INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA - INPA
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA
DOUTORADO EM BOTÂNICA

Taxonomia, biologia e produção de semente-inóculo de *Panus strigellus*, um cogumelo comestível da Amazônia

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Manaus, Amazonas
Setembro, 2012

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Tese apresentada ao Instituto Nacional de Pesquisas da Amazônia como parte dos requisitos para obtenção do título de Doutor em BOTÂNICA, área de concentração em DIVERSIDADE, CONSERVAÇÃO E USO DA FLORA AMAZÔNICA.

Manaus, Amazonas

Setembro, 2012

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V297 Vargas-Isla, Ruby
Taxonomia, biologia e produção de semente-inóculo de *Panus strigellus*, um cogumelo comestível da Amazônia / Ruby Vargas-Isla.--- Manaus : [s.n.], 2012.
xiii, 124 f. : il. color.

Tese (doutorado) --- INPA, Manaus, 2012
Orientador : Noemia Kazue Ishikawa
Coorientador : Ricardo Marenco
Área de concentração : Diversidade, conservação e uso da Flora Amazônica

1. Etnomicologia. 2. Ciclo de vida. 3. *Mating type*. 4. Semente-inóculo 5. Taxonomia. 6. *Lentinus strigellus*. I. Título.

CDD 19. ed. 589.22

Sinopse:

Estudaram a taxonomia, as características biológicas e a produção de semente-inóculo de *Panus strigellus*, um cogumelo comestível da Amazônia.

Palavras-chave:
Etnomicologia; Ciclo de vida; *Mating type*; Semente-inóculo; Taxonomia; *Lentinus strigellus*

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Dedico este trabajo a mis padres Angela E. y Víctor Manuel y a mis hermanos Angela Vanessa y Marcos Alberto Daniel, por ser los más amorosos, los más hábiles, los más valientes, los más fuertes y sobretodo los más sabios, que en todo momento me dieron fortaleza para conseguir la victoria que tanto anhelamos.

ꕥ

AGRADECIMENTOS

À Deus que sempre protege e guia meu caminho.

À Natureza por me permitir enxergar suas maravilhas.

Ao Instituto Nacional de Pesquisas da Amazônia (INPA) por abrir suas portas e contribuir na minha formação profissional em Botânica. À antiga Coordenação de Pesquisas em Tecnologia de Alimentos (CPTA) e à Coordenação de Biodiversidade (CBIO) do INPA.

Ao curso de Botânica pelo apoio efetivo na minha formação acadêmica.

Ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) pela bolsa de estudo.

À Dra. Noemia Kazue Ishikawa pela orientação, atenção, oportunidades, confiança, apoio e ajuda no desenvolvimento deste trabalho.

Ao meu coorientador, Dr. Ricardo Marenco pelo apoio e por ter partilhado conhecimentos importantes à minha formação.

Aos professores do curso de Botânica pelas oportunidades e inúmeros ensinamentos que contribuíram enormemente para minha formação profissional.

Ao Instituto de Botânica do Estado de São Paulo, na pessoa da Dra. Marina Capelari pelo ensino e apoio no desenvolvimento da minha pesquisa.

Ao Instituto Micológico de Tottori no Japão, por permitir realizar estágio de aperfeiçoamento nos temas relacionados a sistemas de cruzamento e taxonomia de basidiomicetos, nas pessoas do Diretor Yukitaka Fukumasa e os Professores Keisuke Tokimoto e Eiji Nagasawa.

À Universidade de Tottori no Japão por permitir realizar a capacitação na análise molecular da espécie trabalhada, na pessoa do Professor Nitaro Maekawa.

Agradeço aos Professores Doutores que participaram da avaliação do projeto de tese, aula de qualificação e/ou defesa da tese.

Aos estudantes e estagiários do Laboratório de Microbiologia de Alimentos do Grupo de Pesquisas de Cogumelos da Amazônia, cujos momentos compartilhados têm sido muito especiais.

Minha profunda gratidão aos familiares, amigos, colegas e pessoas que de uma forma ou outra contribuíram na execução deste trabalho.

Aos meus irmãos Vanessa e Marcos pelo exemplo da alegria e habilidade, pelos bons pensamentos e energias positivas, por estarem sempre presentes acompanhando meus avanços e dividindo as alegrias.

Aos meus pais Angela e Manolo pelo exemplo de valentia e de força, pelo amor e apoio constante e desmedido e pelos conselhos.

Muito obrigada!

APRESENTAÇÃO

Neste manuscrito são apresentados os resultados obtidos durante o curso de Doutorado em Botânica, realizado no Instituto Nacional de Pesquisas da Amazônia, no período de outubro de 2008 a setembro de 2012.

O texto está dividido em: Introdução; Capítulo 1 – Etnomicologia da Amazônia; Capítulo 2 – Taxonomia de *Panus strigellus*; Capítulo 3 – Elaboração de semente-inóculo de *P. strigellus*; Capítulo 4 – Características biológicas de *P. strigellus*; e Considerações finais.

A Introdução e as Considerações finais estão redigidas na forma de texto, sob as normas para apresentação de trabalhos de conclusão de curso do INPA. Os capítulos 1, 2, 3 e 4 estão redigidos na forma de artigo científico, de acordo com as normas das revistas às quais foram e/ou serão submetidos os artigos.

RESUMO

A Amazônia apresenta condições climáticas que favorecem o crescimento de um grande número de espécies de cogumelos. Uma dessas espécies é *Panus strigellus* com potencial de uso na indústria alimentícia. Este trabalho apresenta estudos sobre a taxonomia, biologia e produção de semente-inóculo de *P. strigellus*. Esta espécie comestível apresenta características macroscópicas semelhantes a *P. lecomtei*. Para tanto, evidências combinadas de estudos morfológicos e moleculares foram utilizadas para confirmar a identificação das espécies. Detalhes de caracteres morfológicos para *P. strigellus*, assim como a ocorrência no Estado do Amazonas são descritas pela primeira vez. Também a distribuição geográfica nas Américas para ambas as espécies foi revisada. A produção de semente-inóculo conhecida como *Spawn* em inglês, *Tanekin* em Japonês e *Blanc* em francês é o primeiro passo para o cultivo de cogumelos. Neste trabalho foram obtidas com sucesso formulações para semente-inóculo de *P. strigellus* utilizando-se resíduos agroflorestais regionais. Também foram descritas características biológicas de *P. strigellus*, tais como a preservação micelial, a atividade enzimática extracelular, o ciclo de vida, incluindo o tipo de cruzamento (*mating type*). Para tanto, quatro métodos para a manutenção da cultura micelial foram estudados. Isolados desta espécie apresentaram atividade enzimática extracelular de amilase, celulase, esterase e lipase, em meio sólido específico. O ciclo de vida foi completado em condições laboratoriais e o tipo de cruzamento de *P. strigellus* foi confirmado como tetrapolar. Experimentos de cruzamento entre monocários de *P. strigellus* e de *P. lecomtei* foram realizados pela primeira vez, sendo que estes apresentaram incompatibilidade.

Palavras-chave: Etnomicologia, Ciclo de vida, *Mating type*, Semente-inóculo, *Lentinus strigellus*

ABSTRACT

The Amazon climate favours the growth of numerous mushroom species. One of these species, *Panus strigellus*, has potential applications in food industries. This work presents studies about the taxonomy, biology and spawn's production of *P. strigellus.* This edible mushroom presented macroscopic characteristics similar to those of *P. lecomtei*. Combined evidences of morphological and molecular studies were used to confirm species identification. Morphological characters details for *P. strigellus* as well as its occurrence to Amazonas State are described at the first time. Geographical distribution in the Americas was reviewed for both species. The spawn production, known as Spawn in English, *Tanekin* in Japanese and *Blanc* in French, is the first step for mushrooms cultivation. In this work the spawn formulations of *P. strigellus* were obtained successfully using regional agroforestry residues. Also, the biological characteristics of *P. strigellus* were described, such as mycelial preservation, extracellular enzymatic activity, life cycle including the mating type. For this, four methods to mycelial culture maintenance were studied. Isolates of this species showed amylase, cellulase, esterase and lipase extracellular enzymatic activity in specific solid media. The life cycle under laboratory conditions was completed and the tetrapolar mating system of *P. strigellus* was confirmed. Experiments of monokaryons crossing between *P. strigellus* and *P. lecomtei* were accomplished at the first time, these species showed incompatibility.

Keywords: Ethnomycology, Life cycle, Mating type, Spawn, *Lentinus strigellus*

Sumário

INTRODUÇÃO

INTRODUÇÃO

A importância dos fungos para a manutenção das florestas e seu potencial de uso biotecnológico é indiscutível. Entretanto, o número de pesquisas desenvolvidas na área de micologia na Amazônia está em descompasso com a enorme diversidade de espécies existentes na Região Amazônica. Estima-se que existam mais de 1,5 milhão de espécies de fungos desconhecidas para a ciência (Hawksworth 2001; Webster e Weber 2007). Destes, vários pesquisadores acreditam que a maior parte encontra-se em ecossistemas tropicais (Hawksworth 2001; Hyde 2001; Webster e Weber 2007), como a floresta amazônica. De acordo com dados do Catálogo de Plantas e Fungos do Brasil de 2010, existem 519 espécies de fungos reportadas para a Amazônia (Forzza *et al.* 2010).

A comestibilidade de espécies de cogumelos da Amazônia tem sido reportada em estudos etnomicológicos de povos indígenas como os Yanomami, Nambiquara, Caiabi, Txicão e Txucurramãe no Brasil (Fidalgo 1965, 1968; Prance 1972, 1973, 1984; Fidalgo e Prance 1976; Fidalgo e Hirata 1979); indígenas Uitoto, Muinane e Andoke na Colômbia (Vasco-Palacios *et al.* 2008) e indígenas Hotï na Venezuela (Zent *et al.* 2004).

Relatos da comestibilidade de algumas espécies do gênero *Lentinus sensu lato* (Pegler 1983) foram descritos nos trabalhos de etnomicologia. Embora não descrito na literatura, existe um raro registo sobre o relato da comestibilidade de *Panus strigellus* (Berk.) Overh., que pertence ao subgênero *Panus* (Pegler 1983), se encontra em uma amostra coletada por Prance em 1973 no Estado de Roraima (Brasil), depositado no Herbário do Instituto Nacional de Pesquisas da Amazônia (INPA), onde anotações do *voucher* indican que *P. strigellus* é cozido e consumido pelos Yanomami, e os indígenas não o distinguem de *Panus lecomtei* (Fr.) Corner.

Deste modo, dando continuidade aos estudos iniciados em 2006, este trabalho teve como objetivo realizar estudos taxonômicos de *P. strigellus* e descrever a sua biologia e produção de semente-inóculo desta espécie.

Assim, no Capítulo 1, se apresenta uma compilação de dados com base na etnomicologia dos povos da Amazônia, bem como a atualização dos nomes científicos e as espécies que foram coletadas na região de Manaus.

A taxonomia e distribuição geográfica de *P. strigellus* é descrita no Capítulo 2, mostrando as diferenças entre *P. strigellus* e *P. lecomtei* utilizando as características

morfológicas e análises moleculares, uma vez que ambas as espécies ocorrem no mesmo hábitat e apresentam características macroscópicas similares.

A produção de semente-inóculo conhecida como *Spawn* em inglês, *Tanekin* em Japonês e *Blanc* em francês é o primeiro passo para o cultivo comercial de cogumelos comestíveis, sendo a produção da semente-inóculo o principal desafio para os produtores de cogumelos comerciais. No Capítulo 3 são descritas as formulações de substratos para obtenção da semente-inóculo de *P. strigellus*.

As características biológicas de *P. strigellus* tais como a manutenção da cultura micelial, a produção de enzimas extracelulares, o ciclo de vida da espécie e o estudo de *mating type* foram descritas no Capítulo 4.

OBJETIVOS

Objetivo geral

Realizar estudos taxonômicos de *P. strigellus* e a sua biologia e produção de semente-inóculo.

Objetivos específicos

- Realizar um levantamento das espécies de cogumelos comestíveis da Amazônia com base nos relatos de etnoconhecimento;
- Realizar estudos taxonômicos de *P. strigellus* com auxílio de características morfológicas e análises moleculares;
- Avaliar o uso de resíduos agroflorestais da Amazônia Central para a elaboração de formulações para semente-inóculo de *P. strigellus*;
- Descrever as características biológicas de *P. strigellus*.

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Capítulo 1: ETNOMICOLOGIA DA AMAZÔNIA

Na Amazônia Brasileira, estudos etnomicológicos a partir das décadas de 60 e 70 relatam o consumo de espécies de cogumelos por grupos indígenas como os Yanomami, Tucanos, Nambiquara, Caiabi, Txicão e Txucurramãe. Estudos mais recentes relatam o etnoconhecimento de indígenas Uitoto, Muinane e Andoke da Amazônia colombiana, indígenas Hotï da Amazônia venezuelana, assim como povos rurais e ribeirinhos da Amazônia peruana. Neste trabalho, foram atualizados os nomes científicos dos relatos etnomicológicos compilados.

ARTIGO 1

Vargas-Isla R, Ishikawa NK, Py-Daniel V. Etnomicologia dos povos indígenas da Amazônia.

Manuscrito foi submetido a Ciência & Ambiente em 03 de setembro de 2012

ETNOMICOLOGIA DOS POVOS INDÍGENAS DA AMAZÔNIA

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Etnomicologia é um ramo da etnologia (Wasson 1957), que estuda a relação e as interações no contexto biológico, econômico e social, os usos históricos e o conhecimento dos fungos por diferentes etnias, raças ou nacionalidades. Em etnomicologia os estudos se concentram em macrofungos comestíveis e venenosos.

O conhecimento etnomicológico do leste europeu e asiático do passado permite-nos desfrutar das principais espécies de cogumelos hoje encontrados nos mercados e restaurantes do mundo. No entanto, é pouco divulgado que em países Amazônicos também existem espécies de cogumelos consumidos pelas etnias e povos ribeirinhos.

O Tratado Internacional sobre os Recursos Fitogenéticos para a Alimentação e a Agricultura (FAO 2001) relata que durante milênios, os homens utilizavam mais de 10 mil espécies de plantas, no entanto atualmente este número reduziu-se para 150 espécies cultivadas, destas, apenas quatro espécies, o arroz, o trigo, o milho e a batata, satisfazem as nossas necessidades energéticas. Acreditamos que o mesmo acontece com os cogumelos. Sabe-se por literatura (Sánchez 2004) que cerca de 2 mil espécies são comestíveis, no entanto apenas quatro espécies, Agaricus bisporus, Lentinula edodes, Pleurotus spp. e Auricularia sp., são cultivadas em diversos países. Vendo-se por este ângulo, a situação é pessimista. Entretanto, nas últimas décadas, o mercado gastronômico vem buscando com inquietude novos sabores, o que tem levado ao resgate e/ou encontro de novas opções de espécies alimentícias, inclusive alguns cogumelos.

Neste sentido, aqui revisamos e atualizamos as informações sobre o conhecimento etnomicológico dos povos da Amazônia.

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Introdução

Existem mais de 200 gêneros de macrofungos utilizados por populações no mundo, principalmente pelas suas propriedades comestíveis e cerca de 100 espécies de cogumelos nativos podem ser cultivadas (Boa 2004).

As principais categorias de uso dos macrofungos têm origens muito antigas e estão relacionadas com a ingestão de algumas espécies.

Uso gastronômico – compreende a procura e o consumo de espécies comestíveis que complementam a dieta de muitos povos.

Uso medicinal – desenvolvido principalmente no extremo oriente e associado à Polyporales e espécies afins. Assume uma enorme relevância nas práticas tradicionais da medicina para tratamento de doenças crônicas, como combate ao envelhecimento e regularização de funções vitais.

Uso alucinógeno – relacionado com a presença de substâncias que atuam no sistema nervoso, alterando a percepção sensorial, tanto em relação a estímulos externos como em relação ao próprio corpo, e cujo consumo é tradicionalmente associado a ritos xamânicos em muitas culturas da Ásia, da África e da América.

Toxicidade – relacionado ao conhecimento adquirido devido a confusões das espécies comestíveis nativas com espécies tóxicas ou misturas acidentais.

Etnomicologia da Amazônia

Na década de 60 os povos indígenas foram considerados como não micófilos, mas que utilizavam termos específicos para diferenciar os fungos de outros micro-organismos. O trabalho do botânico brasileiro Oswaldo Fidalgo publicado em 1965, intitulado "*Conhecimento micológico dos índios brasileiros*", é considerado o ponto de partida para os registros de etnomicologia no Brasil. Importantes contribuições neste campo no Brasil foram realizadas pelo botânico e ecólogo inglês Guillean Tolmie Prance as quais foram publicadas junto a trabalhos de Botânica em 1972, 1973 e 1984. Os trabalhos de O. Fidalgo e G.T. Prance em 1976 e O. Fidalgo e José Hirata em 1979, que relatam o consumo de espécies de cogumelos por grupos indígenas, como os Yanomami da Amazônia brasileira.

Recentemente a bióloga Aída Marcela Vasco-Palacios e colaboradores em trabalhos de etnomicologia de 2008 relatam o consumo de cogumelos por indígenas Uitoto, Muinane e Andoke da Amazônia colombiana. Também há revisões de Egleé Zent e colaboradores em 2004 do consumo de cogumelos pelos indígenas Hotï da Amazônia venezuelana e relatos do Engenheiro Florestal Luis Bardales em 1997 pelos povos rurais e ribeirinhos da Amazônia peruana.

As espécies relatadas nos trabalhos acima citados estão listadas na Tabela 1.

Atualização dos nomes científicos

Os fungos pertencem a um grupo monofilético, quer dizer que têm origem em um ancestral comum, apresentando variabilidade morfológica. O caráter monofilético foi confirmado por estudos avançados de filogenia utilizando as informações contidas no DNA (Hibbett e Vilgalys 1993, 1995; Moncalvo 2000), o que levou a mudança nos nomes

científicos e o rearranjo das espécies em níveis hierárquicos maiores. Com isso, o uso e a aplicação dos nomes atualizados das espécies de cogumelos beneficia a ciência, reduzindo a confusão e a duplicação de esforços, melhorando a confiabilidade dos resultados publicados.

Neste sentido, atualizamos os nomes científicos dos relatos etnomicológicos compilados utilizando a classificação do Index Fungorum (http://www.indexfungorum.org) e Mycobank (http://www.mycobank.org).

Foram listadas 34 espécies de cogumelos comestíveis (Tabela 1). Destas, dez espécies foram encontradas na região de Manaus: *Auricularia delicata* (Mont.) Henn., *Auricularia polytricha* (Mont.) Sacc., *Lentinula raphanica* (Murrill) Mata & R.H. Petersen, *Lentinus crinitus* (L.) Fr., *Panus lecomtei* (Fr.) Corner, *Panus velutinus* (Fr.) Sacc., *Pleurotus* sp., *Polyporus tenuiculus* (P. Beauv.) Fr., *Polyporus* sp. e *Pycnoporus sanguineus* (L.) Murrill. Os gêneros mais relatados pelos grupos indígenas e ribeirinhos da Amazônia foram *Auricularia*, *Lentinula*, *Lentinus sensu stricto* Pegler (1983), *Panus, Pleurotus* e *Polyporus*. Considerando-se os nomes relatados a percentagem de atualização dos nomes científicos dos fungos alcançou um total de 59%.

Tabela 1 – Nomes científicos de cogumelos comestíveis consumidos por grupos étnicos da Amazônia

País	Grupo étnico	Espécies reportadas	Nome científico atual	Autor
Brasil	Yanomami	*Favolus brasiliensis* (Fr.) Fr.	*Polyporus tenuiculus* (P. Beauv.) Fr.	Prance 1972;
	(Waukás -	*Favolus tessellatus* Mont.	*Polyporus tenuiculus* (P. Beauv.) Fr.	Prance 1973;
	Roraima)	*Neoclitocybe bissiseda* (Bres.) Sing.	*Neoclitocybe byssiseda* (Bres.) Sing.	Prance 1984
		Polyporus stipitarius Berk. & M.A.Curtis	*Polyporus tricholoma* Mont.	
Brasil	Yanomami	*Coriolus zonatus* (Nees) Quélet	*Trametes ochracea* (Pers.) Gilb. & Ryvarden	Fidalgo e Prance
	(Sanuma -	*Favolus brasiliensis* (Fr.) Fr.	*Polyporus tenuiculus* (P. Beauv.) Fr.	1976; Prance 1984
	Roraima)	*Favolus brunneolus* Berk & M.A.Curtis	*Echinochaete brachypora* (Mont.) Ryvarden	
		Favolus striatulus Ellis & Everh.	*Polyporus alveolaris* (DC.) Bondartsev. & Sing.	
		Favolus tessellatus Mont.	*Polyporus tenuiculus* (P. Beauv.) Fr.	
		Gymnopilus hispidellus Murrill	-	
		Hexagona subcaperata (Murr.) Sing.	*Polyporus tenuiculus* (P. Beauv.) Fr.	
		Hydnopolyporus palmatus (Hook.) O. Fidalgo	-	
		Lactocollybia aequatorialis Singer	-	
		Lentinus crinitus Fr.	*Lentinus crinitus* (L.) Fr.	
		Lentinus glabratus Mont.	-	
		Lentinus sp.	-	
		Lentinus velutinus Fr.	*Panus velutinus* (Fr.) Sacc.	

