Melissa Palacio Pulgarín

ESTUDOS TAXONÔMICOS E FILOGENÉTICOS DO COMPLEXO POLYPORUS DICTYOPUS MONT. (POLYPORACEAE, BASIDIOMYCOTA)

Dissertação submetida ao Programa de Graduação Biologia Pós em de Fungos, Algas e Plantas da Universidade Federal de Santa Catarina para a obtenção do Grau de mestre em Biologia de Fungos, Algas e Plantas.

Orientador: Prof. Dr. Elisandro Ricardo Drechsler dos Santos. Coorientador: Dr. Gerardo Lucio Robledo.

Florianópolis

2016

Ficha de identificação da obra elaborada pelo autor, através do Programa de Geração Automática da Biblioteca Universitária da UFSC.

Palacio, Melissa Estudos Taxonômicos e Filogenéticos do complexo Polyporus dictyopus Mont. (Polyporaceae, Basidiomycota) / Melissa Palacio ; orientador, Elisandro Ricardo Drechsler Santos ; coorientador, Gerardo Lucio Robledo. -Plorianópolis, SC, 2016. 63 p.

Dissertação (mestrado) - Universidade Pederal de Santa Catarina, Centro de Ciências Biológicas. Programa de Pós Graduação em Biologia de Pungos, Algas e Plantas.

Inclui referências

 Biologia de Fungos, Algas e Plantas. 2. Sistemática.
 Filogenética . 4. Polyporus 5. Fungos poliporoides. I. Drechsler-Santos , Elisandro Ricardo. II. Robledo, Gerardo Lucio. III. Universidade Pederal de Santa Catarina. Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas. IV. Título.

"Estudos taxonômicos e filogenéticos do complexo Polyporus dictyopus Mont. (Polyporaceae, Basidiomycota)"

por

Melissa Palacio Pulgarín

Dissertação julgada e aprovada em sua forma final pelos membros titulares da Banca Examinadora (Port. 05/PPGFAP/2016) do Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas - UFSC, composta pelos Professores Doutores:

1	HU S
Prof. Dr. Elisar (Pr	ndro Ricardo Drechsler dos Santos residente/CCB/UFSC)
C	C-1.+ Colle
Prof. D	r. Gilberto Coelho (UFSM)
file	realially
Profa. Dra. Mar	ria Alice Neves (BOT/CCB/UFSC)
Wayara Ka	osi mala: Cooldal
ON	Λ Λ \prec
Profa, Dra. Mai	ria Alice Neves (BOT/CCB/UFSC) oxi mlai C.cddah a Krasinski Caddah (BOT/CCB/UFSC)

1

Florianópolis, 01 de abril de 2016.

AGRADECIMENTOS

A Silvia, Fernando y Mario, mi família, por el incondicional y desproporcionado amor, por ser siempre el motor de todo, por estar siempre presentes y animándome en cada paso, por las palabras, abrazos, cariño y afecto de siempre.

A Ricardo y Gerardo, mis profesores, gracias infinitas por acogerme como su estudiante, por mostrarme la pasión por la micología, por su paciencia y cariño al enseñarme, por su amistad, por permitirme hacer parte de sus caminos.

A Mateus por la inagotable paciencia y disponibilidad para enseñarme, y principalmente por su amistad.

Al MICOLAB, a mis compañeros y amigos por las enseñanzas de todos los días, por las risas, las salidas, los abrazos, por la amistad, por el amor! a los que ya no están en el laboratorio Alti, Jaime, Fer, Caio, Gesi, Ari, Diogo, Cele, Salo, Raque, Carlos, a los que están ahora MariD, Maruquita, Fê, Mary, Pam, Cauê, Ba, Marília, Lina, Duda, Samuel, Gustavo, Felipe y Genis por tantos días lindos en el laboratorio, a todos, especialmente a Maria Alice por hacer de un laboratorio una segunda familia.

A Mari, Ellie, Thais, Gesi y Mayara por el apoyo y amor desde el primer día de clases, por hacerme parte de sus vidas y familias, por darme siempre la mano en los momentos que más lo necesité, por su amistad. A Gra, Ana, Lu, Ba, Gabi, Deb y Tainá, las chicas de la Fenda do Bikini, por el cariño y paciencia.

A mis amigas de Colombia, mis amores Lau, Val, Eli, Yossa, Mari y Nata, siempre presentes.

A Ricardo Callejas mi mejor amigo y maestro, por su apoyo y afecto siempre.

A todos mis colegas de maestría, profesores, a la secretaría de PPGFAP. A CAPES via PPGFAP-UFSC por la beca que permitió que este trabajo fuera realizado, a Aristóteles Góes Neto y el proyecto "Indentificação Molecular de Fungos do Brasil" por proporcionar los medios para realizar los análisis filogenéticos.

A todos un millón de gracias!

RESUMO

Polyporus dictyopus é um táxon com ampla variação morfológica caracterizada principalmente por apresentar basidiomas estipitados, com uma cutícula negra no estipe, a superfície do píleo vinácea, castanho a castanho amarelada, himenóforo poroide, sistema hifal dimítico, com hifas esqueleto-ligadoras e basidiósporos cilíndricos a elipsoides, hialinos de parede fina e lisa. Polyporus dictyopus é causador de podridão branca, apresenta uma distribução pantropical e reune pelo menos 16 sinônimos heterotípicos, propostos a partir de materiais coletados na América. Revisões taxonômicas de P. dictyopus, a partir de estudos morfológicos, já foram realizadas. No entanto, a hipótese de que este táxon represente um complexo de espécies filogenéticas delimitadas ainda não foi testada. Este trabalho apresenta análises macro e micromorfológicas detalhadas, assim como filogenéticas moleculares de materiais previamente identificados como P. dictyopus. Ao todo, foram revisados 45 espécimes, incluindo os tipos de alguns sinônimos. Foram obtidas 62 sequências (ITS, LSU e RPB2), sendo 32 de materiais do complexo P. dictyopus, e 30 de táxons relacionados. Os resultados das análises filogenéticas revelam que as amostras identificadas como P. dictyopus constituem dois clados independentes, correspondentes aos gêneros aqui tratados taxonomicamente: Atroporus e Neodictyopus gen. nov. ad int. Além disso, Neodictvopus atlanticus sp. nov. ad int., N. gugliottae sp. nov. ad int., N. dictyopus comb. nov. ad int., e A. rufoatratus comb. nov. ad int. são apresentados. Descrições detalhadas, ilustrações e uma chave são apresentadas para as espécies de Atroporus e Neodictyopus. Considerando os resultados obtidos nesse estudo, fica claro que reavaliações de outros grupos morfológicos e de complexos de espécies tradicionalmente tratados em Polyporus são necessárias para uma classificação menos artificial, inclusive do próprio gênero.

Palavras-chave: Polyporus, Polyporus dictyopus, estudo de tipos, taxonomia, filogenia, fungos degradadores de madeira.

ABSTRACT

Polyporus dictyopus is a taxon with a wide morphological variation, characterized by stipitate basidiomata, with a black cuticle on the stipe, a vinaceous, brown to yellowish brown pilear surface, poroid hymenophore, dimitic hyphal system, with skeletal-binding hyphae, cylindrical to ellipsoid, hyaline, thin-walled and smooth basidiospores. Polyporus dictyopus causes white root, presents pantropical distribution and at least sixteen heterotypic synonyms were described based on samples from America. Taxonomic revisions of P. dictyopus from morphological studies have already been carried out. However, the hypothesis that this taxon is a complex of phylogenetic species has not been tested yet. This study presents detailed macro- and micromorphological analysis and phylogenetic analysis with specimens previously identified as P. dictyopus. Around 45 specimens, including some types specimens, were examined. About 62 sequences (ITS, LSU, and RPB2) were achiev9ed, 32 of P. dictyopus complex, and 30 of related taxa. The results of the phylogenetic analysis revealed that specimens identified as *P. dictyopus* constitute two independent clades, corresponding to the genera here examined taxonomically: Atroporus and Neodictyopus gen. nov. ad int. Furthermore, Neodictyopus atlanticus sp. nov. ad int., N. gugliottae sp. nov. ad int., N. dictyopus comb. nov. ad int., and A. rufoatratus comb. nov. ad int. are presented. Detailed descriptions, illustrations and a key are prrovided for Atroporus and *Neodictyopus* species. Considering the results obtained in this study it is clear that revisions of other morphological groups and species complexes traditionally treated in Polyporus are needed for a more natural classification, even the genre itself.

Keywords: Polyporus, Polyporus dictyopus, type study, taxonomy, phylogeny, wood decaying fungi.

SUMÁRIO

1. INTRODUÇÃO	9
2. OBJETIVOS	12
2.1 Objetivo Geral	12
2.2 Objetivos Específicos	12
3. MATERIAL E MÉTODOS	13
3.1 Coleções	13
3.2 Análises Morfológicas	13
3.3 Análises Moleculares	14
4. RESULTADOS E DISCUSSÃO	15
Capítulo I	17
5. CONSIDERAOES FINAIS	61
REFERÊNCIAS	62

1. INTRODUÇÃO

Polyporaceae Fr. ex. Corda (Polyporales, Basidiomycota) é uma família de ampla distribuição geográfica e ampla variação morfológica. Apresenta espécies principalmente lignícolas causadoras de podridão branca, com basidiomas sazonais a perenes, ressupinados a pileados e estipitados, himenóforo tubular a lamelar, e basidiósporos globosos a alantoides (Kirk & Cannon 2008). Polyporus Micheli ex Adans, gênero tipo da família, é caracterizado por apresentar espécies com basidiomas pileados, central à excentricamente estipitados, himenóforo tubular, cuja superfície inferior se torna poroide, sistema hifal dimítico com hifas esqueleto-ligadoras e basidiósporos cilíndricos a elipsoides, hialinos, de parede fina e lisa. Polyporus compreende espécies saprófitas (raramente parasitas ex. P. rhizophilus Pat.) que crescem em troncos mortos de angiospermas principalmente (Nuñez & Ryvarden 1995), degradando a lignina, celulose e hemicelulose do substrato (Ejechi et al 1996). Existem poucas espécies com hospedeiros específicos, como por exemplo, P. gavanus Lév. e P. melanopus (Pers.) Fr., que crescem em madeira de Nothofagus Blume (Nuñez & Ryvarden 1995, Silveira & Wrigth 2005). A grande maioria das espécies do gênero é generalista com respeito ao substrato. De acordo com a literatura, apresenta uma distribuição cosmopolita (Nuñez & Ryvarden 1995), com espécies cuja ocorrência já foi registrada para África (Ryvarden & Johansen 1980), Europa (Ryvarden & Gilbertson 1993), Ásia (Nuñez & Ryvarden 2001), América do Norte (Gilbertson & Ryvarden 1987), Central (Carranza & Ruiz-Boyer 2005) e do Sul (Silveira & Wright 2005).

Com respeito à morfologia, *Polyporus* apresenta uma ampla variação e, tradicionalmente, tem sido dividido em grupos morfológicos infragenéricos, que variam de acordo com diferentes autores (Ryvarden & Johansen 1980, Nuñez & Ryvarden 1995, Silveira & Wright 2005). A classificação mais seguida atualmente inclui seis grupos morfológicos infragenéricos sem uma categoria taxonômica definida: "Admirabilis", "Dendropolyporus" [= Dendropolyporus (Pouzar) Jülich], "Favolus" (= Favolus Fr.), "Polyporellus" (= Polyporellus P. Karst.), "Melanopus" (= Melanopus Pat.) e "Polyporus" (Nuñez & Ryvarden 1995). Recentes análises filogenéticas revelaram que os grupos morfológicos não representam grupos naturais e que *Polyporus* é, portanto, polifilético (Krüger et al. 2006, Sotome et al. 2008, 2011, Dai et al 2014, Seelan et al. 2015).

Alguns destes grupos infragenéricos têm sido reconhecidos como gêneros independentes, mas de modo geral, são atualmente considerados sinônimos de *Polyporus*. Em particular, os grupos Favolus e Melanopus receberam uma maior atenção em estudos recentes (Krüger et al. 2006, Sotome et al. 2011, Sotome et al. 2013, Dai et al. 2014). Em um destes trabalhos, baseado em análises filogenéticas e morfológicas, o gênero *Favolus* Fr. foi resgatado e recircunscrito, e *Neofavolus* Sotome & T. Hatt. foi segregado como um novo gênero independente (Sotome et al. 2013).

