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HYGROCYBE SENSU LATO (HYGROPHORACEAE, AGARICALES) NA AMAZÔNIA BRASILEIRA

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Manaus, Amazonas
Março de 2020

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Sinopse:

Estudou-se o gênero de cogumelos *Hygrocybe* s.l. na Amazônia brasileira. Foram feitos novos registros e descrições de novas espécies para a ciência, incluindo análises filogenéticas e chaves de identificação dicotômicas.

Palavras-chave: taxonomia, micologia, cogumelos, ITS, filogenia

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Este trabalho é dedicado à Funga
que junto com a Fauna e Flora
merecem respeito e admiração

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#respiraFunGo

"What do you do when your world starts to fall apart? I go for a walk, and if I'm really lucky, I find mushrooms. Mushrooms pull me back into my senses, not just—like flowers—through their riotous colors and smells but because they pop up unexpectedly, reminding me of the good fortune of just happening to be there. Then I know that there are still pleasures amidst the terrors of indeterminacy."

Anna Tsing, 2015

RESUMO

Hygrocybe é um gênero de macrofungos agaricoides da família Hygrophoraceae, subfam. Hygrocyboideae (Agaricales, Basidiomycota). A circunscrição do gênero *Hygrocybe* foi recentemente alterada no trabalho de filogenia de Hygrophoraceae proposto em 2013 e os gêneros segregados formam o clado *Hygrocybe* sensu lato, com exceção de *Cuphophyllus*. Membros de *Hygrocybe* s.l. são reconhecidos pela coloração vibrante, lamelas espessas, cerosas e espaçadas, esporada branca e estipe sem véu remanescente. Sabendo que a maior diversidade de fungos está concentrada nos trópicos, os trabalhos de taxonomia de fungos na Amazônia são considerados de extrema importância. O objetivo do trabalho foi conhecer a diversidade de *Hygrocybe* sensu lato na Amazônia brasileira, correlacionando dados morfológicos e moleculares. Para isto, foram realizadas coletas em áreas de Floresta Amazônica. As descrições macroscópicas foram feitas seguindo literaturas tradicionais, e amostras dos materiais foram armazenadas para extração de DNA. Os espécimes foram secos a 40°C e devidamente armazenados, e, posteriormente, analisados em microscópio óptico a partir de cortes em lâmina. As exsicatas foram depositadas no Herbário INPA ou no Herbário e Fungário FLOR. O marcador universal ITS foi sequenciado de espécimes selecionados e principalmente utilizado como barcode, identificação e comparação *pairwise* com dados do GenBank. Análises filogenéticas de Máxima Verossimilhança e Bayesiana foram feitas no Capítulo 3. Sessenta espécimes foram analisados ao microscópio óptico e 46 sequências de ITS foram obtidas. Foram identificados 12 táxons distribuídos em 3 gêneros. Dez são espécies novas para a ciência e são propostas neste trabalho, cinco em *Hygrocybe* subgen. *Hygrocybe*, três em *Hygrocybe* subgen. *Pseudohygrocybe*, uma em *Humidicutis* e uma em *Neohygrocybe*. *Hygrocybe* é relatado pela primeira vez para Roraima e Pará; *Humidicutis* é relatado pela primeira vez para Amazonas e Floresta Amazônica; *Neohygrocybe* é relatado pela primeira vez para o Brasil. *Hygrocybe rubroalba* é primeiro registro para Mato Grosso e Floresta Amazônica; *Hygrocybe hololeuca*, primeiro registro para Pará e novo registro para Amazonas.

Palavras-chave: Taxonomia, micologia, cogumelos, ITS, filogenia.

ABSTRACT

Hygrocybe is a genus of agaricoid macrofungi in the family Hygrophoraceae, subfam. Hygrocyboideae (Agaricales, Basidiomycota). The circumscription of *Hygrocybe* was recently altered in the phylogeny of Hygrophoraceae proposed in 2013 and the segregated genera form the *Hygrocybe* sensu lato clade, with the exception of *Cuphophyllus*. Members of *Hygrocybe* s.l. are recognized for their vibrant colours, thick, waxy and well-spaced lamellae, white sporeprint and stipe without remanescent veil. Knowing that the greatest diversity of fungi is concentrated in the tropics, studies on fungal taxonomy in the Amazon are considered of extreme importance. The objective of this work was to know the diversity of *Hygrocybe* s.l. in the Brazilian Amazon through taxonomy, correlating morphological and molecular data. Collections were obtained in areas of the Amazon Forest. Macroscopic descriptions were made following traditional literature and samples of the material were stored for DNA extraction. The specimens were dried at 40°C and properly stored. Then, the materials were analysed under an optical microscope. The exsiccatas were deposited at INPA Herbarium or at FLOR Herbarium and Fungarium. The universal marker ITS was sequenced from selected specimens and mainly used for barcoding, identification and pairwise comparison with data in GenBank. Maximum Likelihood and Bayesian phylogenetic analyses were conducted in Chapter 3. Sixty specimens were analysed in optical microscope and 46 ITS sequences were obtained. Twelve taxa were identified, distributed in three genera. Ten are new species proposed in this work, five in *Hygrocybe* subgen. *Hygrocybe*, three in *Hygrocybe* subgen. *Pseudohygrocybe*, one in *Humidicutis* and one in *Neohygrocybe*. *Hygrocybe* is reported for the first time for Roraima and Pará; *Humidicutis* is reported for the first time for Amazonas and the Amazon Rainforest; *Neohygrocybe* is reported for the first time for Brazil. *Hygrocybe rubroalba* is the first record for Mato Grosso and the Amazon Forest; *Hygrocybe hololeuca*, first record for Pará and new record for Amazonas States.

Keywords: Taxonomy, mycology, mushrooms, ITS, phylogeny.

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LISTA DE ABREVIAÇÕES E SIGLAS

FLOR – Herbário e Fungário da Universidade Federal de Santa Catarina
INPA – Instituto Nacional de Pesquisas da Amazônia

aff. – affinis
cf. – conferatus
diam. – diâmetro, diameter
e.g. – por exemplo, for example
et al. – e colaboradores
Ha. – Hygroaster
Hc. – Hygrocybe
mm – milímetros
p. – página
pers. comm. – comunicação pessoal
Q – Quociente comprimento/largura dos basidiósporos
s.l. – sensu lato
s.s. – sensu stricto
sect. – seção, section
sp. – espécie, species
subfam. – subfamília, subfamily
subgen. – subgênero, subgenus
subsect. – subseção, subsection
µm – micrômetros

1. Introdução

1.1. Reino Fungi

Os fungos são organismos ubíquos, participando na dinâmica de todos os ecossistemas terrestres através de uma série de interações (Blackwell, 2011; Peay *et al.,* 2008). Como decompositores, os fungos possuem um importante papel na ciclagem de nutrientes do solo, sendo essenciais ao ciclo do carbono (Peay *et al.,* 2008). Interagem com diversos grupos de organismos, podendo ser patógenos de plantas, animais e outros fungos, ou mutualistas com cianobactérias e algas (líquens), plantas (micorrizas e fungos endofíticos) e animais (por exemplo, formigas e cupins) (McLaughlin *et al.,* 2009). Hawksworth & Lücking (2017) estimam que existem de 2,2 a 3,8 milhões de espécies de fungos, mas apenas 144.000 espécies já foram descritas (Willis, 2018), o que significa que apenas 3 a 8% dos fungos são conhecidos pela ciência. O reino Fungi possui atualmente 19 filos (Wijayawardene *et al*., 2020), sendo que os organismos que produzem estruturas reprodutivas visíveis ao olho nu, comumente chamados de macrofungos, se concentram em dois principais filos: Ascomycota e Basidiomycota. Agaricales (Fungi, Basidiomycota) é a maior ordem de macrofungos com aproximadamente 17.291 espécies distribuídas em 508 gêneros (He *et al.,* 2019).

1.2. Hygrophoraceae

Hygrophoraceae Lotsy é uma das mais diversas famílias em Agaricales, com 600 espécies em 26 gêneros (Lodge *et al*., 2013). Além da diversidade de espécies, a família possui uma ampla variedade de cores, formas e hábitos, incluindo táxons formadores de líquens (basidioliquens), mutualistas com briófitas, pteridófitas e gramíneas, parasitas de briófitas, lignícolas e ectomicorrízicos (Lodge *et al.,* 2013). Alguns táxons, anteriormente considerados saprófitos, permanecem incertos quanto à ecologia, como ocorre em *Hygrocybe* sensu lato. Estudos recentes com isótopos estáveis de nitrogênio e carbono marcados indicam que *Hygrocybe* e gêneros relacionados são biotróficos, obtendo sua nutrição através de alguma associação com plantas, sendo potencialmente micorrízicos ou endofíticos (Griffith *et al.,* 2002; Griffith, 2004; Halbwachs *et al.,* 2013a; 2013b; 2018; Seitzman *et al.,* 2011). Entretanto, a aquisição de nitrogênio desse grupo de fungos permanece longe do padrão de

outros cogumelos micorrízicos, e Halbwachs *et al*. (2018) sugerem que *Hygrocybe* s.l. pode estar obtendo nitrogênio a partir da fauna do solo.

Há registros de comestibilidade de nove espécies no mundo (Boa, 2004), além de estudos que indicam potencial medicinal de cogumelos do grupo (Chittaragi *et al*., 2013; Chong *et al*., 2014).

1.3. *Hygrocybe* s.l.

Hygrocybe (Fr.) P. Kumm. é um gênero agaricoide – formato comum aos cogumelos, com píleo, lamelas e estipe – da família Hygrophoraceae, subfam. Hygrocyboideae Padamsee & Lodge, com cerca de 350 espécies (Tabela 1) (Lodge, pers. comm., 2020). *Hygrocybe* e gêneros relacionados (sensu lato) se distinguem de outros cogumelos pelas cores vibrantes, lamelas espessas, cerosas e espaçadas, esporada branca e estipe sem véu remanescente (Babos *et al.,* 2011). O aspecto ceroso das lamelas é, sem dúvida, um dos critérios mais utilizados para a identificação de espécimes no campo (Young, 2005). Microscopicamente, se distinguem pelos basídios longos que podem ser até 7 vezes mais compridos do que os basidiósporos.

Tabela 1. Número (N) estimado de espécies de *Hygrocybe* s.l. para o mundo.

Gênero	N estimado de spp.	Referências
Hygroaster	2	Lodge *et al.* (2013)
Hygrocybe	350	Lodge pers. comm.
Gliophorus	11	Lodge pers. comm.
Humidicutis	8	Lodge pers. comm.
Porpolomopsis	2	Lodge pers. comm.
Gloioxanthomyces	3	Lodge pers. comm.
Chromosera	6	Lodge pers. comm.
Neohygrocybe	10	Lodge pers. comm.
Sinohygrocybe	1	Wang *et al*. (2018)
Total	393	

Com exceção de *Cuphophyllus*, os gêneros citados acima formam um clado monofilético com a tribo Hygrocybeae (*Hygroaster* Singer + *Hygrocybe* s.s.) e poderiam continuar a ser tratados como gênero *Hygrocybe*, se *Hygroaster* for reduzido a subgênero (Lodge *et al.,* 2013). Este clado configura a subfam. Hygrocyboideae, que corresponde a *Hygrocybe* sensu lato (Figura 1) (Lodge *et al.,* 2013). O gênero *Sinohygrocybe* C.Q. Wang, Ming Zhang & T.H. Li, recentemente descrito para a China, pertence a tribo Chromosereae da subfam. Hygrocyboideae e, portanto, também pertence ao clado *Hygrocybe* s.l. (Wang *et al*., 2018).

No entanto, para manter a monofilia do gênero *Hygrocybe, Cuphophyllus* deve ser reconhecido como gênero distinto, pois está posicionado em um clado não relacionado com *Hygrocybe* e pertence ao grado "cuphophylloid", um grupo de posicionamento incerto em Hygrophoraceae s.s. (Lodge *et al.,* 2013). Se *Cuphophyllus* não for segregado de *Hygrocybe*, todos os gêneros de Hygrophoraceae, incluindo *Hygrocybe*, teriam que ser recombinados para *Hygrophorus*, que é o gênero tipo da família e o mais antigo, portanto tendo prioridade nomenclatural (Lodge *et al.,* 2013; Lodge, 2014; Turland *et al*., 2018), para que Hygrophoraceae seja considerada como um grupo natural. Portanto, a classificação dos gêneros segregados proposta por Lodge *et al.* (2013) é aceita neste trabalho, considerando que as diferenças morfológicas e moleculares entre os táxons são suficientes para separá-los em gêneros distintos.

1.3.1. Histórico de classificação

O primeiro registro do nome *Hygrocybe* foi feito por Fries (1821, p. 101) como "subtribo" *Hygrocybi*, "tribo" *Clitocybe* do gênero *Agaricus* e logo foi transferido para uma "tribo" dentro do gênero *Hygrophorus* (Fries, 1838, p. 329). O conceito de "tribo" usado por Fries não é válido, pois era uma classificação infragenérica e não infrafamiliar (Lodge *et al.,* 2013). Posteriormente, o táxon *Hygrocybe* foi elevado a subgênero de *Hygrophorus* também por Fries (1849, p. 308). *Hygrocybe* foi reconhecido como um gênero pela primeira vez por Kummer (1871, p. 26). Segundo Babos *et al.* (2011), a maioria dos micólogos considera *Hygrocybe* e *Hygrophorus* como gêneros distintos dentro da família Hygrophoraceae, mas há controvérsias a respeito do reconhecimento dos demais gêneros segregados de *Hygrocybe* s.l.

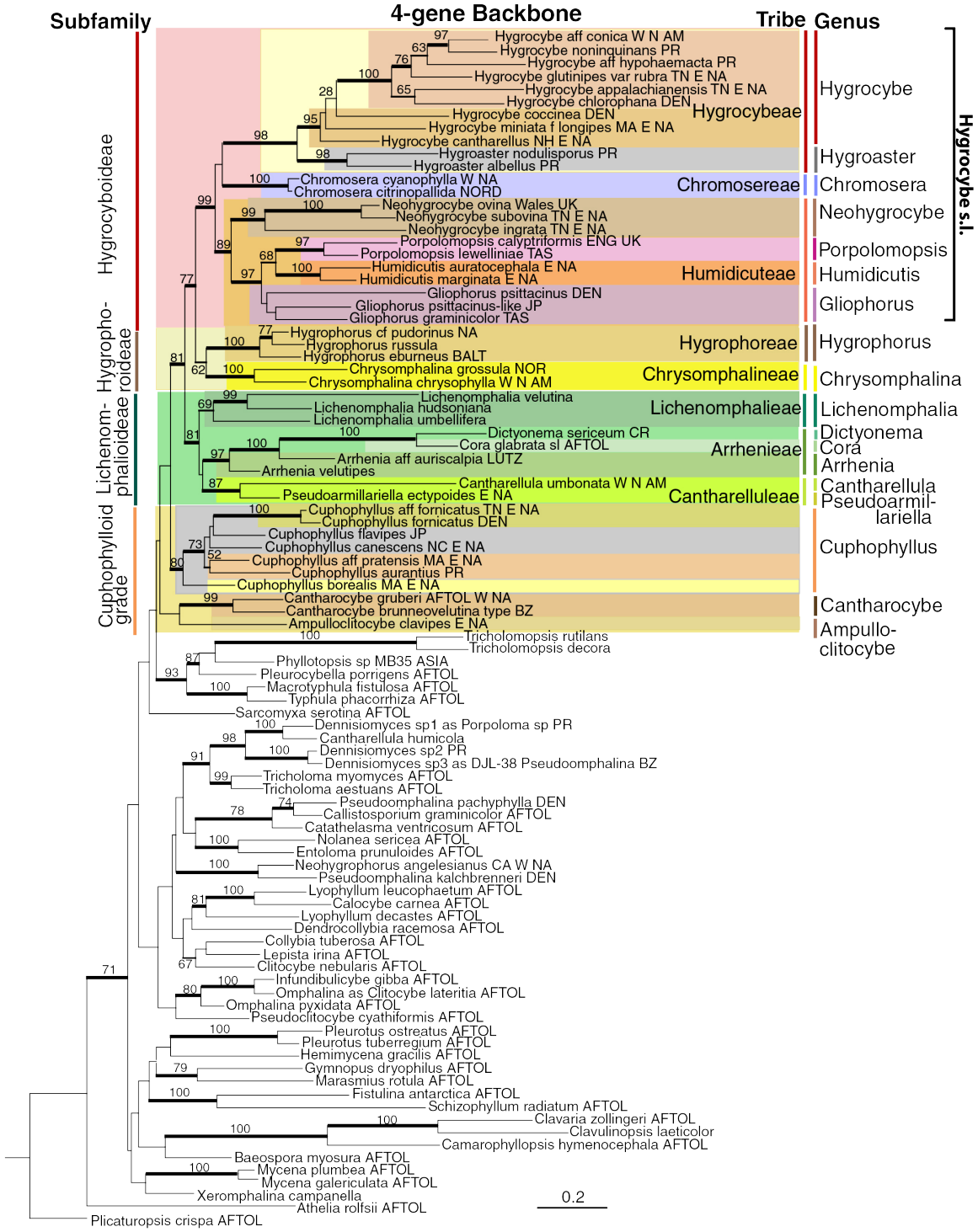

Figura 1. Árvore de Máxima Verossimilhança de Hygrophoraceae, representantes do clado hygrophoroide e representantes de grupos externos em Entolomataceae, Marasmiaceae, Mycenaceae, Pleurotaceae e Tricholomataceae s.s., enraizada com *Plicaturopsis crispa*. Os genes analisados foram ITS (ITS1, 5.8S & ITS2), LSU, SSU e RPB2 (entre os domínios 6 e 7). As diferentes cores correspondem às subfamílias, tribos e gêneros indicadas na filogenia. Valores de máxima parcimônia com *bootstrap* ≥ 50 % aparecem acima dos ramos. Ramos em negrito forte possuem suporte de máxima parcimônia com *bootstrap* ≥ 70 % e, em negrito fraco, 50–69 % de suporte (adaptado de Lodge *et al.,* 2013).

Singer (1986) dividiu Hygrophoraceae em três tribos: Hygrophoreae, incluindo o gênero *Hygrophorus*; Hygroastreae incluindo os gêneros *Hygroaster* e *Omphaliaster* [Insertae sedis (He *et al.,* 2019)]; e Hygrocybeae, incluindo os gêneros *Camarophyllus* (= *Hygrophorus*), *Humidicutis* (incluindo espécies de *Porpolomopsis*)*, Hygrocybe* (incluindo as espécies de *Gliophorus* e *Neohygrocybe*), *Hygrotrama* [= *Camarophyllopsis*, que atualmente está classificado em Clavariaceae (Matheny *et al.,* 2006)] e *Neohygrophorus* (= *Hygrophorus*).

Young (2005) dividiu as espécies de Hygrophoraceae que ocorrem na Austrália em duas tribos, Hygrophoreae P.Henn., para os táxons com trama da lamela divergente (*Hygrophorus*), e Hygrocybeae P.Henn., para os táxons com trama da lamela regular a irregular, mas nunca divergente, contando três gêneros: *Camarophyllopsis*, *Humidicutis* e *Hygrocybe*. Young (2005) aceitou o gênero *Humidicutis* para espécies de *Hygrocybe* s.l. sem fíbulas em quase todo o basidioma, mas com fíbulas toroidais na base dos basídios (Figura 2). Young (2005) dividiu o gênero *Hygrocybe* em 3 subgêneros, *Cuphophyllus* [= gênero *Cuphophyllus* (Lodge *et al.* 2013)], *Hygrocybe* [= *Hygrocybe* subgen. *Hygrocybe* (Lodge *et al.,* 2013)] e *Pseudohygrocybe* (incluindo espécies de *Hygrocybe* subgen. *Pseudohygrocybe*, *Gliophorus* e *Gloioxanthomyces*).

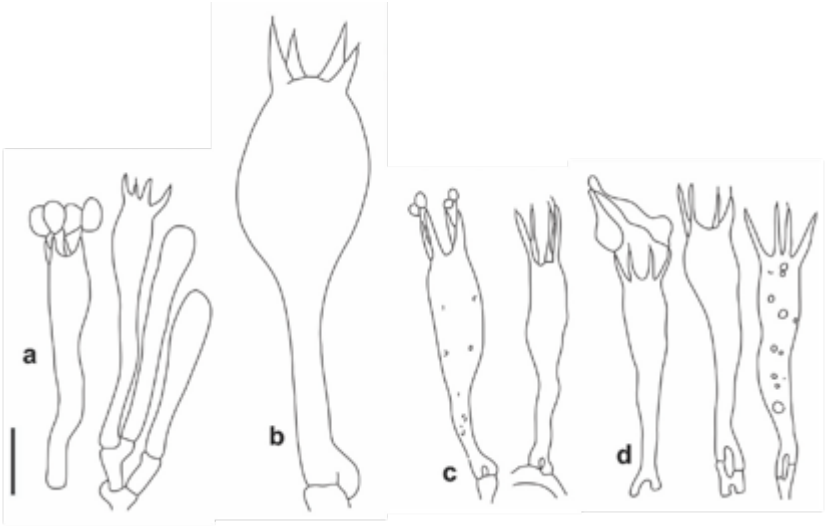

Figura 2. Diferentes tipos de fíbulas encontradas na base dos basídios em espécimes de *Hygrocybe* s.l. **a.** Sem fíbulas, *Hygrocybe* sp. **b.** Fíbulas regulares, *Hygrocybe* sp. **c.** Fíbulas em medalhão, *Gliophorus* cf. *laetus*. **d.** Fíbulas toroidais, *Humidicutis* sp. Escala = 10µm.

Boertmann (2010) dividiu *Hygrocybe* em quatro subgêneros: subg. *Cuphophyllus* Donk (incluindo espécies de *Cuphophyllus* e *Chromosera*), subg. *Pseudohygrocybe* Bon (incluindo espécies de *Cuphophyllus*, *Neohygrocybe*, *Gliophorus, Gloioxanthomyces* e

Hygrocybe s.s.), subg. *Hygrocybe* (incluindo espécies de *Hygrocybe* s.s.) e subg. *Humidicutis* (Sing.) Boertm. (incluindo espécies de *Humidicutis* e *Porpolomopsis*). Boertmann (2010) reconheceu que sua classificação era artificial, mas a sustentou para facilitar os trabalhos de micólogos e amadores, até que uma classificação mais estável e com suporte filogenético fosse proposta.

1.3.2. Classificação genérica e infragenérica de *Hygrocybe* s.l.

A atual classificação é baseada em Lodge *et al.* (2013), que utilizou a morfologia, ecologia, pigmentação e dados moleculares para dividir *Hygrocybe* s.l. [= Subfamília Hygrocyboideae] em tribos, gêneros, seções e subseções designadas abaixo. Para maiores detalhes, ver Lodge *et al.* (2013) e Wang *et al.* (2018). Fotografias da maioria desses grupos estão disponibilizadas na Figura 3, adaptado de Lodge *et al*. (2013).

Tribo Hygrocybeae Kühner, Bull. Soc. Linn. Lyon 48: 621 (1979)
Gênero tipo: *Hygrocybe* (Fr.) P. Kumm., Führ. Pilzk. (Zwickau): 26 (1871)

Gênero *Hygrocybe* (Fr.) P. Kumm., Führ. Pilzk. (Zwickau): 26 (1871)
≡ *Hygrophorus* subg. *Hygrocybe* Fr.
Espécie tipo: *Hygrocybe conica* (Schaeff.) P. Kumm.
≡ *Hygrophorus conicus* (Schaeff.) Fr.

Subgênero *Hygrocybe* [autônimo] (1976)
Espécie tipo: *Hygrocybe conica* (Schaeff.) P. Kumm.
≡ *Hygrophorus conicus* (Schaeff.) Fr.

Seção *Hygrocybe* [autônimo] (1889)
Espécie tipo: *Hygrocybe conica* (Schaeff.) P. Kumm.
≡ *Hygrophorus conicus* (Schaeff.) Fr.

Subseção *Hygrocybe* [autônimo] (1951)
Espécie tipo: *Hygrocybe conica* (Schaeff.) P. Kumm.
≡ *Hygrophorus conicus* (Schaeff.) Fr.

Subseção *Macrosporae* R. Haller Aar. ex Bon, Doc. Mycol. 24(6): 42 (1976)
Espécie tipo: *Hygrocybe acutoconica* (Clem.) Singer (como "Hygrocybe acuticonica" Clem.)

Figura 3. Fotografias de exemplares de Hygrophoraceae. **a–s.** Subfam. Hygrocyboideae. **a–f.** *Hygrocybe* subg. *Hygrocybe* **a.** *H. conica*. **b.** *H. acutoconica*. **c.** *H.* aff. *hypohaemacta*. **d.** *H. appalachianensis*. **e.** *H. citrinovirens*. **f.** *H. chlorophana*. **g–j.** *Hygrocybe* subg. Pseudohygrocybe. **g.** *H. coccinea*. **h.** *H. reidii*. **i.** *H. turunda*. **j.** *H. firma*. **k.** *Hygroaster nodulisporus*. **l.** *Humidicutis marginata*. **m.** *Neohygrocybe ovina*. **n.** *N. nitrata*. **o.** *Porpolomopsis calyptriformis*. **p.** *Gliophorus psittacinus*. **q.** *G. laetus*. **r.** *Chromosera cyanophylla*. **s.** *Gloioxanthomyces vitellinus.* **t.** Grado "cuphophylloid", *Cuphophyllus pratensis*. **u.** Subfam. Hygrophoroideae, *Hygrophorus eburneus*. Escala = 1 cm (prancha adaptada de Lodge *et al.,* 2013).

≡ *Hygrocybe persistens* (Britzelm.) Singer

Seção *Velosae* Lodge, Ovrebo & Padamsee, Fungal Diversity 64: 28 (2013) [2014]

Espécie tipo: *Hygrocybe hypohaemacta* (Corner) Pegler & Fiard

≡ *Hygrophorus hypohaemactus* Corner

Seção *Pseudofirmae* Lodge & Padamsee, Fungal Diversity 64: 28 (2013) [2014]

Espécie tipo: *Hygrocybe appalachianensis* (Hesl. & A.H. Sm.) Kronaw. (como 'appalachiensis')

≡ *Hygrophorus appalachianensis* Hesl. & A.H. Sm.

Seção *Microsporae* Boertm., The genus *Hygrocybe*. Fungi of Northern Europe (Greve) 1: 16 (1995)

Espécie tipo: *Hygrocybe citrinovirens* (J.E. Lange) Jul. Schäff.

Seção *Chlorophanae* (Herink) Arnolds ex Candusso, Hygrophorus. Fungi europ. (Alassio 6: 464 (1997)

Espécie tipo: *Hygrocybe chlorophana* (Fr.) Wünsche

≡ *Agaricus chlorophanus* Fr. : Fr.

Subgênero *Pseudohygrocybe* Bon, Doc. Mycol. 6 (24): 42 (1976)

Espécie tipo: *Hygrocybe coccinea* (Schaeff.) Fr.

≡ Agaricus coccineus Schaeff.

Seção *Coccineae* Fayod, Proc. Hist. Nat. Agar. Ann. Scient. Nat. 7(9): 309 (1889)

≡ *Hygrocybe* sect. *Puniceae* Fayod

≡ *Hygrocybe* sect. '*Inopodes*' Singer

Espécie tipo: *Hygrocybe coccinea* (Schaeff.) Fr.

≡ *Agaricus coccineus* Schaeff.

Subseção *Coccineae* (Bataille) Singer, Lilloa 22: 152 (1951) [1949]

≡ *Hygrocybe* subsect. *Puniceae* (Fayod) Arnolds ex Candusso

≡ *Hygrocybe* subsect. '*Inopodes*' Singer

Espécie tipo: *Hygrocybe coccinea* (Schaeff.) Fr.

≡ *Agaricus coccineus* Schaeff.

Subseção *Siccae* Boertm., The genus *Hygrocybe*. Fungi of Northern Europe - Vol. 1: 15 (1995)

Espécie tipo: *Hygrocybe reidii* Kühner

Subseção *Squamulosae* (Bataille) Singer, Lilloa 22: 152 (1951)[1949]

≡ *Hygrocybe* subsect. *Turundae* (Herink) Bon

Espécie tipo: *Hygrocybe turunda* (Fr.) P. Karst.

≡ *Hygrophorus turundus* (Fr.: Fr.) Fr.

≡ *Agaricus turundus* Fr.

Seção *Firmae* Heinem.

Espécie tipo: *Hygrocybe firma* (Berk. & Broome) Singer

≡ *Hygrophorus firmus* Berk. & Broome

Gênero *Hygroaster* Singer 1955, Sydowia 9(1‑6): 370

Espécie tipo: *Hygroaster nodulisporus* (Dennis) Singer

≡ *Hygrophorus nodulisporus* Dennis

Tribo Humidicuteae Padamsee & Lodge, Fungal Diversity 64: 28 (2013) [2014]

Gênero tipo: *Humidicutis* (Singer) Singer

Gênero *Humidicutis* (Singer) Singer, Sydowia 12(1‑6): 225 (1959) [1958]

Espécie tipo: *Humidicutis marginata* (Peck) Singer

≡ *Hygrophorus marginatus* Peck

Gênero *Neohygrocybe* Herink Sb., Severocesk. Mus., Prír. Vedy 1: 71 (1959)

Espécie tipo: *Neohygrocybe ovina* (Bull. : Fr.) Herink

≡ *Hygrophorus ovinus* (Bull. : Fr.) Fr.

≡ *Agaricus ovinus* Bull.

Seção *Neohygrocybe* [autônimo]

≡ *Neohygrocybe* sect. “Ovinae” Herink

Espécie tipo: *Neohygrocybe ovina* (Bull. ex Fr.) Herink

≡ *Hygrocybe ovina* (Bull.) Kühner

≡ *Hygrophorus ovinus* (Bull. : Fr.) Fr.

≡ *Agaricus ovinus* Bull.

Seção *Tristes* (Bataille) Lodge & Padamsee Fungal Diversity 64: 28 (2013) [2014]

Basiônimo: *Hygrocybe* sect. *Tristes* (Bataille) Singer

≡ *Hygrophorus* Fr. subgen. *Hygrocybe* Fr. [sem classificação] Tristes Bataille

≡ *Neohygrocybe* sect. “Nitratae” Herink

Lectótipo: *Hygrocybe nitrata* (Pers.) Wünsche

≡ *Agaricus nitratus* Pers.

≡ *Neohygrocybe nitrata* (Pers.) Kovalenko

≡ *Neohygrocybe nitrata* (Pers.) Herink

Gênero *Porpolomopsis* Bresinsky, Regensb. Mykol. Schr. 15: 145 (2008)

Espécie tipo: *Porpolomopsis calyptriformis* (Berk.) Bresinsky

≡ *Hygrocybe calyptriformis* (Berk.) Fayod

≡ *Agaricus calyptriformis* Berk.

Gênero *Gliophorus* Herink, Sb. Severocesk. Mus., Prír. Vedy 1: 80 (1959)

Espécie tipo: *Gliophorus psittacinus* (Schaeff. : Fr.) Herink

≡ *Hygrocybe psittacina* (Schaeff. : Fr.) P. Kumm., Führ. Pilzk. (Zwickau): 112 (1871),

≡ *Hygrophorus psittacinus* (Schaeff.) Fr.

≡ *Agaricus psittacinus* Schaeff. : Fr.

Seção *Gliophorus* [autônimo] (1958)

≡ *Gliophorus* sect. "Psittacinae" (Bataille) Herink

Espécie tipo: *Gliophorus psittacinus* (Schaeff.) Herink

≡ *Hygrocybe psittacina* (Schaeff.) P. Kumm.

≡ *Hygrophorus psittacinus* (Schaeff.) Fr.

Seção *Glutinosae* (Kühner) Lodge & Padamsee, Fungal Diversity 64: 28 (2013) [2014]

Basiônimo: *Hygrocybe* sect. *Glutinosae* Kühner

≡ *Gliophorus* sect. *Laetae* (Bataille) Kovalenko

Lectótipo: *Gliophorus laetus* (Pers. : Fr.) Herink

≡ *Hygrocybe laeta* (Pers. : Fr.) P. Kumm.

≡ *Hygrophorus laetus* (Pers.) Fr.,

≡ *Agaricus laetus* Pers.

Seção *Unguinosae* Herink, Sb. Severocesk. Mus., Prír. Vedy 1: 81 (1959)

Espécie tipo: *Gliophorus unguinosus* (Fr.) Kovalenko

≡ *Agaricus unguinosus* Fr. : Fr.

