

UNIVERSIDADE FEDERAL DO PARANÁ

CRISTIANE SEGER

**REVISÃO TAXONÔMICA DO GÊNERO *STROPHARIA* SENSU LATO
(*AGARICALES*) NO SUL DO BRASIL**

CURITIBA

2016

CRISTIANE SEGER

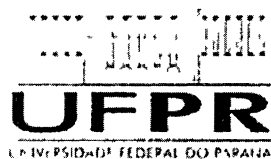
**REVISÃO TAXONÔMICA DO GÊNERO *STROPHARIA* SENSU LATO
(*AGARICALES*) NO SUL DO BRASIL**

Dissertação apresentada ao Programa de Pós-Graduação em Botânica, área de concentração em Taxonomia, Biologia e Diversidade de Algas, Líquens e Fungos, Setor de Ciências Biológicas, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Botânica.

Orientador: Prof. Dr. Vagner G. Cortez

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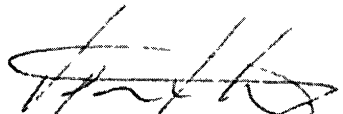
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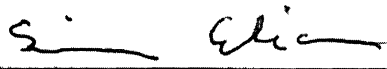
Ata de Julgamento da Dissertação de Mestrado da pos-graduanda **Cristiane Seger** Aos 13 dias do mês de maio do ano de 2016, as nove horas, por meio de videoconferencia, na presença da Comissão Examinadora, composta pelo Dr Vagner Gularte Cortez, pela Drª Paula Santos da Silva e pela Drª Sionara Eliasaro como titulares, foi aberta a sessão de julgamento da Dissertação intitulada "REVISAO TAXONÔMICA DO GÊNERO STROPHARIA SENSU LATO (AGARICALES) NO SUL DO BRASIL" Após a apresentação perguntas e esclarecimentos acerca da Dissertação, a Comissão Examinadora **APROVA O TRABALHO DE CONCLUSÃO** do(a) aluno(a) **Cristiane Seger** Nada mais havendo a tratar, encerrou-se a sessão da qual foi lavrada a presente ata, que, após lida e aprovada, foi assinada pelos componentes da Comissão Examinadora



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Dra. Sionara Eliasaro (UFPR)



UNIVERSIDADE FEDERAL DO PARANÁ

Setor de Ciências Biológicas
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Titulo **Mestre em Ciências Biológicas - Área de Botânica**
Dissertação **“REVISÃO TAXONÔMICA DO GÊNERO STROPHARIA SENSU LATO (AGARICALES) NO SUL DO BRASIL”.**

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Curitiba, 13 de maio de 2016



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UNIVERSIDADE FEDERAL DO PARANA

Setor de Ciências Biológicas
Programa de Pós-Graduação em Botânica



**“REVISÃO TAXONÔMICA DO GÊNERO STROPHARIA SENSU
LATO (AGARICALES) NO SUL DO BRASIL”.**

por

Cristiane Seger

Dissertação aprovada como requisito parcial
para obtenção do grau de Mestre no Programa
de Pós-Graduação em Botânica, pela Comissão
formada pelos doutores

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Curitiba, 13 de maio de 2016

Dedico este trabalho aos meus pais, Lídia e Aloísio Seger, meus maiores exemplos de vida e persistência.

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RESUMO

São tratados como *Stropharia* s.l. espécies pertencentes a este gênero, caracterizadas principalmente pela presença de acantócitos, e também de *Protostropharia*, que compreende espécies muito similares e que por muito tempo foram tratadas também como *Stropharia*, tendo sido infragenericamente separadas pelo hábito coprófilo e estipe glutinoso. O número de espécies conhecidas no Brasil é maior em áreas de Mata Atlântica, em especial em Floresta Ombrófila Mista do Estado do Rio Grande do Sul, onde novas espécies vêm sendo descritas e, portanto, se revela uma área importante para o desenvolvimento deste grupo. Assim sendo, este trabalho visou investigar locais pouco estudados a fim de melhor conhecer a riqueza e distribuição das espécies de *Stropharia* s.l., abrangendo os três estados do sul brasileiro. Os espécimes coletados, e também aqueles já depositados em herbários, foram analisados e, no caso das *Protostropharia* spp., foi sequenciada a região ITS do rDNA de cinco espécimes a fim de resolver problemas taxonômicos pontuais. Com a revisão de *Stropharia*, foi possível identificar dezessete espécies, dentre as quais *S. alvesiana*, *S. coelhoi*, *S. ombrophila*, *S. versiformis* e *S. watlingii* são novas para a ciência, *S. pseudocyanea* é novo registro para a América do Sul, *S. apiahyna* é nova para o Paraná e *S. coronilla*, *S. rugosoannulata* e *S. venusta* são citadas pela primeira vez para Santa Catarina. Além destas, ocorrem na área estudada *S. acanthocystis*, *S. aeruginosa*, *S. agaricoides*, *S. araucariae*, *S. earlei*, *S. melanosperma*, *S. rugosoannulata* e *S. trinitensis*. No estudo de *Protostropharia*, foram geradas cinco sequências da região ITS, as quais formam um clado consistente na análise filogenética. Uma destas amostras corresponde a *P. dorsipora* e as outras quatro são de espécimes de *P. alcis* ssp. *austrobrasiliensis* com variações morfológicas, tais como a presença de pseudoesclerócio e basídios bispóricos. Além destas duas, é conhecida para a região *P. semiglobata*, cuja ocorrência é baseada em trabalhos anteriores. Quanto à distribuição, *P. alcis* ssp. *austrobrasiliensis* é bem representada nos três estados do sul, sendo registrada pela primeira vez para Santa Catarina, enquanto *P. dorsipora* era conhecida para o Rio Grande do Sul e agora também para o Paraná. Para facilitar as futuras identificações das espécies destes dois gêneros, são apresentadas chaves dicotômicas, descrições e ilustrações (quando houveram novas observações), e ainda é discutida a taxonomia e características destas.

Palavras-chave: *Basidiomycota*, microbiota, *Strophariaceae*, taxonomia.

ABSTRACT

Stropharia s.l. comprises agaricoid fungi with acanthocytes, and also coprophilous species with glutinous basidiomata, now classified in the genus *Protostropharia*. The number of species is best known in the Atlantic Forest, especially in mixed rain forest of Rio Grande do Sul, in south Brazil, where new taxa have been described and, therefore, it is an important area for the development of this group. Thus, our work aims at investigating places understudied in order to better understand the richness and distribution of *Stropharia* s.l. at the three states of southern Brazil. Specimens collected, and also those already deposited in herbaria, were analyzed and analysis of the rDNA ITS region of five *Protostropharia* spp. was performed to resolve specific taxonomic problems. With the revision of *Stropharia*, seventeen species were identified, among which *S. alvesiana*, *S. coelhoi*, *S. ombrophila*, *S. versiformis* and *S. watlingii* are new species, *S. pseudocyanea* is new record to South America, *S. apiahyna* is new to Paraná and *S. coronilla*, *S. rugosoannulata* and *S. venusta* are registered for the first time to Santa Catarina. Additionally, there are in the study area *S. acanthocystis*, *S. aeruginosa*, *S. agaricoides*, *S. araucariae*, *S. earlei*, *S. melanosperma*, *S. rugosoannulata* and *S. trinitensis*. In the research of *Protostropharia* has generated five sequences of the ITS region, which form a clade well-supported in the phylogenetic tree. One of these corresponds to *P. dorsipora* and others four are specimens of the *P. alcis* ssp. *austrobrasiliensis* with morphological variations, such as the pseudosclerotia and bisporic basidia presence. Addition to these, *P. semiglobata* is reported to region, whose occurrence is based on previous work for the reason that the materials are scarce and often misidentified. Regarding the distribution, *P. alcis* ssp. *austrobrasiliensis* is recorded for first time for Santa Catarina State and are well known for all Southern Brazil, while *P. dorsipora* was known to the Rio Grande do Sul State and now also for Paraná State. Identification keys of two genera are supplied, as well as descriptions and illustrations (when had new observations) and also taxonomic discussion about these.

Key words: *Basidiomycota*, mycobiota, *Strophariaceae*, taxonomy.

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1 INTRODUÇÃO

1.1 A FAMÍLIA STROPHARIACEAE SINGER & A.H. SM.

Strophariaceae Singer & A.H. Sm, está classificada na ordem *Agaricales* Underw., filo *Basidiomycota* Whittaker ex Moore. A família foi formalmente descrita por Singer e Smith (1946) que, da mesma forma, reconheceram duas subfamílias: *Pholiotoideae* Imai e *Stropharioideae* Singer, as quais até então eram inclusas em *Cortinariaceae* R. Heim e *Coprinaceae* Overeem & Weese, respectivamente. Estas duas subfamílias de *Strophariaceae* eram separadas basicamente pela cor da esporada, onde *Pholiotoideae* abrange espécies com esporada marrom ferrugínea, marrom canela a marrom tabaco; e em *Stropharioideae* a esporada varia de marrom chocolate, marrom vináceo a negra (SINGER, 1949).

Com a ascensão do uso de dados moleculares na filogenia, a classificação das espécies nesta família vem sofrendo algumas modificações. Moncalvo et al. (2002) apresentam uma filogenia com aproximadamente um décimo das espécies conhecidas e classificadas em *Agaricales*. Nesta, estão inclusos no clado “stropharioid” espécies dos gêneros *Hypholoma* (Fr.) P. Kumm., *Pachylepyrium* Singer, *Phaeonematoloma* (Singer) M. Bon, *Pholiota* (Fr.) P. Kumm, *Leratiomyces* Bresinsky & M. Binder, *Stropharia* (Fr.) Quél. e *Weraroa* Singer. Nesta mesma linha, a pesquisa de Matheny et al. (2006) insere na família os gêneros *Agrocybe* Fayod, *Hypholoma*, *Kuehneromyces* Singer & A.H. Smith, *Pholiota*, *Stropharia*, e ainda algumas espécies de *Psilocybe* (Fr.) P. Kumm. e o gênero gasteroide *Nivatogastrium* Singer & A.H. Sm. Ainda, apresenta como grupos irmãos os clados /*Strophariaceae* e /*Hymenogastraceae*, este compreendido por espécies de *Psilocybe*, *Galerina* Earle, *Phaeocollybia* R. Heim, *Anamika* K.A. Thomas, Peintner, M.M. Moser & Manim., *Hebeloma* (Fr.) P. Kumm., *Alnicola* Kühner e *Flammula* (Fr.) P. Kumm.. Com um estudo mais específico do grupo, em especial de *Psilocybe*, Ramirez-Cruz et al. (2013) apresentam uma filogenia onde também mostram a relação entre estas duas famílias, onde incluem em *Strophariaceae* os gêneros *Deconica* (W.G. Sm.) P. Karst., *Flammula*, *Hypholoma*, *Kuehneromyces*, *Pholiota* e *Stropharia* (*Agrocybe* não foi incluso neste estudo).

Como visto, ainda não é consenso a delimitação exata da família, mas de um modo geral, ela compreende espécies com basidioma pequeno a grande, véu

presente ou ausente, esporada com tons de marrons a violáceo (ou até mesmo negro), esporos lisos ou ligeiramente ornamentados, com poro germinativo quase sempre presente, superfície do píleo formado por uma cútis ou ixocútis, raramente apresentando elementos terminais diferenciados (NOORDELOOS, 2011).

As espécies de *Strophariaceae* podem ser encontradas nos cinco continentes, ocorrendo no interior e na borda de mata, em campos abertos, pastos, jardins e banhados. Elas se desenvolvem sobre uma grande variedade de substratos, tais como madeira viva ou em decomposição, solo, serapilheira, musgos, raízes expostas e esterco. Desta forma, apesar de alguns serem parasitas, grande parte atua na natureza como decompositor, fazendo a ciclagem dos materiais orgânicos. Segundo Singer (1986), os fungos dessa família não formam ectomicorrizas.

Alguns destes fungos são utilizados como alimento para a fauna. Exemplo disso são besouros do gênero *Oxyporus* (Coleoptera: Staphylinidae), cujas larvas e adultos crescem e se alimentam das lamelas de cogumelos, entre eles *Stropharia hardii* G.F. Atk. e espécies de *Pholiota* (WEBSTER; DE MERCHANT, 2012). Mitchell (2001) cita também *Pholiota*, e ainda *Hypholoma*, sendo utilizados na alimentação do esquilo voador do norte (*Glaucomys sabrinus fuscus*). No sul do Brasil, Sulzbacher et al. (2007) observaram marcas em basidiomas de *S. rugosoannulata* Farlow coletadas no Rio Grande do Sul, e sugerem que em ambiente natural esta espécie sirva na dieta de aves.

Stropharia rugosoannulata é também utilizada na alimentação humana, sendo produzida e comercializada para este fim (PEGLER, 2001; BOA, 2004). Ainda, alguns povos utilizam na alimentação *S. semiglobata* (Batsch) Quél. [= *Protostropharia semiglobata* (Batsch) Redhead, Moncalvo & Vilgalys] e também *S. coronilla* (Fr.) Quél, esta por vezes utilizada como medicinal, apesar de algumas culturas considerar a espécie tóxica (BOA, 2004).

Com o desenvolvimento de novas pesquisas com o grupo, vem sendo descobertas outras propriedades de espécies de *Strophariaceae* que possibilitam sua utilização em diferentes setores. Na agricultura, *Stropharia* spp. vem sendo testada no controle de nematóides, uma vez que produz estruturas (acantócitos) com capacidade de predação destes animais (LUO et al., 2006; KONG et al., 2013). Já voltado para a saúde humana, os pesquisadores descobriram ação antioxidante de *Pholiota* e *Naematoloma* P. Karst. (ELGORASHI et al., 2008).

1.2 STROPHARIA SENSU LATO

Stropharia é o gênero tipo da família *Strophariaceae*, e foi originalmente descrito como um subgênero do gênero *Agaricus* L. por Fries (1849 - FIGURA 1), sendo posteriormente elevado ao status genérico por Quélet (1872). Uma vez reconhecido como um gênero autônomo, *Stropharia* foi subdividida em grupos infragenéricos por diferentes autores, os quais consideraram distintas estruturas morfológicas para o embasamento de suas classificações.

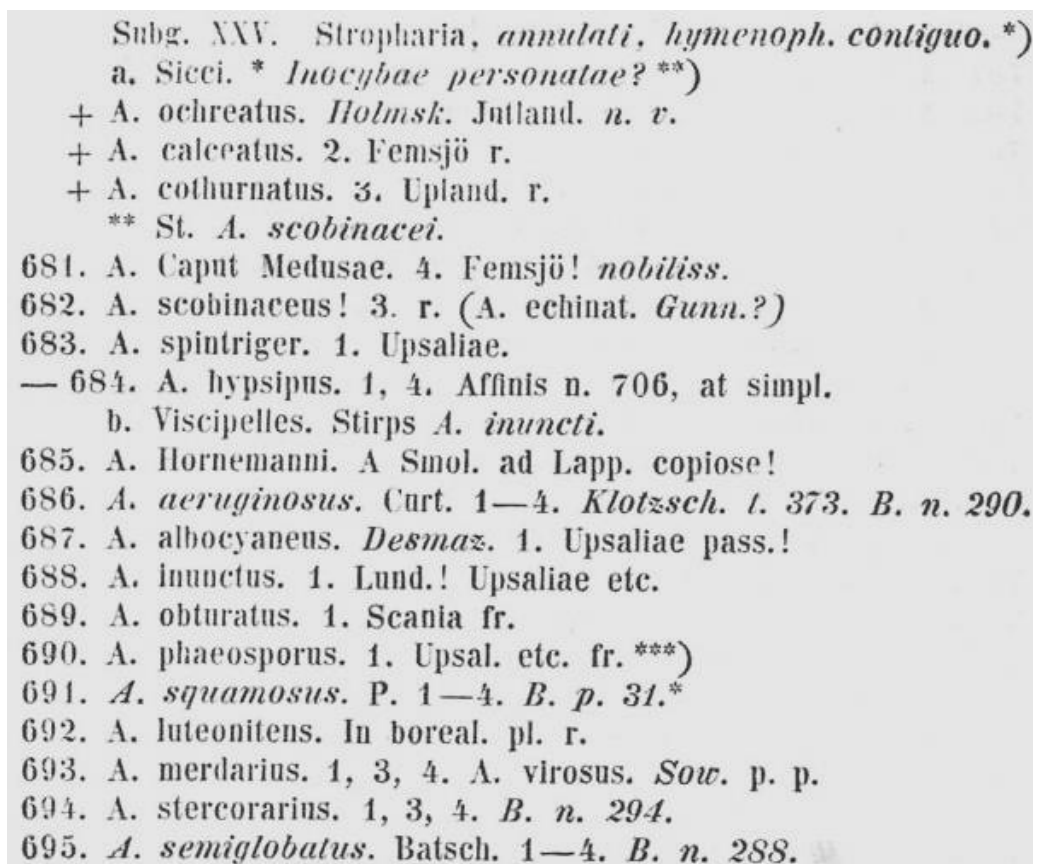


FIGURA 1 – DESCRIÇÃO DE *AGARICUS* SUBG. *STROPHARIA*, POR FRIES (1849).

Singer (1949) propôs três seções para o gênero *Stropharia*: *Mundae* (Fr.) Konr. & Maubl., compreendendo espécies com píleo subvíscido ou úmido; *Pholiotideae* (Fr.) Singer, com espécies de píleo verdadeiramente víscido a glutinoso; e *Stercophila* (Romagnesi) Singer (*nom. nud.*), também com píleo víscido a glutinoso, além de estipe com estas mesmas características e única seção com basidiomas crescendo em esterco e em campos e prados adubados. Posteriormente, Singer

(1962) propôs a substituição da seção *Pholiotideae* por *Stropharia*, onde o autor também reconheceu espécies com píleo subvísido, víscido a glutinoso, e esta classificação foi adotada por inúmeros pesquisadores desde então (SINGER, 1975; 1986; PEGLER, 1977; WATLING; GREGORY, 1987).

Levando em consideração a classificação de Singer e outras peculiaridades ecológicas e biológicas, Wasser e Grodzinskaya (1996) propuseram dois subgêneros para *Stropharia*: *Stropharia* e *Stercophila* S. Wasser & A. Grodz., cuja principal diferença é o substrato, onde as espécies do primeiro crescem sobre solo ou material vegetal, enquanto que o segundo desenvolve-se somente em esterco. Ainda de acordo com a classificação proposta, o subgênero *Stropharia* foi subdividido em duas seções: *Stropharia* e *Mundae*, correspondendo, em linhas gerais, às seções *Stropharia* e *Mundae* de Singer (1962), tendo como espécies-tipo *S. aeruginosa* (Fr.) Quél. e *S. coronilla*, respectivamente.

Noordeloos (1999), com base em ideias desenvolvidas inicialmente por Smith (1979) e Kühner (1980), tratou *Strophariaceae* de uma forma mais ampla, considerando apenas os gêneros *Psilocybe* e *Pholiota*. Mais uma vez, a cor da esporada foi utilizada como caractere determinante para distinguir os táxons, sendo em *Psilocybe* marrom avermelhado, marrom púrpura a cinza violáceo e em *Pholiota* marrom amarelado, marrom tabaco a marrom canela ou marrom ferrugem. Ainda, foi levado em consideração a parede do esporo, que sempre é liso em *Psilocybe* e liso ou raramente ornamentado em *Pholiota*, e também o substrato, onde nos primeiros é variado (crescem em solo, húmus, musgos, grama, madeira) enquanto os segundos normalmente estão em madeira ou mesmo são parasitas.

Nessa proposta, Noordeloos (1995; 1999) considerou em *Psilocybe* s.l. as espécies previamente classificadas nos gêneros *Deconica*, *Hypholoma*, *Melanotus* Pat., *Psilocybe* e *Stropharia*. Consequentemente, as espécies atribuídas a *Stropharia* foram então consideradas em *Psilocybe*, e distribuídas nos subgêneros *Stercophila* (Romagn.) Noordel. e *Stropharia* (Fr.) Noordel. – este último subdividido nas seções *Stropharia* (Fr.) Noordel. e *Mundae* (Fr.) Konr. & Maublanc. Noordeloos (1999) também chama a atenção para a presença de acantócitos nas rizomorfias como característica das espécies do subgênero *Stropharia*, porém sem atribuir qualquer valor taxonômico a este caractere. Acantócitos são células espinhosas que se agrupam e cobrem as rizomorfias dos basidiomas (FIGURA 2), crescendo também no

micélio em cultura (FARR, 1980); são estruturas constituídas de cristais de cálcio, possuem parede fina e são ocos, o que os torna frágeis (CLÉMENÇON, 2002).

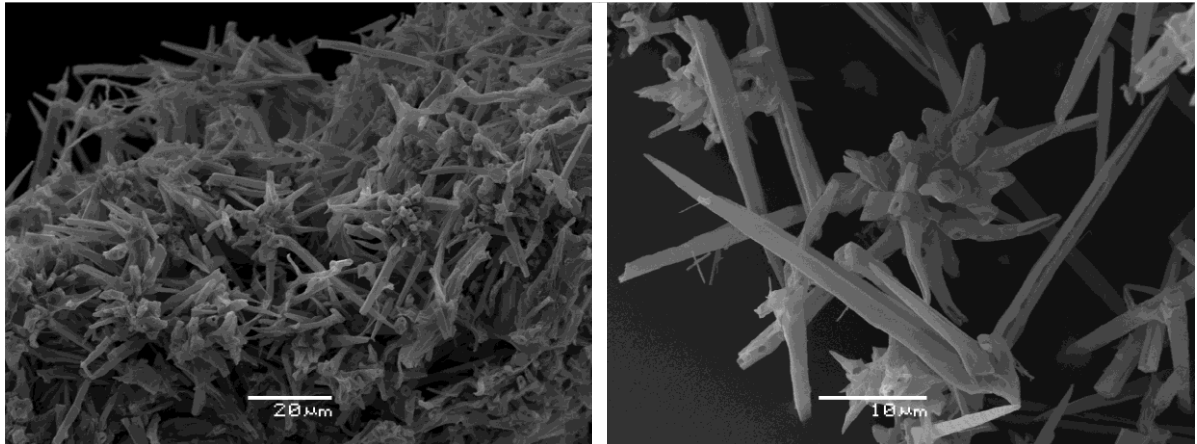


FIGURA 2 – MICROSCOPIA ELETRÔNICA DE VARREDURA (MEV) DE RIZOMORFAS DE *STROPHARIA* SP., EVIDENCIANDO OS ACANTÓCITOS (IMAGENS: C. SEGER).

As espécies coprófilas de *Stropharia* têm sido reconhecidas como um grupo distinto, seja como subgênero ou seção, inicialmente baseado no tipo de substrato (coprófilo) e superfície glutinosa do estipe, e posteriormente devido à ausência de acantócitos. Em nível molecular, como demonstrado por Moncalvo et al. (2002), este aparece como um grupo consistente e bem suportado, onde as espécies de *Stropharia* (seções *Mundae* e *Stropharia*) aparecem agrupadas no clado “stropharia”, enquanto as espécies da seção *Stercophila* aparecem separadamente, constituindo o clado “semiglobata”. Essa posição já havia sido sustentada, porém de forma nomenclaturalmente inválida, por Romagnesi (1936), que propôs o gênero *Stercophila* Romagn. Finalmente, Redhead (2013) propôs o gênero *Protostropharia* Redhead, Moncalvo & Vilgalys para acomodar as espécies do clado “semiglobata” (MONCALVO et al. 2002) e que não possuem acantócitos. Segundo Redhead (2013), as espécies de *Protostropharia* apresentam astrocistídios no micélio basal, que são ramos hifais curtos de porção apical globosa e afiada, com cristais hialinos grossos.

A presença de acantócitos tem sido documentada e adotada como caractere diagnóstico na taxonomia de *Stropharia*, de modo que as espécies previamente classificadas em *Pholiota* e *Hypholoma* também foram posteriormente re combinadas para este gênero (NORVELL; REDHEAD, 2000; CORTEZ, 2008a; 2008b).