		Panus rudis Fr.	*Panus lecomtei* (Fr.) Corner	
		Pholiota bicolor (Speg.) Singer	-	
		Pleurotus concavus (Berk.) Singer	-	
		Pleurotus sp.	-	
		Polyporus aquosus Henn.	-	
		Polyporus sp.	-	
		Polyporus tricholoma Mont.	-	
Brasil	Yanomami	*Collybia pseudocalopus* (Henn.) Singer	-	Prance 1984
	(Tootobi -	*Collybia subpruinosa* (Murrill) Dennis	-	
	Amazonas)	*Favolus brasiliensis* (Fr.) Fr.	*Polyporus tenuiculus* (P. Beauv.) Fr.	
		Gymnopilus earlei Murrill	-	
		Lentinus sp.	-	
		Lepiota cheimonoceps (Berk. & M.A.Curt.) Sacc.	*Leucocoprinus cepistipes* (Sowerby) Pat.	
Brasil	Tucano (Alto rio	*Agaricus* sp.	-	Berkeley 1856
	Negro)	*Fistulina* sp.	-	
Brasil	Nambiquara	*Gloeoporus conchoides* Mont.	*Gloeoporus thelephoroides* (Hook.) G. Cunn.	Fidalgo 1965;
	(Mato Grosso)	*Polyporus pes-simiae* Berk.	-	Fidalgo 1968
		Polyporus sapurema Möller	-	
		Polyporus sp.	-	

Brasil	Txicão	*Auricularia fuscosuccinea* (Mont.) Sacc.	*Auricularia fuscosuccinea* (Mont.) Henn.	Fidalgo e Hirata
		Lentinus crinitus (L.) Fr.	-	1979
Brasil	Txucarramãe	*Auricularia fuscosuccinea* (Mont.) Sacc.	*Auricularia fuscosuccinea* (Mont.) Henn.	Fidalgo e Hirata
		Pycnoporus sanguineus (L.) Murrill	-	1979
		Trametes cubensis (Mont.) Sacc.	-	
		Trichaptum trichomallum (Berk. & Mont.) Murrill	-	
Colômbia	Uitoto, Muinane	*Auricularia delicata* (Mont.) Henn.	-	Vasco-Palacios *et*
	e Andoke	*Lentinula raphanica* (Murrill) Mata & R.H. Petersen	-	*al.* 2008
	(Caquetá)	*Lentinus crinitus* (L.) Fr.	-	
		Lentinus scleropus (Pers.) Fr.	-	
		Lentinus strigosus (Schwein.) Fr.	*Panus lecomtei* (Fr.) Corner	
Peru	População rural	*Auricularia polytricha* (Mont.) Farl.	*Auricularia polytricha* (Mont.) Sacc.	Bardales 1997
	(Loreto)	*Collybia subpruinosa* (Murrill) Dennis	-	
		Coriolus zonatus (Nees) Quél.	*Trametes ochracea* (Pers.) Gilb. & Ryvarden	
		Favolus brasiliensis (Fr.) Fr.	*Polyporus tenuiculus* (P. Beauv.) Fr.	
		Favolus brunneolus Berk & M.A.Curtis	*Echinochaete brachypora* (Mont.) Ryvarden	
		Favolus tesselatus Mont.	*Polyporus tenuiculus (P. Beauv.) Fr.*	
		Lentinus crinitus (L.) Fr.	-	
		Lentinus glabratus Mont.	-	

		Lentinus velutinus Fr.	*Panus velutinus* (Fr.) Sacc.	
Peru	Comunidade do rio Itaya	*Polyporus tenuiculus* (P. Beauv.) Fr.	*Polyporus tenuiculus (P. Beauv.) Fr.*	Vargas-Flores e Gordiano comunicação pessoal
Venezuela	Hotï (Amazonas)	*Agaricus* sp. *Amaroderma* cf. *omphalodes* (Berk.) Torrend *Auricularia delicata* (Fr.) Henn. *Auricularia polytricha* (Mont.) Farl. *Datronia caperata* (Berk.) Ryvarden *Lentinus crinitus* (L.: Fr.) Fr. *Lenzites acuta* Berk. *Macrocybe titans* (H.E. Igelow & Kimbr.) Pegler, Lodge & Nakasone *Pleurotus* sp. *Polyporus tenuiculus* Beauv.: Fr. *Pycnoporus sanguineus* (Fr.) Murr. *Thamnomyces chordalis* Fr.	- *Amauroderma* cf. *omphalodes* (Berk.) Torrend *Auricularia delicata* (Mont.) Henn. *Auricularia polytricha* (Mont.) Sacc. *Coriolopsis caperata* (Berk.) Murrill *Lentinus crinitus* (L.) Fr. *Daedalea flavida* Lév. - - *Polyporus tenuiculus* (P. Beauv.) Fr. *Pycnoporus sanguineus* (L.) Murrill -	Zent *et al.* 2004

(-) não houve mudanças

Panus strigellus e *Panus lecomtei*

As espécies *P. strigellus* (Berk.) Overh. e *P. lecomtei* (Fr.) Corner pertencem ao Filo Basidiomycota, Subfilo Agaricomycotina, Classe Agaricomycetes, Ordem Polyporales e Família Polyporaceae. O gênero *Panus* apresenta 25 espécies amplamente distribuídas no mundo.

Para a atualização de *P. strigellus*, sinonímia de *Lentinus strigellus* Berk., o epíteto "*strigellus*" é retido como o mais antigo validando a combinação de *P. strigellus*. No entanto, foi necessária a mudança do epíteto "strigosus" da espécie *Lentinus strigosus* (Schwein.) Fr., uma vez que *P. strigosus* Berk. & M.A. Curtis é a sinonímia de *Lentinus levis* (Berk. & M.A. Curtis) Murrill. Considerando que para as sinonímias a prioridade do nome mais antigo publicado, *P. lecomtei* é atualmente aceito. Vale lembrar que nos trabalhos realizados na Amazônia nos anos 1960 e 1970, *P. lecomtei* foi registrado como *Panus rudis* Fr.

Nas Américas, *P. strigellus* e *P. lecomtei* ocorrem no mesmo hábitat, sob vegetação densa e em áreas abertas. A similaridade das características macromorfológicas podem levar a confusão na identificação das mesmas. No passado, *P. strigellus* era considerado como sinônimo de *P. lecomtei* (Pegler, 1983). Entretanto, com base nas características micromorfológicas Pegler (1983) as considerou como duas espécies distintas.

A comestibilidade de *P. lecomtei* é conhecida em vários países. No Japão é conhecido como *Aragekawakitake*. Em Roraima (Brasil), nas aldeias Yanomami do Auaris e Xitei/Xidea, o cogumelo *P. lecomtei* é conhecido como *Shio-koni-amo* e *Kasikoirima*, respectivamente.

Por outro lado, um dos raros registros sobre a comestibilidade de *P. strigellus* se encontra em uma amostra coletada por Prance em 1973 no Estado de Roraima (Brasil) e depositada no Herbário do Instituto Nacional de Pesquisas da Amazônia (INPA), onde foi

encontrado um *voucher* número 20016A indicando que *P. strigellus* cresce sobre tronco morto, é cozido e consumido pelos Sanuma do povo Yanomami, e os indígenas não o distinguem de *P. lecomtei*. Em amostras coletadas na Amazônia realizaram-se estudos taxonômicos e os dados morfológicos, moleculares e biológicos levaram a confirmação das espécies *P. lecomtei* e *P. strigellus*, descrevendo novas caracterícticas microscópicas de *P. strigellus*. O estudo biológico levou a confirmação do sistema de cruzamento tetrapolar das espécies acima mencionadas.

Sobre os povos "micófilos"

Na etnomicologia dois termos são utilizados: micofilia e micofobia, que literalmente significam "afinidade com fungos" e "medo de fungos", respectivamente. Nas sociedades ou culturas micófilas os fungos são apreciados e existe uma forte tradição de uso popular. Já as sociedades micofóbicas apresentam uma menor estima pelos fungos e geralmente os vêem com certo receio.

Os povos micófilos que vivem nas florestas buscam incluir os cogumelos na dieta pelas suas propriedades nutricionais, funcionais e gastronômicas. Os registros etnomicológicos demonstram que existem doze grupos étnicos na Amazônia que consomem esporadicamente cogumelos, assim como as populações rurais e ribeirinhas.

Outras revisões de literatura focando o conhecimento dos povos indígenas do Brasil consideram estes como povos não micófilos, mas parecem reconhecer os fungos como um grupo diferente de organismos, sugerindo que existe uma denominação para os fungos como um táxon de nível superior (Góes-Neto e Bandeira 2002).

Também foi comparada a importância cultural e ecológica dos fungos entre os povos Mesoamericanos e da Amazônia, considerando micófilos e micofóbicos, respectivamente

(Mapes *et al.* 2002). O reconhecimento dos fungos pelos indígenas como um táxon foi corroborado e foi registrado que os mesmos termos são utilizados para mais de uma espécie ou gênero (Cardoso *et al*. 2010).

Povos micófilos da Amazônia

G. T. Prance e O. Fidalgo realizaram visitas a vários grupos Yanomami registrando o consumo de espécies de cogumelos. Os grupos foram: do rio Uraricoera, do rio Auaris, do Surucucu e do Toototobi. As coletas foram depositadas no Herbário do Instituto de Botânica em São Paulo, no Jardim Botânico de Nova Iorque e no Herbário do INPA.

Os Yanomami – Os grupos Yanomami de diferentes locais de Roraima, estudados por Prance (1972) têm pouco contato com missionários que vivem entre eles. Eles têm algumas plantas alucinógenas extremamente interessantes que são importantes na cultura do Yanomami (Prance 1972).

Os Tucano – os cogumelos foram coletados pelo naturalista explorador Richard Spruce no rio Negro e foram identificados pelo botânico e micólogo britânico Reverendo Miles Joseph Berkeley em 1856 (Berkeley 1856). As amostras foram entregues junto com uma carta indicando alguns detalhes interessantes, hábitat e localidade onde foram coletados. Berkeley (1856) acredita que as amostras foram depositadas em Cayena. Os Tucano se encontram localizados no rio Negro e constituem um grupo indígena nativo das florestas do Departamento de Vaupés - Colômbia e do Estado do Amazonas - Brasil.

Os Nambiquara – localizado na Serra do Norte no Município de Aripuanã em Mato Grosso. Em 1999, somavam 1 145 indivíduos (Prance 1965, 1968). Seus costumes são a caça e a coleta e quase nunca tiveram contato com a civilização até 1965, quando suas terras foram invadidas para o garimpo e para a extração ilegal de madeira (Prance 1965, 1968).

Os Caiabi, Txicão e Txucarramãe – Fidalgo e Hirata (1979) realizaram um levantamento sobre os termos micológicos indígenas e o uso dos fungos dividindo estes, principalmente, em comestíveis e não comestíveis. As coletas identificadas foram depositadas no Herbário "Maria Eneyda P. Kauffmann Fidalgo" do Instituto de Botânica de São Paulo. As etnias brasileiras estão localizadas no Parque do Xingu em Mato Grosso.

A. M. Vasco-Palacios e colaboradores visitaram os Andoke, Muinane e Uitoto em 2005 durante um período de 12 meses, registrando o conhecimento etnoecológico dos fungos. Os exemplares foram depositados no Herbário Nacional Colombiano e no Herbário da Universidade de Antioquia na Colômbia.

Os Andoke – antes dos seringais, eles habitavam um vasto território que se estende desde as cabeceiras do rio Quinche e Aduche até Monochoa, afluente do rio Caquetá. Após o etnocídio nos seringais, os sobreviventes retornaram ao seu território ancestral, estabelecendo-se no rio Aduche e no rio Caquetá.

Os Muinane – pertencem à família linguística Bora. Famílias se encontram no meio Caquetá, Colômbia, pertencentes aos clãs Kíyeyimɨ, Kɨmejo, Chuumojo e Nejegaimjo. Outras famílias habitam o alto Cahuinarí, Colômbia.

Os Uitoto – é uma das mais numerosas e está conformada por 5 mil indígenas dispersos pela Amazônia colombiana, peruana e brasileira. A sociedade Uitoto está dividida em quatro dialetos: Mika, Bue, Nipode e Minika, formando grupos independentes.

Os Hotï – E.L. Zent e colaboradores visitaram pessoas desta etnia nos períodos de maio de 1996 e outubro de 1999 e as visitas realizadas por E.L. Zent e S. Zent foram em setembro de 2001 e janeiro de 2002. O trabalho reporta o conhecimento e usos dos cogumelos. Foram considerados pelo menos 31 *taxa* de fungos distribuídos em uso alimentício, para caça, protetores de magia, uso medicinal ou enfeites corporais. As coletas foram depositadas em Ovalles, no Herbário Nacional em Caracas, no Herbário Guanare na Venezuela e St. Louis no Missouri nos Estados Unidos da América. Os Hotï apresentam contato direto com a sociedade ocidental, o qual foi estabelecida em 1969. Apesar de algumas mudanças culturais que inevitavelmente resultaram deste encontro, eles continuam a ser um grupo economicamente independente. É um grupo relativamente pequeno, base interfluvial de índios, com uma população de menos de mil pessoas que habitam na Serra Maigualida, localizada na fronteira entre os estados do Amazonas e Bolívar da Venezuela.

Sobre o consumo

Os índios Yanomami (Sanuma) fervem os fungos na água antes de serem consumidos e poucas espécies são consumidas assadas em folhas de bananeira (Fidalgo e Prance 1976). O. Fidalgo e G.T. Prance experimentaram três espécies: *Polyporus tenuiculus* (P. Beauv.) Fr. e *Lentinus* sp. fervidos na água durante 30 minutos e *Polyporus aquosus* Henn. assado, e segundo estes autores, os fungos não apresentaram um sabor específico.

Segundo relatos de O. Fidalgo e J.M. Hirata, os índios Caiabis preparam um pirão com a mistura do cogumelo não identificado socado no pilão junto com farinha de mandioca. Também amassam e fazem mingau de duas espécies de fungos coletadas não identificadas. Os Txicão comem os cogumelos frescos e usualmente os assam sobre cinza e brasa embrulhados em folhas verdes. Já o grupo indígena Txucarramãe somente utilizam os cogumelos na ausência de outros alimentos. Os fungos são desidratados ao sol ou assados na chapa de fazer biju.

Para a etnia Hotï é comum o consumo de fungos, sendo onze as espécies utilizadas na dieta alimentar (Tabela 1). Os cogumelos são consumidos de diferentes formas: frescos, envoltos em folhas de *Heliconia* e assado em brasa, ou cozidos em sopas com outros ingredientes, como banana (*Musa* sp.) amassada ou frutos ralados da palmeira *Attalea macrolepsis* Mart. (Zent *et al.* 2004).

Parte da coleta de basidiocarpos de *Panus strigellus,* realizada em março de 2007, no Campus III do INPA foi degustada por Vargas-Isla, R. e Ishikawa, N.K., após preparada ao *sautéed* com margarina e um pouco de sal. Também, a equipe sempre experimenta ao *sautéed* as coletas abundantes de cogumelos na floresta (*Lentinula raphanica* (Murrill) J.L. Mata & R.H. Petersen, *P. strigellus* e *Auricularia* sp.) e os cogumelos cultivados no laboratório (*P. strigellus*).

Agradecimentos

Aos taxonomistas Marina Capelari, André August Remi de Meijer, Adriana de Mello Gugliotta, Eiji Nagasawa e Takashi Shirouzu pela fundamental contribuição na identificação das espécies de fungos. À Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), ao Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)

pela bolsa de estudo de R. Vargas-Isla e à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo Programa PNADB.

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Capítulo 2: TAXONOMIA

A disponibilidade de trabalhos de sistemática de fungos é diversa e extensa. O uso e aplicação de nomes atualizados de cogumelos é benéfico para a Micologia, uma vez que reduziria a confusão e duplicação de esforços, melhorando a confiança das publicações.

ARTIGO 2

Vargas-Isla R, Capelari M, Menolli N Jr, Nagasawa E, Tokimoto K, Ishikawa NK. Relationships of *Panus lecomtei* and *P. strigellus* inferred from morphological and molecular characters.

Manuscrito em preparação para Mycoscience

FULL PAPER

Relationships of *Panus lecomtei* and *P. strigellus* inferred from morphological and molecular characters

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Text 21 pages; Tables 3; Figures 7

Abstract *Panus strigellus* is recorded from Amazonas State for the first time. This edible mushroom presented macroscopic characteristics similar to *P. lecomtei*. Combined evidences of morphological and molecular studies used to confirm the identification of *P. strigellus*, and show differences between these sympatric species of *Panus*. In addition, this paper discusses and reports some morphological characters that have not yet been described for *P. strigellus*. The geographical distribution in the Americas is presented for both species.

Keywords Edible mushroom · *Lentinus strigellus* · *Lentinus strigosus* · *Panus rudis*

Introduction

In a sample number 20016A collected by Prance in 1973 in the Roraima State (Brazil) deposited in the Instituto Nacional de Pesquisas da Amazônia (INPA) Herbarium was also found a note indicating that *Panus strigellus* (Berk.) Overh. grown on dead log, it is boiled and eaten by Sanama group of the Yanomami people; who not distinguish *P. strigellus* from *P. lecomtei* (Fr.) Corner. Being this note a one of the rare register about the edibility of *P. strigellus*. There are reports of the consumption of *P. lecomtei* in South America by the Yanomami people in Brazil (Fidalgo and Prance 1976; Fidalgo and Hirata 1979; Prance 1984) and in Colombia by the Uitoto, Muinane and Andoke people (Vasco-Palacios et al. 2008). In the Uauaris village is known as Shio-koni-amo (Fidalgo and Prance 1976; Prance 1984), and in the Xitei/Xidea village as Kasikoirima (Victor Py-Daniel personal communication).

Lentinus group as defined by Pegler (1983) now comprise four genus based on morphological and molecular data: *Lentinus* Fr., *Panus* Fr., *Neolentinus* Redhead & Ginns and *Heliocybe* Redhead & Ginns (Redhead and Ginns 1985; Hibbett and Vilgalys 1993; Hibbett and Donoghue 1995; Thorn et al. 2000). Regarding the morphology *Panus* can be differentiated from *Lentinus* mainly by the dimitic hyphal system composed by skeletal thick-walled hyphae, typically unbranched, and absence of hyphal pegs. *Panus* also differs from *Neolentinus* and *Heliocybe* mainly because they present brown rot and bipolar mating system.

In the Americas *P. lecomtei* and *P. strigellus* are commonly recorded for the same habitat and due to their macro-morphological similarities they could be confused on the identification. In the past, *P. strigellus* was placed as a synonym of *P. lecomtei* (Pegler 1983), but based on micro-morphological characters (Pegler 1983) they are considered as distinct species.

In this way, a lentinoid fungus from Amazonas State, Brazil was erroneously identified as *Lentinus strigosus* (Schwein.) Fr. in a previous paper reporting its thermophilic characteristic (Vargas-Isla and Ishikawa 2008). Now, this material was revised and re-identified as *P. strigellus,* but it is known that *P. lecomtei* also occurs in the same region (Sales-Campos and Andrade 2011 as *L. strigosus*).

Thus, we conducted a broad study to recognize *P. lecomtei* and *P. strigellus* supported by combined evidences of morphological and molecular studies. In addition, it is presented the geographical distribution for both species in the Americas.

Material and methods

Sample collection

This study was conducted with the material collected in Amazonas State - Brazil allied to additional collection from United States of America (USA) and Japan (Table 1). The basidiomata used for intersterility study were obtained from the cultures TMIC35103 and INPACM1464 cultivated in *Simarouba amara* Aubl. sawdust and rice bran (5:1; w/w).

Morphological studies

For the microscopic analyses the dried basidiomata were rehydrated in distilled water followed by 2.5% KOH and 1% Congo Red. All microscopic illustrations were made with the aid of a drawing tube. Q represents the range of the length/width quotient for all the measured spores, avQ represents the average of all computed Q values for all the measured

basidiospores and avL (avW) represents the average length (width) of the measured basidiospores.

Molecular studies

The DNA was obtained from lyophilized mycelia grown in PDB (Potato dextrose broth, Difco) using the procedures of Justo et al. (2011) and from dried basidiomata using the Forensic DNA mini kit (Omega Bio-Tek). The nLSU gene was amplified using LR0R and LR5 primer set (Moncalvo et al. 2000), and the ITS region was amplified using ITS1F and ITS4 primer set (White et al. 1990, Gardes and Bruns 1993). PCR reactions containing 0.025 U/µl of Platinum® Taq DNA Polymerase-Brazil (Invitrogen), PCR buffer 1 X, 0.2 mM of each dNTP, 1.5 mM of $MgCl_2$, and 0.5 µM of each primer of the selected region were performed in an Eppendorf thermocycler (Mastercycler) using the following program: 95°C for 2 min, followed by 34 cycles at 94°C for 45 s, 50°C for 1 min and 10 s, and 72°C for 2 min, and then a final extension at 72°C for 10 min. Amplification products were purified using the PureLink PCR Purification Kit (Invitrogen), except for TENN55993 and TENN56192 which were purified using the AxyPrep PCR Clean-up Kit (Axygen).

The nLSU and the ITS sequencing were performed on the DYEnamic ET Dye Terminator Kit in a MegaBACE 1000 DNA sequencer (GE Healthcare) according to the manufacturer's instructions. The samples were sequenced in both directions with the same primers used for amplification. The sequences were deposited in GenBank (Table 2).

The nLSU sequences generated in this study were analyzed with other 32 sequences obtained from GenBank, including 21 of *Panus*, nine of *Lentinus* and two of *Tyromyces chioneus* (Fr.) P. Karst. as outgroup. ITS sequences were analyzed with other 18 obtained from GenBank, including two of *Panus*, 14 of *Lentinus*, and also two of *T. chioneus* as

outgroup (Table 2). The nLSU and ITS sequences were aligned using the Clustal W version (Thompson et al. 1994) in the BioEdit version 7.0.5.3 (Hall 1999). Alignments have been deposited in TREEbase.