Por outro lado, o grupo Melanopus, que é caracterizado tradicionalmente por apresentar espécies com basidiomas coriáceos e cutícula preta no estipe (Nuñez & Ryvarden 1995), a partir de análises filogenéticas (ITS e LSU) mostrou-se como um grupo artificial (Dai et al. 2014). Enquanto algumas espécies [P. admirabilis Peck, P. americanus Vlasák & Y.C. Dai, P. austroandinus Rajchenb. & Y.C. Dai, P. badius (Pers.) Schwein., P. conifericola H.J. Xue & L.W. Zhou, P. fraxineus (Bondartsev & Ljub.) Y.C. Dai, P. melanopus (Pers.) Fr., P. rhizophilus (Pat.) Sacc., P. submelanopus H.J. Xue & L.W. Zhou, P. taibaiensis Y.C. Dai, P. tubaeformis (P. Karst.) Ryvarden & Gilb.] ficaram agrupadas em um clado ("melanopus clade"), sem categoria taxonômica definida, outras como P. leprieurii Mont., P. guianensis Mont. and P. dictyopus Mont. não estão relacionadas filogeneticamente (Dai et al. 2014). Para P. leprieurii e P. guianensis é possível hipotetizar que por apresentarem píleos que variam de cor marrom pálido a bege estariam distantemente relacionados ao "melanopus clade". O que não é possível fazer com P. dictyopus, já que, de modo geral, compartilha os mesmos caracteres das espécies do clado melanopus.

Polyporus dictyopus foi descrita por Montagne em 1835 na "Flora Fernandesiana", a partir de espécimes coletados na Ilha Juan Fernández, localizada na costa pacífica do Chile, tendo como características principais a presença de uma cutícula negra e superfície reticulada no estipe. Juntamente com outras 11 espécies [*P. badius, P. blanchettianus* Berk. & Mont., *P. diabolicus* Berk., *P. doidgeae* Wakef., *P. guianensis*, *P. hemicapnodes* Berk. & Broome, *P. infernalis* Berk., *P. leprieurii, P. melanopus, P. varius* (Pers.) Fr., *P. virgatus* Berk. & M.A. Curtis] forma um grupo morfológico ("P. dictyopus group"), que compartilha a cutícula negra na superfície do estipe (Ryvarden & Johansen 1980). Posteriormente, *P. dictyopus* foi acomodado no grupo melanopus (Nuñez & Ryvarden 1995) e neste táxon foram sinonimizados aproximadamente 25 táxons (Nuñez & Ryvarden 1995). Posteriormente, a partir de uma revisão taxonômica de espécies de *Polyporus* com ocorrência na América do Sul, Silveira & Wright (2005) confirmam a ocorrência de *P. dictyopus* na Argentina, Bolívia, Brasil, Chile, Cuba, Guayana Francesa, Guiana, Panamá, Paraguai e Venezuela, segundo os conceitos morfológicos e mantendo os sinônimos apresentados por Nuñez & Ryvarden (1995). Alguns destes sinônimos heterotípicos apresentam uma distribuição geográfica disjunta e por si só são macro e micro-morfologicamente muito variáveis. Com a sinonimização de pelo menos 16 táxons que foram propostos a partir de amostras da América, a presença de rizomorfos (originalmente descrita em *P. rhizomorpha* Mont.) e de elementos setoides dextrinoides (originalmente descritos em *P. infernalis* Berk. e *P. diabolicus* Berk.) é agora considerado na circunscrição de *P. dictyopus*.

Baseado na revisão de tipos de *P. diabolicus* e *P. infernalis* foi feita a proposição do gênero *Atroporus* Ryvarden (Ryvarden 1973), caraterizado por apresentar cistídios com protuberâncias e ápices pontiagudos. Posteriormente, com a reinterpretação dos cistídios como sendo modificações das hifas esqueleto-ligadoras, *Atroporus diabolicus* Berk. ($\equiv P. diabolicus$) e *A. infernalis* Berk. ($\equiv P. infernalis$) também foram novamente sinonimizados em *P. dictyopus*, consequentemente o gênero *Atroporus* foi também sinonimizado em *Polyporus* (Nuñez & Ryvarden 1995).

Polyporus dictyopus tem sido apontado como um complexo taxonômico, com base na morfologia (Nuñez & Ryvarden 1995, Nuñez & Ryvarden 2001) e estudos de *mating type* (Nuñez & Ryvarden 2001). No entanto, até o presente, nenhum estudo filogenético foi feito para testar essa hipótese e consequentemente resolver o hipotético complexo taxonômico. Sendo assim, um tratamento taxonômico consistente, considerando a morfologia detalhada e análises filogenéticas a partir de marcadores moleculares, poderiam contribuir para a delimitação taxonômica de *P. dictyopus* s.s., de espécies relacionadas e táxons envolvidos e de suas posições taxonômicas dentro de *Polyporus*.

2. OBJETIVOS

2.1 OBJETIVO GERAL

Revisar o conceito de *Polyporus dictyopus* através de estudos morfológicos e moleculares de espécimes da região neotropical

2.2 OBJETIVOS ESPECÍFICOS

- Revisar macro e micromorfologicamente espécimes morfologicamente determinados como *P. dictyopus*, assim como espécimes tipo e de refência, incluindo dos sinônimos heterotípicos e espécies relacionadas;
- Realizar coletas de espécimes, morfologicamente relacionados a *P. dictyopus* na região Neotropical, principalmente em ecossistemas do território brasileiro;
- Obter e disponibilizar sequências das regiões ITS, LSU e RPB2 dos materiais coletados;
- Construir hipóteses filogenéticas das espécies, a partir de análise moleculares, levando em consideração as informações morfológicas;
- Descrever e divulgar as novidades científicas.

3. MATERIAL E MÉTODOS

3.1 COLEÇÕES

Foram estudados espécimes de *P. dictyopus* e de algumas espécies relacionadas coletados na Amazônia, Cerrado e Mata Atlântica do Brasil e Argentina. Também foram analisados materiais tipo e de referência depositados nos herbários BPI, CORD, FLOR e NY. Os materiais coletados foram desidratados por 12 a 24 horas em estufa (30° a 35°C) e armazenados em sacolas plásticas para posteriores análises morfológicas. Para as análises moleculares foram desidratados com sílica pequenos fragmentos dos basidiomas em *eppendorfs*, a fim de preservar o DNA. Os especímes coletados foram depositados nos herbários CORD e FLOR. Os espécimes estudados são listados na Tabela I. Os acrônimos dos herbários seguem a base de dados *Index Herbariorum* (Thiers, atualizado continuamente).

3.2 ANÁLISES MORFOLÓGICAS

Para as descrições macroscópicas, foram observadas e anotadas informações sobre hábito, sazonalidade, tamanho e coloração da superfície superior, himenóforo, estípite, contexto e tubos. Assim também foram anotadas as informações do hospedeiro/substrato como possível identificação e condição viva ou morta. Os códigos de cores (ex. 5YR 8/4 a 7/3) seguiram a tabela de cores Soil Color Chart (Munsell, 1975).

Para as descrições microscópicas, foram realizados cortes a mão livre dos basidiomas (superficie do píleo, contexto, tubos e estipe) para o estudo do sistema hifal, elementos estereis, basídios e basidíosporos. Os cortes foram montados entre lâminas e lamínulas em KOH 2-3% (hidratante para observação da coloração das estruturas), Floxina 1% (corante para observar as hifas generativas, elementos do himênio e basidiósporos), reagente de Melzer, azul de Cresyl, ácido lático e azul de algodão (para observar as possíveis reações das hifas e basidiósporos). Também foram incubados (40°C) por 24-48 horas em NaOH 3% fragmentos do estipe, contexto e tubos. Posteriormente foram cuidadosamente dissecados em estereomicroscópio (Leica EZ4) segundo a metodologia de Decock (2010). A observação e mensuração (n=40) foram realizadas em microscópio óptico (Olympus CX21) com ocular micrométrica. Foram feitas pranchas ilustrativas a partir de desenhos e fotografias de materiais dos taxons aqui tratados.

3.3 ANÁLISES MOLECULARES/FILOGENÉTICAS

As análises moleculares serão apresentadas no item "Materials (pag. 21) no artigo "TAXONOMIC and methods" AND PHYLOGENETIC STUDIES OF THE POLYPORUS DICTYOPUS **NEOTROPICS:** RECOVERY **COMPLEX** IN THE OF **ATROPORUS** RYVARDEN AND **SEGREGATION** OF NEODICTYOPUS GEN. NOV." nos RESULTADOS E DISCUSSÃO.

4. RESULTADOS E DISCUSSÃO

Foram analisados morfologicamente 35 espécimes, produto de coletas realizadas para este trabalho, de coletas e nove da revisão de herbários (BPI e NY), provenientes de Amazônia, Cerrado e Mata Atlântica (Argentina e Brasil). Foram obtidas 62 sequências das regiões ITS, LSU e RPB2, 32 de amostras previamente determinadas como *P. dictyopus* e 30 de espécies de grupos relacionadas (*Echinochaete sp., Favolus brasiliensis* (Fr.) Fr., *Favolus sp., Mycobonia flava* (Sw.) Pat., *P. leprieurii, Polyporus sp.* e *P. tricholoma* Mont.). A lista completa das informações de sequências obtidas e localidades são apresentadas na Tabela I.

Nas análises filogenéticas, foram incluidas sequências geradas para este estudo de grupos relacionados a *Polyporus* como *Favolus*, *Mycobonia* Pat., "Polyporellus" e outros clados de *Polyporus s.l.* Algumas dessas amostras podem representar novidades científicas como *Favolus sp.* (DS1677 e DS1700) e *Polyporus sp.* (DS599), mas por não fazerem parte dos objetivos do estudo de *P. dictyopus*, não serão tratadas neste trabalho.

O tratamento taxonômico dos resultados, bem como a discussão são apresentados no formato de artigo científico.

TABELA I: lista das sequências (ITS, LSU e RPB2) geradas (**X**) neste trabalho a partir de amostras previamente determinadas como *P. dictyopus* (*) e de espécies relacionas.

Espécie	Espécime	Localidade	ITS	LSU	RPB2
Atroporus diabolicus	DS1266*	Amazonas, Brasil	Х	Х	-
	GAS679*	São Paulo, Brasil	Х	-	-
A. rufoatratus	DS1311*	Santa Catarina, Brasil	Х	Х	-
	DS816*	Santa Catarina, Brasil	Х	Х	Х
	LDA139*	Santa Catarina, Brasil	-	Х	-
	LDA140*	Santa Catarina, Brasil	-	Х	-
	MP153*	Santa Catarina, Brasil	Х	Х	-
Echinochaete sp.	DS1625	Pará, Brasil	Х	-	-
Favolus brasiliensis	DS1656	Pará, Brasil	Х	Х	-
Favolus sp. nov.	DS1677	Pará, Brasil	Х	Х	-
	DS1700	Pará, Brasil	Х	Х	-
Mycobonia flava	GAS625	Santa Catarina, Brasil	Х	Х	Х
Neodictyopus atlanticus	GAS622*	São Paulo, Brasil	Х	Х	Х
	G97*	Misiones, Argentina	Х	Х	-
N. gugliottae	DS1284*	Santa Catarina, Brasil	-	Х	-
	DS1285*	Santa Catarina, Brasil	Х	Х	Х
	DS1286*	Santa Catarina, Brasil	Х	Х	Х
	FB351*	Santa Catarina, Brasil	Х	Х	Х
N. dictyopus	GAS60*	Mato Grosso, Brasil	Х	Х	-
	GAS272*	Mato Grosso, Brasil	Х	Х	Х
	GAS281*	Mato Grosso, Brasil	Х	Х	Х
	VFL18*	Mato Grosso, Brasil	Х	-	-
Polyporus leprieurii	DS1581	Pará, Brasil	Х	Х	-
	DS1615	Pará, Brasil	Х	Х	-
	DS1696	Pará, Brasil	Х	Х	-
	MP154	Santa Catarina, Brasil	Х	Х	Х
	MP155	Santa Catarina, Brasil	Х	Х	Х
Polyporus sp.	DS599	Santa Catarina, Brasil	Х	X	-
P. tricholoma	DS1627	Pará, Brasil	Х	Х	-
			26	26	10
				Total	62

TAXONOMIC AND PHYLOGENETIC STUDIES OF THE POLYPORUS DICTYOPUS COMPLEX IN THE NEOTROPICS: RECOVERY OF ATROPORUS RYVARDEN AND SEGREGATION OF NEODICTYOPUS GEN. NOV.