Além dos acantócitos e astrocistídios, as espécies de *Stropharia* s.l. caracterizam-se pela presença de esporada marrom ocráceo a marrom violáceo,

esporos com parede lisa e espessa, geralmente com poro germinativo bem desenvolvido e truncado, e superfície pilear formada por hifas gelatinizadas prostradas (SINGER, 1986; CORTEZ; SILVEIRA, 2008). Os queilocistídios estão normalmente presentes e os pleurocistídios são crisocistídios, ou seja, cistídios himeniais que, com a maturidade, passam a apresentar conteúdo amorfo refringente em seu interior, possivelmente no vacúolo. Por vezes, este conteúdo pode tomar todo o citoplasma do cistídio, deixando-o totalmente amarelo-refringente (CLÉMENÇON, 1995). Ainda, *Stropharia* s.l. apresenta características macroscópicas importantes, tais como lamelas adnatas ou adnexas (nunca livres), presença de véu parcial e rizomorfos geralmente bem desenvolvidos (SINGER, 1986; CORTEZ; SILVEIRA, 2008).

1.3 DISTRIBUIÇÃO E RIQUEZA DAS ESPÉCIES

Pesquisas com fungos macroscópicos realizadas em vários países relatam a presença de *Stropharia* em todos os continentes, com exceção das regiões polares. Na América do Norte, 38 espécies deste gênero são citadas, porém grande parte destas corresponde a nomes inválidos ou sinônimos, em especial aqueles oriundos de trabalhos do início do século passado (HARPER, 1914; MURRILL, 1912; 1918; 1922; 1943; SMITH, 1941; MITCHEL; SMITH, 1978; BARONI, 1981; NORVELL; REDHEAD, 2000; DESJARDIN; HEMMES, 2001; CESKA, 2012).

Com poucas pesquisas realizadas, são conhecidas na África cinco espécies de *Stropharia* (PEGLER, 1977; MALENÇON, 1979). Cinco espécies também são conhecidas na Austrália (GRGURINOVIC, 1997; CHANG et al. 2006; RATKOWSKY et al. 2013). Levantamentos na Ásia citam 13 espécies (BAU; MENG, 2008; SENTHILARASU; SINGH, 2013; TIAN; BAU, 2014) e especificamente na Turquia são 11, entre elas *S. aurantiaca* (Cooke) S. Imai, que atualmente é conhecida como *Leratiomyces ceres* (Cooke & Masee) Spooner & Bridge (SESLI; DENCHEV, 2008).

Em revisão de *Strophariaceae* para a Europa, Noordeloos (2011) relata a ocorrência de 19 espécies de *Stropharia* s.l., incluindo sete espécies recentemente transferidas para o gênero *Protostropharia*. Bon e Roux (2003) desenvolveram uma chave dicotômica na qual apresentam 19 espécies, três variedades e uma forma. Na América do Sul e Central, 28 espécies e quatro variedades foram registradas, sendo os principais trabalhos realizados em território brasileiro e argentino (COIMBRA, 2015).

Apesar de aparentemente ser um gênero relativamente conhecido, é importante destacar que ainda não foi realizada uma revisão do gênero em nível mundial, tornando assim o conhecimento do táxon escasso e disperso.

1.4 STROPHARIA SENSU LATO NO BRASIL

Segundo a Lista da Flora do Brasil (CAPELARI et al., 2015), são conhecidas 14 espécies e uma variedade de *Stropharia* s.l. no país, ocorrendo principalmente em áreas de Mata Atlântica do sul e sudeste brasileiro, mas também com registros esparsos na Amazônia e no Pantanal. Em termos de distribuição geográfica, é conhecida uma espécie para Pernambuco, uma no Mato Grosso do Sul, sete no Estado de São Paulo, quatro no Paraná (PR) e 13 espécies e uma variedade no Rio Grande do Sul (RS).

No RS, estado com maior número de espécies de *Stropharia* conhecidas no país, os trabalhos de coleta e identificação de fungos macroscópicos começaram já no início do século XX, principalmente com o Padre Johannes Rick, o qual identificou nove espécies para este estado (RICK, 1961). Estas coletas foram posteriormente reanalisadas e novas coletas realizadas. São aceitas no estado as espécies *Stropharia acanthocystis* Cortez & R.M. Silveira, *S. aeruginosa*, *S. agaricoides* P.S. Silva, Cortez & R.M. Silveira, *S. apiahyna* (Speg.) Cortez & R.M. Silveira, *S. araucariae* Cortez & R.M. Silveira, *S. coronilla*, *S. earlei* Norvell & Redhead, *S. melanosperma* (Fr.) Gillet, *S. rugosoannulata*, e *S. venusta* P.S. Silva, Cortez & R.M. Silveira (CORTEZ; COELHO, 2004; 2008; SOBESTIANSKY, 2005; SILVA et al., 2006; 2008; 2009; 2012; SULZBACHER et al., 2007; CORTEZ; SILVEIRA, 2008). Há ainda as espécies que passaram a ser denominadas *Protostropharia*, com ocorrência registrada de *P. alcis* ssp. *austrobrasiliensis* (Cortez & R.M. Silveira) C. Hahn, *P. dorsipora* (Esteve-Rav. & Barrasa) Redhead e *P. semiglobata*.

Para o Paraná, Meijer (2006; 2010) cita a ocorrência de *Stropharia* cf. *albivelata* A.H. Sm. & Hesler, *S. coronilla*, *S. rugosoannulata* e *S. trinitensis* (Dennis) Cortez (como *Pholiota trinitensis* Dennis) e *Protostropharia semiglobata* (como *S. semiglobata*). Ainda, Putzke (1994), em levantamento bibliográfico, cita *S. coronilla* e *P. semiglobata* (= *S. semiglobata*) com ocorrência em Pernambuco e São Paulo, respectivamente. Bononi et al. (2008) menciona *S. trinitensis* [como *Hypholoma*

trinitense (Dennis) Pegler] para Mato Grosso do Sul e Cortez (2008a) relata *S. varzeae* (Singer) Cortez para o estado do Amazonas.

Apesar da circunscrição relativamente bem delimitada do grupo, a proximidade com gêneros como *Pholiota* e *Hypholoma* tem tornado a identificação e classificação de algumas espécies difícil, e sua taxonomia vem sofrendo alterações nos últimos anos. Exemplos disso são *Stropharia albivelata*, *S. apiahyna*, *S. trinitensis* e *S. varzeae*, ambas a partir do gênero *Pholiota* (NORVELL; REDHEAD, 2000; CORTEZ 2008a; b; CORTEZ & SILVEIRA, 2008)

Da mesma forma, espécies antes incluídas em *Stropharia* foram transferidas para outros gêneros, como é o caso de *Leratiomyces ceres*, mais bem conhecida como *S. aurantiaca* ou ainda *Hypholoma aurantiacum* (Cooke) Faus, (BRIDGE et al., 2008)

Novas espécies foram descritas recentemente para o Brasil, mais especificamente no Estado do Rio Grande do Sul, sendo elas *Stropharia acanthocystis*, *S. agaricoides*, *S. araucariae* e *S. venusta*, além da variedade *S. alcis* var. *austrobrasiliensis* Cortez & R.M. Silveira (= *P. alcis* ssp. *austrobrasiliensis*), o que evidencia o desconhecimento da diversidade do gênero em terras brasileiras (CORTEZ; SILVEIRA, 2007; 2008; SILVA et al. 2009).

Assim, o objetivo geral deste trabalho é revisar as espécies sul-brasileiras de *Stropharia* s.l., com base na revisão dos espécimes depositados em herbários e realização de novas coletas, afim de buscar novos espécimes e ampliar o conhecimento da sua distribuição geográfica no sul do Brasil.

2 MATERIAL E MÉTODOS

Para a revisão morfotaxonômica do gênero *Stropharia* s.l. foram analisados espécimes oriundos dos três estados do sul do Brasil, estes depositados nos herbários do Instituto de Biociências, Universidade Federal do Rio Grande do Sul (ICN), do Departamento de Biologia, Universidade Federal de Santa Maria (SMDB), do Departamento de Botânica, Universidade Federal de Santa Catarina (FLOR) e do Museu Botânico Municipal de Curitiba (MBM). Paralelamente, foram realizadas coletas em distintas regiões fisionômicas a fim de aumentar o esforço amostral na área de estudo, por considerar-se que a amostragem era ainda insuficiente.

Todos os espécimes coletados foram depositados no Herbário do Campus Palotina (HCP) da Universidade Federal do Paraná (UFPR), e os holótipos no herbário ICN ou do Departamento de Botânica da UFPR (UPCB).

2.1 METODOLOGIA DE COLETA

A área de estudo é a região sul do Brasil, formada pelos estados do Paraná (PR), Santa Catarina (SC) e Rio Grande do Sul (RS), e está compreendida no Bioma Mata Atlântica. As formações florestais presentes nesta região são a Floresta Estacional Decidual (FED), Floresta Estacional Semidecidual (FES), Floresta Ombrófila Mista (FOM) e Floresta Ombrófila Densa (FOD). As florestas estacionais estão sujeitas a uma estação desfavorável quanto a captação de água (baixas temperaturas e/ou precipitação), momento em que os componentes vegetais do dossel da mata perdem suas folhas como forma de evitar a perda de água para o ambiente. Já as florestas ombrófilas possuem precipitação elevada e melhor distribuída, o que mantém o ambiente úmido durante todo o ano. A FOD apresenta espécies vegetais de grande e médio porte e grande densidade de epífitas e lianas lenhosas, enquanto a FOM tem como principal característica a presença de *Araucaria angustifolia* (Bertol.) O. Kuntze. (IBGE, 2012; VIBRANS et al., 2012).

As coletas começaram no primeiro semestre de 2014 e se estenderam até julho de 2015, com 30 saídas a campo, em 18 cidades (MAPA 1) e 22 pontos de coleta (TABELA 1). O esforço amostral foi maior nos estados do PR e SC pois são regiões com carência de dados acerca do grupo estudado.

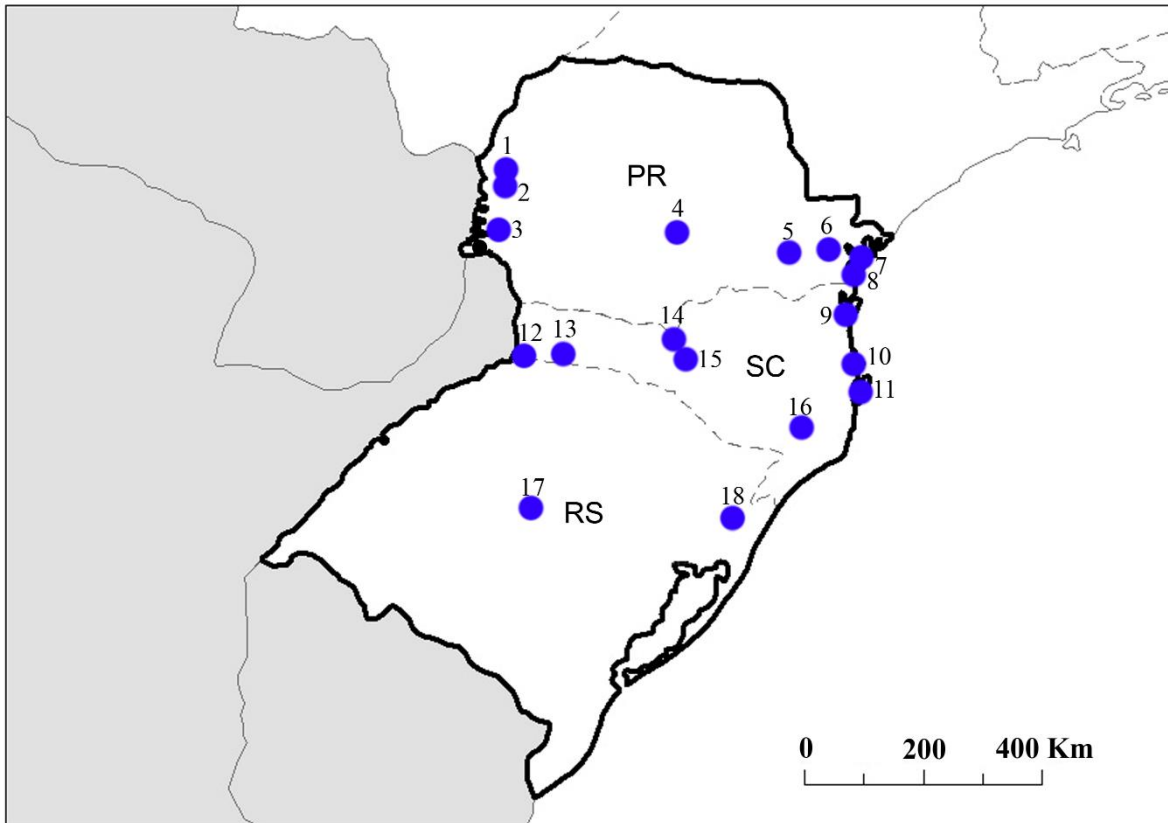


FIGURA 3 – REGIÃO SUL DO BRASIL, MOSTRANDO OS MUNICÍPIOS DE COLETA NOS ESTADOS DO PARANÁ (PR), SANTA CATARINA (SC) E RIO GRANDE DO SUL (RS). (1: TERRA ROXA; 2: PALOTINA; 3: FOZ DO IGUAÇÚ; 4: GUARAPUAVA; 5: SÃO LUIZ DO PURUNÃ; 6: MORRETES; 7: PONTAL DO PARANÁ; 8: MATINHOS; 9: ITAPOÁ; 10: BOMBINHAS; 11: FLORIANÓPOLIS; 12: ITAPIRANGA; 13: SÃO CARLOS; 14: ÁGUA DOCE; 15: JOAÇABA; 16: URUBICI; 17: SANTA MARIA; 18: SÃO FRANCISCO DE PAULA).

Os espécimes foram coletados no período da manhã e início da tarde, devido à umidade do ar que tende a ser mais elevada. As coletas seguiram a metodologia de Fidalgo e Bononi (1989), os quais sugerem a retirada dos basidiomas com faca ou espátula, trazendo junto parte do substrato. Posteriormente, os espécimes foram embalados individualmente e acomodados em caixas organizadoras de plástico, para que não quebrassem ou misturassem os esporos. Todo o material coletado foi fotografado ainda no local de coleta.

A secagem dos espécimes ocorreu em estufa com circulação de ar, à temperatura aproximada de 50°C, ou por desidratação em sílica gel. Depois de secos, para evitar reidratação do material, os espécimes ficam envoltos por papel alumínio e acomodados individualmente em sacos plásticos com fecho hermético.

TABELA 1 – RELAÇÃO DOS ESTADOS, MUNICÍPIOS E LOCAIS DE COLETA DE MACROFUNGOS, COM RESPECTIVAS FORMAÇÕES VEGETAIS. (FED: FLORESTA ESTACIONAL DECIDUAL; FES: FLORESTA ESTACIONAL SEMIDECIDUAL; FLONA: FLORESTA NACIONAL; FOD: FLORESTA OMBRÓFILA DENSA; FOM: FLORESTA OMBRÓFILA MISTA; PARNA: PARQUE NACIONAL).

Estado	Município	Localidade	Vegetação
PR	Foz do Iguaçu	Parna do Iguaçu	FES
PR	Guarapuava	Parque Municipal das Araucárias	FOM
PR	Matinhos	Parque Estadual Rio da Onça	Restinga
PR	Morretes	Morro Mãe Catira	FOD
PR	Palotina	Parque Estadual São Camilo	FES
PR	Pontal do Paraná	Pontal do Sul	Restinga
PR	São Luiz do Purunã	Morro do Cristo	Campos
PR	Terra Roxa	Fazenda Açú	FES
RS	Santa Maria	Morro da Caturrita	FES
RS	Santa Maria	Morro do Elefante	FES
RS	Santa Maria	Parque de Exposições UFSM	Pastagem
RS	São Francisco de Paula	Flona São Francisco de Paula	FOM
SC	Água Doce	Fazenda Quiben	FOM
SC	Bombinhas	Morro do Macaco	Restinga
SC	Florianópolis	Lagoa do Peri	FOM
SC	Itapiranga	Linha Becker	FED
SC	Itapiranga	Linha Dois Saltinhos	FED
SC	Itapiranga	Linha Sede Capela	FED
SC	Itapoá	Reserva Volta Velha	FOD
SC	Joaçaba	Parque Natural Municipal do Vale do Rio do Peixe	FOM/FED
SC	São Carlos	Fragmento florestal urbano	FOM/FED
SC	Urubici	Parna São Joaquim	FOM

2.2 ANÁLISE MACROSCÓPICA

A descrição dos basidiomas foram realizadas a olho nu logo após a coleta, uma vez que a desidratação provoca modificações na coloração e forma dos mesmos. Nos fungos ainda frescos foram observados: a. Píleo: coloração, forma, consistência, superfície, dimensões e presença de vestígios de véu na margem; b. Contexto: coloração, consistência; c. Lamelas: coloração, inserção, distância; d. Estipe: coloração, forma, consistência, tipo de superfície, presença de anel, volva, micélio basal ou rizomorfos e dimensões (LARGENT, 1986). A identificação das cores seguiu a carta de Kornerup e Wanscher (1978) e as medidas foram obtidas com paquímetro.

Após as mensurações e descrições, parte dos píleos foi destacada e colocada com a porção fértil (lamelas) sobre um pedaço de papel branco, a fim de coletar a esporada do fungo, esta de importância taxonômica (FIDALGO; BONONI, 1989).

2.3 ANÁLISE MICROSCÓPICA

Para a análise das microestruturas, foram realizados cortes histológicos do píleo, contexto, lamelas e estipe, estes feitos a mão livre utilizando-se lâmina de aço inoxidável sob estereomicroscópio. As lâminas foram então montadas com solução de hidróxido de potássio (KOH) 3%, Vermelho Congo 1% e hidróxido de amônia (NH₄OH) 10% (LARGENT et al., 1977).

As imagens de microscopia óptica foram obtidas com câmera Moticom 2500 acoplada ao microscópio Moticom[®], disponível no Laboratório de Biodiversidade Fúngica (LaBFun) da UFPR – Setor Palotina. As dimensões das microestruturas foram baseadas na medida de pelo menos 25 elementos, obtidas utilizando o programa Moticom Image Plus 2.0[®], que acompanha o equipamento.

2.4 IDENTIFICAÇÃO

Somente com os dados macroscópicos e microscópicos completos, torna-se possível a identificação taxonômica das espécies. Para isto, foram consultadas literaturas específicas sobre o tema, em especial Singer e Digilio (1952), Watling e Gregory (1987), Cortez e Silveira (2008) e Noordeloos (2011).

Muitos dos espécimes analisados foram também estudados por Cortez e Silveira (2008) e por eles descritos e ilustrados. Assim, as espécies que não possuem novas coletas, ou que foram analisadas mas não houveram observações morfológicas relevantes, foram apenas citadas e discutidas. Todas as demais espécies identificadas são descritas macro e microscopicamente, ilustradas e discutidas. Para posterior identificação, os artigos de revisão contam com uma chave dicotômica das espécies do sul do Brasil.

2.5 MICROSCOPIA ELETRÔNICA DE VARREDURA (MEV)

A análise por MEV foi realizada a fim de detalhar a morfologia de basidiósporos e acantócitos, estruturas microscópicas importantes para a identificação. Para isto, a porção de interesse (lamela ou rizomorfias) é destacada e fixada por fita adesiva de carbono em suportes de alumínio (“*stubs*”). Os “*stubs*” são submetidos à metalização por três a cinco minutos em metalizador Balzers SCD030 –

Balzers Union FL9496, revestindo o material com uma camada de 150 Å de ouro. Finalmente, o material é observado ao MEV sob tensão de 15-20 Kv e magnificação variável (2.000-30.000x). As análises foram realizadas no Centro de Microscopia Eletrônica (CME) da UFPR, em Curitiba, PR.

2.6 EXTRAÇÃO DE DNA

A fim de resolver alguns problemas taxonômicos e definir os limites morfológicos em *Protostrophia*, cinco espécimes foram analisados molecularmente. A extração do DNA foi efetuada a partir de um fragmento do fungo herborizado, contendo parte do contexto e lamelas, utilizando a metodologia descrita por De Hoog et al. (2005) com pequenas modificações. Para isto, o material fúngico foi fragmentado utilizando lâminas de aço inoxidável sobre papel alumínio, ambos novos para cada amostra, e então transferidos para microtubos de 1,5 mL devidamente identificados. À amostra, foram adicionados 400 µL de tampão de lise (Tris-HCl 50 mM pH 8, EDTA 5 mM pH 8, NaCl 100 mM e SDS 1%) e mantido a -20°C por 10 minutos, seguida da adição de pérolas de vidro, e duas repetições de agitação mecânica em vórtex por 30 segundos intercaladas por 30 segundos em banho de gelo. Feito isto, foram adicionados 5 µL de Proteinase K 20 mg/mL (Invitrogen®) e as amostras incubadas a 60°C por 30 minutos, e posteriormente a 65°C por 10 minutos com 162 µL de tampão CTAB (Tris HCl 200 mM pH 7,5, EDTA 200 mM pH 8, NaCl 8,2% e CTAB 2%). Após, foram adicionados aos tubos 570 µL de clorofórmio/álcool isoamílico (24:1), incubação a 0°C durante 30 minutos, seguida de centrifugação por 10 minutos a 13.200 r.p.m. O sobrenadante obtido foi recolhido e transferido para um novo tubo onde foi acrescido de uma fração de 10% do volume total de uma solução de acetato de sódio 3 M, incubado a 0°C por 30 minutos e centrifugado por 10 minutos a 13.200 r.p.m. Novamente o sobrenadante foi transferido para outro tubo e então acrescido 50% do volume de isopropanol e centrifugado por 5 minutos a 13.200 r.p.m. O sobrenadante foi descartado por inversão, adicionado 200 µL de etanol 70% ao tubo, que novamente foi centrifugado por 5 minutos a 13.200 r.p.m e novamente invertido para descarte do sobrenadante. Os tubos foram mantidos abertos em temperatura ambiente para completa evaporação do etanol por aproximadamente duas horas. As amostras foram então ressuspensas em 50 µL de tampão TE (Tris-HCl 10 mM e EDTA 1 mM) e armazenadas a -20°C até o momento do uso. Todos os procedimentos de extração, e

posterior amplificação, foram realizados no Laboratório de Diagnóstico Biotecnológico (Labiotec), da UFPR - Setor Palotina.

2.7 AMPLIFICAÇÃO, SEQUENCIAMENTO E ANÁLISE FILOGENÉTICA

Para a caracterização molecular de *Protozoopharia*, foi realizada a amplificação da região ITS1-5.8S-ITS2 do rDNA, utilizando os primers ITS1 - *forward* (5'-TCCGTAGGTGAACCTGCGG-3') e ITS4 - *reverse* (5'-TCCTCCGCTTATTGATATGC-3') (WHITE et al, 1990). A Reação em Cadeia da Polimerase (PCR) foi realizada com volume final de 25 µL contendo 1x tampão de PCR (20 mM Tris-HCl pH 8.4 e 50 mM KCl), MgCl₂ 3 mM, Mix dNTP 0,4 mM, 0,2 µM de cada *primer* e 1U da *Taq Platinum* DNA Polimerase (Invitrogen®). A PCR foi realizada com 5 minutos iniciais a 94°C seguida de 25 ciclos de 94°C por 30 segundos, 56°C por 45 segundos e 72°C por 1 minuto e extensão final a 72°C por 7 minutos.

