	Lat	Host
Phellinotus piptadeniae (Teixeira) Drechsler-Santos & Robledo			
SC1 (Brazil, SC, Criciúma)			
*SGE_117	-49,359590	-28,689340	*Piptadenia gonoacantha*
*SGE_113	-49,358620	-28,688920	*Piptadenia gonoacantha*
*SGE_115	-49,359620	-28,689140	*Piptadenia gonoacantha*
*SGE_111	-49,358670	-28,688480	*Piptadenia gonoacantha*
SC2 (Brazil, SC, Tubarão)			
AGS_48 (FLOR 51451)	-49,032320	-28,485414	*Piptadenia gonoacantha*
SC3 (Brazil, SC, Florianopolis)			
SGE_127	-48,479250	-27,595417	*Piptadenia gonoacantha*
SGE_126	-48,479222	-27,595333	*Piptadenia gonoacantha*
SGE_124	-48,474583	-27,590278	*Piptadenia gonoacantha*
SGE_125	-48,478111	-27,593444	*Piptadenia gonoacantha*
SGE_123	-48,475889	-27,591111	*Piptadenia gonoacantha*
MABS_136 (FLOR 39574)	-48,549200	-27,596700	*Piptadenia gonoacantha*
MABS_135 (FLOR 39573)	-48,549200	-27,596700	*Piptadenia gonoacantha*
MABS_106 (FLOR 39571)	-48,549200	-27,596700	*Piptadenia gonoacantha*
PR (Brazil, PR, Maringá)			
*SGE432	-51,932007	-23,426426	*Piptadenia gonoacantha*
*SGE417	-51,932007	-23,426426	*Piptadenia gonoacantha*
*SGE406	-51,938746	-23,404635	*Piptadenia gonoacantha*
*SGE444	-51,932007	-23,426426	*Piptadenia gonoacantha*
SP1 (Brazil, SP, Caraguatatuba)			
*SGE_226	-45,429810	-23,596850	*Piptadenia gonoacantha*
*SGE_222	-45,429200	-23,595860	*Piptadenia gonoacantha*
*MAR_1178_16	-45,429623	-23,593581	*Piptadenia gonoacantha*
*SGE_225	-45,429810	-23,596640	*Piptadenia gonoacantha*
*SGE_243	-45,429805	-23,595831	*Piptadenia gonoacantha*
*SGE_224	-45,429730	-23,596700	*Piptadenia gonoacantha*
*SGE_245	-45,429737	-23,595862	*Piptadenia gonoacantha*
*SGE_242	-45,429607	-23,596028	*Piptadenia gonoacantha*
*SGE_221	-45,429190	-23,595860	*Piptadenia gonoacantha*
*SGE_220	-45,428640	-23,592760	*Piptadenia gonoacantha*
SP2 (Brazil, SP, Botucatu)			
MF_043	-48,429889	-22,836417	*Piptadenia gonoacantha*
MF_032a	-48,426250	-22,836417	*Piptadenia gonoacantha*
MF_046	-48,426250	-22,836417	*Piptadenia gonoacantha*
MF_031	-48,426250	-22,836417	*Piptadenia gonoacantha*
MF_044	-48,424889	-22,837056	*Piptadenia gonoacantha*
MF_040	-48,425028	-22,837444	*Piptadenia gonoacantha*
MF_035	-48,429306	-22,836972	*Piptadenia gonoacantha*