Title

Taxonomic and phylogenetic studies of the *Polyporus dictyopus* complex in the neotropics: recovery of *Atroporus* Ryvarden and segregation of *Neodictyopus* gen. nov.

Melissa Palacio^{*}

Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas, Departamento de Botânica, Universidade Federal de Santa Catarina, Campus Universitário Trindade, CEP: 88040-900, Florianópolis, Santa Catarina, Brasil.

Gerardo Lucio Robledo^{*}

Instituto Multidisciplinario de Biología Vegetal, Universidad Nacional de Córdoba, C.C.495, 5000, Córdoba, Argentina. Fundación FungiCosmos, Av. General Paz 154, 4° piso, oficina 4, Córdoba, Argentina.

Mateus Arduvino Reck

Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas, Departamento de Botânica, Universidade Federal de Santa Catarina, Campus Universitário Trindade, CEP: 88040-900, Florianópolis, Santa Catarina, Brasil.

Emanuel Grassi

Laboratorio de Micología Experimental-Aplicaciones de Hongos ligninolíticos. Departamento de Biodiversidad y Biología Experimental. Facultad de Ciencias Exactas y Naturales Universidad de Buenos Aires - Ciudad Universitaria - Núñez - Ciudad Autónoma de Buenos Aires -Buenos Aires – Argentina.

Elisandro Ricardo Drechsler dos Santos

Programa de Pós-Graduação em Biologia de Fungos, Algas e Plantas, Departamento de Botânica, Universidade Federal de Santa Catarina, Campus Universitário Trindade, CEP: 88040-900, Florianópolis, Santa Catarina, Brazil.

*Corresponding authors

M. Palacio: melissapalacio@gmail.com;

G. Robledo: glrobledo@yahoo.com.

Main message

Morphological and phylogenetic studies on *Polyporus dictyopus* complex of species revelead a hidden diversity in Neotropics. In order to accommodate it, here is proposed the segregation of three species in a new genus, *Neodictyopus*, including the new combination *N. dictyopus* and two new species (*N. gugliottae* sp. nov. and *N. atlanticus* sp. nov.), and the recircunscription of the genus *Atroporus* Ryvarden with description and comments on *A. diabolicus* and the new combination of *A. rufuatratus*. Additional comments on other species which should be taxonomically studied are presented and discussed.

Keywords

Atroporus, Neodictyopus, type studies, taxonomy, phylogeny, wood decaying fungi.

Abstract

Polyporus dictyopus is traditionally considered a species complex characterized by wide morphological variation and a large number of heterotypic synonyms. Based on a detailed macro- and micro-morphological examination of neotropical specimens, including types, and multigene phylogenetic analyses (ITS, LSU, RPB2 markers) we found that specimens from Amazonia, Cerrado and the Atlantic Forest previously identified as *P. dictyopus* form two distinct unrelated clades, corresponding to different genera and species. *Atroporus* Ryvarden is recircunscribed and *Neodictyopus* gen. nov. is proposed to accommodate this segregated diversity. Our study confirms that at least five distinct species were passing under the name *P. dictyopus*. Detailed descriptions, pictures, illustrations, and a key are provided for *Atroporus* and *Neodictyopus* species.

Introduction

.

Polyporus P. Micheli ex Adans has been traditionally characterized by presenting stipitate basidiomata, poroid hymenophore, a dimitic hyphal system with skeletal-binding hyphae and cylindrical to ellipsoid basidiospores (Nuñez & Ryvarden 1995, Silveira & Wright 2005), and considered as having a wide global distribution (Ryvarden & Johansen 1980, Gilbertson & Ryvarden 1987, Ryvarden & Gilbertson 1994, Nuñez & Ryvarden 1995, Nuñez & Ryvarden 2001, Silveira & Wright 2005). Due the macroscopical morphology heterogeneity and also for practical use, *Polyporus* was divided into six non-taxonomical

morpho-groups: "Polyporus", "Favolus", "Melanopus", "Polyporellus", "Admirabilis", and "Dendropolyporus" (Nuñez & Ryvarden 1995).

Recent phylogenetic analyses revealed that Polyporus morphogroups do not comprise entirely independent monophyletic lineages. Aditionally, *Polyporus* has been shown to be polyphyletic (Krüger et al. 2006, Sotome et al. 2008, 2011, Dai et al 2014, Seelan et al. 2015). Based on phylogenetic and morphological analysis of "Favolus group", Favolus Fr. was recovered and recircunscribed, and Neofavolus Sotome & T. Hatt. segregated as an independent genus (Sotome et al. 2013). On the other hand, "Melanopus group", which has been characterized by having coriaceous basidiomata with a black cuticle in the stipe (Nuñez & Ryvarden 1995) was recovered as an artificial group, based on phylogenetic analysis of ITS and nucLSU DNA (Dai et al. 2014); several species are grouped and constitute the current "melanopus clade", treated recently as Picipes Zmitr. & Kovalenko (Zmitrovich & Kovalenko 2016), some other taxa traditionally considered into "Melanopus group", such as Polyporus leprieurii Mont., P. guianensis Mont. and P. dictyopus Mont., are not phylogenetically related. Polyporus guianensis and P. leprieurii, which have pale brown tan to beige pileus, are morphologically distinct, however, P. dictyopus share the same general characters those presented by "melanopus clade" members.

Polyporus dictyopus has been indicated as a species complex based on morphology and mating type data (Nuñez & Ryvarden 1995, 2001). The current concept of *P. dictyopus* involves a wide variation in the pilear surface color (chesnut to purplish black), stipe insertion (laterally to centrally stipitate), and basidiospores size and shape (ellipsoid to cylindrical) and a large number of heterotypic synonyms, at least 16 are known from tropical and subtropical America (Nuñez & Ryvarden 1995, Gugliotta et al. 1996).

Polyporus diabolicus Berk. and *P. infernalis* Berk., some of heterotypic synonyms of *P. dictyopus*, were accommodated in *Atroporus* Ryvarden, being characterized by having cystidia with protuberances and "sharply pointed apex" (Ryvarden 1973). Then, *Atroporus* cystidia were reinterpreted as modified binding hyphae (Ryvarden 1976) and proposed the synonymization of *Atroporus* in *Polyporus*, with *P. diabolicus* and *P. infernalis* considered as heterotypics synonyms of *P. dictyopus* (Nuñez & Ryvarden 1995). This idea was followed by further

studies (Gugliotta et al. 1996, Núñez & Ryvarden 2001, Silveira & Wright 2005, Louza & Gugliotta 2007, Gomes-Silva et al. 2012).

The morphological heterogeneity and the global wide distribution of *P. dictyopus* strongly suggest that there is a hidden and underestimated taxonomic diversity under *P. dictyopus* name. In order to test if there are species supported by morphological, phylogenetic, and distribution evidences within *P. dictyopus*, we performed detailed morphological and molecular analysis with specimens identified as *P. dictyopus* from the Neotropics.

Materials and methods

Collections and morphological studies

Specimens were collected in the Boreal Brazilian dominion, Cerrado dominion, Parana dominion, and South eastern Amazonian dominion, in the Brazilian and Chocoan subregions in the states of Amazonas, Bahia, Santa Catarina, São Paulo (Brazil), and province of Misiones (Argentina). Voucher specimens were deposited in FLOR and CORD. We also examined several other reference specimens, including types, held in NY and BPI (herbarium acronyms follow Thiers, continuously updated). Color descriptions were given according to Munsell (1975). Microscopic observations were made from freehand cross sections of dried materials mounted in Melzer's reagent, 5% KOH, 1% phloxine, lactophenol, cresyl blue and cotton blue (CB). To observe the hyphal system, we follow the technique described by Decock et al. (2013). Basidiospores measurements were made in Melzer's reagent. The meanings of abbreviations are as follow: IKI+= dextrinoid, IKI-= inamyloid and indextrinoid, CB+/- = cyanophilous/acyanophilous, ave = arithmetic mean and Q = the ratio of length/width of basidiospores. In presenting the size range of several microscopic elements 5% of the measurements at each end of the range are given in parenthesis, when relevant. We followed Stalpers (1996) and the Stalpers database (http://www.cbs.knaw.nl/russulales/) for the basidiospores shape terminology. For the species distribution, we used the Neotropical regionalization proposed by Morrone (2014).

DNA extraction, PCR amplification and sequencing

DNA was extracted from dried specimens using Doyle & Doyle (1987) protocol adapted by Góes-Neto et al. (2005). The partial regions

of nuclear ribosomal internal transcribed spacer (ITS), nuclear ribosomal large subunit (nucLSU), and RNA polymerase II second subunit (RPB2) were amplified by PCR. The primers for amplification used were ITS8F - ITS6R (Dentinger et al. 2010), LR0R-LR7 (Vilgalys & Hester 1990) and fRPB2-5F and bRPB2-7.1R (Frøslev et al. 2005, Matheny 2005), respectively. The PCR products were sequenced with BigDye Terminator 3.1 Cycle Sequencing Kit following manufacturer procedures, using the primers ITS8F - ITS6R for ITS, LR0R - LR5 primer for LSU, and fRPB2-5F, bRPB2-6F and bRPB2-7.1R for RPB2 at FIOCRUZ-MG (Brazil) as part of the FungiBrBol project (www.brbol.org). The sequences and chromatograms were manually checked and edited with Geneious 6.1.8 (Kearse et al. 2012). Sequences newly generated in this study were submitted to GenBank (Table 1).

Phylogenetic analyses

Two distinct datasets were constructed: the first based on three molecular markers (ITS, nucLSU, and RPB2), and the second based on two (ITS and nucLSU). The ITS, nucLSU and RPB2 sequences, including related sequences downloaded from GenBank (Table 1), were aligned using Mafft v.7 (Katoh & Standley, 2013) under the Q-INS-I strategy for ITS and G-INS-i strategy for nucLSU and RPB2 for both datasets. The alignments were manually examined and adjusted with MEGA 6 (Tamura et al. 2013).

We coded the ITS and nucLSU indels present in the datasets as binary characters following the simple indel coding method (SIC, Simmons and Ochoterena 2000), performed in the SeqState software (Müller 2005). An intron in RPB2 were separated and analyzed as a distinct partition. The first dataset was subdivided into nine partitions: ITS1, 5.8S, ITS2, nucLSU, RPB2 -1st, -2nd, -3rd codon positions, RPB2 intron, and ITS and LSU Indels; the second was subdivided into five partitions, excluding the partitions related to RPB2. The best-fit evolutionary model for every partition was selected using iModelTest v. 1.6 (Guindon and Gascuel 2003, Posada 2008) following the Bayesian Information Criterion (BIC). The final alignments were deposited at TreeBASE (http://www.treebase.org/treebase/index.html) (ID to be provided). Two distinct analyzes were performed for each dataset: Bayesian Inference (BI) and Maximum Likelihood (ML). Bayesian Inferences were conducted using MrBayes 3.2.6 as available in CIPRES Science Gateway 3.1 (Miller et al. 2010), and implemented with two

independent runs, each one with four chains and starting from random trees. The runs performed 20.000.000 generations and trees were sampled every 1000th generation. The 25% of sampled trees were discarded as burn-in, while the remaining ones were used for calculating a 50% majority consensus tree and Bayesian Posterior Probabilities (BPP). ML trees were obtained using RAxML v.8.1.4 (Stamatakis, 2014). CIPRES science gateway (Miller et in 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, with no proportion of invariant sites and all other parameters estimated by the software. We provided a partition file to force RAxML software to search for a separate evolution model for each dataset. Bootstrap support values (BS) were obtained with multi-parametric bootstrapping replicates under the same model, allowing the program halts bootstrapping automatically by the autoMRE option. A node was considered to be strongly supported if it showed a BPP ≥ 0.95 and/or BS $\ge 90\%$, while moderate support was considered BPP < 0.95 and/or BS < 90%. Trametes hirsuta (Wulfen) Lloyd and Trametes versicolor (L.) Lloyd were used as outgroup based on previous studies (Sotome et al. 2013, Dai et al. 2014).