Os produtos da reação de amplificação (3 µL) foram submetidos a eletroforese em gel de agarose 1,5% em tampão TBE 1x, e corados em solução de brometo de etídio para posterior visualização em transiluminador de luz ultra-violeta do Laboratório de Melhoramento Vegetal e Biotecnologia da UFPR - Setor Palotina. Os DNAs resultantes da reação de amplificação foram quantificados em equipamento NanoDrop, e então 30 a 60 ng do DNA-molde e mais 4,5 pmol de *primer* (*forward* e *reverse*, separadamente) foram encaminhados para sequenciamento na ACTGene Análises Moleculares (Alvorada, Rio Grande do Sul, Brasil). As amostras foram então marcadas com o reagente BigDye (Applied Biosystems) e então sequenciados por eletroforese capilar em Sequenciador ABI PRISM® 3500 Genetic Analyser (Applied Biosystems).

As sequências obtidas foram inicialmente avaliadas pelo programa Phred/Phrap (<http://asparagin.cenargen.embrapa.br/phph/>) e manualmente editadas e corrigidas pelo programa BioEdit versão 7.2.5 (HALL, 1999), simultaneamente com o FinchTV versão 1.4 (<http://www.geospiza.com/Products/finchtv.shtml>). A homologia das sequências foi comparada com as sequências depositadas em bases públicas de dados (GenBank) utilizando o programa BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). As sequências nucleotídicas foram alinhadas com sequências de outras espécies da família *Strophariaceae* com ClustalW residente no BioEdit, o qual também foi utilizado para a construção da matriz de identidade. Para

a inferência filogenética a árvore foi construída pelo método da Máxima Verossimilhança (ML - *Maximum Likelihood*) utilizando o programa MEGA versão 6.0 (TAMURA et al., 2013). Foi empregado o modelo evolutivo “General Time Reversible” (GTR) com distribuição gama e sítios invariantes (GTR + I + G). A robustez da inferência filogenética sugerida foi avaliada através do método de *bootstrapping* (com base em 1000 réplicas).

3 RESULTADOS

Estas coletas resultaram em 22 espécimes de *Stropharia* e 12 de *Protostropharia*. Dos materiais depositados em herbários (ICN, MBM, FLOR e SMDB), recebemos e analisamos 61 espécimes de *Stropharia* e 12 de *Protostropharia*. Ainda, 11 espécimes são oriundos de coletas anteriores no RS mas que, por falta de identificação, não haviam sido depositados em herbário, e outras quatro são materiais da Suécia enviados por S. Jacobsson, e que agora encontram-se no Herbário ICN. Assim sendo, foram analisados um total de 122 espécimes.

Entre as 20 espécies identificadas (17 *Stropharia* e três de *Protostropharia*), cinco são novas para a ciência: *S. alvesiana*, *S. coelhoi*, *S. ombrophila*, *S. versiformis* e *S. watlingii*. Uma espécie corresponde a novo registro para a América do Sul, três são citadas pela primeira vez para Santa Catarina, uma para o Paraná e uma para o oeste do Paraná.

A análise molecular resultou ainda em cinco sequências da região ITS1-5.8S-ITS2, sendo estas de espécimes de *P. dorsipora* e as outras quatro de *P. alcis* ssp. *austrubrasiliensis*. As sequências foram depositadas no GenBank e correspondem às primeiras sequências de materiais sul-americanos do gênero.

Os resultados obtidos são aqui apresentados na forma de capítulos que correspondem a um artigo já aceito para a publicação e dois artigos que ainda serão submetidos, como dispostos a seguir:

Capítulo 1: ***Stropharia coelhoi* (Basidiomycota): a new species from Brazil**

Este artigo encontra-se aceito para publicação à revista Darwiniana (Argentina) e está em processo de editoração. Nele é descrita uma nova espécie de *Stropharia*, onde sua taxonomia é discutida e é apresentada uma prancha para ilustrar a espécie e auxiliar na identificação. Este artigo conta com a colaboração do pesquisador Marcelo A. Sulzbacher (Universidade Federal de Santa Maria). O texto segue a formatação recomendada pelas normas da revista, disponível em: <http://www.ojs.darwin.edu.ar/index.php/darwiniana/about/submissions>.

Capítulo 2: **The genus *Stropharia* s.s. (Agaricales) in Southern Brazil.**

O artigo será submetido à revista Kew Bulletin (Reino Unido), e apresenta a revisão das espécies de *Stropharia* s.s. do sul do Brasil. Neste artigo, são propostas quatro espécies novas, e contém ainda uma chave dicotômica para a identificação das 17 espécies de *Stropharia* spp. da região estudada, descrições, pranchas e discussões acerca destas. A formatação segue as normas da revista, disponível no endereço eletrônico: <http://www.kew.org/publications/kewbulletin.html>.

Capítulo 3: Taxonomy of the Southern Brazilian species of *Protostropharia* (*Strophariaceae*, *Agaricales*)

Este artigo será submetido à revista Rodriguésia (Brasil). Com base em investigações a nível molecular da região ITS do rDNA, são apresentadas e discutidas descrições e ilustrações atualizadas de *Protostropharia* identificadas para o sul do Brasil. Ainda, é discutida a distribuição do fungo cosmopolita *P. semiglobata* no país. Este trabalho conta com a colaboração das pesquisadoras Elisabete Takiuchi e Jéssica Cristhine Gallego, da Universidade Federal do Paraná, que auxiliaram na realização das análises moleculares. A formatação segue as normas da revista, disponível no endereço eletrônico: <http://rodriguesia.jbrj.gov.br/>.

4 REFERÊNCIAS

- BARONI, T. J. Collections in the Farlow Herbarium: new species of *Melanophyllum* and *Gastrocybe*, type studies on *Armillaria* and *Stropharia*. **Mycologia**, v. 73, p. 181-197, 1981.
- BAU, T.; MENG, T. *Strophariaceae* of China (II) *Stropharia*. **Journal of Fungal Research**, v. 6, n. 1, p. 7-34, 2008.
- BOA, E. Wild edible fungi a global overview of their use and importance to people. **Technical Paper Non-Wood Forest Products**, 17. FAO, Rome. 2004.
- BON, M; ROUX, P. Clé analytique de la famille *Strophariaceae* Singer & A.H. Smith. **Documents mycologiques**, v. 129, p. 3-56, 2003.
- BONONI, V. L. R.; OLIVEIRA, A. K. M.; QUEVEDO, J. R.; GUGLIOTTA, A. M. Fungos macroscópicos do Pantanal do Rio Negro, Mato Grosso do Sul, Brasil. **Hoehnea**, v. 35, n. 4, p. 489-511, 2008.
- BRIDGE, P. D.; SPOONER, B. M.; BEEVER, R. E.; PARK, D.-C. Taxonomy of the fungus commonly known as *Stropharia aurantiaca*, with new combinations in *Leratiomyces*. **Mycotaxon**, v. 103, p. 109-121, 2008.
- CAPELARI, M.; CORTEZ, V. G.; NEVES, M. A.; BASEIA, I. G.; WARTCHOW, F.; MENOLLI JÚNIOR, N.; KARSTEDT, F.; OLIVEIRA, J. J. S.; URREA-VALENCIA, S. *Agaricales* in **Lista de Espécies da Flora do Brasil**. Jardim Botânico do Rio de Janeiro. 2015. Disponível em: <<http://floradobrasil.jbrj.gov.br/jabot/floradobrasil/FB12>>. Acesso em: 07 abr. 2015.
- CESKA, O. **A Survey of Macrofungi on Observatory Hill: Spring 2011 and Winter 2011/2012**. Canadá, mar. 2012.
- CHANG, Y. S.; GATES, G. M.; RATKOWSKY, D. A. Some new species of the *Strophariaceae* (*Agaricales*) in Tasmania. **Australasian Mycologist**, v. 24, n. 3, p. 53-68, 2006.
- CLÉMENÇON, H. Chrysocystides. **Documents Mycologiques**, v. 98-100, p. 129-133, dec. 1995.

CLÉMENÇON, H. Die Stachelzellen der Träuschlinge: Les cellules épineuses des Strophaires. (*Stropharia*). **Schweiz. Z. Pilzkunde** v. 80, p. 120, 2002.

COIMBRA, V. R. M. Checklist of Central and South American *Agaricales* (*Basidiomycota*) II: *Strophariaceae*. **Mycosphere**, v. 6, n. 4, p. 441-458, 2015.

CORTEZ, V. G. Type studies on South American *Strophariaceae*: 1. *Pholiota varzeae* from the Brazilian Amazon. **Mycotaxon**, v. 103, p. 137-140, jan.-mar. 2008a.

CORTEZ, V. G. Type studies on South American *Strophariaceae*: 2. *Pholiota trinitensis* is transferred to *Stropharia*. **Mycotaxon**, v. 105, p. 7-10, jul.-sep. 2008b.

CORTEZ, V. G.; COELHO, G. The *Stropharioideae* (*Strophariaceae*, *Agaricales*) from Santa Maria, Rio Grande do Sul, Brazil. **Mycotaxon**, v. 89, n.2, p. 355-378. apr./jun. 2004.

CORTEZ, V.G.; COELHO, G. Occurrence of the Rare Agaric *Stropharia melanosperma* (*Strophariaceae*) in Brazil. **Boletín de la Sociedad Micológica de Madrid**, v. 32, p. 31-37, 2008.

CORTEZ, V. G.; SILVEIRA, R. M. B. A new species of *Stropharia* with hymenial acanthocytes. **Mycologia**, v. 99, n. 1, p. 135-138, 2007.

CORTEZ, V. G.; SILVEIRA, R. M. B. The agaric genus *Stropharia* (*Strophariaceae*, *Agaricales*) in Rio Grande do Sul State, Brazil. **Fungal Diversity**, v. 32, p. 31-57, 2008.

DE HOOG, G. S.; GÖTTLICH, E.; PL ATAS, G.; GENILLOUD, O.; LEOTTA, G.; BRUMMELEN, J. V. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. **Studies in Mycology**, v. 51, p. 33-76, 2005.

DESJARDIN, D. E.; HEMMES, D. E. *Agaricales* of the Hawaiian islands: 7. Notes on *Volvariella*, *Mycena*, sect. *Radiatae*, *Physalacria*, *Porpoloma* and *Stropharia*. **Harvard papers in Botany**, v. 6, n. 1, p. 85-103, 2001.

ELGORASHI, E. E.; MAEKAWA, N.; SATOH, H. In Vitro Anti-inflammatory Activity of Selected Japanese Higher Basidiomycetes Mushrooms. **International Journal of Medicinal Mushrooms**, v. 10, n. 1, p: 49-53, 2008.

FARR, D. F. The acanthocyte, a unique cell type in *Stropharia* (*Agaricales*). **Mycotaxon**, v. 11, p. 241-249, 1980.

FIDALGO, O.; BONONI, V. L. R. **Técnicas de coleta, preservação e herborização de material botânico**. Série Documentos. Instituto de Botânica, São Paulo, jan. 1989. 62 p.

FRIES, E. M. **Summa Vegetabilium Scandinaviae**. Sectio posterior, 1849.

GRGURINOVIC, C. A. **Larger Fungi of South Australia**. Adelaide: The Botanic Gardens of Adelaide and State Herbarium: Flora and Fauna of South Australia Handbooks Committee. 1997.

HALL, T. A. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. **Nucleic Acids Symposium Series**, v. 41, p. 95-98, 1999.

HARPER, E. T. Species of *Pholiota* and *Stropharia* in the Region of the Great Lakes. **Wisconsin Academy of Sciences, Arts and Letters**, v. 17, p. 1011-1026, 1914.

IBGE (Instituto Brasileiro de Geografia e Estatística). **Manuais Técnicos em Geociências número 1: Manual Técnico da Vegetação Brasileira**. Rio de Janeiro, 2012.

KONG, C.; ZHAI, C.; LI, J.; ZHANG, J.; ZHANG, K.; LIU, Y. Evaluation of *Stropharia* sp. 1.2052 nematicidal effects against *Meloidogyne incognita* on tomato. **African Journal of Microbiology Research**, v. 7, p. 5737-5741. 2013.

KORNERUP, A.; WANSCHER, J. H. **Methuen Handbook of Colour**. 3 ed. London: Eyre Methuen. 1978.

KÜHNER, R. **Les Hymenomycetes Agaricoïdes: Étude Generale et Classification**. Lyon: Société Mycologique de Lyon. 1980.

LARGENT, D. L. **How to Identify Mushrooms to Genus I: Macroscopic Features**. Eureka: Eureka Publishing. 1986.

LARGENT D. L; JOHNSON, D.; WATLING, R. **How to Identify Mushrooms to Genus III: Microscopic Features**. Eureka, Eureka Publishing. 1977.

LUO, H.; LI, X.; LI, G.; PAN, Y.; ZHANG, K. Acanthocytes of *Stropharia rugosoannulata* function as a nematode-attacking device. **Applied and Environmental Microbiology**, v. 72, p. 2982-2987, 2006.

MALENÇON, G. Champignons du Maroc. **Beihefte zur Sydowia**, v. 8, p. 258-267, 1979.

MATHENY, P. B.; CURTIS, J. M.; HOFSTETTER, V.; AIME, M. C.; MONCALVO, J-M.; GE, Z-W., YANG, Z-L.; SLOT, J. C.; AMMIRATI, J. F.; BARONI, T. J.; BOUGHER, N. L.; HUGHES, K. W.; LODGE, D. J.; KERRIGAN, R.W; SEIDL, M. T.; AANEN, D. K.; DENITIS, M.; DANIELE, G. M.; DESJANDIN, D. E.; KROPP, B. R.; NORVELL, L. L.; PARKER, A.; VELLINGA, E. C.; VILGALYS, R.; HIBBETT, D. S. Major clades of Agaricales: a multilocus phylogenetic overview. **Mycologia**, v. 98, n. 6, p. 982-995, 2006.

MEIJER, A. A. R. Preliminary List of the Macromycetes From the Brazilian States of Paraná. **Boletim do Museu Botânico Municipal**, Curitiba, Paraná, n. 68, p. 1-55, 2006.

MEIJER, A. A. R. Preliminary list of the macromycetes from the Brazilian state of Paraná: corrections and updating. **Boletim do Museu Botânico Municipal**, Curitiba, Paraná, n. 72, 2010.

MITCHELL, D. Spring and Fall Diet of the Endangered West Virginia Northern Flying Squirrel. **The American Midland Naturalist**, v. 146, n. 2, 439-443. 2001.

MITCHELL, D. H; SMITH, A. H. Notes on Colorado Fungi III: new and interesting mushrooms from the aspen zone. **Mycologia**, v. 70, p. 1040-1063, 1978.

MONCALVO, J-M; VILGALYS, R.; REDHEAD, S. A.; JOHNSON, J. E.; JAMES, T. Y.; AIME, M. C.; HOFSTETTER, V.; VERDUIN, S. J. W.; LARSSON, E.; BARONI, T. J.; THORN, R. G.; JACOBSSON, S.; CLÉMENÇON, H.; MILLER J. R, O. K. One hundred and seventeen clades of euagarics. **Molecular Phylogenetics and Evolution**, v.23, p. 357-400, 2002.

MURRILL, W. A. The *Agaricaceae* of the pacific coast - III. **Mycologia**, v. 4, n. 6, p. 294-308, nov. 1912.

MURRILL, W. A. The *Agaricaceae* of Tropical North America - VIII. **Mycologia**, v. 10, p. 62-85, 1918.

MURRILL, W. A. Dark-spored agarics II. *Gomphidius* and *Stropharia*. **Mycologia**, v. 14, p. 121-142, 1922.

MURRILL, W. A. Additions to Florida Fungi. **Mycologia**, v. 35, p. 529-537, 1943.

NOORDELOOS, M. E. Notulae ad Floram Agaricinam Neerlandicam XXIII - *Psilocybe* and *Pholiota*. **Persoonia**, v. 16, p. 127-129, 1995.

NOORDELOOS, M. E. *Strophariaceae*, Vol. 4. In: BAS, C.; KUYPER, T. W.; NOORDELOOS, M. E.; VELLINGA, E. (Ed.). **Flora Agaricina Neerlandica**. Rotterdam: A. A. Balkema, 1999. p. 27-107.

NOORDELOOS, M. E. *Strophariaceae* s.l. Fungi Europaei 13. Alassio: Edizioni Candusso. 2011.

NORVELL, L. L.; REDHEAD, S. A. *Stropharia albivelata* and its basionym *Pholiota albivelata*. **Mycotaxon**, v. 76, p. 315-320, 2000.

PEGLER, D. N. A preliminary agaric flora of East Africa. **Kew Bulletin Additional Series**, v. 6, p. 1-615, 1977.

PEGLER, D. N. Useful Fungi of the World: Wine Cap - The Gardener's Mushroom. **Mycologist**, v. 15, n. 2, 2001.

PUTZKE, J. Lista de fungos *Agaricales* referidos para o Brasil. **Caderno de Pesquisa**. Série Botânica. Santa Cruz do Sul, v. 6, n. 2, p. 3-186, 1994.

QUÉLET, L. Les champignons du Jura et des Vosges. **Mémoires de la Société d'Émulation de Montbéliard**. Paris: J.B. Baillière et Fils, 1872.

RAMÍREZ-CRUZ, V., GUZMÁN, G., VILLALOBOS-ARÁBOLA, A. R., RODRÍGUEZ, A., MATHENY, P. B., SÁNCHEZ-GARCIA, M. & GUZMÁN-DÁVALOS, L. Phylogenetic inference and trait evolution of the psychedelic mushroom genus *Psilocybe* sensu lato (Agaricales). **Botany**, v. 91, p. 573-591, 2013.

RATKOWSKY, D. A; GATES, G. M.; CHANG, Y. S. Two new combinations in the *Strophariaceae* (*Agaricales*) of Tasmania. **Australasian Mycologist**, v. 31, p. 39-40, 2013.

REDHEAD, S. A. Nomenclatural novelties. **Index Fungorum**, n. 15, p. 1-2, 2013.
Disponível em:
<<http://www.indexfungorum.org/Names/IndexFungorumPublicationsListing.asp>>.
Acesso em: 02 ago. 2013.

RICK, J. Basidiomycetes Eubasidii in Rio Grande do Sul – Brasília. 5. *Agaricaceae*. **Iheringia**, Série Botânica, v. 8, p. 296-450, 1961.

ROMAGNESI, H. Essai d'une classification générique des agarics lanthinosporés et Melanosporés. **Revue de Mycologie**, v. 1, p. 27-36, 1936.

SENTHILARASU, G.; SINGH, S. A new species of *Stropharia* from Western Ghats, India. **Mycotaxon**, v. 123, p. 213-220, jan.-mar. 2013.

SESLI, E.; DENCHEV, C. M. Checklists of the *Myxomycetes*, larger *Ascomycetes*, and larger *Basidiomycetes* in Turkey. **Mycotaxon**, v.106, p. 65-67, 2008.

SILVA, P. S; CORTEZ, V. G.; SILVEIRA, R. M. B. The mycobiota of Itapuã Park, Rio Grande do Sul, Brazil - I. Species of *Strophariaceae* (*Agaricales*). **Mycotaxon**, v. 97, p. 219-229 july./sept. 2006.

SILVA, P. S; CORTEZ, V. G.; SILVEIRA, R. M. B. *Strophariaceae* (*Agaricales*, *Basidiomycota*) no Parque Estadual de Itapuã, Viamão, Rio Grande do Sul: chave para identificação das espécies. **Revista Brasileira de Biociências**, Porto Alegre, v. 6, n. 3, p. 253-258, jul./set. 2008.

SILVA, P. S; CORTEZ, V. G.; SILVEIRA, R. M. B. New species of *Stropharia* from *Araucaria angustifolia* forests of southern Brazil. **Mycologia**, v. 101, n. 4, p. 539-544, 2009.

SILVA, P. S; CORTEZ, V. G.; SILVEIRA, R. M. B. Synopsis of the *Strophariaceae* (*Basidiomycota*, *Agaricales*) from Floresta Nacional de São Francisco de Paula, Rio Grande do Sul State, Brazil. **Hoehnea**, v. 39, n. 3, p. 479-487, 2012.

SINGER, R. The *Agaricales* in Modern Taxonomy. **Lilloa**, v. 22, p. 1-832, 1949.

SINGER, R. **The Agaricales in Modern Taxonomy**, 2th ed. Weinheim: J. Cramer. 1962.

SINGER, R. **The Agaricales in Modern Taxonomy**, 3th ed. Vaduz: J. Cramer. 1975.

SINGER, R. **The Agaricales in modern taxonomy**. 4th ed. Koenigstein: Koeltz Scientific Books. 1986.

SINGER, R.; DIGILIO, A. P. L. Pródromo de La Flora Agaricina Argentina. **Lilloa**, v. 25, p. 5-462, 1952.

SINGER, R; SMITH, A. H. The taxonomic positions of *Pholiota mutabilis* and related species. **Mycologia**, v. 38, n. 5, p. 500-523, 1946.

SMITH, A. H. Studies of North American Agarics – I. **Contributions from the University of Michigan Herbarium**, n. 5, 1941.

SMITH, A. H. Generic relationship within the *Strophariaceae* of the *Agaricales*. **Taxon**, v. 28, p. 19-21, 1979.

SOBESTIANSKY, G. Contribution to a Macromycete Survey of the States of Rio Grande do Sul and Santa Catarina in Brazil. **Brazilian Archives of Biology and Technology**, v. 48, n. 3, p. 437-457, 2005.

SULZBACHER, M. A.; PUTZKE, J.; PUTZKE, M. T. L.; CORTEZ, V. G . Nota sobre os fungos *Agaricales* (*Basidiomycota*) da Estação Ecológica de Aracuri, Esmeralda, Rio Grande do Sul, Brasil. **Biociências**, Porto Alegre, v. 15, n. 1, p. 143-153, jan. 2007.

TAMURA, K.; STECHER, G.; PETERSON, D.; FILIPSKI, A.; KUMAR, S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. **Molecular Biology and Evolution**, v. 30, p. 2725-2729, 2013.

TAN, E.; BAU, T. *Stropharia jilinensis*, a new species (*Strophariaceae*, *Agaricales*) from China. **Nova Hedwigia**, v. 99, p. 271-276, aug. 2014.

VIBRANS, Alexander Christian et al. **Inventário Florístico Florestal de Santa Catarina** – Diversidade e Conservação dos Remanescentes Florestais. Blumenau: Edifurb, v. 1, 2012. 344 p.

WASSER, S. P.; GRODZINSKAYA, A. A. Contributions to the taxonomy of the family *Strophariaceae* Sing. et A.H. Sm. (Higher Basidiomycetes). In: **Botany and Mycology for the Next Millennium**. (ed. WASSER, S. P.). Kholodny Institute of Botany/National Academy of Sciences of Ukraine. Kiev: 372-405, 1996.

WATLING, R.; GREGORY, N. M. **British Fungus Flora. 5: Strophariaceae & Coprinaceae** p. p. Edinburg: Royal Botanic Gardens/HMSO. 1987.

WEBSTER, R. P.; DE MERCHANT, I. New Staphylinidae (Coleoptera) records with new collection data from New Brunswick, Canada: Oxyporinae. **Zookeys**, v. 186, p. 263-271. 2012.

WHITE, T. J.; BRUNS, T.; LEE, S.; TAYLOR, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: INNIS, M. A.; GELFAND, D. H.; SNINSKY, J. J.; WHITE, T. J. (Ed). **PCR protocols: a guide to methods and applications**. San Diego: Academic Press. p. 315-322, 1990.

5 CAPÍTULO 1: Artigo aceito para publicação pela revista Darwiniana.

Stropharia coelhoi (Basidiomycota): a new species from Brazil

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Stropharia coelhoi: a new species from Brazil

Abstract. Seger, C.; M. A. Sulzbacher & V. G. Cortez. 2016. *Stropharia coelhoi* (Basidiomycota): a new species from Brazil. *Darwiniana*, nueva serie 4(1): xx-xx.

A new species of *Stropharia* is described from the Atlantic Forest of Rio Grande do Sul State, in southern Brazil. *Stropharia coelhoi* is distinguished by the smooth dark brown pileus, the presence of hymenial acanthocytes, cheilocystidia and pleurocystidia as chrysocystidia and the basidiospores size. The new species is described, illustrated and its taxonomy is discussed.