MF_037	-48,430056	-22,836944	*Piptadenia gonoacantha*
RJ (Brazil, RJ, Rio de Janeiro)			
*SGE349	-43,252902	-22,972153	*Piptadenia gonoacantha*
*SGE348	-43,251111	-22,972687	*Piptadenia gonoacantha*
*SGE353	-43,250617	-22,972776	*Piptadenia gonoacantha*
*SGE354	-43,252687	-22,972390	*Piptadenia gonoacantha*
*SGE350	-43,249703	-22,973318	*Piptadenia gonoacantha*
*SGE253	-43,250057	-22,973012	*Piptadenia gonoacantha*
*MAR_836_14	-43,252458	-22,957049	*Piptadenia gonoacantha*
DF (Brazil, DF, Brasilia)			
*SGE385	-47,884307	-15,740762	*Piptadenia gonoacantha*
*SGE386	-47,886101	-15,740958	*Piptadenia gonoacantha*
*SGE387	-47,886031	-15,740957	*Piptadenia gonoacantha*
*SGE389	-47,886031	-15,740957	*Piptadenia gonoacantha*
*SGE388	-47,885253	-15,740742	*Piptadenia gonoacantha*
*SGE390	-47,886765	-15,741583	*Piptadenia gonoacantha*
PE1 (Brazil, PE, Serra Talhada)			
DS139PE (URM 80768)	-38,301389	-7,900278	*Piptadenia sp.*
DS97PE (URM 80360)	-38,304722	-7,891389	*Mimosa sp.*
PE2 (Brazil, PE, Barro Branco)			
DS300 (URM 80595)	-37,247176	-8,592380	*Piptadenia stipulacea*
PE3 (Brazil, PE, Caruaru)			
DS110PE (URM 80345)	-35,920000	-8,231389	*Senegalia sp.*
DS109PE (URM 80322)	-35,920000	-8,231389	*Mimosa sp.*
DS163PE (URM 80766)	-35,920556	-8,230556	*Mimosa sp.*
DS128PE (URM 80361)	-35,920278	-8,230833	*Senegalia sp.*
Phellinotus teixeirae Salvador-Montoya, Galvão-Elias & Drechsler-Santos			
AR (Argentina, Corrientes, Corrientes)			
*Popoff_Dichtiar_34J	-58,829640	-27,473137	
PERU (Perú, Piura, Piura)			
CS 457b (FLOR 16945)	-80,664234	-5,184876	*Libidibia glabrata*
CS_377 (FLOR 7554)	-80,664234	-5,184876	*Pithecellobium excelsum*
CS 454b (FLOR 16944)	-80,664234	-5,184876	*Libidibia glabrata*
SE (Brazil, SE, Niterói)			
DS44PE (URM 80403)	-37,462500	-9,755000	
PE (Brazil, PE, Buíque)			
DS257 (URM 80889)	-37,247176	-8,592380	*Pityrocarpa moniliformis*
DS108 (URM 80636)	-37,247176	-8,592380	*Piptadenia* sp.

* collections performed in this study.

Table 2. Polymorphic sites in the collapsed haplotypes. Hyphen (-) indicate indel position.

Haplotype	Sequence												
Hap_1	T	C	C	G	G	T	A	G	T	T	-	T	C
Hap_2	.	.	.	.	.	.	G	.	.	.	-	.	.
Hap_3	C	.	T	.	.	.	G	.	.	.	-	.	.
Hap_4	C	.	.	.	.	.	G	.	.	.	-	.	.
Hap_5	.	.	.	A	.	.	G	.	.	.	-	.	.
Hap_6	.	.	.	.	A	.	G	.	.	.	C	.	.
Hap_7	.	.	T	.	.	.	G	.	.	.	-	.	.
Hap_8	C	T	T	.	.	.	G	.	.	.	-	.	.
Hap_9	C	.	.	.	.	.	G	.	.	.	-	.	A
Hap_10	.	.	.	.	A	.	G	.	.	.	-	.	.
Hap_11	C	.	T	.	.	C	G	.	.	C	-	.	.
Hap_12	.	.	.	.	.	.	G	T	C	.	-	.	.
Hap_13	.	.	.	.	.	.	.	.	.	.	-	C	.

Table 3. Genetic cluster identified in the Structure analysis. Bold values indicate high probability to locality belong to respective k group. N, number of specimens sampled in the population, h, number of haplotypes, and Pi, nucleotide diversity.