Parsimony tree was obtained by heuristic searches with simple sequence addition in 1000 replicates, employing tree-bisection-reconnection (TBR) branch-swapping algorithm. Characters from the extreme 5′ and 3′ ends of the sequences were deleted from all taxa to obtain individual datasets that had identical start and end positions, gaps were treated as missing, all characters were unordered and equally weighted, and multistate taxa was interpreted as uncertainty. Starting trees were obtained via stepwise addition, with one tree held at each step during stepwise addition and the steepest descent option not in effect. Also, the initial MaxTrees were set to auto-increase, branches of zero length were collapsed (creating polytomies), and MulTrees options were in effect. Branch and branch node supports were determined using 1000 BS replicates. Estimated levels of homoplasy and phylogenetic signal (retention and consistency indexes) were determined.

Results and discussions

Morphological studies

According to Pegler (1983) *P. lecomtei* presents a uniform and densely villose to hispid-tomentose pileus, with an excentric to lateral or more rarely central stipe (Figure 1a), and abundant to occasional metuloids on both sides and edges of the lamellae (Pegler 1983). On the other hand, *P. strigellus* presents a glabrescent pileus with scattered squamules and a thinner context, frequently with a central stipe (Figure 1b), and the gloeocystidia are present on lamella-edge and sometimes over lamella surface.

Our morphological studies showed that the main difference between *P. lecomtei* and *P. strigellus* is the presence of abundant metuloids (Table 3). However, in the Amazonian collections of *P. strigellus* (Figure 2 and 3) herein studied were observed some differences from the description of Pegler: cylindric-clavate cheilocystidia (Fig. 2c' and 3a), clavate pleurocystidia (Fig. 2d'and 3b); 3) hyphae degenerate from hymenium and surrounding pleurocystidia (Fig. 2d and 3c); and 4) the squamules were persistent over the pileus surface.

Molecular analysis

The most parsimonious tree generated from the nLSU and ITS sequences data revealed a well-supported clade (100% BS) including *Panus* and *Lentinus* species clustered in two major clades (Fig. 4 and 5).

In the nLSU analysis (Fig. 4), the *Panus* clade was divided in four groups: *P. velutinus* complex, *P. fasciatus/ciliatus* complex, *P. strigellus* clade and *P. lecomtei* clade. The *P. velutinus* complex (58% BS) and *P. fasciatus/ciliatus* complex (67% BS) showed similar topologies when compared to the Bayesian analyses of Douanla-Meli and Langer (2010). The Amazonian collections of *P. strigellus* clustered (60% BS) in a clade with other two *P. strigellus* from USA. Although in Douanla-Meli and Langer (2010) *P. strigellus* was placed as a sister branch of the *P. velutinus* complex, in our analysis *P. strigellus* was revealed as a separated clade. All sequences of *P. lecomtei* clustered (91% BS) in the same clade. In Douanla-Meli and Langer (2010) sequences of *P. lecomtei* (including *P. rudis* Fr.) were positioned in a sister clade of *P. fasciatus/ciliatus* complex.

The segregation between *P. lecomtei* and *P. strigellus* was also shown in the ITS analysis which includes them in a clade (73% BS) subdivided in two well-supported branches including all *P. lecomtei* (100% BS) and *P. strigellus* (98% BS) sequences (Fig. 5).

Geographical distribution

This study emphasizes the morphological differences between *Panus lecomtei* and *P. strigellus*, supported by molecular studies. The both species are well distributed in the Americas and they are frequently found in the same region (Fig. 6).

This paper reported for the first time the combined multiple evidences and occurrence of *P. lecomtei* and *P. strigellus* from Amazonas State, which has approximately 1.57 million km^2 and represents the most extensive State of Brazil. Previous papers recorded them under many names including *Lentinus lecomtei* Fr., *L. strigosus*, *L. villosus* Klotzch and *P. rudis* for *P. lecomtei* and *L. strigellus* Berk. and *L. subglaber* Lloyd for *P. strigellus*.

Panus lecomtei is known from Canada (Lloyd 1913; Pegler 1983), Ecuador - Galapagos (Reid et al. 1980; Pegler 1983), USA, Mexico, Cuba, Santo Domingo, Dominica, Trinidad, French Guiana, Venezuela, Argentina (Pegler 1983), Colombia (Pegler 1983; Guzmán et al. 2004; Vasco-Palacios et al. 2008) and Peru (Espinoza et al. 2006). In Brazil *P. lecomtei* was reported from States of Santa Catarina (Pazschke 1892), São Paulo (Hennings 1904; Sydow and Sydow 1907; Teixeira 1946), Minas Gerais (Sydow and Sydow 1907; Teixeira 1946; Pegler 1983), Roraima (Fidalgo and Prance 1976), Mato Grosso (Pegler 1983), Amazonas (Pegler 1983; Sales-Campos and Andrade 2011), Rio Grande do Sul (Pegler 1983; Sobestiansky 2005), and Paraná (Meijer 2001).

Panus strigellus was reported from Peru (Berkeley and Curtis 1868; Pegler 1983), Paraguay (Singer 1951; Pegler 1983), Mexico (Pegler 1983; Grand et al. 2011), El Salvador, Cuba, Guadaloupe, Martinique, Colombia and Venezuela (Pegler 1983), Argentina (Pegler 1983; Lechner et al. 2006), and USA (herein studied). From Brazilian States *P. strigellus* is known from São Paulo (Hennings 1904; Sydow and Sydow 1907; Pegler 1983), Rio Grande do Sul (Rick 1930), Pará (Pilát 1936), and Santa Catarina (Trierveiler-Pereira et al. 2009).

Conclusions

Morphological and molecular studies corfirm the identification of *P. strigellus* and , and the paper reports some morphological characters that have not yet been described for *P. strigellus*. *Panus strigellus* is recorded from Amazonas State for the first time. In the same way, the inclusions of the sequences of Amazonian collections were accomplished in GenBank.

Acknowledgments The authors thank the curator of TENN for the loan of *P. strigellus* to morphological and molecular studies. Poliana de Oliveira Ventura and Tatiane Asai are thanking for laboratorial technical support. R. Vargas-Isla is grateful for a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). N.K. Ishikawa is grateful for a scholarship from Japan Society for the Promotion of Science (JSPS). This research was financed by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

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Figure legends

Fig. 1 Mushrooms cultivated on substrate formulated with *Hymenolobium petraeum*, *Bactris gasipaes* internal sheath and rice bran (5:5:1). **a** *Panus lecomtei* mushroom. **b** *P. strigellus* mushroom. *Bars* 1 cm

Fig. 2 *Panus strigellus* microscopic structures of wild basidiomata. **a** Basidiospores. **b** Basidia. **c** Cheilocystidia. **c'** subfusiform. **c"** cylindric-clavate. **d** Pleurocystidia (arrow indicate hyphae degenerate from hymenium). **d'** cylindric-clavate. **d"** subfusiform. **e** Hyphae of pileus hairs. *Bars* 10 µm for spores and 20 µm for other structures

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Fig. 6 Geographic distribution of *Panus lecomtei* and *P. strigellus* in the Americas

Table 1 Collections used to morphological and molecular studies.

Species	Herbarium number	Culture number [a]	Locality
Panus strigellus	INPA222827	INPACM1464	Brazil, AM, Manaus, INPA – *Campus* III
	INPA239979	INPACM1530 / CCIBt3396	Brazil, AM, Manaus, Puraquequara community
	INPA243941[b]	INPACM1530	Cultivated material
	INPA243940	INPACM1531 / CCIBt3399	Brazil, AM, Manaus, Puraquequara community
	INPA243943	INPACM1532	Brazil, AM, Manaus, INPA – *Campus* III
	TENN55993	-	USA, Louisiana, East Baton Rouge, Baton Rouge
	TENN56192	-	USA, Louisiana, West Feliciana, Matinquoin
Panus lecomtei	-	TMIC35103	Japan, Tottori
	INPA239978[b]	TMIC35103	Cultivated material
	-	INPACM1466	Brazil, AM, Manaus, Estrada AM 10, Km 10

[a] INPACM = Coleção de Micro-organismos de Interesse Agrossilvicultural of Instituto Nacional de Pesquisas da Amazônia (INPA), CCIBt = Coleção de culturas de Algas, Cianobactérias e Fungos do Instituto de Botânica, TMIC = Culture collection of the Tottori Mycological Institute

[b] Basidiomata obtained to cultivated material

Table 2 Taxon information and GenBank accession numbers

Taxon	GenBank accession number		Herbarium/culture/ collector number	Geographic origin
	nLSU	ITS		
Tyromyces chioneus	AF393080		-	-
T. chioneus	EU522817		-	Canada
T. chioneus		AY636061	-	-
T. chioneus		FJ467367	-	-
Lentinus sp.		GQ849478	-	-
L. scleropus		GU207310	TENN59704	Mexico
L. squarrosulus	EU908178		DMC178	-
L. squarrosulus		GU001951	-	-
L. squarrosulus		GQ849475	-	-
L. squarrosulus		AB478883	-	Japan
L. tigrinus	AY615977		FB11746	Iran
L. tigrinus	AY615973		TENN59833	Austria
L. tigrinus	AY615974		TENN54918	USA
L. tigrinus		GU207274	TENN59710	USA
L. tigrinus		GU207273	-	USA
L. tigrinus		GU207272	-	USA
L. tigrinus		GU207271	-	USA
L. cf. *crinitus*		JQ955723	INPA243944	Brazil
L. crinitus	AY615981		TENN58775	USA
L. crinitus	AY615979		TENN59659	USA
L. crinitus		GU207300	-	Puerto Rico
L. crinitus		GU207299	-	Puerto Rico
L. crinitus		GU207298	TENN54876	USA
L. bertieri	AY615986		FB11755	USA
L. bertieri	AY615985		FB11756	USA
L. bertieri	AY615984		TENN59773	Dominican Republic
L. bertieri		GU207307	TENN59781	Dominican Republic
L. bertieri		GU207306	TENN59770	Dominican Republic
L. bertieri		GU207305	TENN58997	Argentina
Panus sp.		HM245784	-	-
P. rudis	AF287878		DSH-92-139	-
P. lecomtei	AY615994		TENN51805	USA
P. lecomtei	JQ955730	JQ955721	INPACM1466	Brazil
P. lecomtei	JQ955733	JQ955726	TMIC35103	Japan
P. strigellus	JQ955729	JQ955722	INPA222827	Brazil
P. strigellus	JQ955731	JQ955724	INPA239979	Brazil
P. strigellus	JQ955732	JQ955725	INPA243940	Brazil
P. strigellus	AY616002	JQ955727	TENN56192	USA

P. strigellus	AY616001	JQ955728	TENN55993	USA
P. ciliatus	AY616008		FB11755	USA
P. ciliatus	AY616007		TENN59786	Thailand
P. ciliatus	AY616006		TENN59785	Thailand
P. cf. *fasciatus*	EU908181		DMC696	-
P. fasciatus	EU908180		DMC184	-
P. similis	EU908182		DMC189	-
P. velutinus var. *glabrior*	EU908183		DMC174	-
P. velutinus var. *glabrior*	EU908184		DMC188	-
P. fulvus	AY615996		TENN58776	USA
P. similis	AY616000		TENN59008	Argentina
P. similis	AY615999		TENN59829	Argentina
P. similis	AY615998		TENN58995	Argentina
P. velutinus	GQ487335		NAL318	-
P. velutinus	EU908185		DMC683	-
P. velutinus	EU908186		DMC734b	-
P. velutinus	EU908187		DMC694	-
P. velutinus	EU908188		DMC695	-

(-) do not informed

Table 3 Microscopic characteristics description of *Panus lecomtei* and *P. strigellus*, edible mushrooms of the Brazilian Amazon

Microscopic structures	*Panus lecomtei*			*Panus strigellus*		
	Shape	Description of Pegler (1983)	Herein study[a]	Shape	Description of Pegler (1983)	Herein study[b]
Cheilocystidia	subfusiform	18–35 × 4–6 µm; difficult to observe	(12.5–)21.2–43.7(–58.8) × 7.5-15 µm	subfusiform	22–28 × 7–9 µm	(22.2–)27–69(–90) × (5–)6.2-13.7(–15) µm
		-	-	cylindric-clavate	non described	(20–)25–52(–67) × (5–)6.2–12.8(–13.8) µm
Pleurocystidia	metuloid	25–55 × 9–13 µm; abundant to occasional on both sides and edges of the lamellae	43.8–68.7(–71.3) × (8.8–)10–13.7(–17.5) µm	subfusiform	35–70 × 6–14 µm; numerous on lamellae-edge, sometimes sparse over lamellae surface	(25.2–)33.2–81.6(–112) × 6.2–16 µm
		-	-	clavate	non described	26.2–52.5(–85) × 6.2–16.2 µm
Spores		4.5–6 × 2.5–3.7 µm, Q=1.65, ovoid to ellipsoid	3.75–6.25 × 2.5–3.75 µm, Q=1.5–2, avQ=1.79, avL=5.36, avW=3.04, ovoid to ellipsoid		4.7–7 × 3–3.7 µm, Q=1.76, ovoid to ellipsoid	5–8.75 × 2.5–6.25 µm, Q=1.33–3, avQ=1.77, avL=6.14, avW=3.54, ovoid to ellipsoid

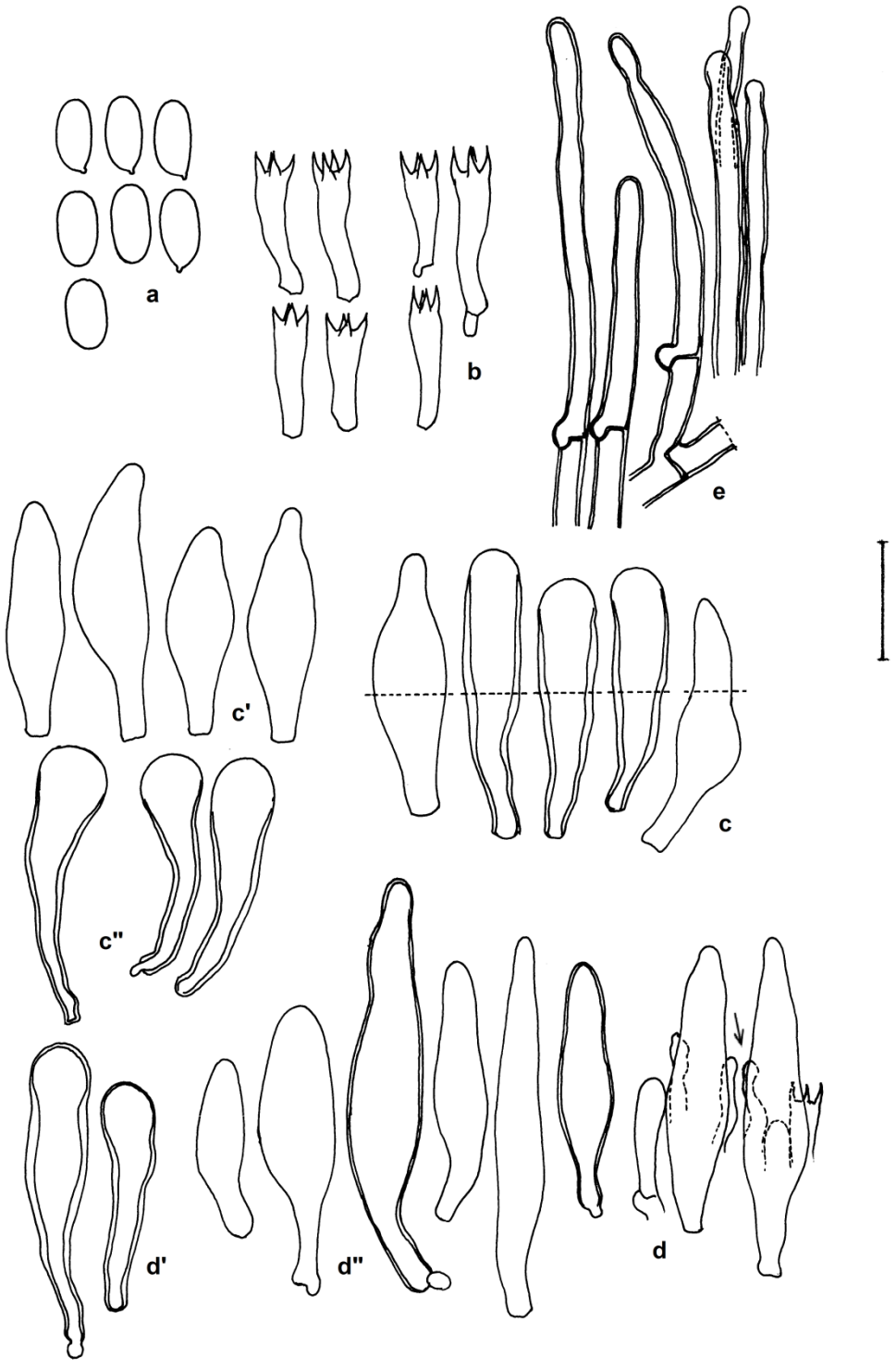
Other characteristic	-	-	Degenerated hyphae	non described	basidiomata shows hyphae degenerated from hymenium and surrounding pleurocuystidia
	-	-	Basidiomata surface	finely striate, glabrous except for minute, scattered, spinose-squarrose squamules	the squamules were persistent over the pileus surface

Material examined: [a] INPA239978; [b] INPA222827, INPA239979, INPA 243940, INPA243941, INPA243943, TENN55993 and TENN56192. The following abbreviations are used in the descriptions: avL for average length, avW for average width, Q for quotient of length and width and avQ for average quotient.

Fig. 1

Fig. 1 Mushrooms cultivated on substrate formulated with *Hymenolobium petraeum*, *Bactris gasipaes* internal sheath and rice bran (5:5:1). **a** *Panus lecomtei* mushroom. **b** *P. strigellus* mushroom. *Bars* 1 cm

Fig. 2



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Designs prepare by Professor Eiji Nagasawa, Tottori Mycological Institute-Japan.