Keywords: Agaricales, *Araucaria angustifolia*, mycobiota, Neotropical fungi

Resumen. Seger, C.; M. A. Sulzbacher & V. G. Cortez. 2016. *Stropharia coelhoi* (Basidiomycota): nueva especie para el Brasil. *Darwiniana*, nueva serie 4(1): xx-xx.

Se describe una nueva especie de *Stropharia* para el Bosque Atlántico del estado de Rio Grande do Sul, en el sur del Brasil. *Stropharia coelhoi* se distingue por su píleo liso, de color castaño oscuro, presencia de acantocitos en las láminas, queilocistidios y pleurocistidios del tipo crisocistidios y el tamaño de las esporas. Se describe e ilustra la nueva especie y se discute su taxonomía.

Palabras clave: Agaricales, *Araucaria angustifolia*, hongos neotropicales, micobiota

INTRODUCTION

Stropharia (Fr.) Quél. is a genus of agaricoid, mushroom-forming fungi, which are characterized by the presence of partial veil usually forming an annulus or leaving remnants at the pileus margin, adnate to adnexed lamellae and well-developed rhizomorphs on stipe base, which are densely covered by spiniform crystals called acanthocytes – the latter considered a diagnostic feature of the genus (Farr, 1980; Cortez & Silveira, 2008).

Members of the genus are distributed worldwide, being recorded from all continents; however, currently a number of 25-30 species has been accepted, and such knowledge is presumed as being too limited yet. Recent taxonomic studies revealed unknown species from Asia (Senthilarasu & Singh, 2013; Tian & Bau, 2014), Australia (Chang et al., 2006), and America (Desjardin & Hemmes, 2001; Bandala et al., 2005). In Brazil, the genus is best known from the Atlantic Forest areas of southern and southeastern Brazil (Maia et al., 2015) and recent research in the southernmost state of Rio Grande do Sul resulted on the discovery of new taxa (Cortez & Silveira, 2007, 2008; Silva et al., 2009). In the course of our ongoing studies on southern Brazilian agaricoid fungi, we found a new member of *Stropharia*, whose description is the subject of the present paper.

MATERIALS AND METHODS

Specimens were collected in two areas of native ombrophilous and seasonal forests, from the state of Rio Grande do Sul, southern Brazil. In both places, the presence of *Araucaria angustifolia* (Brazilian or Paraná pine) was remarkable, as dominant element in the landscape of mixed ombrophilous forest (Esmeralda) or as relict in the seasonal deciduous forest (Santa Cruz do Sul) (Fig. 1). The specimens were analyzed macro- and microscopically following standard procedures for agaricoid fungi; color names in macroscopic features descriptions are from Kornerup & Wanscher (1978). Microstructures were analyzed with 3% KOH solutions and 1% Congo Red. Q is the ratio between the basidiospores length and width, Q_m is the average spore quotient and n is the number of measured basidiospores/number of analyzed basidiomata/number of collections. Holotype is preserved at the herbarium ICN (Department of Botany of Universidade Federal do Rio Grande do Sul – Thiers, 2016) and additional specimens at the HCP herbarium (Universidade Federal do Paraná, Campus Palotina).

RESULTS

Stropharia coelhoi C. Seger, Sulzbacher & Cortez **sp. nov.**

MycoBank MB 816934

TYPE: Brazil, Rio Grande Do Sul, Esmeralda, Estação Ecológica do Aracuri, 5-VI-2004, M.A. Sulzbacher, J. Putzke & M.T.L. Putzke 40 (holotype ICN). Fig. 2A–I.

Distinguished from other species of Stropharia by the smooth and dark brown pileus, abundant veil remnants on the pileus margin, basidiospores $10.5\text{--}13 \times 6.5\text{--}8 \mu\text{m}$, pleuro- and cheilocystidia as chrysocystidia, and presence of scattered acanthocytes in the hymenium and stipe apex.

Pileus 12–24 mm, convex to broadly convex, slightly umbonate, surface smooth, glabrous, wet when fresh, dark brown (7F4) to brown (7E4) on mature basidiomata, margin decurved with white velar remnants abundant. *Lamellae* adnate with decurrent tooth, near, greyish brown (6D3) becoming dark brown (6F6) in age, lamellulae present, edges straight and conspicuously white (6A1). *Stipe* 35–51 \times 3–8 mm, central, cylindrical to slightly broader at base; surface dry, slightly fibrillose to scabrose, squamulose below the annulus, white (28A1) at the top to greenish grey (28B2) near base. *Veil* present covering up the gills in young basidiomata, then producing abundant remnants on the pileus margin of mature specimens, as well also a floccose annulus on the stipe apex, white (28A1), often fragile and collapsing. *Rhizomorphs* pale gray (1B1), up to 20 mm long, present at base and sometimes involving the substrate. *Context* fleshy, orange white

(5A2). *Spore print* greyish brown (11F3) to violet brown (11F4), as seen on the stipe apex.

Basidiospores 10.5–14 (–14.5) × (6–) 6.5–8 (–9) μm, $Q= 1.47–1.87$ (–2), $Q_m= 1.66$, $n= 83/2/2$, ellipsoid to oblong in face and side view, apically truncated by a large germ pore ≤ 2.5 μm, rare slightly eccentric in side-view, brown in KOH, smooth, thick-walled.

Basidia 25–38 × 8–12 μm, clavate, mainly four-spored but two-spored basidia also present, sterigmata ≤ 3 μm long, thin-walled, hyaline; basidioles pyriform becoming clavate to broadly clavate, rounded to subcapitate apex; scattered basidia present in the gill edge. *Pleurochrysocystidia* 28.5–65 × 6–13 μm, clavate, clavate-fusiform, some lanceolate or fusoid, apex rounded or acute, less commonly mucronate (≤ 4 μm long), thin-walled, abundant in the sides of the gills. *Cheilochrysocystidia* (25–) 30–66.5 × 7–12.5 μm, fusiform to subcylindrical, rounded to acute apex, or with short mucronate apex, (≤ 2 μm long), thin-walled, common on gill edge. *Pileipellis* a cutis, slightly gelatinized, composed of cylindrical, prostrate to anticlinal hyphae, hyaline to yellowish brown in mass, thin-walled, with smooth or incrustated walls, 1.5–9 μm diam. *Pileal context* formed by cylindrical to inflated hyphae, interwoven, hyaline, smooth and thin-walled, 5–22 μm diam. *Lamella trama* subregular, slightly gelatinized, hyaline hyphae, thin-walled to slightly thick-walled, 3.5–11.5 μm diam. *Stipitipellis* with thin, smooth and hyaline hyphae, 3–6.5 μm diam., bearing bundles of caulocystidia. *Stipe trama* with cylindrical,

subparallel, hyaline hyphae, thin- to slightly thick-walled, 2.5–10 μm diam. *Cauloleptocystidia* 20–44 \times 6.5–11 μm , clavate to cylindrical, with rounded apex, thin-walled. *Chrysovessels* present, but rare in the pileus and stipe trama, filled with golden yellow content. *Acanthocytes* present, abundant on rhizomorphs and scattered in the hymenium and stem. *Clamp connections* present.

Etymology. Named after Dr. Gilberto Coelho (Universidade Federal de Santa Maria, UFSM), in recognition of his contributions to the knowledge of southern Brazilian macrofungi, especially the polypores.

Habitat and substrate. Solitary or in couples, on soil or rotting wood, in the *A. angustifolia* (Brazilian pine) forest.

Observations. The proposed new species is another *A. angustifolia* forest inhabitant and, as other recently discovered taxa from this region, it shares some morphological similarities (Cortez & Silveira, 2007, 2008; Silva et al., 2009). The presence of a brown pileus, basidiospores features and presence of cauloleptocystidia remind those of *S. araucariae* Cortez & R.M. Silveira, but the latter species differs in the fleshy and grooved annulus, shape of pleurochrysocystidia and cheilochrysocystidia, which are broadly fusoid and bear a rostrate apex, presence of cheiloleptocystidia and pileipellis strongly gelatinized; in addition, it is a more robust mushroom (Cortez & Silveira, 2008).

Stropharia acanthocystis Cortez & R.M. Silveira and *S. apiahyna* (Speg.) Cortez & R.M. Silveira also present hymenial acanthocytes, however their basidiospores are much smaller (up to $8 \times 5 \mu\text{m}$), yellowish under KOH and with a reduced and inconspicuous germ pore (Cortez & Silveira, 2008). The presence of hymenial acanthocytes, presumed by Cortez & Silveira (2007) as an exclusive feature in *S. acanthocystis*, has now been found in other southern Brazilian species of the genus. They can be much scantier or even rare in some species such as *S. coelhoi* and *S. apiahyna*, or very common and conspicuous as in *S. acanthocystis*.

Some brownish forms from the northern hemisphere *S. inuncta* (Fr.) Quéf. are also comparable, since they also exhibit a smooth pileus, partial veil producing a membranous annulus or a fibrillose annular zone, two or four spored basidia, as well similar pleuro- and cheilocystidia (Noordeloos, 2011). However, they differ in the lack of velar remnants on pileus margin, basidiospores slightly smaller with a reduced germ pore, lack of cheilochrysocystidia, and pileipellis with a thick gelatinized layer (Noordeloos, 2011).

Stropharia hornemannii (Fr.: Fr.) Lund. & Nannf., another species from northern hemisphere, presents umbonate pileus, with abundant velar remnants on margin and producing a floccose annulus; microscopically, it has similar shape and size of the basidiospores and pleurocystidia. The major differences among both species are the more robust and larger basidiomata with yellowish to violaceous tinges of *S. hornemannii*, as

well the absence of cheilochrysocystidia and pileipellis strongly gelatinized (Noordeloos, 2011).

Specimens examined. BRAZIL. **Rio Grande do Sul**. Esmeralda, Estação Ecológica do Aracuri, 05-VI-2004, *M.A. Sulzbacher, J. Putzke & M.T L. Putzke 40* (ICN). Santa Cruz do Sul, Parque da Gruta dos Índios, 28-VII-2007, *M.A. Sulzbacher & V.G. Cortez 146/07* (HCP).

DISCUSSION

In terms of infrageneric classification, *Stropharia* requires a profound revision, since the proposed taxonomic concepts for subgenera and sections are based mostly on European and North American members (e.g. Singer, 1986; Noordeloos, 2011). In the light of recent data and new described species, some Neotropical taxa (some previously classified in *Pholiota*) cannot be placed in any of the proposed subgenera/sections. According to Singer (1986) and Noordeloos (2011), *Stropharia* is divided into three main groups: *Stropharia*, *Mundae* and *Stercophila* - the latter now recognized as an independent genus: *Protostropharia* Redhead, Moncalvo & Vilgalys (Redhead, 2013). *Stropharia* Sect. *Stropharia* Fr. according to both Singer (1986) and Noordeloos (2011), is distinguished by subviscid to glutinous pileus, stipe dry, annulus never strongly grooved, may be absent, and chrysocystidia always present. The same authors defined *Stropharia* Sect. *Mundae*

(Fr.) Konr. & Maubl. by the subviscid to dry pileus, never glutinous, deeply grooved annulus and chrysocystidia present or absent.

Based on the current classification, *S. coelhoi* is not appropriately classified in any of the known sections. The surface of the pileus would include this species in Sect. *Mundae*, but the lack of fleshy and grooved annulus leads to Sect. *Stropharia*.

Other members of the genus (e.g. *S. acanthocystis*, *S. agaricoides* and *S. earlei*) are not accordingly classified in the proposed subgenera/sections, since they share morphological features common to both groups. Future studies, including molecular data and the comparison of worldwide materials, are needed to improve the infrageneric classification of *Stropharia*.

With the discovery of this additional species, we reinforce that diversity of strophariaceous fungi in *A. angustifolia* forests of southern Brazil remains poorly explored, and the number of species is probably higher than expected. Ongoing studies on *Stropharia* in southern Brazil will certainly reveal a more diverse mycobiota.

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BIBLIOGRAPHY

- Bandala, V. M., L. Montoya & D. Jarvio. 2005. Agarics from coffee plantations in Eastern Mexico: two new records. *Fungal Diversity* 20: 17–29.
- Chang, Y. S., G. M. Gates & D. A. Ratkowsky. 2006. Some new species of the Strophariaceae (Agaricales) in Tasmania. *Australasian Mycologist* 24: 53–68.
- Cortez, V. G. & R. M. B. Silveira. 2007. A new species of *Stropharia* with hymenial acanthocytes. *Mycologia* 99: 135–138.
- Cortez, V. G. & R. M. B. Silveira. 2008. The agaric genus *Stropharia* (Strophariaceae, Agaricales) in Rio Grande do Sul State, Brazil. *Fungal Diversity* 32: 31–57.
- Desjardin, D. E. & D. E. Hemmes. 2001. Agaricales of the Hawaiian Islands: 7. Notes on *Volvariella*, *Mycena* sect. *Radiatae*, *Physalacria*, *Porpoloma* and *Stropharia*. *Harvard Papers in Botany* 6: 85–103.
- Farr, D. F. 1980. The acanthocyte, a unique cell type in *Stropharia* (Agaricales). *Mycotaxon* 11: 241–249.
- Kornerup, A. & K. H. Wanscher. 1978. Methuen Handbook of Colour. 3rd ed. London: Eyre Methuen.

- Maia, L. C., A. A. Carvalho Júnior, L. H. Cavalcanti, A. M. Gugliotta, E. R. Drechsler-Santos, A. L. M. A. Santiago, M. S. Cáceres, T. B. Gibertoni, A. Aptroot, A. J. Giachini, A. M. S. Soares, A. C. G. Silva, A. C. Magnago, B. T. Goto, C. R. S. Lira, C. A.S. Montoya, C. L. A. Pires-Zottarelli, D. K. A. Silva, D. J. Soares, D. H. C. Rezende, E. D. M. N. Luz, E. L. Gumboski, F. Wartchow, F. Karstedt, F. M. Freire, F. P. Coutinho, G. S. N. de Melo, H. M. P. Sotão, I. G. Baseia, J. Pereira, J. J. S. Oliveira, J. F. Souza, J. L. Bezerra, L. S. Araujo Neta, L. H. Pfenning, L. F. P. Gusmão, M. A. Neves, M. Capelari, M. C. W. Jaeger, M. P. Pulgarín, N. Menolli Junior, P. S. Medeiros, R. C. S. Friedrich, R. S. Chikowski, R. M. Pires, R. F. Melo, R. M. B. Silveira, S. Urrea-Valencia, V. G. Cortez & V. F. Silva. 2015. Diversity of Brazilian fungi. *Rodriguésia* 66(4): 1033–1045.
- Noordeloos, M. E. 2011. Strophariaceae s.l. Fungi Europaei 13. Alassio: Edizioni Candusso.
- Redhead, S. A. 2013. Nomenclatural novelties. *Index Fungorum* 15: 1–2.
- Senthilarasu, G. & S. Singh. 2013. A new species of *Stropharia* from Western Ghats, India. *Mycotaxon* 123: 213–220.
- Silva, P. S., V. G. Cortez & R. M. B. Silveira. 2009. New species of *Stropharia* from *Araucaria angustifolia* forests of southern Brazil. *Mycologia* 101: 539–544.
- Singer, R. 1986. The Agaricales in Modern Taxonomy. 4th ed. Koeltz, Koenigstein.

- Thiers, B. [continuing updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>. Accessed: March, 2016.
- Tian, E. & T. Bau. 2014. *Stropharia jilinensis*, a new species (Strophariaceae, Agaricales) from China. *Nova Hedwigia* 99: 271–276.

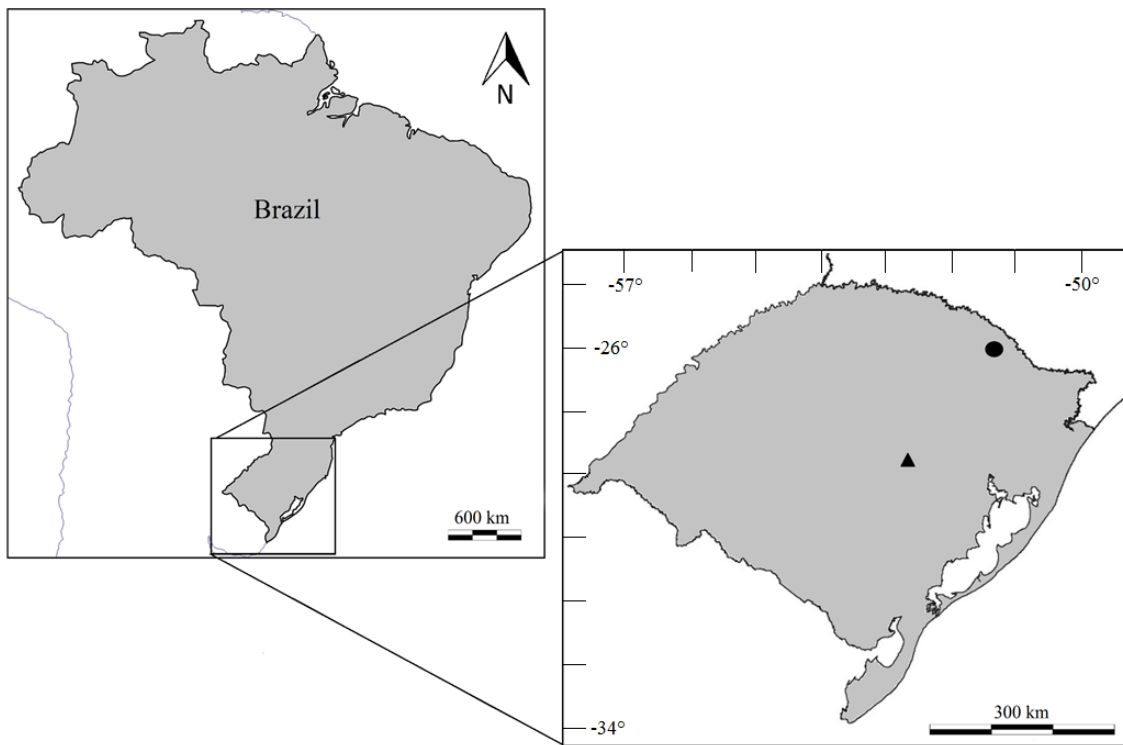


Fig. 1. Distribution of *Stropharia coelhoi*: Brazil, Rio Grande do Sul State, Esmeralda (●) and Santa Cruz do Sul (▲).

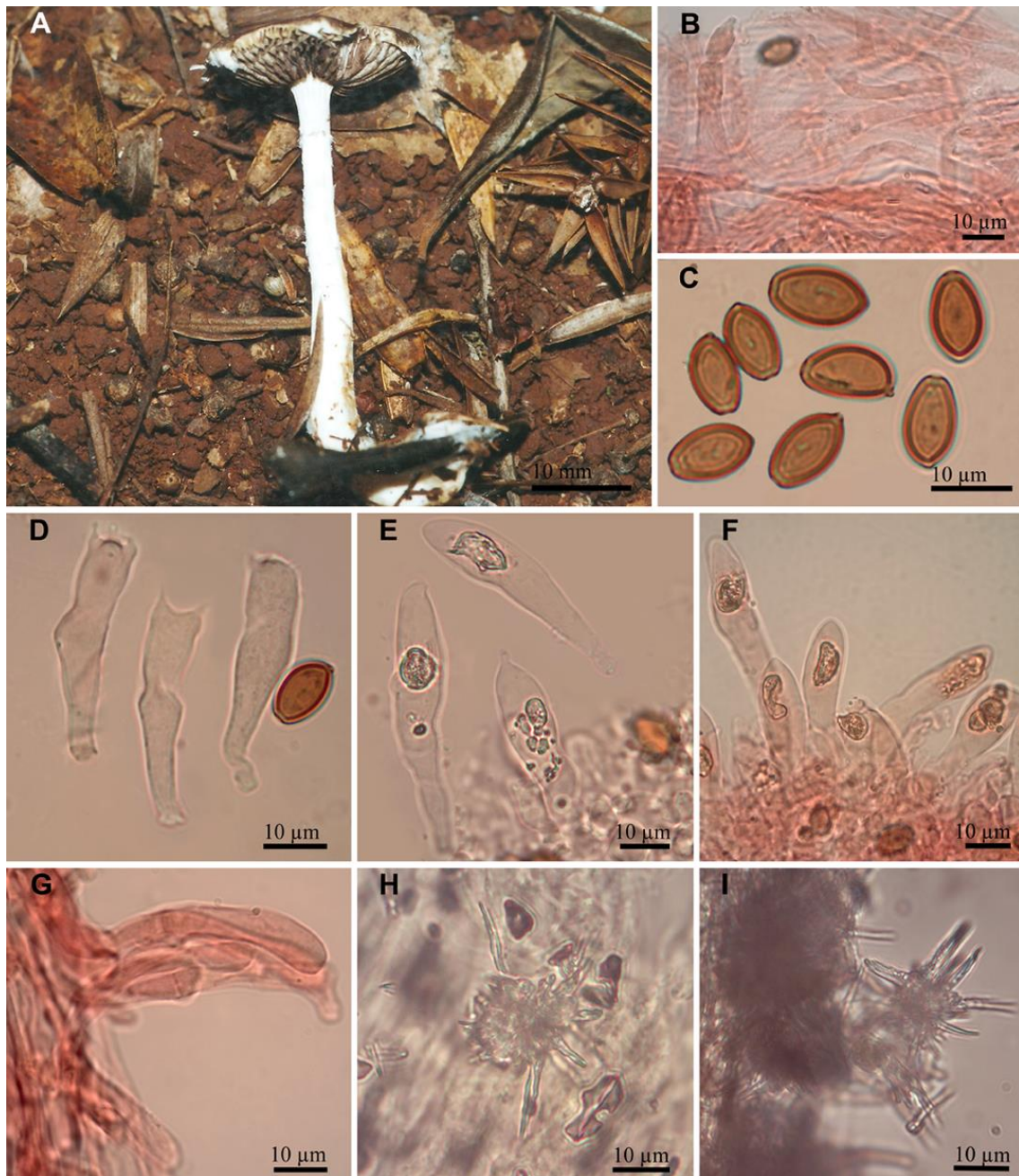


Fig. 2. *Stropharia coelhoi*. **A**, basidioma. **B**, pileipellis. **C**, basidiospores. **D**, basidia. **E**, pleurocystidia. **F**, cheilocystidia. **G**, caulocystidia. **H**, hymenial acanthocytes. **I**, acanthocytes on rhizomorphs. From Sulzbacher et al. 40 (ICN).

6 CAPÍTULO 2: Artigo a ser submetido à revista Kew Bulletin.

THE GENUS *STROPHARIA* S.S. (AGARICALES) IN SOUTHERN BRAZIL

Cristiane Seger¹ & Vagner G. Cortez²

Summary. The species of *Stropharia* (Strophariaceae) occurring in the Southern Brazil are revised. Seventeen species are recognized in the area, including four new taxa: *Stropharia acanthocystis*, *S. aeruginosa*, *S. agaricoides*, *S. alvesiana* sp. nov., *S. apiahyna*, *S. araucariae*, *S. coelhoi*, *S. coronilla*, *S. earlei*, *S. melanosperma*, *S. ombrophila* sp. nov., *S. pseudocyanea*, *S. rugosoannulata*, *S. trinitensis*, *S. venusta*, *S. versiformis* sp. nov. and *S. watlingii* sp. nov. Descriptions and illustrations are furnished for most studied species, including images of scanning electron micrographs showing details of the acanthocytes, also accompanied by distribution and taxonomic discussion, as well key for identification.

Key Words: Agaricales, Atlantic Forest, dark-spored agarics, Neotropical mycobiota, taxonomy.