Results

Phylogenetic analysis

A total of thirty one sequences were newly generated in this study (12 ITS, 12 nucLSU, and seven RPB2). The first dataset included 45 specimens representing 28 putative species, including Datronia, Echinochaete, Favolus, Mycobonia, Neodatronia, Polyporus, and Trametes species and the final alignment consisted of 2521 bp long, with 214 indels recoded, resulting in 2735 characters. The second dataset included 77 specimens representing 42 putative species, including Echinochaete, Favolus. Datronia, Lentinus. Mycobonia.Neodatronia, Polyporus, Pseudofavolus Pat., and Trametes species and the final alignment consisted of 1482 bp long, with 324 indels recoded, resulting in 1806 characters. The best-fit evolutionary model selected for every partition and related information was summarized in the Table 2. The topology of the BI and ML of the first and second dataset analyzes showed no inconsistency in any supported clades, so is shown the BI tree (Fig. 1). For the second dataset is shown

the topology of the ML analyzes, which has not inconsistency with the BI tree (Fig. 2). The bootstopping criteria of RAxML indicated 360 pseudo replicates as sufficient to access the internal branch support for the first dataset, and 204 for the second dataset.

All phylogenetic analysis performed showed that specimens of *P. dictyopus* complex were grouped into two distinct strongly supported clades, Atroporus clade and Neodictyopus clade.

Within Neodictyopus clade (BS = 100, BPP = 1) two clades can be observed. One group of neotropical species (BS = 77, BPP = 0.85), including three species: *P. dictyopus sp1* (BS = 98, BPP= 1), *P. dictyopus sp2* (BS = 100, BPP = 1), and *P. dictyopus sp3* (BS = 100, BPP = 1). The second clade grouped specimens from paleotropics (subtropical Asia). Within atroporus clade (BS = 100, BPP = 1) two species can be distinguished, *P. dictyopus sp4* (BS= 100, BPP = 1) and *P. dictyopus sp5*.

Polyporus tuberaster (Jacq. ex Pers.) Fr., the generic type of *Polyporus*, was placed in a clade moderately supported (BS = 1, BPP = 81) with some *Datronia* Donk, *Neodatronia* B.K. Cui, Hai J. Li & Y.C. Dai, *Polyporus*, *Mycobonia* Pat. and *Echinochaete* Reid species. *Datronia*, *Echinochaete*, *Favolus*, *Mycobonia*, *Neofavolus* and *Neodatronia* were each supported as monophyletic, well as Melanopus clade sensu Dai et al. (2014).

Atroporus and Neodictyopus clades have distinct morphological characters that separate them from *Polyporus* as distinct genera. We accept *Atroporus* Ryvarden representing Atroporus clade as recircunscribed here and we propose *Neodictyopus* gen. nov. for Neodictyopus clade. *Atroporus* and *Neodictyopus*, as well as their respective species, are described and illustrated below.

Taxonomy

Atroporus Ryvarden, Norw. Jl Bot. 20: 2 (1973), emend. Palacio, Robledo, Reck & Drechsler-Santos

Basidiomata annual to biannual, centrally to eccentrically stipitate; pileus circular; pilear surface glabrous, radially striate to finely wrinkled, dark purplish red to blackish; margin sterile, with a black cuticle. Pores circular. Context homogenous, light brown. Stipe cylindrical, solid, bearing a black cuticle. Hyphal system dimitic with generative and skeletal-binding hyphae; generative hyphae with clamp connections; skeletal-binding hyphae from the context and stipe usually dominating, arboriform, hyaline, IKI–; skeletal-binding hyphae in the trama of tubes IKI+, with differentiated and wide stalk, and sharply pointed apex. Basidia clavate, 4-sterigmate. Basidiospores ellipsoid, thin-walled, smooth, hyaline, IKI–.

Type species. Atroporus diabolicus (Berk.) Ryvarden.

Remarks: Basidiospores descriptions and Melzer reagent reaction of the skeletal-binding hyphae are new information to the genus. *Atroporus* could be compared with *Polyporus sensu lato* and *Echinochaete* Reid, however, the combination of ellipsoid basidiospores, strongly dextrinoid skeletal-binding hyphae with a differentiated apex, and the black cuticle on the pileus are unique to the group *Atroporus*. All the species grow on dead wood, typically dead fallen branches of relative thin diameter (up to 10 cm diam) and produces white rot on the substrate. So far the genus is only known from the Neotropics.

Atroporus diabolicus (Berk.) Ryvarden, Norw. Jl Bot. 20: 2 (1973) (Figs. 3e, 3e1, 3f, 3f1, 4a, 4b)

 \equiv *Polyporus diabolicus* Berk. Hooker's J. Bot. Kew Gard. Misc. 8: 174 (1856)!.

= *Polyporus vernicosus* Berk. Hooker's J. Bot. Kew Gard. Misc. 8: 175 (1856)!.

Basidiomata annual to biannual, central to eccentrically stipitate, solitary; pileus circular, up to 3.2 cm in diameter and 4 mm thick; pilear surface reddish black (10R2.5/1) to very dark red (2.5YR2.5/2), glabrous, radially striate to finely wrinkled; margin rounded/truncate, sterile, with a black cuticle. Pore surface light brown (7.5YR6/4) to dark brown (7.5YR3/2), in some specimens a black cuticle covering the surface; pores circular, regular, 5–8 per mm, 90–140(–150) μ m (ave=111.5 μ m, n=80/2); dissepiments entire, 30–100.5(–120) μ m thick, (ave=51.1 μ m, n=80/2). Tubes concolorous with pore surface, not stratified to stratified into 3 layers up to 7 mm long each one. Context homogeneous, ligth brown (7.5YR6/4), 1.5 mm thick. Stipe cylindrical, solid, glabrous, longitudinally striate, bearing a black cuticle up to 3.2 cm long, up to 5 mm diam, with a robust appearance. Hyphal system

dimitic with generative hyphae and skeletal-binding hyphae. Generative hyphae with clamps, hyaline, thin-walled, 2-3 µm thick, difficult to observe. Skeletal-binding hyphae of two types; arboriform type, present in stipe and contex, up to 230 µm long, 2.5-4 µm wide, thick-walled, with a short unbranched stalk (17.5-48 µm), 4-6 branches with an alternating arrangement, and shorten as approaching the trama of the tubes, hyaline to yellowish in KOH and water, nondextrinoid (Figs. 9a, 10a). In the trama of the tubes, they differ in the second type of hyphae (Figs. 9b, 10c), skeletal-binding hyphae short (41-75 µm long) and "prickly" always with acute apex that is projected above hymenium. These skeletal-binding "prickly" hyphae are golden vellow in KOH and water, strongly dextrinoid changing to dark brown in Melzer reagent, thick-walled, just after the septa $(3-5 \mu m \text{ wide})$ developed a stalk, that is considerably enlarged at the central portion (7–11 µm wide) between the middle portion and apical hyphal ending in an acute apex as a small spines, from the stalk arise from 2 to 6 branches (1-3 µm wide) at angles 75° – 90° , generally longer towards the base, which can reach up to 76 µm long, sometimes with dichotomous branches. Basidia clavate, 4-sterigmate, clamped, $19-22 \times 6-8$ µm. Cystidia and chlamydospores absent. Basidiospores ellipsoid, thin-walled, hyaline, smooth, IKI-, CB-, $(5-)6(-7) \times (2-)3-3.5 \mu m$, (ave=6 × 3 μ m), Q=1.7-2.3(-2.5) μm (ave=2µm, n=40) (Fig. 4b, 9c1, 10e).

Distribution: *Atroporus diabolicus* is know from Brazilian and Chacoan subregions, in the Boreal Brazilian, Parana, and South-eastern dominions, including the Atlantic, Imer, and Xingu-Tapajos provinces (Fig. 11).

Specimens examined: BRAZIL, Amazonas, Panuré, Feb 1853, Spruce 195 (NY 730627, syntype of *Polyporus diabolicus*); Collector unspecified s.n. (NY 731050, type of *P. vernicosus*); Novo Airão, Parque Nacional de Anavilhanas, Igarapé Santo Antônio, 02°24'227"S, 60°58'215"W, 25 m elevation, on dead twig on the ground, 6 Dec 2013, ER. Drechsler-Santos DS1266 (FLOR); Bahia, Wenceslau Guimarães, Estação Ecológica Wenceslau Guimarães, 14 Aug 2008, J. Pereira JAD3 (FLOR); São Paulo, Iporanga, Parque Estadual Turístico do Alto Ribeira, Morro do Santana, 14 Dec 2014, G. Alves-Silva GAS679 (FLOR); Pará, Belterra, Floresta Nacional de Tapajós, BR 163-KM 117, 03°21'213"S, 54°56'595"W, 29 Jan 2015, ER. Drechsler-Santos DS1695 (FLOR).

Remarks: *Atroporus diabolicus* is characterized by the presence of strongly dextrinoid skeletal-binding "prickly" hyphae with a pointed apex in the trama of the tubes that arise above the hymenium, the rounded/truncate and sterile margin and the robust appearance of the basidiomata. *Atroporus dibolicus* is similar to *A. rufoatratus* and *A. infernalis*, however *A. rufoatratus* has rounded apex of the skeletal-binding hyphae from the tubes, and *A. infernalis* has a short and lateral stipe.

Atroporus rufoatratus (Berk.) Palacio, Reck & Robledo, comb. nov. (Figs. 3j, 3j1, 3k, 3k1, 5a, 5b)

Mycobank number to be provided.

 \equiv *Polyporus rufoatratus* Berk. Hooker's J. Bot. Kew Gard. Misc. 8: 174 (1856)!

Basidiomata annual, centrally stipitate, solitary; pileus circular, depressed to slightly infundibuliform, up to 2.6 (-4) cm in diameter and 1.5 mm thick; pilear surface dark reddish brown (2.5YR2.5/4), glabrous, radially striate; margin deflexed to inflexed, steril, with a black cuticle. Pore surface brownish yellow (10YR6/6); pores circular 4-7 per mm, 90-220(-250) μ m (ave=144.4 μ m, n=240/6); disseptiments entire to slightly lacerate, (20–) 30-70(-90) µm thick, (ave = 49.2 µm, n=240/6). Tubes concolorous with the context, not stratified, up to 0.8 mm long, decurrent to free. Context homogeneous, yellow (10YR7/6), up to 1 mm thick. Stipe cylindrical, solid, glabrous, smooth to slightly striate, bearing a black cuticle, up to 3.7 cm (-9.8 cm) cm long and 3 mm in diam. Hyphal system dimitic with generative hyphae and skeletalbinding hyphae. Generative hyphae with clamps, hyaline, thin-walled, 2-3 µm thick, IKI-, CB-; skeletal-binding hyphae of two types. Arboriform skeletal-binding hyphae present in the context and the stipe, up to 160 µm long, 2-4.5 µm wide, straight to geniculated, thin to thickwalled, branched, with a short unbranched stalk (30-45 µm), 5-7 branches (up to 210 μ m long and 1–3 μ m wide) with an alternating arrangement and shorten as approaching the trama of the tubes, hvaline to yellowish in KOH, water, and lactofenol, not dextrinoid. In the trama of the tubes they differ in the second type of hyphae (Fig. 5a), skeletalbinding hyphae with a wider main stalk (49–93 µm long) developed just after the clamp scar (2–3 µm wide) that is enlarged specially in the central portion (4–8 µm wide), between the middle and apical portion arise from 2 to 5 branches (1–3 µm wide), up to 76 µm long, with dichotomous branches, thin to thick-walled, the hyphal apex is round and projected above hymenium, hyaline to yellowish in KOH, water, and lactofenol, strongly dextrinoid changing to dark brown. Pileipellis as an anamorph matrix, 20–28 µm thick, pale yellow to dark orange. Cystidioles subulate, 13–20 × 5–7 µm, clampled; basidia clavate, 4sterigmate, clamped, 17–21 × 6–8 µm. Basidiospores narrowly ellipsoid to rarely subcylindrical, thin-walled, hyaline, smooth, IKI–, CB–, 5–7 × 3–4 µm, (ave=5.8 × 3.3 µm), Q= 1.8–2.3 µm (ave= 1.9 µm, n= 120/6) (Fig. 5b, 9c2, 10f).

Distribution: Widely distributed in the Brazilian and Chacoan subregions including the Parana and Boreal Brazilian dominion in the Atlantic, Imer, Pantepui, and Parana Forest and provinces (Fig. 11).