Population	K1	K2	K3	K4	N	h	Pi
SC1	0,024	0,296	**0,665**	0,015	8	3	0,0833
SC2	0,032	0,046	**0,884**	0,037	2	1	0,0000
SC3	0,013	**0,526**	**0,448**	0,012	16	4	0,0736
PR	0,007	0,000	**0,993**	0,000	8	2	0,0417
SP2	0,016	**0,860**	0,109	0,015	18	7	0,1765
SP1	0,012	0,066	**0,912**	0,011	20	4	0,0811
RJ	0,016	0,141	**0,830**	0,013	14	5	0,0815
DF	0,020	0,070	**0,892**	0,019	12	2	0,0884
PE1	0,023	0,023	0,474	**0,480**	4	2	0,0556
PE2	0,016	0,017	0,040	**0,927**	2	1	0,0000
PE3	0,012	0,014	0,029	**0,944**	8	1	0,0000
Outgroup	**0,974**	0,007	0,012	0,007	-	-	-

Table 4. Summary of Analysis of Molecular Variance outputs from the three models includes in this analysis. Groups included in each model are closed between parentheses. df = degrees of freedom, SQ = sum of squares, F_{OBS} = F statistic observed, H_0 = null hypothesis of the permutation test.

AMOVA Model/Source of variation	df	SQ	Var. Component	% var.	F_{OBS}	H_0	Sign.
Model 1 (SC3,SP2) | (SC1,SC2,SP1,RJ,PR,DF) | (PE1,PE2,PE3)							
Among groups	2	9,526	0,13513	32,06	0,3206	FCT(Rd>=Obs.)	0,0009
Among populations Within groups	8	3,549	0,0187	4,44	0,0653	FSC(Rd>=Obs.)	0,0001
Within populations	101	27,032	0,26764	63,50	0,3650	FST(Rd<=Obs.)	0,0008
Total	111	40,107	0,42147				
Model 2 (SP2) | (SC1,SC2,SC3,SP1,RJ,PR,DF) | (PE1,PE2,PE3)							
Among groups	2	9,251	0,16251	35,88	0,3588	FCT(Rd>=Obs.)	0,0009
Among populations Within groups	8	3,824	0,02278	5,03	0,0784	FSC(Rd>=Obs.)	0,0089
Within populations	101	27,032	0,26764	59,09	0,4091	FST(Rd<=Obs.)	0,0000
Total	111	40,107	0,42147				
Alternative Model (SC1,SC2,SC3,SP1,SP2,RJ,PR,DF) | (PE1,PE2,PE3)							
Among groups	1	5,74	0,20769	39,23	0,3923	FCT(Rd>=Obs.)	0,0059
Among populations Within groups	9	7,336	0,05403	10,21	0,1680	FSC(Rd>=Obs.)	0,0000
Within populations	101	27,032	0,26764	50,56	0,4944	FST(Rd<=Obs.)	0,0000
Total	111	40,107	0,52936				

Table 5. Summary statistics for ITS of *P. piptadeniae*.genetic analysis.

n	h	Pi	hd	D		Fs		R2	
56	12	0,1029	0,722	-1,19	ns	-4,68	*	0,16	***

ns = $p > 0.05$, * = $p < 0.05$, *** = $p < 0.001$, n = Number of individuals, h = Haplotype number, hd = Haplotype diversity, D = Tajima's test, Fs = Fu's test, R2 = Ramos-Onsins & Rozas's test

Figure 1. Kernel Density map based in *P. gonoacantha* distribution. Orange regions indicate high density regions of population at 5km of influence radius. Green rhombus indicates specimens published by Salvador-Montoya et al (2015) and yellow stars indicate collections performed by Teixeira (1950).