Introduction

Stropharia (Fr.) Quél. is the type genus of Strophariaceae Singer & A H. Sm. (Agaricales), characterized by ochraceous brown to violet brown spore print, smooth and thick-walled

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basidiospores, which are apically truncated by a well-developed germ-pore, pileipellis formed by prostrate and more or less gelatinized hyphae, cheilocystidia usually present, pleurocystidia as chrysocystidia (cystidia with amorphous yellow-refractive content). Moreover, *Stropharia* spp. present important macroscopic features such as adnate or adnexed gills, presence of partial veil and well developed rhizomorphs (Singer 1986; Cortez & Silveira 2008; Noordeloos 2011). It has been adopted as a diagnostic feature of the genus the presence of acanthocytes, so that species previously classified into *Pholiota* (Fr.) P. Kumm. and *Hypholoma* (Fr.) P. Kumm. were subsequently recombined into this genus (Norvell & Redhead 2000; Cortez 2008a; 2008b). Acanthocytes are fascicles of spiny cells that cover the rhizomorphs of the basidiomata, and also grow in the mycelium in culture (Farr 1980). Coprophilous species of *Stropharia* [= Section *Stercophila* (Romagn.) Singer or Subgenus *Stercophila* Romagn. ex Noordel.], which do not produce acanthocytes, were transferred to a new genus: *Protostropharia* Redhead, Moncalvo & Vilgalys (Redhead 2013).

Researchers have reported *Stropharia* from all continents, except Polar Regions. Surveys of Strophariaceae reported 12 *Stropharia* s.s. from Europe (Noordeloos 2011), around 10 from North America (Bessette *et al.* 1997) and 32 taxa in South and Central America (Coimbra 2015). In Brazil, 11 species of *Stropharia* s.s. are known, mostly occurring in Atlantic Forest areas of south and southeastern Brazil (Maia *et al.* 2015).

In spite of being considered a supposedly well studied genus, its knowledge is limited and the affinities to other genera of Strophariaceae led to redefinition of taxonomic concepts in recent years. The aim of this work is improve the knowledge on *Stropharia* species occurring in Southern Brazil.

Material and methods

Studied specimens were collected in the Atlantic Forest, from Brazilian States of Rio Grande do Sul, Santa Catarina and Paraná. Specimens preserved at the herbaria ICN (Instituto de Biociências, Universidade Federal do Rio Grande do Sul), SMDB (Departamento de Biologia, Universidade Federal de Santa Maria), FLOR (Departamento de Botânica, Universidade Federal de Santa Catarina) and MBM (Museu Botânico Municipal, Curitiba) were also examined. The macroscopic description is based on fresh material (whenever possible), and color names and codes follows Kornerup & Wanscher (1978). For microscopic analysis, slides were mounted with 3% KOH solution and 1% Congo Red. Measurements of microscopic features were obtained using Moticam 2500 camera attached to Motic[®] microscope and dimensions by the Motic Image Plus 2.0[®] program. Scanning electron micrographs of the acanthocytes were obtained from a Jeol JSM-6360LV scanning electron microscope at Universidade Federal do Paraná; the procedure consisted of removal of a small portion of rhizomorph (ca. 2 mm long), which were coated by a 150 Å of gold-palladium in a Balzers SCD030 – Balzers Union FL9496, and examined under variable magnifications. All collected specimens are deposited at the Herbário do Campus Palotina, Universidade Federal do Paraná (HCP) and type specimens at the Herbário do Departamento de Botânica, Universidade federal do Paraná (UPCB). Descriptions and discussions for the most of the Southern Brazilian species of *Stropharia* are presented; specimens just described and illustrated by Cortez & Silveira (2008) are not re-described, unless new observations of taxonomic importance were added.

Key to the species of *Stropharia* in Southern Brazil

1. Basidiospores 5 – 9 (– 10) µm long, germ-pore inconspicuous or, if conspicuous, never truncated.....2
1. Basidiospores 9 – 16 µm long, with a conspicuous and apically truncated germ-pore..... 11

2. Pileus surface subviscid, viscid or glutinous; pileipellis an ixocutis with hyphae embedded in gelatinized layer.....3
2. Pileus surface dry or subviscid, never glutinous; pileipellis a cutis, none or slightly gelatinized5
3. Pileus brown to light brown, membranous light orange annulus, cauloleptocystidia and caulochrysocystia present.....**11. *S. ombrophila***
3. Pileus distinctly green or with greenish tones4
4. Spore print dark brown, caulocystidia as chrysocystidia.....**2. *S. aeruginosa***
4. Spore print brown, caulocystidia as leptocystidia.....**12. *S. pseudocyanea***
5. Acanthocytes numerous in the hymenium and stipe apex, annulus absent.....
.....**1. *S. acanthocystis***
5. Acanthocytes absent or very rare in the hymenium and stipe apex; annulus present, but sometimes ephemeral6
6. Cheiloleptocystidia clavate to pyriform or subglobose, caulochrysocystidia presente.....7
6. Cheiloleptocystidia clavate to broad-clavate, rare pyriform, caulochrysocystidia absent...9
7. Lamellae adnate, cheilochrysocystidia absent, cauloleptocystidia absent....**14. *S. trinitensis***
7. Lamellae adnexed, chrysocystidia and leptocystidia present on gill edge and stipe apex...8
8. Pileus orange with reddish tones, cheiloleptocystidia and cauloleptocystidia with rounded apex, < 10 µm in wide.....**3. *S. agaricoides***
8. Pileus yellowish brown, cheiloleptocystidia and cauloleptocystidia frequently with neck or projection at the apex, < 26 µm in wide.....**16. *S. versiformis***
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Species descriptions

Stropharia acanthocystis Cortez & R.M. Silveira (2007: 135). Type: Brazil, Rio Grande do Sul State, *V. G. Cortez* 053/05 (holotype ICN).

DESCRIPTION: see Cortez & Silveira (2007; 2008). Fig. 1 A & B.

DISTRIBUTION. Brazil: Rio Grande do Sul State (Cortez & Silveira 2008).

SPECIMENS EXAMINED: BRAZIL. Rio Grande do Sul State: São Francisco de Paula, FLONA SFP, 14 May 2005, *V. G. Cortez* 055/05 (ICN 139.133); 19 May 2006, *P. S. Silva* 146/06 (ICN 139.193); *P. S. Silva* 147/06 (ICN 139.194); 18 May 2007, *P. S. Silva* 169/07 (ICN 139.363); 18 May 2009, *P. S. Silva* 220/09 (ICN 154.249).

HABITAT: Gregarious or solitary, on rotten wood and less frequent in the ground, in mixed ombrophilous forest vegetation.

CONSERVATION STATUS. Not evaluated.

NOTES. *Stropharia acanthocystis* was described from the ombrophilous mixed forest of Rio Grande do Sul State and, until now, is known only from the type locality. This mushroom is recognized by the convex, slightly humid and red to yellowish red colored pileus, abundant veil present in the margin of the pileus and not producing a ring on stipe, the pileipellis is a cutis, basidiospores are yellowish brown, with inconspicuous germ-pore, $5.5 - 9 \times 3.5 - 5.5$ μm , pleurochrysocystidia clavate, cheiloleptocystidia pyriform to clavate, caulocystidia are lepto- and chrysocystidia, and acanthocytes present like cystidia both on stipe apex as hymenium (Cortez & Silveira 2007; 2008). After revising specimens collected after the original description, we noted that some materials presented yellowish brown basidiospores and more rounded tips (ICN 154.363), while others are brown and usually with a more tapered tips (ICN 139.194 and ICN 139.133). In dried specimens it is somewhat difficult to find cheilocystidia and caulocystidia, but other features (pleurocystidia, pileus surface and

especially hymenial acanthocytes) matches well the protologue of *S. acanthocystis*. First described as gregarious growing on wood, new collections indicate that it can be found also solitary on soil.

Stropharia aeruginosa (Curtis) Quél. (Quélet 1872: 141). Type: not designated.

DESCRIPTION: see Cortez & Silveira (2008). Fig. 1 E & F.

DISTRIBUTION. Widespread in Europe and North America (Noordeloos 2011). In South America, it is known from Argentina (Singer 1969) and Brazil (Rio Grande do Sul State) (Cortez & Silveira 2008).

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: Santa Maria, Morro do Elefante, 04 May 2002, *F. Wartchow & V. G. Cortez* 035/02 (SMDB 9.573, destroyed specimen); 037/02 (SMDB 9.575, destroyed specimen); 12 April 2003, *V. G. Cortez* 007/03 (SMDB 9.604, destroyed specimen); 11 April 2005, *V. G. Cortez* 011/05 (ICN 139.098).

ADDITIONAL SPECIMENS EXAMINED: SWEDEN. Hallands: Vallda, 25 Oct. 1976, *S. Jacobsson* 76.247 (ICN) – *Stropharia aeruginosa*. Bohuslän: Hogsam, 01 Oct. 1994, *S. Jacobsson* (ICN) – *Stropharia cyanea*.

HABITAT: Solitary to scattered, on much rotten wood, in seasonal semideciduous forest. Elsewhere, this fungus grows on soil in parks and urban places (Noordeloos 2011).

CONSERVATION STATUS. Not evaluated.

NOTES. This is the type species of the *Stropharia* and a blue-green species these genus, characterized yet by the white and well-developed annulus (Noordeloos 2011). However, Brazilian specimens lack that blue shades, and instead they are dark grayish green to grayish green with the margin fading yellowish, and the annulus is fugacious. All other features match well the descriptions of European and North American materials: a viscid to glutinous surface, white remnants at veil on the pileus edge, spore print dark brown, basidiospores pale brown

with inconspicuous germ pore, $6 - 8 (-9) \times 3.5 - 5 \mu\text{m}$, lamella edge sterile, with cylindrical to clavate cheiloleptocystidia, caulochrysocystidia present and pileipellis an ixocutis. The examined materials are the same reported by Cortez & Silveira (2008), since our attempts to recollect the mushroom were unsuccessful. A molecular comparison of Southern Brazilian collections with Northern Hemisphere specimens is necessary, which may prove to be taxonomically distinct.

Stropharia agaricoides P.S. Silva, Cortez & R.M. Silveira (2009: 101). Type: Brazil, Rio Grande do Sul State, *P.S. Silva* 191/07 (holotype ICN!).

Pileus 26 – 39 mm, flat to slightly depressed in age, dry surface, smooth to slightly squamulose toward the edge, brownish orange/reddish golden (6C3 – 6C4) to pastel red (7A4) on the margin and reddish orange (7A6) centrally; margin crenulate, slightly hygrophanous when wet. *Context* soft, thick centrally, gradually thinner to the margin, white. *Lamellae* adnexed, near, pastel grey (1C1) becoming greyish brown (7F3) in age, concolorous edge. *Stipe* 20 – 40 \times 3 – 4 (apex) \times 7 (base) mm, central, clavate flexuous; surface dry, smooth to slightly longitudinally striate, yellowish grey (4B2). *Veil* present like remnants scarce in the pileus margin and on the stipe apex it produces a fragile, ephemeral annulus. *Rhizomorphs* present at base. *Spore print* greyish brown (7E3 – 7F3).

Basidiospores (5.5 –) $6 - 7.5 (-9) \times (3.5 -) 3.5 - 5 (-5.5) \mu\text{m}$, $Q = 1.31 - 1.78$, $Q_m = 1.53$, $n = 45$, ellipsoid to oblong in face-view, amygdaliform in side view, inconspicuous germ pore, brown, smooth and thick-walled. *Basidia* $18.5 - 24.5 \times 6 - 7.5 \mu\text{m}$, clavate, tetrasporic (bisporic rare); sterigmata $\leq 4 \mu\text{m}$, thin-walled, hyaline. *Pleurochrysocystidia* $24 - 43 \times 7 - 11.5 (-14.5) \mu\text{m}$, fusiform, some broadly fusiform, acute to mucronate apex ($\leq 3.5 \mu\text{m}$ long), thin-walled, abundant. *Cheiloleptocystidia* $16.5 - 35 \times 5.5 - 8.5 \mu\text{m}$, clavate to pyriform or subglobose, rounded apex, thin-walled, common on gill edge. *Cheilochrysocystidia* $20.5 - 44$

× 7.5 – 10.5 µm, fusiform to pyriform, rounded to acute apex, rarely mucronate, thin-walled, uncommon. *Pileipellis* a cutis composed of cylindrical, prostrate, thin-walled hyphae, 4 – 11 µm diam., hyaline to light brown in mass, walls smooth. *Pileus context* formed by interwoven, hyaline, smooth and thin-walled hyphae, 4.5 – 13 µm diam. *Hymenophoral trama* subregular, with thin-walled, hyaline hyphae, 5 – 14 µm diam. *Cauloleptocystidia* 15.5 – 33.5 × 5 – 10 µm, clavate to pyriform, rounded apex, thin-walled, uncommon on stipe apex. *Caulochrysocystidia* 17 – 28.5 × 8 – 12 µm, clavate to subfusiform, rounded to acute apex, thin-walled, uncommon. *Stipitipellis* with parallel, hyaline and thin-walled hyphae, 3 – 17 µm diam. *Chryso-vessels* present. *Acanthocytes* present on rhizomorphs. *Clamp connections* present. Fig. 1 C & D & Fig. 2 A & B.

DISTRIBUTION. Brazil: Rio Grande do Sul State (Silva *et al.* 2009).

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: São Francisco de Paula, FLONA SFP, 30 June 2007, *P. S. Silva* 191/07 (ICN 139.385); 18 May 2009, *P. S. Silva* 239/09 (ICN 154.352, destroyed specimen); 13 April 2014, *C. Seger* 082 (HCP 899); 14 April 2014, *C. Seger* 083 (HCP 900).

HABITAT: Solitary, growing on soil and leaf litter in forests with *Araucaria angustifolia*.

CONSERVATION STATUS. Not evaluated.

NOTES. *Stropharia agaricoides* was described from *Araucaria angustifolia* Forest in Rio Grande do Sul State (Silva *et al.* 2009), with a few specimens deposited in herbarium. Based on herbarium review (including the holotype) and new collections at the type locality, we could note the presence of caulocystidia as leptocystidia and chrysocystidia, both not described in the protologue. These structures are, in fact, scattered in the examined specimens and sometimes difficult to find, but have taxonomic significance. *Stropharia agaricoides*, as other members in the genus, is only known from the Atlantic Forest of the Southern Brazilian plateau in altitude around 900 m.

Stropharia alvesiana C. Seger & Cortez **sp. nov.** Type: Brazil, Santa Catarina State, C.

Seger & C.R. Alves 133 (UPCB – holotype here designated).

MycoBank: NNNNN

Pileus 48 – 78 mm, broadly convex, depressed, viscid, translucent striate margin, olive yellow (3D8) centrally, olive brown (4E3) in margin, translucent striate dark grey (1F1). *Context* fleshy to soft, concolor with the pileus. *Lamellae* adnate, near, greyish brown (6F3), concolor edge. *Stipe* 89 – 93 × 5 – 7 mm, central, cylindrical, slightly thickened base; surface dry, longitudinally striate and with whitish fibrils, pale orange (5A3) to reddish blond (5C4). *Veil* present like remnants scarce on the pileus margin and on the stipe apex it produces a fragile, ephemeral annulus, leaving a scar in older basidiomes. *Rhizomorphs* present at stipe base. *Spore print* greyish brown (6E3).

Basidiospores (10.5 –) 11 – 15 (– 16) × 6 – 8 (– 9) μm, Q= 1.62 – 2.18 (– 2.59), Q_m= 1.86, n= 60, oblong to cylindrical in face and side-view, apically truncated by a germ-pore ≤ 2.5 μm, brown in KOH, with thick-walled, smooth. *Basidia* 19.5 – 27.5 × 7.5 – 8.5 μm, subclavate to hyphoid and centrally constricted, thin-walled, hyaline, tetrasporic; sterigmata ≤ 4.5 μm long. *Pleurochrysocystidia* 36 – 66.5 × 9 – 13.5 μm, fusiform, mucronate to rostrate apex (≤ 8.5 μm), content subhyaline to pale yellow, thin-walled, abundant. *Cheilocystidia* absent. *Pileipellis* a cutis, composed of prostrate, thin-walled hyphae, walls smooth, hyaline to slightly yellow in mass, 4 – 8.5 μm diam. *Pileal context* formed by interwoven, hyaline, smooth and thin-walled hyphae, 6 – 13.5 μm diam. *Hymenophoral trama* subregular with thin-walled, hyaline hyphae, 6.5 – 14 μm diam. *Cauloleptocystidia* 28.5 – 57.5 × 5 – 11 μm, cylindrical to lageniform, with rounded to subcapitate apex, thin-walled. *Stipitipellis* with parallel, hyaline and thin-walled hyphae, 6 – 13 μm diam. *Chryso-vessels* present. *Acanthocytes* present on rhizomorphs. *Clamp connections* present. Fig. 3 A – K & Fig. 9 C.

RECOGNITION. This fungus differs by olive yellow to olive brown pileus with translucent striate margin, veil forming an ephemeral annulus, leaving a scar in older basidiomes, large basidiospores ($11.5 - 14.5 \times 6.5 - 7.5 \mu\text{m}$), pleurochrysocystidia fusiform with mucronate to rostrate apex, cheilocystidia absence and long cylindrical cauloleptocystidia.

DISTRIBUTION. Brazil: Santa Catarina State.

SPECIMEN EXAMINED: BRAZIL. Santa Catarina State: Urubici, PARNA São Joaquim, 11 June 2014, *C. Seger & C.R. Alves* 133 (UPCB).

HABITAT: Scattered, growing on soil among grasses on the *Araucaria angustifolia* Forest.

CONSERVATION STATUS. Not evaluated.

ETYMOLOGY. Named after Brazilian mycologist Camila Ribeiro Alves, co-collector of the type specimen.

NOTES. This material was collected only once and in conditions of high humidity, showing a strongly glutinous pileus surface. In spite of a single collection, composed of two basidiomata in distinct stages of development, the absence of cheilocystidia of any type (lepto- or chrysocystidia) is a very uncommon feature in *Stropharia*, and therefore considered diagnostic for the proposal of a new taxon. The Mexican *S. cifuentesii* Bandala, Montoya & Jarvio is another species lacking cheilocystidia, but basidiomata are smaller (pileus < 30 mm diam., stipe < 37 mm long), reddish-brown to orange-brown color in pileus and has markedly smaller basidiospores ($5.5 - 7 \times 3.5 - 4.5 \mu\text{m}$, Bandala *et al.* 2005).

Stropharia araucariae (to be discussed as further) has similar size and shape, same habitat, as well size and shape of basidiospores, pleurocystidia and caulocystidia, but in this species there is abundant veil on pileus margin, a remarkable fleshy and grooved annulus and dimorphic cheilocystidia (Cortez & Silveira 2008), both absent in our material.

Stropharia alpina (M. Lange) M. Lange and *S. caerulea* Kreisel, two Northern Hemisphere species, also have pileus with green and yellow tinges, ephemeral annulus and

pleurochrysocystidia mucronate to rostrate, but both species present smaller basidiospores (8 – 9 μm in *S. caerulea*, 9 – 11 μm long in *S. alpina*), pileipellis an ixocutis and cheilocystidia are present (Noordeloos 2011).

Thus, based on the absence of cheilocystidia and combination of other features (cystidia, pileipellis and basidiospores), we propose it as a new species of *Stropharia*.

Stropharia apiahyna (Speg.) Cortez & R.M. Silveira (2008: 41). Type: Brazil, São Paulo, *Puiggari* 56 (LPS).

Pileus 14 – 23 mm, convex becoming plane with depressed center, surface glabrous to subtomentose, dark brown (6F6) to reddish brown (8E6). *Context* up to 3 mm, fleshy, pinkish white (9A2). *Lamellae*, adnate to adnexed, near, dark brown (6F6 – 8A2), with a white to concolorous edges. *Stipe* 19 – 39 \times 3 – 4 (apex) \times 5 (base) mm, central, cylindrical to clavate; surface dry with scattered white recurved scales specially below the annulus, pale orange (5A3) on apex and pale orange (5A4) to reddish orange (5C3) near at base. *Veil* present like white remnants on pileus margin and on the stipe upper half it produces a flocculose-membranous annulus, slightly furrowed on upper surface. *Rhizomorphs* white (1A1) present at base and involving some substrate. *Spore print* not seen.

Basidiospores (5–) 5.5 – 7 (–8) \times 3 – 5 μm , Q: 1.2 – 2, $Q_m = 1.59$, n= 80, ellipsoid to ovoid in face-view, reniform in side-view, inconspicuous germ-pore, some slightly tapered toward the germ-pore, pale yellow to yellowish brown in KOH, with smooth and thick-walled. *Basidia* (12 –) 14 – 24 \times 5 – 8 μm , clavate, some subcylindrical, tetrasporic; sterigmata \leq 5.5 μm , thin-walled, hyaline; clavate basidioles. *Pleurochrysocystidia* 21 – 40 \times 8 – 12 μm , fusiform to broadly fusiform, rounded to mucronate apex (\leq 5 μm long), thin-walled, abundant. *Cheiloleptocystidia* (18 –) 23 – 42.5 \times 9 – 21 μm , broadly clavate to pyriform, rounded apex, common on gill edge. *Pileipellis* a cutis, composed by cylindrical prostrate with clumps

anticlinally arranged hyphae, internal brown pigment present or some with brownish pigment incrustated in walls, smooth and thick-walled, 6 – 13 μm diam; some short and inflated elements on hypodermium zone. *Pileal context* formed by cylindrical to inflated, interwoven, hyaline, smooth and thin- to slightly thick-walled hyphae, 5.5 – 15 μm diam. *Hymenophoral trama* subregular, with cylindrical, thin-walled, hyaline hyphae, 6 – 11.5 μm diam. *Cauloleptocystidia* 12 – 52 \times 4 – 21.5 μm , clavate, fusiform or pyriform, some sphaeropedunculate, common on stipe apex. *Stipitipellis* with parallel, hyaline and thin-walled hyphae, 6 – 14 μm diam. *Chryso-vessels* present. *Acanthocytes* present on rhizomorphs. *Clamp connections* present. Fig. 1 G – L, Fig. 2 C & D. & Fig. 9 A & B.

DISTRIBUTION. Brazil: Paraná, Rio Grande do Sul (Cortez & Silveira 2008) and São Paulo States (Spegazzini 1919). Colombia: Cali (Singer 1978).

SPECIMEN EXAMINED: BRAZIL. Paraná State: Céu Azul, PARNA do Iguacú, 17 June 2015, *C. Seger* 206 (HCP 902). Palotina, PESC, 15 Aug. 2012, *L. S. Lettrari* 004 (HCP 903); 20 May 2014, *C. Seger & C. R. Alves* 122 (HCP 901). São Mateus do Sul, 23 May 1990, *A.A.R. de Meijer* 1747 (MBM 329.889, as *Stropharia earlei*).

HABITAT: Solitary, growing on soil or on rotten wood, in mixed ombrophilous and seasonal semideciduous forests.

CONSERVATION STATUS. Not evaluated.

NOTES. The studied materials of *S. apiahyna* previous to this study were reported by Singer (1978, as *Pholiota apiahyna*) and Cortez & Silveira (2008). Due to old material, there are some doubts on microscopic features, as caulocystidia, which not described in any of these papers. We based the identification of material from Paraná State on the habitat, shape and color of the pileus, basidiospore size and color, pileipellis, hypodermium structure, and cheilocystidia.

Stropharia ealei Norvell & Redhead and *S. trinitensis* (Dennis) Cortez are considered related species, sharing the pileus color, flocculose annulus, squamulose to glabrous surface, basidiospore size, pileipellis and cheiloleptocystidia. However, both fungi have larger basidiomata and the annulus is typically evanescent, and also, in *S. earlei* the cheilocystidia are variable in shape, basidiospores are brown and pileipellis is slightly gelatinized. *Stropharia trinitensis* lacks pleurocystidia with mucronate apex, presents globose and smaller cheilocystidia and caulochrysocystidia are present (Pegler 1983; Cortez & Silveira 2008).

In Brazil, *S. apiahyna* was known from the states of São Paulo and Rio Grande do Sul (Cortez & Silveira 2008) and our new collections expand its distribution to the Deciduous Forests of Paraná.