Fig. 3

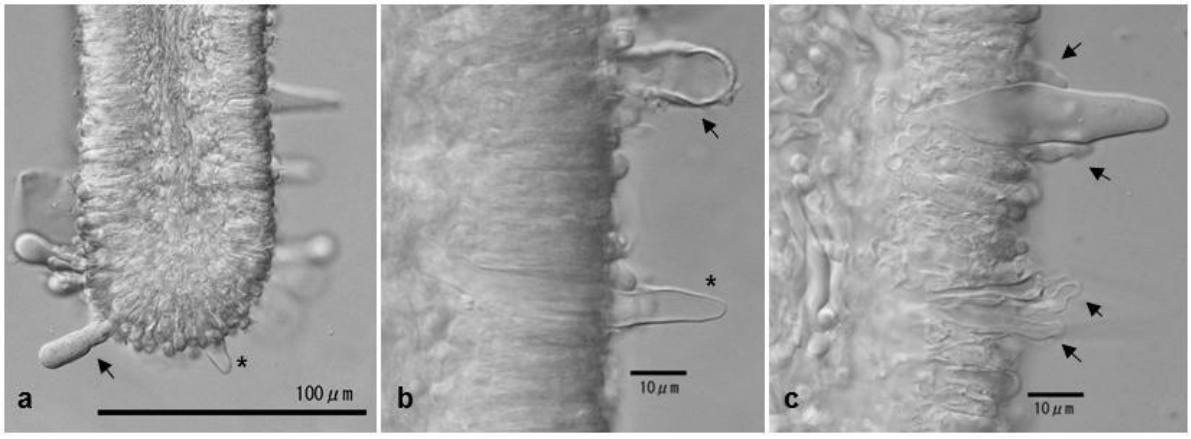

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Fig. 4

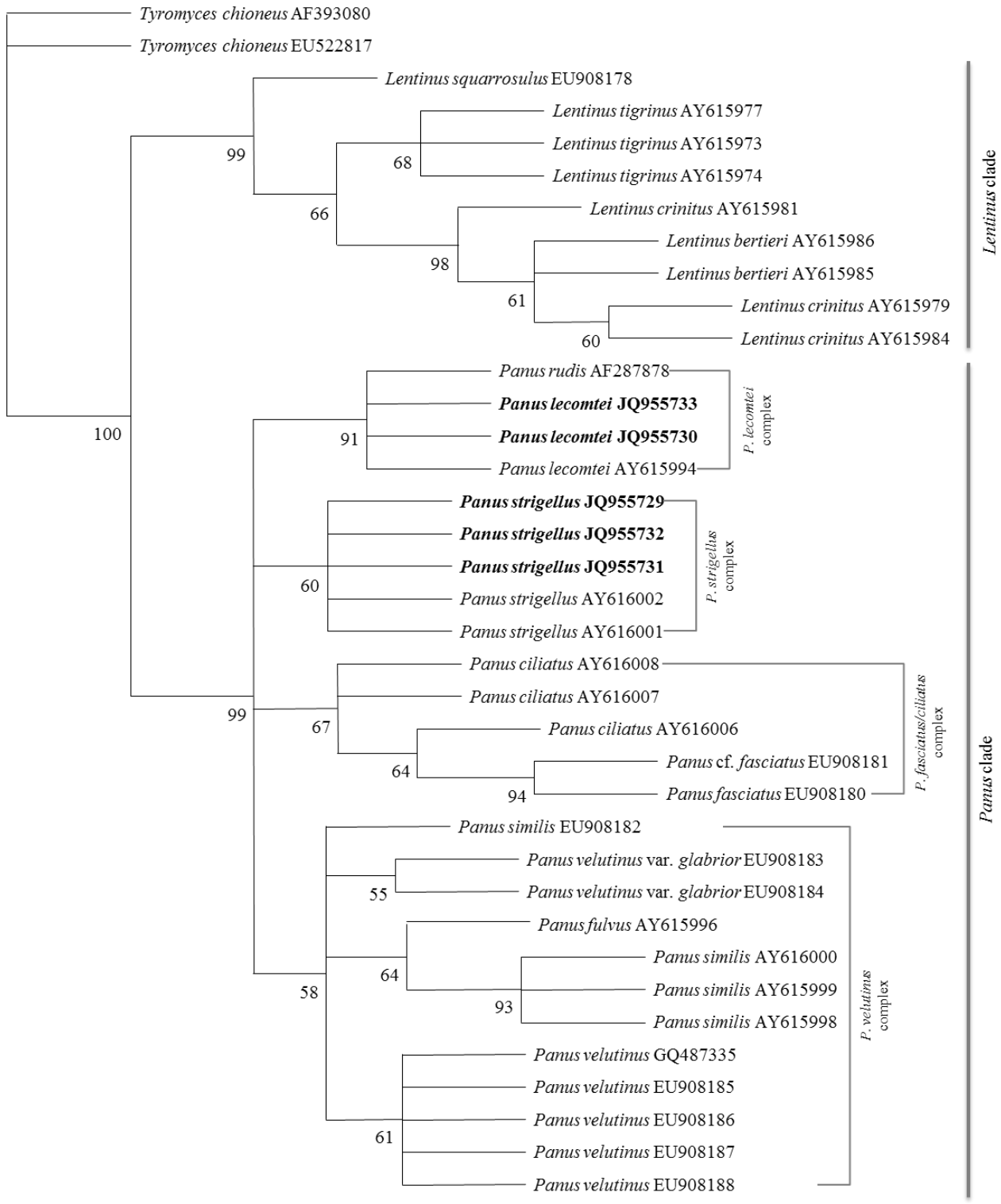

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Fig. 5

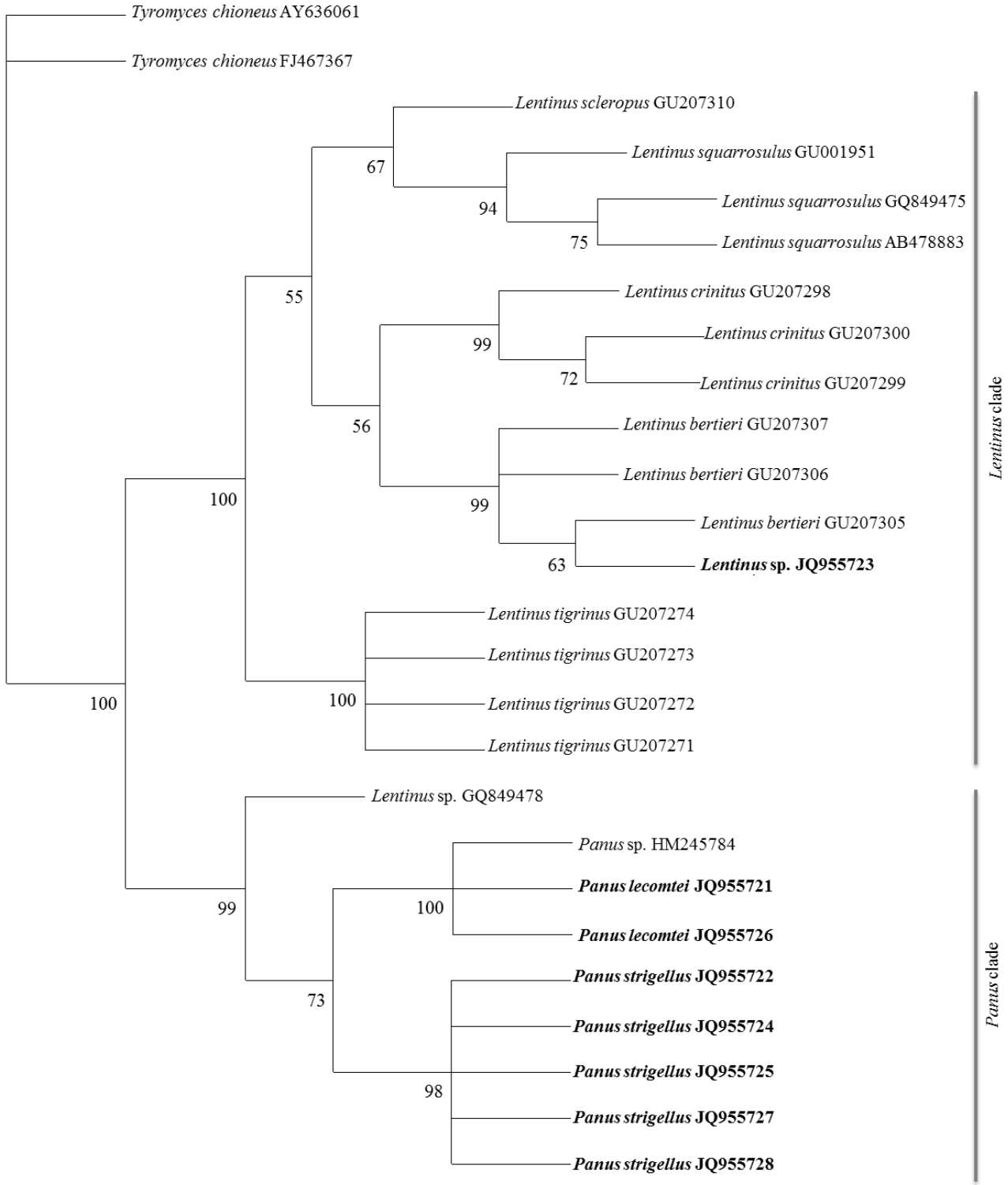

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Fig. 6

Fig. 6 Geographic distribution of *Panus lecomtei* and *P. strigellus* in the Americas

Capítulo 3: PRODUÇÃO DE "SEMENTE-INÓCULO" (*SPAWN*)

A base para o cultivo comercial de cogumelos comestíveis é a semente-inóculo, sendo a produção desta o principal desafio para os produtores de cogumelos comerciais. No Brasil não existe uma empresa para a produção de semente-inóculo de cogumelos da Amazônia, é oportuno a criação de uma empresa ou estabelecimento que possa produzir e disponibilizar a semente-inóculo aos futuros produtores de cogumelos.

ARTIGO 3

Vargas-Isla R, Hanada RE, Ishikawa NK. 2012. Sawdust and fruit residues of Central Amazonian for *Panus strigellus* spawn's production. *Pesquisa Florestal Brasileira* 32 (70): 123-128 p.

Publicado em 29 de junho de 2012.

Sawdust and fruit residues of Central Amazonian for *Panus strigellus* spawn's production

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Serragem e resíduos de frutos da Amazônia Central para produção de semente-inóculo de *Panus strigellus*

Resumo

Neste trabalho objetivou-se realizar uma triagem de resíduos de espécies florestais da Amazônia Central para o preparo da semente-inóculo do cogumelo comestível *Panus strigellus.* Foram testados substratos de serragem de 11 espécies florestais. Em seguida, suplementação com levedura de cerveja, farelos de cereais e resíduos de frutas regionais foram avaliados na relação serragem:suplemento (5:1 e 10:1). O crescimento micelial de *P. strigellus* ocorreu em todos os substratos formulados com espécies florestais da Amazônia, apresentando potencial de uso na formulação da semente-inóculo e/ou cultivo deste fungo comestível. Entre estes, o substrato formulado com serragem de *Simarouba amara* promoveu maior crescimento micelial ($P<0,05$). A formulação de *S. amara* suplementada com farelo da casca do fruto de *Astrocaryum aculeatum* (10:1) apresentou a melhor alternativa de suplementação entre os resíduos de frutos regionais. Três tipos de embalagens para o preparo da semente-inóculo foram avaliados e o saco de polipropileno (32×45 cm) foi considerado a embalagem mais adequada. Serragem de *S. amara* e casca do fruto de *A. aculeatum* são de fácil disponibilidade na região Norte e os resultados demonstram que estes resíduos podem substituir a serragem de *Eucalyptus* sp. e farelo de arroz comumente utilizado nas regiões Sul e Sudeste de Brasil para a produção de semente-inóculo de cogumelos.

Termos para indexação: Basidiomicetos; Cogumelo comestível; *Lentinus strigellus*; *Lentinus strigosus*; *Simarouba amara*; *Astrocaryum aculeatum*

Sawdust and fruit residues of Central Amazonian for *Panus strigellus* spawn's production

Abstract

The objective of this work was to perform a screening of residues of forest species of the Central Amazon to prepare spawn of the edible mushroom *Panus strigellus*. Sawdust substrates from 11 forest species were tested. Then supplementation with beer yeast, cereal bran and regional fruit residues in sawdust:supplementation relation (5:1 and 10:1) were evaluated. Mycelial growth of *P. strigellus* occurred in all the substrates composed of the Amazonian forests species, suggesting that all have potential for use in spawn formulation and/or cultivation of this edible mushroom. Among these species the substrate formulated with *Simarouba amara* sawdust promoted higher mycelial growth ($P<0.05$). The formulation of *S. amara* supplemented with *Astrocaryum aculeatum* fruit shell bran (10:1) presented the best supplementation alternative among regional fruit residues. Three types of packaging for spawn preparation were evaluated, and the polypropylene sack (32×45 cm) was considered the most appropriate. *Simarouba amara* sawdust and *A. aculeatum* fruit shell are readily available in the North region, and the results demonstrating that these residues might substitute *Eucalyptus* sp. sawdust and rice bran, commonly used in the South and Southeast of Brazil for mushroom spawn production.

Index terms: Basidiomycetes; Edible mushroom; *Lentinus strigellus*; *Lentinus strigosus*; *Simarouba amara*; *Astrocaryum aculeatum*

Introduction

The most cultivated mushrooms species worldwide are *Agaricus bisporus* (J.E. Lange) Imbach, *Lentinula edodes* (Berk.) Pegler, *Pleurotus* spp., *Auricularia auricula-judae* (Bull.) Quél., *Flammulina velutipes* (Curt. ex Fr.) Sing. and *Volvariella volvacea* (Bull.) Singer (Sánchez, 2004). The diversity of species used for cultivation of fungi is influenced by the consumption preferences of the producing countries. In Brazil, the main edible mushrooms produced in the South and Southeast are *A. bisporus*, *L. edodes* and *Pleurotus* spp. These are originated from temperate climates places. Since the 90's in Brazil began using agroforestry waste as a substrate for *L. edodes* mushroom production, added to *Eucalyptus* spp. sawdust supplemented with agricultural residues easily found in the region. The Amazon Region has interesting potential for the development of mushroom cultivation, having abundance and diversity of native edible mushrooms species as well as agroforest residues that might be used as lignicolous substrates to produce organic products of high nutritional, medicinal, and gastronomic value.

Ethnomycological studies of indigenous groups such as the Yanomami in Brazil (Fidalgo & Prance, 1976; Fidalgo & Hirata, 1979; Prance, 1984) and the Uitoto, Muinane and Andoke in Colombia (Vasco-Palacios et al., 2008) have described the edibility of various mushrooms. In 2008, we publish the thermophilic characteristic of the INPACM 1464 isolated (Coleção de Micro-organismos de Interesse Agrossilvicultural of the Instituto Nacional de Pesquisas da Amazônia-INPA) collected in a lignicolous substrate in the Central Amazon (Vargas-Isla & Ishikawa, 2008). At that time, the isolated was identified like *Lentinus strigosus* (Schwein.) Fr. (*Panus lecomtei* (Fr.) Corner, current name), however after re-examination of exsiccate, the microscopic characteristics and molecular analyses demonstrated that the species is *Panus strigellus* (Berk.) Overh. (= *L. strigellus* Berk.).

The specimens presented mycelial growth from 25 to 45 ºC, with the optimum temperature being 35 ºC. The broad temperature range suitable for mycelial growth of this species is an advantage for its cultivation in the Amazon region that has average annual temperatures of 30 to 33.4 ºC in the shade year-round. In the sun (the condition in which the mushroom was collected), temperatures can reach 40–45 °C. For edible mushrooms production, the search to substrates formulation for spawn production is an important step. The objective was carry out a substrate screening using sawdust of Amazon forest species and search supplements options available in the region for *P. strigellus* spawn preparation.

Material and methods

Microorganism

The isolated of *P. strigellus* (INPACM 1464) utilized in this study was collected in a lignicolous substrate on Campus III of the INPA, Manaus, AM, Brazil. The stock culture was maintained on Sabouraud Dextrose Agar (SDA; Becton Dickinson) slants at 25 °C, in dark. Mycelia of the stock culture were cultivated at 35 °C in Petri plates (90 mm diameter) containing SDA medium. After five days of growth, disks of the mycelia (10 mm diameter) were removed and used as the inoculum for the experiments.

Sawdust screening

The sawdust type were selected from the main Amazon forest species harvested for timber in Manaus, AM, Brazil, as described in a technical report by Vianez & Barbosa (2002). Two exotic species, *Eucalyptus* sp. and *Quercus acutissima* Carr., were also included because *Eucalyptus* sp. sawdust is commonly used to cultivate edible mushrooms such as *L. edodes* in Southern and Southeastern Brazil (Paula et al., 2001; Queiroz et al., 2004; Silva et al., 2005;

Shiomi et al., 2007; Ishikawa, 2008) and *Q. acutissima* is used for mushroom cultivation in Asia (Przybylowicz & Donoghue, 1990; Quimio et al., 1990).

The substrates were formulated separately from sawdust from each of the following trees: *Aniba rosaeodora* Ducke, *Astronium lecointei* Ducke, *Bertholletia excelsa* Bonpl., *Bombacopsis quinata* (Jacq.) Dugand., *Caryocar* sp., *Cedrela odorata* L., *Eucalyptus* sp., *Hymenaea courbaril* L., *Hymenolobium petraeum* Ducke, *Hura crepitans* L., *Ocotea cymbarum* Kunth, *Q. acutissima*, and *Simarouba amara* Aubl. The sawdust, sifted at 3mm mesh sieve from different timbers was screened, then dried in an oven with air circulation at 65 °C until constant weight and stored in plastic bags at room temperature. The sawdust samples were mixed with rice (*Oryza sativa* L.) bran (sawdust:supplement = 5:1; w/w) and with distilled water until approximately 60% hydration (w/v).

Two additional experiments were conducted. The first one was to exam how supplements commonly used in mushroom cultivation affected *P. strigellus* mycelial development. Rice bran, soy (*Glycine max* (L.) Merrill) fiber, soy extract, wheat (*Triticum aestivum* (L.) Thell.) fiber, wheat germ, and beer yeast were added to *S. amara* (sawdust:supplement = 5:1; w/w), selected in the sawdust screening. Pure *S. amara* sawdust was used as a control.

In the second additional experiment, *Astrocaryum aculeatum* G.Mey. (common name = tucumã) fruit shell, *Carapa guianensis* Aubl. (common name = andiroba) seed shell, *Euterpe oleracea* Mart. (common name = açaí) seed, and *Passiflora edulis* Sims fruit shell were examined. Each residue was dried at 65 °C, crushed and sifted. These materials were separately supplemented to *S. amara* sawdust in weight ratios of 5:1 and 10:1 (sawdust:supplement).

The formulation preparation (sawdust:supplement) was then distributed in five Petri plates (15 ± 1 g/plate) and sterilized twice an hour with interval of 24 h at 121 °C. Following sterilization, one mycelial disk was deposited in the center of the plate containing the

formulation and incubated at 35 °C without light. Mycelial growth was evaluated by the index of mycelial growth rates (IMGR), calculated as $\sum$ (D – Da)/N, where D is the diameter of the colony on the observation day (measurement in cm), Da is the diameter of the colony on the previous day (measurement in cm), and N is the number of days after inoculation.

In addition to the IMGR, the colony vigor was visually evaluated and classified under three vigor levels of (+) thin, (++) medium, and (+++) dense (see details on Figure 1).

Spawn production and substrate inoculation

Three kinds of polypropylene packing: (1) flask, 15 cm height × 9 cm diameter, with the capacity to hold 600 g of wet substrate with screw cap. Two holes of 1 cm in diameter were made in the cap for gas exchange, and they were covered with adhesive tape (microporous filters), (2) transparent plastic bags, 23 cm wide × 36 cm height, and holding capacity of 800 g of wet substrate. For gas exchange is was necessary to create a respirator using a ring of PVC tubing with 3 cm height × 5 cm diameter and hydrophobic cotton, and (3) transparent bag, 32 cm wide × 45 cm height with 1200 g of substrate holding capacity and a hole of 4.5 cm diameter covered with filter paper for gas exchange (see details on Figure 2).

As a first step, mycelia were placed on wheat grains to multiply. The grain was washed and immersed in water for 24 h. Soon after, 250 g of wheat grain was put in each of ten 500 ml glass flasks and sterilized in an autoclave at 121 °C for 1 h following the methodology described by Stamets (1993). Ten mycelial disks of *P. strigellus* was then transferred into each flask and incubated at 35 °C for 15 days.

Next, sawdust from *S. amara*, *H. petraeum*, and *A. lecointei*, which had shown good results for mycelial growth, was separately evaluated for spawn growth. The sawdust supplemented with rice bran (5:1) was placed in the polypropylene packing. The substrates were sterilized twice an hour with interval of 24 h at 121 °C. For each 100 g of substrate, we added 3.5 g of

colonized wheat grains and incubated the material at 35 °C for 25 days in the absence of light in Biologic Oxygen Demand (BOD – TE-390/TECNAL). After this period, the colonized substrates were taken from the packing materials and cut obliquely into blocks for visual observation of the colonization. Each type of packing material was tested in five replications.

The experiments in Petri plates were tested in five replications and two repetitions. We used analysis of variance (ANOVA) to examine the results of the experiments and compared the averages using the Scott–Knott test at the 5% level of significance.

Results and discussion

The substrates formulated with *B. quinata* and *S. amara* provided the highest *P. strigellus* IMGR values ($P < 0.05$; Table 1), with the colonies reaching the border of the Petri plates (90 mm diameter) in five days after inoculation. This growth was fast compared to that of other edible mushrooms such as *L. edodes* and *F. velutipes* (Ishikawa, 2001). Substrates composed of *C. odorata*, *H. crepitans*, and *O. cymbarum* sawdust showed the lowest IMGR values ($P < 0.05$), with colonies reaching the Petri plate borders in 10 days. However, this growth is considered common for other edible mushrooms. Mycelial growth of *P. strigellus* occurred in all the substrates composed of the Amazonian forests species as well as *Eucalyptus* sp. and *Q. acutissima*, suggesting that all have potential for use in spawn formulation and/or cultivation of this edible mushroom. *Simarouba amara* sawdust was chosen as substrate for the supplementation experiments for its rate of mycelial growth and also because, the specie is frequently used for lumber making and its sawdust is readily available.

Table 1. Effect of forest species sawdust substrates on *Panus strigellus* mycelial growth.

Substrate[(1)]	Mean[(2)]	Standard deviation
Bombacopsis quinata	1.197 a	± 0.019
Simarouba amara	1.175 a	± 0.018
Quercus acutissima	1.084 b	± 0.016
Astronium lecointei	1.050 b	± 0.026
Hymenaea courbaril	1.038 b	± 0.042
Hymenolobium petraeum	1.036 b	± 0.017
Eucalyptus sp.	0.923 c	± 0.045
Aniba rosaeodora	0.898 c	± 0.010
Bertholletia excelsa	0.891 c	± 0.084
Caryocar sp.	0.837 d	± 0.019
Cedrela odorata	0.774 e	± 0.014
Hura crepitans	0.756 e	± 0.019
Ocotea cymbarum	0.724 e	± 0.021

(1) All substrate was composed of a five-to-one (w/w) mixture of sawdust and rice bran. (2)Average of five replications and two repetitions of index of mycelial growth rates values-IMGR (cm/day). Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test.

Rice bran, soybean fiber, wheat fiber and germ supplementations in spawn production produced the highest *P. strigellus* IMGR values ($P < 0.05$) and improved the colony vigor compared to the control (Table 2). Beer yeast and soybean extract presented lower IMGR values but also improved the colony vigor. Sales-Campos et al. (2008) obtained the highest mycelial growth to *Pleurotus ostreatus* (Jacq.) P. Kumm. using *S. amara* sawdust supplemented with soybean bran. Also *S. amara* residue, rice and wheat bran, and $CaCO_3$ formulation was used for *P. lecomtei* mushroom production (Sales-Campos & Andrade, 2011).

However, while these supplements are generally inexpensive in cereal-producing areas of Brazil, rice, wheat, and soybean cultivation is scarce to nonexistent in the Central Amazon. Thus acquiring large amounts of these supplements would elevate the costs of the substrate in the Amazon region.

Other regional agroforestry residues, however, are produced in large amounts and rarely used. For example, the pulp of *A. aculeatum* fruit is widely consumed in regional dishes throughout the year; the fruit shell residue is generally not used for other purposes and is easy to acquire. Likewise, the shells of *C. guianensis* are an unused residue of oil extraction for cosmetic and therapeutic products, while *E. oleracea* seed shells and *P. edulis* fruit shells are left over from the production of açaí and passion fruit juices.

Table 2. Effect of supplementing *Simarouba amara* sawdust substrate with beer yeast and cereal bran on *Panus strigellus* mycelial growth.