Specimens examined: ARGENTINA, Misiones: Oberá, Campo Ramon, Centro de Investigación Antonia Ramos (CIAR), 27°26' S, 54°55' W, 300-500 m elevation, Feb 2015, N. Gómez NG134 (FLOR); 1 Dec 2011, E. Grassi MEX0138 (CORD). BRAZIL, Amazonas, Panuré, collector unspecified s.n. (NY 730938, type of *Polyporus rufoatratus*); Roraima, Caracaraí, Estrada Manaus-Caracaraí, Km 513, Ac. Novo Paraíso, 21 Nov 1977, I. Araujo 651 (NY1972060); Km 328, 16 Nov 1977, I. Araujo 494 (NY1972061); Km 360, 19 Nov 1977, I. Araujo s.n. (NY1972065); Santa Catarina, Santo Amaro da Imperatriz, 21 Mar 2015, M. Palacio MP153 (FLOR); Plaza Caldas da Imperatriz, Trilha da Cascata, 27 Feb 2014, L. Dalpaz LDA 129 (FLOR); LDA 138 (FLOR); LDA 139 (FLOR). Florianópolis, Lagoa do Peri, 08 Jan 2014, J. Prata JP1 (FLOR), 15 Feb 2014 ER. Drechsler-Santos DS 1311 (FLOR); Naufragados, 10 Jan 2014, J. Prata JP10 (FLOR); 15 Mar 2014, L. Dalpaz LDA 140 (FLOR); 23 Feb 2016, MP 158 (FLOR); Unidade de Conservação Ambiental Desterro, 2 Jun 2012, ER. Drechsler-Santos DS 816 (FLOR).

Remarks: This species is well characterized by the ellipsoid basidiospores and the skeletal-binding hyphae of the trama, strongly dextrinoid, with a wider main stalk and a round apex projected above the hymenium; macroscopically it is characterized by its centrally stipitate basidiomata, infundibuliform dark reddish brown pilei, and slender stipe. *Atroporus diabolicus* is a related species, but it has a pointed apex of skeletal-binding hyphae on the trama, and a more robust appearance of the basidiomata.

Comments on other taxa related to Atroporus

Atroporus infernalis (Berk.) Ryvarden, Norw. Jl Bot. 20: 2 (1973)! Pore surface brown (10YR5/3); pores circular (5–)6–7 per mm; dissepiments entire to sligthly lacerate, 20–50(–70) µm thick, (X = 32.8 µm, n=40/1). Hyphal system dimitic. Generative hyphae thin-walled, hyaline, with clamp connections, up to 4 µm in diam. Skeletal-binding hyphae thick-walled to solid, branched, hyaline (similar to *A. rufoatratus*), IKI+, up to 6 µm in diam. Basidiospores not seen.

Remarks: the type specimen is damaged, only a pilear fragment remaining in the exsiccate. Berkley described *P. infernalis* based on a collection from Minas Gerais (Brazil) as an allied species of *P. varius* (Pers.) Fr. and *P. dictyopus*, but as a "very distinct species". *Polyporus infernalis* was later transferred to *Atroporus* (Ryvarden 1973), based on the IKI+ and modified skeletal-binding hyphae in the trama of the tubes. After our type revision we confirm the presence of this feature, endorsing that this species belongs to *Atroporus*; we also observed the sterile margin as mentioned in the protologue. *Atroporus infernalis* is related to *A. rufoatratus* but it differ for having a short and lateral stipe, and flabelliform pileus (protologue information, Berkeley 1856). Unfortunately, we did not observed basidiospores and the poor condition of the type did not allow us to compare to other specimens. Specimen examined: Brazil. Minas Gerais: Arraial des Merces, Oct.

1840 (NY 730749, type of *Polyporus infernalis*)

Other species possibly included in the genus *Atroporus Fomes holomelanus* Berk. ex Cooke, Grevillea 15(no. 74): 51 (1886). *Polyporus atroumbrinus* Berk., Hooker's J. Bot. Kew Gard. Misc. 8: 199 (1856).

Neodictyopus Palacio, Robledo, Reck & Drechsler-Santos gen. nov. Mycobank number to be provided.

Etymology. Neo (Lat.): new; dictyopus (Gre.): reticulate stipe surface of *Polyporus dictyopus* s.l.; the new dictyopus, in reference to the recognition of a new genera segregated from *P. dictyopus* complex. Basidiomata annual, lateral to eccentrical, rarely centrally stipitate; pileus reniform to flabelliform; pilear surface glabrous, radially striate, dark reddish brown; margin irregular, wavy, and lobed to decurved and entire. Pores circular. Context homogenous, yellow to light brown. Stipe cylindrical, solid, reticulated to longitudinally striate, bearing a black cuticle. Hyphal system dimitic; generative hyphae clamped, hyaline, thin-walled, branched skeletal-binding hyphae dominating, arboriform, hyaline, IKI– to slightly dextrinoid (only in mass) in the trama of the tubes. Basidia clavate, 4-sterigmate. Basidiospores cylindrical, thin-walled, smooth, hyaline, IKI–.

Type species. Neodictyopus gugliottae Palacio & Drechsler-Santos.

Remarks: *Neodictyopus* is characterized by its cylindrical basidiospores, reniform to spatulate pileus, and skeletal-biding hyphae of arboriform type, slightly dextrinoid (when in mass) in the trama of the tubes. So far, the genus is Neotropical, but probably pantropical, since some specimens from paleotropics clustered together with Neodictyopus clade. All the species grow on dead wood, typically dead fallen branches of relative thin diameter (up to 10 cm diam) and produces white rot on the substrate. *Neodictyopus* is microscopically similar to *Polyporus*; however, *P. tuberaster*, the type species of *Polyporus*, has fleshy (when fresh) basidiomata, and pileus upper surface whitish to ochraceous covered with scales. Macroscopically, *Neodictyopus* is similar to *Atroporus*, but the ellipsoid basidiospores and strongly dextrinoid skeletal-biding hyphae from the trama of the tubes are unique to *Atroporus*.

Neodictyopus atlanticus Palacio, Grassi & Robledo, sp. nov. (Figs. 3d, 3d1, 6a, 6b).

Mycobank number to be provided.

Holotype: Brazil, Santa Catarina, Joaçaba, Parque Ecológico Municipal Rio do Peixe. G. Alves-Silva 622, 27 Sep 2014, (FLOR).

Etymology: atlanticus (Latin) Atlantic, referring to the species type locality, placed in Atlantic province.

Basidiomata annual, laterally stipitate, solitary; pileus flabelliform to slightly spathulate, up to 1.5 cm in diameter and 2 mm thick; pilear surface reddish brown 2.5YR (4/4) to dark reddish brown (2.5YR2.5/4), radially striate, glabrous; margin decurved and entire. Pore surface yellow 10YR (7/6); pores circular to slightly radially elongated (5-)6-7 129.5–190.5(–200) µm, per mm. (120 -)(ave=161.8. n=40): disseptiments entire, $(20-)30-60(-70) \mu m$ thick, (ave=44.8 μm , n=40/1). Tubes concolorous with the pore surface, not stratified, up to 1 mm long. Context homogeneous, yellow (10YR8/8), up to 1 mm thick. Stipe cylindrical, solid, slender, longitudinally striate, glabrous, bearing a black cuticle, up to 2.3 cm long and 2 mm in diam. Hyphal system dimitic with generative hyphae and skeletal-binding hyphae. Generative hyphae with clamps, hyaline, thin-walled, 2-3 µm thick, IKI-, CB-, more easily to observed in the tubes. Skeletal-binding hyphae hyaline to yellowish in KOH and water, IKI-, CB-. Stipe, context and trama of the tubes composed mainly of skeletal-binding hyphae with a loose arboriform branching pattern, up to 310 µm long, 3-5 µm wide, thickwalled, geniculated, with a short unbranched stalk (92-155 µm) and then with 2-4 branches (up to 190 µm long) with an alternating arrangement. Skeletal-binding hyphae from the tubes are shorter (up to 120 µm) than in stipe and context, and becoming shorter (up to 90 µm) as approaching the dissepiments where have more (3-6) and shorter ramifications (Fig. 6a). Cystidiole subulate, $12-15 \times 3-5$ µm, clampled. Basidia clavate, 4-sterigmate, $21-23 \times 5-6$ um. Basidiospore narrowly cylindrical, thin-walled, hyaline, smooth, IKI–, CB–, $6-9 \times 2-2.5 \mu m$, $(ave=7.6 \times 2.1 \ \mu m), Q= 2.8-4.5 \ \mu m (ave = 3.6 \ \mu m, n= 40/1)$ (Fig. 6b,

9f3, 10g).

Distribution: *Neodictyopus atlanticus* so far is only known from Araucaria and Parana Forest provinces in Brazil and Argentina (Fig. 11).

Specimens examined: ARGENTINA, Misiones, Oberá, Campo Ramon, Centro de Investigación Antonia Ramos (CIAR), 27°26' S, 54°55' W, 300–500 m elevation, 10 Dec 2011, E. Grassi CI110 (CORD, FLOR). BRAZIL, Santa Catarina, Joaçaba, Parque Ecológico Municipal Rio do Peixe. G. Alves-Silva 622, 27 Sep 2014, (FLOR).

Remarks: *N. atlanticus* is characterized by the narrowly cylindrical basidiospores, the eccentrically stipitate basidiomata with a circular pileus. *Neodictyopus gugliotae* has a similar slender and developed stipe

but it differs in its irregular, wavy and lobed pileus margin and shorter basidiospores in average.

Neodictyopus dictyopus (Mont.) Palacio, Robledo & Drechsler-Santos comb. nov. (Figs. 3b, 3b1, 3c, 3c1, 7a, 7b, 7b1).

Mycobank number to be provided.

Basionym: *Polyporus dictyopus* Mont. Annls Sci. Nat., Bot., sér. 2 3: 349 (1835)!

Basidiomata annual, laterally stipitate, solitary to clustered; pileus round, reniform to flabeliform, up to 7.5 cm in diameter and 2.5 mm thick; pilear surface dark reddish brown (5YR3/2) to vellowish red (5YR5/8), radially striate, glabrous; margin irregular, wavy and lobed. Pore surface brown (10YR5/3); pores circular 6-9 per mm, 90-130(-150) μ m, (ave=108.3 μ m, n=120/3); disseptiments entire to slightly lacerate 20-70(-80) µm thick, (ave=36.7 µm, n=120/3). Tubes concolorous with the pore surface, not stratified, up to 0.8 mm long, decurrent and irregularly attach to the stipe. Context homogeneous, yellow (10RY7/8), up to 1 mm thick. Stipe cylindrical, solid, glabrous, reticulated, bearing a black cuticle, short up to 1.5 cm long and 8 mm in diam. Hyphal system dimitic with generative hyphae and skeletalbinding hyphae. Generative hyphae with clamps, hyaline, thin-walled, 1-3 µm thick, IKI-, CB-, more easily to observed in the tubes. Skeletalbinding hyphae hyaline to yellow in KOH and water, nondextrinoid to ocasionally weakly dextrinoid, CB-. Stipe, context and trama of the tubes composed mainly by skeletal-binding hyphae with a loose arboriform branching pattern, up to 250 µm long, 2.5-5 µm wide, thickwalled, geniculated, with a short unbranched stalk (25–75 μ m) and then with 2-5 branches (up to 250 µm long) with an alternating arrangement. In the trama the skeletal-binding hyphae are shorter (up to 107 µm) than those of stipe and context, and more shorter (up to 84 µm) as approaching the disseptiments where have more (4-7) and shorter ramifications (up to 85 µm long) (Fig. 7a). Cystidioles subulate, 17-21 \times 4–5 µm, clampled. Basidia clavate, 4-sterigmate, 15–21 \times 5–7 µm. Basidiospores subcylindrical, thin-walled, hyaline, smooth, IKI-, CB-, $(6-)6.5-8 \times 2-3 \mu m$, (ave=7 × 2.6 μm), Q = 2.5-3.3 μm (ave=2.81 μm , n= 120/3) (Figs. 7b, 7b1, 9f1, 9f2).

Distribution: This species was originally described from temperate forest of the Juan Fernández archipelago (Chile) it was also found in Cerrado province of the Mato Grosso state (Brazil) (Fig. 11).