Stropharia araucariae Cortez & R.M. Silveira (2008: 42). Type: Brazil, Rio Grande do Sul State, V. G. Cortez 044/05 (holotype ICN).

DESCRIPTION: see Cortez & Silveira (2008). Fig. 4 A & B.

DISTRIBUTION. Brazil: Rio Grande do Sul State (Cortez & Silveira 2008).

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: São Francisco de Paula, FLONA SFP, 14 May 2005, V. G. Cortez 050/05 (ICN 139.128); V. G. Cortez 056/05 (ICN 139.134).

HABITAT: Solitary or dispersed, growing on soil into *Araucaria* Forest.

CONSERVATION STATUS. Not evaluated.

NOTES. Distinguished by the robust basidioma, with larger pileus (35 – 65 mm) and stipe (70 – 110 mm), dark grayish brown pileus with velar remnants at the margin, annulus fleshy and strongly grooved, basidiospores ellipsoid to ovoid, 10 – 13 µm long, with a broad germ-pore, pleurochrysocystidia fusiform-rostrate, dimorphic cheilocystidia and cauloleptocystidia

present. The examined materials are the same as reported by Cortez & Silveira (2008) and, as new collections were not made, *S. araucariae* remains known only from the type locality.

Stropharia coelhoi C. Seger, Sulzbacher & Cortez (2016: in press). Type: Brazil. Rio Grande Do Sul State, *Sulzbacher et al.* 40 (holotype ICN!).

DESCRIPTION: see Seger *et al.* (2016). Fig. 2 E & F.

DISTRIBUTION. Brazil: Rio Grande do Sul State (Seger *et al.* 2016).

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: Esmeralda, Estação Ecológica do Aracuri, 05 June 2004, *M.A. Sulzbacher et al.* 40 (ICN). Santa Cruz do Sul, Parque da Gruta dos Índios, 28 July 2007, *M.A. Sulzbacher & V.G. Cortez* 146/07 (HCP 892).

HABITAT: Solitary or in couples, on rotting wood or soil, in the *Araucaria* Forest.

CONSERVATION STATUS. Not evaluated.

NOTES. It is a small species of *Stropharia*, recently described from *Araucaria angustifolia* forests of Rio Grande do Sul State, Brazil (Seger *et al.* 2016). Its pileus is up to 24 mm, smooth and dark brown and have abundant veil at pileus margin, the stipe is up to 51 mm and presents a floccose and apical annulus, basidiospores are ellipsoid to oblong apically truncated by a large germ-pore, $10.5 - 14 \times 6.5 - 8 \mu\text{m}$, only chrysocystidia present on lamellae sides and edge, while caulocystidia as leptocystidia. For a detailed full description and discussion, see Seger *et al.* (2016).

Stropharia coronilla (Bull.) Quélet. (Quélet 1872: 110). Type: not existing.

DESCRIPTION: see Cortez & Silveira (2008).

DISTRIBUTION. Widely distributed in all continents (Noordeloos 2011; Coimbra 2015).

SPECIMEN EXAMINED: BRAZIL. Paraná State: Curitiba, São José dos Pinhais, Reserva Biológica Cambui, 27 March 1980, *A.A.R. de Meijer* (MBM 325.688); 4 April 1980, *A.A.R.*

de Meijer (MBM 325.689). Palotina, Campus UFPR, 20 Feb. 2015, *C. Seger* 154 (HCP 889); 20 May 2015, *C. Seger* 190 (HCP 890); 26 Nov. 2015, *C. Seger* 209 (HCP 891). Paranaguá, Pontal do Sul, 1992 (MBM 397.340). Rio Grande do Sul State: Caçapava do Sul, Guaritas, 30 April 2005, *V. G. Cortez* 028/05 (ICN 139.110); 17 Feb. 2008, *V. G. Cortez* 034/08 (HCP). Dom Pedro de Alcântara, 20 May 2005, *V. G. Cortez* 066/05 (ICN 139.144). Porto Alegre, Campus of Universidade Federal do Rio Grande do Sul, 06 May 2004, *V. G. Cortez* 015/04 (ICN 139.003). Santa Maria, Camobi, 26 Feb. 2003, *V. G. Cortez* 003/03 (SMDB 9.600); Campus of Universidade Federal de Santa Maria, 27 March 2002, *V. G. Cortez* 017/02 (SMDB 9.557); 08 May 2008, *V. G. Cortez* 077/08 (HCP); Silva Jardim Street, 23 Feb. 2009, *V. G. Cortez* 005/09 (HCP); Tancredo Neves, 12 April 2005, *V. G. Cortez* 012/05 (ICN 139.099); 07 April 2002, *V. G. Cortez* 027/02 (SMDB 9.566); Três Barras, 14 March 2008, *V. G. Cortez* 061/08 (HCP); Vila Caramelo, 02 May 2001, *V. G. Cortez* 021/01 (SMDB 9.243). Viamão, Itapuã State Park, 08 May 2004, *P. S. Silva* 063/04 (ICN 139.075); *P. S. Silva* 064/04 (ICN 139.076); *P. S. Silva* 065/04 (ICN 139.077); *P. S. Silva* 067/04 (ICN 139.078); 09 April 2005, *P. S. Silva* 099/05 (ICN 139.072); *P. S. Silva* 098/05 (ICN 139.073); *P. S. Silva* 100/05 (ICN 139.074); 16 April 2005, *P. S. Silva* 117/05 (ICN 139.071); *P. S. Silva* 114/05 (ICN 139.079); 27 Jan. 2006, *M.S. Rother* 111/06 (ICN 139.350); 27 April 2006, *M.S. Rother* 137/06 (ICN 139.351). Santa Catarina State: Vargem Bonita, 18 March 2013, *C. Seger* 032 (HCP 667).

HABITAT: Scattered to gregarious, growing on lawns, gardens and pastures.

CONSERVATION STATUS. Not evaluated.

NOTES. This is a mushroom commonly found in lawns, gardens and field areas, identified by yellow to pale yellow and dry pileus, short stipe with a grooved membranous to fleshy annulus, basidiospores brown with a small but visible germ-pore, but not apically truncated, 5.5 – 9 (– 10) μm long, cheiloleptocystidia clavate to broad-clavate, cauloleptocystidia present and the pileipellis a cutis (Noordeloos 2011). Distributed worldwide, in Brazil it known from

the Northeast (Batista & Bezerra 1960) to South, where it is more common (Meijer 2006; Cortez & Silveira 2008) and now it is recorded for the first time to Santa Catarina State and seasonal forest of the western Paraná.

Stropharia earlei Norvell & Redhead (2000: 317). Type: Cuba: Prov. De la Halana, *F. S. Earle* 159 (isotype MICH).

= *Pholiota cubensis* Earle (1906: 242) non *Stropharia cubensis* Earle [= *Psilocybe cubensis* (Earle) Singer 1948: 37]

DESCRIPTION: see Cortez & Silveira (2008). Fig. 4 C & D.

DISTRIBUTION. Central and South America (Coimbra 2015), Brazil: São Paulo (Pegler 1997) and Rio Grande do Sul States (Cortez & Silveira 2008).

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: Itaara, Balneário Pinhal, 23 Fev. 2002, *V. G. Cortez* 007/02 (SMDB 9.550); 14 April 2005, *V. G. Cortez* 014/05 (ICN 139.000). Santa Maria, Morro do Elefante, 24 April 2004, *V. G. Cortez* 008/04 (ICN 139.001).

HABITAT: On soil or wood debris, inside of the forest.

CONSERVATION STATUS. Not evaluated.

NOTES. Characterized by medium to large sized basidiomata, pileus 30 – 120 mm diam., stipe 30 – 75 mm long, dry to subviscid, yellowish brown to dark brown pileus, with surface floccose-scaly, membranous to floccose, median and evanescent annulus, basidiospores 6 – 8 µm long, ovoid in face view and reniform in side view, yellowish brown in KOH, cheilocystidia and caulocystidia as leptocystidia, and pileipellis a cutis, which is not gelatinized (Smith & Hesler 1968; Pegler 1983; Cortez & Silveira 2008). These features placed this and other taxa (now placed in *Stropharia*) in *Pholiota* Sect. *Albivelata* A.H. Sm. & Hesler. Also belonging to this group, *S. apiahyna* differs in the smaller basidiomata (pileus up to 3 cm), cheiloleptocystidia broadly fusiform to ventricose and slightly smaller

basidiospores (5.5 – 7 µm). *Stropharia ombrophila*, *S. varzeae* and *S. trinitensis* present caulochrysocistidia; the former has pileipellis of ixocutis type, *S. varzeae* has cheilochrysocistidia and *S. trinitensis* has little pigmented (pale yellow) basidiospores and pyriform to globoid cheilocystidia, while in *S. earlei* they are clavate to cylindrical (Pegler 1983).

Three materials from Paraná State have been identified as *S. earlei* (Meijer 2010). However, we have concluded that one of these (MBM 329.889) corresponds to *S. apiahyna* because of its small basidiomata (pileus dry with 1 cm), pale yellow basidiospores and slightly smaller (5.5 – 7 × 3 – 5 µm). Likewise, the other two specimens (MBM 397.338 and MBM 397.339) have smaller basidiospores (5 – 7.5 × 3.5 – 5.5 µm) and both have caulochrysocistidia, but the identification of these materials was inconclusive. Thus, *S. earlei* is reported in Brazil for the states of São Paulo (Pegler 1997) and Rio Grande do Sul (Cortez & Silveira 2008).

Stropharia melanosperma (Fr.) Gillet (1874: 579). Type: not existing.

DESCRIPTION: see Cortez & Coelho (2008). Fig. 4 E.

DISTRIBUTION. Eurasia, poorly known in America: United States, Costa Rica, Argentina and Brazil (only in Rio Grande do Sul State - Murril 1922; Noordeloos 2011; Coimbra 2015).

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: Santa Maria, Morro do Elefante, 23 June 2006, *G. Coelho & V. G. Cortez* 056/06 (SMDB 10.725).

HABITAT: Solitary or in small groups, terrestrial on litter. In Southern Brazil occurs in seasonal semideciduous forest.

CONSERVATION STATUS. Not evaluated.

NOTES. *Stropharia melanosperma* is a rare fungus and is registered only once in Brazil (Cortez & Coelho 2008), whose material was revised here. The species is recognized by virtue of the robust and white basidiomata, deep violet lamellae and fleshy double annulus (striated

above and smooth below), basidiospores $10 - 12.5 (- 13.5) \times 6.5 - 7.5 (- 9) \mu\text{m}$, ellipsoid to oblong in side view, subhexagonal in face-view, truncated by apical germ pore, with dark brown and thick wall, cheilochrysocystidia and cauloleptocystidia. Subhexagonal basidiospores are also produced by *S. rugosoannulata* Farlow, which shares other macro- and microscopic features with *S. melanosperma* (e.g. shape of the basidiomata, annulus, lamellae color, chrysocystidia and leptocystidia), but they differ because *S. rugosoannulata* has reddish brown pileus and chrysocystidia on lamellae edge (Noordeloos 2011).

Stropharia ombrophila C. Seger & Cortez **sp. nov.** Type: Brazil, Paraná State, C. Seger 199 (UPCB – holotype here designated).

Mycobank: NNNNN

Pileus 25 – 50 mm, broadly conic (or convex) papillate when young becoming plane in age, subviscid to viscid layer that can be easily removed, scales scarce on surface of dry basidiomata, brown (7E5) centrally and light brown (7D5) in the margin. *Context* 3 mm, white (1A1), fleshy. *Lamellae* 3 – 6 mm, adnate with a decurrent tooth, near, greyish brown (6D3), concolorous to white edge. *Stipe* 55 – 85 \times 4 – 6 mm, central, clavate flexuous, stuffed; surface dry, smooth above at annulus and fibrillose scarce below that, pale orange (5A3) to light orange (5A4) for the most part and white (5A1) to orange white (5A2) near of apex. *Veil* present like remnants scarce in the pileus surface and at margin and on the stipe it produces a membranous, single edge annulus, concolorous with a stipe. *Rhizomorphs* present at base. *Spore print* yellowish brown (5E8).

Basidiospores (5.5 –) 6.5 – 8.5 (– 10) \times 3.5 – 5 (– 5.5) μm , $Q = 1.27 - 2.19$, $Q_m = 1.69$, $n = 147$, ellipsoid to oblong in face-view, ellipsoid to amygdaliform in side view, inconspicuous germ pore, yellowish brown with thick-walled, smooth. *Basidia* (13 –) 15 – 27 \times 4.5 – 8 μm , clavate, tetrasporic; sterigmata $\leq 5 \mu\text{m}$, thin-walled, hyaline. *Pleurochrysocystidia* 26 – 50 \times 8 – 16

µm, fusiform to broadly fusiform, acute to mucronate apex (≤ 5.5 µm long) thin-walled, abundant. *Cheiloleptocystidia* 20 – 39 × 4 – 13 µm, cylindrical to clavate flexuous, some lageniform, rounded apex, thin-walled, abundant on gill edge. Lamellae edge sterile. *Pileipellis* an ixotricoderm, composed of gelatinized, thin and cylindric hyphae, anticlinally arranged, smooth and slightly thick-walled, with internal yellowish pigment, 3 – 5 µm diam. *Pileal context* formed by interwoven, hyaline, smooth and thin-walled hyphae, some inflated, 6 – 25 µm diam. *Hymenophoral trama* subregular to regular, with thin-walled, hyaline hyphae, 9 – 13 µm diam. *Cauloleptocystidia* 34 – 40 × 5 – 10 µm, cylindrical, clavate, fusiform to utriform, with rounded apex, thin-walled, common. *Caulochrysocystidia* 26 – 34 × 6 – 10 µm, cylindric to clavate, some fusiform, with rounded or square apex, thin-walled, abundant on stipe apex. *Stipitipellis* with parallel, hyaline and thin-walled hyphae, 7 – 14 µm diam. *Chryso-vessels* present. *Acanthocytes* abundant on rhizomorphs. *Clamp connections* present. Fig. 5 A – K & Fig. 9 D.

RECOGNITION. The species distinguished by membranous and pale to light orange annulus, spore print yellowish brown, cheiloleptocystidia cylindrical to clavate flexuous and abundant on gill edge, pileipellis an ixotricoderm and dimorphic caulocystidia are present.

DISTRIBUTION. Brazil: Rio Grande do Sul, Santa Catarina and Paraná States.

SPECIMEN EXAMINED: BRAZIL. Paraná State: Colombo, Ribeira Street km 111, 18 May 1999, A. A. R. de Meijer & M. A. L. A. Amazonas 3610 (MBM 397.336). Guarapuava, Parque Natural Municipal das Araucárias, 01 June 2015, C. Seger 191 (HCP 893); C. Seger 199 (UPCB). Rio Grande do Sul State: São Francisco de Paula, CPCN Pró Mata, 10 June 2005, V. G. Cortez 074/05 (ICN 139.152). São Francisco de Paula, FLONA SFP, 13 April 2014, C. Seger 077 (HCP 894). Santa Catarina State: Itapoá: RPPN Volta Velha, 09 May 2014, C. Seger & C. R. Alves 102 (HCP 896). Urubici, PARNA São Joaquim, 22 April 2015, C. Seger & C. R. Alves 169 (HCP 895).

HABITAT: Scattered in *Araucaria angustifolia* forest (Mixed Ombrophilous Forest) and subtropical rain forest (Ombrophilous Dense Forest), growing on wood or leaf litter, including in *Araucaria angustifolia* branches and needles.

CONSERVATION STATUS. Not evaluated.

ETYMOLOGY. Referring to the ombrophilous (rainy) forest habitat.

NOTES. The light brown spore print, phaseoliform and yellowish brown basidiospores with thin walls would place this species in *Pholiota* Sect. *Albivelata*, but most species in this group have now been transferred to *Stropharia* due to the presence of acanthocytes on rhizomorphs. In this way, *Stropharia albivelata* (= *Pholiota albivelata*) seems to be the most closely related species, as it has basidiomata with approximately equal size (reaching 5.5 cm broad), slightly fibrillose stipe and fixed annulus, but differs by longer and narrower basidiospores ($9 - 11 \times 4 - 5 \mu\text{m}$) and the annulus shape, being very broad, white and furrowed by the lamellae (Murrill 1912). With further details of the microscopic characters, Smith & Hesler (1968) described chrysocystidia on the sides of the lamellae and its edge sterile by filamentose-subcapitate cheiloleptocystidia, and the most different thing: the presence of scattered cauloleptocystidia and lack of chrysocystidia on stipe apex.

Stropharia varzeae, only known from Amazonia where it grows on wood, is similar by basidiomata size, it has convex umbonate pileus, membranous annulus, equal basidiospores and presence of caulochrysocystidia. However, it differs of our species by dry pileus, stipe proportionally shorter compared to pileus, cheilocystidia as chrysocystidia with mucronate apex and pileipellis a trichodermium not gelatinized (Cortez 2008a). *Stropharia trinitensis* has similar basidiomata and basidiospores, but differs by fibrillose to floccose veil, lageniform to ventricose and larger pleurochrysocystidia ($38 - 59 \times 10 - 14.5 \mu\text{m}$), cheiloleptocystidia pyriform to globose and lack of gelatinized pileipellis (Cortez 2008b). *Stropharia aeruginosa* and *S. pseudocyanea* resembles microscopically by basidiospores, gelatinized pileipellis and

long thin cheiloleptocystidia (subcapitate in these species). However, they differ easily by pileus with green hues and evanescent annulus (Noordeloos 2011).

Stropharia ombrophila seems to be well distributed in the ombrophilous forests of Southern Brazil, since it was found in the three states of the region. Like other species of Southern Brazilian *Stropharia*, it is better represented in *Araucaria angustifolia* forest, but also found in Tropical Rainforest.

Stropharia pseudocyanea (Desm.) Morgan (1908: 74). Type: not existing.

= *Stropharia albocyanea* (Fr.) Quélet (Quélet 1872: 255);

= *Pholiota subcaerulea* A.H. Sm. & Hesler (Smith & Hesler 1968: 147);

= *Stropharia ochrocyanea* M. Bon (1972: 28);

= *Stropharia procera* (Kreisel) Contu (1999: 33).

Pileus 20 mm, convex with distinct rounded umbo, very viscid with gelatinous pellicle when moist, greyish green (30E5) becoming olive yellow (3C8 – 3D8) when dry; velar remnants scarce on the pileus and more densely in the margin. *Context* fleshy, white. *Lamellae* adnate, with small decurrent tooth, brownish grey (7C2) becoming light brown (5D5) with age, slightly white edge. *Stipe* 33 × 4 mm, central, cylindrical-flexuous, slightly thickened base; surface slight fibrillose/tomentose, white (28A1), greenish grey near base (28B2). *Rhizomorphs* present at base. *Spore print* brown (6E4).

Basidiospores (6.5 –) 7 – 7.5 μm × 4 – 4.5 (– 5) μm, Q= 1.46 – 1.90, Qm= 1.64, n= 30, ellipsoid to oblong in face-view, amygdaliform in side view, with a minute germ pore, yellowish brown/melleous in KOH, smooth, thick-walled. *Basidia* 17.5 – 26 × 6.5 – 7.5 μm, clavate, tetrasporic; thin-walled, hyaline. *Pleurochrysocystidia* 26 – 41 × 7 – 10 μm, fusiform, mucronate apex; thin-walled, abundant. *Cheiloleptocystidia* 34.5 – 51.5 × 7.5 – 10 μm,

cylindrical with middle part flexuous subcapitate to capitate apex, thin-walled. *Pileipellis* an ixotrichoderm, composed of cylindrical, anticlinal to prostrate, hyaline to yellowish, thin-walled hyphae, with smooth or incrustated walls by yellowish pigment, 2 – 5.5 µm diam. *Pileal context* formed by interwoven, hyaline, smooth and thin-walled hyphae, some inflated, 6 – 18 µm diam. *Hymenophoral trama* subregular with thin-walled, hyaline hyphae, 2 – 16 µm diam. *Cauloleptocystidia* 22.5 – 40.5 × 6.5 – 11.5 µm, cylindrical with middle part flexuous, some lageniform or clavate, with rounded to capitate apex, thin-walled. *Stipitipellis* with parallel, hyaline and thin-walled hyphae, 4.5 – 11 µm diam. *Chryso-vessels* present. *Acanthocytes* present on rhizomorphs. *Clamp connections* present. Fig. 2 G & H & Fig. 4 G – L.

DISTRIBUTION. Europe (Noordeloos 2011). North America: Canada and Mexico (Redhead 1984; Bandala & Montoya 1993). South America: Brazil (Santa Catarina State).

SPECIMEN EXAMINED: BRAZIL. Santa Catarina State: Joaçaba, Parque Natural Municipal do Vale do Rio do Peixe, 06 July 2013, *C. Seger* 043 (HCP 670).

ADDITIONAL SPECIMENS EXAMINED: *Stropharia cyanea*. SWEDEN. Bohuslän: Hogsam, 01 Oct. 1994, *S. Jacobsson* (ICN).

HABITAT: Solitary, growing on rotting wood, indoor forest.

CONSERVATION STATUS. Not evaluated.

NOTES. *Stropharia pseudocyanea* is one of the blue-green *Stropharia* species, as well as *S. aeruginosa* and *S. caerulea*. It differs of the latter by lacking chrysocystidia on gill edge, and from *S. aeruginosa* in the more robust basidiomata, well-developed and persistent annulus, and presence of caulochrysocystidia in the stipe apex (Noordeloos 2011).

Stropharia pseudocyanea usually occurs in small groups and have been collected in the meadow or sandy soil (Noordeloos 2011). Unlike, our material was found growing solitary in the forest, on rotten wood; these differences in the substrate may be significant and cleared with molecular analysis, however, on a morphological basis we have not differences enough

to consider it distinct from Northern Hemisphere taxon. Rarely found on the American continent, it was cited just in Mexico (Bandala & Montoya 1993) and Canada (Redhead 1984), and the material here reported is considered the first record from Brazil.

Stropharia rugosoannulata Farlow (Murrill 1922: 139). Type: United States, Massachusetts, *G. B. Fessenden* (NY).

DESCRIPTION: see Cortez & Silveira (2008). Fig. 2 J & K & Fig. 4 F.

DISTRIBUTION. Eurasia (Noordeloos 2011), North (Murril 1922) and South America (Coimbra 2015): Brazil: São Paulo, Paraná, Santa Catarina and Rio Grande do Sul States (Cortez & Silveira 2008).

SPECIMEN EXAMINED: BRAZIL. Paraná State: Curitiba, São José dos Pinhais, Reserva Biológica Cambui, 6 Dec. 1979, *A.A.R. de Meijer* (MBM 323.936); 14 May 1980, *A.A.R. de Meijer* (MBM 325.628). Rio Grande do Sul State: Dom Pedro de Alcântara, 20 May 2005, *V. G. Cortez* 062/05 (ICN 139.140). Esmeralda, Est. Ecol. Aracuri, 24 Nov. 2004, *V. G. Cortez* 060/04 (ICN 139.031). Santa Maria, Morro da Caturrita, 26 May 2009, *G. Coelho* (HCP 888); 23 April 2014, *C. Seger & G. Coelho* 097 (HCP 887); Morro do Elefante, 09 May 2001, *V. G. Cortez* 027 (SMDB 9.247); *V. G. Cortez* 028 (SMDB 9.248); 04 May 2002, *V. G. Cortez* 039/02 (SMDB 9.577); Tancredo Neves, Passo dos Ferreiros, 17 April 2001, *V. G. Cortez* 018 (SMDB 9.241). São Francisco de Paula, FLONA SFP, 13 May 2005, *V. G. Cortez* 060/05 (ICN 139.138); 01 June 2007, *P. S. Silva* 185/07 (ICN 139.379). Viamão, Parque Natural Municipal Saint-Hilaire, 13 July 1978, *M.H. Homrich & R.T. Guerreiro* (ICN 102.124). Santa Catarina State: Água Doce, Fazenda Quiben, 07 July 2013, *C. Seger* 044 (HCP 671). Florianópolis, 18 June 2012, col. *A.C. Magnago* 366 (FLOR 47.955). Joaçaba, Parque Natural Municipal do Vale do Rio do Peixe, 06 Aug. 2012, *C. Seger* 001 (HCP 661); Campus UNOESC, 26 May 2013, *C. Seger* 039 (HCP 668).