Supplement[(1)]	**Mean**[(2)]	**Standard deviation**	**Colony vigor**[(3)]
Wheat fiber	1.22 a	± 0.02	+++
Rice bran	1.21 a	± 0.02	+++
Soy fiber	1.21 a	± 0.02	+++
Wheat germ	1.19 a	± 0.09	+++
Soy extract	1.15 b	± 0.07	+++
Beer yeast	1.13 b	± 0.05	+++
Control (without supplementation)	1.13 b	± 0.02	+

(1)The substrate was composed of a five-to-one (w/w) mixture of *Simarouba amara* sawdust and the supplement. Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test. (2)Average of five replications and two repetitions of index of mycelial growth rates values-IMGR (cm/day). (3)Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

Regarding supplementation with regional agroforest residues, all of the supplements improved the IMGR and/or colony vigor of *P. strigellus* in *S. amara* sawdust compared to the control (Table 3). Considering both IMGR and colony vigor, the 10:1 mixture of *S. amara* sawdust with *A. aculeatum* presented the best alternative.

Table 3. Effect of supplementing *Simarouba amara* sawdust substrate with Central Amazon fruit residues on *Panus strigellus* mycelial growth

Substrate	Amount	Mean[(1)]	Standard deviation	Colony vigor[(2)]
Euterpe oleracea seed	5:1	1.15 a	± 0.04	+
Carapa guianensis seed shell	10:1	1.14 a	± 0.03	+
Euterpe oleracea seed	10:1	1.13 a	± 0.05	+
Astrocaryum aculeatum fruit shell	10:1	1.13 a	± 0.03	++
Simarouba amara (control)	10:0	1.08 b	± 0.04	+
Carapa guianensis seed shell	5:1	1.08 b	± 0.04	++
Astrocaryum aculeatum fruit shell	5:1	1.08 b	± 0.06	++
Passiflora edulis fruit shell	10:1	1.04 c	± 0.02	++
Passiflora edulis fruit shell	5:1	0.97 d	± 0.02	+++

(1)Average of five replications of index of mycelial growth rates values-IMGR (cm/day). Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test. (2)Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

For spawn production: *S. amara*, *H. petraeum*, and *A. lecointei* substrates had been totally colonized by *P. strigellus* after 25 days of incubation at 35 ºC in the three types of packing materials tested (Figure 2). However, several aspects should be considered, including the costs of the packing materials, the time required for the spawn run, the transportation viability, and the feasibility of mycelial inoculation of the substrate. From packing materials examined here, type 1 (the flask) was more feasible for inoculation and easier to transport. The flask, however, was also the most expensive packing material and visually checking whether colonization had occurred was difficult because the flask was opaque (Figure 2A), and it could prevent observation of contaminants during the incubation. Packing material 2 (the bag without a filter) was of intermediate cost and allowed for good visibility of colonization. Nevertheless, it was also fragile and required the use of two bags; furthermore, it was necessary to create a respirator using a ring of PVC tubing and hydrophobic cotton (Figure 2B). Packing material 3 (the bag with a filter) held a larger amount of substrate, resulting in lower cost. Produced specifically for the production of *L. edodes* spawn, the bag

contains a filter for gaseous change, is resistant enough for transport, and allows for visual checking of colonization (Figure 2C). This polypropylene sack was considered the most appropriate packing by representing smaller cost for the spawn production. However, few distributors of this material operate in Brazil.

Conclusion

Simarouba amara sawdust and *Astrocaryum aculeatum* fruit shell are readily available in the North region, these residues showed potential as substitute of *Eucalyptus* sp. sawdust and rice bran, commonly used in the South and Southeast of Brazil, for *P. strigellus* spawn production.

Acknowledgments

This research was financed by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) PIPT Program, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by PNADB Program. R. Vargas-Isla is grateful for a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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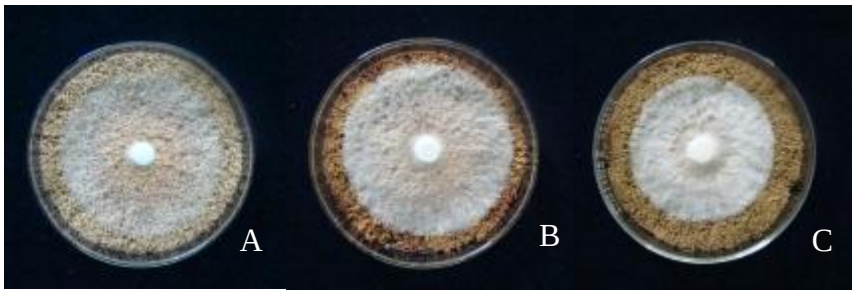

Figure 1. Classification of three colony vigor levels of *Panus strigellus*. (A) thin (+); (B) medium (++); and (C) dense (+++). Fonte: R. Vargas-Isla

Figure 2. *Panus strigellus* spawn in sawdust of *Simarouba amara*. The polypropylene packing material: (A) 15 × 9 cm flask; (B) 23 × 36 cm bag with an added respirator; and (C) 32 × 46 cm bag with a filter. Fonte: R. Vargas-Isla.

ARTIGO 4

Vargas-Isla R, Yuyama LKO, Aguiar JPL, Ishikawa NK. Potential use of internal sheath of peach palm for *Panus strigellus* spawn production. *Pesquisa Florestal Brasileira*.

Manuscrito submetido a Pesquisa Florestal Brasileira em 09 de outubro de 2012

Potential use of internal sheath of peach palm for *Panus strigellus* spawn production

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Potencial de uso da bainha interna da pupunheira para produção de semente-inóculo de *Panus strigellus*

Resumo

Panus strigellus, apresenta crescimento micelial em temperaturas elevadas favorecendo o desenvolvimento do cultivo em regiões tropicais. Obter substratos disponíveis localmente é o primeiro passo para cultivo rentável de cogumelos. A pupunha, *Bactris gasipaes*, é cultivado nas agroindústrias de palmito no Amazonas; um dos resíduos da palmeira é sua bainha interna (PPIS) protetora. Neste trabalho, foi avaliado o potencial de uso do PPIS para a produção de semente-inóculo de *P. strigellus*. Em placas de Petri foram analisados o efeito da umidade do substrato e níveis de suplementação de serragens por PPIS. O crescimento micelial foi avaliado pela medida da colônia em cm dia^{-1} e vigor. A umidade de 60% e *S. amara* suplementada com PPIS na proporção 10:1 foi considerada o melhor resultado. Esta formulação foi aplicada na elaboração da semente-inóculo em sacos de polipropileno. Obteve-se com sucesso a semente-inóculo de *P. strigellus* após 25 dias de incubação a 35 ºC.

Termos para indexação: *Bactris gasipaes*; *Simarouba amara*; *Lentinus strigellus*

Potential use of internal sheath of peach palm for *Panus strigellus* spawn production

Abstract

Panus strigellus presents mycelial growth at elevated temperatures, making it promising for cultivation in tropical regions. The use of locally available substrates is the first step in cost-effective mushroom production. Peach palm, *Bactris gasipaes* is grown in palm agribusinesses in Amazonas State; one of the waste products of this palm is its protective internal sheath (PPIS). In this study, we evaluated the potential use of the PPIS for *P. strigellus* spawn production. The effects of substrate moisture and supplementation levels of sawdust were analyzed in Petri dishes. Mycelial growth was evaluated by measuring the vigor and growth of the colony (cm day^{-1}). A humidity of 60% and supplementation of *Simarouba amara* sawdust with PPIS in a 10:1 ratio was considered the most suitable combination. This formulation was applied for spawn preparation in polypropylene bags. Spawn production of *P. strigellus* was successfully achieved after 25 days of incubation at 35 °C.

Index terms: *Bactris gasipaes*; *Simarouba amara*; *Lentinus strigellus*

Introduction

In Brazil, heart of palm is extracted from several genera and species of palm. Although *Euterpe edulis* Mart. (common name = juçara) and *E. oleracea* Mart. (common name = açaí) have been used for heart of palm production, more heart of palm has been produced using *Bactris gasipaes* Kunth (common name = peach palm, *pupunha*) (Clement and Bovi 2000).

Bactris gasipaes is the only domesticated neotropical palm whose starchy–oily fruits are subsistence products, and heart of palm production is an expanding agribusiness (Silva and Clement 2005). The micro-businesses of peach palm preserves in the Amazonas State, including the Manaus region, produced 905 tons of heart of palm in 2007 (IDAM 2007). In heart of palm production from *B. gasipaes*, the protective internal sheath of the palm is discarded, generating large amounts of residues that are not used. Some studies have examined the use of these residues in mushroom cultivation. Tonini *et al.* (2007) investigated the use of *E. edulis* sheaths as a medium for *Lentinula edodes* (Berk.) Pegler axenic culture and obtained success in the basidiomata production. Furthermore, Sales-Campos and Andrade (2010) studied mycelia growth of *P. lecomtei* (Fr.) Corner on *B. gasipaes* stipe.

Panus strigellus (Berk.) Overh. mycelial growth in tropical climates at temperatures of 35–40 °C, as well as its accentuated *umami* taste, is different from other mushrooms (Vargas-Isla and Ishikawa 2008). Given these characteristics, *P. strigellus* is a very interesting prospect for the development of mushroom cultivation in Amazonas State. Screening has been conducted using 11 forest species and regional fruit residues of the Central Amazon to produce the spawn of *P. strigellus*; among these, *Simarouba amara* Aubl. (common name = *marupá*) sawdust and *Astrocaryum aculeatum* G.Mey. (common name = *tucumã*) fruit shell formulations presented the best alternatives for *P. strigellus* spawn production (Vargas-Isla *et al.* 2012). However, the fruit shell residues are only produced in small amounts by fruit

processing places and are thus unlikely to be available on the scale required for spawn production.

The spawn constitute the base for the commercial cultivation of edible mushrooms, and their production is the main challenge of commercial mushroom producers. In Brazil does not exist, until the time, commercial spawn from Amazonian mushrooms. The current study evaluated formulations using the *B. gasipaes* internal sheath (PPIS) as an alternative for use in a substrate for *P. strigellus* spawn.

Material and methods

Microorganism

The INPACM 1464 culture from the Coleção de Micro-organismos de Interesse Agrossilvicultural – Instituto Nacional de Pesquisas da Amazônia (INPA) was used. The stock culture and inocula for the experiments were the same as reported by Vargas-Isla *et al.* (2012).

Residues for substrate formulation

Bactris gasipaes stipe (1.50 m height; $n = 5$) was cut on Campus III of the INPA to obtain PPIS. The samples were weighed and divided into stipe, leaves, external and internal sheath, and heart of palm. Following dehydration at 65 °C, PPIS was triturated in an industrial blender (LSP-04, SIEMSEN, Ltd., Brusque-SC, Brazil) and then transferred and sifted (Willye TE-680, TECNAL, Piracicaba-SP, Brazil) using a 0.5 mm-mesh sieve. Sawdust of *S. amara* and *Hymenolobium petraeum* Ducke (common name = *angelim-pedra*), *A. aculeatum* fruit shell (TFS), and rice (*Oryza sativa* L.) bran were used for comparison. Each residue was dried in an oven at 65 °C with air circulation and stored in plastic bags at room temperature.

Centesimal analysis

The centesimal composition analysis of the residues was conducted in accordance with the AOAC (1998) methodology (n = 3).

Substrate humidity

The percentage of substrate humidity was determined. Sawdust of *H. petraeum* mixed with rice bran (sawdust:supplement, 10:1, w/w) was used for the humidity measurement, and distilled water was added to the formulation to reach 30, 40, 50, 60 and 70% hydration (w/v) prior to its distribution in Petri plates (15 ± 1 g/plate). The incubation temperature for the experiments was 35 ºC.

The colony diameter was evaluated by the mycelial growth measurement (cm day^{-1}). In addition to mycelial growth, the colony vigor was visually evaluated and classified as (+) thin, (++) medium, or (+++) dense.

Substrate formulations

The experiment with different substrate formulations was carried out in two stages. First, the PPIS and *S. amara* sawdust were mixed and supplemented separately with TFS and rice bran in proportions of 10:0, 10:1, and 5:1 (w/w) and mixed with distilled water to approximately 60% hydration (w/v). Second, we prepared sawdust of *H. petraeum* and *S. amara* mixed separately with PPIS and rice bran (1:1 and 10:1, w/w), with distilled water added to approximately 60% hydration (w/v). The formulations were then distributed on Petri plates (15 ± 1 g/plate). The experiment was evaluated using the same criteria as the substrate humidity test after five days of mycelial growth.

Statistical analysis

Each experiment was conducted twice using five replicates. Analysis of variance (ANOVA) was used to examine the results of the experiments, and the averages were compared using the Tukey test at the 1% level of significance.

Spawn production

Simarouba amara sawdust supplemented with PPIS (10:1) was placed in polypropylene packing (23 × 36 cm) with 800 g of wet substrate (Vargas-Isla *et al.* 2012). After 25 days, the colonized substrates were taken from the packing and cut for visual observation (n = 5).

Results and discussion

Table 1 lists the quantity of residue used to obtained heart of palm of *B. gasipaes.* The total residue is equivalent to 97.5%, of which the internal sheath residue represents 8.5%. Considering these data and the 2007 production of heart of palm of *B. gasipaes* in the Manaus region, we estimated that over 3,000 tons of residue has been discarded, making this reside highly available.

Table 1. Distribution of fresh material obtained of *Bactris gasipaes*, Manaus-AM

	Parts of palm				
	Stipe	Leaves	External sheath	Internal sheath	Heart of palm
Fresh weight (kg)	17.9[(1)]	5.5	1.7	2.4	0.7
Percentage	63.5	19.5	6.0	8.5	2.5

[(1)]Data shown represent the average of five samples with approximately 1.5 m height.

Supplementation with a nitrogen source is necessary because pure *S. amara* sawdust contains only 1.34% protein (Table 2). The PPIS contained more protein (3.65%) than *S.*

amara sawdust but less protein than rice bran (13.07%). The nutritional supplements were added to increase the levels of nitrogen and useable carbohydrates because nitrogen levels in the sawdust were low, which may be a limiting factor for decay.

Table 2. Composition (% dry matter) of the residues obtained in Manaus-AM

Residues	Ash	Protein	Lipids
Astrocaryum aculeatum fruit shell	3.28 ± 0.049[(1)]	5.55 ± 0.041	24.29 ± 0.081
Bactris gasipaes internal sheath	3.79 ± 0.040	3.65 ± 0.046	0.88 ± 0.062
Oryza sativa bran	3.28 ± 0.407	13.07 ± 0.701	14.76 ± 0.002
Simarouba amara sawdust	0.13 ± 0.012	1.34 ± 0.034	0.42 ± 0.0003

[(1)]Data shown represent the average of three samples according to AOAC (1998) methods.

Considering both criteria of mycelial growth, the best substrate condition was provided by 60% humidity ($p<0.01$; Figure 1).

The substrates formulated with *S. amara* sawdust provided the highest radial mycelial growth (cm day^{-1}) of *P. strigellus* ($p<0.01$; Figure 2). However, both rice bran and TFS supplementation improved the colony vigor compared with the control *S. amara* sawdust. Although the mycelial growth on PPIS formulations supplemented with rice bran and TFS was lower, the colony vigor was at the highest level; thus, in the second experiment PPIS was used as a supplementation.

The effect of the supplementing *H. petraeum* and *S. amara* sawdust with PPIS on *P. strigellus* mycelial growth was tested (Figure 3). Formulations with both sawdust and PPIS (1:1) presented the lowest radial mycelial growth (cm day^{-1}). Considering both criteria (i.e., radial mycelial growth and colony vigor) and the additional costs to obtain PPIS, the sawdust:PPIS (10:1) formulation presented the best alternative.

Conclusion

Regarding spawn production, *Simarouba amara* substrates (800 g) had been totally colonized by *Panus strigellus* after 25 days of incubation at 35 ºC in the polypropylene bag. This result suggests that the internal sheath of *Bactris gasipaes* is an alternative for the spawn production of *P. strigellus*.

Acknowledgments

The authors thank Dr. Kaoru Yuyama for providing specimens of *B. gasipaes*. R. Vargas-Isla is grateful for a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). This research was financed by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by the PNADB Program.

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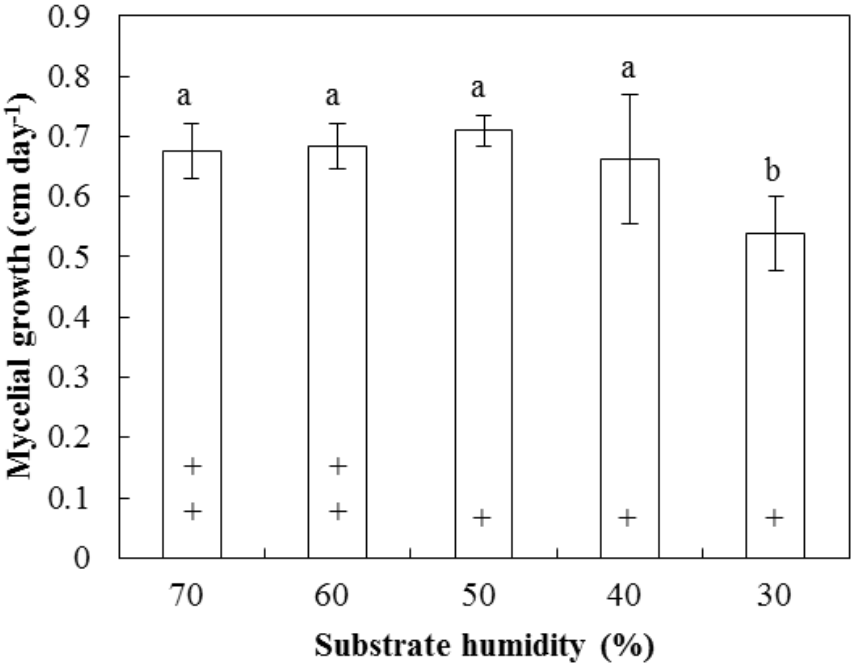

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Figure 1. Effect of substrate humidity on *Panus strigellus* mycelial growth. The substrate formulation was a mixture of *Hymenolobium petraeum* sawdust and rice bran (10:1; w/w). Data shown represent the average of five replications and two repetitions of mycelial growth (cm day^{-1}). Means indicated by the same letter(s) are not significantly different ($p<0.01$) by the Tukey test. Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

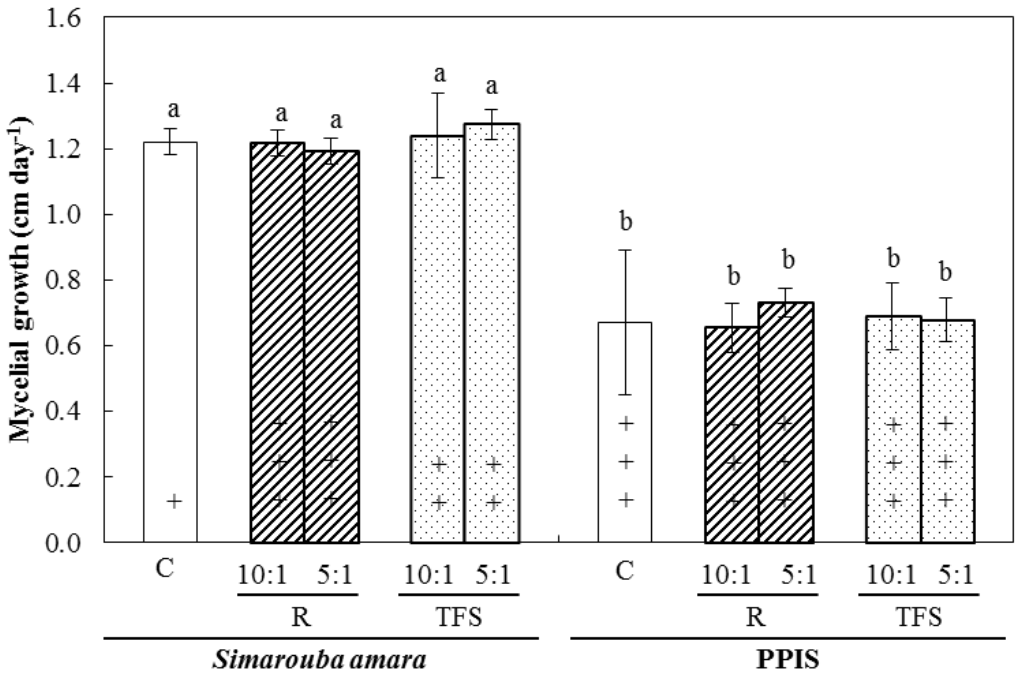

Figure 2. Effect of supplementing *Bactris gasipaes* internal sheath and *Simarouba amara* sawdust on *Panus strigellus* mycelial growth at 35 °C. C, control; R, rice bran; TFS, *Astrocaryum aculeatum* fruit shell; PPIS, *B. gasipaes* internal sheath. Substrate composition was 10:1 and 5:1 (sawdust:supplement, w/w). Data shown represent the average of five replications and two repetitions of mycelial growth (cm day^{-1}). Means indicated by the same letter(s) are not significantly different ($p<0.01$) by the Tukey test. Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

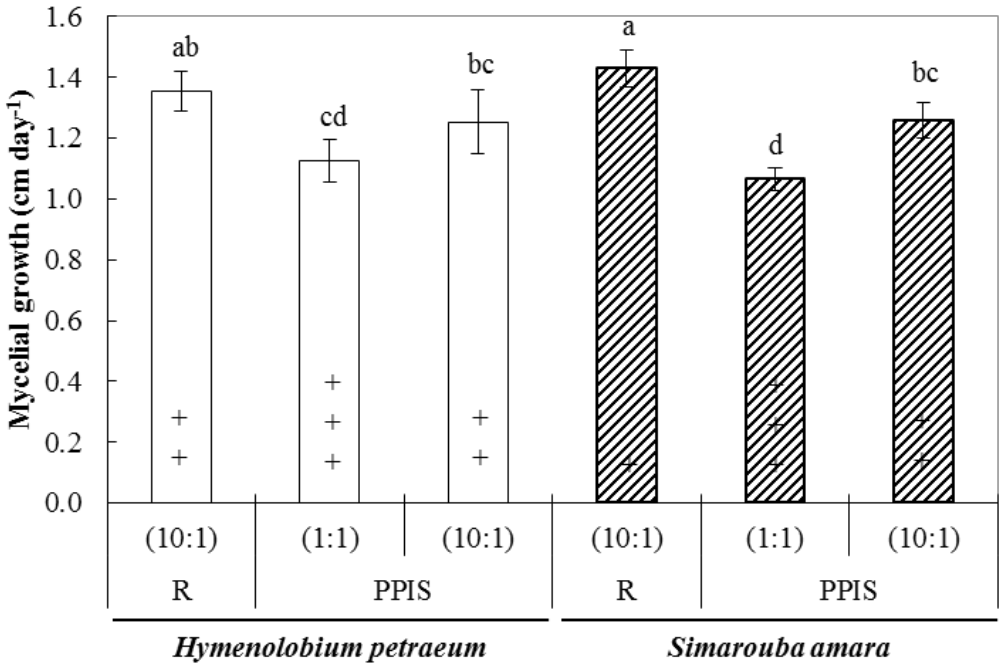

Figure 3. Effect of supplementing sawdust with *Bactris gasipaes* internal sheath on *Panus strigellus* mycelial growth at 35 °C. R, rice bran; PPIS, *B. gasipaes* internal sheath. Substrate was composed of 10:1 and 1:1 (sawdust:supplement, w/w). Data shown represent the average of five replications and two repetitions of mycelial growth (cm day^{-1}). Means indicated by the same letter(s) are not significantly different ($p<0.01$) by the Tukey test. Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

Capítulo 4: CARACTERÍSTICAS BIOLÓGICAS DE *Panus strigellus*

O estudo das características biológicas inclui o ciclo de vida dos cogumelos. A elucidação de um ciclo sexual oferece uma ferramenta valiosa para a análise genética clássica e proporciona detalhes sobre o papel ou os sistemas de reprodução sexual de espécies de fungos como Panus strigellus.