Specimens examined: BRAZIL, Mato Grosso, Cuiabá, Chapada dos Guimarães, Parque Nacional da Chapada dos Guimarães, 15°24'28.3"S, 55°50'00.3"W, 27 Nov 2011, G. Alves-Silva GAS60 (FLOR); 15°24'30.0"S, 55°49'57.5"W, 05 Aug 2012, G. Alves-Silva GAS272 (FLOR); G. Alves-Silva GAS281 (FLOR); Véu da Noiva, 15°24'25"S, 55°50'17"W, 19 Jun 2011, V. Ferreira-Lopes VFL18 (FLOR). CHILE, Juan Fernandez, Bertero 1683 (BPI US207664, type of *P. dictyopus*).

Remarks: *Neodictyopus dictyopus* is characterized by having basidioma laterally stipitate, with short, robust, black, and reticulated stipe, margin irregular, wavy and lobed, variable pilear surface color, and cylindrical basidiospores. The Brazilian specimens here examined are linked to the type specimen by morphological comparison, besides the disjunct distribution. To better define the circumscription and distribution of *N. dictyopus*, more collections from the type locality are needed.

Neodictyopus gugliottae Palacio, Robledo, Reck & Drechsler-Santos, sp. nov. (Figs. 3a, 3a1, 8a, 8b).

Mycobank number to be provided.

Holotype: Brazil, Santa Catarina, Santo Amaro de Imperatriz, Caldas da Imperatriz, ER. Drechsler-Santos DS1285, 15 November 2013, (FLOR). Etymology: in honor for Dr. Adriana Gugliotta, a Brazilian expert in polypores, for its contributions to our knowledge of polypores fungi diversity.

Basidiomata annual, laterally to eccentrically stipitate, tipically gregariuos, up to four basidiomata in 10 cm of wood; pileus reniform, up to 4.1 cm in diameter and 1.5 mm thick; pilear surface strong brown (7.5YR5/8) to dark reddish brown (2.5YR2.5/4), radially striate, glabrous; margin irregular, wavy and lobed. Pore surface brownish yellow (10YR6/8) to grayish brown (10YR5/2); pores circular 5–9 per mm, (80–)90–170(–180) μ m (ave=121.3 μ m, n=160/4); dissepiments entire to slightly lacerated, 20–90(–100) μ m thick, (ave=48.3 μ m, n=160/4). Tubes concolorous with the pore surface, not stratified, up to 0.5 mm long, decurrent and irregularly attach to the stipe. Context

homogeneous, light brown (7.5YR6/4), up to 1 mm thick. Stipe cylindrical, solid, slender, glabrous, longitudinally striated, bearing a black cuticle, up to 2 cm long and 2 mm in diam. Hyphal system dimitic with generative hyphae and skeletal-binding hyphae. Generative hyphae with clamps, hyaline, thin-walled, 1-2.5 µm thick, IKI-, CB-, more easily to observed in the tubes. Skeletal-binding hyphae hyaline to yellowish in KOH or water, nondextrinoid to ocasionally weakly dextrinoid, CB-. Stipe, context and trama of the tubes composed mainly of skeletal-binding hyphae with a loose arboriform branching pattern (Fig 9d, Fig 10b), up to 350 µm long, 2.5-5 µm wide, thick-walled, geniculated, with a short unbranched stalk (20-90 µm) and then with 2-5 branches (up to 550 µm long) with an alternating arrangement. Skeletal-binding hyphae in the trama (Fig. 9e) shorter (80-150 µm) than those at stipe and context, becoming shorter (up to 90 µm) as approaching the dissepiments where have more (5-9) and shorter ramifications (Fig. 8a, 10d). Cystidiole subulate, $14-20 \times 4-5 \mu m$, clampled. Basidia clavate, 4-sterigmate, $19-21 \times 5-6$ um. Basidiospore narrowly cylindrical, thin-walled, hyaline, smooth, IKI-, CB-, $(6-)6.5-8 \times 2-3 \ \mu\text{m}$, (ave = $6.3 \times 2.1 \ \mu\text{m}$), Q=2-3.5 $\ \mu\text{m}$, (ave=3 $\ \mu\text{m}$, n=160/4) (Fig. 8b, 9f4, 10h).

Distribution: *Neodictyopus gugliottae* is only known from the Atlantic province in the Parana dominion (Fig. 11).

Specimens examined: BRAZIL, Santa Catarina, Blumenau, Parque Nacional da Serra do Itajaí, Trilha da Chuva, 27°03'073" S, 49°04'5320" W, 17 Jan 2015, F. Bittencourt FB351 (FLOR); Santo Amaro da Imperatriz, Caldas da Imperatriz, Hotel Caldas da Imperatriz; 15 Nov 2013, ER. Drechsler-Santos DS1284 (FLOR); DS1285 (FLOR); DS1286 (FLOR).

Remarks: *Neodictyopus gugliottae* is well characterized by lateral to eccentrically stipitate basidiomata, well developed and slender stipe, reniform pileus with irregular, wavy and lobed margin, and the gregarious habit. *Neodictyopus dictyopus* can be differentiated from *N. gugliottae* by the short, robust, and lateral stipe.

Comments on taxa related to Neodictyopus

Polyporus blanchetianus Berk. & Mont., Annls Sci. Nat., Bot., sér. 3 11: 238 (1849)

Pore surface brown (10YR5/3); pores circular 6–7 (–8) per mm; dissepiments entire, (20–)30–50(–60) μ m thick, (ave = 41.9 μ m, n=40/1). Hyphal system dimitic. Generative hyphae thin-walled, hyaline, with clamp connections, up to 5 μ m in diam. Skeletal-binding hyphae from the tubes thick-walled to solid, branched, hyaline, IKI–, up to 5 μ m in diam. Basidiospores cylindrical, thin-walled, hyaline, smooth, IKI–, CB–, 6–6.5 × 2 μ m Q= 2.8–4.5 μ m (ave = 3.6 μ m, n= 20/1).

Remarks: Type specimen damaged, only a pilear fragment remaining in the exsiccate. Based on the cylindrical basidiospores and skeletalbinding hyphae IKI-, it is possible to recognize *P. blanchetianus* as a *Neodictyopus* member; however, given the poor condition of the holotype, we prefer to consider *P. blanchetianus* as a dubious species. Specimen examined: Brazil. Bahia, Blanchet s.n. (NY 730532, type of *Polyporus blanchetianus*).

Other species possibly included in the genus *Neodictyopus Melanopus scabellus* Pat., Bull. Soc. mycol. Fr. 16: 178 (1901).

Polyporus nephridis Berk., Hooker's J. Bot. Kew Gard. Misc. 8: 195 (1856)!

Polyporus parvimarginatus Speg., Anal. Soc. Cient. Argent. 16(6): 280 (1883).

Polyporus rhizomorphus Mont., Annls Sci. Nat., Bot., sér. 2 13: 202 (1840).

Polystictus puiggarii Speg., Boln Acad. Nac. Cienc. Córdoba 11(4): 441 (1889).

Discussion

Inferences from previous phylogenetic studies including specimens identified as P. dictyopus have been limited by their small number of sequences and did not link the results with morphological studies (Krüger et al. 2008, Sotome et al. 2008, Dai et al. 2014). Our reconstructions revealed that P. dictyopus as currently understood, in the sense of Nuñez & Ryvarden (1995), Gugliotta et al. (1996), and Silveira & Wright (2005), is polyphyletic and includes species belonging to two well defined clades: *Atroporus* and *Neodictyopus*, which present distinct morphological features that support them as independent genera.

Atroporus includes A. diabolicus, the generic type species, and A. rufoatratus. This clade is strongly supported by BI and MP analysis (BPP = 1.00, BS = 100%, Fig. 1). One sample of A. diabolicus from Imerí province (Amazonas, Brazil), and three samples of A. rufoatratus from Atlantic province (Santa Catarina, Brazil) formed two highly supported lineages (Fig 1). Both species have ellipsoid basidiospores, basidiomata centrally to eccentrically stipitate, and skeletal-binding hyphae from the trama of tubes strongly dextrinoid.

Neodictyopus is strongly supported by both BI and MP analysis (BPP = 1.00, BS = 100%, Fig. 1) and is formed by four lineages. One clade strongly supported (BPP = 1.00, BS = 98%) composed by three specimens from Atlantic province (Santa Catarina, Brazil) of N. gugliottae, the type species of the genera. Neodictyopus dictyopus clade (BPP = 1.00, BS = 100%) is composed by three specimens from Cerrado province (Mato Grosso, Brazil). Another clade strongly supported (BPP = 1.00, BS = 100%) is formed by two specimens of N. atlanticus from Araucaria and Paraná Forest provinces (São Paulo, Brazil and Misiones, Argentina). Finally, an Asian clade (BPP = 1.00, BS = 100%) with three samples from subtropical Asia. *Neodictvopus* gugliottae atlanticus. N. *dictyopus*, and *N*. share cylindrical basidiospores, reniform pileus, and lateral to occasionally eccentrical stipe.

We identified and associated the specimens collected in Cerrado (Fig. 3b) with N. dictyopus type on morphological basis (Fig. 3c). The type and Cerrado specimens share the same macromorphological features: the reticulated stipe surface (Fig. 3b1, 3c1), the short (up to 1.5 cm) and wide (up to 8 mm) stipe, and the flabeliform Micro-morphological features pileus. are also identical. such basidiospores shape and size (Fig. 7b, 7b1), as well as skeletal-binding hyphae with a loose arboriform branching pattern and weakly dextrinoid present only in mass of dissepiment. Despite our phylogenetic analysis did not include sequences from the N. dictyopus type, or specimens from the type locality, the morphological similarity allow us infer that Cerrado specimens (GAS60; GAS272; GAS281, VFL18) represent the same species.

Atroporus and Neodictyopus share similar hyphal system in the context of pileus and stipe, with generative hyphae with clamps and dominant skeletal-binding hyphae. Both genera have basidiomata with a dark reddish brown cuticle on the pilear surface, except by A. diabolicus which can be darker. Atroporus species can be differentiated by its ellipsoid basidiospores, and strongly dextrinoid skeletal-binding hyphae in the trama of tubes with projected apex, and basidiomata centrally to eccentrically stipitate. In contrast, Neodictyopus species have cylindrical basidiospores, nondextrinoid to weakly dextinoid (only in mass) skeletal-binding hyphae, and lateral to eccentric stipitate basidiomata.

However, the distinct skeletal-binding hyphae of the trama are typical of Atroporus were once considered as cystidia (Ryvarden 1973) and/or as modified skeletal-binding hyphae (Ryvarden 1973, 1976, Nuñez & Ryvarden 1995, Gugluita et al. 1996). Meticulous examinations of the hyphal system (according Decok et al. 2013) allow us to observe and describe whole hyphae, and then reinterpret as a unique type of skeletal-binding hyphae exclusive of *Atroporus*.

In this study, *Neodictyopus* was recovered as sister group of *Picipes*. Our results, also bring new phylogenetic information about *Atroporus*, which appears as a sister clade of the remaining *Neodictyopus* and *Picipes*. This three genera formed a strongly supported clade (BPP=1.00, BS=98%, Fig. 1), in which all the species share the black cuticle in the stipe, the principal character that define *Melanopus sensu* Patouillard and Melanopus group *sensu* Nuñez & Ryvarden (1995). However, another species (e.g. *P. leprieurii, P. guianensis*, and *P. varius*) that present the same cuticle are not related to those clades, so Melanopus, as previously pointed out, is an artificial group. We could not identify morphological evidence to maintain *Neodictyopus, Picipes*, and *Atroporus* species as a single genus, then we prefer maintain them as separated genera.

Another white rot polypores genera share characters with *Atroporus* and *Neodictyopus* but can be easily morphologically differentiated. *Lentinus* Fr. and *Panus* Fr. have also stipitate basidiomata, dimitic hyphal system, and cylindrical to subellipsoid, smooth, and inamyloid basidiospores (Hibbet & Vilgalys 1993, Seelan 2015), but produce gilled basidiomata. *Pseudofavolus* Pat. also produce stipitate and poroid basidiospores (more than 10 um), the gelatinous subhymenium and the presence of dendrohyphidia differentiated this genus (Nuñez & Ryvarden 1995). *Datronia* share similar microscopic characters, but produce effused-reflexed basidiomata with dendrohyphidia (Li et al 2014). *Echinochaete*

has a dimitic hyphal system similar to *Atroporus*, with dextrinoid arboriform skeletal-binding hyphae and generative hyphae with clamps, however the former has spinulose setoid elements on the pilear surface and in the hymenium (Sotome et al. 2009). *Polyporus melanopus* is a morphologically related species to *Neodictyopus* species with similar brownish pilear surface, but this species only grows on *Nothofagus* Blume in temperate zones (Silveira & Wright 2005). *Polyporus austroandinus* (Pers.) Fr., has also basidiomata with a stipe bearing a black cuticle similar to *Neodictyopus* species, nevertheless *P. austroandinus* has larger pores (4–5 per mm) and basidiospores [(–8)9– 11.5 × 3–3.8(–4)], and grows in the southern forest of Andes (Dai et al. 2014).