≡ *Gliophorus unguinosus* Herink

≡ *Hygrocybe unguinosa* (Fr.: Fr.) P. Karst

≡ *Hygrocybe irrigata* (Pers.: Fr.) Bon

≡ *Gliophorus irrigatus* (Pers.) A.M. Ainsw. & P.M. Kirk

Tribo Chromosereae Vizzini, Lodge Norvell & Redhead, Fungal Diversity 64: 28 (2013) [2014]

Gênero tipo: *Chromosera* Redhead, Ammirati & Norvell

Gênero *Chromosera* Redhead, Ammirati & Norvell, Beih. Sydowia 10: 161 (1995), emend. Vizzini & Ercole, Micol. Veget. Medit. 26(2): 97 (2012) [2011]

Espécie tipo: *Chromosera cyanophylla* (Fr.) Redhead, Ammirati & Norvell

≡ *Agaricus cyanophyllus* Fr.

Subgênero *Chromosera* [autônimo]

Espécie tipo: *Chromosera cyanophylla* Redhead, Ammirati & Norvell

≡ *Agaricus cyanophyllus* Fr.

Subgênero *Oreocybe* (Boertm.) Vizzini, Lodge & Padamsee, Fungal Diversity 64: 28 (2013) [2014]

Basiônimo: *Hygrocybe* sect. *Oreocybe* Boertm., Nordic Jl. Bot. 10(3): 315 (1990)

≡ *Hygrocybe* subg. *Oreocybe* (Boertm.) Beis.

Espécie tipo: *Chromosera citrinopallida* (A.H. Sm. & Hesler) Vizzini & Ercole

≡ *Gliophorus citrinopallidus* (A.H. Sm. & Hesler) Kovalenko

≡ *Hygrocybe citrinopallida* (A.H. Sm. & Hesler) Kobayasi

≡ *Cuphophyllus citrinopallidus* (A.H. Sm. & Hesler) Bon

≡ *Hygrophorus citrinopallidus* A.H. Sm. & Hesler

Subgênero *Subomphalia* Vizzini, Lodge & Padamsee, Fungal Diversity 64: 28 (2013) [2014]

Espécie tipo: *Chromosera viola* (J. Geesink & Bas) Vizzini & Ercole

≡ *Hygrocybe viola* J. Geesink & Bas

≡ *Cuphophyllus viola* (J. Geesink & Bas) Bon

Gênero *Gloioxanthomyces* Lodge, Vizzini, Ercole & Boertm, in Lodge *et al.* Fungal Diversity 64: 28 (2013) [2014]

Espécie tipo: *Gloioxanthomyces vitellinus* (Fr.) Lodge, Vizzini, Ercole & Boertm.

≡ *Hygrophorus vitellinus* Fr.

Gênero *Sinohygrocybe* C.Q. Wang, Ming Zhang & T.H. Li, in Wang, Zhang, Li, Liang & Shen, MycoKeys 38: 67 (2018)
Espécie tipo: *Sinohygrocybe tomentosipes* C.Q. Wang, Ming Zhang & T.H. Li

1.3.3. Caracterização de *Hygrocybe* s.l.

Uma breve caracterização é indicada para cada gênero, adaptada de Lodge *et al.* (2013) para a maioria dos gêneros e de Wang *et al.* (2018) para *Sinohygrocybe*. As características morfológicas que diferenciam os gêneros em *Hygrocybe* s.l., no entanto, são inconsistentes, o que leva a necessidade de haver uma grande combinação de características diagnósticas para separar cada grupo.

Hygrocybe (Fr.) P. Kumm., Führ. Pilzk. (Zwickau): 26 (1871)

Basidiomas geralmente de cores brilhantes, vivas, ou menos comumente brancos, às vezes com reações de mudança de cor para preto ou cinza; véu verdadeiro ausente; píleo normalmente cônico, umbonado, convexo ou umbilicado, lamelas geralmente presentes, espessas, com aparência cerosa; basidiósporos inamiloides, de parede fina, com gotículas oleaginosas, hialinos, lisos ou raramente com espinhos, mono- ou dimórficos em tamanho e formato; basídios clavados, clavado-estipitado ou cilindro-clavados, geralmente com 4 esterigmas, razão entre o comprimento dos basídios para os basidiósporos de 3 a 7; raramente com cistídios verdadeiros; trama da lamela regular a subregular; fíbulas presentes em todas as estruturas; hábito terrestre, raramente em madeira ou arbóreo, crescendo em florestas ou em gramados, biotrófico, mas não formam micorrizas.

Hygroaster Singer 1955, Sydowia 9(1‑6): 370

Basidiomas geralmente secos, de cores fuscas ou branco; lamelas espessas, decorrentes e distantes ou subdistantes; basidiósporos subglobosos a globosos, espinosos, com espinhos cônicos longos e ápices agudos, inamiloides, hialinos, razão entre o comprimento dos basídios para os basidiósporos >5; trama da lamela subregular, levemente divergente nas laterais, com

elementos curtos, parte central geralmente pigmentada; fíbulas ausentes em todo o basidioma; hábito terrestre, em florestas tropicais, conhecido somente para a região neotropical.

Humidicutis (Singer) Singer, Sydowia 12(1‑6): 225 (1959) [1958]

Píleo convexo, umbonado ou cônico, geralmente higrófano, úmido, raramente viscoso, coloração geralmente brilhante, laranja, amarelo, rosa, roxo ou verde, ou menos comumente oliváceo ou branco; lamelas espessas, sinuadas, adnatas, ou com um dente decorrentes; estipe seco, liso; basidiósporos inamiloides, de parede fina, com gotículas oleaginosas, hialinos, lisos, elipsoides; basídios clavados ou cilindro-clavados, geralmente com esterigmas longos, com fíbulas toroidais na base dos basídios, razão entre o comprimento dos basídios para os basidiósporos ≥5; trama da lamela regular ou subregular; pileipellis cútis, normalmente com pigmentos encrustrados; fíbulas ausentes em todas as estruturas, exceto na base dos basídios.

Neohygrocybe Herink Sb., Severocesk. Mus., Prír. Vedy 1: 71 (1959)

Píleo hemisférico ou campanulado, normalmente umbonado e margem incurvada quando jovem à plano-convexo com margem elevada, seco ou úmido, fibriloso, tomentoso ou escamuloso, frequentemente rimoso, normalmente marrom acizentado, podendo ter reações de mudança de cor para vermelho e em seguida marrom escuro; lamelas adnexas, sinuadas ou adnatas, espessas, cerosas, distantes e frágeis, estipe frequentemente compresso ou sulcado, liso, contexto preenchido ou oco; normalmente com odor de nitrato, cloro ou frutado; basidiósporos inamiloides, de parede fina, com gotículas oleaginosas, hialinos, lisos, oblongos ou elipsoides, às vezes subglobosos; basídios clavados, com 2 ou 4 esterigmas, razão entre o comprimento dos basídios para os basidiósporos >5; trama da lamema regular ou subregular; pseudocistídios comuns, emanando da trama, às vezes com conteúdos amarronzados; pileipellis frequentemente com pigmentos amarronzados; fíbulas presentes em todas as estruturas.

Porpolomopsis Bresinsky, Regensb. Mykol. Schr. 15: 145 (2008)

Píleo cônico, cônico-campanulado, convexo-umbonado ou cuspidado, úmido, margem geralmente partindo radialmente, de modo que as lamelas também se partem, rosa, rosa-

salmão, lilás, roxo ou branco; lamelas geralmente livres ou quase livres; basidiósporos inamiloides, de parede fina, hialinos, lisos, elipsoides; basídios clavados, com fíbulas toroidais na base, razão entre o comprimento dos basídios para os basidiósporos ≥5; trama da lamela regular, com elementos longos; hifas da pileipellis arranjadas radialmente, fusiformes; fíbulas ausentes ou muito raras, presentes apenas na base dos basídios.

Gliophorus Herink, Sb. Severocesk. Mus., Prír. Vedy 1: 80 (1959)

Píleo e estipe glutinosos, com pigmentações brilhantes ou opacas, azul, violeta, rosa, rosa-salmão, verde, amarelo ocre, amarelo, vermelho-amarronzado, cinza-amarronzado; lamelas sinuadas ou decorrentes, às vezes com margem gelatinosa; basidiósporos inamiloides, de parede fina, com gotículas oleaginosas, hialinos, lisos, elipsoides; basídios com 4 esterigmas, frequentemente com fíbulas em medalhão ou toroidais na base, razão entre o comprimento dos basídios para os basidiósporos ≅5; trama da lamela subregular, com elementos curtos, subhimênio às vezes gelatinoso; ixoqueilocistídios normalmente presentes; pilepellis tricodermal com camada gelatinosa e fíbulas em medalhão; fíbulas presentes em todas as estruturas.

Chromosera Redhead, Ammirati & Norvell, Beih. Sydowia 10: 161 (1995), emend. Vizzini & Ercole, Micol. Veget. Medit. 26(2): 97 (2012) [2011]

Basidiomas onfalinoides (de *Omphalina* sp.) de colorações rosadas, violeta, roxo ou amarelo, geralmente viscosos, com píleo diminuto e lamelas decorrentes ou arqueada-decorrentes, basidiósporos inamiloides, de parede fina, hialinos, lisos; basídios pequenos em relação aos basidiósporos, razão entre o comprimento dos basídios para os basidiósporos <5; trama da lamela subregular a irregular; reações dextrinoides efêmeras comuns no contexto; pilepellis com camada gelatinosa, com pigmentos extracelulares efêmeros; fíbulas presentes em todas as estruturas, às vezes em medalhão; crescem em solo, madeira de coníferas e possivelmente associado a gramíneas e briófitas, ocorrem em regiões árticas e alpinas.

Gloioxanthomyces Lodge, Vizzini, Ercole & Boertm, in Lodge *et al.* Fungal Diversity 64: 28 (2013) [2014]

Basidiomas de colorações amarelas ou amarelo-alaranjadas, viscosos; lamelas arqueadas-decorrentes, com margem gelatinosa, translúcida; basidiósporos inamiloides, de parede fina, hialinos, lisos, com gotículas, com apêndice hilar largo, elipsoides ou subglobosos Q ≅ 1.2–1.3; basídios com 4 esterigmas, com fíbulas em medalhão na base, pequenos, razão entre o comprimento dos basídios para os basidiósporos 4–5; trama da lamela subregular, com elementos cilíndricos à subglobosos, subhimênio de hifas estreitas, entrelaçadas; queilocistídios clavados, simples ou lobados; pileipellis e stipitipellis com camada gelatinosa; fíbulas presentes em todas as estruturas, às vezes em medalhão.

Sinohygrocybe C.Q. Wang, Ming Zhang & T.H. Li, in Wang, Zhang, Li, Liang & Shen, MycoKeys 38: 67 (2018)

Basidiomas subcespitosos, de tamanho médio, amarelo à alaranjado; píleo convexo a aplanado, levemente depresso no centro, seco ou subviscoso, mas não glutinoso; lamelas adnatas ou decorrentes, com lamélulas furcadas e intervenosas; basidiósporos inamiloides, de parede fina, hialinos, lisos, com gotículas, com apêndice hilar largo, elipsoides a oblongos, Q = 1.6–1.7; basídios com 4 esterigmas, razão entre o comprimento dos basídios para os basidiósporos >5; trama da lamela subregular; pileipellis e stipitipellis cútis; fíbulas presentes em todas as estruturas.

1.4. Distribuição

Hygrocybe s.l. é amplamente distribuído no mundo, tendo ocorrência para todos os continentes, exceto Antártica, mas há registros para as regiões árticas e subárticas da Europa, América do Norte e Rússia (Boertmann, 2010; Borgen & Ohenoja, 2013; Kovalenko, 1999). Na região neotropical, ocorre na Argentina, Belize, Brasil, Chile, Colômbia, Costa Rica, Cuba, Equador, Estados Unidos da América (Califórnia e Flórida), Guadalupe, Guiana Francesa, Ilhas Virgens Americanas, Jamaica, Martinica, México, Panamá, Porto Rico, República Dominicana, Trinidad e Venezuela, sendo que a maior parte das ocorrências é da região caribenha da América Central (Laessoe & Boertmann, 2008; Cantrell & Lodge, 2000; 2001; 2004; Dennis, 1953; 1961; 1970; Furci, 2013; 2018; Hesler & Smith, 1963; Lodge & Pegler, 1990; Magnago *et al.*, 2015; Montoya *et al.*, 2005; Neves *et al.*, 2013; Niveiro & Albertó, 2012; Pegler, 1983; 1997; Pegler & Fiard, 1978; Raithelhuber, 1992; Singer, 1965;

1989; Vasco-Palacios & Franco-Molano, 2013).

No Brasil, há 21 espécies de *Hygrocybe* s.s. e duas de *Hygroaster* registradas segundo a Flora do Brasil *in Agaricales* 2020 (em construção). Na rede *species*Link (http://www.splink.org.br/), há 831 registros de *Hygrocybe* s.s. (Figura 4), 15 de *Hygroaster*, cinco de *Humidicutis*, três de *Neohygrocybe* e um registro para *Gliophorus*. Vale ressaltar que dos 831 registros de *Hygrocybe*, 546 estão identificados apenas até o nível genérico. Putzke & Putzke (2017) em sua revisão de Agaricales relatam 35 espécies de *Hygrocybe* s.s. para o Brasil. Na revisão bibliográfica realizada neste trabalho, foram levantadas 37 espécies de *Hygrocybe* s.s. e duas de *Hygroaster* para o Brasil, sendo 26 dos registros com ocorrência apenas para a Mata Atlântica, quatro com ocorrência para Mata Atlântica e Amazônia, uma para Mata Atlântica e região semiárida, cinco com ocorrência apenas para a Amazônia e uma para o Cerrado.

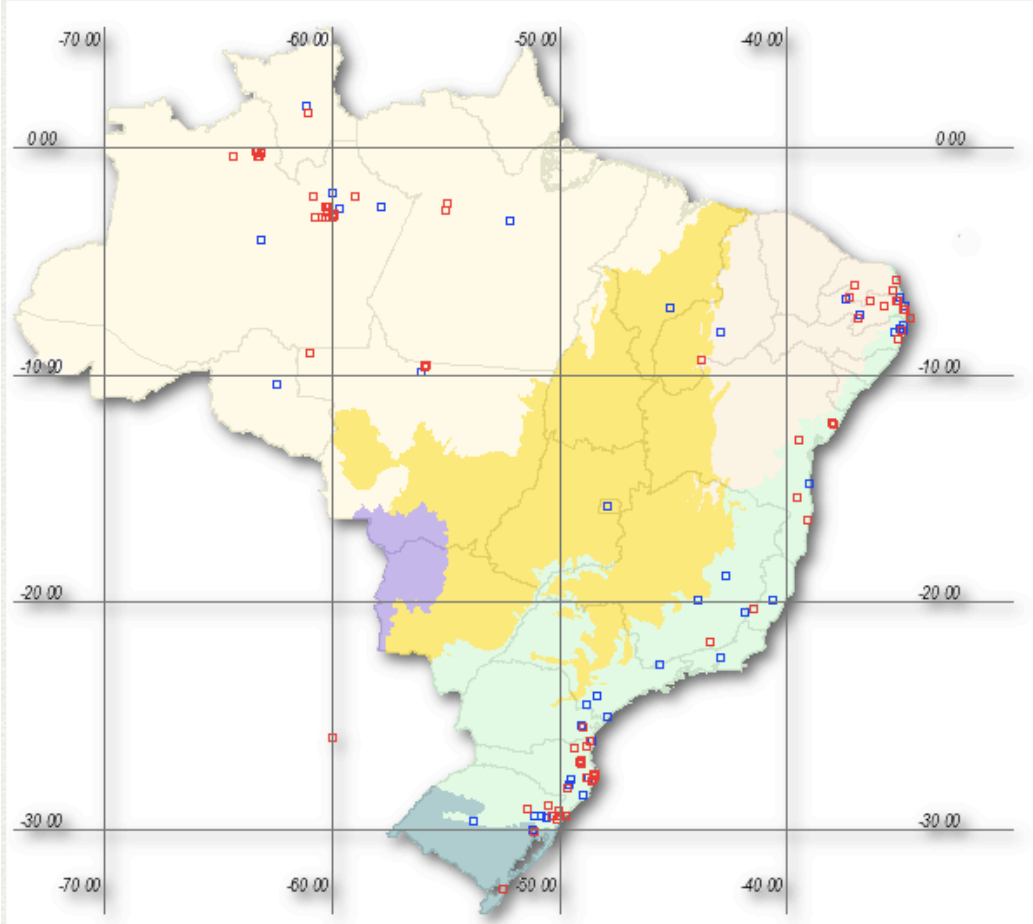

Figura 4. Mapa de distribuição das coletas de *Hygrocybe* s.s. no Brasil gerado pela rede *speciesLink* (http://www.splink.org.br/) no dia 8/03/2020. Os pontos em vermelho são das coordenadas originais das coletas e os pontos em azul são coordenadas dos municípios. As diferentes cores no mapa representam os biomas brasileiros.

1.5. Amazônia brasileira

Os primeiros trabalhos com macrofungos Agaricales na Amazônia brasileira foram os de Singer (1965; 1973; 1989), Singer & Araújo (1979), Singer *et al.,* (1983) e Capelari & Maziero (1988b), que contribuíram tanto para o conhecimento da diversidade quanto da ecologia destes organismos. Recentemente, outros trabalhos de caráter ecológico e de levantamento de espécies têm citado a ocorrência de macrofungos Agaricales na Amazônia Central (Braga-Neto *et al.,* 2008; Komura *et al.,* 2017; Souza & Aguiar, 2004).

Segundo a literatura consultada, há ocorrência de uma espécie de *Hygroaster* e oito espécies de *Hygrocybe* s.s. para a Amazônia brasileira (Tabela 2). *Hygroaster nodulisporus* (Dennis) Singer foi descrita para Trinidad (Dennis, 1953) e há ocorrência para o Paraná (Meijer, 2008). Apesar de ser um gênero de ocorrência neotropical (Lodge *et al.,* 2013), ainda não há registros de *Ha. nodulisporus* para a Amazônia brasileira. Capelari & Maziero (1988a) descreveram uma espécie de *Hygrocybe* para Rondônia e Singer (1989) descreveu quatro espécies de *Hygrocybe* e uma de *Hygroaster* para o Amazonas. *Hygrocybe siparia* e *Hc. trinitensis* são citadas para o Amazonas (Singer, 1965; Komura *et al.,* 2017) e *Hc. occidentalis* é citado para Amazonas e Rondônia (Capelari & Maziero, 1988b; Souza & Aguiar, 2004). No trabalho de Souza & Aguiar (2004), em um levantamento de Agaricales para uma área de terra-firme na Amazônia Central, são citadas 11 espécies de *Hygrocybe*: *Hc.* cf. *megistospora* Singer, *Hc.* aff. *miniceps* (Stev.) Horak, *Hc. occidentalis* (Dennis) Pegler var. *scarletina* Pegler & Fiard, e mais oito táxons que não foram identificados ao nível especí-

Tabela 2. *Hygrocybe* s.l. previamente registradas para a Amazônia brasileira

Táxon	Estado	Referências
Hygroaster albellus Singer	AM	Singer (1989)
Hygrocybe amazoniensis Singer	AM	Singer (1989)
Hygrocybe campinaranae Singer	AM	Singer (1989)
Hygrocybe hololeuca Singer	AM	Singer (1989)
Hygrocybe mutabilis Singer	AM	Singer (1989)
Hygrocybe occidentalis (Dennis) Pegler	AM, RO	Capelari & Maziero (1988a); Souza & Aguiar (2004)
Hygrocybe siparia (Berk.) Singer	AM	Singer (1965)
Hygrocybe trinitensis (Dennis) Pegler	AM	Komura *et al.* (2017)
Hygrocybe viridis Capelari & Maziero	RO	Capelari & Maziero (1988b)

fico. Outro estudo realizado na Amazônia Central (Komura *et al.,* 2017) relata a ocorrência de uma espécie de *Hygroaster* e três de *Hygrocybe*, sendo somente uma (*Hc. trinitensis*) identificada ao nível específico. Esta carência de identificações de espécimes de *Hygrocybe* s.l. ao nível específico demonstra a dificuldade de estudar este grupo na Amazônia brasileira.

Tendo em vista que a maior diversidade de fungos está provavelmente concentrada nos trópicos (Hawksworth & Rossman, 1997), os trabalhos de taxonomia de fungos na Amazônia são de extrema importância. Estima-se que a Amazônia abrigue 50.000 espécies de plantas (Hubbell *et al.,* 2008) e levando em conta a estimativa de 9,8 espécies de fungos para cada espécie de planta apresentada por Hawksworth & Lücking (2017), teríamos um total de 490.000 espécies de fungos somente para a bacia da Amazônia. Segundo o projeto Flora do Brasil 2020 (em construção), há ocorrência de 1051 espécies de fungos para a Amazônia brasileira e, destes, 133 espécies pertencem à ordem Agaricales. Esse cenário ilustra a falta de estudos micológicos nesta região, e isso se dá principalmente pela falta de micólogos taxonomistas na Amazônia, enquanto que a maior parte dos estudos estão concentrados nas áreas de Mata Atlântica das regiões sul, sudeste e nordeste do país (Maia *et al.,* 2015).

2. Objetivo Geral

Conduzir estudos taxonômicos de *Hygrocybe* sensu lato (Lodge *et al.*, 2013) na Amazônia brasileira, correlacionando dados morfológicos e moleculares.

2.1. Objetivos Específicos

- Realizar nova amostragem de espécimes de *Hygrocybe* s.l. na Amazônia brasileira;
- Conduzir estudos macro- e micromorfológicos para taxonomia integrativa;
- Identificar os espécimes coletados ao nível específico ou descrever novas espécies integrando dados morfológicos e de sequências de ITS;
- Obter novas sequências de espécimes brasileiros para inclusão na filogenia de *Hygrocybe* s.l.

3. Material e Métodos

3.1. Áreas de amostragem

Os locais de coleta foram distribuídos pela Amazônia brasileira, buscando amostrar diferentes regiões e fitofisionomias. As áreas de coleta foram, principalmente: Reserva Florestal Adolpho Ducke – RFAD (Manaus, AM, 2°57'48.3"S 59°55'38.8"W), Museu da Amazônia – MUSA (Manaus, AM, 3°00'20.4"S 59°56'25.8"W), Base do Alto Cuieiras INPA (Manaus, AM, 2°34'06.7"S 60°19'15.2"W), Parque Nacional do Viruá (Caracaraí, RR, 1°17'22.51"N 61°11'23.85"W), Floresta Nacional do Tapajós (Belterra, PA, 2°45'26.3"S 55°01'10.1"W) e Reserva Particular do Patrimônio Nacional Cristalino (Alta Floresta, MT, 9°35'51.0"S 55°55'52.8"W). Um número menor de coleções (mais relacionado ao menor esforço de amostragem) foram obtidas também em: Arquipélago Mariuá (Barcelos, AM, 0°22'06.4"S 64°20'22.3"W), Reserva de Desenvolvimento Sustentável Rio Negro (Iranduba, AM, 3º3'41.47"S 60º45'3.63"W), Campus da Universidade Federal do Amazonas – UFAM (Manaus, AM, 3°05'47.4"S 59°58'40.0"W), Campus I do Instituto Nacional de Pesquisas da Amazônia – INPA (Manaus, AM 3°05'40.7"S 59°59'15.2"W) e Ramal do Novo Amanhecer (Ramal do Pau Rosa, Manaus, AM, 2°50'49.1"S 60°14'13.9"W). Algumas coletas cedidas por colaboradores também foram analisadas, coletadas em: Estação Científica do Uatumã (São Sebastião do Uatumã, AM, 2°08'32"S 59°00'05"W) e próximo a represa de Balbina (Presidente Figueiredo, AM, 1°56'34.7"S 59°27'58.3"W) (Figura 5).

3.2. Coleta

A coleta dos basidiomas foi feita por busca não estruturada ao longo das trilhas. Para as florestas de áreas alagáveis, foi respeitado o pulso de inundação dos rios e as coletas foram feitas em períodos de seca. Para as florestas de terra-firme e campinaranas, as coletas foram feitas em períodos de alta pluviosidade.

Os materiais foram fotografados com uso de escala e, em seguida, coletados manualmente com o auxílio de um canivete, e armazenados em caixas plásticas contendo divisórias de modo que não houvesse contato entre os espécimes. As informações relevantes de substrato e habitat foram anotadas no caderno de coleta. As posições geográficas dos locais de coleta foram feitas através do sistema de posicionamento global (GPS) quando possível, ou

inferidas através do *Google Maps*. Para a extração de DNA, uma porção do píleo foi retirada do material ainda fresco e armazenada em sílica ou em *FTA® Cards*.

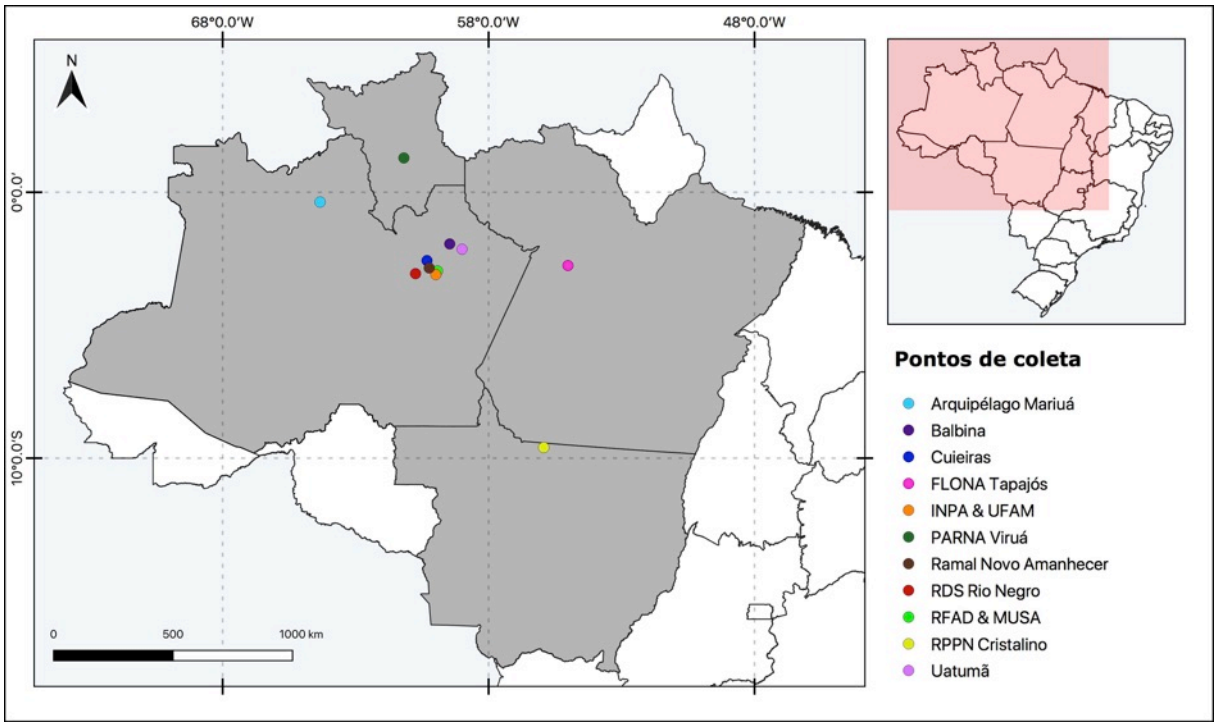

Figura 5. Recorte do mapa do Brasil com ênfase nas áreas que foram amostradas, cujos estados brasileiros estão destacados em cinza.

3.3. Análises morfológicas

A descrição macroscópica dos basidiomas foi feita ao final de cada coleta com base no material ainda fresco, utilizando literatura tradicional para descrição de cogumelos (Largent, 1986). As variáveis analisadas para píleo e estipe dos basidiomas foram as dimensões, formato, coloração e características da superfície. Para o himenóforo foram analisadas a inserção das lamelas, as dimensões, a coloração, o formato, a presença ou ausência de lamélulas e o tipo de espaçamento entre as lamelas. O código das cores faz referência ao guia de cores *Online Auction Color Chart* (Kramer, 2004). Após a descrição macroscópica, os materiais foram desidratados em uma secadora de frutas com circulação de ar a até 40°C e armazenados em sacos plásticos com fecho hermético (tipo *ziplock*) para conservação. Os espécimes já desidratados e devidamente armazenados foram colocados em freezer a -20°C por sete dias para a eliminação completa de contaminantes ou insetos.

A observação microscópica do material foi feita com os basidiomas já desidratados a partir de cortes com lâminas de aço inoxidável, montados entre lâmina e lamínula para observação em microscópio óptico. Para a reidratação dos cortes, foi utilizado álcool 70% e hidróxido de potássio 5%. O corante Vermelho Congo foi utilizado para realizar as medições das estruturas microscópicas e o reagente de Melzer para testar se havia reação amiloide ou dextrinoide (Largent *et al.,* 1977). As estruturas microscópicas foram fotografadas através de câmera LEICA EC3 acoplada ao microscópio e medidas com o programa LAS EZ v3.3.0. Ilustrações das estruturas microscópicas diagnósticas foram feitas com base nas fotografias. As características analisadas foram: comprimento × largura dos basidiósporos (n = 30) e basídios (n = 20), largura das hifas da trama lamelar (n = 10), hifas terminais da pileipellis e stipitipellis (n = 10), disposição das hifas da trama lamelar, da pileipelis e da estipitipelis e presença ou ausência de cistídios. Os cistídios, quando presentes, foram medidos (comprimento × largura) e seus formatos foram anotados. Para os basidiósporos, foi incluído o quociente comprimento/largura (Q) e as médias. As médias aparecem sempre entre as medidas mínimas e máximas de cada estrutura, em itálico, incluso a média do quociente Q. Todas as informações relevantes ao grupo e estruturas conspícuas foram anotadas na descrição de cada espécime. Chaves de identificação dicotômicas foram feitas para cada subgrupo.

3.4. Procedimentos de biologia molecular

As amostras reservadas para realização de estudos moleculares foram devidamente cadastradas na plataforma do Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado – SisGen, com o número de cadastro A76D0E6, e enviadas ao exterior conforme a Lei 13.123/2015.

Os procedimentos de extração de DNA, amplificação em PCR, purificação e sequenciamento Sanger foram feitos no Laboratório de Sistemática Molecular do Royal Ontario Museum (ROM) de acordo com Dentinger *et al*. (2010). O DNA foi extraído de fragmentos de basidiomas secos em sílica ou armazenados em *FTA® Cards*. Para amplificação em PCR do marcador molecular universal ITS (*Internal Transcribed Spacer* – Figura 6), foram utilizados os *primers* ITS1F/ITS4 (Gardes & Bruns 1993, Lodge *et al.,* 2013, White *et al.* 1990). Como produto final desta etapa, foram obtidas sequências de ITS.

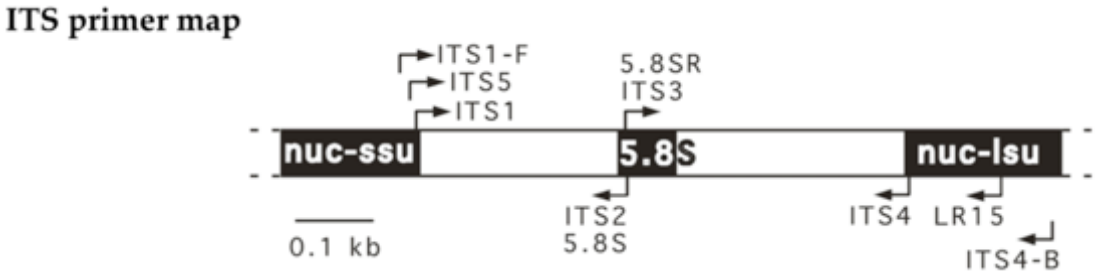

Figura 6. Mapa de *primers* para obtenção de sequências ITS. Retirado de Binder & Hibbett (2003). Disponível online em: https://www2.clarku.edu/faculty/dhibbett/Protocols_Folder/Primers/Primers.pdf (Acesso em 25/08/2020).

3.5. Processamento de dados

A identificação dos táxons foi feita utilizando literaturas específicas do grupo e chaves de identificação, com ênfase nas literaturas disponíveis para a região neotropical (Cantrell & Lodge, 2000; 2001; 2004; Capelari & Maziero, 1988b; Dennis, 1953; 1961; 1970; Franco-Molano *et al.,* 2000; Lodge & Ovrebo, 2008; Lodge & Pegler, 1990; Lodge *et al.,* 2013; Meijer, 2008; Neves *et al.,* 2013; Pegler, 1983; 1997; Pegler & Fiard, 1978; Raithelhuber, 1992; Rick, 1938; Silva-Filho *et al.*, 2019; Singer, 1965; 1973; 1989; Vizzini *et al*., 2015). Como parte da taxonomia integrativa, a identificação molecular foi feita através de BLAST no banco de dados *online GenBank* (NCBI). As espécies novas foram nomeadas de acordo com o Código Internacional de Nomenclatura de Algas, Fungos e Plantas (ICN) (Turland *et al.,* 2018) e serão inseridas no banco de dados *online* para fungos *MycoBank* (Robert *et al*. 2005). As exsicatas foram depositadas no Herbário INPA e, para as coletas realizadas na RPPN Cristalino, no Herbário e Fungário FLOR. As sequências obtidas serão inseridas no *GenBank* (NCBI), também servindo para identificação molecular como códigos de barra de espécies.