HABITAT: Solitary to gregarious, on fallen leaves or wood chips, into or near forests.

CONSERVATION STATUS. Not evaluated.

NOTES. *Stropharia rugosoannulata* has the largest basidiomata in the genus, the pileus is brown to reddish brown, has double, fleshy and with grooved above annulus, dimorphic cheilocystidia, cauloleptocystidia and the basidiospores are dark brown, truncated by apical germ pore, (9 –) 10 – 12.5 (– 14.5) μm long and some are subhexagonal in face-view. It is an edible mushroom and now cultivated worldwide, in spite of naturally occurring in many countries. In Brazil, it is known from the States of São Paulo (Pegler 1997), Paraná (Meijer 2006) and Rio Grande do Sul (Silva et al. 2012), and now we recorded for the first time from Santa Catarina State. These mushrooms are relatively common in Southern Brazil, especially in areas of ombrophilous forests.

Stropharia trinitensis (Dennis) Cortez (2008: 105). Type: Trinidad Isl., Sta. Augustine, R.W.G. Dennis 78 (K).

Pileus 20 mm, convex, glabrous, yellowish brown (5D5) when dry; velar remnants scarce on the pileus margin. *Context* 2 mm, fleshy. *Lamellae* adnate, golden brown (5D7), with concolour edge. *Stipe* 50 mm, central, clavate-flexuous, brown (6E5) becoming brownish grey at near base (6C2). *Rhizomorphs* present at base. *Spore print* not seen.

Basidiospores 6 – 7 \times 3.5 – 4.5 μm , Q= 1.44 – 1.78, Qm= 1.60, n= 25, ellipsoid to oblong in face-view, slightly amygdaliform in side view, with a minute germ pore, brownish yellow in KOH, smooth, thick-walled. *Basidia* 14 – 18 \times 5.5 – 6.5 μm , clavate, tetrasporic, sterigmata \leq 2.5 μm thin-walled, hyaline. *Pleurochrysocystidia* 29 – 43 \times 9 – 15 μm , clavate, fusiform to broadly fusiform, acute to rounded apex or with broadly neck, thin-walled, abundant. *Cheiloleptocystidia* 17 – 21.5 \times 10.5 – 12.5 μm , pyriform to subglobose, rounded apex, thin-

walled, scarce on dry basidiomata. *Pileipellis* a cutis, composed of cylindrical, prostrate, slightly gelatinized, hyaline to yellowish, thin-walled hyphae, 3.5 – 8.5 μm diam. *Pileal context* formed by interwoven, hyaline, smooth and thin-walled hyphae, some inflated, 4.5–22.5 μm diam. *Hymenophoral trama* subregular with thin-walled, hyaline hyphae, 5 – 14.5 μm diam. *Caulochrysocystidia* 22.5 – 34 \times 9.5 – 14 μm , clavate to fusiform, with rounded apex, thin-walled. *Stipitipellis* with parallel, hyaline to yellow, thin-walled hyphae. *Chryso-vessels* not seen. *Acanthocytes* present on rhizomorphs. *Clamp connections* present. Fig. 6 A & B.

DISTRIBUTION. Central America: Trinidad Island and South America (Coimbra 2015): Argentina and Brazil: Mato Grosso do Sul (Bononi *et al.* 2008), São Paulo (Pegler 1997) and Paraná States (Meijer 2006).

SPECIMEN EXAMINED: BRAZIL. Paraná State: Antonina, Reserva Natural do Rio Cachoeira, 28 June 2003, A.A.R. de Meijer 4227 (MBM 397.337, as *Pholiota* aff. *trinitensis*).

HABITAT: Solitary, on decayed dicot trunk, in dense ombrophilous forest.

CONSERVATION STATUS. Not evaluated.

NOTES. The above description is based exclusively on dried specimen from Paraná State, for this reason the macroscopic description is much simplified. *Stropharia trinitensis* was first described by Dennis (1970) in the genus *Pholiota*, and was also treated as *Hypholoma* (Pegler 1983) and *Naematoloma* (Singer 1986). When reviewed the type, Cortez (2008b) found acanthocytes on rhizomorphs of basidiomata, diagnostic feature of *Stropharia*. Basidiospores are similar in color, size and shape to other species previously classified in *Pholiota* Sect. *Albivetae*. However, *S. trinitensis* is unique among these by glabrescent pileus, partial veil forming a fibrillose to floccose and ephemeral annulus, globose to pyriform cheiloleptocystidia, pleurochrysocystidia without mucronate apex and caulochrysocystidia with same shape (Pegler 1983). Wright *et al.* (2008) reported *Hypholoma* aff. *trinitensis* in

subtropical rain forest of Misiones, Argentina, region at the boundary with Brazil, but their material presented longer basidiospores (6 – 9.5 μm) and squamulose pileus, and may be distinct. In Brazil, it recorded from Mato Grosso do Sul (Bononi *et al.* 2008, as *H. trinitense*), São Paulo (Pegler 1997, as *H. trinitense*) and Paraná States (Meijer 2006, as *P. aff. trinitensis*).

Stropharia venusta P.S. Silva, Cortez & R.M. Silveira (2009: 543). Type: Brazil, Rio Grande do Sul State, P.S. Silva 130/06 (holotype ICN!).

Pileus 28 – 48 mm, broadly convex to plane, viscid with a thin glutinous layer, reddish brown (8D4) to light brown (7D5), yellowish brown (5D8) centrally. *Context* fleshy, yellowish white (1A2). *Lamellae* adnate, near, violet grey (17C2) becoming dull violet (17E3) in age, smooth, concolor to whitish edge. *Stipe* 52 – 104 \times 4 – 8 mm, central, cylindrical to clavate; dry, longitudinally striate surface with white squamules below the annulus, orange white (6A2) to pale orange (6A3). *Veil* present like abundant remnants on the pileus margin and on the stipe apex it produces a membranous annulus, ridged above. *Rhizomorphs* present at base. *Spore print* greyish brown (10E3).

Basidiospores (10.5 –) 11 – 13 \times 6 – 7 μm , Q= 1.64 – 1.91, Q_m= 1.76, n= 38, oblong in face and side-view, apically truncated by a germ-pore \leq 2 μm , brown in KOH, with thick-walled, smooth. *Basidia* 27.5 – 43 \times 7.5 – 9.5 μm , clavate, thin-walled, hyaline, tetrasporic; sterigmata \leq 5 μm . *Pleurochrysocystidia* 30 – 56.5 \times 8 – 11.5 μm , fusiform to clavate, acute to mucronate apex (\leq 3.5 μm long) containing subhyaline to yellowish contents; thin-walled, abundant. *Cheiloleptocystidia* (15 –) 24.5 – 46 (– 60.5) \times 5 – 10.5 μm , dimorphic: clavate, lageniform, fusiform or broadly fusiform, rounded or with one to three spherical protuberances, some branched; thin-walled. *Cheilo-chrysocystidia* 40 – 58 \times 7.5 – 11 μm , fusiform, rounded to mucronate apex, thin-walled. *Pileipellis* composed of two layers: a) *suprapellis* an ixotrichoderm, composed of cylindrical, anticlinal to prostrate, hyaline, thin-walled hyphae,

with smooth or uncommon encrusted walls by yellowish pigment, 3 – 6 µm diam. b) *subpellis* a cutis, composed of prostrate hyaline, thin-walled hyphae, smooth walls, slightly yellow in mass, 5 – 8.5 µm diam. *Pileal context* formed by interwoven, hyaline, smooth and thin-walled hyphae, 4.5 – 13 µm diam. *Hymenophoral trama* subregular, with thin-walled, hyaline hyphae, 6 – 9 µm diam. *Cauloleptocystidia* 11 – 24.5 × 6.5 – 10 µm, cylindrical to sphaeropedunculate, with rounded apex, thin-walled. *Caulochrysocystidia* 18.5 – 36.5 × 6.5 – 14 µm, fusiform or clavate to ventricose, rounded to acute apex, thin-walled, rare. *Stipitipellis* with parallel, hyaline and thin-walled hyphae, some with granules, 4.5 – 7.5 µm diam. *Chryso-vessels* present. *Acanthocytes* present on rhizomorphs. *Clamp connections* present. Fig. 2 L & M, Fig. 6 C – F & Fig. 9 E.

DISTRIBUTION. Brazil: Rio Grande do Sul (Silva *et al.* 2009) and Santa Catarina States.

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: São Francisco de Paula, FLONA SFP, 14 May 2005, V. G. Cortez 052/05 (ICN 139.130); 12 May 2006, P. S. Silva 130/06 (ICN 139.177); 19 May 2006, P. S. Silva 145/06 (ICN 139.192), P. S. Silva 153/06 (ICN 139.200); 18 May 2007, P. S. Silva (ICN 139.365); 01 June 2007, P. S. Silva 184/07 (ICN 139.378). Santa Catarina State: Urubici, Parna São Joaquim, 12 June 2014, C. Seger & C. R. Alves 141 (HCP 897); 142 (HCP 898).

HABITAT: Scattered, growing on soil, into the *Araucaria* Forest.

CONSERVATION STATUS. Not evaluated.

NOTES. *Stropharia venusta* was described from the State of Rio Grande do Sul, in Southern Brazil, and since then it has been found in the *Araucaria angustifolia* forests, now also from Santa Catarina State. It draws attention by robust basidiomata, glutinous to subviscid pileus with pink tones, veil abundant on pileus margin and also on stipe apex where it forms a membranous annulus. In the protologue, Silva *et al.* (2009) did not reported chrysocystidia in the gill edge, but we noted these structures, with fusiform shape and rounded to mucronate

apex, similar to pleurochrysocystidia. Moreover, the material of Santa Catarina State has cheiloleptocystidia with spherical projections to irregular at the apex, sometimes forked, whereas in the holotype there is a predominance of rounded apex. Although these differences, there is no reason to believe both specimens correspond to distinct species. *Stropharia venusta* is comparable with Tasmanian *Stropharia formosa* Y.S. Chang, A.K. Mills, G.M. Gates & D.A. Ratkowsky but the latter differs by more scaly-squamulose stipe, evanescent annulus (in contrast to Brazilian species, which is persistent), and small differences in the cystidia shape (Chang *et al.* 2006; Ratkowsky *et al.* 2013).

Stropharia versiformis C. Seger & Cortez **sp. nov.** Type: BRAZIL. Rio Grande do Sul State, Santa Maria, C. Seger & G. Coelho 086 (UPCB – holotype here designated).

MycoBank: NNNNN

Pileus 27 mm, broadly convex, depressed centrally, scaly-squamulose surface, wet, with thin layer viscid, yellowish brown (5D5). *Context* 3.5 mm, fleshy, white (5A1) to orange white (5A2). *Lamellae* 4 mm, adnexed, near, yellowish brown (5F4), with a concolorous to whitish edges. *Stipe* 31 × 3 mm, central, cylindrical to clavate; surface dry, longitudinally striate, yellowish white (4A2) on apex becoming yellowish grey (4B3) toward the base. *Veil* present like remnants scarce in the pileus margin and on the stipe apex it produces a fleshy annulus, grooved and brownish beige (6E3) on upper surface, yellowish grey (4B3) below, can be absent in older basidiomata. *Rhizomorphs* present at base. *Spore print* greyish brown (11F3). *Basidiospores* 5.5 – 8.5 × (3.5 –) 4 – 5 μm, Q= 1.33 – 1.98, Q_m= 1.58, n= 38, ellipsoid to oblong in face-view, amigdaliform in side-view, apex narrowed with an inconspicuous germ-pore, yellowish brown in KOH, with smooth and thick-walled. *Basidia* 16.5 – 31 × 5 – 8 μm, clavate, tetrasporic, thin-walled, hyaline; sterigmata ≤ 2.5 μm long. *Pleurochrysocystidia* 20 – 49.5 × 9 – 16 μm, fusiform to broadly fusiform, rounded to acute apex, rarely mucronate (≤

5 μm long), thin-walled, abundant. *Cheiloleptocystidia* (16.5 –) 23.5 – 52 \times (6.5 –) 12.5 – 21.5 μm , with different shapes: clavate, broadly clavate, pyriform, sphaeropedunculate, rounded to rostrate apex, sometimes with two branches, rostrum \leq 12 μm , thin-walled. *Cheilochrysocystidia* 23 – 42 \times 7.5 – 16.5 μm , fusiform, some lageniform and sphaeropedunculate, rounded to acute apex, thin-walled, uncommon. *Pileipellis* a cutis, composed of cylindrical, prostrate, slightly gelatinized, hyaline to yellow, thin-walled hyphae, 2.5 – 14 μm diam. *Pileal context* formed by interwoven, hyaline, smooth and thin-walled hyphae, 5 – 24 μm diam. *Hymenophoral trama* subregular, with thin-walled, hyaline hyphae, 3.5 – 25.5 μm diam. *Cauloleptocystidia* 17 – 47 \times 7.5 – 26.5 μm , clavate, broadly clavate to sphaeropedunculate, some halteriform, with rounded apex or with a neck, thin-walled. *Caulochrysocystidia* 21.5 – 29.5 \times 9.5 – 11.5 μm , fusiform, rounded apex, thin-walled, uncommon. *Stipitipellis* with parallel, hyaline and thin-walled hyphae, 2 – 12.5 μm diam. *Chryso-vessels* present. *Acanthocytes* present on rhizomorphs. *Clamp connections* present. Fig. 7 A – L & Fig. 9 E.

RECOGNITION. Pileus surface viscid, yellowish brown, lamellae adnexed, grooved annulus, basidiospores yellowish brown and small (5.5 – 8.5 \times 4 – 5 μm), cheiloleptocystidia with different shapes including with two branches, cauloleptocystidia present, chrysocystidia found on the sides and edge of the lamellae, and on stipe apex too.

DISTRIBUTION. Brazil: Rio Grande do Sul State.

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: Santa Maria, Morro do Elefante, 22 April 2014, *C. Seger & G. Coelho* 086 (UPCB).

HABITAT: Solitary, growing on soil in seasonal decidual forest

CONSERVATION STATUS. Not evaluated.

ETYMOLOGY. Refers to the variable shapes of cheilocystidia.

NOTES. This species distinguished by the several shapes of cheilocystidia, with long and often branched rostri, which we consider an unusual feature in the genus. *Stropharia rubrobrunnea* Senthil. & S.K. Singh also has cheiloleptocystidia with these features (although slightly thinner), cheilochrysocystidia present, mucronate pleurocystidia and basidiospores narrow towards the germ pore (Senthilarasu & Singh 2013), but differs by pileus strongly hygrophanous, pinkish white becoming yellowish white and by dark brown, with truncate germ pore and slightly larger ($7.5 - 10 \times 5 - 6.5 \mu\text{m}$) basidiospores.

The pileus diameter and length of the stipe, presence of fleshy grooved annulus, yellowish brown basidiospores with inconspicuous germ pore, and cheiloleptocystidia clavate and pyriform relate it to *S. coronilla* and/or *S. agaricoides*. The former differs from *S. versiformis* by the yellow pileus, slightly longer basidiospores ($7.5 - 9 \mu\text{m}$), cheilocystidia without any projection at the apex, lack of caulochrysocystidia and also by habit among grasses on gardens and meadows (Noordeloos 2011). *Stropharia agaricoides* differs by pileus with orange to reddish tones, white to pinkish white annulus, smaller and especially thinner cheiloleptocystidia ($16.5-35 \times 5.5-8.5 \mu\text{m}$) and cauloleptocystidia ($15.5 - 33.5 \times 5 - 10 \mu\text{m}$), both without neck or projecting apex (Silva *et al.* 2009). In spite of a single collection, the morphological variation is distinct enough from other *Stropharia* to propose it as a new taxon.

Stropharia watlingii C. Seger & Cortez **sp. nov.** Type: Brazil, Rio Grande do Sul State, V. G. Cortez 079/05 (ICN – holotype here designated).

Mycobank: NNNNN

Pileus 39 mm, broadly convex to plane, surface wet and smooth, yellowish grey (4B3) centrally and yellowish white (4A2) in the margin, light orange (5A6) at the dry basidiomata. *Context* 4 mm, orange white (5A2), fleshy. *Lamellae* sinuate, near, violet grey (17D2) becoming brown (7E4), whitish and eroded edge. *Stipe* 88×6 mm, central, cylindrical, surface

dry, striated above at annulus and tomentose below that, more fibrillose near the base, white (4A1) for the most part and yellowish white (4A2) near of base. *Veil* present like white remnants abundant in the pileus margin and on the stipe it produces a cottony and grooved annulus, concolorous with a stipe becoming violet grey (17D2) on upper surface with spore deposit. *Rhizomorphs* present at base. *Spore print* probably violet grey (17D2), seen only in the ring.

Basidiospores $10 - 11 (-11.5) \times 5.5 - 7 \mu\text{m}$, $Q = 1.55 - 1.83$, $Q_m = 1.71$, $n = 25$, oblong in face and side-view, apically truncated by a germ-pore $\leq 1.5 \mu\text{m}$, brown in KOH, smooth and thick-walled. *Basidia* $22 - 27.5 \times 7.5 - 8.5 \mu\text{m}$, clavate to clavate-ventricose, tetrasporic, thin-walled, hyaline; sterigmata $\leq 3 \mu\text{m}$; basidioles clavate to distinctly clavate-ventricose, sometimes with neck. *Pleurochrysocystidia* $30.5 - 56.5 \times 8 - 13 \mu\text{m}$, fusiform, rounded to acute apex, rarely mucronate ($\leq 2 \mu\text{m}$ long), thin-walled, abundant. *Cheiloleptocystidia* $18.5 - 33 \times 6.5 - 11 \mu\text{m}$, clavate, lageniform, utriform to pyriform, rounded or with neck on the apex, thin-walled, common on gill edge. *Cheilochrysocystidia* $27 - 44 (-60) \times 7.5 - 11.5 \mu\text{m}$, clavate, fusiform or broadly fusiform, acute to mucronate apex, uncommon on gill edge. *Pileipellis* composed of two layers: a) *suprapellis*: an ixotrichoderm, composed of gelatinized, thin and cylindric hyphae, anticlinally arranged, smooth and thin-walled, $5 - 7 \mu\text{m}$ diam.; b) *Subpellis*: a cutis with prostrate and cylindrical hyphae, yellowish brown in mass, with smooth or incrustated walls by brownish pigment, thin-walled $4 - 11 \mu\text{m}$ diam. *Pileal context* formed by interwoven, hyaline, smooth and thin-walled hyphae, $7 - 16 \mu\text{m}$ diam. *Hymenophoral trama* subregular, with hyaline hyphae, $5.5 - 13 \mu\text{m}$ diam., thin-walled. *Cauloleptocystidia* $17.5 - 52.5 \times 3.5 - 9 \mu\text{m}$, cylindrical to clavate, with rounded apex, thin-walled, common on stipitipellis. *Stipitipellis* a trichoderm, with abundant cylindrical and loosely arranged hyphae, $5.5 - 8.5 \mu\text{m}$ diam. *Stipe trama* with parallel hyphae, hyaline and thin-walled, $4 - 11 \mu\text{m}$ diam.

Chryso-vessels not seen. *Acanthocytes* abundant on rhizomorphs. *Clamp connections* present.

Fig. 8 A – K.

RECOGNITION. Macroscopically it can be recognized by basidiomata with light yellowish tones to white, stipe tomentose, abundant white remnants at veil in the pileus margin and cottony to grooved annulus, and microscopically by the length (10 – 11 μm) and shape of the basidiospores, cheilocystidia dimorphic, cauloleptocystidia and pileipellis has a suprapellis like ixotrichoderm and subpellis a cutis.

DISTRIBUTION. Brazil: Rio Grande do Sul State.

SPECIMEN EXAMINED: BRAZIL. Rio Grande do Sul State: São Francisco de Paula, CPCN Pró Mata, 11 June 2005, V.G. Cortez 079/05 (ICN 139.157).

HABITAT: Solitary, growing on soil and involving leaf litter.

CONSERVATION STATUS. Not evaluated.

ETYMOLOGY. Named after Prof. Roy Watling, Edinburgh (UK), in honor to his contributions on taxonomy of macrofungi, especially the dark-spored mushrooms, including the Strophariaceae.

NOTES. This species is easily distinctive of others Southern American *Stropharia* by the yellowish white pileus, fibrillose to squamulose stipe and cottony and grooved annulus. *Stropharia venusta* and the Tasmanian *S. formosa* also have same stature, abundant veil remnants on pileus margin, squamulose stipe and equal basidiospores, but both have pileus with red or vinaceous tinge (Chang *et al.* 2006; Silva *et al.* 2009).

The yellowish white fungus *S. melanosperma* is a related species, but the stipe has reduced white scales, the annulus is thick and well-developed, larger subhexagonal basidiospores, the shape of pleurocystidia are clavate-mucronate and lack cheiloleptocystidia (Cortez & Coelho 2008). Some macroscopic features as the size and color of basidiomata, veil on the pileus margin, annulus substrate to flocculose, stipe fibrillose to squamulose are reminiscent of *S.*

albonitens (Fr.) Quél., also by gelatinized pileipellis and clavate cauloleptocystidia, but this mushroom has smaller basidiospores ($6.5 - 8 \times 4 - 5 \mu\text{m}$), pleurocystidia with mucronate apex and cheilochrysocystidia absent (Noordeloos 2011).

Acknowledgements

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References

- Bandala, V. M. & Montoya, L. (1993). Nuevos registros de hongos del Estado de Veracruz, V: Nuevos Aphyllophorales y Agaricales. *Rev. Mex. Mic.* 9: 85 – 118.
- _____, ____ & Jarvio, D. (2005). Agarics from coffee plantations in Eastern México: two new records. *Fungal Div.* 20: 17 – 29.
- Batista, A. C. & Bezerra, J. L. (1960). Basidiomycetes vulgares em o Nordeste brasileiro. *Publ. Inst. Micol. Univ. Recife* 294: 1 – 30.
- Bessette, A. E, Bessette, A. R. & Fischer, D. W. (1997). *Mushrooms of Northeastern North America*. Syracuse: Syracuse University Press.
- Bon, M. (1972). Macromycètes du littoral boulonnais. *Doc. Mycol.* 1: 9 – 46.

- Bononi, V. L. R., Oliveira, A. K. M., Quevedo, J. R. & Gugliotta, A. M. (2008). Fungos macroscópicos do Pantanal do Rio Negro, Mato Grosso do Sul, Brasil. *Hoehnea* 35: 489 – 511.
- Chang, Y. S., Gates, G. M. & Ratkowsky, D. A. (2006). Some new species of the Strophariaceae (Agaricales) in Tasmania. *Austral. Mycol.* 24: 53 – 68.
- Coimbra, V. R. M. (2015). Checklist of Central and South American Agaricales (Basidiomycota) II: Strophariaceae. *Mycosphere* 6: 441 – 458.
- Contu, M. (1999). Fungi Mediterranei rariores. *Micol. Veg. Medit.* 14: 33.
- Cortez, V. G. (2008a). Type studies on South American Strophariaceae: 1. *Pholiota varzeae* from the Brazilian Amazon. *Mycotaxon* 103: 137 – 140.
- _____ (2008b). Type studies on South American Strophariaceae: 2. *Pholiota trinitensis* is transferred to *Stropharia*. *Mycotaxon* 105: 7 – 10.
- _____ & Coelho, G. (2008). Occurrence of the rare agaric *Stropharia melanosperma* (Strophariaceae) in Brazil. *Bol. Soc. Micol. Madrid* 32: 31 – 37.
- _____ & Silveira, R. M. B. (2007). A new species of *Stropharia* with hymenial acanthocytes. *Mycologia* 99: 135 – 138.
- _____ & _____ (2008). The agaric genus *Stropharia* (Strophariaceae, Agaricales) in Rio Grande do Sul State, Brazil. *Fungal Div.* 32: 31 – 57.
- Dennis, R. W. G. (1970). Fungus Flora of Venezuela and adjacent countries. *Kew. Bull. Add. Ser.* 3: 1 – 531.
- Earle, F. S. (1906). Algunos hongos cubanos. *Inf. An. Est. Cent. Agron. Cuba* 1: 225 – 242.
- Farr, D. F. (1980). The acanthocyte, a unique cell type in *Stropharia* (Agaricales). *Mycotaxon* 11: 241 – 249.