The reexamination of morph-groups and species complex within *Polyporus* is required in order to classify the genus in a less artificial way. Independent inspection of the hyphal system from the trama of the tubes, context, and pileus, and basidiospores shapes comparison (from the Q value), can assist the detection of morphological patterns within clades already recognized as Melanopus clade sensu Dai et al. (2015).

Key to species of Atroporus and Neodictyopus

- Basidiospores ellipsoid, skeletal-binding hyphae from the tubes strongly dextrinoid with a well differentiated apex protruding into the hymenium. *Atroporus* 2
- Basidiospores cylindrical, skeletal-binding hyphae from the tubes IKI- to occasionally weakly dextrinoid, without differentiated apex Neodictyopus 3
- 2. Skeletal-binding hyphae from the tubes with a sharply pointed apex, basidiomata robust, generally with 2-3 tube layers, sometimes in old specimens with a black cuticle covering the hymenophore, stipe robust (up to 3.2 cm long \times 0.5 cm diam.) *Atroporus diabolicus*
- Skeletal-binding hyphae from the tubes with a rounded apex, basidiomata slender, always with one tube layer, stipe slender (up to 9.8 cm long × 0.3 cm diam.) Atroporus rufoatratus
- 3. Pilear margin regular, decurved, and entire;

Neodictyopus atlanticus

4

3. Pilear margin irregular, wavy, and lobed;

- Basidiomata eccentrically stipitate, stipe perpendicular to the pileus (aprox. 90°), slender (up to 2 mm in diameter), up to 2 cm long, pileus reniform Neodictyopus gugliottae
- Basidiomata laterally stipitate, stipe horizontal to the pileus (aprox. 180°), robust (up to 10 mm), up to 1.5 cm long, pileus reniform to flabelliform *Neodictyopus dictyopus*

AKNOWLEDGMENTS

The authors thank the Estação Ecológica Wenceslau Guimarães (Bahia, Brazil), Floresta Nacional de Tapajós (Pará, Brazil), Parque Estadual Turístico do Alto Ribeira (São Paulo, Brazil), Parque Nacional Anavilhanas (Amazonas, Brazil), Parque Nacional da Chapada dos Guimarães (Mato Grosso, Brazil), Parque Nacional da Serra do Itajaí (Santa Catarina, Brazil), and Unidade de Conservação de Desterro (Santa Catarina, Brazil) for permission to sample collections and herbaria mentioned (BPI, CORD, FLOR, and NY); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for providing a master's scholarship to M. Palacio; Fiocruz for performing the molecular sequencing; PPGFAP/UFSC and BrBOL for partial financing of the research. Mateus A. Reck thanks CAPES (PNPD Institucional 2011—23038.007790/2011-93) for scholarship and funding.

REFERENCES

Berkeley MJ. 1856. Decades of Fungi LI-LIV, Rio Negro Fungi. Hooker's London Journal of Botany & Kew Garden Miscellany 8: 174.

Dai YC, Xue HJ, Vlasák J, Rajchenberg M, Wang B, Zhou LW. 2014. Phylogeny and global diversity of Polyporus group Melanopus (Polyporales, Basidiomycota). Fungal Diversity 64:133–144.

Decock C, Amalfi M, Robledo G, Castillo G. 2013. Phylloporia nouraguensis, an undescribed species on Myrtaceae from French Guiana. Cryptogamie Mycol 34:15–27.

Dentinger BTM, Margaritescu S, Moncalvo JM. 2010. Rapid and reliable high-throughput methods of DNA extraction for use in barcoding and molecular systematics of mushrooms. Molecular Ecology Resources 10: 628–633.

Doyle JJ, Doyle JL. 1987. A rapid isolation procedure for small quantities of fresh tissue. Phytochemical Bulletim 19:11-15.

Frøslev TG, Matheny PB, Hibbett DS. 2005. Lower level relationships in the mushroom genus Cortinarius (Basidiomycota, Agaricales): a comparison of RPB1, RPB2, and ITS phylogenies. Molecular Phylogenetics and Evolution 37: 602–618.

Gilbertson RL, Ryvarden L. 1987. North American polypores 2. Fungiflora, Oslo

Góes-Neto A, Loguercio-Leite C, Guerrero RT, 2005. DNA extraction from frozen field-collected and dehydrated herbarium fungal basidiomata: performance of SDS and CTAB-based methods. Biotemas 18(2): 19-32.

Gomes-Silva, A. C., L. Ryvarden, P. S. Medeiros, H. M. P. Sotão, and T. B. Gibertoni. 2012. Polyporus (Basidiomycota) in the Brazilian Amazonia, with notes on Polyporus indigenus I.J. Araujo & M.A. de Sousa and P. sapurema A. Möller. Nova Hedwigia 94: 227–238.

Guindon S, Gascuel, O. 2003. A simple, fast and accurate method to estimate large phylogenies by maximum-likelihood. Systematic Biology 52: 696-704.

Gugliotta AM., Capelari M., Bononi VLR. 1996. Estudo taxonômico e sinomização das espécies do grupo Polyporus dictyopus Mont. (Polyporaceae, Aphyllophorales). Revista Brasileira de Botânica 19: 185-192.

Hibbett DS, Vilgalys. 1993. Phylogenetic relationships of *Lentinus* (Basidiomycotina) inferred from molecular and morphological characters. Syst Bot 18:409–433.

Katoh K, Standley DM. 2013. MAFFT. Multiple sequence alignment software 7: improvements in performance and usability. Mol Bio Evol 30:772–780.

Kearse M., Moir R., Wilson A., Stones-Havas S., Cheung M., Sturrock S., Buxton S., Cooper A., Markowitz S., Duran C., Thierer T., Ashton B., Meintjes P., Drummond A. 2012. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649.

Krüger D, Petersen RH, Hughes KW. 2006. Molecular phylogenies and mating study data in Polyporus with special emphasis on group "Melanopus" (Basidiomycota). Mycological Progress 5: 185–206.

Li, H.J., Cui, B.K., Dai, Y.C. (2014) Taxonomy and multi-gene phylogeny of Datronia (Polyporales, Basidiomycota). Persoonia 32: 170–182

Louza, GSG., Gugliotta, AM. 2007. Polyporus Fr. (Polyporaceae) no Parque Estadual das Fontes do Ipiranga, São Paulo, SP, Brasil. Hoehnea 34:367-384.

Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). Molecular Phylogenetics and Evolution 35: 1–20.

Miller MA, Pfeiffer W. Schwartz T. 2010. Creating the CIPRES science gateway for inference of large phylogenetic trees. In: Proceedings of the Gateway Computing Environments Workshop (GCE). 14 Nov 2010, New Orleans, Louisiana p 1–8.

Morrone JJ. 2014. Biogeographical regionalization of the Neotropical region. Zootaxa 3782 (1): 1–110.

Munsell. 1975. Soil Colors Charts. Baltimore, Maryland: Munsell Color.

Müller K. 2005. SeqState—primer design and sequence statistics for phylogenetic DNA datasets, Appl Bioinf 4: 65–69.

Núñez M, Ryvarden L. 1995. Polyporus (Basidiomycotina) and related genera. Synopsis Fungorum 10:1–85

Núñez M, Ryvarden L. 2001. East Asian polypores. Synopsis Fungorum 14:170–522

Posada D. 2008. jModelTest: phylogenetic model averaging. Mol Biol Evol. 25:1253–1256

Ryvarden L. 1973. New genera in the Polyporaceae. Norw. J. Bot. 20:1-5

Ryvarden L. 1976. Type studies in the Polyporaceae, 7. Species described by J.M. Berkeley from 1836 to 1843. Kew Bull. 31: 81-103

Ryvarden L, Johansen I. 1980. A preliminary polypore flora of East Africa. Fungiflora, Oslo

Ryvarden L, Gilbertson RL. 1994. European polypores. Part 2. Synop Fungorum 7:394–743.

Seelan, J.S.S., Justo, A., Nagy, L.G., 2015. Phylogenetic relationships and morphological evolution in *Lentinus*, *Polyporellus* and *Neofavolus*, emphasizing southeastern Asian taxa. Mycologia 107 (3): 460-474.

Silveira RMB., Wright, JE. 2005. The taxonomy of *Echinochaete* and *Polyporus s. str.* in Southern South America. Mycotaxon 93:1-59.

Simmons MP, Ochoterena H. 2000. Gaps as characters in sequencebased phylogenetic analyses. Syst. Biol.;49:369-381.

Sotome K, Hattori T, Ota Y, To-anun C, Salleh B, Kakishima M. 2008. Phylogenetic relationships of Polyporus and morphologically allied genera. Mycologia 100:603–615.

Sotome K, Hattori T, Ota Y, Kakishima M. 2009. Second report of Polyporus longiporus and its phylogenetic position. Mycoscience 50:415–420.

Sotome K, Hattori T, Ota Y (2011) Taxonomic study on a threatened polypore, Polyporus pseudobetulinus, and a morphologically similar species, P. subvarius. Mycoscience 52:319–326.

Sotome K, Akagi Y, Lee SS, Ishikawa NK, Hattori T (2013) Taxonomic study of Favolus and Neofavolus gen. nov. segregated from Polyporus (Basidiomycota, Polyporales). Fungal Divers 58:245–266.

Stalpers, JA (1996) The aphyllophoraceous fungi II. Keys to the species of the Hericiales. – Stud Mycol 40: 1-185.

Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogeneis. Bioinformatics 30:1312–1313.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0. Molecular Biology and Evolution 30: 2725-2729.

Thiers B Index Herbariorum: a global directory of public herbaria and associated staff. New York Garden's Virtual Herbarium. In: New York Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several Cryptococcus species. J Bacteriol 172:4238–4246.

Zmitrovich IV &. Kovalenko AE. 2016. Lentinoid and Polyporoid Fungi, Two Generic Conglomerates Containing Important Medicinal Mushrooms in Molecular Perspective. 18(1): 23–38.

FIGURE LEGENDS

Fig. 1 Phylogenetic relationships of members of the *Atroporus* and *Neodictyopus* clades inferred from ITS, nucLSU, and RPB2 sequences. Topology is from Bayesian Inference analysis. Bootstrap support values (before the slash markers) and Bayesian posterior probabilities (after the slash markers) are indicated. The Indicate type species of the genus.

Fig. 2 Phylogenetic relationships of members of the *Atroporus* and *Neodictyopus* clades inferred from ITS and nucLSU sequences. Topology is from Maximum Likelihood analysis. Bayesian posterior probabilities (before the slash markers) and Bootstrap support values (after the slash markers) are indicated. \checkmark Indicate type species of the genus.

Fig. 3 Basidiomata of *Neodictyopus* and *Atroporus* species. **a**. *N. gugliottae* (DS1284). **a1**. pores. **b**. *N. dictyopus* (GAS272). **b1**. pores and reticulated stipe. **c**. *N. dictyopus* type (Bertero 1683). **c1**. pores and reticulated stipe. **d**. *N. atlanticus* (GAS622). **d1**. pores. **e**. *A. diabolicus* (DS1266). **e1**. context and tubes. **f**. *A. diabolicus* type (NY 730627). **f1**. context and tubes. **j**. *A. rufoatratus* (LDA138). **j1**. pores. **k**. *A. rufoatratus* type (NY 730938). **k1**. pores. Scale white bar = 1 cm. Scale black bar = 1 mm.

Fig. 4 Microscopical features of *Atroporus diabolicus*. **a**. tramal hyphae (DS1266). **b**. ellipsoid basidiospores (GAS679). \emptyset = clamp scar. Pointed apex of the hyphae. Scale bars=10 µm.

Fig. 5 Microscopical features of *Atroporus rufoatratus*. **a**. tramal hyphae (LDA138). **b**. ellipsoid basidiospores (LDA139). \emptyset = clamp scar. Pointed apex of the hyphae. Scale bars=10 µm.