Capítulo 1

Studies in *Hygrocybe* subgen. *Hygrocybe* sect. *Pseudofirmae* (Hygrophoraceae, Agaricales) from the Brazilian Amazon: new species and one new record

Abstract

Hygrocybe subgen. *Hygrocybe* is characterized by brightly coloured basidiomes, that can stain black or grey with bruise or age, free to adnexed or uncinate lamellae, regular lamellar trama with long parallel hyphae, basidia that are 3 to 5 times the length of their basidiospores and mono- or dimorphic basidia and basidiospores. *Hygrocybe* sect. *Pseudofirmae* is one of the 5 sections within *Hygrocybe* subg. *Hygrocybe* and includes species with dimorphic basidiospores and basidia. In this study, we propose five new species in sect. *Pseudofirmae* for the Southern Brazilian Amazon and one new record for *Hygrocybe hololeuca*, including the first record for Pará state. The new species are proposed based on morphology and molecular identification using the universal marker ITS.

Key words: ITS, barcode, fungi, taxonomy, Mato Grosso, Cristalino.

Introduction

Hygrocybe subgen. *Hygrocybe* (Fr.) P. Kumm. is currently known to include *Hygrocybe* (Fr.) P. Kumm. species with brightly coloured basidiomes, which can sometimes stain black or grey with bruise or age, with free to adnexed or uncinate lamellae, lamellar trama with long parallel elements and basidia that is usually 3 to 5 times the length of their basidiospores (Lodge *et al*. 2013). Species in *Hygrocybe* subg. *Hygrocybe* can be mono- or dimorphic in their basidia and basidiospores sizes and are classified in 5 sections: *Hygrocybe* (Fr.) P. Kumm., *Chlorophanae* (Herink) Arnolds ex Candusso, *Microsporae* Boertm., *Pseudofirmae* Lodge, Padamsee & S.A. Cantrell and *Velosae* Lodge, Ovrebo & Padamsee. The dimorphism of basidia and basidiospores was first mentioned by Petch (1917) and was soon recognised by various tropical agaricologists (Lodge *et al*., 2013). These species were classified all together in one section of *Hygrocybe*, viz. *Firmae* Heinemann (1963). Later, in a

multilocus phylogenetic analysis, Lodge *et al*. (2013) recognised that the dimorphism had multiple origins, occurring in other two sections of *Hygrocybe* subg. *Hygrocybe*, viz. *Pseudofirmae* and *Velosae*, while sect. *Firmae* is now classified in subg. *Pseudohygrocybe*.

Section *Pseudofirmae* groups tropical dimorphic species characterized by an often perforated pileus that is smooth to tomentose or squamulose, macrobasidia usually broadly clavate or clavate-stipitate, which are conspicuously wider than the microbasidia, regular lamellar trama often with interwoven refractive hyphae, pileipellis can vary from cutis to trichoderm with or without a gelatinous layer (Lodge *et al*., 2013).

The Brazilian Amazon is a vast territory but very little is known about its funga. With the increase of deforestation, especially in Southern Amazon, an area known as the Arc of Deforestation (IPAM, 2020), extinction of species is expected to occur (Stropp *et al.*, 2020), and many undescribed species are put at risk of becoming extinct without even having a name. From August 2018 to July 2019, 1,702 km^2 of Amazon forest were deforested in Mato Grosso state, which represents 16.8% of the total loss of Amazon forest in the Brazilian territory in this period of time (INPE, 2020).

The Cristalino Reserve is an important conservation area in this fragile region. Together with other conservation units such as Cristalino State Park, Juruena National Park, Serra do Cachimbo Springs Biological Reserve, the Indigenous Lands and the Brazilian Air Force Base they form a protection layer against the constantly raising deforestation in the region (Fundação Ecológica Cristalino, 2020). The Reserve is managed by the NGO Cristalino Ecological Foundation (Fundação Ecológica Cristalino – FEC) and one of their projects is the Cristalino Fungi Project, created in 2015, started by the fungal parataxonomist and citizen scientist Susanne Sourell. So far, more than 1,000 collections of fungi have been documented within this project and are stored at FLOR Herbarium and Fungarium (Neves *et al*., 2018). The main goal of the project is to bring awareness about conservation of fungi, showing the importance of protecting the forest, especially in the constantly threatened Southern Amazon.

In this study we propose five new species of subgen. *Hygrocybe* sect. *Pseudofirmae* from Mato Grosso state, based on morphology and molecular identification using ITS. One new record to Pará state of *H. hololeuca* Singer is indicated together with a discussion regarding its placement among subg. *Hygrocybe*. An identification key to the species of *Hygrocybe* subg. *Hygrocybe* sect. *Pseudofirmae* occurring in Brazil is provided.

Material and Methods

Study area

The specimens were collected in four localities: Cristalino Private Reserve of National Heritage (RPPN), Tapajós National Forest, Adolpho Ducke Forest Reserve (RFAD) and *Museu da Amazônia* (MUSA). RPPN Cristalino is located along the Cristalino River in Mato Grosso State, between Alta Floresta and Novo Mundo, in Southern Amazon (9°35'51.0"S, 55°55'52.8"W). It is an important preserved area close to the "Arc of Deforestation", known as a conflict zone mainly due to the expansion of monoculture of soybeans and cattle breeding (IPAM, 2020). The area is characterised mostly by clayish soils typical of *terra-firme* (upland) forest in the Amazon (Pires & Prance, 1985). The Tapajós National Forest is a federal conservation area with sustainable exploitation activities and research stations, located mostly in Belterra and adjacent cities in Pará state, along the Tapajós River (3°25'08.5"S, 55°04'18.2"W). The area consists mainly of *terra-firme* forest with periodically flooded areas (Pires & Prance, 1985). The soils are mostly clayish but near the Tapajós River margin, the soils are a mix of clay and sand, and sometimes it can be predominantly sandy (ICMBio, 2020a). Adolpho Ducke Forest Reserve is an area of well-protected forest just next to the urban area of Manaus, Amazonas (3°00'21.9"S, 59°56'25.3"W) that is under management of the National Institute for Amazonian Research (INPA). The forest type is a typical *terra-firme* forest with clayish soils and tall trees, with a few lowland areas with sandy soils (Pires & Prance, 1985). MUSA is the visitors' part of the Reserve, an open museum of natural amazon forest. An additional collection made by D.L. Komura in São Sebastião do Uatumã, Estação Científica do Uatumã (2°08'32"S, 59°00'05"W) was also analysed in this study.

Morphological analysis

Specimens were photographed in the field and described while still fresh. Specimens were dried at 40ºC and preserved in zipped plastic bags. Morphological descriptions were conducted using traditional methodology based on Largent (1986) and Largent *et al*. (1977). Colour codes follow the *Online Auction Color Chart* (Kremer 2004). Micromorphological features were observed in hand-cut sections of different parts of the basidiomata mounted in KOH 3% or 5% and Congo Red 1% and another section mounted with Melzer's reagent.

Thirty basidiospores of each specimen were measured, excluding the hilar appendage. The basidiospore quotient (Q, ratio of length/width) was calculated for each specimen. The mean values for basidiospore dimensions and Q are indicated in between the measurements in italic font. Specimens were deposited at FLOR and INPA Herbaria and new names were registered in the MycoBank database.

Sequencing and sequence editing

A piece of each specimen was preserved in FTA Cards for DNA extraction. The DNA extraction, amplification of the ITS region in PCR, purification and sequencing procedures were performed at the Molecular Systematics Laboratory of the Royal Ontario Museum (ROM) following Dentinger *et al*. (2010) protocols. The DNA was extracted from fragments stored in FTA® Cards. ITS1F/ITS4 primers were used to amplify the Internal Transcribed Spacer (ITS) (Gardes & Bruns 1993, Lodge *et al*., 2013, White *et al*. 1990). As a final product of this step, three ITS sequences were obtained. Sequences were edited in Geneious R7 program for combining forward and reverse pairs, to edit base pairs with low resolution or ambiguities, generate the consensus sequence and trimming of the ends. The sequences were submitted to BLASTn searches for barcode identification and compared with the sequences available in the GenBank database. The sequences obtained were deposited in the online database GenBank.

Results

Taxonomy

Hygrocybe cristalinensis J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 7 a, e and 8)

Etymology: From the Cristalino River.
Holotype: BRAZIL, Mato Grosso, Alta Floresta, Reserva Particular do Patrimônio Natural Cristalino, Trilha do Francisco, 4 January 2019, *Santos, C.* CJL647 (FLOR67406, holotype!).
Diagnosis: Differs from *Hygrocybe prieta* Lodge & Pegler in the lack of purple, red and orange colourations in the basidiomata and presence of cheilocystidia.

Description:

Pileus 10–36 mm diam., convex when young, then becoming umbilicate, often perforated at the centre, smooth, radially fibrillose, moist to lubricous, intense dark green (oac103) or sometimes leaf green (oac89), with flushes of olive green (oac866) to brownish green (oac831), light green (oac75) to yellowish-green (oac24, oac10) at the centre, translucent-striate towards margin; margin straight to slightly incurved, with a whitish to very pale green (oac51) minutely appendiculate fringe. *Lamellae* decurrent to uncinate, subdistant, narrow to broad, up to 6 mm broad, green (oac82, oac112); edge entire or eroded, very pale green (oac67) to pale yellow (oac858); lamellulae of one or two lengths. *Stipe* (20–) 49–104 × 2–7 mm, central, regular to flexuous, tapering towards the base, dry to lubricous, silky, fibrillose, vivid green (oac103) at the apex, then fading to lighter hues of green (oac89, oac68, oac77) base yellow (oac5, oac857) to whitish, hollow.

Basidiospores and *basidia* dimorphic. *Macrospores* 11.1–*13.70*–15.9 (–17.6) × 6.2–*7.89*– 9.8 µm, Q = 1.640–*1.740*–1.839, oblong, smooth, thin-walled, hyaline, inamyloid, guttulate. *Microspores* 4.8–*5.91*–7.7 × 2.8–*3.57*– 4.6 µm, Q = 1.640–*1.655*–1.671, oblong, smooth, thin-walled, hyaline, inamyloid, guttulate. *Macrobasidia* 32.8–55.3 × 10.8–18.4 µm, clavate, thin-walled, hyaline, inamyloid, guttulate, with basal clamp connections, 4-spored, sterigmata up to 9.7 µm. *Microbasidia* 27.7–39.6 × 4.6–7.9 µm, cylindro-clavate, thin-walled, hyaline, inamyloid, guttulate, with basal clamp connections, 4-spored, sterigmata up to 6.5 µm. *Lamellar edge* fertile. *Cheilocystidia* 20.6–89.7 × 3.9–13.0 µm, ventricose-rostrate, mucronate, papillate, oval or ogival, frequently with apical hyphoid projections or with digitiform appendix, thin-walled, hyaline, with basal clamp connections. *Lamellar trama* regular, with parallel inflated hyphae, 9.7–15.0 µm diam., clamp connections present. *Pileipellis* a cutis with a thin layer of uplifted hyphae, 69.9–171.7 × 7.8–9.0 µm, encrusted pigments present but not abundant, clamp connections present. *Stipitipellis* a cutis with clusters of erect interwoven hyphae, terminal hyphae very thin, 2.8–4.9 µm diam., contorted, interlaced, with many clamp connections.

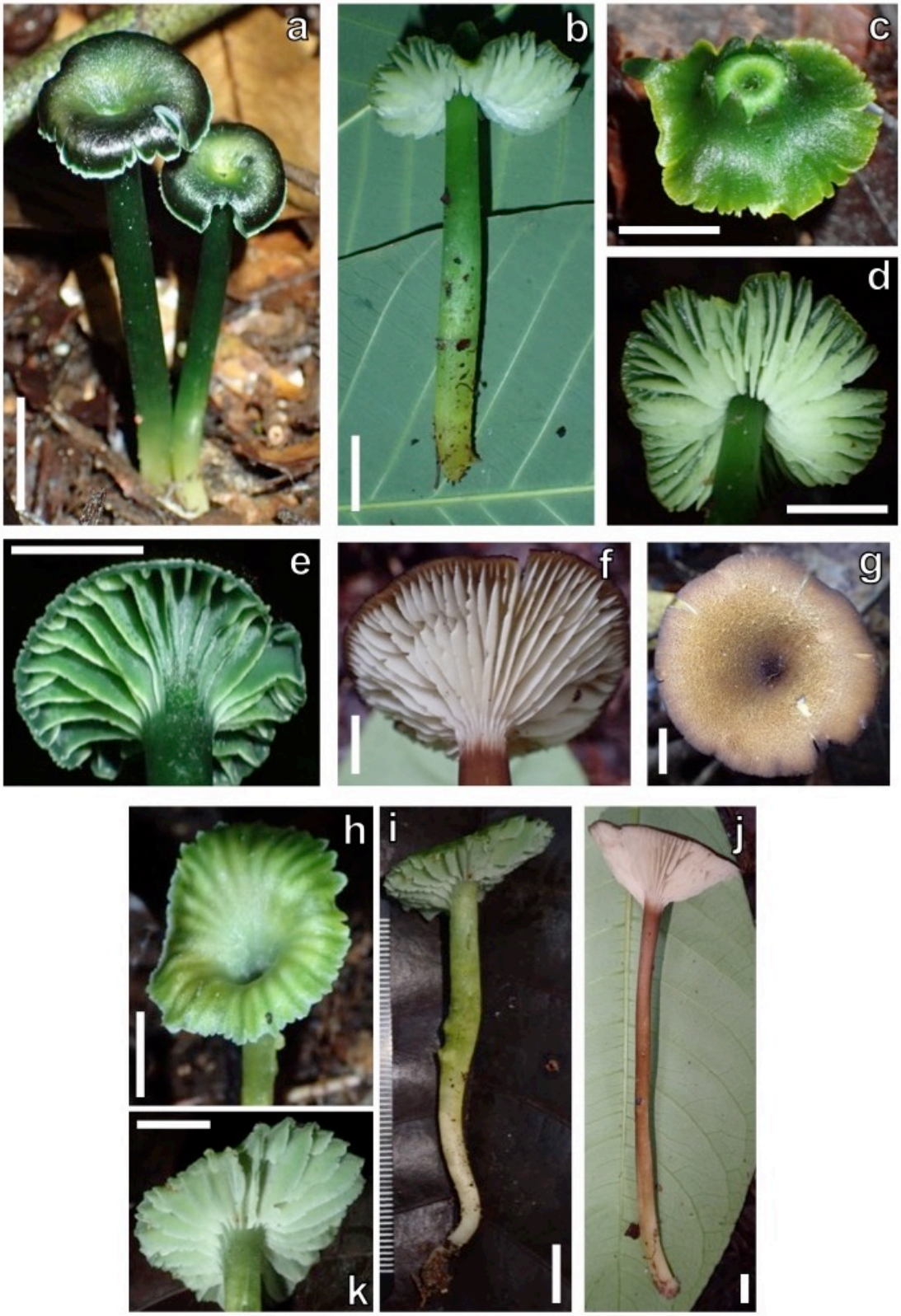

Figure 7. a, e. *Hygrocybe cristalinensis* (JS251). b, c, d. *Hygrocybe viridilacerata* (CJL648). f, g, j. *Hygrocybe pegleri* (JS561). h, i, k. *Hygrocybe sourellae* (JS192). Scales = 10mm. Photos from a, b, c, d, e, h, i, k by S. Sourell.

Specimens examined: BRAZIL, Mato Grosso, Alta Floresta, Reserva Particular do Patrimônio Natural Cristalino, Trilha do Francisco, 4 January 2019, *Santos, C.* CJL647 (FLOR67406); *Santos, C.* CJL652 (FLOR67408); 10 January 2019, *Cardoso J.S. & Furtado A.N.M.* 605 (FLOR67462); Trilha da Serra Nova, 12 January 2018, *Ribeiro, R.S.* CJL554 (FLOR63512); Trilha do Dr. Haffer, 6 January 2019, *Cardoso J.S. & Furtado A.N.M. 556* (FLOR67424); Trilha do Cajá, 7 January 2019, *Cardoso J.S. & Furtado A.N.M.* 570 (FLOR67438); Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Torre 2, 24 January 2018, *Cardoso, J.S.* 249 (FLOR63509); *Cardoso, J.S.* 251 (FLOR63511); Trilha da Castanheira, 5 January 2019, *Cardoso J.S. & Furtado A.N.M. 545* (FLOR67414).
Distribution: Known only from the type locality, in the Southern Amazon.
Habitat: *Terra-firme* forest.

Comments: This is the most common green *Hygrocybe* at RPPN Cristalino. There are three green dimorphic *Hygrocybe* species previously described for the neotropics: *H. chloochlora* Pegler & Fiard from Martinique, *H. prieta* Lodge & Pegler from Puerto Rico and *H. viridis* Capelari & Maziero from Rondônia State, Brazil (Pegler & Fiard, 1978; Lodge & Pegler, 1990; Capelari & Maziero, 1988). The morphology of the basidioma of *H. cristalinensis* is very similar to *H. prieta*, but it differs in the lack of purple and red pigments in the pileus, that is consistently deep dark green in *H. cristalinensis* (Lodge & Pegler, 1990). *Hygrocybe cristalinensis* lamellae are always deep dark green and never purplish, and the stipe is predominantly green rather than yellow-orangish and without waxy squamules, like in *H. prieta* (Lodge & Pegler, 1990). In microscopy *H. cristalinensis* differs from *H. prieta* in the presence of cheilocystidia and by having microspores that are slightly wider (2.5–3.6 µm, Q = l.78–1.95 in *H. prieta*), which are more elongate in *H. prieta* (Lodge & Pegler, 1990). The second most closely related taxon, *H. chloochlora*, differs by having lighter green colours on the pileus, lamellae that are white to very pale green and a stipe that is yellowish green (Pegler & Fiard, 1978). Microscopically, the macrospores of *H. chloochlora* are bigger (17.5 × 10.0 µm) and the cheilocystidia are hyphoid to narrowly clavate or cylindrical rather than predominantly ventricose-rostrate with apical projections as found in *H. cristalinensis* (Lodge & Pegler, 1990). The other green dimorphic species, *H. viridis*, was described for the Brazilian Amazon (Capelari & Maziero, 1988). *Hygrocybe viridis* differs in having an all green basidioma, with no traces of yellow, much smaller macrospores (8.4–11.0 × 4.8–6.0

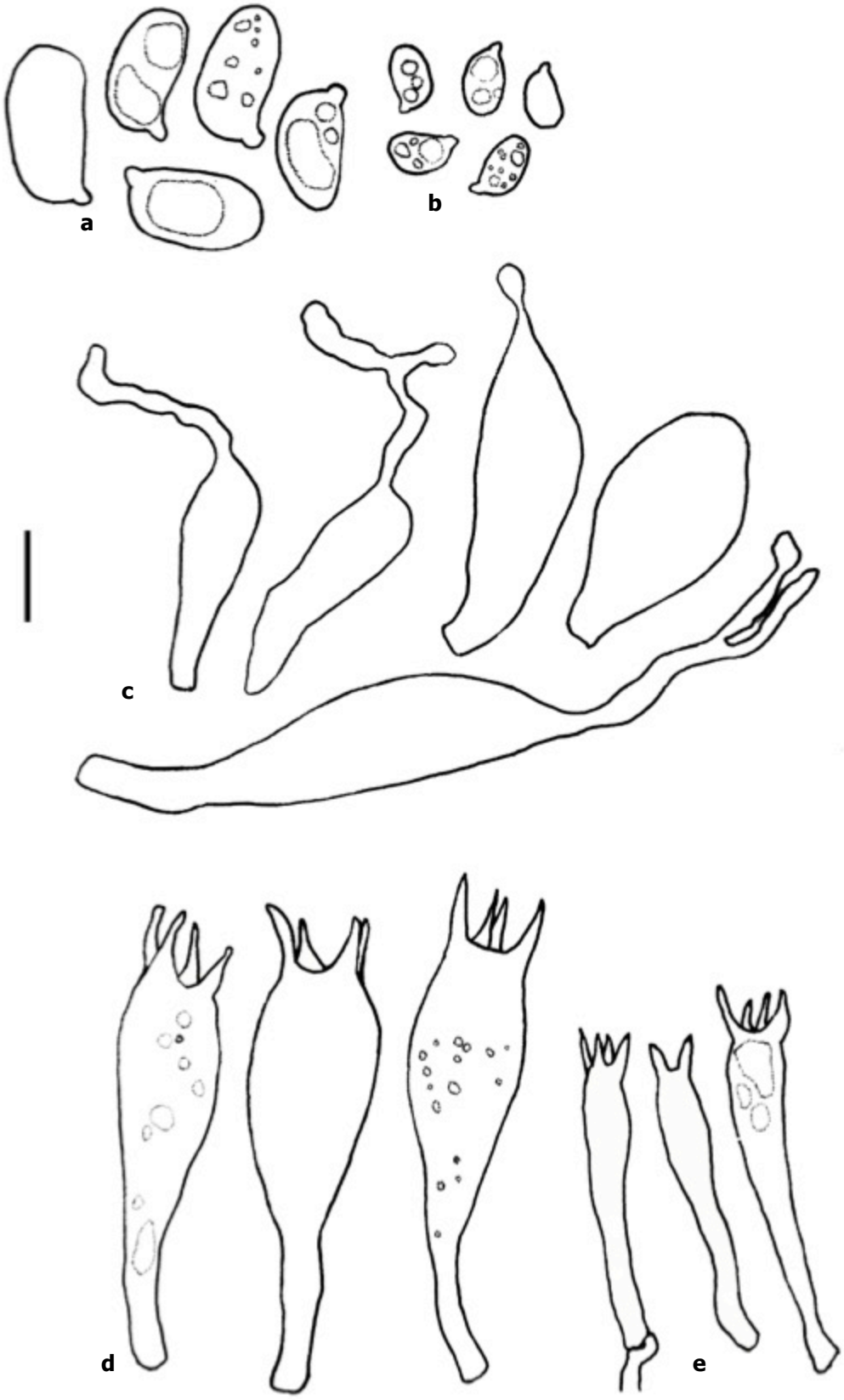

Figure 8. *Hygrocybe cristalinensis* (CJL647). a. macrospores, b. microspores, c. cheilocystidia, d. macrobasidia, e. microbasidia. Scale = 10 µm.

µm), narrower macrobasidia (8.4–9.6 µm) and absence of cheilocystidia (Capelari & Maziero, 1988b). Finally, *H. cristalinensis* differs from the other two green dimorphic species described in this work by the following: *Hygrocybe viridilacerata* has a conic-convex to umbonate pileus with a star shaped perforation in the centre, the pileus is vivid green, never dark green, the lamellae is white or pale green and almost free, has slightly bigger microspores (7.6 × 4.8) and the microbasidia are predominantly 2-spored and lacks cheilocystidia. *Hygrocybe sourellae* has a translucent-striate pileus that is light green, pale green lamellae, an eccentric stipe and lacks cheilocystidia.

In a BLASTn search, the higher score is to a sequence named as "Hygrocybe poena" (GenBank: MN419324.1), an unpublished *Hygrocybe* species from Ecuador, with 80.44% similarity and a query cover of 76%, and secondly to a *Hygrocybe* aff. *prieta* sequence from Belize (GenBank: KF291168.1) with 79.20% similarity and a query cover of 86%. We include *H. cristalinensis* in sect. *Pseudofirmae* based on morphology and preliminary molecular evidences.

Hygrocybe viridilacerata J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 7 b, c, d, 9 and 10)

Etymology: From the Latin "viridis" = green and "lacerata" = referring to the ragged pileus centre.

Holotype: BRAZIL, Mato Grosso, Alta Floresta, Reserva Particular do Patrimônio Natural Cristalino, Trilha do Francisco, 8 January 2018, *Cardoso, J.S. & Furtado A.N.M.* 583 (FLOR67446, holotype!).

Diagnosis: Pileus umbonate and perforated like a star-shaped rupture at the centre, vivid green; lamellae white; stipe green to yellow near the base; microbasidia predominantly 2-spored.

Description:

Pileus 11–37 mm diam., conic to convex when young, umbonate or plane-convex at maturity, ragged like a star-shaped rupture at the centre, smooth, fibrillose, moist to lubricous, translucent-striate towards margin, vivid green (oac110, oac117) to olive green (oac82, oac38) with brownish-green (oac866) to brown (oac719) tints when old; margin straight, decurved or uplifted, transluscent-striate, irregular, plicate to eroded, often splitting, light

green (oac32) to yellow (oac854, oac896). *Lamellae* adnexed or uncinate, sometimes free, subdistant, broad to ventricose, up to 6 mm broad, white to very pale green (oac30, oac67) with greenish tints (oac50, oac51, oac97, oac90); margin eroded, concolour; lamellulae of two or three lengths. *Stipe* 41–83 × 3–6 mm, central, rarely eccentric, regular to flexuous, smooth, dry to moist, green (oac47, oac68, oac83) to pale yellow (oac855, oac856) at the base, hollow. Solitary to gregarious on clay soils.

Basidiospores and *basidia* dimorphic. *Macrospores* (9.7–) 10.6–*13.54*–17.6 (–18.4) × 5.8–*7.64*–9.5 (–10.0) µm, Q = 1.672–*1.771*–1.853, oblong, smooth, thin-walled, hyaline, inamyloid, guttulate. *Microspores* 5.6–*7.65*–9.7 × 3.7–*4.85*– 6.6 µm, Q = 1.533–*1.578*–1.635, ellipsoid to oblong, smooth, thin-walled, hyaline, inamyloid, guttulate. *Macrobasidia* 30.3–47.7 (–51.1) × 10.4–17.7 (–19.4) µm, broadly clavate, thin-walled, hyaline, predominantly 4-spored, few 1–3-spored, sterigmata up to 9 µm, with basal clamp connections. *Microbasidia* (19.7–) 21.9–34.3 (–39.1) × 4.4–9.5 (–10.8) µm, clavate, predominantly 2-spored, or 1–3–4-spored, thin-walled, hyaline, sterigmata up to 8.8 µm, with basal clamp connections. *Lamellar edge* fertile, microbasidia more abundant. *Cheilocystidia* and *pleurocystidia* absent, but *pseudocystidia* sometimes present (Figure 10), as hyphae projecting from the lamellar trama up to 80 µm above the hymenium, cylindrical, tapering towards the apex, with rounded apex. *Lamellar trama* regular, with inflated parallel elements 19.3–54.7 µm diam., with interwoven refractive hyphae 2.1–4.3 µm diam., clamp connections present. *Pileipellis* a cutis of repent hyphae, 6.5–31.4 µm diam., inflated, sometimes with encrusted pigments, refractive hyphae present, but not abundant, clamp connections present. *Stipitipelli*s a cutis, 7.6–19.0 µm diam., inflated hyphae with encrusted pigments, similar to the pileipellis, clamp connections present.

Specimens examined: BRAZIL, Mato Grosso, Alta Floresta, Reserva Particular do Patrimônio Natural Cristalino, Trilha do Francisco, 4 January 2019, *Santos, C.* CJL648 (FLOR67407); *Santos, C.* CJL654 (FLOR67378); 8 January 2018, *Cardoso, J.S. & Furtado A.N.M.* 583 (FLOR67446); Trilha do Dr. Haffer, 25 January 2018, *Cardoso, J.S.* 273 (FLOR63560); 30 December 2018, *Santos, C.* CJL606 (FLOR67388); 6 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 554 (FLOR67422); *Cardoso, J.S. & Furtado, A.N.M.* 555 (FLOR67423); Trilha da Torre 1, 31 December 2018, *Santos, C.* CJL613 (FLOR67392); Trilha do Saleiro Novo, 16 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 538 (FLOR67409).

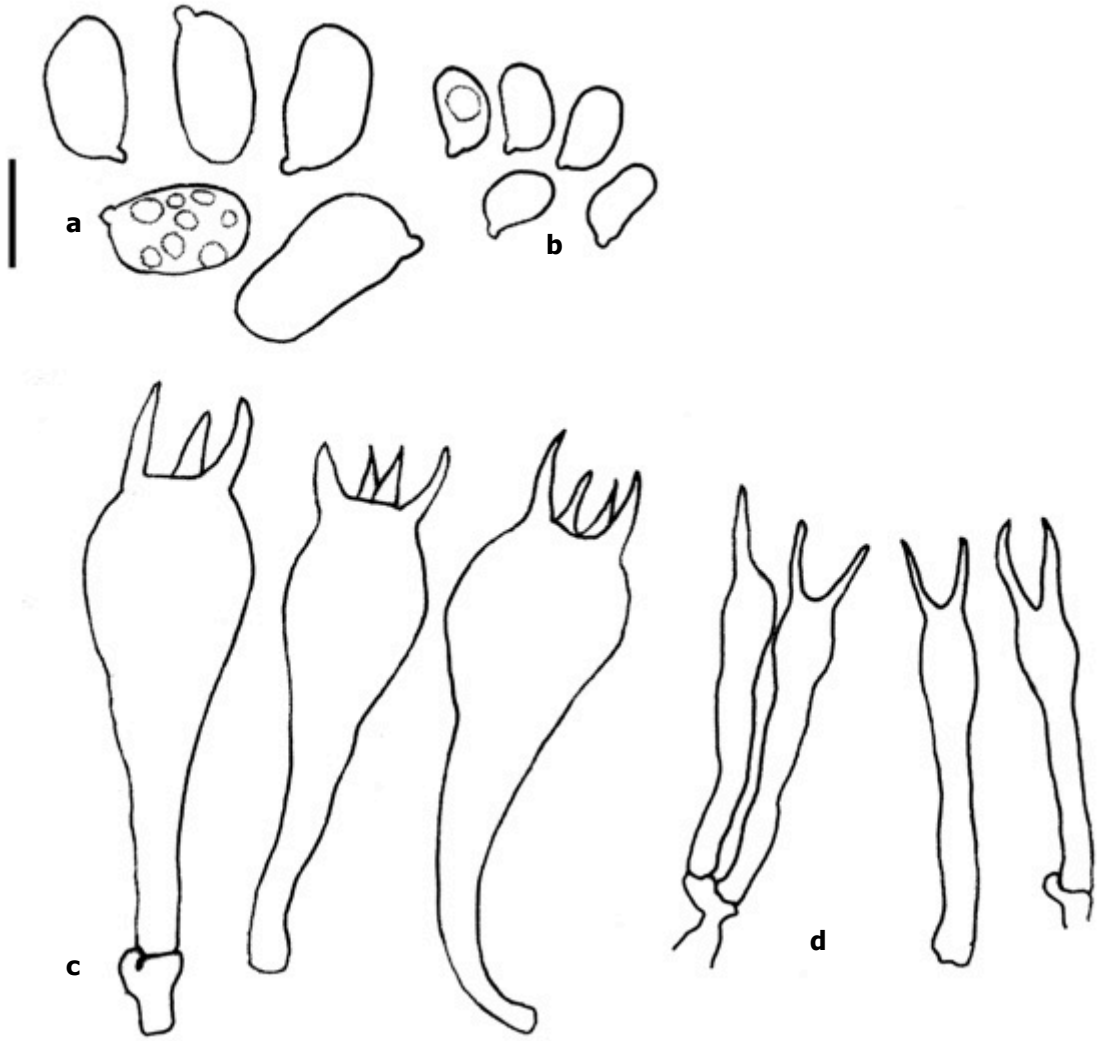

Figure 9. *Hygrocybe viridilacerata* (JS583). a. macrospores, b. microspores, c. macrobasidia, d. microbasidia. Scale = 10µm.

Additional specimen examined: BRAZIL, Santa Catarina, Lages, Coxilha Rica, 28°00'18.3"S 50°19'05.3"W, 916m alt., 28 February 2016, *Santos, B.B.* 001.

Distribution: Known from the Southern Brazilian Amazon in Mato Grosso state and ranges to the Atlantic Rainforest in Santa Catarina.

Habitat: Growing in *terra-firme* forest in the Amazon and in rural areas with remnants of *Araucaria angustifolia* mixed forests in Santa Catarina.