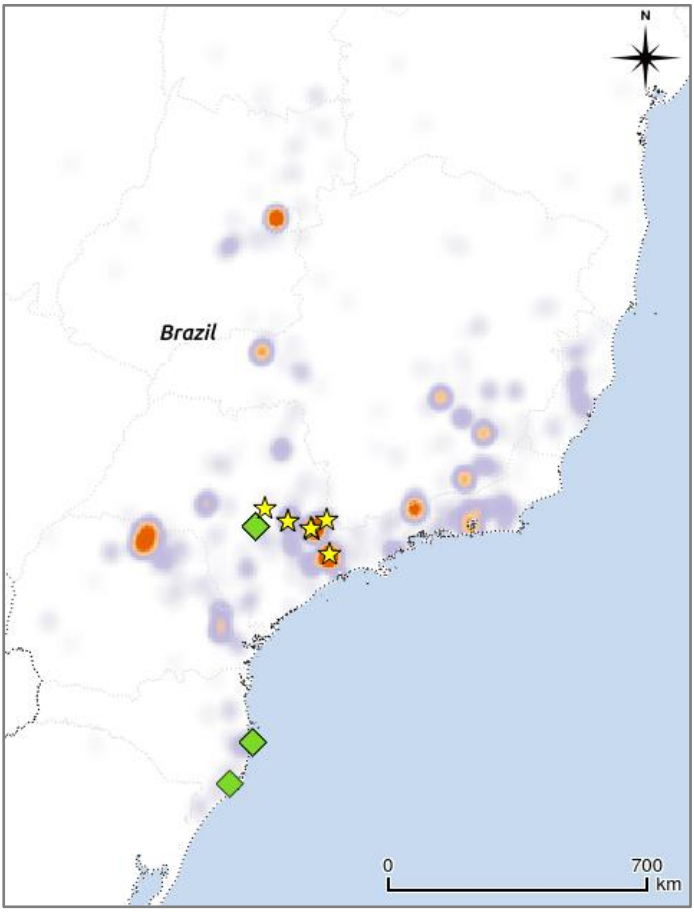


Figure 2. The geographical distribution of the estimated haplotypes (A), the size of the color in each circle represents your relative frequency on the sampled populations. The haplotype-network for ITS (B), each circle represents a unique haplotype and the size represents a relative frequency of the haplotype. In the figure B asterisks (*) indicate the haplotype exclusive of the locality, and when colored of grey indicate a singleton haplotype. Hatched area indicate SDTF potential distribution sensu Särkinen et al. (2011).

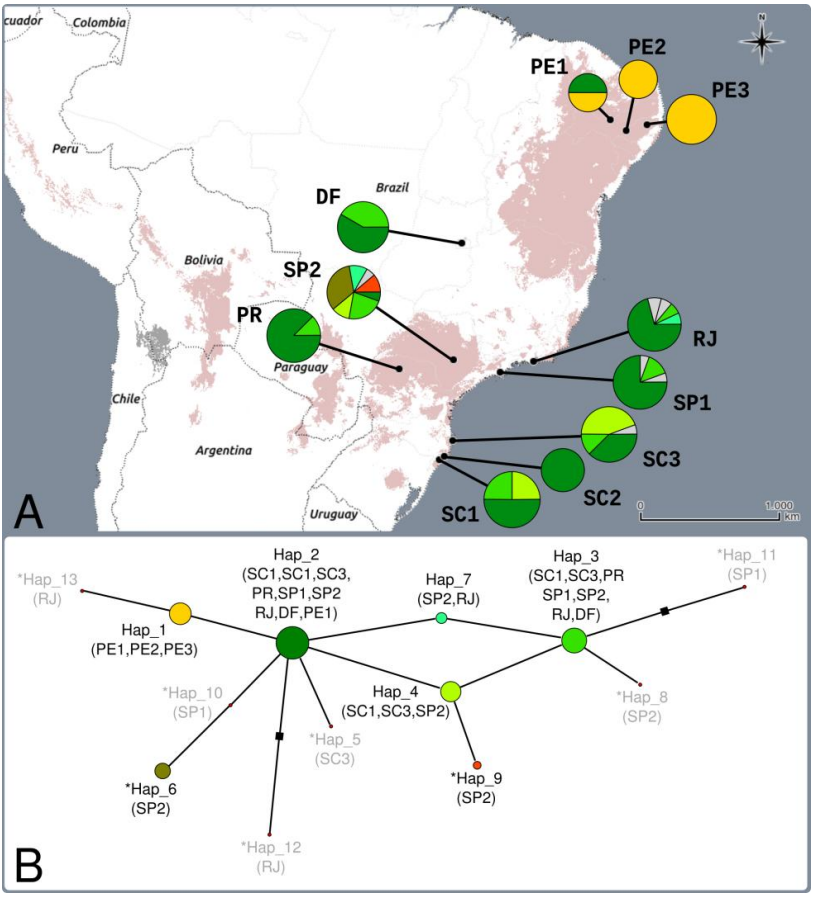
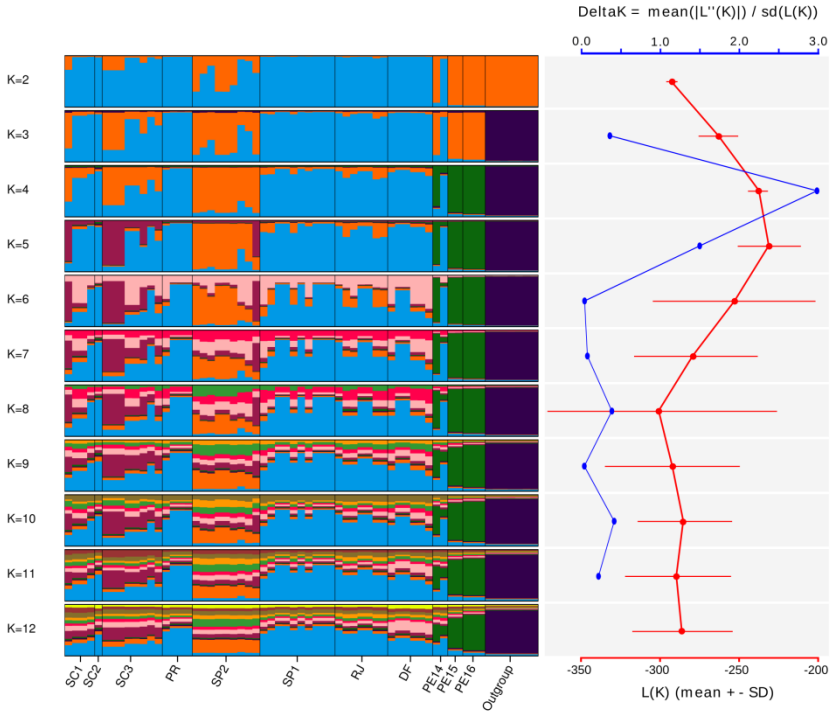