ARTIGO 5

Vargas-Isla R, Tokimoto K, Ishikawa NK. Características biológicas de *Panus strigellus*.

Manuscrito formatado nas normas da Acta Amazonica

Biological characteristics of *Panus strigellus*

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12 Abstract

13

14 The Amazon climate favours the growth of numerous mushroom species. One
15 of these species, *Panus strigellus*, has potential applications in food industries. In this
16 paper, we describe the biological characteristics of *P. strigellus*, including mycelial
17 preservation, enzymatic activity, and the life cycle (including mating type). Four
18 methods of mycelial culture maintenance during 360 days were compared, and the
19 silica gel method worked efficiently at 4 °C. Isolates of this species showed amylase,
20 cellulase, esterase, and lipase extracellular enzymatic activity in specific solid media.
21 Also, the tetrapolar mating system of *P. strigellus* and the incompatibility between *P.*
22 *strigellus* and *P. lecomtei* was confirmed.

23

24 Keywords: *Lentinus strigellus*; Mycelium preservation; Extracellular enzyme; Fruit-body;
25 Tetrapolar system

26

Características biológicas de *Panus strigellus*

Resumo

A Amazônia apresenta condições climáticas que favorecem o crescimento de um grande número de espécies de cogumelos. Uma destas espécies é *Panus strigellus*, com potencial de uso na indústria alimentícia. Neste trabalho, foram descritas as características biológicas de *P. strigellus*, incluindo a preservação do micélio, a atividade enzimática e o ciclo de vida (incluindo o *mating system*). Foram comparados quatro métodos para a manutenção da cultura micelial durante 360 dias e o método de sílica-gel foi mais eficiente para a manutenção da cultura de *P. strigellus* a 4 °C. Os isolados desta espécie apresentaram atividade enzimática extracelular de amilase, celulase, esterase e lipase em meio sólido específico. Também foi confirmado o sistema tetrapolar de cruzamento de *P. strigellus* e a incompatibilidade entre *P. strigellus* e *P. lecomtei*.

Palavras-chave: *Lentinus strigellus*; Preservação de micélio; Enzima extracelular; Corpo de frutificação; Sistema tetrapolar

45 Introduction

46

47 The Amazon climate favours the growth of numerous mushroom species including
48 several edible species, such as *Panus strigellus* [Berk.] Overh., (= *Lentinus strigellus* Berk.)
49 with potential applications in food industries and for cultivation.

50 The basidiomata of an edible mushroom *P. strigellus* were collected in 2006 in
51 Manaus, Brazil. This sample had thermophilic characteristics, with a mycelial growth
52 capacity in a temperature range of 25–45 °C and an optimal temperature of 35°C,
53 demonstrating its potential use for mushroom cultivation in tropical climates (Vargas-Isla and
54 Ishikawa 2008). This lentinoid species is commonly found in tropical and South America
55 (Pegler 1983). However, mycelial culture storage was difficult when using common
56 techniques such as storage in a refrigerator at 4 ± 1°C. Thus, in this study we developed a
57 novel *P. strigellus* culture preservation method.

58 The study of mushroom biology includes every aspect of taxonomy, growth, nutrition,
59 physiology, genetics, medicinal and tonic attributes, edibility, and toxicity (Chang 1993). In
60 this study, *P. strigellus* enzymatic activity, life cycle, and mating type were examined.

61 The enzymatic activities of fungi have been applied in many industries (primarily food
62 industries), such as the brewing, dairy, starch, paper, and biofuel industries; they have also
63 been used as biological detergents (Carlile *et al*. 2001). The biotechnology industry is always
64 seeking lineages that produce larger amounts of enzyme. In previous studies in which we
65 examined novel formulations and ingredients as substrates for the production of spawn and/or
66 mushroom cultivation, information on the enzymes produced by different fungi was important
67 for optimizing substrate usage. In this study, common assays to examine extracellular enzyme
68 production were used.

Characterizations of sexual cycles are invaluable in classical genetic analyses. We used this to investigate the role of sexual reproduction in *P. strigellus*. Because *P. strigellus* is a wild species, knowledge of the life cycle is important to ensure its correct isolation and understand its mating system for future genetic improvement. Because *P. strigellus* is frequently confused with *P. lecomtei* (Fr.) Corner (= *L. strigosus* [Schwein.] Fr.), the monokaryon of both species were isolated to demonstrate incompatibility.

Materials and methods

Microorganism

The isolated used in this study were obtained from the basidioma collected in Amazonas State – Brazil (Table 1). The stock culture was maintained on potato dextrose agar (PDA; HiMedia Laboratories Pvt. Ltd., India) slants at 25 ºC in the dark. Mycelia of the stock culture were cultivated at 35 ºC in Petri plates (90 mm diameter) containing PDA medium. After five days of growth, fragments of mycelia (2 × 2 mm) were removed and used as inoculum for the experiments.

Mycelium maintenance

Four maintenance methods to preserve *P. strigellus* isolates (INPACM1464 and INPACM1532) were used: (1) culture medium slants (Figure 1A), in which isolates were maintained in tubes containing Sabouraud dextrose agar (SDA; Difco, USA); (2) mineral oil (Figure 1B), in which cultures were grown in tubes containing SDA medium slants covered

with sterilized mineral oil; (3) Castellani's method (Figure 1C), in which fragments of mycelia were transferred to Eppendorf tubes containing sterile distilled water; and (4) silica gel (Figure 1D), in which colonized wood pieces (1.5 cm length) of tooth sticks with the isolates were transferred to tubes covered with threads containing grains of sterile silica gel and hydrophobic cotton. All samples were stored in the dark at 25 °C and 4 °C. The cultures were renewed every 120, 240, and 360 days to verify mycelia viability.

Enzymatic activity analysis

The extracellular enzymatic activity of *P. strigellus* isolates (INPACM1464 and INPACM1531) was evaluated in specific solid culture media.

To evaluate aminolitic activity, we used culture medium containing 23 g nutrient agar (Difco, USA), 2 g soluble starch (VETEC, Brazil), and 1000 mL distilled water, adjusted to pH 6. The plates were inoculated with fragments of mycelial and maintained at 35 °C for 2 days. Alcoholic iodine solution 1% (10 mL) (1 g iodine resublimed, VETEC, Brazil; 100 mL absolute alcohol, NUCLEAR, Brazil) was added to each plate (Pandolfo *et al*. 2004).

For cellulolitic activity, we used minimum medium as described by Pontecorvo *et al.* (1953) containing 6 g $NaNO_3$ (CRQ, Brazil), 1.5 g KH_2PO_4 (NUCLEAR, Brazil), 0.5 g KCl (NUCLEAR, Brazil), 0.01 g $MgSO_4.7H_2O$ (VETEC, Brazil), 0.01 g $FeSO_4.7H_2O$ (Synth, Brazil), 0.01 g $ZnSO_4.7H_2O$ (Quimex, Brazil), 15 g agar (Difco, USA), 10 g carboximetilcellulose (NUCLEAR, Brazil), and 1000 mL distilled water, adjusted to pH 6. The plates with fragments of mycelial were maintained at 35 °C for 2 days.For enzymatic activity evaluation, 5 mL 1% Congo red (NUCLEAR, Brazil) solution (10 g/L) was added to each colony over 15 min. Next, the Congo red solution was discarded and the plates were

117 washed using 4M NaCl (CRQ, Brazil) solution (234 g/L) over the colony of several minutes
118 (Pallu 2010).
119 For esterase and lipolitic activity, we used 10 g peptone (Difco, France), 5 g NaCl
120 (CRQ, Brazil), 0.1 g $CaCl_2.2H_2O$ (VETEC, Brazil), 20 g agar (Difco, USA), and 1000 mL
121 distilled water, adjusted to pH 6.0. A total of 10 mL sterilized Tween 80 (VETEC, Brazil) and
122 Tween 20 (VETEC, Brazil) was added separately to the medium. The plates were inoculated
123 with fragments of mycelial and maintained for 2 days at 35 °C. Once colonies formed, the
124 plates were stored at 10 °C for 7 days to allow for crystal halo formation (Pandolfo *et al*.
125 2004).
126 Samples were evaluated in two phases. First, the enzymatic index measuring the
127 diameter of the halo formed around the colony divided by the diameter of the colony. Second,
128 we prepare others Petri plates with specific solid medium to measurement: the mycelial
129 growth (cm/day) and the biomass as described by Vargas-Isla and Ishikawa (2008). The
130 experimental design was completely randomized with three replications of the enzymatic
131 index and five repetitions of the mycelial growth and biomass. Analysis of variance
132 (ANOVA) was used to examine the results of the experiments, and the averages were
133 compared using the Tukey test at the 1% significance level.
134
135 *Basidiomata production*
136
137 Initially, fragments of *P. strigellus* mycelia were placed on sterilized wheat grains
138 (*Triticum aestivum* (L.) Thell.) at 121 °C for 1 h, to multiply and were incubated at 35 °C for
139 15 days. Next, following the methodology described by Vargas-Isla *et al*. (2012), *Simarouba*
140 *amara* Aubl. sawdust supplemented with rice (*Oryza sativa* L.) bran (10:1, w/w) was placed

141 in polypropylene packing (23 × 36 cm) with 800 g wet substrate. Next, we added 3 ± 1 g
142 colonized wheat grains and incubated at 35 °C for 25 days, in dark. The colonized substrates
143 were used as spawn for the mushroom fructification study.

144 The substrate formulation to fructification was made from the sawdust of
145 *Hymenolobium petraeum* Ducke, *Bactris gasipaes* Kunth (common name = peach palm,
146 *pupunha*) internal sheath, and rice bran (results of the Chapter 3), and was irrigated with
147 distilled water until reaching approximately 60% hydration (w/v; n = 20). Polypropylene bags
148 (5 cm wide × 15 cm height) were filled with 100 g wet substrate. For gas exchange, the bags
149 were stoppered with hydrophobic cotton plugs held by polyvinylchloride (PVC) cylinders and
150 the blocks were sterilized for 1 h at 121 °C. After cooling, the bags were inoculated with 1.5 g
151 spawn from strains INPACM1530 and INPACM1531, and incubated in the dark at 35 °C
152 until the mycelium completely colonized the substrate. Spawning run time and time of
153 primordium initiation were recorded.

154 After the bags were completely colonized by the mycelium, the blocks were incubated
155 at 20 °C for 24 h. Next, the blocks underwent cold soaking for 4 h until fructification. Then
156 the blocks were moved to the fruiting room in the greenhouse and were placed inside
157 transparent plastic boxes (47 cm large × 31 cm wide × 20 cm height) to maintain humidity.
158 The temperature (min 22.9 °C, max 35.6 °C, average 27.1 °C) and humidity (min 50.1%, max
159 93.9%, average 81.4%) were measured using a thermohigrometer/datalogger (ICEL HT-4000,
160 Manaus, Brazil). The natural photoperiod (744–3330 lux) of the greenhouse was determined
161 using an illuminance meter (IM-5, Topcon, Japan). Spray watering for 5 min three times was
162 manually provided and bags were removed after primordium initiation.

Obtention of monosporic cultures

After collecting the basidioma, the basidiospores obtained from the spore print were re-suspended in sterile distilled water and agitated. Next, serial dilutions of the distilled water were obtained and an aliquot was spread over the PDA surface in the Petri dish and incubated at 25 °C for 12–14 h. Each individual germinated basidiospore was selected under an optical microscope and transferred to a new Petri dish of PDA after obtaining monosporic cultures, incubated at 25 °C, and verified for the absence of clamp connections.

Self-crosses

The mating types were determined by pairing the monosporic isolates in all possible combinations and placing mycelia plugs 1 mm apart on PDA. The cultures were incubated at 25 °C for 24–48 h and the formation of a clamp connection was observed under a microscope.

Mating studies

To evaluate the intersterility of *P. strigellus* and *P. lecomtei*, monosporic isolate plugs (2 × 2 mm approximately) of each mating type were paired on a PDA plate, incubated at 25 °C for 24–48 h, and then observed under a microscope.

183 Results and Discussion

184

185 *Culture maintenance*

186

187 All methods preserved the viability of culture mycelium during 120 days (Table 2).
188 Three maintenance methods resulted in 100% culture viability at specific temperatures in 240
189 days, but only SDA with mineral oil preserved all samples at 4 ℃ and 25 ℃ during 240 days.
190 Culture maintenance at 4 °C during 360 preservation days, which is normally used to
191 preserve edible mushrooms cultures, the unique silica gel method maintained the viability of
192 *P. strigellus* isolates (Table 2). This method combined low temperatures and dehydration,
193 resulting in the fungi entering a "latency period". Mycelium viability was lost after storage at
194 25 ℃ using the silica gel method. Some wood-inhabiting basidiomycetes and ascomycetes
195 can be stored on wood chips for up to 10 years at 4 °C (Nakasone *et al*. 2004).

196 Also, SDA with mineral oil using the Castellani method preserved the viability of
197 isolates at 25 °C in 360 preservation days (Table 2). During storage, the temperature and
198 relative humidity influenced spore viability to sporulant fungi (Cárdenas 2010). Preservation
199 of microorganisms is important to ensure viability and morphologic, physiologic, and genetic
200 integrity of strain cultures, and to maintain their original characteristics (Chang 1993).

201 The silica gel method at 4 ℃ was more feasible for renewing the isolates and it could
202 reduce contamination during preservation. The appropriate combination of method and
203 temperature allowed for the maintenance and viability of thermophilic fungi such as *P.*
204 *strigellus*.

205 *Enzymatic activity*

206

207 *Panus strigellus* isolates (INPACM1464 and INPACM1531) showed amylase,
208 cellulase, esterase, and lipase extracellular enzymatic activity. The area degraded by amylase
209 around the colony was yellow and the area degraded by cellulose was opaque against the red
210 background compared to a zone not degraded by carboximetilcellulose. The esterase and
211 lipase activity resulted from the deposition of calcium salt crystals formed by the liberation of
212 oily acids from the enzymatic activity. However, there were significant differences between
213 isolates in most of the treatments ($p<0.01$; Figure 2).

214 Figure 3 shows mycelial growth (cm/day) of *P. strigellus* isolates in specific solid
215 media. There were significant differences between isolates on starch and tween culture
216 medium. The highest dry biomass was obtained on starch culture medium ($p<0.01$; Figure 4).
217 Decomposition of some biopolymers, cellulose, starch, and hemicellulose provided carbon
218 and energy for growth (Baldrian 2008). The hyphae secrete enzymes that break down
219 otherwise insoluble materials (such as cellulose and lignin in wood) and convert them into
220 simple sugars that diffuse back to the fungal hyphae, where they are absorbed (Przybylowicz
221 and Donoghue 1990).

222 The success of *P. strigellus* mycelial growth in *S. amara* sawdust supplemented with
223 *Astrocaryum aculeatum* G.Mey. fruit shell, *Carapa guianensis* Aubl. seed shell or *Euterpe*
224 *oleracea* Mart. seed (Vargas-Isla *et al*. 2012) could be due the esterase and lipase activity
225 (Figure 2). Although it is known that these fruit species contain lipids, further studies are
226 required.

Life cycle

Figure 5 depicts the life cycle of *P. strigellus*. Mushroom biology is the basis of mushroom cultivation and production, and the core of mushroom biotechnology (Chang 1993).

The life cycle of *P. strigellus* (Figure 5) begins when a mature fruiting body sheds basidiospores (which are thin-walled) into the air and they are dispersed by the wind. Those that land on a suitable substrate may, under proper conditions, germinate and establish a new colony. When the basidiospore geminates, it grows into primary mycelium (monokaryons). To develop secondary mycelium, two primary hyphae (monokaryons), containing compatible nuclei, grow together to form a dikaryon. Not all combinations of primary mycelial of *P. strigellus* are compatible. *Panus strigellus* has four mating types that are compatible only under certain combinations (Figure 5). The mating system of *P. strigellus* is heterothallic (two genetically different spores must mate) and tetrapolar (four different mating types). The secondary mycelium resulting from this mating can produce fruiting bodies and complete the life cycle. In this vegetative stage (secondary mycelium), the mycelium colonizes the substrate, absorbing and storing nutrients in preparation for fruiting. Unless sufficient nutrients have been stored, the fungus cannot progress to the fruiting stage (Przybylowicz and Donoghue 1990).

Fruiting bodies are produced in response to environmental signals such as temperature, humidity, and illumination (Figure 5), which often stress the mycelium. If the environment is favourable, they will continue to expand and develop into mature fruiting bodies. The change from the assimilative state (mycelial growth) to the reproductive state (basidiome formation) depends on complex factors such as disponibility substrate, hyphae age, and hyphae energy level, and specific environmental signals (Kendrick, 2000).

The fertile hymenium on the surface of the lamellae is covered with basidia and basidiospores (Figure 5). Fusion of the dikaryotic nuclei occurs within the basidia, which is immediately followed by a reduction division (meiosis) that results in four genetically different monokaryotic basidiospores. The mature spores fall from the hymenium onto surrounding areas or are picked up by air currents and are carried to new substrates.

Mating type study

Our results confirm the tetrapolar mating system for *P. strigellus* and *P. lecomtei* as observed by Petersen *et al.* (1997). For *P. strigellus*, 20 haploid isolates were used to define the mating system, and the tetrapolar system was defined using 7 monokaryous (Figure 6). For *P. lecomtei*, 12 haploid isolates were used to define the tetrapolar system (Figure 7). *Panus strigellus* and *P. lecomtei* were confirmed to be separate biological species by pairing experiments which showed crossing incompatibility (intersterility groups) as judged by the absence of clamp connections between-region pairings and distal sides of the inoculum plugs (Figure 8).

Conclusions

The silica gel method was optimal for preserving *P. strigellus* mycelial culture at 4 °C for extended periods (360 days). The enzyme activities differed significantly between isolates of this species and showed amynolitic, cellulolytic, esterase, and lipolityc activity. Also, the tetrapolar mating system for *P. strigellus* and *P. lecomtei* were confirmed and the incompatibility between species was observed.

278 Acknowledgments

279 R. Vargas-Isla is grateful for a scholarship from Conselho Nacional de
280 Desenvolvimento Científico e Tecnológico (CNPq). This research was financed by Fundação
281 de Amparo à Pesquisa do Estado do Amazonas (FAPEAM), and the Coordenação de
282 Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by PNADB Program.

283

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319 Table 1 – Isolates used is this study.

Species	Herbarium number	Culture number*	Locality
Panus strigellus	INPA222827	INPACM1464	Brazil, AM, Manaus, INPA – *Campus* III
	INPA239979	INPACM1530	Brazil, AM, Manaus, Puraquequara community
	INPA243941	INPACM1530	Cultivated material
	INPA243940	INPACM1531	Brazil, AM, Manaus, Puraquequara community
	INPA243943	INPACM1532	Brazil, AM, Manaus, INPA – *Campus* III
P. lecomtei	INPA239978	TMIC35103	Japan, Tottori. Cultivated material

320 *INPACM = Coleção de Micro-organismos de Interesse Agrossilvicultural of
321 Instituto Nacional de Pesquisas da Amazônia (INPA); TMIC = Culture collection of
322 the Tottori Mycological Institute.