Comments: This species is easily distinguished from the other green dimorphic *Hygrocybe* species due to its pileus shape which is never umbilicate, but somewhat umbonate to plane-convex at maturity and the umbo seems to tear up forming a star shaped rupture in the centre

of the pileus. The lamellae is whitish and almost free, while the other taxa have broadly attached lamellae that are greener. The microscopy does not show many distinct characteristics, expect that the microbasidia are often predominantly 2-spored, a feature not reported for the closely related taxa.

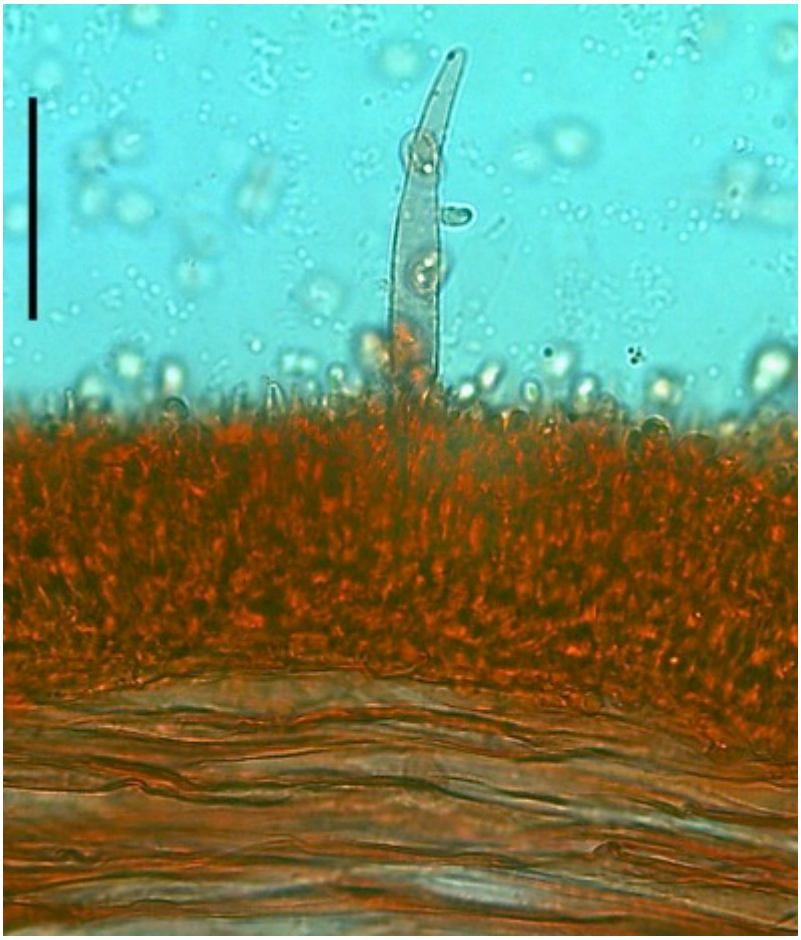

Figure 10. *Hygrocybe viridilacerata* (JS583). Detail of the hymenium showing the pseudocystidia emerging from the lamellar trama. Scale = 50µm.

In a BLASTn search, the highest score is to *Hygrocybe occidentalis* var. *occidentalis* (Dennis) Pegler (GenBank: EU435151.1, from Puerto Rico), with 85.29% similarity and a query cover of 92%. H. *occidentalis* var. *occidentalis* is a dimorphic species that is predominantly yellow with shades of orange and red that belongs to sect. *Pseudofirmae* in which *H. viridilacerata* is also included based on morphological characteristics and preliminary molecular evidences.

Hygrocybe sourellae J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 7 h, i, k and 11)

Etymology: in honour of Susanne Sourell who gave innumerous assistance to the first author, and who created the Cristalino Fungi Project at Cristalino Reserve around the Cristalino River, an important protected Southern Amazon forest area close to the "Arc of deforestation".

Type: BRAZIL, Mato Grosso, Alta Floresta, RPPN Cristalino, Trilha do Francisco, 18 January 2018, *Cardoso, J.S.* 192 (FLOR67427, holotype!).

Diagnosis: Differs from *Hygrocybe chloochlora* Pegler & Fiard in the smooth and glabrous pileus, eccentric fibrillose stipe, smaller macrospores and absence of cystidia.

Description:

Pileus 18–27mm, umbilicate, usually perforated at the centre, smooth, glabrous, translucent-striate, moist to lubricous but not viscid, vivid green (oac873, oac880, oac874, oac881) with shades of brown (oac867, oac838); margin eroded, crenate, slightly sulcate, pale green (oac67). *Lamellae* uncinate to decurrent, distant, 3–6 mm broad, light olive green (oac57, oac59); edge eroded, pale greyish green (oac60); lamellulae of one length. *Stipe* 34–70 × 3–5 mm, eccentric, flexuous, tapering towards the base, fibrillose, silky, dry, olive green (oac70) to yellow (oac855) towards the base, hollow, base whitish. Solitary on clay soils. *Basidiospores* and *basidia* dimorphic. *Macrospores* (9.9–) 10.9–*13.05*–15.3 × 6.5–*7.90*–9.7 µm, Q = 1.629–*1.652*–1.667, oblong, smooth, thin-walled, hyaline, inamyloid, guttulate. *Microspores* 5.1–*6.15*–7.6 × 3.2–*3.91*–4.9 µm, Q = 1.537–*1.572*–1.622, ellipsoid, smooth, thin-walled, hyaline, inamyloid, guttulate. *Macrobasidia* 40.7–61.7 × 9.3–20 µm, clavate, thin-walled, hyaline, inamyloid, guttulate, with basal clamp connections, 4-spored, sterigmata up to 10.6 µm. *Microbasidia* 29.3–42.8 × (4.4–) 5.3–8.5 µm, clavate, thin-walled, hyaline, inamyloid, guttulate, with basal clamp connections, 4-spored, sterigmata up to 7.2 µm. *Lamellar edge* fertile. *Cystidia* absent. *Lamellar trama* regular, with parallel inflated hyphae, 67.6–210.1 × 4.7–32.1 µm diam., some with encrusted pigments, refractive hyphae 2–3 µm diam., not abundant, clamp connections present. *Pileipellis* a cutis of parallel, undifferentiated hyphae 75.5–231.2 × 5.4–24.8 µm, refractive hyphae present, 2–4.9 µm diam., interwoven, with many branching, clamp connections present. *Stipitipellis* a cutis of parallel,

undifferentiated hyphae, 36.4–117.4 × 4.6–7.1 µm, with clusters of interwoven hyphae, clamp connections present.

Specimens examined: BRAZIL, Mato Grosso, Alta Floresta, Reserva Particular do Patrimônio Natural Cristalino, Trilha do Francisco, 18 January 2018, *Cardoso, J.S.* 192 (FLOR63533), Trilha da Torre 1, 31 December 2018, *Santos, C.* CJL612 (FLOR67391), Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Torre 2, 5 January 2019, *Cardoso J.S. & Furtado A.N.M.* 550 (FLOR67418).

Distribution: Known only from the type locality, in the Southern Amazon.

Figure 11. *Hygrocybe sourellae* (JS192). a. macrospores, b. microspores, c. macrobasidia, d. microbasidia. Scale = 10µm.

Habitat: On *terra-firme* forest in the Amazon.

Comments: This is the less frequent green dimorphic *Hygrocybe* at RPPN Cristalino, with only three collections along the three field expeditions of the Cristalino Fungi Project. It is very closely related to *H. chloochlora* based on the macromorphology, but differs in having a smooth and glabrous pileus rather than radially fibrillose and rimose, an eccentric rather than equal stipe, which lacks any waxy furfuraceous squamules and a white basal mycelium (Lodge & Pegler, 1990). Furthermore, in the original description and colour photographs of *H. chloochlora*, the pileus and stipe are more yellowish (Pegler & Fiard, 1978). In the microscopy *H. sourellae* differs in having smaller macrospores (up to 25 µm length in *H. chloochlora*), and by having a fertile lamellar edge with no cystidia (Lodge & Pegler, 1990).

Hygrocybe pegleri J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 7 f, g, j and 12)

Etymology: in honour of David N. Pegler, who described and studied many neotropical *Hygrocybe* species.

Type: BRAZIL, Mato Grosso, Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 6 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 561 (FLOR67428, holotype).

Diagnosis: Differs from *Hygrocybe firma* var. *firma* (Berk. & Broome) Singer by having a smaller pileus, taller stipe, with no colour changes on the basidiomata, fertile lamellar edge and branched hyphae with brown pigments in the pileipellis.

Description:

Pileus 32–47 mm diam., umbilicate, depressed, squamulose, squamules more abundant in the centre and margin, moist to dry, pale yellow (oac899, oac857) to pale orange (oac812, oac810) with brown (oac733, oac734) squamules; margin arched or straight, entire, sometimes splitting, concolorous to pileus, but with an aspect of being darker due to the concentrated squamules. *Lamellae* decurrent to uncinate, ventricose, up to 10 mm broad, subdistant, pale yellow (oac855) to almost white (oac899), margin eroded, concolorous; lamellulae of 3 lengths. *Stipe* 102–155 × 4–8 mm, central, sometimes eccentric, equal, usually with longitudinal fissure, tomentose, dry, yellow (oac855, oac857), covered with brown (oac734) microsquamules, hollow. Solitary on clay soils.

Basidiospores and *basidia* dimorphic. *Macrospores* 12.4–*15.06*–20.0 × 7.5–*9.67*–10.9 (–12.0) µm, Q = 1.487–*1.597*–1.775, ellipsoid to oblong, smooth, thin-walled, hyaline, inamyloid, multiguttulate. *Microspores* 5.7–*7.14*–9(–9.5) × 3.4–*4.40*–5.0(–5.5) µm, Q = 1.533–*1.622*–1.735, ellipsoid to oblong, smooth, thin-walled, hyaline, inamyloid, multiguttulate. *Macrobasidia* 49.3–70.2 (–82.0) × 11.7–18.9 (–25.0) µm, ventricose-clavate, thin-walled, hyaline, 4-spored, sterigmata up to 14.2 µm, with basal clamp connections. *Microbasidia* 30.8–51.0(–55.0) × 5.6–8.6(–10.0) µm, clavate, thin-walled, hyaline, 4-spored, sterigmata up to 8.6 µm, with basal clamp connections. *Lamellar edge* fertile. *Cystidia* absent. *Lamellar trama* regular, with parallel inflated hyphae, 76.8–282.8 × 12.8–30.7 µm, refractive hyphae present, interwoven, clamp connections present. *Pileipellis* a trichoderm with clusters of brown pigmented terminal hyphae (14–)22–67.7(–81) × 7–17.9(–23) µm, branched, forming a pyramidal structure, clamp connections present. *Stipitipellis* a trichoderm with clusters of interwoven brown hyphae, 14.5–49.4 × 3.3–9.3 µm, clamp connections present.

Specimens examined: BRAZIL, Mato Grosso, Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 6 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 561 (FLOR67428, holotype!); Trilha da Torre 2, 5 January 2019, *Cardoso J.S. & Furtado A.N.M.* 551 (FLOR67419); Alta Floresta, Reserva Particular do Patrimônio Natural Cristalino, Trilha do Francisco, January 2018, *Cardoso, J.S.* 268 (FLOR63558).

Additional specimens examined: BRAZIL, Santa Catarina, Parque Estadual da Serra do Tabuleiro, Santo Amaro da Imperatriz, Plaza Caldas da Imperatriz, Trilha da Cachoeira, 27°44'24.7"S 48°48'25.0"W, 10 April 2014, *Magnago, A.C. & Cardoso, J.S.* 1029 (FLOR 57243).

Distribution: Southern Amazon in Mato Grosso state and Atlantic Rainforest in Santa Catarina state.

Habitat: Growing on soils amongst litter in *terra-firme* forest in the Amazon and on hilly areas of well-preserved remnants of Atlantic Rainforest.

Comments: This taxon is similar to *Hygrocybe firma* var. *firma* (Berk. & Broome) Singer in Pegler (1983) based on collections from the Antilles, but it differs significantly in the colour photographs and illustrations provided in previous publications (e.g. Heinemann, 1966; Pegler & Fiard, 1978; Pegler, 1983). In the macromorphology, *H. firma* var. *firma* differs by having a wider pileus [(20–)40–70 mm vs. 32–47 mm diam. in *H. pegleri*], a shorter stipe (up to 110 mm vs. 155 mm height in *H. pegleri*), and colour changes on bruising or with time.

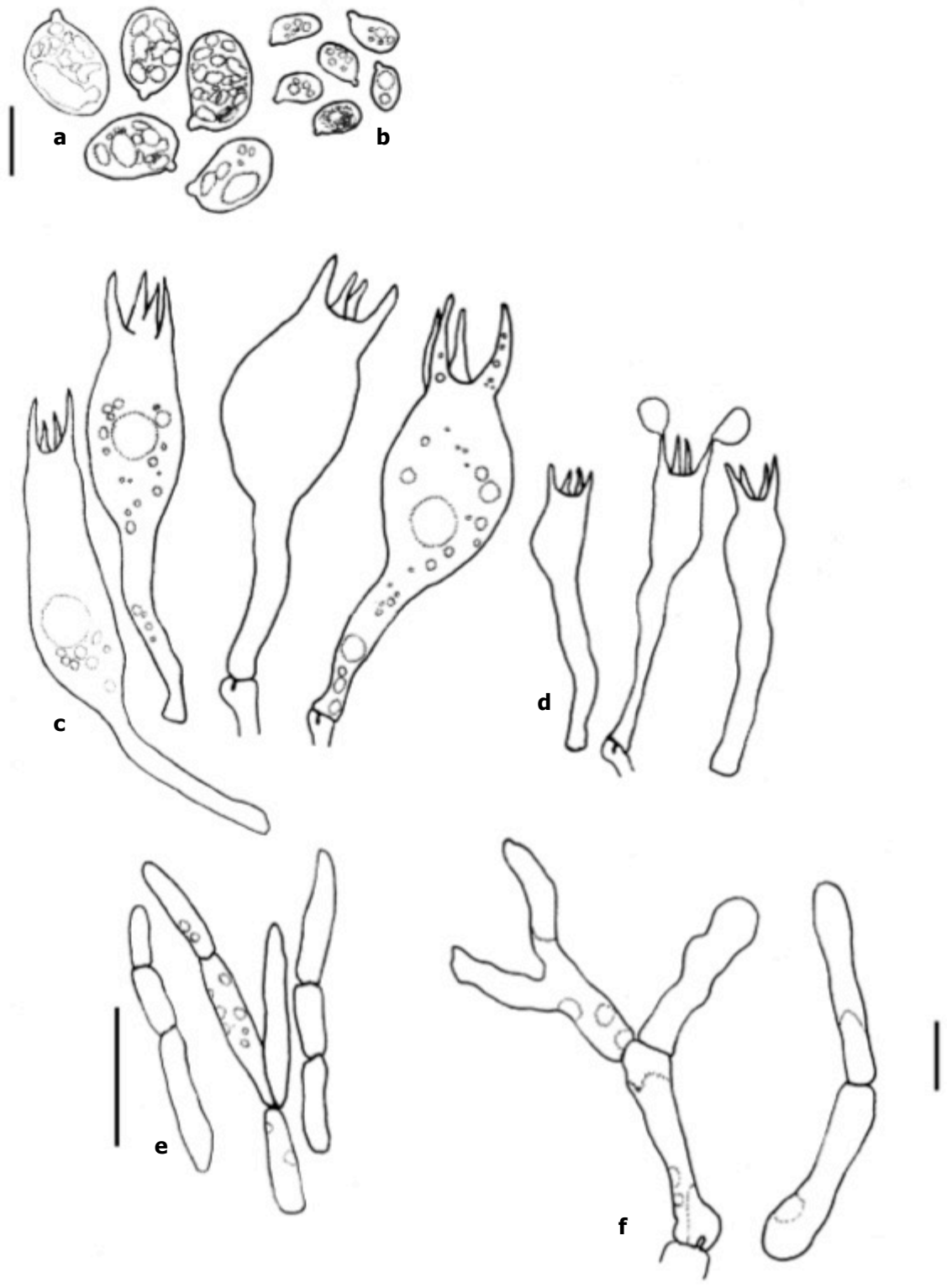

Figure 12. *Hygrocybe pegleri* (JS561). a. macrospores, b. microspores, c. macrobasidia, d. microbasidia, e. pilepellis terminal hyphae, f. stipitipellis hyphae. a, b, c, d: scale = 10µm; e, f: scale = 50µm.

Microscopically, *H. firma* var. *firma* differs by having a sterile lamellar edge with basidioles, smaller macrobasidia [50–60 × 14–16 µm vs. 49.3–70.2 (–82.0) × 11.7–18.9 (–

25.0) µm in *H. pegleri*] and unbranched hyphae with no pigments in the pileipellis (Pegler, 1983). Other closely related taxa with squamulose pileus and dimorphic basidia and basidiospores are *Hygrocybe brunneosquamosa* Lodge & S.A. Cantrell and *Hygrocybe neofirma* Lodge & S.A. Cantrell (Cantrell & Lodge, 2001). *Hygrocybe brunneosquamosa* also has brown pigmented hyphae in the trichodermal pileipellis, but has an entire brown pileus that lacks yellow or orange pigments, the lamellae is cinnamon brown, and the macrospores are oblong to cylindrical (Cantrell & Lodge, 2001). *Hygrocybe neofirma* differs from *H. pegleri* by the presence of staining reactions, a much shorter stipe, and by the absence of brown pigmented pileipellis hyphae (Cantrell & Lodge, 2001).

In a BLASTn search, *H. pegleri* has 82.23% in similarity to the type material of *Hygrocybe sangayensis* A. Barili, C.W. Barnes, J.A. Flores & Ordoñez (GenBank: NR_166798.1), a dimorphic species described from Ecuador (Crous *et al*., 2017), and 81.18% to a sequence of *H. occidentalis* var. *occidentalis* from Puerto Rico, which can demonstrate the proximity of these taxa that are all placed in sect. *Pseudofirmae*.

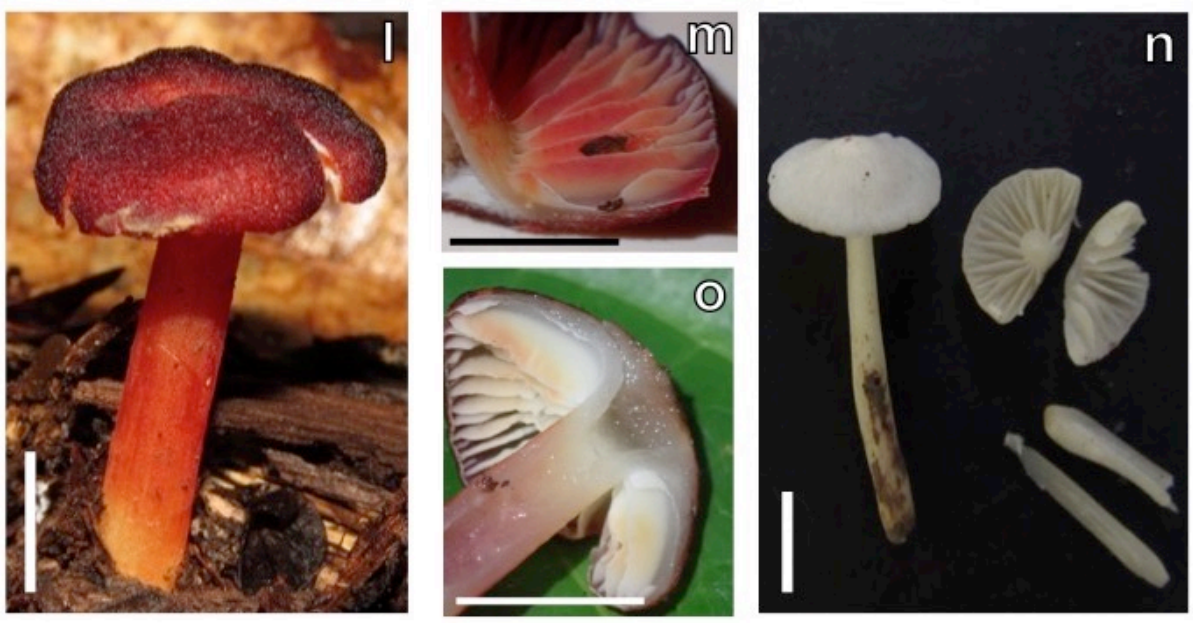

Figure 13. l, m, o. *Hygrocybe vinaceosquamulosa* (JS186). n. *Hygrocybe hololeuca* (JS342). Scales = 10mm. Photo from item l by R.C. Hoyer.

Hygrocybe vinaceosquamulosa J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 13 l, m, o and 14)

Etymology: From Latin “vinaceus” = purple and “squamulosus” = referring to the squamules on the pileus.

Type: BRAZIL, Mato Grosso, Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 6 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 543 (FLOR67412, holotype).

Diagnosis: Differs from *Hygrocybe siparia* (Berk.) Singer in having a convex pileus, presence of pink shades in the lamellae, a fibrillose stipe with pink staining reaction at the base and oblong to cylindrical macrospores with a middle constriction.

Description:

Pileus 19–38 mm diam., convex, tomentose-squamulose, moist to dry, dark cherry red (oac563, oac600, oac601, oac607), crimson (oac579, oac586), pink (oac617), sometimes with flushes of orange (oac632) covered with dark purple small squamules; margin entire, decurved, slightly incurved, concolourous or paler. *Context* translucent to white, gelatinous. *Lamellae* adnate to slightly sinuate, up to 4 mm broad, pale yellow (oac857, oac898) to orangish (oac812) or beige (oac766, oac 814) with shades of dark pink (oac594), or stained with orangish pink (oac615) towards the edge; edge entire to finely fimbriate, sometimes eroded, concolourous; lamellulae of two lengths. *Stipe* 28–55 × 4–6 mm central, regular, fibrillose, moist to dry, yellowish orange (oac789, oac812) wish shades of cherry red (oac614), or pink (oac572) stained with pale orange (oac632), base pale yellow (oac858), sometimes with dark pink (oac558) stain reaction, hollow.

Basidiospores and *basidia* dimorphic. *Macrospores* (11.4–) 12.2–*14.75*–19 (–20.7) × 5.5–*7.38*–8.7 µm, Q = 1.728–*2.014*–2.300, oblong to cylindrical, often constricted in the middle, smooth, thin-walled, hyaline, inamyloid, multiguttulate. *Microspores* 6.0–*7.52*–9.5 (–10.9) × 3.2–*4.26*–5.1 µm, Q = 1.622–*1.762*–1.902, oblong, often constricted in the middle, smooth, thin-walled, hyaline, inamyloid, multiguttulate. *Macrobasidia* 41.7–61.2 × 11.2–16.2 (–17.1) µm, clavate, thin-walled, hyaline, predominantly 4-spored, or 1–2–3-spored also common, sterigmata up to 13.5 µm, with basal clamp connections. *Microbasidia* 29.0–44.3 (–54.3) × 6.0–8.3 (–10.2) µm, cylindro-clavate, thin-walled, hyaline, predominantly 4-spored, 1–2–3-spored also common, 5-spored rare, sterigmata up to 10.0 µm, with basal clamp connections. *Lamellar edge* fertile. *Cystidia* absent. *Lamellar trama* regular to subregular, inflated hyphae with pigments, 4.9–22.1 µm diam., clamp connections present, interlaced with refractive hyphae, 4.2–5.3 µm diam., with clamp connections of the medallion type. *Pileipellis* a

trichoderm, with pigmented hyphae forming chains arranged in pyramidal form, therefore creating the squamules, terminal elements 15.9–116.0 × 6.6–12.3 µm, branching, with clamp connections, brown in KOH. *Pileus trama* with abundant interwoven refractive hyphae. *Stipitipellis* a trichoderm of thin hyphae 2.6–7.1 µm diam., slightly brownish in KOH.

Specimens examined: BRAZIL, Mato Grosso, Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 6 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 543 (FLOR67412); 17 January 2018, *Cardoso, J.S.* 186 (FLOR63504); 7 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 566 (FLOR67434); 1 January 2019, *Santos, C.* CJL624 (FLOR67398).

Distribution: Only known from the type locality.

Habitat: Growing solitary or gregarious on clay soils in *terra-firme* forest.

Comments: This species is very remarkable due to its cherry colours and dark purple squamules in the pileus. It resembles *Hygrocybe siparia* (Berk.) Singer, which was first described by Berkeley (1856, p. 134) based on a collection from the Upper Rio Negro region, in the Uaupés River in Amazonas state. It was later recollected by Singer (1965) in Pernambuco state and also re-examined by Pegler (1997) from collections found in São Paulo state. *Hygrocybe siparia* differs in the pileus shape, which is umbilicate, lack of pink shades in the lamellae and a stipe that is smooth rather than fibrillose with no pink staining reaction at the base. In the microscopy, *H. siparia* has ellipsoid macrospores, which are smaller in length (up to 13 µm, Q = 1.53) and the microspores are also smaller in length which are also ellipsoid (up to 6.7 µm, Q = 1.42).

Hygrocybe macrosiparia A. Barili, C.W. Barnes, J.A. Flores & Ordoñez is another morphologically similar species that was recently described from Ecuador (Crous *et al*. 2017), but it differs in the lack of pink or purple colours in the basidiomata, macrospores sizes (10.5 × 6.5 µm), and the much shorter sterigmata in the basidia (up to 6.5 µm vs. in macrobasidia). Unfortunately, the authors did not provide Q values for the basidiospores and the pileipellis description is not very detailed. *Hygrocybe vinaceosquamulosa* is included in sect. *Pseudofirmae* based on morphological characters.

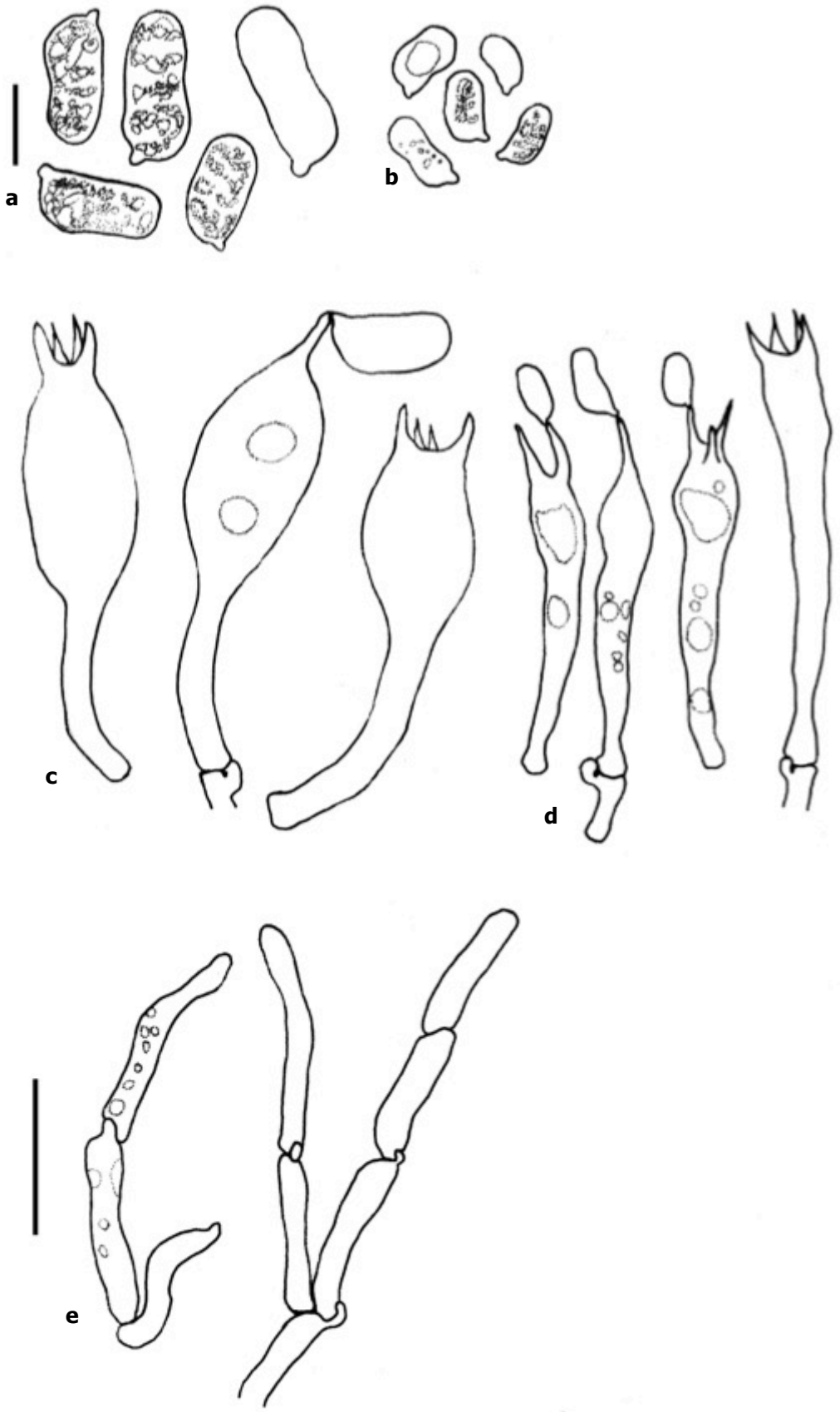

Figure 14. *Hygrocybe vinaceosquamulosa* (JS543). a. macrospores, b. microspores, c. macrobasidia, d. microbasidia, e. pilepiellis terminal hyphae. a, b, c, d: scale = 10µm; e: scale = 50µm.

Hygrocybe hololeuca Singer, *Fieldiana, Bot*. 21: 4 (1989) (Figures 13 o and 15)

Pileus 6–13 mm diam., conic-convex, convex or convex-umbonate, smooth to finely fibrillose, transluscent-striate, silky, dry to moist, white (oac900, oac899); margin entire or slightly fimbriate. *Lamellae* adnexed to free, or sinuate, narrow, up to 2 mm broad, white, with lamellulae of 2 lengths. *Stipe* 16–32 × 1–2 mm, central, smooth, silky, dry, white, fistulose. Solitary or in groups, on clay soils.

Basidiospores 4.4–*5.41*–6.8 × 4.0–*4.86*–6.0 µm, Q = 1.103–*1.113*–1.119, subglobose, smooth, hyaline, inamyloid, thin-walled, guttulate. *Basidia* (22.2–) 24.4–38.6 (–46.6) × 5–11.3 (–14.1), clavate, thin-walled, 4-spored, sterigmata up to 8 µm, with basal clamp connections. *Lamellar trama* regular, of parallel hyphae 4–21 µm diam., terminal hyphae with rounded apices and tapering ends, up to 212.7 µm long, sometimes with oblique septa, refractive hyphae 2.8–5.8 µm diam., more abundant near the hymenium, clamp connections present. *Lamellar edge* fertile. *Cystidia* absent. *Pileipellis* a cutis of parallel hyphae, 3–13.2 µm diam., slightly rugulose, with oblique septa and medallion clamp connections. *Stipitipellis* a cutis of parallel hyphae, 1.8–5.6 µm diam., with encrusted pigments and medallion clamp connections.

Specimens examined: BRAZIL, Amazonas, Manaus, Reserva Florestal Adolpho Ducke, 4 March 2018, *Cardoso, J.S.* 309 (INPA285592); Museu da Amazônia (MUSA), 15 May 2018, *Cardoso, J.S. & Oliveira, J.J.S.* 344; *Oliveira, J.J.S. & Cardoso, J.S.* 1032; São Sebastião do Uatumã, Estação Científica do Uatumã, 2°08'32"S, 59°00'05"W, 27 Feb. 2019, *Komura, D.L.* 2441 (INPA285149); Pará, Belterra, Floresta Nacional do Tapajós, Km 17, Grid M1, 9 April 2019, *Cardoso, J.S. & Oliveira, J.J.S.* 686 (INPA285665).

Distribution: Known from Central Amazon in Amazonas state and for Eastern Amazon in Pará state.

Habitat: On *terra-firme* forest soil, amongst litter.

Comments: This is the first record of *H. hololeuca* for Pará state. It was previously known only to the Manaus region in Amazonas state. This taxon is probably better placed on subgen. *Hygrocybe* due to its long parallel hymenophoral trama hyphae, that exceeds 140 µm long with tapered ends and oblique septa, and a basidia/basidiospore length of about 5.5. Some of the basidia examined were much bigger than the regular ones (Figure 15.c), indicating it could be placed in sect. *Pseudofirmae*, with only a vague dimorphism of spores and basidia (Lodge

pers. comm.), but more studies are needed to confirm its sectional placement, including molecular analysis. *Hygrocybe hololeuca* remains in uncertain placement in subg. *Hygrocybe* until further analysis.