Figure 3. Results of Bayesian cluster analysis. Each of the 53 individuals included in the analysis is represented by a vertical bar. The x axis of the colored box (the left side of the figure) indicate the populations that each individual belong, and y axis the k group included in the analysis. The line chart (right side of the figure) indicate the Likelihood score (red line and scale bar) of the respective k-group tested and the DeltaK statistic (blue line and scale bar) for the respective group.



6 CONSIDERAÇÕES FINAIS E RECOMENDAÇÕES PARA TRABALHOS FUTUROS

Com a adição das duas novas espécies descritas na presente dissertação, *Phellinotus* passa a incluir quatro espécies. Excetuando-se *P. magnoporatus*, as espécies desse gênero associam-se a diversos gêneros de leguminosas.

Considerando a possível ocorrência/prevalência de *Phellinotus teixeirae* sp. nov. Ad int. nos bosques secos da América do Sul, novas expedições de campo devem ser realizadas, assim como a revisão de coleções de herbário, devem ser feitas com a finalidade de identificar a real distribuição geográfica da espécie com base em coletas realizadas nessas regiões.

A distribuição geográfica da leguminosa *P. gonoacantha* representa um bom preditor da distribuição geográfica de *Phellinotus piptadeniae* na Mata Atlântica e no Cerrado, desse modo, na Caatinga, a distribuição geográfica dos demais hospedeiros (*Piptadenia* spp., *Senegalia* spp. e *Minosa* spp.) poderia ser utilizada na predição das regiões de potencial ocorrência do fungo.

Apesar de *P. piptadeniae* estar altamente associado a *P. gonoacantha* e outras espécies de leguminosas, a identificação dos mecanismos de interação entre fungo-planta ainda carecem de ser descritos. Possivelmente estudos anatômicos proveriam importantes informações acerca desses mecanismos.

No presente estudo apenas parte da região ITS foi utilizada nas inferências filogeográficas de *P. Piptadeniae*. Desse modo, a inclusão de mais marcadores moleculares, pertencentes aos genômas nucleares e mitocondriais, torna-se necessária para elucidar o provável histórico

evolutivo de *P. piptdeniae*. Igualmente, informações filogeográficas dos hospedeiros representam importantes evidências.

Considerando a baixa variabilidade genética observada no marcador mitocondrial ATP6 (dados não apresentados), outros marcadores mitocondriais devem ser testados.

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