323

324 Table 2 – Maintenance methods to preserve *Panus strigellus* isolates

Maintenance culture method	Temperature	Period (days)*		
	(°C)	120	240	360
Sabouraud Dextrose Agar (SDA) slants	25	100	75	0
	4	100	50	50
SDA slants + mineral oil	25	100	100	100
	4	100	100	0
Castellani's method	25	100	100	100
	4	100	75	50
Silica gel	25	100	50	0
	4	100	100	100

325 * Results represent in percentage

Figure 1 – *In vitro* culture preservation of *Panus strigellus* (A) Culture medium slants; (B) mineral oil; (C) Castellani's method; and (D) silica gel.

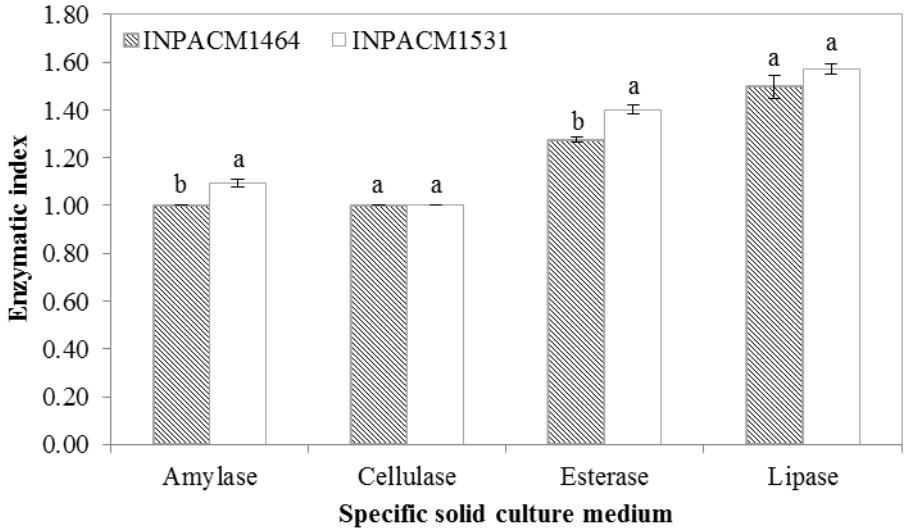
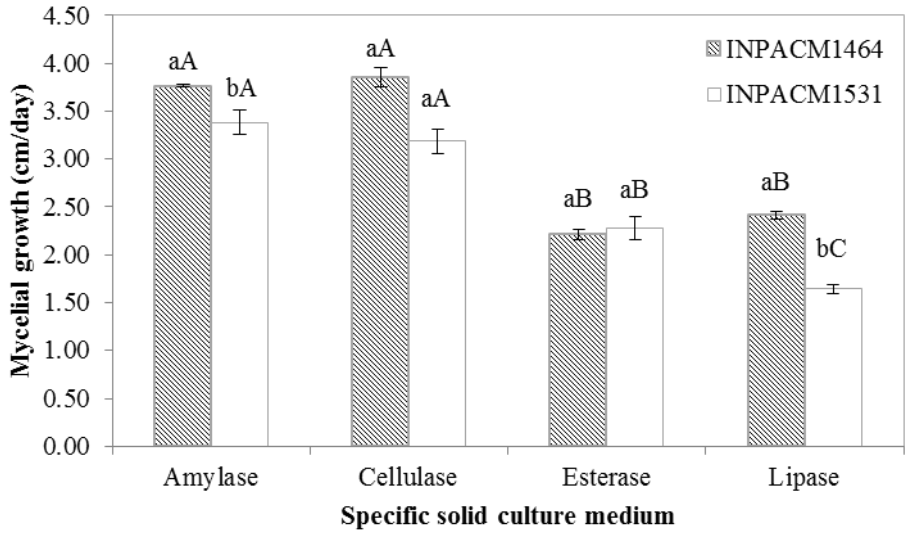
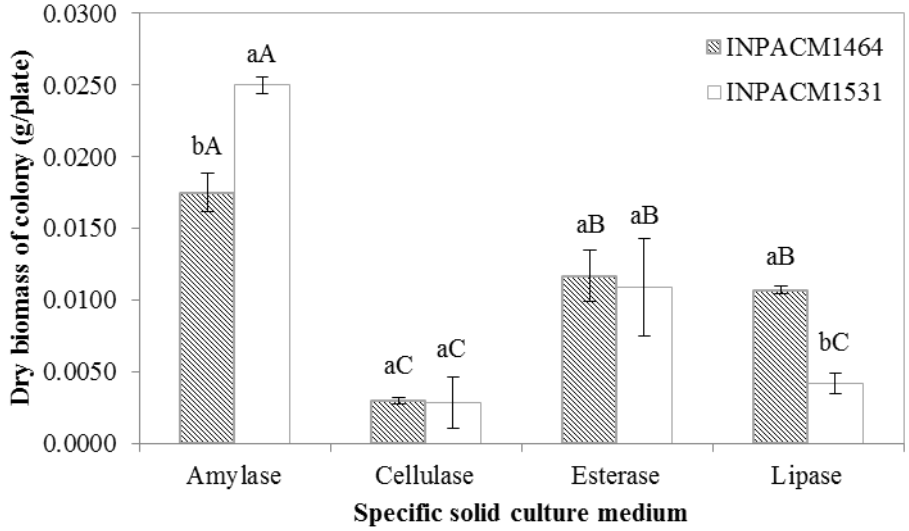


Figure 2 – Enzymatic index of *Panus strigellus* isolates using solid enzymatic culture media. Three days of mycelial growth at 35 °C. Results represent the average of three replicates. Means with the same letter(s) are not significantly different between isolates ($p<0.01$) by the Tukey test.



342

343 Figure 3 – Mycelial growth of *Panus strigellus* isolates using solid enzymatic culture media.
344 Five days of mycelial growth at 35 °C. Results represent the average of five replicates. Means
345 with the same minuscule letter(s) are not significantly different between isolates and means
346 with the same capital letter(s) are not significantly different between enzymatic culture media
347 ($p<0.01$) by the Tukey test.



348

349 Figure 4 – Dry biomass of *Panus strigellus* isolates using solid enzymatic culture media. Five
350 days of mycelial growth at 35 °C. Results represent the average of five replicates. Means with
351 the same minuscule letter(s) are not significantly different between isolates and means with

the same capital letter(s) are not significantly different between enzymatic culture media ($p<0.01$) by the Tukey test.

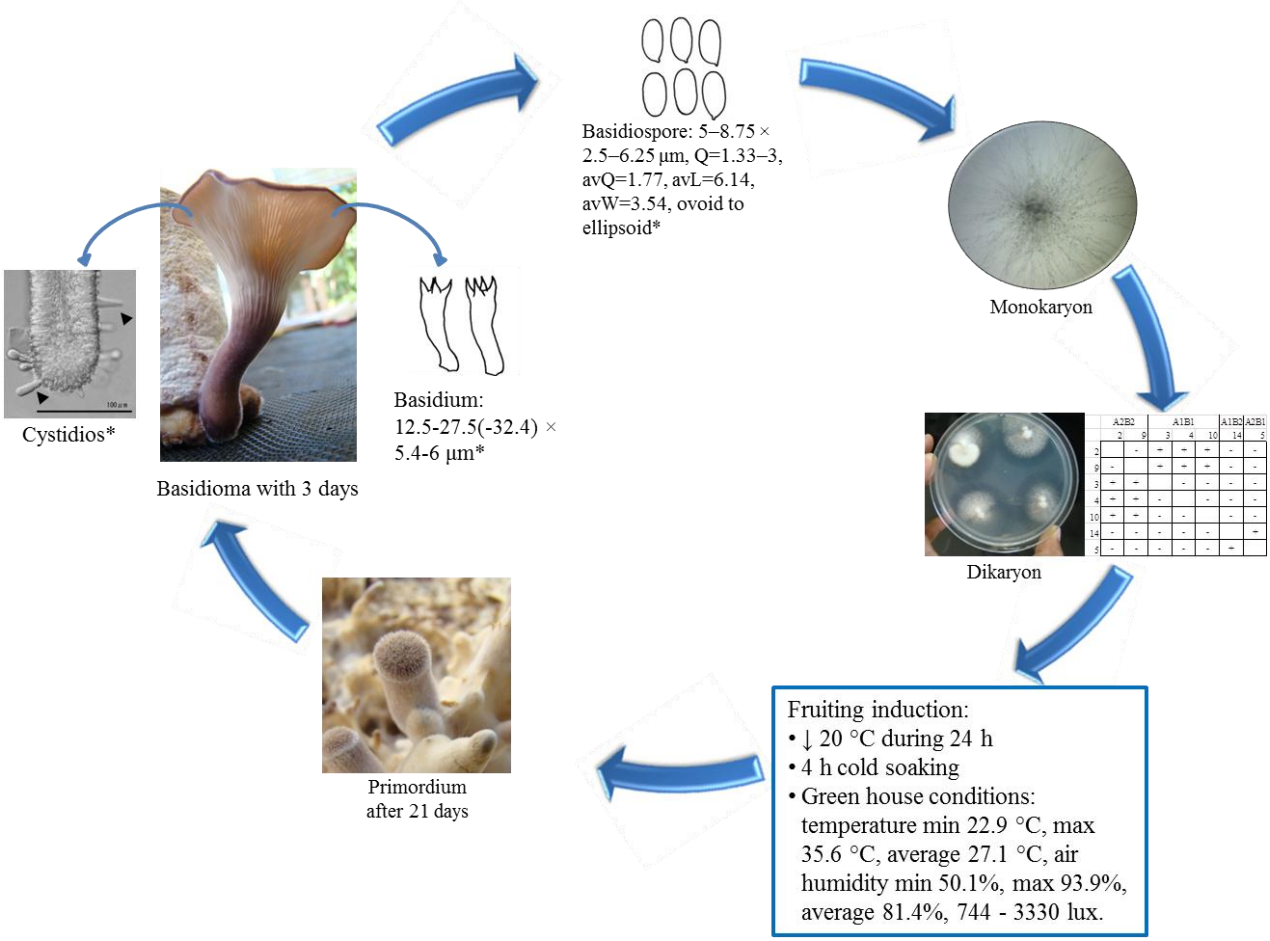


Figure 5 – The life cycle of *Panus strigellus*, showing the developmental stages of the fungus during one generation. *Microscopic data described in Chapter 2 page 44.

	A2B2		A1B1			A1B2	A2B1
	2	9	3	4	10	14	5
2		-	+	+	+	-	-
9	-		+	+	+	-	-
3	+	+		-	-	-	-
4	+	+	-		-	-	-
10	+	+	-	-		-	-
14	-	-	-	-	-		+
5	-	-	-	-	-	+	

359

360 Figure 6 – Haploid isolates of *Panus strigellus* (INPA243941). Self-cross grid between seven

361 haploid isolates. (+) compatibility mating; (-) incompatible mating.

	A1B1			A2B2		A2B1			A1B2			
	3	6	12	9	11	7	10	8	1	2	4	5
3		-	-	+	+	-	-	-	-	-	-	-
6	-		-	+	+	-	-	-	-	-	-	-
12	-	-		+	+	-	-	-	-	-	-	-
9	+	+	+		-	-	-	-	-	-	-	-
11	+	+	+	-		-	-	-	-	-	-	-
7	-	-	-	-	-		-	-	+	+	+	+
10	-	-	-	-	-	-		-	+	+	+	+
8	-	-	-	-	-	-	-		+	+	+	+
1	-	-	-	-	-	+	+	+		-	-	-
2	-	-	-	-	-	+	+	+	-		-	-
4	-	-	-	-	-	+	+	+	-	-		-
5	-	-	-	-	-	+	+	+	-	-	-	

362

363 Figure 7 – Haploid isolates of *Panus lecomtei* (INPA239978). Self-cross grid between twelve

364 haploid isolates. (+) compatibility mating; (-) incompatible mating.

Panus lecomtei	*Panus strigellus** 2	3	5	14
1	-	-	-	-
3	-	-	-	-
7	-	-	-	-
9	-	-	-	-

365

366 Figure 8 – Crosses between *Panus strigellus* (INPA243941) and *P. lecomtei* (INPA239978). *

367 haploid isolated number; (-) incompatible mating.

CONSIDERAÇÕES FINAIS

CONSIDERAÇÕES FINAIS

- Realizou-se uma revisão sobre os estudos etnomicológicos da Amazônia com a atualização de 59% dos nomes científicos das espécies comestíveis relatadas;

- Os estudos taxonômicos levaram a confirmação das espécies *Panus lecomtei* e *P. strigellus* com auxilio de características morfológicas e análises moleculares, descrevendo novas características microscópicas de *P. strigellus*. Neste trabalho a primeira ocorrência de *P. strigellus* para o Estado do Amazonas foi relatada. Assim como, nove sequências de DNA de coletas de fungos da Amazônia foram incluídas no GenBank;

- Avaliou-se o uso de resíduos agroflorestais da Amazônia Central para a elaboração de formulações para semente-inóculo de *P. strigellus*. O isolado INPACM1464 apresentou crescimento micelial em serragem de onze espécies florestais testadas, a serragem de *Simarouba amara* (nome comum = marupá) foi considerado a melhor opção de serragem regional. A casca de tucumã (*Astrocaryum aculeatum*) e a bainha interna da pupunheira (*Bactris gasipaes*) foram consideradas como potenciais suplementos para substituir o farelo de arroz (*Oryza sativa*);

- Descreveu-se as caracterícticas biológicas de *P. strigellus*, tais como: 1) Método de armazenamento de cultura em sílica gel a 4 °C e nos métodos de Castellani e meio de cultura adicionado de óleo mineral a 25 °C foram considerados as melhores técnicas para manutenção da cultura micelial de *P. strigellus*; 2) A atividade enzimática diferiu entre isolados de *P. strigellus* e estes mostraram atividade aminolítica, celulolítica, esterase e lipolítica; 3) Realizou-se a confirmação do sistema tetrapolar de cruzamento para *P. lecomtei* e *P. strigellus*. Assim como a confirmação da incompatibilidade biológica entre as duas espécies.

FINAL CONSIDERATIONS

- In this study was updated 59% of the scientific names of edible mushrooms species reported in ethnomycologic studies of the Amazon;

- The taxonomic studies led us to confirmation of the species *Panus lecomtei* and *P. strigellus* with help of morphological and molecular analyses, and describing new microscopic characterisctics for *P. strigellus*. In this study, the first occurrence of *P. strigellus* for the Amazonas State-Brazil was reported. And nine ADN sequences from Amazon fungi collections were included in the GenBank;

- The use of agroforestry residues of the Central Amazon for the preparation of formulations for *P. strigellus* spawn was evaluated. This isolate INPACM1464 showed mycelial growth in sawdust of eleven forestry species evaluated, the sawdust of *Simarouba amara* (commom name = *marupá*) was consider the best regional sawdust option. The tucumã (*Astrocaryum aculeatum*) shell and internal sheath of peach palm (*Bactris gasipaes*) were considered as potential supplements to replace rice bran (*Oryza sativa*);

- Biological caracterícticas of *P. strigellus* were described, such as: 1) Preservation of *P. strigellus* mycelium culture on silica gel method at 4 °C, Castellani's method and culture medium added with mineral oil method at 25 °C, was consider the best techniques of maintenance mycelium culture of *P. strigellus*; 2) The enzymatic activity differed between isolates of *P. strigellus* and these showed aminolitic, cellulolytic, lipolytic and esterase activity; 3) Mating system was performed and confirm the tetrapolar system for *P. lecomtei* and *P. strigellus*. Likewise the biological incompatibility between both species was confirmed.

ANEXOS

ANEXO A - RAPID COLOR GUIDE

Vargas-Isla R, Capelari M, Ishikawa NK. 2011. Common Mushrooms of the Brazilian Amazon Part 1. The Field Museum, Chicago, IL, USA

Disponível em <http://fm2.fieldmuseum.org/plantguides/guide_pdfs/308%20Mushrooms-b1.pdf>
Publicado em julho de 2011

Common MUSHROOMS of the BRAZILIAN AMAZON Part 1

1

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Fotos: Ruby Vargas-Isla, Noemia Kazue Ishikawa, Kamila Tomoko Yuyama, Alisson dos Reis Castro; with support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
Produced by: Juliana Philipp, T. Wachter, R. Foster; with support from Connie Keller, Ellen Hyndman Fund and Andrew Mellon Foundation.

Rapid Color Guide # 308 version 1 07/2011

1 *Agaricus* 2 *Agaricus* 3 *Agaricus* 4 *Auricularia delicata*

5 *Auricularia delicata* 6 *Chlorophyllum molybdites* 7 *Chlorophyllum molybdites* 8 *Chlorophyllum molybdites*

9 *Chlorophyllum molybdites* 10 *Cookeina speciosa* 11 *Cookeina tricholoma* 12 *Dacryopinax*

13 *Daldinia eschscholzii* 14 *Lentinula raphanica* 15 *Lentinula raphanica* 16 *Panus strigellus*

Common MUSHROOMS of the BRAZILIAN AMAZON Part 1 **2**

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Fotos: Ruby Vargas-Isla, Noemia Kazue Ishikawa, Kamila Tomoko Yoyama, Alfinete dos Reis Canto; with support from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).
Produced by: Juliana Philipp, T. Wachter, R. Foster; with support from Connie Keller, Ellen Rynd man Fund and Andrew Mellon Foundation.

Rapid Color Guide # 305 version 1 07/2011

17 *Panus strigellus* 18 *Panus strigellus* 19 *Panus velutinus* 20 *Panus velutinus*

21 *Phallus indusiatus* 22 *Phallus indusiatus* 23 *Phallus indusiatus* 24 *Phallus indusiatus*

25 *Phlebopus brasiliensis* 26 *Phlebopus brasiliensis* 27 *Phlebopus brasiliensis* 28 *Pleurotus djamor*

29 *Polyporus tenuiculus* 30 *Polyporus tenuiculus* 31 *Pycnoporus sanguineus* 32 *Tremella*

ANEXO B - ARTIGO

Vargas-Isla R, Hanada RE, Ishikawa NK. 2012. Sawdust and fruit residues of Central Amazonian for *Panus strigellus* spawn's production. *Pesquisa Florestal Brasileira* 32 (70): 123-128 p.

Pesquisa Florestal Brasileira
Brazilian Journal of Foresty Research
www.cnpf.embrapa.br/pfb

Sawdust and fruit residues of Central Amazonian for *Panus strigellus* spawn's production

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Termos para indexação:
Basidiomicetos
Cogumelo comestível
Lentinus strigellus
Lentinus strigosus
Simarouba amara
Astrocaryum aculeatum

Index terms:
Basidiomycetes
Edible mushroom
Lentinus strigellus
Lentinus strigosus
Simarouba amara
Astrocaryum aculeatum

Histórico do artigo:
Recebido em 19/08/2011
Aprovado em 23/04/2012
Publicado em 29/06/2012

doi: 10.4336/2012.pfb.32.70.07

Abstract - The objective of this work was to perform a screening of residues of forest species of the Central Amazon to prepare spawn of the edible mushroom *Panus strigellus*. Sawdust substrates from 11 forest species were tested. Then supplementation with beer yeast, cereal bran and regional fruit residues in sawdust supplementation relation (5:1 and 10:1) were evaluated. Mycelial growth of *P. strigellus* occurred in all the substrates composed of the Amazonian forests species, suggesting that all have potential for use in spawn formulation and/or cultivation of this edible mushroom. Among these species the substrate formulated with *Simarouba amara* sawdust promoted higher mycelial growth ($P<0.05$). The formulation of *S. amara* supplemented with *Astrocaryum aculeatum* fruit shell bran (10:1) presented the best supplementation alternative among regional fruit residues. Three types of packaging for spawn preparation were evaluated, and the polypropylene sack (32×45 cm) was considered the most appropriate. *Simarouba amara* sawdust and *A. aculeatum* fruit shell are readily available in the North region, and the results demonstrating that these residues might substitute *Eucalyptus* sp. sawdust and rice bran, commonly used in the South and Southeast of Brazil for mushroom spawn production.

Serragem e resíduos de frutos da Amazônia Central para produção de semente-inóculo de *Panus strigellus*

Resumo - Neste trabalho objetivou-se realizar uma triagem de resíduos de espécies florestais da Amazônia Central para o preparo da semente-inóculo do cogumelo comestível *Panus strigellus*. Foram testados substratos de serragem de 11 espécies florestais. Em seguida, suplementação com levedura de cerveja, farelos de cereais e resíduos de frutas regionais foram avaliados na relação serragem: suplemento (5:1 e 10:1). O crescimento micelial de *P. strigellus* ocorreu em todos os substratos formulados com espécies florestais da Amazônia, apresentando potencial de uso na formulação da semente-inóculo e/ou cultivo deste fungo comestível. Entre estes, o substrato formulado com serragem de *Simarouba amara* promoveu maior crescimento micelial ($P < 0,05$). A formulação de *S. amara* suplementado com farelo da casca de *Astrocaryum aculeatum* (10:1) apresentou a melhor alternativa de suplementação entre os resíduos de frutos regionais. Três tipos de embalagens para o preparo da semente-inóculo foram avaliados e o saco de polipropileno (32×45 cm) foi considerado a embalagem mais adequada. Serragem de *S. amara* e casca de *A. aculeatum* são de fácil disponibilidade na região Norte e os resultados demonstram que estes resíduos podem substituir a serragem de *Eucalyptus* sp. e farelo de arroz comumente utilizado no Sul e Sudeste de Brasil para a produção de semente-inóculo de cogumelos.