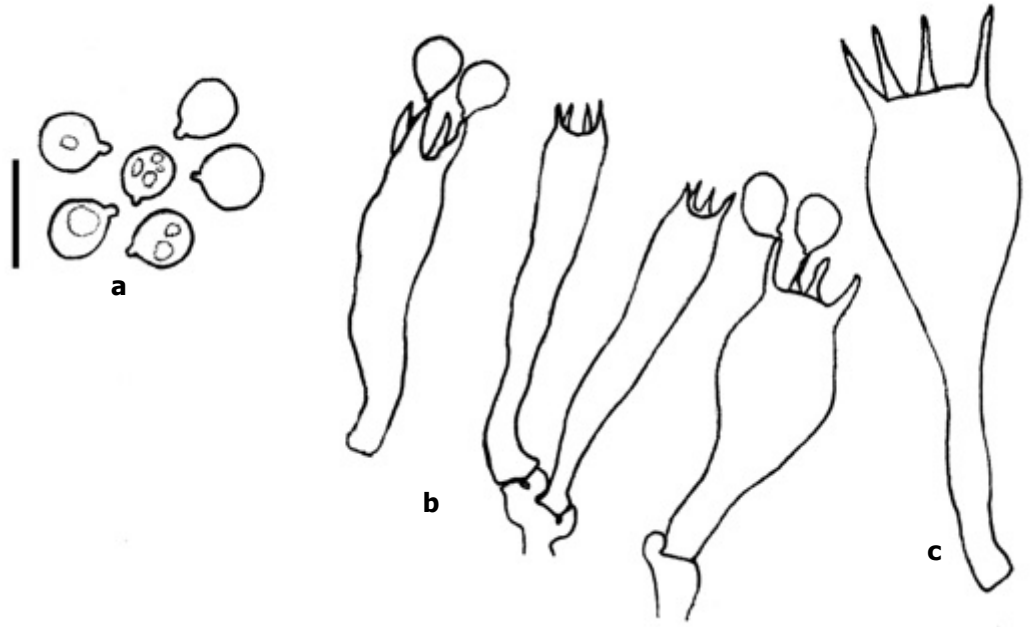

Figure 15. *Hygrocybe hololeuca* (JS309). a. basidiospores, b. basidia, c. potencial macrobasidia. Scale = 10µm.

Identification Key to Sections of Hygrocybe *subg.* Hygrocybe

1. Lamellae broadly attached, often decurrent, lamellar trama subregular with short elements, not exceeding 140 µm long, basidia long, cylindro-clavate with a mean ratio of basidiospore to basidia length usually bigger than 5…….....Subg. *Pseudohygrocybe*

1. Lamellae free, adnexed or with a decurrent tooth, lamellar trama regular, with long parallel hyphae with tapered ends, basidia clavate, mean ratio of basidiospores to basidia length 3 to 5…….Subg. *Hygrocybe*, 2

2. Basidiospores and basidia monomorphic……3

2. Basidiospores and basidia dimorphic……..5

3. Basidiomata stain black or slightly grey in the stipe……..Sect. *Hygrocybe*

3. Basidiomata without colour reactions……4

4. Pileus conical or conico-campanulate, dry, tomentose, squamulose or fibrillose, basidiospores mostly less than 10 µm long, pileipellis a trichoderm……...Sect. *Microsporae*

4. Pileus convex-applanate, sometimes umbonate, viscid to glutinous, smooth, basidiospores can reach more than 10 µm long, pileipellis an ixotrichoderm……Sect. *Chlorophanae*

5. Basidiomata glutinous, red or pink, pileus not perforated in the centre, usually with a glutinous annulus in the stipe, lamellar edge with pseudocystidia, basidiospores globose, subglobose to broadly ellipsoid, macrobasidia clavate……...Sect. *Velosae*

5. Basidiomata not glutinous, but pileus can be viscid to glutinous, usually red, orange, yellow or green, pileus often perforated in the centre, without annulus in the stipe, pseudocystidia sometimes present, basidiospores usually ellipsoid, oblong or cylindrical, macrobasidia broadly clavate or clavate-stipitate……Sect. *Pseudofirmae*

Identification Key for Hygrocybe *subg.* Hygrocybe *sect.* Pseudofirmae *in Brazil*

1. Basidiomes predominantly green or with significant shades of green in pileus, lamellae and stipe……2

1. Basidiomes predominantly red, orange, yellow, pink or purple……....6

2. Pileus convex to umbilicate, perforated in the centre, entirely green or with shades of green in pileus, lamellae or stipe……3

2. Pileus conic-convex, umbonate, ragged in the centre, like a rupture, vivid green ……*H. viridilacerata*

3. Basidiomata with shades of yellow, red, orange and/or purple……..4

3. Basidiomata entirely green, without shades of yellow ……*H. viridis*

4. Pileus conspicuously green, with or without fringed margin, lamellae green without shades or purple, stipe mostly green, without squamules……...5

4. Pileus a mixture of crimson, dark red and green, with a white fringe, lamellae green turning purplish, stipe green to orange-yellow, turning purplish, with furfuraceous waxy squamules……...*H. prieta*

5. Pileus deep dark green, with a pale green fringed margin, lamellae deep green, stipe central, equal, cheilocystidia 20.6–89.7 × 3.9–13.0 µm..……...*H. cristalinensis*

5. Pileus vivid green, margin eroded, lamellae greyish green, stipe often eccentric, tapering towards the base, cheilocystidia absent……*H. sourellae*

6. Pileus glabrous or finely fibrillose……..7

6. Pileus tomentose to squamulose……...13

7. Basidiomata predominantly yellow……8

7. Basidiomata predominantly red, orange or violaceous……...9

8. Basidiomata entirely yellow, pileus convex, glabrous, slightly depressed in centre, moist to vaguely viscid, macrospores 16.5–22 × 11–14 µm, cheilocystidia 100 × 7–12 µm……...*H. megistospora*

8. Basidiomata predominantly yellow, pileus convex-applanate, finely fibrillose, translucent-striate, perforated at the centre, dry to moist, macrospores (9–)11–15 × 6.3–10(–12) µm, cheilocystidia 37–60 × 5–20 µm……*H. occidentalis* var. *occidentalis*

9. Basidiomata entirely red……...10

9. Basidiomata orange or violaceous carmine……..12

10. Pileus ≤10 mm diam., convex-umbilicate with a yellow margin, macrospores 10–13 × 6–7.5 µm……*H. trinitensis*

10. Pileus >10 mm diam, pileus convex-depressed, macrospores usually bigger in width.……11

11. Pileus translucent-striate, finely fibrillose, perforated at the centre, macrospores (9–)11–15 × 6.3–10(–12) µm, cheilocystidia 37–60 × 5–20 µm, basidiomata growing in soil……*H. occidentalis* var. *scarletina*

11. Pileus glabrous, smooth, not perforated, macrospores 11.5–15 × 7.3–11.5 µm, cheilocystidia 19 × 3.5 µm, basidiomata found on well-decayed pieces of wood in white sand forests……*H. campinaranae*

12. Pileus 9–10 mm diam., orange, margin yellow, lamellae orange with yellow margin, stipe orange, macrospores 16.5–18.5 × 10–12 µm……...*H. amazoniensis*

12. Pileus 10–15 mm diam., violaceus carmine, lamellae pinkish vinaceous, stipe yellow with pinkish vinaceous apex, macrospores 10–14 × 6.5–8 µm……...*H. naranjana*

13. Pileus yellow to orange with brown to black squamules……14

13. Pileus red to crimson or violaceous, tomentose to squamulose……..15

14. Pileus not perforated, yellow with brown small squamules, basidiomata without stain reactions, stipe 102–155 mm height, tomentose, macrospores 12.4–20 × 7.5–10.9(–12) µm, pileipellis a trichoderm with brown pigmented hyphae……*H. pegleri*

14. Pileus with a star-shaped perforation in the centre, yellow-orange with blackish-brown staining squamules, lamellae and stipe with purple-brown stain reaction, stipe up to 22 mm height, fibrillose, macrospores 12.8–17.6 × 8–10.4 µm, pileipellis a trichoderm without pigmented hyphae……. *H. neofirma*

15. Pileus convex-umbilicate, red to crimson, tomentose to furfuraceous, lamellae pale yellow, stipe glabrous, macrospores 10–13 × 6–8.5 µm, pileipellis a disrupted trichoderm without pigmented hyphae……*H. siparia*

15. Pileus convex, crimson with dark purple squamules, lamellae pale yellow with shades of pink, stipe fibrillose, macrospores (11.4–)12.2–19(–20.7) × 5.5–8.7 µm, pileipellis a trichoderm with brown pigmented hyphae……...*H. vinaceosquamulosa*

Capítulo 2

New species and new records of *Hygrocybe* subgenus *Pseudohygrocybe* (Hygrophoraceae, Agaricales) from the Brazilian Amazon

Abstract

Hygrocybe subg. *Pseudohygrocybe* accommodates species with a rather subregular lamellar trama with short elements not exceeding 140 µm long and long basidia, with a mean ratio of basidiospore to basidia length usually bigger than five times. Subgenus *Pseudohygrocybe* is divided into two sections: *Coccineae*, to include species with monomorphic basidiospores and basidia and *Firmae* to include species with dimorphic basidiospores and basidia. In this study, collections of fresh material were performed in four different localities of Brazilian Amazon forest, with sequencing of the ITS region. Three new species are proposed in sect. *Coccineae* and one new record in sect. *Firmae* is confirmed for the Amazon. When possible, the new species were further supported by comparison of the ITS barcodes via BLAST searches in the GenBank database.

Key words: Taxonomy, ITS, barcode, fungi, Tapajós, Viruá, Cristalino.

Introduction

Hygrocybe subgenus *Pseudohygrocybe* Bon was first proposed to accommodate species with subregular lamellar trama of short hyphal elements and this concept was used by different authors (e.g. Young, 2005 and Boertmann, 2010). Later, Lodge *et al*. (2013) proposed a more comprehensive diagnosis for subg. *Pseudohygrocybe*, adding that the lamellar trama hyphae segments would not exceed 140 µm long, with right-angle septa, with basidia that are mostly cylindro-clavate in shape, and with a mean of basidiospore/basidia length usually bigger than 5 times (Lodge *et al.*, 2013). Basidia and basidiospores in subg. *Pseudohygrocybe* are mono- or dimorphic and spinose basidiospores can rarely occur (Lodge *et al.*, 2013). Basidiomata are typically brightly coloured with no bruising reactions (Lodge *et al*., 2013).

There are currently two sections in *Hygrocybe* subg. *Pseudohygrocybe*: sect. *Coccineae* Fayod, accommodating the taxa with monomorphic basidiospores and basidia; and sect. *Firmae* Heinem., to accommodate tropical species with dimorphic basidiospores and basidia (Lodge *et al.*, 2013). Section *Coccineae* has three valid subsections: Subsect. *Coccineae* (Bataille) Singer, with brightly coloured basidiomata that are usually lubricous or viscid, having a gelatinous layer in the pileipellis; subsect. *Siccae* Boertm., with dry to slightly lubricous basidiomata, smooth pileus and stipe, that occasionally produce characteristic odours; and subsect. *Squamulosae* (Bataille) Singer with often depressed to infundibuliform pileus which is dry and squamulose, or tomentose, stipe dry and smooth, the lamellae often arcuate-decurrent and a trichodermal pileipellis (Lodge *et al*., 2013).

No species in subg. *Pseudohygrocybe* were described for the Brazilian Amazon since Singer (1989) described *H. mutabilis* Singer. Here three new species of *Hygrocybe* subg. *Pseudohygrocybe* sect. *Coccineae* are described and one new record of sect. *Firmae* is documented for the Brazilian Amazon. The new species proposal and identification were based on morphology and molecular data, using the ITS region as DNA barcode.

Material and Methods

Study area

The specimens were collected in four localities: Tapajós National Forest, Viruá National Park, Cristalino Private Reserve of National Heritage (RPPN) and *Museu da Amazônia* (Amazon Museum – MUSA). The Tapajós National Forest is a federal conservation area with sustainable exploitation activities and research stations, located mostly in Belterra and adjacent municipalities in Pará state, along the Tapajós River (3°25'08.5"S, 55°04'18.2"W). The area consists mainly of *terra-firme* forest with periodically flooded areas (Pires & Prance, 1985). The soils are mostly clayish, but near the Tapajós River margin the soils are a mix of clay and sand, and sometimes it is predominantly sandy (ICMBio, 2020a). Viruá National Park is another federal conservation area located in Caracaraí, Roraima (1°17'28.7"N, 61°09'08.2"W). The area includes upland forests and periodically flooded white sand forests (ICMBio, 2020b). RPPN Cristalino is located along the Cristalino River in Mato Grosso State, close to the border with Pará State, in Southern Amazon (9°35'51.0"S, 55°55'52.8"W). It is a well-protected area near the "Arc of Deforestation", where the

agricultural border is expanding immensely due to the monoculture of soybeans and cattle breeding mainly (IPAM, 2020). The area is characterised mostly by clayish soils typical of *terra-firme* (upland) forest in the Amazon (Pires & Prance, 1985). MUSA is an open museum of natural amazon forest, which is the visitors' part of the Adolpho Ducke Forest Reserve (under management of the National Institute for Amazonian Research - INPA), an area of well-protected forest just next to the urban area of Manaus, Amazonas (3°00'21.9"S, 59°56'25.3"W). The forest type is a typical *terra-firme* forest with clayish soils and tall trees (Pires & Prance, 1985). Additional collections from Presidente Figueiredo, Amazonas state, close to the Balbina dam (1°56'34.7"S, 59°27'58.3"W), were also analysed in this study.

Morphological analysis

Specimens were photographed in field and described while still fresh. A fragment of each specimen was preserved in FTA ® Cards for DNA extraction. Then, specimens were dried at 40ºC and preserved in zipped plastic bags. Morphological descriptions were conducted following Largent *et al*. (1977) and Largent (1986). Colour codes follow the *Online Auction Color Chart* (Kramer 2004). Micromorphological features were observed in hand-cut sections of different parts of the basidiomata mounted in KOH 3% or 5% and Congo Red 1% and another section mounted with Melzer's reagent. Thirty basidiospores of each specimen were measured, excluding the hilar appendage. The spore quotient (Q, ratio of length/width) was calculated for each specimen. The mean values for spore dimensions and Q are indicated in between the measurements in italic font. Specimens were deposited at FLOR Fungarium or INPA Herbarium, and names were registered in the MycoBank database.

Sequencing and sequences editing

The samples were properly registered on the Brazilian platform of the National System for the Management of Genetic Heritage and Associated Traditional Knowledge - SisGen, with the registration number A76D0E6, and sent abroad in accordance with Brazilian Biodiversity Law 13.123/2015.

The DNA extraction, PCR amplification of the ITS region, purification and sequencing procedures were performed at the Molecular Systematics Laboratory of the Royal

Ontario Museum (ROM) following Dentinger *et al*. (2010) protocols. The DNA was extracted from samples in FTA Cards. ITS1F/ITS4 primers were used to amplify the Internal Transcribed Spacer (ITS) (Gardes & Bruns, 1993; Lodge *et al*., 2013; White *et al*., 1990). As a final product, two complementar reads of the ITS were obtained, assembled into sequences and edited in Geneious R7 program, with verification of base pairs with low resolution or ambiguities and trimming of ends. The sequences were compared with the data available in the GenBank database via BLASTn searches. The sequences obtained were deposited in the GenBank database.

Results

Taxonomy

Hygrocybe spinosispora J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 16 a and 17)

Etymology: From Latin "spinosus" = spiny and "spora" = spore.
Holotype: BRAZIL, Mato Grosso, Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 5 Jan. 2019, *Cardoso, J.S. & Furtado, A.N.M.* 548 (FLOR67417, holotype).
Diagnosis: Basidiomata orange with sweet odour, with occasional spinose basidiospores, hyphoid cheilo- and pleurocystidia and hyphae frequently with encrustations.

Description:
Pileus (5–)9–27 mm diam., convex to plano-convex, smooth, dull, moist to dry, hygrophanous, deep orange (oac712, oac629, oac691), orange (oac679) or less frequently light orange (oac790) at the centre, then fading to lighter orange (oac761, oac789, oac790); margin sulcate to slightly crenulate, often translucent-striate, pale orange (oac812) to faint yellow (oac855, oac857). *Lamellae* decurrent to uncinate, or adnate, subdistant, narrow, sometimes broad, 1–4 mm broad, pale yellow (oac855, oac857) to cream-coloured (oac683, 681, oac793, oac794), orangish (oac791) near the attachment to the pileus; edge entire, concolorous; lamellulae of two or three lengths, sometimes intervenose and forking near the pileus edge. *Stipe* 11–41 × 2–7 mm, central, regular to flexuous, sometimes tapering towards

the base, often compressed and with a longitudinal fissure, smooth, silky, moist to dry, deep orange (oac712, oac761), orange (oac790, oac791) or yellowish orange (oac810, oac853), fading to light orange (oac812), pale yellow (oac855) at the base, hollow. *Odour* sweet, *Cantharellus*-like, only distinctive when cut. In clusters on clay soil.

Basidiospores 6.2–*7.39*–9.2(–10.6) × 3.8–*4.85*–5.9 µm, Q = 1.495–*1.524*–1.551, ellipsoid, thin-walled, hyaline, inamyloid, guttulate, predominantly smooth, but with sparse spinose basidiospores, spines conical with acute apices, up to 1.4 µm long. *Basidia* 41.7–59.4 × (5.4–)6.1–8.4 µm, cylindro-clavate, thin-walled, guttulate, some with encrusted pigments, clamp connections present, predominantly 4-spored; sterigmata long, up to 15.4 µm. *Lamellar edge* fertile. *Cystidia* present, irregularly distributed throughout the hymenium, as cheilo- and pleurocystidia, 22.6–59.6 × 2.6–5.6 µm, hyphoid, sometimes forked at the apex, with basal clamp connections. *Lamellar trama* subregular, consisting of inflated elements 29.3–100.8 × 9.6–12.8 µm, with few encrustations, clamp connections present. *Pileipellis* a cutis of slightly interwoven hyphae, 24.0–81.8 × 7.6–9.4 µm, some hyphae with encrusted pigments, terminal elements with rounded apices, clamp connections present. *Stipitipellis* a cutis, 24.8–88.8 × 2.6–3.7 µm, terminal elements with rounded apices, sometimes forking, clamp connections present.

Specimens examined: BRAZIL, Mato Grosso, Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 15 Jan. 2018, *Cardoso, J.S.* 152 (FLOR63526); *Cardoso, J.S.* 153 (FLOR63527); *Cardoso, J.S.* 161 (FLOR63529); *Cardoso, J.S.* 164 (FLOR 63530); 17 Jan. 2018, *Cardoso, J.S.* 180 (FLOR63531); 21 Jan. 2018 *Cardoso, J.S.* 217 (FLOR63542); *Cardoso, J.S.* 218 (FLOR63543); 24 Jan. 2018, *Cardoso, J.S.* 260 (FLOR63556); 5 Jan. 2019 *Cardoso, J.S. & Furtado, A.N.M.* 548 (FLOR67417); 6 Jan. 2019, *Cardoso, J.S. & Furtado, A.N.M.* 560 (FLOR67427); Alta Floresta, Reserva Particular do Patrimônio Natural Cristalino, Trilha do Dr. Haffer, 6 Jan. 2019, *Cardoso, J.S. & Furtado, A.N.M.* 553 (FLOR67421).

Distribution: Known only from the type locality, in the Southern Amazon.

Habitat: On clay soils of *terra-firme* forest.

Comments: This is a very distinct species due to its orange colours, sweet smell, occasional spinose basidiospores, hyphoid cheilo- and pleurocystidia and hyphae frequently with encrustations. It is similar to *Hygrocybe zonata* S. A. Cantrell & Lodge from Puerto Rico due to the orange colours and the zonation of colours in the pileus, but *H. zonata* differs in the

absence of spinose spores, cystidia and encrusted hyphae (Cantrell & Lodge, 2004). The macroscopy reminds of *Hygrocybe reidii* Kühner, a species described for France with wide distribution throughout Europe (Boertmann, 2010). *Hygrocybe reidii* is also reported for North America having orange colours, dull pileus, with a margin often undulate or crenate and sometimes translucent-striate, and smells like honey (Boertmann, 2010). It differs from *H. spinosispora* due to the absence of scattered spinose basidiospores and hyphoid cystidia.

Figure 16. a. *Hygrocybe spinosospora*; b-d. *Hygrocybe cantharellula*. b. DLK2635; c. JS733; d. JS506; e-f. *Hygrocybe musaensis* (JS750); g-h. *Hygrocybe rubroalba* (JS599). Scale = 10mm. Photo b by D.L. Komura.

The presence of scattered spinose basidiospores mixed with regular smooth basidiospores was recorded in literature before and occurs in three species: *H. insipida* (J.E. Lange) M.M. Moser, *H. anomala* A.M. Young and *H. kouskosii* A.M. Young (Boertmann, 2010). *Hygrocybe insipida* from Europe is characterized by yellow to orange colours with neutral odour, oblong to cylindrical basidiospores and an ixotrichoderm in the pileipellis (Boertmann, 2010). *Hygrocybe anomala* from Australia is characterized by yellow to orange or red pileus, cream-coloured lamellae and orange-red stipe, with no distinct odours and without cystidia (Young, 2005). Basidiomata of *H. kouskosii*, also from Australia, have brown pileus, white lamellae, yellow stipe and no distinct odours (Young, 2005).

In a BLASTn search, *H. spinosispora* is 86.74% similar with a query cover of 87% to sequences named as *Hygrocyb*e aff. *reidii* (GenBank: KF291196.1), *Hygrocybe* sp. (GenBank: HM020688.1) and *Hygrocybe punicea* (Fr.) P. Kumm. (GenBank: HM020682.1), all from the USA, and 87.34% similar and query cover of 84% to *H. reidii* from Florida, USA (GenBank: MH211855.1). The new species is included in sect. *Coccineae* subsect. *Siccae* together with *H. reidii*, which seems to be a closely related taxon.

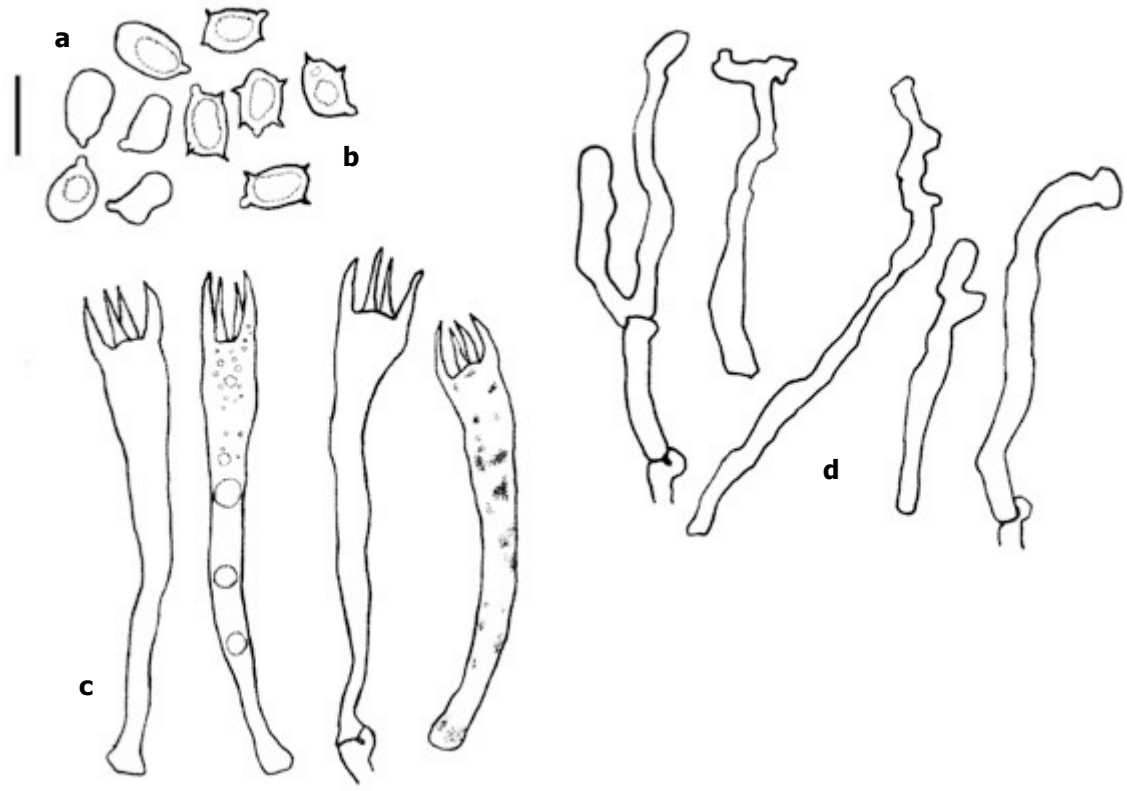

Figure 17. *Hygrocybe spinosispora*. a. smooth basidiospores, b. spinose basidiospores, c. basidia, d. cystidia. a, b, c: JS548; d: JS560. Scale = 10 µm.

Hygrocybe cantharellula J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 16 b, c, d and 18)

Etymology: Refers to the small sized basidiomata (from Latin suffix "-ula") that is close related to *Hygrocybe cantharellus* (Schwein.) Murrill.

Holotype: BRAZIL, Pará, Belterra, Floresta Nacional do Tapajós, Maguary Community, 13 April 2019, *Cardoso, J.S. & Oliveira, J.J.S.* 733 (INPA285675, holotype).

Diagnosis: Basidiomata minute, orange, hymenophore lamellate to pseudolamellate, lamellar trama irregular and presence of scattered spinose basidiospores

Description:

Pileus 2–12 mm diam., convex, to plano-convex when old, tomentose, dry to moist, deep orange (oac678) to light orange (oac761, oac789); margin decurved, sulcate, yellowish (oac810, oac811). *Hymenophore* lamellate, lamellae decurrent, distant, narrow, edge entire; or pseudolamellae folds, smooth near insertion to the stipe, pale orange (oac793); lamellulae sometimes present, or like pseudolamellulae small folds. *Stipe* 4–15 × 1 mm, central, regular, smooth, moist to lubricous, orange (oac691, oac761, oac714, oac649), reddish orange at the apex (oac670), base pale orange (oac763), hollow, with a white basal mycelium. In groups on white sand soils, growing from little pieces of litter or amongst bryophytes.

Basidiospores 5.9–*7.75*–10.1 × 5.1–*6.96*–9.1 µm, Q = 1.058–*1.118*–1.152, globose to subglobose, smooth, or rarely spinose, spines inconspicuous, 0.5–0.9 µm long, with obtuse apices, 5 to 6 in lateral view, hyaline, inamyloid, thin-walled, guttulate. *Basidia* 36.8–52.3 × 7.7–12.7 µm, cylindro-clavate, thin-walled, 2–4-spored, predominantly 4-spored, sterigmata long, up to 13.9 µm, with basal clamp connections. *Lamellar edge* fertile. *Cystidia* absent. *Hymenophoral trama* irregular, of short elements, 27.9–152.7(–204.8) × 12.4–27(–36.6) µm, interwoven, inflated, cylindrical to subglobose, sometimes with median constriction, clamp connections present. *Pileipellis* a trichoderm, terminal elements 13.9–159 × 9.8–20.1 µm, cylindrical, obovoid or clavate, sometimes strangulated in the middle, with rounded apices, occasionally with encrusted pigments, clamp connections present. *Stipitipellis* a cutis, repent hyphae with clusters of erect interwoven hyphae, 2.4–3.7 µm diam., clamp connections present.

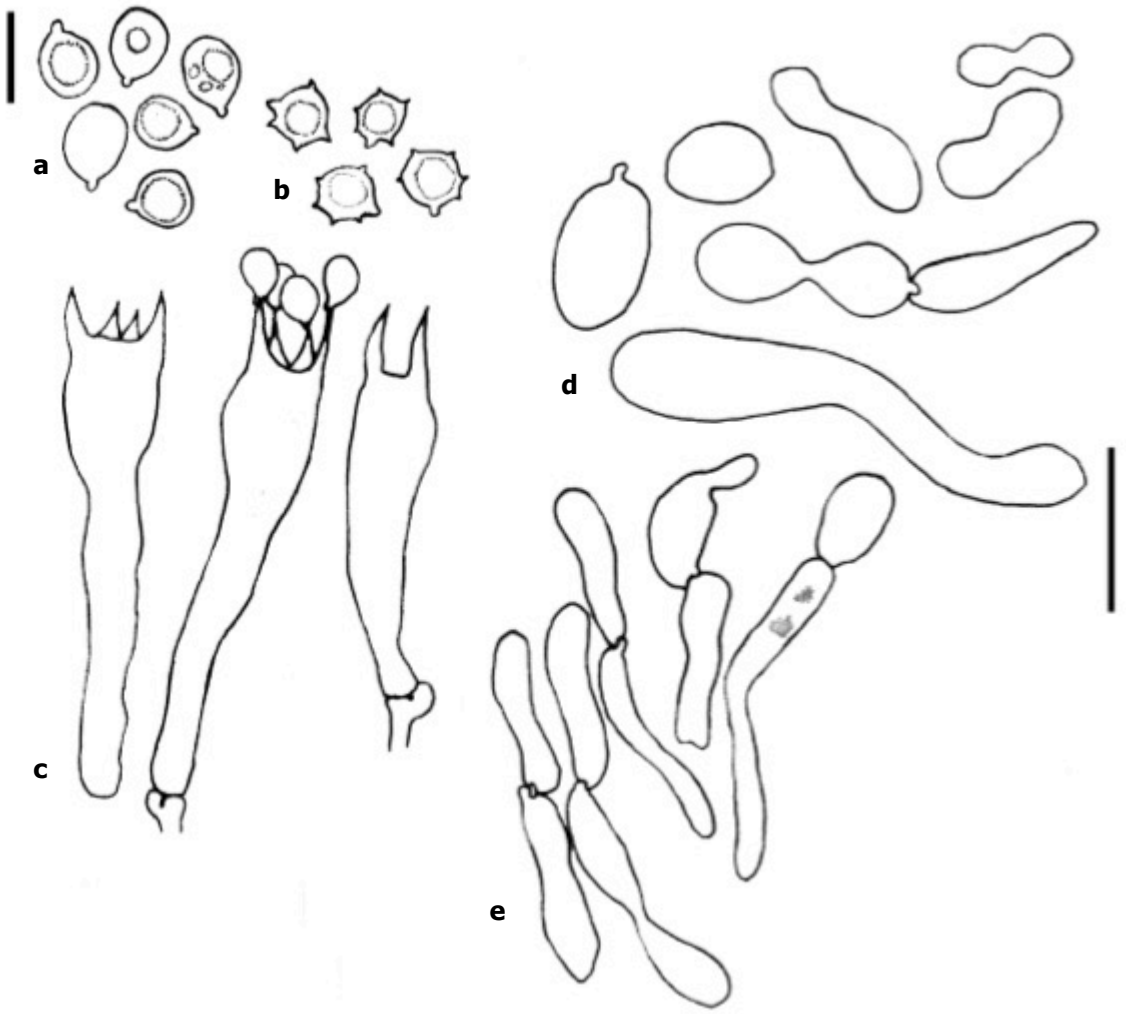

Figure 18. *Hygrocybe cantharellula*. a. smooth basidiospores, b. spinose basidiospores, c. basidia, d. lamellar trama hyphae, e. pileipellis terminal elements. a,b,c and e. JS733; d. DLK2635. Scale = 10 µm for a,b,c; scale = 50 µm for d,e.

Specimens examined: BRAZIL, Roraima, Caracaraí, Parque Nacional do Viruá, campinarana da trilha da perdida, 23 August 2018, *Cardoso, J.S. & Oliveira, J.J.S.* 506 (INPA285630); 26 August 2018, *Cardoso, J.S. & Oliveira, J.J.S.* 534; Pará, Belterra, Floresta Nacional do Tapajós, Maguary Community, 13 April 2019, *Cardoso, J.S. & Oliveira, J.J.S.* 733 (INPA285675); Amazonas, Presidente Figueiredo, Balbina, Ramal da Morena, campina forest, floodable area, 8 May 2019, *Komura, D.L.* 2629; *Komura, D.L.* 2633; *Komura, D.L.* 2634 (INPA285695); *Komura, D.L.* 2635 (INPA285696).

Distribution: Known for the Brazilian Amazon in Roraima, Amazonas and Pará states.

Habitat: Occurs in white sand forests (*campinarana* and *campina*).