Fig. 6 Microscopical features of *Neodictyopus atlanticus*. **a**. tramal hyphae. **b**. cylindrical basidiospores (GAS622). \emptyset = clamp scar. Scale bars=10 µm.

Fig. 7 Microscopical features of *Neodictyopus dictyopus*. **a**. tramal hyphae. **b**. cylindrical basidiospores (GAS281). **b1**. cylindrical basidiospores (BPI US207664, type of *N. dictyopus*). \emptyset = clamp scar. Scale bars=10 µm.

Fig.8 Microscopical features of *Neodictyopus gugliottae*. **a**. tramal hyphae (DS1284). **b**. cylindrical basidiospores (FB351). \emptyset = clamp scar. Scale bars=10 µm.

Fig 9 Microscopical features comparison of *Atroporus* and *Neodictyopus*. Schematic drawings of: **a**. context hyphae of *A*. *diabolicus* (DS1266). **b**. tramal hyphae *A*. *diabolicus* (DS1266). **c1** basidiospores of *A*. *diabolicus* (DS1266). **c2** basidiospores of *A.rufoatratus* (MP153). **d**. context hyphae of *N*. *gugliottae* (DS1284). **e**. tramal hyphae of *N*. *gugliottae* (DS1284). **e**. tramal hyphae of *N*. *gugliottae* (DS1284). Basidiospores of **f1**. *N*. *dictyopus* (GAS281), **f2**. (BPI US207664, type of *N*. *dictyopus*). **f3** *N*. *atlanticus* (GAS622). **f4** *N*. *gugliottae*. Scale black bar = 10 µm.

Fig.10 Microscopical features comparison of *Atroporus* and *Neodictyopus*. Photos of: **a**. context hyphae of *A. diabolicus* (DS1266). **b**. context hyphae of *N. gugliottae* (DS1284). **c**. tramal hyphae *A. diabolicus* (DS1266). **d**. tramal hyphae of *N. gugliottae* (DS1284). **e**. basidiospores of *A. diabolicus* (DS1266). **f**. basidiospores of *A. rufoatratus* (MP153). **g**. basidiospores of *N. atlanticus*. **h**. basidiospores of *N. gugliottae*. Scale black bar = 1 μ m.

Fig. 11 Atroporus and Neodictyopus species distribution based on biogeographical regionalization of Morrone (2014). A. A. diabolicus (Atlantic, Imerí, and Xingu-Tapajos provinces). B. A. rufoatratus (Atlantic, Imer, Pantepui, and Parana Forest provinces). C. N. atlanticus (Araucaria and Parana provinces). D. N. dictyopus (Cerrado province and Juan Fernandez arquipelago). E. N. gugliottae (Atlantic province).

Table 1 List of species, collections, and GenBank accession numbers for the ITS, nucLSU,

	and RPB2 sec	juences use	ed in the	phyloger	netic analys	ses.
--	--------------	-------------	-----------	----------	--------------	------

<u>Grandan</u>	Starsin /Sarahman Na	T analita	GenBank accesion No.				
Species	Strain/Specimen No.	Locality	ITS	nucLSU	RPB2		
Atroporus diabolicus	DS1266	Amazonas, Brazil	To be provided	To be provided	-		
A. rufoatratus	DS1311	Santa Catarina, Brazil	To be provided	To be provided	-		
	DS816	Santa Catarina, Brazil	To be provided	To be provided	To be provided		
	MP153	Santa Catarina, Brazil	To be provided	To be provided	-		
Neodictyopus atlanticus	GAS622	Sao Paulo, Brazil	To be provided	To be provided	To be provided		
	G97	Misiones, Argentina	To be provided	To be provided	-		
N. dictyopus	GAS60	Mato Grosso, Brazil	To be provided	To be provided	-		
	GAS272	Mato Grosso, Brazil	To be provided	To be provided	To be provided		
	GAS281	Mato Grosso, Brazil	To be provided	To be provided	To be provided		
N. gugliottae	DS1285	Santa Catarina, Brazil	To be provided	To be provided	To be provided		
	DS1286	Santa Catarina, Brazil	To be provided	To be provided	To be provided		
	FB351	Santa Catarina, Brazil	To be provided	To be provided	To be provided		
D. stereoides	Holonen	Finland	KC415179	KC415196	KC415202		
Echinochaete russiceps	WD674	Japan	AB462310	AB368065	AB368123		
Favolus brasiliensis	INP241452	Brazil	AB735977	AB735953	-		
	TENN10242	Costa Rica	AB735976	AB368097	-		
F. emerici	WD2343	Japan	AB587626	AB368089	AB368146		
	WD2379	Japan	AB587628	AB587619	AB368147		
F. pseudobetulinus	TFM F-27567	Japan	AB587644	AB587639	-		
-	TFM F-27626	Japan	AB587645	AB587640	-		

	TRTC51022	Canada	AB587629	AB587620	-
F. roseus	PEN33	Malaysia	AB735975	AB368099	AB368156
Mycobonia flava	TENN59088	Argentina	AY513571	AJ487933	-
	TENN57579	Costa Rica	AY513570	AJ487934	-
Neofavolus alveolaris	WD2340	Japan	AB735970	AB368077	AB368135
	WD2358	Japan	AB587624	AB368079	AB368136
Nf. cremeoalbidus	TUMH 50009	Japan	AB735957	AB735980	-
Nf. mikawai	TUMH 50005	Japan	AB735964	AB735944	-
Nedatronia. sinensis	Cui 9434	China	JX559271	JX559282	JX559319
	Dai 11921	China	JX559272	JX559283	JX559320
Polyporus americanus	JV 0809-104	USA	KC572003	KC572042	-
	JV 0509-149	USA	KC572002	KC572041	-
P. badius	WD2341	Japan	AB587625	AB368083	AB368140
P. conifericola	WD1839	Japan	AB587634	AB368101	
P. dictyopus	TENN 58930	Paraguay	AF516562	-	
	TENN 59385	Belize	AF516561	AJ487945	
	WD1845	Japan	-	AB368085	AB368142
	WD2342	Japan	-	AB368086	AB368143
	WD2345	Japan	-	AB368087	AB368144
	UOC MINNP MK68	Sri Lanka	KR907877	-	-
	TENN59089	Argentina	AF518760	-	-
	SFC070618-06	South Korea	-	HM003899	-
	SFC070915-26	South Korea	-	HM003900	-
Poyporus fraxinicola	Dai 2494	China	KC572023	KC572062	-
Polyporus guianensis	TENN58404	Venezuela	AF516566	AJ487948	-
	TENN59093	Argentina	AF516564	AJ487947	-
Polyporus leprieurii	TENN58597	Costa Rica	AF516567	AJ487949	AB368150
Polyporus melanopus	MJ 372-93	Czech	KC572026	KC572065	-
	H 6003449	Finland	JQ964422	KC572064	-

Polyporus squamosus	MUCL 30721	Belgium	AB587630	AB368094	-
	AFTOL ID-704	USA	DQ267123	AY629320	DQ408120
Polyporus tubaeformis	WD1839	Japan	AB587634	AB368101	AB368158
Polyporus tuberaster	WD2382	Japan	AB474086	AB368104	AB368161
Polyporus udus	WD1878	Japan	-	AB368108	AB368165
Polyporus umbellatus	WD719	Japan	-	AB368109	AB368166
Polyporus varius	WD619	Japan	AB587635	AB368110	AB368167
Trametes hirsuta	RLG5133T	USA	JN164941	JN164801	JN164854
Trametes versicolor	FP135156sp	USA	JN164919	JN164809	JN164850

Table 2 Summary of data sets of ITS rDNA, nucLSU rDNA, and RPB2

Properties	First Datasets								
	ITS1	5.8 S	ITS2	nucLSU	rpb2 1st	rpb2 2nd	rpb2 3rd	rpb2 intron	Indels
Model selected	TIM2+G	K80+I	TrN+G	TIM2+I+G	TIM2+G	K80+G	TIM2+I+G	TrNef+G	F81-Like
Likelihood score	2570.1624	- 404.9665	2405.6239	- 3126.5433	- 1556.8426	- 1155.1076	- 5287.9108	- 1218.2834	-
Base frequencies									
Freq. A =	0.2000	Equal	0.2021	0.2588	0.2727	Equal	0.1258	Equal	_
Freq. C =	0.2134	Equal	0.2194	0.1899	0.2484	Equal	0.3391	Equal	_
Freq. G =	0.2417	Equal	0.2124	0.3029	0.3099	Equal	0.3181	Equal	_
Freq. T =	0.3450	Equal	0.3661	0.2484	0.1690	Equal	0.2171	Equal	_
Proportion of invariable sites	_	8.490	_	5.340	_	_	0.0320	_	_
Gamma shape	6.310	_	5.910	4.600	2.370	1.480	4.2260	2.4110	-



Fig 1

0.05

51









Fig 4



Fig 5



Fig. 6



Fig. 7









Fig. 10





5. CONSIDERAÇÕES FINAIS

Polyporus é um gênero polifilético de distribuição pantropical que inclui vários grupos morfológicos e complexos de espécies amplamente citados na literatura. A revisão desses problemas taxonômicos a partir de uma abordagem que integre e reavalie morfologias e aspectos ecológicos (distribuição e substrato) em um contexto filogenético é necessária para reorganizar os membros do grupo e dar um tratamento taxonômico de uma maneira menos artificial.

Análises morfológicas detalhadas que incluam a inspeção do sistema hifal de maneira independente dos tubos, contexto e píleo, usando a separação das hifas com 3% NaOH, assim também como a comparação dos formatos dos basidióporos (valor Q), como foi usado neste estudo taxonômico, pode auxiliar na busca de padrões morfológicos correspondentes a diferentes linhagens nos diferentes clados já reconhecidos como por exemplo "Melanopus".

Levando em consideração que as relações filogenéticas das espécies conhecidas de *Polyporus s.l.* com a espécie tipo do gênero, *P. tuberaster*, não tem sido esclarecidas, também análises filogenéticas *multiloci* são necessárias para o entendimento do grupo e inclusive para a circunscrição de *Polyporus s.s.*

Por fim, sobre o complexo *P. dictyopus*, ainda há a necessidade de revisar os sinônimos heterotípicos propostos a partir de espécimes coletados fora da região Neotropical.

REFERÊNCIAS

Dai YC, Xue HJ, Vlasák J, Rajchenberg M, Wang B, Zhou LW. 2014. Phylogeny and global diversity of Polyporus group Melanopus (Polyporales, Basidiomycota). Fungal Diversity 64:133–144.

Decock C, Amalfi M, Robledo G, Castillo G. 2013. Phylloporia nouraguensis, an undescribed species on Myrtaceae from French Guiana. Cryptogamie Mycol 34:15–27.

Carranza-Velásquez, J. & A. Ruiz-Boyer. 2005. Checklist of polypores of Costa Rica. Revista Mexicana de Micología 20:45–52.

Ejechi BO, Obuekwe CO, Ogbimi AO (1996) Microchemical studies of wood degradation by brown rot and white rot fungi in two tropical timbers. Int Biodeterior Biodegrad 38:119-122.

Gilbertson RL, Ryvarden L. 1987. North American polypores 2. Fungiflora, Oslo.

Kirk PM, Cannon PF, Minter DW, Stalpers JA. 2008. Ainsworth & Bisby's Dictionary of the Fungi. 10rd edition, CAB International, United Kingdom.

Krüger D, Petersen RH, Hughes KW. 2006. Molecular phylogenies and mating study data in Polyporus with special emphasis on group "Melanopus" (Basidiomycota). Mycological Progress 5: 185–206.

Munsell. 1975. Soil Colors Charts. Baltimore, Maryland: Munsell Color.

Núñez M, Ryvarden L. 1995. Polyporus (Basidiomycotina) and related genera. Synop Fungorum 10:1–85.

Núñez M, Ryvarden L (2001) East Asian polypores. Synop Fungorum 14:170–522

Ryvarden L, Johansen I. 1980. A preliminary polypore flora of East Africa. Fungiflora, Oslo.

Ryvarden L, Gilbertson R. L. 1993. European polypores. Part 1. Fungiflora: Oslo, Norway.

Silveira RMB, Wright JE (2005) The taxonomy of Echinochaete and Polyporus s. str. in South America. Mycotaxon 93:1–59.

Thiers B. Index Herbariorum: a global directory of public herbaria and associated staff. New York Garden's Virtual Herbarium. In: New York Garden's Virtual Herbarium. http://sweetgum.nybg.org/ih/.