Introduction

The most cultivated mushrooms species worldwide are *Agaricus bisporus* (J.E. Lange) Imbach, *Lentinula edodes* (Berk.) Pegler, *Pleurotus* spp., *Auricularia auricula-judae* (Bull.) Quél., *Flammulina velutipes* (Curt. ex Fr.) Sing. and *Volvariella volvacea* (Bull.) Singer (Sánchez, 2004). The diversity of species used for cultivation of fungi is influenced by the consumption preferences of the producing countries. In Brazil, the main edible mushrooms produced in the South and Southeast are *A. bisporus*, *L. edodes* and *Pleurotus* spp. These are originated from temperate climates places. Since the 90's in Brazil began using agroforestry waste as a substrate for *L. edodes* mushroom production, added to *Eucalyptus* spp. sawdust supplemented with agricultural residues easily found in the region. The Amazon Region has interesting potential for the development of mushroom cultivation, having abundance and diversity of native edible mushrooms species as well as agroforest residues that might be used as lignicolous substrates to produce organic products of high nutritional, medicinal, and gastronomic value.

Ethnomycological studies of indigenous groups such as the Yanomanni in Brazil (Fidalgo & Prance, 1976; Fidalgo & Hirata, 1979; Prance, 1984) and the Uitoto, Muiname and Andoke in Colombia (Vasco-Palacios et al., 2008) have described the edibility of various mushrooms. In 2008, we publish the thermophilic characteristic of the INPACM 1464 isolated (Coleção de Microrganismos de Interesse Agrosilvicultura of the Instituto Nacional de Pesquisas da Amazônia-INPA) collected in a lignicolous substrate in the Central Amazon (Vargas-Isla & Ishikawa, 2008). At that time, the isolated was identified as *Lentinus strigosus* (Schwein.) Fr. (*Panus lecomtei* (Fr.) Corner, current name), however after re-examination of exsiccate, the microscopic characteristics and molecular analyses demonstrated that the species is *Panus strigellus* (Berk.) Overh. (= *L. strigellus* Berk.).

The specimens presented mycelial growth from 25 to 45 °C, with the optimum temperature being 35 °C. The broad temperature range suitable for mycelial growth of this species is an advantage for its cultivation in the Amazon region that has average annual temperatures of 30 to 33.4 °C in the shade year-round. In the sun (the condition in which the mushroom was collected), temperatures can reach 40–45 °C. For edible mushrooms production, the search to substrates formulation for spawn production is an important step. The objective was to carry out a substrate screening using sawdust of Amazon forest species and search supplements options available in the region for *P. strigellus* spawn preparation.

Material and methods

Microorganism

The INPACM 1464 isolated of *P. strigellus* utilized in this study was collected in a lignicolous substrate on Campus III of the INPA, Manaus, AM, Brazil. The stock culture was maintained on Sabouraud Dextrose Agar (SDA; Becton Dickinson) slants at 25 °C, in dark. Mycelia of the stock culture were cultivated at 35 °C in Petri plates (90 mm diameter) containing SDA medium. After five days of growth, disks of the mycelia (10 mm diameter) were removed and used as the inoculum for the experiments.

Sawdust screening

The sawdust type were selected from the main Amazon forest species harvested for timber in Manaus, AM, Brazil, as described in a technical report by Vianez & Barbosa (2002). Two exotic species, *Eucalyptus* sp. and *Quercus acutissima* Carr., were also included because *Eucalyptus* sp. sawdust is commonly used to cultivate edible mushrooms such as *L. edodes* in Southern and Southeastern Brazil (Paula et al., 2001; Queiroz et al., 2004; Silva et al., 2005; Shioni et al., 2007; Ishikawa, 2008) and *Q. acutissima* is used for mushroom cultivation in Asia (Przybylowicz & Donoghue, 1990; Quimio et al., 1990).

The substrates were formulated separately from sawdust from each of the following trees: *Aniba rosaeodora* Ducke, *Astronium lecointei* Ducke, *Bertholletia excelsa* H.B.K., *Bombacopsis quinata* (Jacq.) Dugand., *Caryocar* sp., *Cedrela odorata* L., *Eucalyptus* sp., *Hymenaea courbaril* L., *Hymenolobium petraeum* Ducke, *Hura crepitans* L., *Ocotea cymbarum* Kunth, *Q. acutissima*, and *Simarouba amara* Aubl. The sawdust was sifted at 3 mm mesh sieve from different timbers was screened, then dried in an oven with air circulation at 65 °C until constant weight and stored in plastic bags at room temperature. The sawdust samples were mixed with rice (*Oryza sativa* L.) bran (sawdust:supplement = 5:1; w/w) and with distilled water until approximately 60% hydration (w/v).

Two additional experiments were conducted. The first one was to exam how supplements commonly used in mushroom cultivation affected *P. strigellus* mycelial development. Rice bran, soy (*Glycine max* (L.) Merrill) fiber, soy extract, wheat (*Triticum aestivum* (L.) Thell.) fiber, wheat germ, and beer yeast were added to *S. amara* (sawdust:supplement = 5:1; w/w), selected in the sawdust screening. Pure *S. amara* sawdust was used as a control.

In the second additional experiment, *Astrocaryum aculeatum* Meyer (common name = tucumã) fruit shell, *Carapa guianensis* Aubl. (common name = andiroba) seed shell, *Euterpe oleracea* Mart. (common name = açaí) seed, and *Passiflora edulis* Sims fruit shell were examined. Each residue was dried at 65 °C, crushed and sifted. These materials were separately supplemented to *S. amara* sawdust in weight ratios of 5:1 and 10:1 (sawdust:supplement).

The formulation preparation (sawdust:supplement) was then distributed in five Petri plates (15 ± 1 g/plate) and sterilized twice an hour with interval of 24 h at 121 °C. Following sterilization, one mycelial disk was deposited in the center of the plate containing the formulation and incubated at 35 °C without light. Mycelial growth was evaluated by the index of mycelial growth rates (IMGR), calculated as $\sum (D - Da)/N$, where D is the diameter of the colony on the observation day (measurement in cm), Da is the diameter of the colony on the previous day (measurement in cm), and N is the number of days after inoculation.

In addition to the IMGR, the colony vigor was visually evaluated and classified under three vigor levels as (+) thin, (++) medium, and (+++) dense (see details on Figure 1).

Spawn production and substrate inoculation

Three kinds of polypropylene packing: (1) flask, 15 cm height × 9 cm diameter, with the capacity to hold 600 g of wet substrate with screw cap. Two holes of 1 cm in diameter were made in the cap for gas exchange, and they were covered with adhesive tape (microporous filters), (2) transparent plastic bags, 23 cm wide × 36 cm height, and holding capacity of 800 g of wet substrate. For gas exchange it was necessary to create a respirator using a ring of PVC tubing with 3 cm height × 5 cm diameter and hydrophobic cotton, and (3) transparent bag, 32 cm wide × 45 cm height with 1200 g of substrate handing capacity and a hole of 4.5 cm diameter covered with filter paper for gas exchange (see details on Figure 2).

As a first step, mycelia were placed on wheat grains to multiply. The grain was washed and immersed in water for 24 h. Soon after, 250 g of wheat grain was put in each of ten 500 ml glass flasks and sterilized in an autoclave at 121 °C for 1 h following the methodology described by Stamets (1993). Ten mycelial disks of *P. strigellus* was then transferred into each flask and incubated at 35 °C for 15 days.

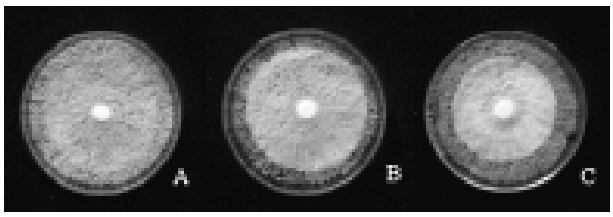

Figure 1. Classification of three colony vigor levels of *Panus strigellus*. (A) thin (+); (B) medium (++); and (C) dense (+++).

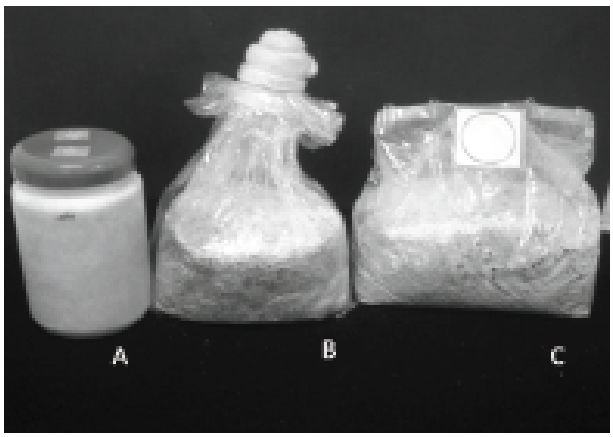

Figure 2. *Panus strigellus* spawn in sawdust of *Simarouba amara*. The polypropylene packing material: (A) 15 × 9 cm flask; (B) 23 × 36 cm bag with an added respirator; and (C) 32 × 46 cm bag with a filter.

Next, sawdust from *S. amara*, *H. petraeum*, and *A. lecointei*, which had shown good results for mycelial growth, was separately evaluated for spawn growth. The sawdust supplemented with rice bran (5:1) was placed in the polypropylene packing. The substrates were sterilized twice an hour with interval of 24 h at 121 °C. For each 100 g of substrate, we added 3.5 g of colonized wheat grains and incubated the material at 35 °C for 25 days in the absence of light in Biologic

Oxygen Demand (BOD – TE-390/TECNAL). After this period, the colonized substrates were taken from the packing materials and cut obliquely into blocks for visual observation of the colonization. Each type of packing material was tested in five replications.

The experiments in Petri plates were tested in five replications and two repetitions. We used analysis of variance (ANOVA) to examine the results of the experiments and compared the averages using the Scott–Knott test at the 5% level of significance.

Results and discussion

The substrates formulated with *B. quinata* and *S. amara* provided the highest *P. strigellus* IMGR values ($P < 0.05$; Table 1), with the colonies reaching the border of the Petri plates (90 mm diameter) in five days after inoculation. This growth was fast compared to that of other edible mushrooms such as *L. edodes* and *F. velutipes* (Ishikawa, 2001). Substrates composed of *C. odorata*, *H. crepitans*, and *O. cymbarum* sawdust showed the lowest IMGR values ($P < 0.05$), with colonies reaching the Petri plate borders in 10 days. However, this growth is considered common for other edible mushrooms. Mycelial growth of *P. strigellus* occurred in all the substrates composed of the Amazonian forests species as well as *Eucalyptus* sp. and *Q. acutissima*, suggesting that all have potential for use in spawn formulation and/or cultivation of this edible mushroom. *Simarouba amara* sawdust was chosen as substrate for the supplementation experiments for its rate of mycelial growth and also because, the specie is frequently used for lumber making and its sawdust is readily available.

Rice bran, soybean fiber, wheat fiber and germ supplementations in spawn production produced the highest *P. strigellus* IMGR values ($P < 0.05$) and improved the colony vigor compared to the control (Table 2). Beer yeast and soybean extract presented lower IMGR values but also improved the colony vigor. Sales-Campos et al. (2008) obtained the highest mycelial growth to *Pleurotus ostreatus* (Jacq.) P. Kumm. using *S. amara* sawdust supplemented with soybean bran. Also *S. amara* residue, rice and wheat bran, and $CaCO_3$ formulation was used for *P. lecomtei* mushroom production (Sales-Campos & Andrade, 2011).

However, while these supplements are generally inexpensive in cereal-producing areas of Brazil, rice, wheat, and soybean cultivation is scarce to nonexistent in the Central Amazon. Thus acquiring large amounts of these supplements would elevate the costs of the substrate in the Amazon region.

Other regional agroforestry residues, however, are produced in large amounts and rarely used. For example, the pulp of *A. aculeatum* fruit is widely consumed in regional dishes throughout the year; the fruit shell residue is generally not used for other purposes and is easy to acquire. Likewise, the shells of *C. guianensis* are an unused residue of oil extraction for cosmetic and therapeutic products, while *E. oleracea* seed shells and *P. edulis* fruit shells are left over from the production of açaí and passion fruit juices.

Regarding supplementation with regional agroforest residues, all of the supplements improved the IMGR and/or colony vigor of *P. strigellus* in *S. amara* sawdust compared to the control (Table 3). Considering both IMGR and colony vigor, the 10:1 mixture of *S. amara* sawdust with *A. aculeatum* presented the best alternative.

For spawn production: *S. amara*, *H. petraeum*, and *A. lecointei* substrates had been totally colonized by *P. strigellus* after 25 days of incubation at 35 °C in the three types of packing materials tested (Figure 2). However, several aspects should be considered, including the costs of the packing materials, the time required for the spawn run, the transportation viability, and the feasibility of mycelial inoculation of the substrate. From packing materials examined here, type 1 (the flask) was more feasible for inoculation and easier to transport. The flask, however, was also the most expensive packing material and visually checking whether colonization had occurred was difficult because the flask was opaque (Figure 2A), and it could prevent observation of contaminants during the incubation. Packing material 2 (the bag without a filter) was of intermediate cost and allowed for good visibility of colonization. Nevertheless, it was also fragile and required the use of two bags; furthermore, it was necessary to create a respirator using a ring of PVC tubing and hydrophobic cotton (Figure 2B). Packing material 3 (the bag with a filter) held a larger amount of substrate, resulting in lower cost. Produced specifically for the production of *L. edodes* spawn, the bag contains a filter for gaseous change, is resistant enough for transport, and allows for visual checking of colonization (Figure 2C). This polypropylene sack was considered the most appropriate packing by representing smaller cost for the spawn production. However, few distributors of this material operate in Brazil.

Table 1. Effect of forest species sawdust substrates on *Panus strigellus* mycelial growth.

Substrate[1]	Mean[2]	Standard deviation
Bombacopsis quinata	1.197 a	± 0.019
Simarouba amara	1.175 a	± 0.018
Quercus acutissima	1.084 b	± 0.016
Astronium lecointei	1.050 b	± 0.026
Hymenaea courbaril	1.038 b	± 0.042
Hymenolobium petraeum	1.036 b	± 0.017
Eucalyptus sp.	0.923 c	± 0.045
Aniba rosaeodora	0.898 c	± 0.010
Bertholletia excelsa	0.891 c	± 0.084
Caryocar sp.	0.837 d	± 0.019
Cedrela odorata	0.774 e	± 0.014
Hura crepitans	0.756 e	± 0.019
Ocotea cymbarum	0.724 e	± 0.021

(1)All substrates were composed of a five-to-one (w/w) mixture of sawdust and rice bran. (2)Average of five replications and two repetitions of index of mycelial growth rates values-IMGR (cm/day). Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test.

Table 2. Effect of supplementing *Simarouba amara* sawdust substrate with beer yeast and cereal bran on *Panus strigellus* mycelial growth.

Supplement[1]	Mean[2]	Standard deviation	Colony vigor[3]
Wheat fiber	1.22 a	± 0.02	+++
Rice bran	1.21 a	± 0.02	+++
Soy fiber	1.21 a	± 0.02	+++
Wheat germ	1.19 a	± 0.09	+++
Soy extract	1.15 b	± 0.07	+++
Beer yeast	1.13 b	± 0.05	+++
Control (without supplementation)	1.13 b	± 0.02	+

(1)The substrate was composed of a five-to-one (w/w) mixture of *Simarouba amara* sawdust and the supplement. (2)Average of five replications and two repetitions of index of mycelial growth rates values-IMGR (cm/day). Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test. (3) Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

Table 3. Effect of supplementing *Simarouba amara* sawdust substrate with Central Amazon fruit residues on *Panus strigellus* mycelial growth.

Substrate	Amount	Mean[1]	Standard deviation	Colony vigor[2]
Euterpe oleracea seed	5:1	1.15 a	± 0.04	+
Carapa guianensis seed shell	10:1	1.14 a	± 0.03	+
Euterpe oleracea seed	10:1	1.13 a	± 0.05	+
Astrocaryum aculeatum fruit shell	10:1	1.13 a	± 0.03	++
Simarouba amara (control)	10:0	1.08 b	± 0.04	+
Carapa guianensis seed shell	5:1	1.08 b	± 0.04	++
Astrocaryum aculeatum fruit shell	5:1	1.08 b	± 0.06	++
Passiflora edulis fruit shell	10:1	1.04 c	± 0.02	++
Passiflora edulis fruit shell	5:1	0.97 d	± 0.02	+++

(1)Average of five replications of index of mycelial growth rates values-IMGR (cm/day).Means with the same letter(s) are not significantly different ($P < 0.05$) by the Scott–Knott test. (2) Colony vigor levels: (+) thin, (++) medium, and (+++) dense.

Conclusion

Simarouba amara sawdust and *A. aculeatum* fruit shell are readily available in the North region and these residues showed potential as substitute of *Eucalyptus* sp. sawdust and rice bran, commonly used in the South and Southeast of Brazil, for *P. strigellus* spawn production.

Acknowledgments

This research was financed by Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM) PIPT Program, and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) by PNADB Program. R. Vargas-Isla is grateful for a scholarship from Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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ANEXO C – ATA DA AULA DE QUALIFICAÇÃO

Aula de qualificação realizada em 23 de março de 2009.

INPA | DIVISÃO DOS CURSOS DE PÓS-GRADUAÇÃO

PIPG BTRN

Ministério da Ciência e Tecnologia

AULA DE QUALIFICAÇÃO

PARECER

Aluno(a): **RUBY VARGAS ISLA**
Curso: BOTÂNICA
Nível: Doutorado
Orientador(a): Noemia Kasue Ishikawa (INPA)

Título:

"ESTRATÉGIAS PARA O DESENVOLVIMENTO DE BASIDIOCARPOS DE *Lentinus strigellus*, UM COGUMELO COMESTÍVEL TERMÓFILO DA AMAZÔNIA"

BANCA JULGADORA:

TITULARES:	**SUPLENTES:**
CRISTINA SAYURI MAKI (UFAM)	
ROGÉRIO EIJI HANADA (INPA)	ROSALEE A. COELHO NETTO (INPA)
VALDELY FERREIRA KINUPP (IFAM)	ADEMIR DE CASTRO E SILVA (UEA)
CECI SALES-CAMPOS (INPA)	
RICARDO ANTONIO MARENCO (INPA)	

EXAMINADORES	PARECER	ASSINATURA
CRISTINA SAYURI MAKI	(X) Aprovado ( ) Reprovado	
ROGÉRIO EIJI HANADA	(X) Aprovado ( ) Reprovado	
VALDELY FERREIRA KINUPP	(X) Aprovado ( ) Reprovado	
CECI SALES-CAMPOS	(X) Aprovado ( ) Reprovado	
RICARDO ANTONIO MARENCO	(X) Aprovado ( ) Reprovado	
ROSALEE A. COELHO NETTO	( ) Aprovado ( ) Reprovado	
ADEMIR DE CASTRO E SILVA	( ) Aprovado ( ) Reprovado	

Manaus(AM), 23 de março de 2009.

OBS: _____

PROGRAMA INTEGRADO DE PÓS-GRADUAÇÃO EM BIOLOGIA TROPICAL E RECURSOS NATURAIS – PIPG BTRN
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ANEXO D – ATA DA DEFESA PÚBLICA DA TESE

Defesa em 14 de setembro de 2012.

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

GOVERNO FEDERAL
BRASIL
PAÍS RICO É PAÍS SEM POBREZA

Curso de Pós-Graduação em Botânica
INPA

ATA DA DEFESA PÚBLICA DA TESE DE DOUTORADO DE DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

Aos quatorze dias do mês de setembro do ano de 2012, às 09h00min, no mini auditório da casa da ciência do INPA, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: **Dr. Luadir Gasparotto,** da Empresa Brasileira de Pesquisa Agropecuária, **Dra. Cristina Sayuri Maki,** da Universidade Federal do Amazonas, **Dr. José Renato Pereira Cavallazzi**, da Universidade Federal do Amazonas, **Dr. Valdely Ferreira Kinupp,** do Instituto Federal do Amazonas e **Dr. Ulisses Brigatto Albino,** da Universidade Federal do Pará, tendo como suplentes **Dra. Rosalee Albuquerque C. Netto**, do Instituto Nacional de Pesquisas da Amazônia e o **Dr. Iuri Goulart Baseia**, Universidade Federal do Rio Grande do Norte, sob a presidência do primeiro, a fim de proceder a argüição pública da **TESE DOUTORADO** da discente **RUBY VARGAS-ISLA**, intitulada "**TAXONOMIA, BIOLOGIA E PRODUÇÃO DE SEMENTE-INÓCULO DE *PANUS STRIGELLUS*, UM COGUMELO COMESTÍVEL DA AMAZÔNIA",** sob a orientação Dra. Noemia Kasue Ishikawa e co-orientação Dr. Ricardo Marenco.

Após a exposição, dentro do tempo regulamentar, o (a) discente foi argüido (a) oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

(X) **APROVADO** ( ) **REPROVADO**

Nada mais havendo, foi lavrado a presente ata, que, após lida e aprovada, foi assinada pelos seguintes membros da Comissão Examinadora:

Dr(a). **Luadir Gasparotto (EMBRAPA)** _____

Dr(a). **Cristina Sayuri Maki (UFAM)** _____

Dr(a). **José Renato Pereira Cavallazzi (UFAM)** _____

Dr(a). **Valdely Ferreira Kinupp (IFAM)** _____

Dr(a). **Ulisses Brigatto Albino (UFPA)** _____

Coordenação do Programa de Pós-Graduação em Botânica do INPA

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