Comments: This is a very unusual *Hygrocybe* species due to its minute size, orange colour, irregular hymenophoral trama and spinose basidiospores. The closest taxon seems to be *H. cantharellus*, but it has more robust basidiomata with an umbilicate pileus, true and deeply decurrent lamellae, ellipsoid to oblong basidiospores (none recorded as spinose) and a regular to subregular lamellar trama (Cantrell & Lodge, 2004). No other morphologically similar species are known. The spinose basidiospores were found only in one of the specimens analysed (JS733) and it is 98% similar to JS506 and 97% to DLK2635 in ITS sequence pairwise comparison. The occurrence of sparse spinose basidiospores in some collections of *Hygrocybe* species has been reported in literature and it is common that not all specimens in that species hold this feature (Lodge *et al*., 2013; Boertmann, 2010; Young, 2005).

Via BLAST searches, one ITS sequence of *H. cantharellus* from Canada (GenBank: MN992416.1) is 86.2% similar to the new species. *Hygrocybe cantarellula* also has 86.52% of similarity to an undetermined *Hygrocybe* species from North Japan (GenBank: LC098738.1) and 86.04% of similarity with a sequence of *Hygrocybe calciphila* Arnolds from Mexico (GenBank: MF156249.1). This species should belong to sect. *Coccineae* subsect. *Squamulosae* based on morphological and molecular data.

Hygrocybe musaensis J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 16 f, g and 19)

Etymology: Based on the type locality, Amazonian Museum (MUSA), located in Manaus, Amazonas.

Holotype: BRAZIL, Amazonas, Manaus, Museu da Amazônia (MUSA) – Jardim Botânico, 25 April 2012, *Komura, D.L.* 370 (holotype).

Diagnosis: Pileus velutinous, scarlet red, stipe smooth, orange, solid, cystidia abundant, up to 148 μm long.

Description:

Pileus 10–19 mm diam., convex to umbilicate, sometimes perforated at the centre, velutinous to somewhat tomentose, moist to dry, hygrophanous, scarlet red (oac656, oac663); margin involute, irregularly sulcate, orangish (oac792); context concolorous with the pileus. *Lamellae* sinuate, subdistant, very thick, narrow, up to 2 mm diam., orange (oac792); lamellulae of two lengths. *Stipe* 15–25 × 1.5–4 mm, central, slightly compressed, smooth, dry, tapering towards the base, solid, orange (oac789, oac790). Solitary or gregarious on clay soils.

Basidiospores 5.0–*6.02*–7.1 × 4.2–*5.28*–6.2 µm, Q = 1.111–*1.140*–1.163, subglobose, smooth, thin-walled, hyaline, inamyloid, guttulate. *Basidia* 42.8–74.3(–86.3) × 6.4–10.8(–16) µm, cylindro-clavate, thin-walled, hyaline, inamyloid, guttulate, with basal clamp connections, 4-spored, sterigmata up to 10.6µm. *Lamellar edge* fertile. *Cheilo- and pleurocystidia* present and without difference in size or format between them, (22–)73–148.5 × 11.1–24.6 µm, ventricose-rostrate to clavate, more abundant in the lamellar edge as cheilocystidia. *Lamellar trama* regular, with short elements, 11.0–82.7 × 4.7–7 µm, clamps abundant, sometimes of the medallion type, some refractive hyphae present, interlaced between regular hyphae. *Pileipellis* a trichoderm of cylindrical hyphae with brown encrustations in KOH, terminal elements 48.0–99.7 × 9.1–19 µm, sometimes swollen or strangulated in the middle, with rounded or sometimes swollen apices, clamp connections present. *Stipitipellis* a trichoderm similar to pileipelis, terminal elements 16.4–112 × 4.4–10.6 µm.

Specimens examined: BRAZIL, Amazonas, Manaus, Museu da Amazônia (MUSA) – Jardim Botânico, 29 January 2013, *Komura, D.L. 370*; 15 May 2018, *Cardoso, J.S. & Krahl, D.R.P.* 342 (INPA285605); 18 April 2019, *Cardoso, J.S. & Dávila, N.* 740 (INPA285678); *Cardoso, J.S. & Komura, D.L.* 750 (INPA285681).

Distribution: Known only from the type locality, in Central Amazon.

Habitat: *Terra-firme* forest, on clay soils, among bryophytes (*Lophocolea* sp.) and/or basidiolichens (*Sulzbacheromyces* sp.).

Comments: *Hygrocybe musaensis* is an uncommon and easily distinguishable *Hygrocybe* species due to its solid stipe and the long and abundant cystidia. No species with this combination of characteristics are known. There is only one described species in sect. *Coccineae* from the Amazon forest, *H. mutabilis* Singer, but it differs significantly in having a pink smooth pileus, white lamellae and stipe, much bigger basidiospores (7.5–12 × 4.5–7.5 µm) and much smaller cystidia (33–35 × 4–6 µm) (Singer, 1989). The morphological characteristics place *H. musaensis* in sect. *Coccineae* subsect. *Squamulosae*, although the basidiospores are narrower than the indicated by Lodge *et al.* (2013), with a Q = 1.140 vs. 1.2–1.7 (−1.8). Unfortunately, sequences from the collections of *H. musaensis* were not obtained, but the species is proposed as new based on its remarkable morphological characteristics.

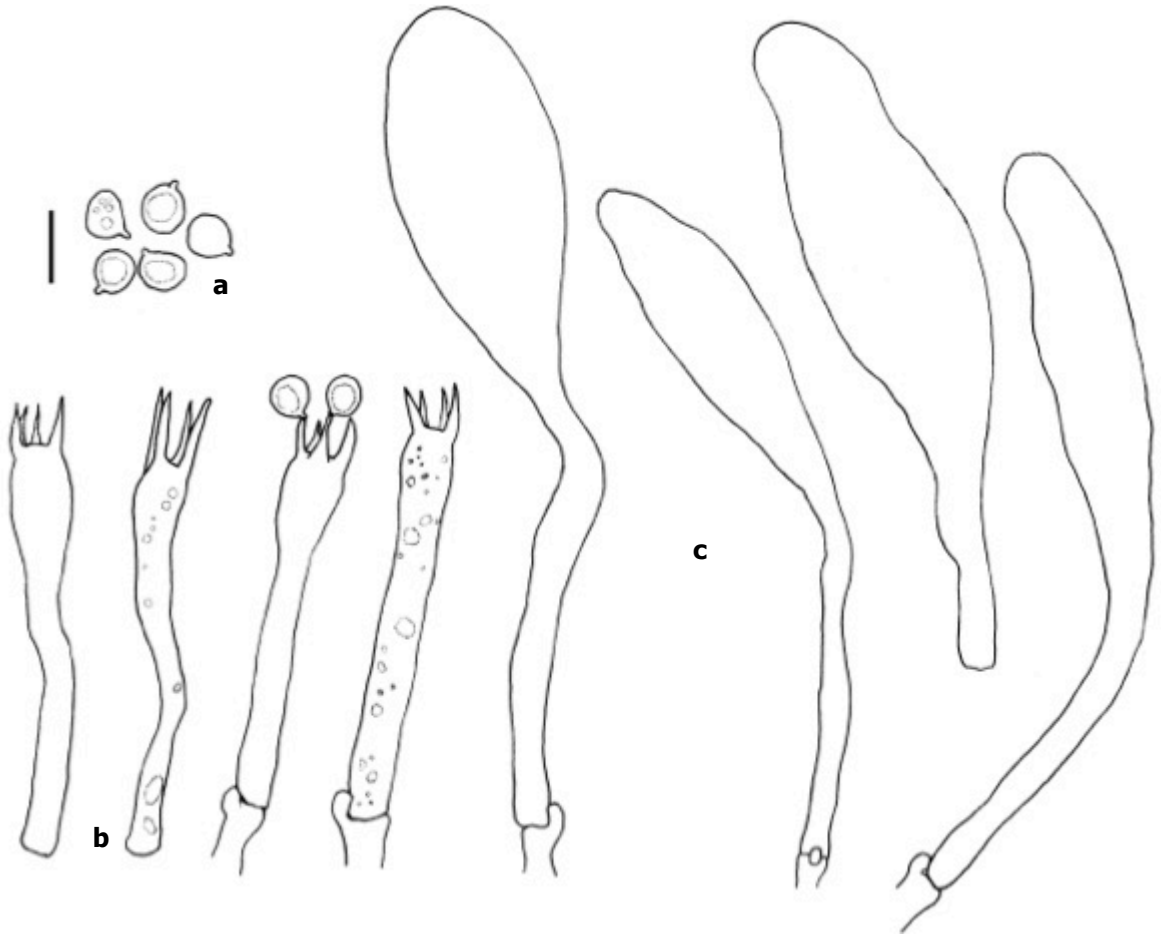

Figure 19. *Hygrocybe musaensis*. a. basidiospores, b. basidia, c. cystidia. a,b. JS342; c. DLK370. Scale = 10µm.

Hygrocybe rubroalba Picciola, Battistin & Vizzini, in Vizzini, Picciola, Battistin & Ercole, *Phytotaxa* 226(1): 22 (2015) (Figures 16g,h and 20)

Description:

Pileus 19–35 mm diam., convex-campanulate, smooth, glabrous, moist but not viscid, faintly translucid-striate towards the margin, pale pink (oac550, oac548, oac597) with pink (oac547, oac587) flushes more concentrated at the centre; margin slightly uplifted, somewhat sulcate, sometimes eroded, white. *Lamellae* uncinate to slightly decurrent, subdistant, up to 8 mm broad, pure white; lamellulae of 3 lengths. *Stipe* 50–52 × 3–5 mm, central, regular, smooth, glabrous, only slightly fibrillose, moist but not viscid, orange (oac789, oac761), sometimes with flushes of reddish orange (oac670), pale orange (oac812) near the hymenophore, hollow. *Basidiomata* caespitose. *Odour* like burned rubber.

Basidiospores and *basidia* dimorphic. *Macrospores* 11.7–*12.81*–13.7 × 6.9–*7.83*–8.6 µm, Q = 1.636, oblong, smooth, thin-walled, hyaline, inamyloid, guttulate. *Microspores* (4.6–)5–*5.87*–7.1 × 3.5–*4.14*–4.8 µm, Q = 1.417, ellipsoid, smooth, thin-walled, hyaline, inamyloid, guttulate. *Macrobasidia* (48–)51.9–62.5 × 10.1–13.8(–14.6) µm, clavate, thin-walled, hyaline, inamyloid, guttulate, with basal clamp connections, 2–4-spored, sterigmata up to 9.5 µm long. *Microbasidia* (31.5–)34.1–43.9 × 5.8–7.3 µm, clavate, thin-walled, hyaline, inamyloid, with basal clamp connections, 4-spored, sterigmata up to 5.5 µm long. *Lamellar trama* subregular, of relatively short elements, 24.4–102.8 × 2.1–22.5 µm, intermixed with refractive hyphae ± 2.0 µm diam., clamp connections present, sometimes of the medallion type. *Lamellar edge* almost sterile, consisting of a few microbasidia intermixed with basidioles. *Cystidia* absent. *Pileipellis* a disrupted cutis, almost forming a trichoderm, with somewhat interwoven hyphae, 2.0–14.6 µm diam., some hyphae with irregular branches and bifurcations, with uplifted hyphae, hyaline in KOH and water, clamp connections present, sometimes of the medallion type. *Stipitipellis* a cutis of more or less interwoven hyphae, 1.5–10.6 µm diam., with many ramifications, some projecting, a few hyphae with encrusted pigments observed, with medallion clamp connections.

Specimen examined: BRAZIL, Mato Grosso, Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 9 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 599 (FLOR67459).

Distribution: Known from states of Paraná and Mato Grosso, Brazil.

Habitat: Caespitose to gregarious in clay soils in *terra-firme* forest and in *Araucaria* mixed forests.

Comments: *Hygrocybe rubroalba* is a dimorphic species that belongs to subgenus *Pseudohygrocyb*e sect. *Firmae* based on morphology and molecular data (Vizzini *et al*., 2015). It was described in 2015 for Paraná State in the Atlantic Mixed Forests of Southern Brazil (Vizzini *et al*., 2015) and is very closely related to *Hygrocybe magnifica* de Meijer. The latter, however, has more elongate macrospores that are subcylindrical, cheilocystidia in the hymenium and intracellular vivid red pigments in the pileipellis hyphae (Meijer, 2008). There are other few species which are morphologically similar to *H. rubroalba*: *H. batistae* Singer, *H. paraibensis* Singer and *H. aurantiomagnifica* Silva-Filho & Wartchow, all compared in Table 3 in their dimorphic basidiospores and basidia. All of them are reddish (that can vary in the intensity from orange, pink to deep red) species with whitish lamellae and dimorphic basidiospores and basidia, and are described for Brazil. *Hygrocybe*

martinicensis Pegler & Fiard is also a closely related species known to occur in Brazil (Pegler, 1997) but with consistently yellow pileus and stipe, and hyphoid cheilocystidia (Pegler & Fiard, 1978). More work to elucidate the relationship of these closely related taxa is needed because their characteristics seem to overlap considerably. For example, the pileipellis of *H. rubroalba* (JS599) resembles the described by Lodge & Pegler (1990) for *H. batistae* based on a specimen collected in Puerto Rico; *H. auratiomagnifica* looks like a dried-out orange version of *H. batistae* and was described based on only one collection (Silva-Filho *et al*., 2019); *H. paraibensis* was described by Singer (1965) based on a collection from Paraíba state and was recollected once by himself in the Amazonas state (*species*Link, 2020). This implies that both *H. paraibensis* and *H. batistae* have a wide distribution.

Our collection matches with the ITS sequence from the type of *H. rubroalba* (GenBank: NR_155167) in 97% via BLASTn search with a query cover of 96.6%. Our collection differs from *H. rubroalba*'s type collection mainly in the pileus colour, which is lighter, very pale pink with pink flushes in the centre, lacking any red colours. Nonetheless, these colour variations in some species of *Hygrocybe* is rather common, especially in the yellow-orange-red spectrum. It is suggested that they are the same species based on morphology and ITS similarity, but with emphasis that more work is needed to elucidate the relationship of the closely related taxa mentioned above.

Table 3. Table comparing taxa in subgen. *Pseudohygrocybe* sect. *Firmae* with occurrence in Brazil. Data from the type publications, unless otherwise stated.

Taxon	Macrospores	Microspores	Macrobasidia	Microbasidia
H. rubroalba	12.0–15.0 × 7.0–9.0 µm	6.1–8.0 × 4.0–5.3 µm	60–80 × 10–15 µm	35–55 × 5–8 µm
***H. rubroalba* (JS599)**	**11.7–13.7 × 6.9–8.6 µm**	**5–7.1 × 3.5–4.8 µm**	**51.9–62.5 × 10.1–13.8 µm**	**34.1–43.9 × 5.8–7.3 µm**
H. batistae (Singer 1965)	10.5–14.5 × 6.5–8 µm	–	57–62.5 × 10–10.8 µm	30–34 × 3.3–4.7 µm
H. batistae (Lodge & Pegler 1990)	10-13.5 × 7.5–9.5 µm	6.3–7.3 × 4.2–5.2 µm	64–74 × 9.5–12.5 µm	40–55 × 5–7.5 µm
H. paraibensis	13.5–16.7 × 7.7–9.5 µm	10–11.7 × 5.8–7.7 µm	54–63 × 12–13 µm	27–44 × 6.5–9.5 µm
H. magnifica	15–18 × 6–8.5 µm	6–8 × 4–4.5 µm	75–81 × 11–14 µm	37–45 × 6.5–8 µm
H. aurantiomagnifica	13–16 × 8–10.5 µm	6–7.5 × 4.0–5.5 µm	60.5–70.5 × 10–17.5 µm	30–35 × 4–6 µm
H. martinicensis	11–15.5 × 7–9 µm	3.5–7.5 × 3.5–5 µm	70–80 × 10–12 µm	38–45 × 6–8 µm

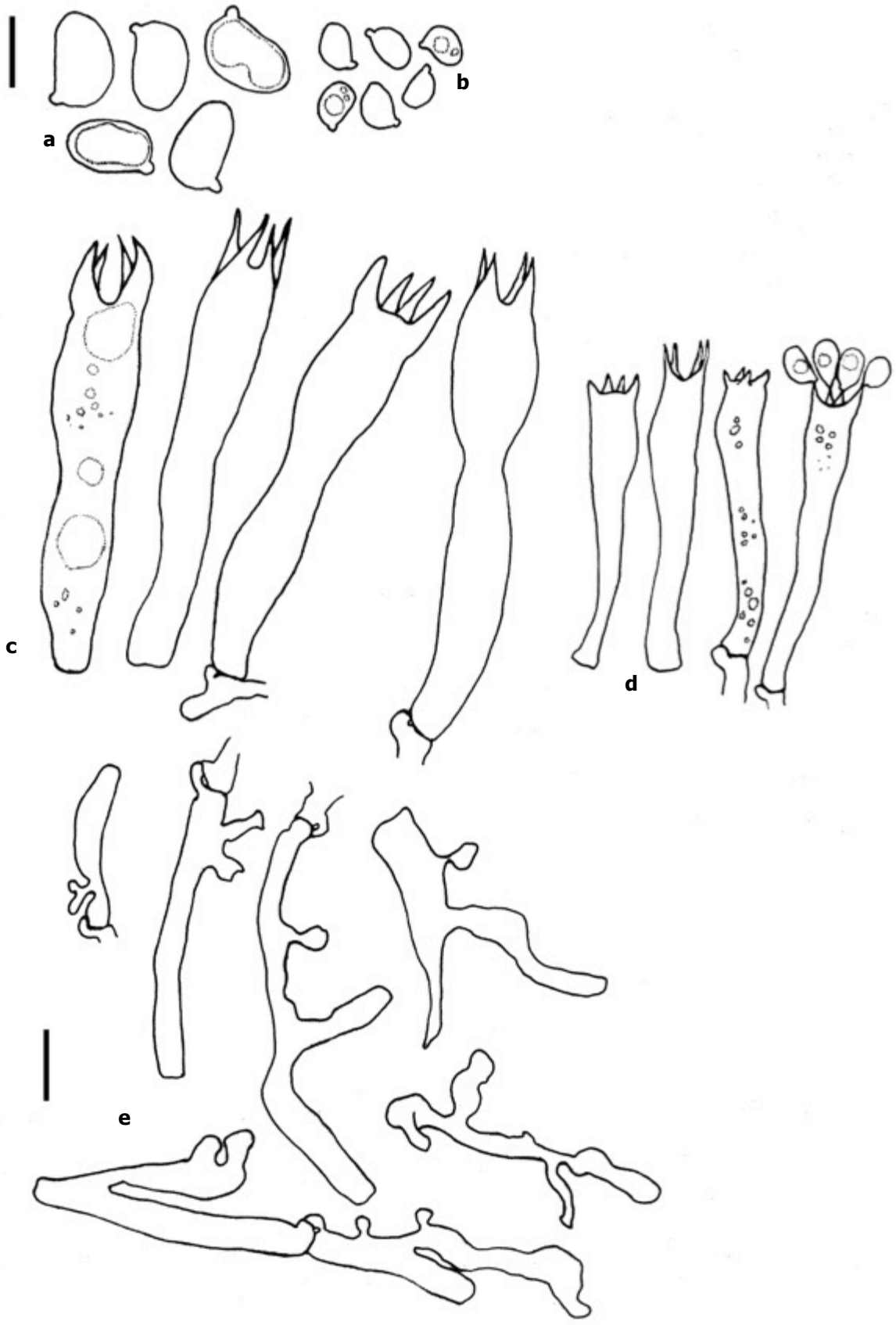

Figure 20. *Hygrocybe rubroalba.* a. macrospores, b. microspores, c. macrobasidia, d. microbasidia, e. differentiated hyphae in the pileipellis. Scale = 10 µm.

Key to Hygrocybe *subg.* Pseudohygrocybe *in Brazil*

1. Lamellae free, adnexed or with a decurrent tooth, lamellar trama regular, with long parallel hyphae with tapered ends, basidia clavate, mean ratio of basidiospores to basidia length 3 to 5……Subg. *Hygrocybe*

1. Lamellae broadly attached, often decurrent, lamellar trama subregular, with short elements not exceeding 140 µm long, basidia long, cylindro-clavate with a mean ratio of basidiospores to basidia length usually bigger than 5……...Subg. *Pseudohygrocybe*, 2

2. Basidiospores and basidia monomorphic……Sect. *Coccineae*, 3

2. Basidiospores and basidia dimorphic…….Sect. *Firmae*, 17

3. Pileus usually smooth, dry, viscid, glutinous or at least subviscid when wet, with an ephemeral ixocutis……..4

3. Pileus tomentose to squamulose, or scurfy in the disc with a smooth margin, pileipellis a trichoderm, at least in the centre of pileus……Subsect. *Squamulosae*, 5

4. Pileus lubricous, viscid or glutinous, usually with a gelatinous layer in the pileipellis…….Subsect. *Coccineae*, 11

4. Pileus usually dry without a gelatinous layer in the pileipellis……Subsect. *Siccae,* 15

5. Pileus distinct red……6

5. Pileus orange, yellow, brown or pink…….8

6. Pileus entirely velutinous, scarlet red, lamellae orange, thick, cystidia abundant……...……*H. musaensis*

6. Pileus tomentose to scurfy in the centre with a smooth margin, lamellae white to yellow, cystidia absent……7

7. Pileus scurfy in centre, scarlet with orange margin, lamellae white to very pale yellow, basidiospores 7.5–8.5(–10) × 4–6 µm, lamellar trama subregular with hyphae 3–6(–12) µm diam……...*H. subcaespitosa*

7. Pileus tomentose in centre, entirely red, lamellae buff yellow, basidiospores 7–9.5 × 4–5.5 µm, lamellar trama regular with hyphae 5.5–20 µm diam……..*H. miniata*

8. Basidiomata minute, pileus ≤ 12 mm diam., tomentose, orange, deeply decurrent lamellae or pseudolamellae, hymenophoral trama irregular, with rare spinose basidiospores…….....*H. cantharellula*

8. Pileus usually ≥ 12 mm diam., not tomentose, different coloured, lamellae adnate to decurrent, hymenophoral trama subregular, basidiospores smooth……9

9. Pileus squamulose, usually perforated in centre, honey yellow to brown-yellow covered with brown squamules, lamellae cream colour, basidiospores 7.8–10(–12) × 5–7(–8) µm……*H. melleofusca*

9. Pileus glabrous to pellucid striate or squamulose, not perforated, pink, lamellae white, basidiospores slightly bigger in length……10

10. Pileus glabrous and slightly pellucid-striate, hygrophanous, stipe whitish, basidiospores 7.5–12.5 × 4.5–7.5 µm, variable in format, pileipellis a cutis……...*H. mutabilis*

10. Pileus squamulose, not hygrophanous, stipe pink, basidiospores 8.5–14 × 6–7.5 µm, ellipsoid to oblong, pilleipellis a trichoderm……..*H. rhodoleuca*

11. Pileus subviscid or glutinous, brightly coloured, yellow, orange or red……12

11. Pileus subviscid, darker in colour, with shades of brown or ferrugineous……14

12. Pileus glutinous, translucent-striate, deep red, lamellae pale lemon yellow, basidiospores 5–6.5 × 2.2–3.5 µm, often constricted, pileipellis an ixotrichoderm……...*H. subminutula*

12. Pileus subviscid, yellow to orange, lamellae yellow or orange, basidiospores different sized, not constricted, pileipellis not an ixotrichoderm……13

13. Pileus yellow-orange, lamellae pale orange, stipe yellow, basidiospores 6.5–8.5 × 2.5–4.5 µm, cheilocystidia absent……*H. parvula*

13. Pileus yellow, lamellae yellow, stipe citrinous, basidiospores 5–7 × 5–6 µm, cheilocystidia present……...*H. flavolutea*

14. Pileus orangish brown, lamellae yellow, stipe orangish brown, basidiospores 11–15 × 5–7.5 µm, cheilocystidia present……...*H. pernambucensis*

14. Pileus ferrugineous, lamellae violaceous, stipe dark brick red, basidiospores 6.5–9 × 3.5–5.5 µm, cheilocystidia absent……*H. troyana*

15. Pileus dry to lubricous, opaque, orange, basidiospores smooth but with scattered spinose basidiospores……..*H. spinosospora*

15. Pileus not viscid, not opaque, scarlet red, all basidiospores smooth……16

16. Pileus not perforated, margin translucent-striate, sulcate, lamellae yellow with pinkish red tints, stipe scarlet red, basidiospores 7–9.5 × 4–7 µm……...*H. mexicana*

16. Pileus perforated in the centre, margin not translucent-striate, undulate, lamellae whitish, stipe pale yellowish, basidiospores 5.5–7.5 × 3.2–4.0 µm……...*H. ignipileata*

17. Pileus predominantly red……18

17. Pileus predominantly orange or yellow……..21

18. Pileus smooth……..19

18. Pileus not completely smooth…….20

19. Pileus glabrous, vivid red, lamellae pure white, macrospores 15–18 × 6–8.5 µm, Q = 2.6, subcylindrical, cheilocystidia present, pileipellis hyphae with reddish contents……*H. magnifica*

19. Pileus glabrous to slightly rugulose under lens, red with purplish and orange-yellow tints, lamellae white with purplish tints, macrospores 12.0–15.0 × 7.0–9.0 µm, Q = 1.7, ellipsoid to oblong, cheilocystidia absent, pileipellis without reddish contents…….*H. rubroalba*

20. Pileus slightly granulose, rugulose to rugose, macrospores 10-13.5 × 7.5–9.5 µm, Q = 1.46, ellipsoid, pileipellis hyphae with irregular diverticulae, somewhat coralloid.....*H. batistae*

20. Pileus glabrous, tomentose in the centre, macrospores 13.5–16.7 × 7.7–9.5 µm, ellipsoid to cylindrical, pileipellis hyphae without diverticulae……*H. paraibensis*

21. Pileus yellow with no presence of orange or red colours, smooth, glabrous, opaque, dry, lamellae white with pinkish tints, stipe yellow, macrospores 11–15.5 × 7–9, Q = 1.6, ellipsoid to oblong, cheilocystidia 30 × 2.5–4 µm……*H. martinicensis*

21. Pileus orange, orange-yellow, fading to yellow, smooth, slightly rugulose, lamellae whitish with pinkish-violet margin, stipe yellow to orange, macrospores 13–16 × 8–10.5 µm, Q = 1.59, ellipsoid to oblong, cheilocystidia absent……..*H. aurantiomagnifica*

Notes on the dichotomic key

Based on morphological data, *Hygrocybe miniata* was kept in sect. *Coccineae* subsect. *Squamulosae* in the species dichotomic key. However, based on molecular data (Lodge *et al*. 2013), the species was excluded from sect. *Coccineae* and remain *incertae sedis* in subgen. *Pseudohygrocybe*. In Hesler & Smith (1963) no clamp connections were observed in *H. flavolutea* [= *Hygrophorus flavoluteus* (Murrill) Murrill]. Pegler (1997) combined it in *Hygrocybe*, but without mention about clamp connections. A revision of the type and Pegler's collections from São Paulo state would be necessary to confirm the presence or absence of

clamp connections in this taxon. Two taxa described for Southern Brazil (Raithelhuber, 1992), *H. lugubris* and *H. subpisittacina*, were not included in the key due to insufficient information to classify them in the subgenera and sections of *Hygrocybe*.

Members of *Hygrocybe* sect. *Firmae* seems to be abundant in Brazil, with many similar species. However, only a few of these species have molecular data available (Silva-Filho *et al*., 2019). A comprehensive and detailed work is needed to understand the limits of each taxon in this group, including more sampling and the use of more molecular markers. Also, there is inconstancy in the diagnosis of the type species of this section, *H. firma* (Berk. & Broome) Singer, which was described as *Hygrophorus firmus* Berk. & Broome (1871) with a yellow, minutely tomentose, umbilicate pileus and strongly decurrent lamellae. Later, Corner (1936) described 17 varieties of *H. firmus*, with many colour variations, and the "typical form" would be *H. firmus* var. *typicus*, with minutely scurfy squamulose pileus to innately fibrillose and striate towards the margin, orange-red or scarlet. The macro- and microbasidia described by Corner (1936) are also not congruent with the description in Lodge *et al*. (2013) for sect. *Firmae*, because they differ significantly in width, being the macrobasidia clavate (50–75 × 12–16 µm) and microbasidia subclavate or subcylindrical (28–40 × 6–8 µm). Then, Singer (1957) combined *Hygrophorus firmus* var. *typicus* sensu Corner (1936) to *Hygrocybe firma* var. *firma,* with red and fibrillose pileus, red lamellae and stipe, separating the basidiospores and basidia into 3 size groups and regular lamellar trama with long fusoid elements. Finally, Pegler & Fiard (1978) commented that most of those varieties were actually different species distinguishable with micromorphological characters. The authors defined *H. firma* var. *firma* with orange to yellow tomentose and squamulose pileus, pale yellow lamellae, broadly clavate macrobasidia (50–60 × 14–16 µm), narrowly clavate microbasidia (35–40 × 6–8 µm) and regular lamellar trama. Based on these evidences, *H. firma* sensu Pegler (1978) would probably be placed in subg. *Hygrocybe*, sect. *Pseudofirmae* and the *H. firma* sensu Lodge (2013) with minutely tomentose red pileus, red lamellae and stipe, and narrow macrobasidia that does not change much in width from the microbasidia, would be another species. *Hygrocybe firma* is recorded for Brazil (Pegler, 1997), but it was excluded from our dichotomic identification key due to the incongruences mentioned above.

Capítulo 3

Studies in *Hygrocybe* s.l. (Hygrocyboideae, Hygrophoraceae) in Brazil: New species of *Humidicutis* and *Neohygrocybe*

Abstract

Humidicutis and *Neohygrocybe* are genera of wax cap mushrooms closely related to *Hygrocybe*, in tribe Humidicuteae, subfam. Hygrocyboideae. Basidiomata are brightly coloured or grey brown, the lamellae are thick and waxy, the basidia are long and the lamellar trama is regular to subregular. This is the first study in *Humidicutis* and *Neohygrocybe* from Brazil, describing *H. pindoramensis* sp. nov. and *N. fumosa* sp. nov. based on morphology and ITS data. Maximum Likelihood and Bayesian analyses were implemented to reconstruct the phylogenetic relationship of the new species and related taxa. The resulting trees support the uniqueness of the new species as well as display how they relate to the taxa of correspondent genera with ITS data available.

Keywords: Fungi, Agaricales, taxonomy, ITS barcode, phylogeny, Amazon.

Introduction

Humidicutis (Singer) Singer and *Neohygrocybe* Herink are agaric genera that belong to the *Hygrocybe* sensu lato clade (Hygrophoraceae Lotsy, subfam. Hygrocyboideae Padamsee & Lodge). They share the same general characteristics as those of *Hygrocybe* s.s., producing basidiomata that are frequently brightly coloured or grey brown, with waxy thick lamellae, long basidia that are more or less 5 times the length of the basidiospores, and regular to subregular lamellar trama (Lodge *et al*. 2013). Both genera were recurrently classified as subgenus or sections of *Hygrocybe* s.s., depending on the author (eg. Boertmann, 2010), but after a comprehensive study of the Hygrophoraceae family (Lodge *et al*. 2013), they are now distinct genera accommodated in tribe Humidicuteae Padamsee & Lodge, together with *Gliophorus* Herink, *Porpolomopsis* Bresinsky and *Gloioxanthomyces* Lodge, Vizzini, Ercole & Boertm., based on molecular phylogeny, morphology and pigment chemistry.

Humidicutis species produce basidiomata with bright or dull colours (e.g. orange, red, yellow, pink, purple, green, bluish-green, brown, greyish-brown, olivaceous and white), pileus dry to moist, rarely viscid, usually convex-umbonate, lamellae often with a decurrent tooth, pileipellis usually with encrusting pigments and clamp connections absent in all hyphae but present at the base of basidia in the toruloid form (Horak 1990, Lodge 2013, Young 2005). *Neohygrocybe* comprises taxa with dull coloured basidiomata, generally grey-brown, usually dry or moist, never viscid or glutinous, often with red to brown staining reactions in lamellae, pileus (and/or context), frequently with distinct nitrous smell, sometimes with pseudocystidia and/or cheilocystidia with fuscous pigments, with usual clamp connections at the basidia base (not toruloid or medallion form) (Lodge *et al*. 2013).

There are about twelve species of *Humidicutis* and ten of *Neohygrocybe* known worldwide (He *et al*., 2019; Lodge pers. comm. 2020); both genera are recorded in America, Europe, Asia and Oceania. Here we describe one new species of *Humidicutis* and one of *Neohygrocybe* for the first time in Brazil based on morphology and ITS-based phylogenetic analyses.

Material and Methods

Study area

The specimens were collected in three localities: Private Reserve of National Heritage (RPPN) Volta Velha, RPPN Cristalino and Rio Cuieiras INPA Reserve. RPPN Volta Velha is a conservation area that holds a remnant of well-protected Atlantic Rainforest in a coastal region of Southern Brazil, in Itapoá, Santa Catarina State. The soil is sandy, typical of the *restinga* landscape. RPPN Cristalino is located along the Cristalino River in the Mato Grosso State, close to the border with Pará State, in Southern Amazon. It is a well-protected area near the "Arc of Deforestation", where the agricultural border is expanding immensely due to the monoculture of soybeans and cattle breeding mainly. It is characterised mainly by clayish soils typical of *terra-firme* (upland) forest type of the Amazon forest. The Rio Cuieiras INPA Reserve is located in the Low Rio Negro basin, in Manaus, Amazonas state. It is a research station managed by the National Institute for Amazonian Research (INPA) and encompasses the *Museu na Floresta* (Museum in the Forest) project, which aims to produce citizen science

knowledge. The area is characterised by having *terra-firme*, *campinaranas* (white sand forest) and *igapó* (periodically flooded forest) forests (Pires & Prance, 1985).

Morphological analysis

Specimens were photographed in field and described while still fresh. A fragment of each specimen was preserved in FTA ® Cards for the DNA extraction. Specimens were dried at 40ºC and preserved in zipped plastic bags. Morphological descriptions were conducted following Largent (1986) and Largent *et al*. (1977). Colour codes are based on the *Online Auction Color Chart* (Kramer 2004). Micromorphological features were observed in hand-cut sections of different parts of the basidiomata mounted in KOH 3% or 5% and Congo Red 1% or in Melzer's reagent. Thirty basidiospores of each specimen were measured, excluding the hilar appendage. The spore quotient (Q, ratio of length/width) was calculated for each specimen. The mean values for spore dimensions and Q are indicated in between the measurements in italic font. Specimens were deposited at FLOR Fungarium or INPA Herbarium. The new names will be registered in the MycoBank database.

Sequencing and sequences editing

The samples were registered on the platform of the National System for the Management of Genetic Heritage and Associated Traditional Knowledge - SisGen, with the registration number A76D0E6, and sent abroad in accordance with Brazilian Law 13.123 / 2015. The DNA extraction, PCR amplification, purification and sequencing procedures were performed at the Molecular Systematics Laboratory of the Royal Ontario Museum (ROM) following Dentinger *et al*. (2010) protocols. The DNA was extracted from samples in FTA Cards. ITS1F/ITS4 primers were used to amplify the Internal Transcribed Spacer (ITS) (Gardes & Bruns 1993, Lodge *et al*., 2013, White *et al*. 1990). As a final product, reads were assembled into ITS sequences and further edited in Geneious R7. The sequences will be deposited in the GenBank database.

Phylogenetic analysis

Firstly, BLAST searches were conducted with the ITS queries JS18 [=JS20], JS19 (*Humidicutis*), JS493 (*Hygroaster*), and JS600 (*Neohygrocybe*) across the GenBank NCBI database. Within the first hundred entries deemed to be the closest in the GenBank listed via BLAST searches of each queries, entries were selected according to each list pattern: "Description" including the correspondent genus with non-determined taxa among them. "Per. Ident.", "E value" and "Query Cover" were observed as references to regard in the entries selection. Secondly, objective searches were conducted to find all ITS sequences entries in the database determined as *Humidicutis*, *Hygroaster* and *Neohygrocybe*. Thirdly, all ITS sequences determined as *Gliophorus* and *Porpolomopsis*, 24 ITS sequences in total, were objectively downloaded from GenBank. Finally, one ITS representative of *Hygrocybe conica* (type-species of *Hygrocybe*) was included in the dataset to complete a *Hygrocybe* s.l. assemblage. All the ITS sequences gathered by these four steps (Table 4) were downloaded from the GenBank database. One ITS sequence determined as *Hygrophorus eburneus* (type-species of *Hygrophorus*) was included as the outgroup. This *Hygrocybe* s.l. dataset (DATASET1) was used to build a backbone phylogenetic tree from which the clades were pinched to form specific subdatasets of *Humidicutis*, *Hygroaster*, *Neohygrocybe*. Since *Hygroaster* has only three ITS representative sequences corresponding to three species (one of them, JS493) and their phylogeny is fully solved in the backbone tree, the specific subdatasets were arranged only to *Humidicutis* (DATASET2) and *Neohygrocybe* (DATASET3). Non-determined taxa were kept in the analyses to fill gaps of diversity of the ITS sampling space.

DATASET1, DATASET2 and DATASET3 were aligned separately via Muscle v3.8.31 (Edgar, 2004). The alignments were processed in Geneious R7 for manual rearrangement of bases and trimming of ends and ambiguous blocks. The best-scored nucleotide substitution models were selected via MrModeltest v.2.3 using AKAIKE Information Criterion (AIC) for each dataset: DATASET1 (GTR+G), DATASET2 and DATASET3 (HKY+G).

For all datasets, Maximum Likelihood analyses (ML) were conducted in RAxML 7.0.4 (Stamatakis, 2006) through fast-bootstrapping including 1,000 pseudoreplicates implementing GTRGAMMAI with GAMMA+P-Invar Model parameters estimated up to an accuracy of 0.001 Log Likelihood units. The best-scored ML tree was chosen after full ML optimization. Bayesian analyses (BA) were performed in MrBayes 3.2.1 (Ronquist *et al*., 2012) consisting of MCMCMC using selected models to determine the parameters Nst = 6 for

DATASET1 and Nst = 2 for DATASETs 2 and 3. The BA implemented two independent runs of 1 000 000 of generations, sampling frequency every 100 generations, 4 independent chains and 2 swap for all datasets. All burnin was set at 10 %. Final BA trees were summarized using the 50 % majority-rule consensus method. Branch lengths were summarized across the 95 % highest posterior density trees. The statistical support values generated by the mentioned analyses were those of bootstrap (BS ≥ 70%) and posterior probability (PP ≥ 0.95), respectively. The trees generated were visualized in software FigTree v1.4.3 and edited in CorelDraw X7.

Table 4. Sequences used in the phylogenetic analyses, including GenBank accession number.

Taxon	Country	Voucher	ITS Accession number
Fungal sp. strain	Tibet	XZQGJ	MK056267
Gliophorus aff. *psittacinus*	Japan	CFMR JP4	KF291079
Gliophorus aff. *psittacinus*	Sweden	TFB12668 TENN62836	KF291068
Gliophorus europerplexus	Wales	K 181246	NR120275
Gliophorus flavoviridis	India	US1367	MF542320
Gliophorus graminicolor	Australia	TJB10048	KF381520
Gliophorus psittacinus	England	KM90029	EU784341
Gliophorus psittacinus	England	KM90674	EU784342
Gliophorus psittacinus	England	KM127194	EU784340
Gliophorus psittacinus	USA	CFMRNY42	KF291082
Gliophorus psittacinus	Denmark	CFMR DEN25	KF291075
Gliophorus sciophanus	Spain	EF4678	KY807674
Gliophorus sp.	India	KD 17-38	MH392196
Gliophorus sp.	India	KD 17-28	MH392195
Gliophorus sp.	USA	AM05	HM020676
Humidicutis auratocephala	USA	DJLTN81 DJL05TN81	KF291103
Humidicutis auratocephala	USA	AJ122	GU256224
Humidicutis auratocephala	USA	AM2 1	HM020678
Humidicutis auratocephala	USA	AJ121	GU256223
Humidicutis auratocephala	USA	BHS2009_14	HM020685
Humidicutis auratocephala	USA	AFTOL1727	DQ490624
Humidicutis dictiocephala	USA	MEN09 02	HM020694
Humidicutis cf. *marginata*	Ecuador	QCAM6000	KY689661
Humidicutis marginata	USA	AFTOL1337	DQ490625
Humidicutis marginata	Canada	ANT018 QFB28582	MN992411
Humidicutis marginata	USA	TFB12230 TENN60232	KF291144
Humidicutis marginata var. *olivacea*	USA	MO #320164	MN089498
Humidicutis marginata var. *olivacea*	USA	ECO764	MG196097
Humidicutis pindoramensis	**Brazil**	**JS18 FLOR57148**	–
Humidicutis pindoramensis	**Brazil**	**JS20 FLOR57150**	–

Taxon	Country	Voucher	ITS Accession number
Humidicutis pura	USA	PBM4191a	MT237512
Humidicutis pura	USA	PBM4191b	MT237511
Humidicutis sp.	Canada	ANT181 QFB28609	MN992410
Humidicutis sp.	Mexico	20184	MK332022
Humidicutis sp.	Mexico	2018 1A	MK332021
Humidicutis sp. 2	Puerto Rico	CFMRPR6524	KF291150
***Humidicutis* sp. 2**	**Brazil**	**JS19 FLOR57149**	–
Humidicutis sp. 3	Belize	CFMRBZ3923	KF291110
Hygroaster* aff. *nodulisporus	**Brazil**	**JS493 INPA285628**	–
Hygroaster albellus	Puerto Rico	AFTOL1997 CFMR6377	KF381521
Hygroaster nodulisporus	Belize	DJLBZ178 CFMR4385	KF291152
Hygrocybe conica	England	KM90423	EU784298
Hygrophorus eburneus	Sweden	LAS94_111	AY463485
Neohygrocybe fumosa	**Brazil**	**JS600 FLOR67460**	–
Neohygrocybe griseonigra	China	GDGM44492	MG779451
Neohygrocybe griseonigra	China	GDGM44493	NR165214
Neohygrocybe ingrata	England	KM126941	EU784316
Neohygrocybe ingrata	England	KM127311	EU784317
Neohygrocybe ingrata	USA	TN62 DJL05TN62	KF381525
Neohygrocybe ingrata	Wales	GWG 23-10-06	KF291225
Neohygrocybe ingrata	USA	ECV4011	KY777401
Neohygrocybe ingrata	USA	MO #308217	MG926553
Neohygrocybe nitrata	Germany	Lueck17	KP965781
Neohygrocybe nitrata	Hungary	H39	FM208885
Neohygrocybe nitrata	England	KM126121	EU784333
Neohygrocybe ovina	Wales	K(M) 187568	KF291228
Neohygrocybe ovina	Wales	GWG	KF291233
Neohygrocybe subovina	USA	DJL04TN16 GRSM77065	KF291140
Neohygrocybe subovina	USA	CFMR NC-61	KF291136
Porpolomopsis aff. *calyptriformis*	USA	ECV4071	KY777398
Porpolomopsis aff. *calyptriformis*	USA	DJL05TN80	KF291246
Porpolomopsis calyptriformis	Hungary	isolate H3	FM208854
Porpolomopsis calyptriformis	England	KM133777	EU784284
Porpolomopsis calyptriformis	Canada	3775	KM248873
Porpolomopsis calyptriformis	USA	MO235549	KX010429
Porpolomopsis calyptriformis	England	KM106775	EU784283
Porpolomopsis calyptriformis	England	CFMRENG3	KF291242
Porpolomopsis calyptriformis	England	KM82282	EU784285
Porpolomopsis lewelliniae	Thailand	TJB10034	KF291238
Uncultured ectomycorrhizal fungus	USA	man56soilD01	GU328579
Uncultured ectomycorrhizal fungus	Madagascar	Trich163388a	KP754118
Uncultured ectomycorrhizal fungus	Madagascar	Trich 62 536a	KP754067
Uncultured fungus clone	Canada	SG035 A02	KP889772
Uncultured fungus clone	USA	OcAMayG06	GU174424

Taxon	Country	Voucher	ITS Accession number
Uncultured fungus clone	USA	AlbAAugH06	GU174318
Uncultured fungus clone	China	NLR34	KF412336
Uncultured fungus clone	China	NLR39	KF412340
Uncultured fungus clone	USA	JR44	KC791061
Uncultured *Humidicutis*	USA	cl6 Bart1231S	HQ021981
Uncultured *Humidicutis*	USA	cl1 Bart677S	HQ021841
Uncultured *Tricholoma* clone	Australia	BH3222F	JF960847
Uncultured *Tricholoma* clone	Australia	BH2039R	JF960848

Results

Taxonomy

Humidicutis pindoramensis J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 21 a, b, c, d, e and 22 a, b, c)

Etymology: Refers to Pindorama, from *tupi-guarani* origin, which is considered the indigenous name for Brazil.

Holotype: BRAZIL, Santa Catarina, Itapoá, Reserva Particular do Patrimônio Natural Volta Velha, Trilha da casa de vidro, 26°05'23.4"S 48°38'18.5"W, 18 November 2012, *Cardoso, J.S. & Neves, M.A.* 18 (FLOR57148, holotype).

Diagnosis: Basidiomata green to orange, pileus umbonate, lamellae with orange edges, stipe with orangish small fibrils, context turning bluish when cut, growing solitary in white sandy soils.

Description:

Pileus 22–35 mm diam., umbonate to papillate or plane with an umbo, sometimes with a rupture at the center, smooth to finely fibrillose, moist to dry, hygrophanous, moss green (oac41) to olive green (oac866, oac867) at the centre, becoming orange (oac838, oac775, oac789) towards the margin, turning light brown (oac777) to brown (oac749) with age; margin even or slightly raised and splitting, sometimes eroded, transluscent-striate, orange (oac761), brown-orangish (oac842) with green tints or light brown (oac799). *Lamellae* uncinate, subdistant, up to 3 mm broad, slightly intervenose, with interveined faces, thick, aspect very waxy, olive green (oac867, oac859) to moss green (oac41) near insertion to

pileus, then yellowish-green (oac887) and yellowish-orange (oac852, oac853) near the edge; edge entire, sometimes forking near insertion to stipe, orange (oac761, oac789); lamellulae of two lengths. *Stipe* 20–50 mm × 3–4 mm, central, regular to irregular, hollow, sometimes with longitudinal fissure, dry, smooth to slightly fibrillose, green (oac40) to light-green (oac67, oac851, oac21) at the apex, to yellowish (oac855) or orange (oac790, oac791) at the base, with orangish (oac845, oac810) fibrils. *Context* of the pileus becoming slightly blue after cut. *Basidiomata* becoming pink-orange when dry, solitary on sandy soils.

Basidiospores 6.6–*7.98*–11.0 × 4.0–*5.61*–7.0 µm, Q = 1.422–*1.450*–1.470, ellipsoid, guttulate, thin walled, hyaline, inamyloid, some germinating when still attached to the sterigmata. *Basidia* (29.0–)34.0–52.1 × 5.9–11.0 µm, cylindro-clavate, funnel-shaped at the base, guttulate, hyaline, 4-spored, sterigmata long, up to 13.0 µm, with conspicuous basal clamp-connections of the toruloid form. *Lamellar edge* fertile. *Cystidia* absent. *Lamellar trama* regular to subregular, with parallel, slightly divergent hyphae, with some inflated elements, 33.0–197.0 × 3.0–40.0 µm, clamp connections absent. *Pileipellis* a cutis composed of slightly interlaced parallel hyphae, 3.0–9.4 µm diam., with encrusted pigments, some protruding, with many branching and occurrence of anastomosis, hyaline in KOH, light yellow in water, clamp connections absent. *Stipitipellis* a cutis of parallel hyphae 3.1–18.0 µm diam., cylindrical, septate, with encrusted pigments, with many anastomosis, upper layer of thin hair-like interwoven hyphae, 1.0–3.2 µm diam., protruding, hyaline in KOH, clamp connections absent.

Specimen examined: BRAZIL, Santa Catarina, Itapoá, Reserva Particular do Patrimônio Natural Volta Velha, Trilha da casa de vidro, 26°05'23.4"S 48°38'18.5"W, 18 Novemeber 2012, *Cardoso, J.S. & Neves, M.A.* 18 (FLOR57148); *Cardoso, J.S. & Neves, M.A.* 20 (FLOR 57150); Amazonas, Manaus, Base do Alto Cuieiras INPA, 2°34'06.7"S 60°19'15.2"O, 12 July 2018, *Cardoso, J.S. & Vieira, S.S.* 485 (INPA285626).

Distribution: Atlantic Rainforest in Santa Catarina state and Amazon Forest in Amazonas state.

Habitat: Growing in white sand forests, found in the *restinga* and *campinarana* vegetation types.

Comments: There are seven green *Humidicutis* species described worldwide, with only one recorded for South America, *Humidicutis multicolor* (Berk. & Broome) E. Horak, which was described for Sri Lanka with records for Tierra del Fuego in Southern Argentina and New

Figure 21. a, b, c, d, e. *Humidicutis pindoramensis*; a, b, c. JS485; d, e. JS18. f, g, h. *Neohygrocybe fumosa* (JS600). Scale = 10mm. Photos d and e by M.A. Neves.

Zealand (Horak, 1979; Horak, 1990). *Humidicutis multicolor* also shows a blue pileus context, but differs by having purple-lilac-blue pigments in the pileus and stipe, and by the much smaller basidiospores (5.5–7 × 4–5 µm) and basidia (20–45 × 6–7 µm) (Horak, 1990). *Humidicutis pindoramensis* is macroscopically similar to *Humidicutis luteovirens* (Horak) Horak from New Zealand but the latter has smaller basidiospores (6–8 × 3.5–4.5 µm) and basidia (25–42 × 6–7 µm) (Horak, 1990). The Australian species *Humidicutis arcohastata*

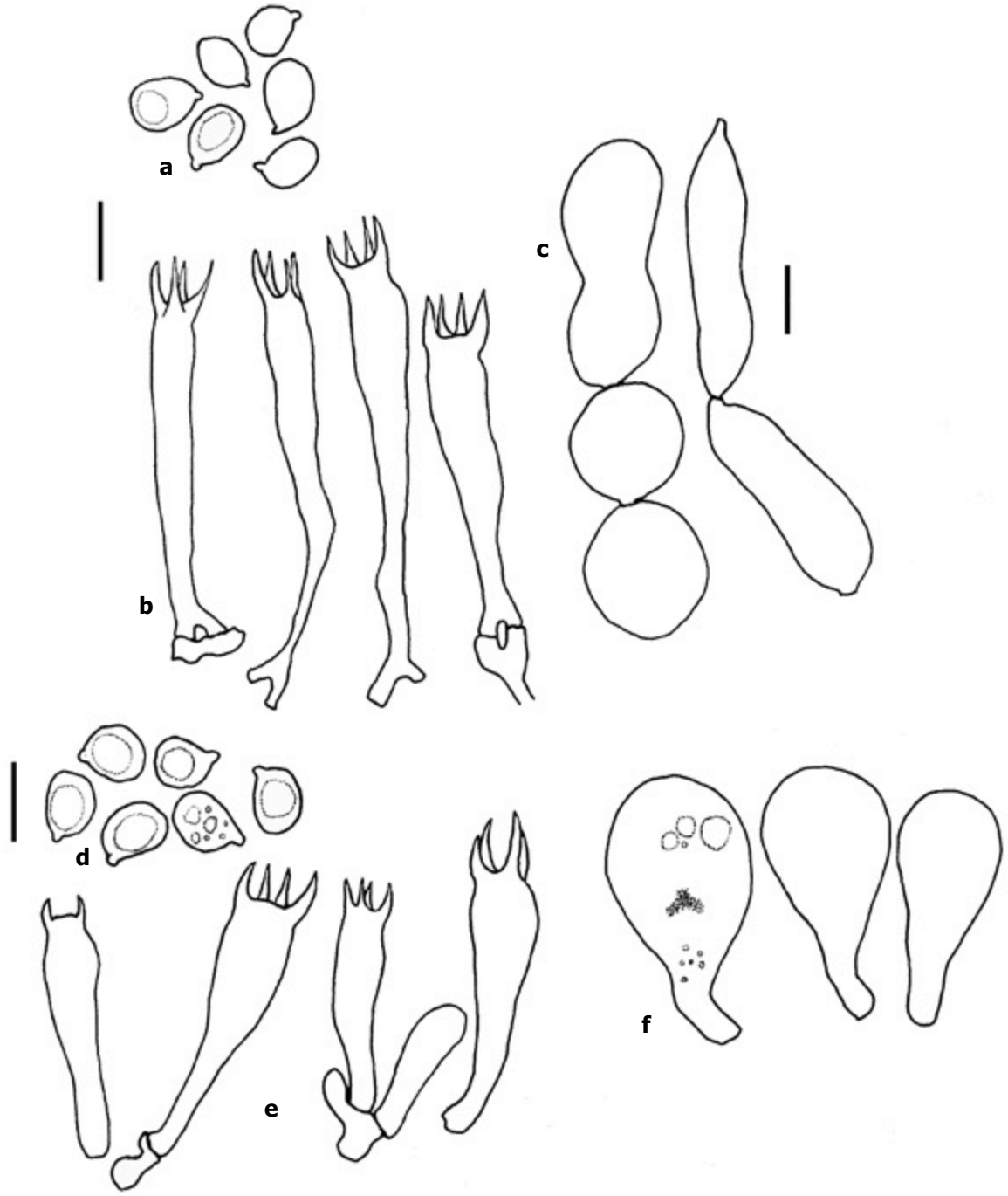

Figure 22. a, b, c. *Hygrocybe pindoramensis* (JS485). a. basidiospores, b. basidia, c. lamellar trama hyphae. d, e, f. *Neohygrocybe fumosa* (JS600). d. basidiospores, e. basidia, f. cheilocystidia. Scale = 10 μm.

(A.M. Young) A.M. Young differs from *H. pindoramensis* in the conical to campanulate pileus and in the presence of purple-mauve tints on the pileus and stipe (Young, 2005). *Humidicutis helicoides* (A.M. Young) A.M. Young has lime-green lamellae rather than green to yellow-orangish lamellae with a deep orange edge (Young, 2005). Also, *H. pindoramensis*

lacks the spiral bands of encrusted pigments found in the pileipellis hyphae of *H. helicoides*. *Humidicutis taekeri* (A.M. Young) A.M. Young is easily distinguished from *H. pindoramensis* by its brilliant orange lamellae and *Humidicutis viridimagentea* A.M. Young & K. Syme differs by the distinctive magenta colouration (Young, 2005; Young & Syme, 2008). Finally, *Humidicutis woodii* (A.M. Young) A.M. Young has a white stipe and lacks the conspicuous toroidal clamp connections (Young, 2005) found at the basidia base of *H. pindoramensis*.

Humidicutis pindoramensis was described based on two specimens from Southern Brazilian Atlantic Rainforest and one from the Amazon forest. Basidiomata were encountered on white sand soils, so this species is probably adapted to soils poor in nutrients. The germinating basidiospores found in one of the specimens (JS20) analysed are very intriguing, and no records of such phenomena were found in literature, except for the specimens studied by Cardoso (2017) and in this present study. This is the second species of *Humidicutis* known for South America, with only one species previously known for Tierra del Fuego in Argentina, collected and described by Horak (1979). *Humidicutis* is now known to occur in Atlantic Rainforest and Amazon biogeographical regions of Brazil, amplifying the distribution range of the genus.

Neohygrocybe fumosa J.S. Cardoso, M.A. Neves & J.S. Oliveira, **sp. nov.** (Figures 21 f, g, h; 22 d, e, f and 23)

Etymology: From Latin "fumosus" = smoky, grey, changing to brown, due to the greyish light brown pileus.

Holotype: BRAZIL, Mato Grosso State, Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 9 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 600 (FLOR67460, holotype).

Diagnosis: Basidiomata dull coloured, pileus umbonate, greyish brown, lamellae pale greyish-brown, stipe light silver grey, basidiomata with a distinct nitrous smell and without colour change reactions.

Description:

Pileus 30–44 mm diam., plane-convex, umbonate, sometimes tearing in the centre, slightly fibrillose, moist to dry, becoming transluscent-striate towards margin, hygrophanous, light

grey brown (oac730, oac729) to brown (oac748, oac749); margin transluscent-striate, uplifted to revolute, undulating, eroded, pale grey (oac732). *Lamellae* uncinate, broad, up to 8mm broad, thick, white with shades of grey brown (oac711, oac718), intervenose, with veins projecting in the lamellar faces; lamellulae of two lengths, anastomosing. *Stipe* 75–83 × 5–7 mm, central, flexuous, hollow, smooth, glabrous, moist to dry, silky, tapering, light silver grey (oac774, oac690) to whitish. *Odour* nitrous. *Solitary* on clay soils.

Basidiospores 6.8–*7.99*–9.1 × 5.2–*6.08*–7.1 µm, Q = 1.315, ellipsoid, smooth, hyaline, inamyloid, thin-walled, guttulate, hilar appendage visible. *Basidia* 26.8–36.4 × 6.3–9.7 (–10.8) µm, clavate, thin-walled, hyaline, 2–4-spored, sterigmata up to 7µm, with basal regular clamp connections. *Lamellar edge* fertile. *Cheilocystidia* 32.8–35.6 × 16.6–22.1 µm, pyriform, like a swollen basidiole, sometimes guttulate. *Pleurocystidia* absent. *Pseudocystidia* 96.7–219.3 × 15.1–23.6 µm, obclavate to ventricose-rostrate, apex sometimes with cellular contents, projecting up to 40 µm above basidia and basidioles (Figure 22). *Lamellar trama* regular, composed of parallel inflated elements, 40.2–233.2 × 8.6–33.8 µm, clamp connections present. *Pileipellis* a cutis, with parallel, undifferentiated hyphae, 3.3–7.0 µm diam., some with encrusted pigments, pale brownish in water, hyaline in KOH, clamp connections present. *Stipitipellis* a cutis, hyphae 2.3–15.9 µm diam., hyaline in KOH and water, with rare encrustations, clamp connections present.

Specimens examined: BRAZIL, Mato Grosso, Alta Floresta, Reserva Particular do Patrimônio Natural Cristalino, Trilha do Dr. Haffer, 9º3”10'S 55º54”53'W, 25 January 2018, *Cardoso, J.S.* 277 (FLOR63574); Novo Mundo, Reserva Particular do Patrimônio Natural Cristalino, Trilha da Castanheira, 9 January 2019, *Cardoso, J.S. & Furtado, A.N.M.* 600 (FLOR67460).

Distribution: Known only from the type locality.

Habitat: On *terra-firme* forest, clay soil.

Comments: *Neohygrocybe* comprises about 10 species (Lodge pers comm.) which are mostly from Europe. There are at least two species from the neotropical region: *Neohygrocybe subovina* (Hesler & A.H. Sm.) Lodge & Padamsee and *Hygrocybe ovinoides* Lodge, S.A. Cantrell & T.J. Baroni (Cantrell & Lodge, 2004). *Hygrocybe ovinoides* is clearly a *Neohygrocybe* species (Lodge pers. comm.), but not formally combined. *Neohygrocybe fumosa* is the first species of the genus described from Brazil. *Neohygrocybe subovina*, from USA, also has cheilocystidia and pseudocystidia projecting from the hymenium, but the basidiomata are much darker in colour, the lamellae bruise black, the basidiospores are

globose to subglobose rather than ellipsoid, and the cheilocystidia is vermiform and cylindrical rather than pyriform (Cantrell & Lodge, 2004). *Hygrocybe ovinoides* produce very small basidiomata also dark in colour, but the pileus has a white margin (Cantrell & Lodge, 2004). In the microscopy, *H. ovinoides* lacks cheilocystidia and has hook-like pileocystidia (Cantrell & Lodge, 2004). Both species mentioned above lack the nitrous odour found in *N. fumosa.* Only two specimens were found over 3 field excursions of the Cristalino Fungi Project, held at RPPN Cristalino, implying that *N. fumosa* is a rather uncommon species.

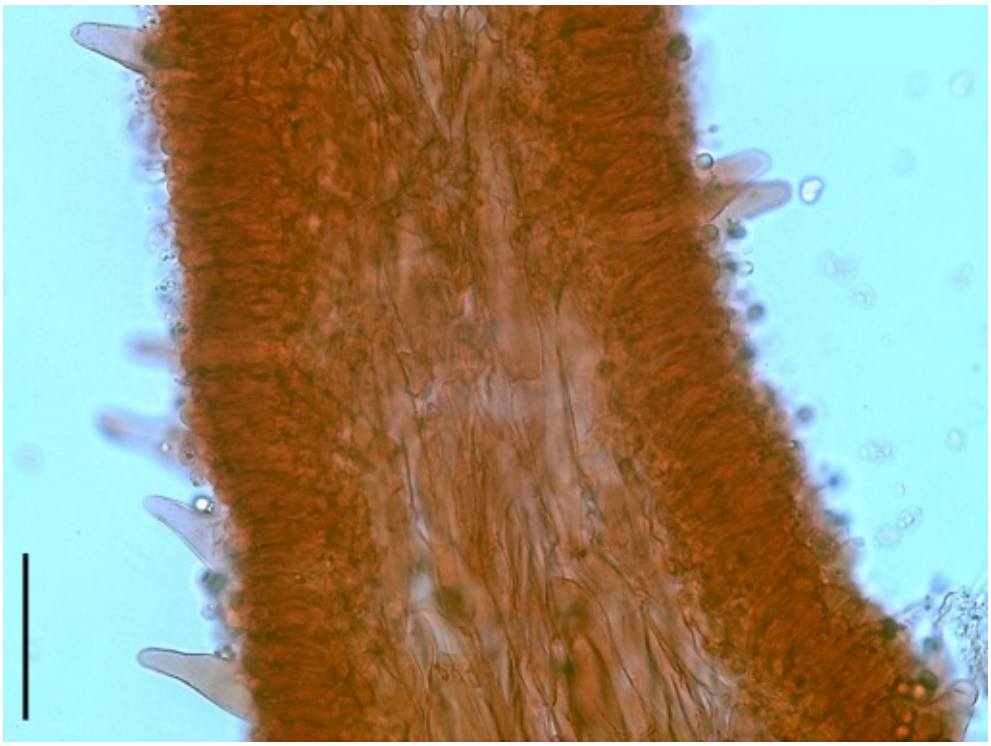

Figure 23. Detail of the lamellar trama showing the pseudocystidia emerging from the hymenium of *N. fumosa* (JS600). Scale = 50µm.

Phylogeny

The phylogenetic analyses conducted in this study resulted in trees that are mostly congruent to those by Lodge *et al.* (2013). The large trees are a backbone (Figures 24 and 25) and, as expected for the ITS alone in DATASET1, the trees are not fully resolved and/or some nodes are unsupported. Despite this fact, all *Humidicutis* taxa + close "undetermined strains" (possibly *Humidicutis* OTUs) formed a clade at the top of both trees (Figures 24 and 25), with strong support (pp = 0.99) in the BA tree (Figure 24). This clade bearing *Humidicutis* is sister

to a clade grouping many *Gliophorus* taxa, but without support. *Gliophorus* also appear polyphyletic in both ML and BA trees, but it is monophyletic with strong support in the 4-gene analyses in Lodge *et al.* (2013). *Neohygrocybe* also did not form a single clade, appearing scattered into multiple branches in a polytomy at the bottom of the DATASET1 trees. In the multilocus analyses as well as in the ITS-LSU analysis conducted by Lodge *et al*. (2013) *Neohygrocybe* appears as a monophyletic clade with strong support, but support is low in the ITS only analysis. *Hygrocybe* grouped as sister to *Hygroaster* (Figures 24 and 25) with strong support and this relationship *Hygrocybe–Hygroaster* (Tribe Hygrocybeae) agrees with Lodge *et al.* (2013). *Humidicutis*, *Gliophorus*, *Porpolomopsis* and *Neohygrocybe* would group together forming a clade (Tribe Humidicuteae) in Lodge *et al.* (2013), which is sister to Tribe Hygrocybeae. Unfortunately, very few sequences of *Humidicutis* and *Neohygrocybe* are available in GenBank, being the most part from European and North American taxa.

Based on the backbone trees of DATASET1, another analysis with *Humidicutis* (DATASET2) taxa display the relationship of *H. pindoramensis* with closely related species (Figure 26). In both ML and BA trees, *Humidicutis* species formed two strongly supported clades and one grade: “Humidicutis marginata” clade, with sequences from USA; “Humicutis auratocephala+neotropical spp.”, with *H. auratocephala* from USA and sequences from Central and South America, including *H. pindoramensis*; and a grade with undetermined taxa from China, Madagascar, Brazil and Australia. It is interesting to note that the neotropical species grouped in the same clade. *Humidicutis pindoramensis* is sister to *H. auratocephala*, but without support, or to the clade comprising species from Belize, Puerto Rico and Ecuador (Figure 26).

In the BA and ML trees of DATASET3 (Figure 27), *Neohygrocybe fumosa* appears independent amongst the closest taxa, but closely related to *N. ingrata* (J.P. Jensen & F.H. Møller) Herink of strains from Europe and USA than to *N. nitrata* (Pers.) Kovalenko of strains from Germany, Hungary and Wales (Europe). Hopefully, with more sequences from the neotropical region, the phylogenetic relationship of *N. fumosa* with other closely related taxa will be elucidated.

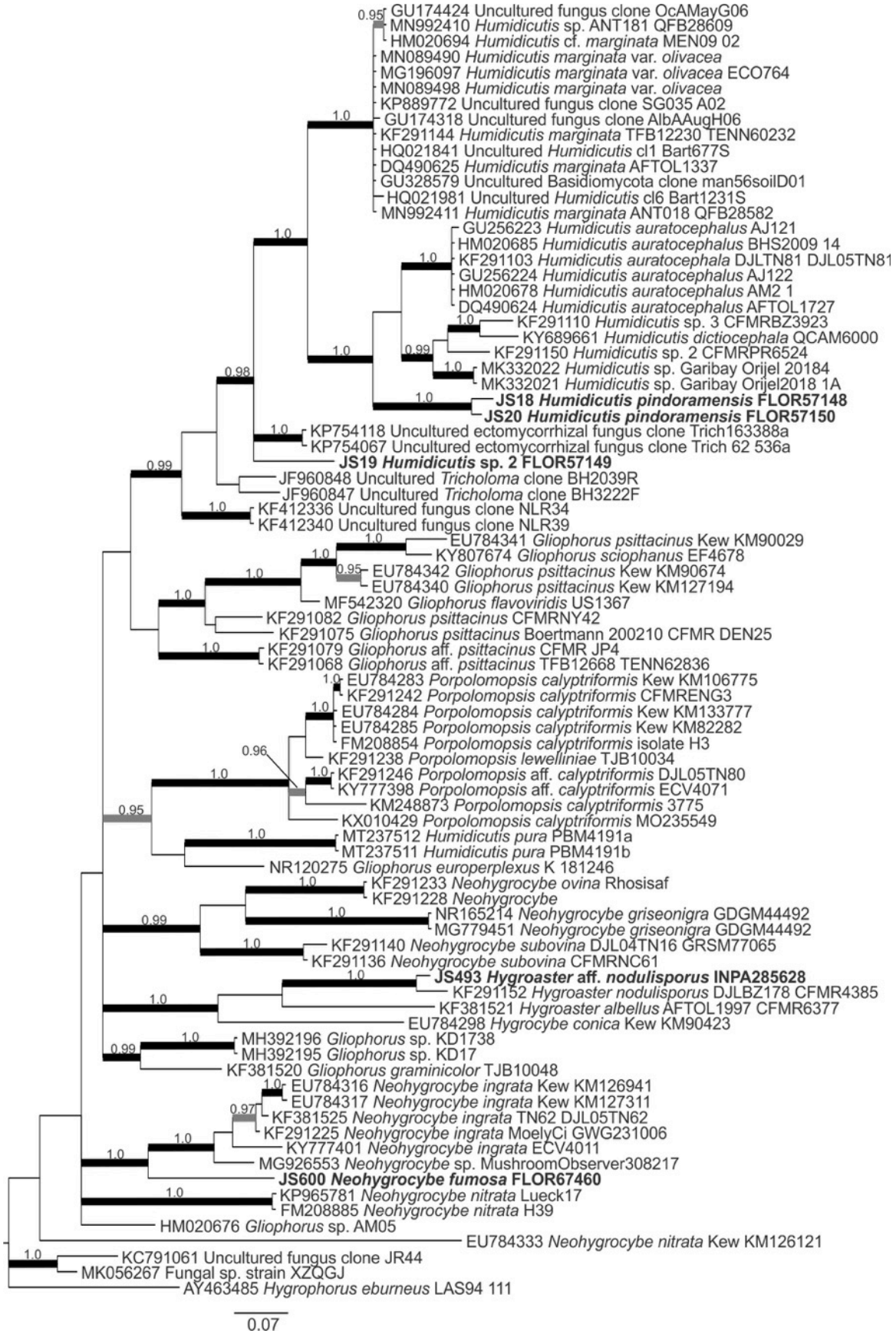

Figure 24. 50 % majority-rule consensus BA phylogenetic tree of *Hygrocybe* s.l. using ITS sequences. Branches in black bold are those with pp > 0.97 and branches in grey bold are those with pp = 0.95–0.97.

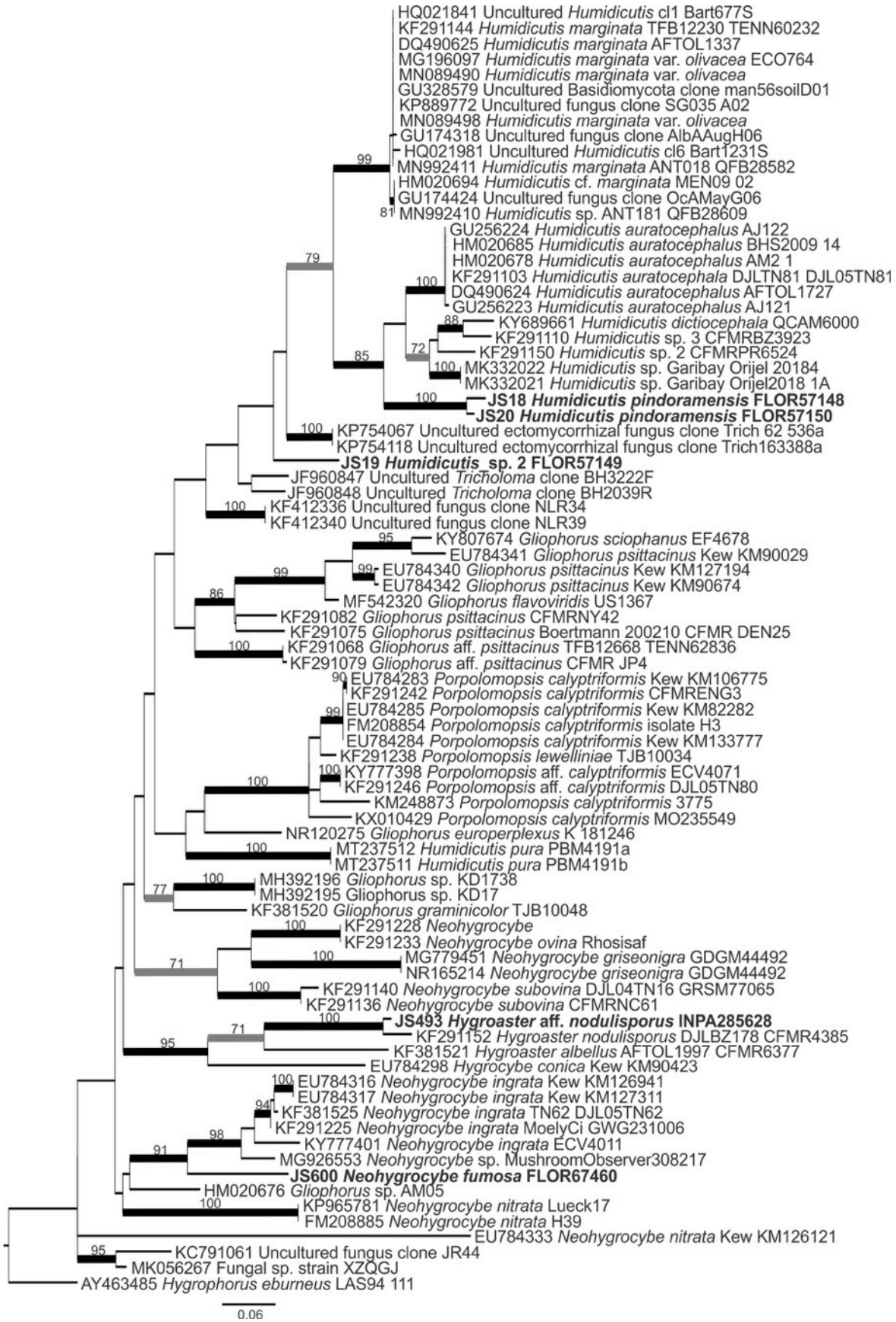

Figure 25. ML phylogenetic tree of *Hygrocybe* s.l. using ITS sequences. Branches in black bold are those with bootstrap > 80%, branches in grey bold are those with bootstrap 70–80%.

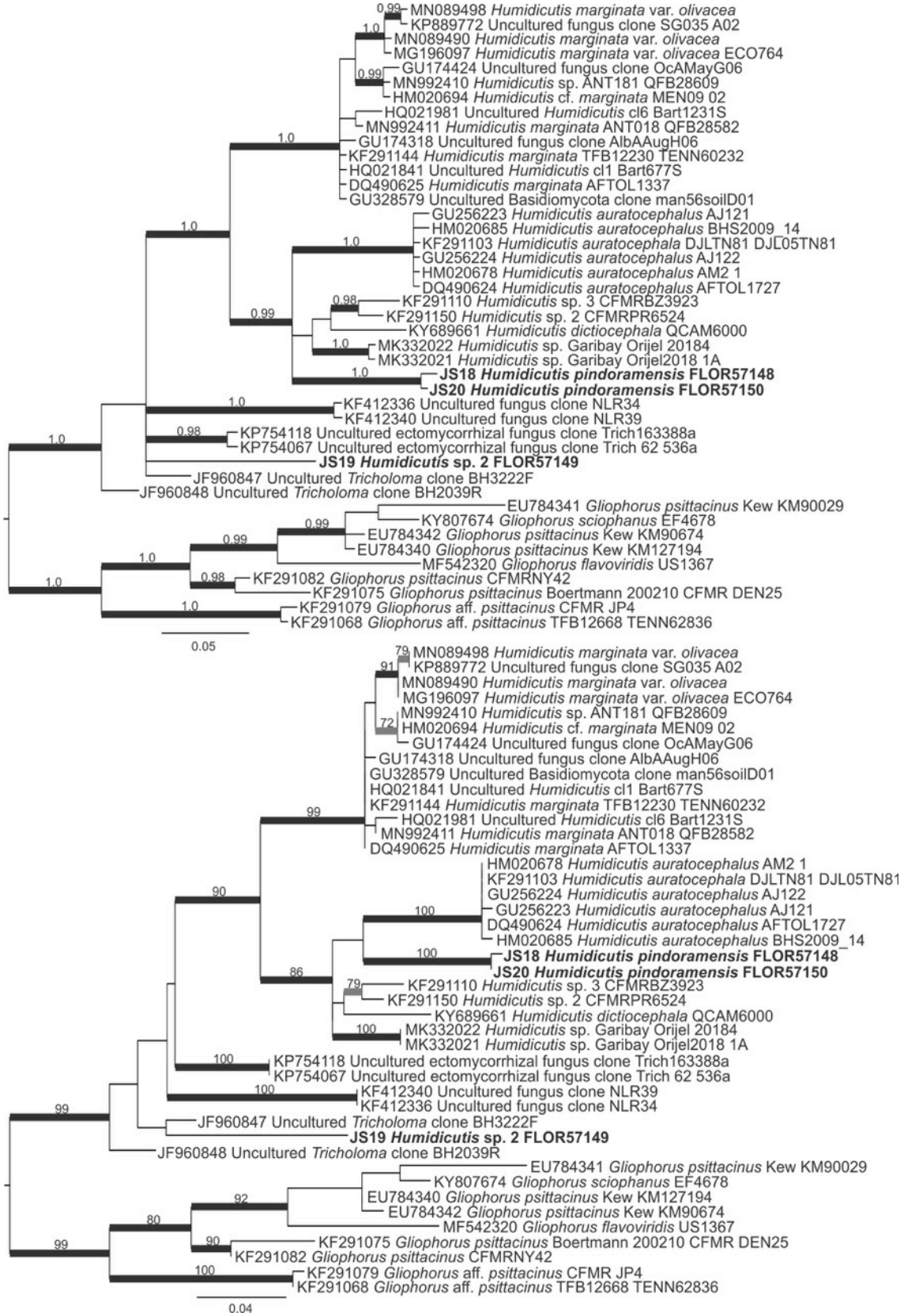

Figure 26. 50 % majority-rule consensus BA (top) and ML (bottom) phylogenetic trees of *Humidicutis* using ITS sequences. Branches in black bold are those with pp > 0.97 and bootstrap > 80%, respectively, branches in grey bold are those with pp 0.95–0.97 and bootstrap 70–80%.

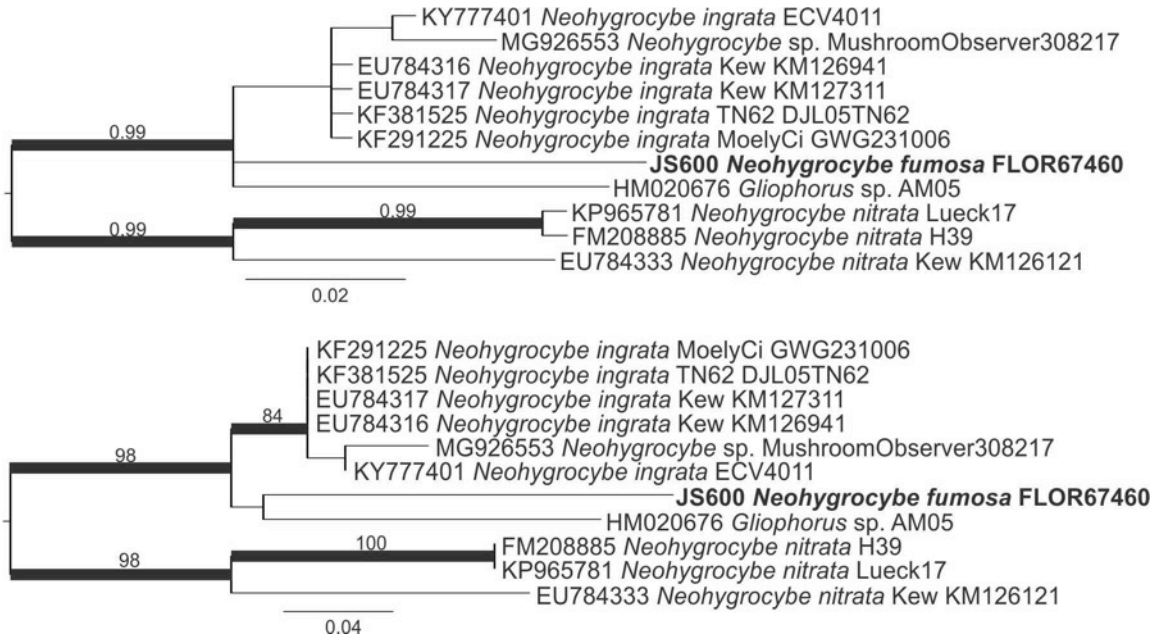

Figure 27. 50 % majority-rule consensus BA (top) and ML (bottom) analyses of *Neohygrocybe* ITS sequences. Branches in bold are those with pp > 0.97 and bootstrap > 70%, respectively.

4. Síntese

Foram coletados 278 espécimes em 11 áreas de Floresta Amazônica totalizando 21 expedições a campo. Dos espécimes coletados, 60 foram observados em microscópio óptico. Das 206 amostras enviadas para procedimentos de biologia molecular, foi possível extrair o DNA de 98 espécimes armazenados em *FTA® Cards*, dos quais foram obtidas 46 sequências de ITS. Os espécimes observados foram identificados em 12 táxons, sendo 10 novas espécies para a ciência, cujos nomes foram propostos neste trabalho, e 2 novos registros foram feitos para os estados de Mato Grosso e Pará (Tabelas 5 e 6).

Tabela 5. Breve síntese dos resultados em números

Síntese dos resultados		
Espécimes coletados		278
Morfotipos		86
Espécies novas descritas		10
Novos registros		2
Análise microscópica		60
Sequências ITS obtidas		46
Áreas de coleta		11
Expedições	Arquipélago Mariuá	1
	Rio Cristalino	2
	FLONA Tapajós	1
	INPA	3
	MUSA	4
	PARNA Viruá	1
	Ramal Pau Rosa	1
	RDS Rio Negro	1
	Reserva Ducke	3
	Rio Cuieiras	3
	UFAM	1
	Total	21

Os nomes para as espécies novas são provisórios e não há intenção em validar sua publicação neste presente trabalho, de acordo com os artigos 30.9 e 36.1 do Código Internacional de Nomenclatura para Algas, Fungos e Plantas (Turland *et al*., 2018). Uma lista das espécies de *Hygrocybe* s.l. para o Brasil foi disponibilizada (Tabela 7). Chaves de identificação são apresentadas nos capítulos 1 e 2 e análises filogenéticas são incluídas no capítulo 3.

Tabela 6. Número de espécimes coletados por gênero e por local de coleta.

Local de coleta	**Gêneros coletados**					**Total**
–	*Humidicutis*	*Hygroaster*	*Hygrocybe*	*Neohygrocybe*	Hygropho-raceae indet	5
Arquipélago Mariuá			8			8
RPPN Cristalino			147	3	3	153
FLONA Tapajós			20			20
INPA			6			6
MUSA			7	1		8
PARNA Viruá		1	27			28
Ramal Pau Rosa			1			1
RDS Rio Negro			3			3
RFAD			12		1	13
Rio Cuieiras	1		35	1		37
UFAM		1				1
Total	1	2	266	5	4	278

Foi também observada a presença de três complexos de espécies, *Hygrocybe* "conica/astatogala group", *Hygrocybe occidentalis* e *Hygrocybe trinitensis*. Devido a necessidade de um estudo mais aprofundado desses táxons, eles não foram inseridos nas descrições deste trabalho. Materiais de outros táxons, como *Hygrocybe* cf. *papillata*, *Hygroaster* aff. *nodulisporus*, *Hygroaster* sp. 2 e *Hygrocybe* aff. *martinicensis*, foram observados, mas igualmente não foram inseridos neste trabalho por necessidade de novas coletas, seja por amostragem insuficiente, por estarem estéreis ou por estarem com basidiósporos e basídios colapsados. Materiais de *Hygrocybe* aff. *hypohaemacta* também foram observados para o Amazonas e Mato Grosso, mas não foram inseridos no trabalho. Este táxon, com ocorrência para América Central e América do Sul é provavelmente uma espécie distinta (Lodge & Ovebro, 2008; Lodge *et al*. 2013) da descrita para Singapura por Corner (1936), e maiores estudos são necessários para descrevê-lo como uma espécie nova. Porém, a sequência obtida neste estudo diverge da sequência disponibilizada no GenBank por D.J. Lodge (GenBank EU435150), com somente 79% de identificação, apesar de mostrar uma cobertura de busca de 100%.

Tabela 7. Lista de espécies de *Hygrocybe* s.l. ocorrentes no Brasil. Em negrito as espécies com ocorrência para Amazônia. FA = Floresta Amazônica, MA = Mata Atlântica, SA = Semi-árido, CR = Cerrado. *Brejo de altitude

Táxon	Estado	Bioma	Referências
Gliophorus psittacinus (Schaeff.) Herink [como *Hygrocybe psittacina* (Schaeff.) P.Kumm.]	PR	MA	Meijer (2008)
***Humidicutis pindoramensis* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**AM, SC**	**FA, MA**	**Presente estudo**
***Hygroaster albellus* Singer**	**AM**	**FA**	**Singer (1989)**
Hygrocybe acutoconica (Clem.) Singer	SC, PB	MA	Cardoso (2017)
***Hygrocybe amazoniensis* Singer**	**AM**	**FA**	**Singer (1989)**
Hygrocybe arnoldsii de Meijer	PR	MA	Meijer (2008)
Hygrocybe atrosquamosa Pegler	PE	MA	Lodge & Pegler (1990)
Hygrocybe aurantiomagnifica Silva-Filho & Wartchow	PB	MA*	Silva-Filho *et al*. (2019)
Hygrocybe batistae Singer	SC, PR, RJ, PE, PB	MA	Singer (1965), Lodge & Pegler (1990), Magnago *et al*. (2015), Cardoso (2017)
***Hygrocybe campinaranae* Singer**	**AM**	**FA**	**Singer (1989)**
***Hygrocybe cantharellula* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**AM, RR, PA**	**FA**	**Presente estudo**
Hygrocybe chlorophana (Fr.) Wünsche	RS	MA	Raithelhuber (1992)
Hygrocybe conica (Schaeff.) P. Kumm.	RS, SC, PR, SP	MA	Karstedt & Stürmer (2008), Meijer (2008), Cardoso (2017)
***Hygrocybe cristalinensis* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**MT**	**FA**	**Presente estudo**
Hygrocybe firma (Berk. & Broome) Singer	SP	MA	Pegler (1997)
Hygrocybe flavolutea (Murrill) Pegler	SP	MA	Pegler (1997)
***Hygrocybe hololeuca* Singer**	**AM, PA**	**FA**	**Singer (1989), presente estudo**
Hygrocybe hypohaemacta (Corner) Pegler	PR, PB	MA, SA	Meijer (2008), Neves *et al*. (2013)
Hygrocybe ignipileata Pegler	SP	MA	Pegler (1997)
Hygrocybe lugubris (Rick) Raithelh.	RS	MA	Raithelhuber (1992)

Táxon	Estado	Bioma	Referências
Hygrocybe magnifica de Meijer	PR	MA	Meijer (2008)
***Hygrocybe martinicensis* Pegler & Fiard**	**SP, AM**	**MA, FA**	**Pegler (1997), Silva-Filho *et al*. (2019)**
Hygrocybe megistospora Singer	GO	CR	Singer (1989)
Hygrocybe melleofusca Lodge & Pegler	PR	MA	Meijer (2008)
Hygrocybe mexicana Singer f. *mexicana*	PR	MA	Meijer (2008)
Hygrocybe miniata (Fr.) P. Kumm.	SP, RS	MA	Pegler (1997), Raithelhuber (1992)
***Hygrocybe musaensis* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**AM**	**FA**	**Presente estudo**
***Hygrocybe mutabilis* Singer**	**AM**	**FA**	**Singer (1989)**
Hygrocybe naranjana Pegler	SP	MA	Pegler (1997)
Hygrocybe neofirma S.A. Cantrell & Lodge	RJ	MA	Albuquerque *et al*. (2007)
***Hygrocybe occidentalis* (Dennis) Pegler**	**SC, PR, SP, RO, AM, PB**	**MA, FA**	**Capelari & Maziero (1988a), Meijer (2008), Pegler (1997), Souza & Aguiar (2004), Magnago *et al*. (2015), Cardoso (2017)**
Hygrocybe paraibensis Singer	PE	MA	Singer (1965)
Hygrocybe parvula (Peck) Pegler	PR, SP	MA	Meijer (2008), Pegler (1997)
***Hygrocybe pegleri* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**MT, SC**	**FA, MA**	**Presente estudo**
Hygrocybe pernambucensis (Bat. & Vital) Putzke	PE	MA	Batista (1957): 127
Hygrocybe prieta Lodge & Pegler	RJ	MA	Albuquerque *et al*. (2007)
Hygrocybe rhodoleuca Singer	PB	MA	Singer (1973)
***Hygrocybe rubroalba* Picciola, Battistin & Vizzini**	**PR, MT**	**MA, FA**	**Vizzini *et al*. (2015), presente estudo**
Hygrocybe silvae-araucariae de Meijer	PR	MA	Meijer (2008)
***Hygrocybe siparia* (Berk.) Singer**	**PE, AM, SP**	**MA, FA**	**Singer (1965), Pegler (1997)**
***Hygrocybe sourellae* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**MT**	**FA**	**Presente estudo**
***Hygrocybe spinosospora* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**MT**	**FA**	**Presente estudo**

Táxon	Estado	Bioma	Referências
Hygrocybe subcaespitosa (Murrill) Lodge & Pegler	PB	MA	Magnago (2015)
Hygrocybe subflavida (Murrill) Pegler	PR	MA	Meijer (2008)
Hygrocybe subminutula (Murrill) Pegler	PE	MA	Putzke & Putzke (2017)
Hygrocybe subpsittacina (Rick) Raithelh.	RS	MA	Raithelhuber (1992)
***Hygrocybe trinitensis* (Dennis) Pegler**	**SP, AM, PB**	**MA, FA**	**Pegler (1997), Komura *et al.* (2017), Magnago *et al.* (2015)**
Hygrocybe troyana (Murrill) Courtec.	PR	MA	Meijer (2008)
***Hygrocybe vinaceosquamulosa* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**MT**	**FA**	**Presente estudo**
***Hygrocybe viridilacerata* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**MT, SC**	**FA, MA**	**Presente estudo**
***Hygrocybe viridis* Capelari & Maziero**	**PR, RO**	**MA, FA**	**Capelari & Maziero (1988b), Meijer (2008)**
***Neohygrocybe fumosa* J.S. Cardoso, M.A. Neves & J.S. Oliveira sp. nov.**	**MT**	**FA**	**Presente estudo**

5. Conclusões

Neste estudo, foram identificados 12 táxons distribuídos em três gêneros. Dez novas espécies são propostas: *Humidicutis pindoramensis*, *Hygrocybe cantharellula*, *Hygrocybe cristalinensis*, *Hygrocybe musaensis*, *Hygrocybe pegleri*, *Hygrocybe sourellae*, *Hygrocybe spinosispora*, *Hygrocybe vinaceosquamulosa*, *Hygrocybe viridilacerata* e *Neohygrocybe fumosa*. Foram obtidas sequências ITS de sete delas, servindo como *barcode*. *Hygrocybe* é relatado pela primeira vez para os estados de Roraima e Pará; *Humidicutis* é relatado pela primeira vez para Amazonas e para a Floresta Amazônica; e *Neohygrocybe* é relatado pela primeira vez para o Brasil. *Hygrocybe rubroalba* é primeiro registro para Mato Grosso e Floresta Amazônica e *Hygrocybe hololeuca* é primeiro registro para Pará e novo registro para o Amazonas. *Humidicutis pindoramensis, Hygrocybe pegleri*, *Hygrocybe rubroalba* e *Hygrocybe viridilacerata* ocorrem tanto na Mata Atlântica quanto na Floresta Amazônica indicando uma conexão biogeográfica para estes táxons. *Hygrocybe cantharellula* e *H. hololeuca* demonstraram ser bem distribuídas na Amazônia central, enquanto que *Hygrocybe*

musaensis foi encontrada apenas em uma localidade, em Manaus, Amazonas. *Hygrocybe cristalinensis*, *H. pegleri*, *H. sourellae*, *H. spinosispora*, *H. vinaceosquamulosa* e *Neohygrocybe fumosa* foram encontradas apenas em Mato Grosso, na Amazônia Meridional.

Com este estudo, foi possível avaliar uma amostragem das espécies de *Hygrocybe* s.l. que ocorrem na Amazônia brasileira, ampliando de nove para 21 o número de espécies conhecidas para esta região e 52 o número de espécies conhecidas para o Brasil. Levando em conta que não foi possível analisar microscopicamente todos os 278 espécimes coletados distribuídos em 86 morfotipos, além da detecção de três grandes complexos de espécies, o número de espécies é certamente muito maior. Esta pesquisa contribui significativamente para o conhecimento da diversidade de *Hygrocybe* s.l. na Amazônia do Brasil e fornece um abrangente e valiosíssimo material para a continuidade do estudo taxonômico e filogenético do gênero.

A nova amostragem de espécimes de *Hygrocybe* s.l. para a Amazônia brasileira realizada neste trabalho contribui muito para o enriquecimento dos herbários e fungários, onde muitas das exsicatas são antigas e encontram-se em mal estado para realização de microscopia. Além disso, é comum a falta de descrições macroscópicas detalhadas ou fotografias dos materiais frescos atreladas às exsicatas. A nova amostragem é importante também pela dificuldade e alto custo de realizar coletas em áreas da Amazônia brasileira .

A região ITS é considerada como um marcador universal para fungos (Schoch *et al.,* 2012) servindo como "código de barras" (*barcode*) na identificação molecular de espécies. Porém, a utilização do ITS para análises filogenéticas é apenas um primeiro passo para a compreensão das relações filogenéticas entre espécies, sendo útil para testar relações entre espécies de um mesmo gênero, mas não para resolver nós mais profundos da filogenia, como a relação entre gêneros, tribos ou famílias, por exemplo. Isso porque a região ITS é muito polimórfica, o que dificulta testar a relação filogenética desses nós mais profundos. Algumas incongruências podem ser observadas nas análises realizadas no capítulo 3. As árvores de BA demonstraram muitas politomias, indicando que mais marcadores são necessários para resolver esses nós mais profundos da filogenia.

Há uma grande lacuna de conhecimento para o estudo de micologia na América do Sul e no Brasil. Muitas espécies são desconhecidas e muitas vezes não há infra-estrutura ou financiamentos necessários para a realização de estudos moleculares, por exemplo. A esmagadora maioria dos dados de *Hygrocybe* s.l. disponíveis na plataforma GenBank é proveniente de sequências do hemisfério norte, mais especificamente, Europa, Estados

Unidos e Canadá. Este trabalho contribuiu com 46 sequências de ITS, aumentando significantemente o número de sequências de *Hygrocybe* s.l. provenientes do Brasil e da Amazônia. As análises filogenéticas para *Hygrocybe* s.s. não foram realizadas neste trabalho, por ser um grupo mais difícil de resolver, e que possui um número de dados muito maior. Porém, as sequências serão disponibilizadas no banco de dados *online GenBank* e utilizadas em futuros trabalhos para a publicação em revistas científicas, conjuntamente com os resultados apresentados neste presente estudo. Com a realização de futuros estudos de filogenia para o grupo, especialmente de *Hygrocybe* s.s., será possível elucidar relações filogenéticas entre as espécies do hemisfério norte e sul, incluindo espécies neotropicais. Além disso, será possível avaliar se as espécies da América do Sul se encaixam nas classificações genéricas e infragenéricas propostas por Lodge *et al.* (2013) ou se novos gêneros, subgêneros, seções ou subseções fornecerão melhora no sistema de classificação.

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P A R E C E R

Aluna: **JULIA SIMON CARDOSO**
Curso: BOTÂNICA
Nível: Mestrado
Orientador: Dr. Jadson J. Souza de Oliveira (INPA) e Coorientadora: Dra. Maria Alice Neves (UFSC)

Título:

"*Hygrocybe sensu lato (Hygrophoraceae, Agaricales) na Amazônia brasileira*"

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Manaus (AM), 12 de Novembro de 2018.

OBS: A banca recomenda que a inclusão dos comentários/sugestões feitos durante a defesa, em especial sobre a metodologia taxonômica e filogenética.

Dr. Michael John Gilbert Hopkins
Coordenador do Programa de Pós-Graduação em Botânica
PO. 116/2017 - DIBOT/INPA

INPA
INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA

DIVISÃO DO CURSO DE PÓS-GRADUAÇÃO EM BOTÂNICA

PÁTRIA AMADA BRASIL GOVERNO FEDERAL

ATA DEFESA PÚBLICA DE DISSERTAÇÃO DE MESTRADO DISCENTE DO PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS (BOTÂNICA) DO INSTITUTO NACIONAL DE PESQUISAS DA AMAZÔNIA.

Aos nove dias do mês de outubro de 2020 às 14:00 horas, através do Google Meet, reuniu-se a Comissão Examinadora da Defesa Pública, composta pelos seguintes membros: Dra. Larissa Trierveiler Pereira, da Universidade Federal de São Carlos (UFSCAR), Dr. Nelson Menolli Junior, do Instituto Federal de Educação, Ciência e Tecnologia de São Paulo (IFSP) e Dra. Tiara Sousa Cabral, do Instituto Nacional de Pesquisas da Amazônia (INPA), tendo como suplentes: Dra. Dirce Leimi Komura, do Instituto Nacional de Pesquisas da Amazônia (INPA) e Dr. Marcelo Aloisio Sulzbacher, sob a presidência do primeiro, a fim de proceder a arguição pública da **DISSERTAÇÃO DE MESTRADO**, intitulada: **"*Hygrocybe* senso lato (Hygrophoraceae, Agaricales) na Amazônia Brasileira"** discente: **Julia Simon Cardoso**, sob orientação: Dr. Jadson José Souza de Oliveira e coorientação: Dra. Maria Alice Neves. Após a exposição, dentro do tempo regulamentar, a discente foi arguida oralmente pelos membros da Comissão Examinadora, tendo recebido o conceito final:

EXAMINADORES	PARECER		ASSINATURA
LARISSA TRIERVEILER PEREIRA	(x) APROVADO	( ) REPROVADO	
NELSON MENOLLI JUNIOR	(x) APROVADO	( ) REPROVADO	
TIARA SOUSA CABRAL	(x) APROVADO	( ) REPROVADO	Tiara Sousa Cabral
DIRCE LEIMI KOMURA	( ) APROVADO	( ) REPROVADO	_____
MARCELO ALOISIO SULZBACHER	( ) APROVADO	( ) REPROVADO	_____

Manaus (AM), 09 de outubro de 2020.

OBS: Os membros da banca irão encaminhar em arquivos separados para orientador e candidata as correções pertinentes na dissertação. Entretanto, membros da banca concordam que o trabalho foi bem realizado e a candidata possui mérito para aprovação.

Nada mais havendo, foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos membros da Comissão Examinadora.

Dr. Michael John Gilbert Hopkins
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PO. 116/2017 - DIBOT/INPA