- Gillet, C. C. (1878). *Les Hyménomycètes, ou, Description de tous les champignons (fungi) qui croissent en France, avec l'indication de leurs propriétés utiles ou vénéneuses*. Alençon: Ch. Thomas.
- Kornerup, A. & Wanscher, J. H. (1978). *Methuen Handbook of Colour*. 3rd ed. London: Eyre Methuen.
- Kreisel, H. (1979). Zur Taxonomie von *Stropharia aeruginosa* sensu lato. *Beih. Sydowia*. 8: 228 – 232.
- Kummer, P. (1871). *Der Führer in die Pilzkunde*. Zerbst: C. Luppe.
- Lange, M. (1980). *Stropharia alpina* in Greenland, Iceland, and the Faroes. *Bot. Tidsskr.* 75: 89 – 91.
- Maia, L. C., Carvalho Júnior, A. A., Cavalcanti, L. H., Gugliotta, A. M., Drechsler-Santos, E. R., Santiago, A. L. M. A., Cáceres, M. S., Gibertoni, T. B., Aptroot, A., Giachini, A. J., Soares, A. M. S., Silva, A. C. G., Magnago, A. C., Goto, B. T., Lira, C. R. S., Montoya, C. A.S., Pires-Zottarelli, C. L. A., Silva, D. K. A., Soares, D. J., Rezende, D. H. C., Luz, E. D. M. N., Gumboski, E. L., Wartchow, F., Karstedt, F., Freire, F. M., Coutinho, F. P., de Melo, G. S.N., Sotão, H. M. P., Baseia, I. G., Pereira, J., Oliveira, J. J. S., Souza, J. F., Bezerra, J. L., Araujo Neta, L. S., Pfenning, L. H., Gusmão, L. F. P., Neves, M. A., Capelari, M., Jaeger, M. C. W., Pulgarín, M. P., Menolli Junior, N., Medeiros, P. S., Friedrich, R. C. S., Chikowski, R. S., Pires, R. M., Melo, R. F., Silveira, R. M. B., Urrea-Valencia, S., Cortez, V. G. & Silva, V. F. (2015). Diversity of Brazilian fungi. *Rodriguésia* 66: 1033 – 1045.
- Meijer, A. A. R. de. (2006). Preliminary List of the Macromycetes From the Brazilian States of Paraná. *Bol. Mus. Bot. Mun., Curitiba* 68: 1 – 55.
- _____. (2010). Preliminary List of the Macromycetes From the Brazilian States of Paraná: corrections and updating. *Bol. Mus. Bot. Mun., Curitiba* 72: 1 – 9.
- Morgan, A. P. (1908). North American Species of Agaricaceae. *J. Mycol.* 14: 64 – 75.

- Murrill, W. A. (1912). The Agaricaceae of the Pacific coast – II. *Mycologia* 4: 231 – 262.
- _____. (1922). Dark-spored agarics–II: *Gomphidius* and *Stropharia*. *Mycologia* 14: 121 – 142.
- Noordeloos, M. E. (2011). *Strophariaceae s.l.* Fungi Europaei 13. Alassio: Candusso.
- Norvell, L. L. & Redhead, S. A. (2000). *Stropharia albivelata* and its basionym *Pholiota albivelata*. *Mycotaxon* 76: 315 – 320.
- Pegler, D. N. (1983). Agaric Flora of the Lesser Antilles. *Kew Bull. Add. Ser.* 9: 1 – 668.
- _____. (1997). *The agarics of São Paulo, Brazil*. London: Royal Botanic Gardens/HMSO.
- Quélet, L. (1872). Les champignons du Jura et des Vosges. *Mém. Soc. d'Émul. Mont.* 5: 43 – 332.
- Ratkowsky, D. A., Gates, G. M. & Chang, Y. S. (2013). Two new combinations in the Strophariaceae (Agaricales) of Tasmania. *Austral. Mycol.* 31: 39 – 40.
- Redhead, S. A. (1984). Additional Agaricales on wetland Monocotyledoneae in Canada. *Can. J. Bot.* 62: 1844 – 1851.
- _____. (2013). Nomenclatural novelties. *Index Fung.* 15: 1 – 2.
- Seger, C., Sulzbacher, M. A., Cortez, V. G. (2016). *Stropharia coelhoi* (Basidiomycota): a new species from Brazil. *Darwiniana*: under review.
- Senthilarasu, G. & Singh, S. (2013). A new species of *Stropharia* from Western Ghats, India. *Mycotaxon* 123: 213 – 220.
- Silva, P. S., Cortez, V.G. & Silveira, R. M. B. (2009). New species of *Stropharia* from *Araucaria angustifolia* forests of Southern Brazil. *Mycologia* 101: 539 – 544.
- _____, _____. & _____. (2012). Synopsis of the Strophariaceae (Basidiomycota, Agaricales) from Floresta Nacional de São Francisco de Paula, Rio Grande do Sul State, Brazil. *Hoehnea* 39: 479 – 487.
- Singer, R. (1948). Diagnosis Fungorum Novorum Agaricalium. *Sydowia* 2: 26 – 42.

_____. (1969). Mycoflora Australis. *Beih. Nova Hedw.* 29: 1 – 405.

_____. (1978). Interesting and new Basidiomycetes from Ecuador II. *Nova Hedw.* 29: 1 – 98.

_____. (1986). *The Agaricales in modern taxonomy*. 4th ed. Koenigstein: Koeltz.

Smith, A. H. & Hesler L. R. (1968). *The North American Species of Pholiota*. New York: Hafner.

Wright, J. E., Lechner, B. E. & Popoff, O. F. (2008). *Atlas pictóricos de los hongos del Parque Nacional Iguazú*. 1st ed. Buenos Aires: L.O.L.A.

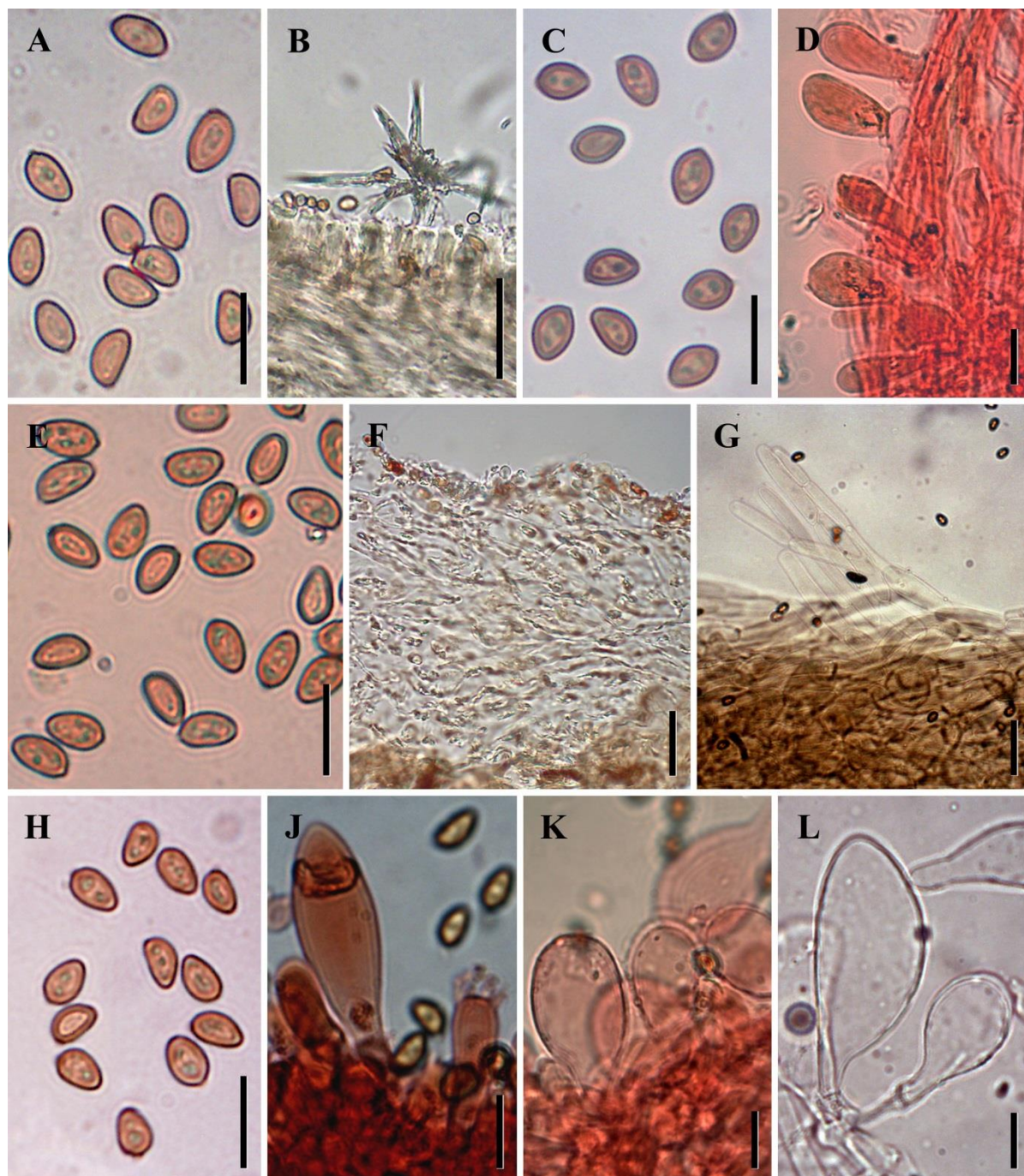


Fig 1. *S. acanthocystis* (ICN 139.193): **A** Basidiospores; **B** Hymenial acanthocytes; *S. agaricoides* (HCP 900): **C** Basidiospores; **D** Caulocystidia; *S. aeruginosa* (ICN 139.098): **E** Basidiospores; **F** Pileipellis. *S. apiahyna* (G – K: HCP 901; L: HCP 903): **G** Pileipellis; **H** Basidiospores; **J** Pleurocystidia; **K** Cheilocystidia; **L** Caulocystidia. (Scale bar: A, C – E, H – L= 10 μ m; B, F & G= 30 μ m). PHOTOS: A – L: C. SEGER.



Fig 2. A & B *S. agaricoides* (HCP 900); C & D *S. apiahyna* (HCP 901); E & F *S. coelhoi* (HCP 892); G & H *S. pseudocyanea* (HCP 670); J & K *S. rugosoannulata* (HCP 887); L & M *S. venusta* (HCP 897). (Scale bar: A – H= 10 mm; J – M= 20 mm). PHOTOS: A – D & G – M: C. SEGER; E – F: V.G. CORTEZ.

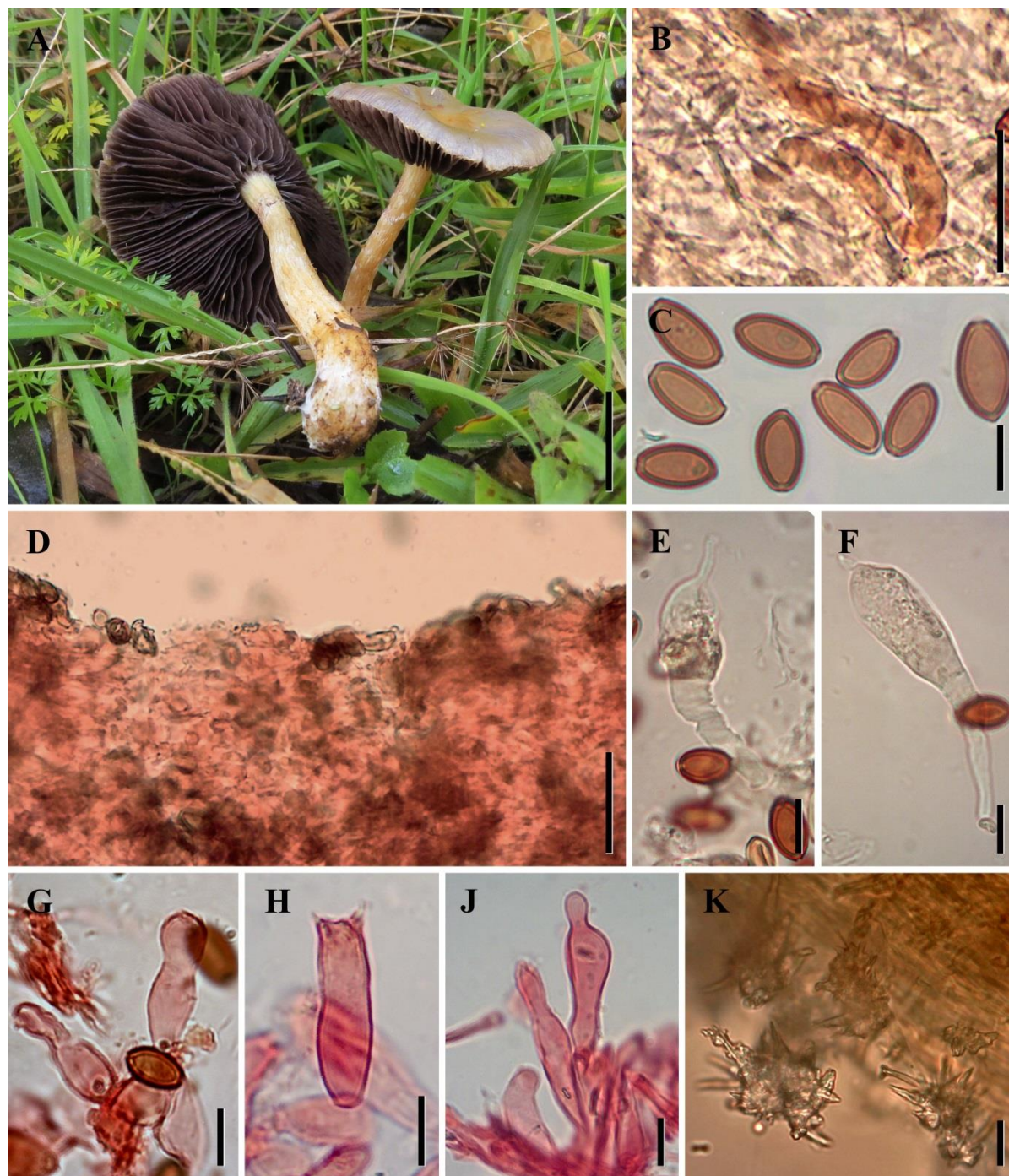


Fig. 3. *S. alvesiana*. **A** Basidiomata; **B** Chryso-vessel on pileal context; **C** Basidiospores; **D** Lamellae edge without cheilocystidia; **E** – **F** Pleurocystidia; **G** Basidiolos; **H** Basidia; **J** Caulocystidia; **K** Acanthocytes on rhizomorphs. All from *C. Seger & C.R. Alves* 133 (UPCB, holotype). (Scale bar: A= 10 mm; B & D= 30 μ m; C, E – K= 10 μ m). PHOTOS: A – K: C. SEGER.

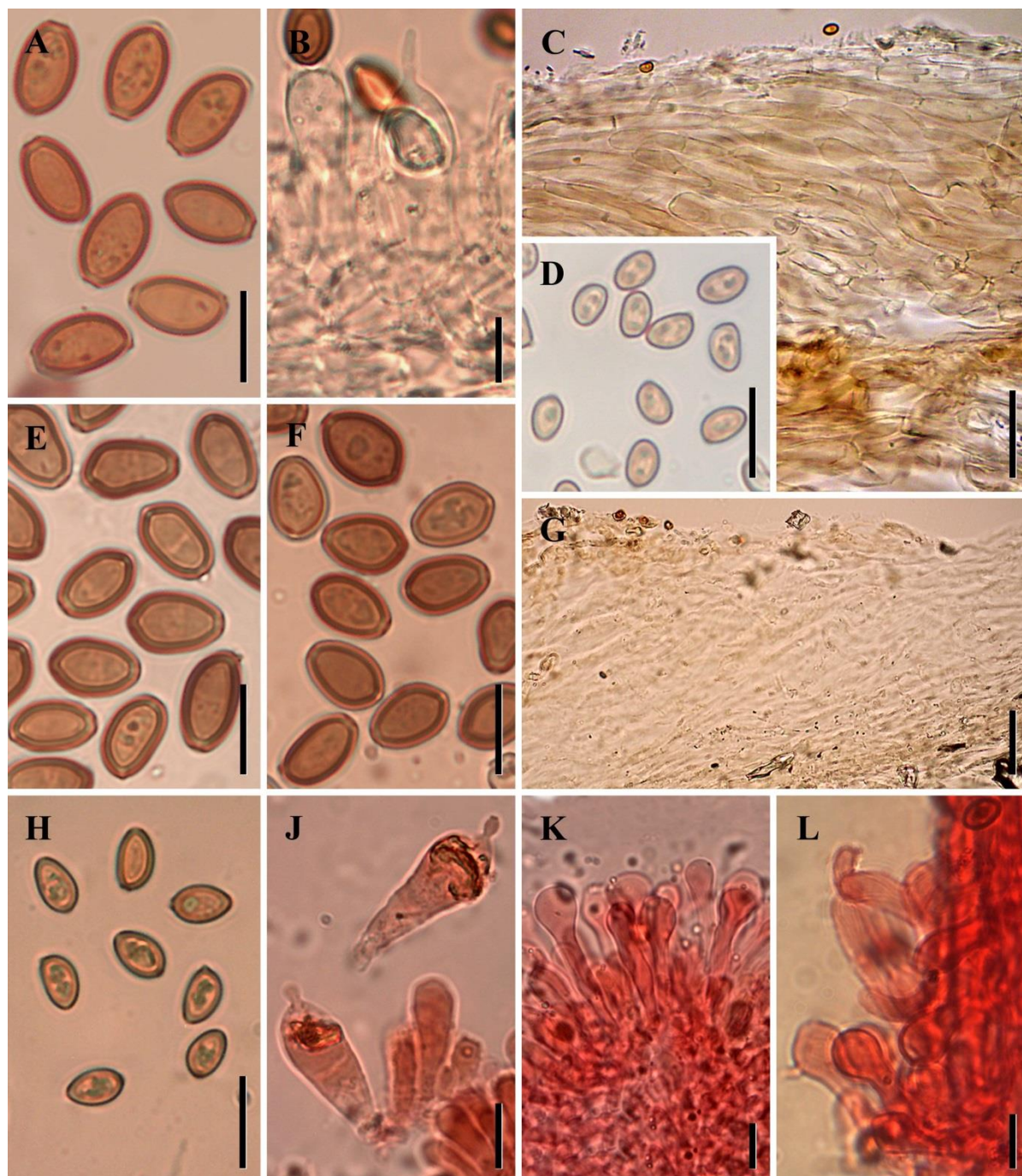


Fig 4. *S. araucariae* (ICN 139.134): **A** Basidiospores; **B** Basidia and pleurocystidia; *S. earlei* (ICN 139.001): **C** Pileipellis; **D** Basidiospores; *S. melanosperma* (SMDB 10.725): **E** Basidiospores; *S. rugosoannulata* (HCP 887): **F** Basidiospores; *S. pseudocyanea* (HCP 670): **G** Pileipellis; **H** Basidiospores; **J** Pleurocystidia; **K** Cheilocystidia; **L** Caulocystidia. (Scale bar: A & B, D – F, H – L= 10 µm; C & G= 30 µm). PHOTOS: A – L: C. SEGER.

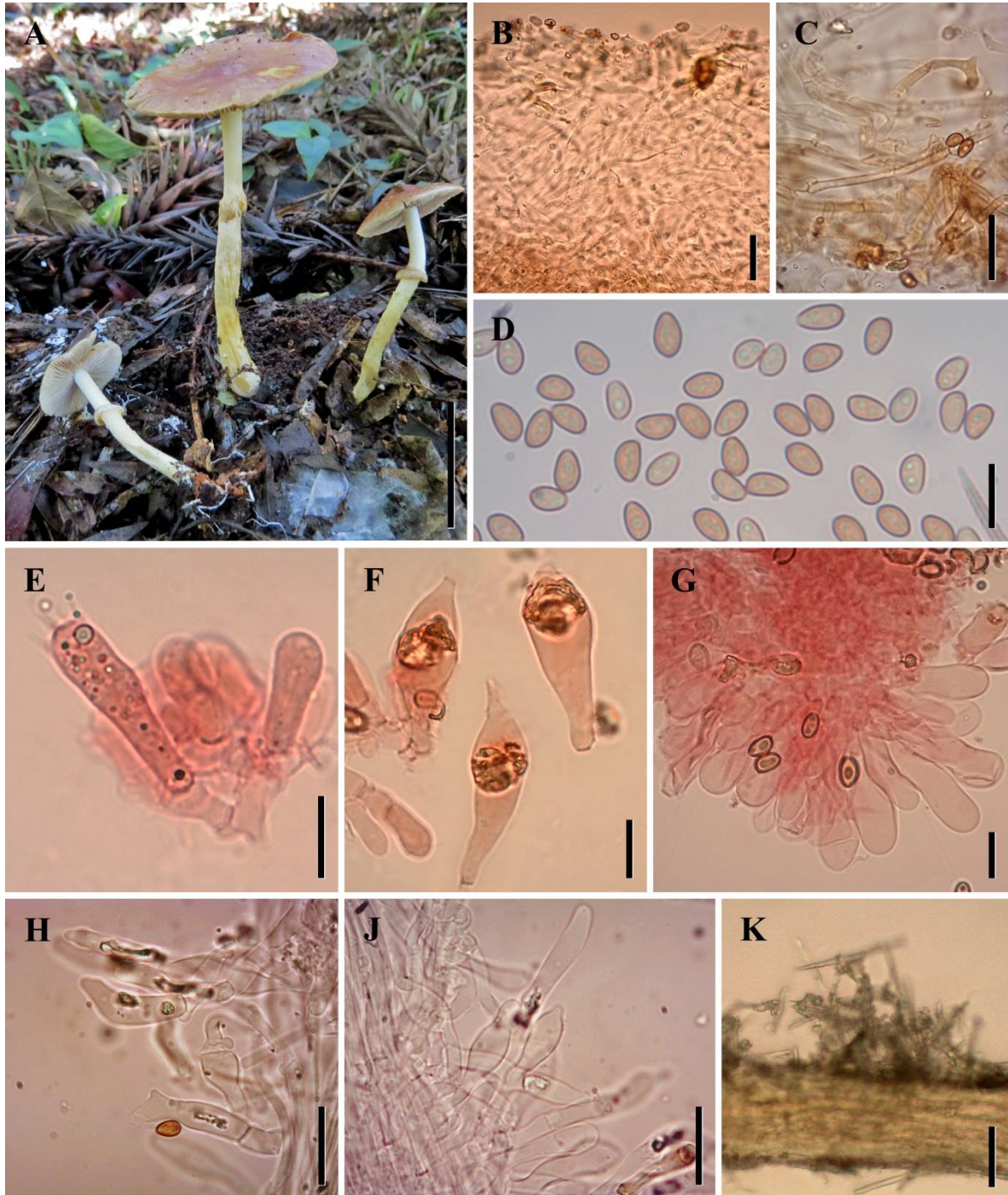


Fig. 5. *S. ombrophila*. **A** Basidiomata; **B** – **C** Pileipellis; **D** Basidiospores; **E** Basidia; **F** Pleurocystidia; **G** Cheilocystidia; **H** – **J** Caulocystidia; **K** Acanthocytes on rhizomorphs. All from *C. Seger* 199 (UPCB, holotype). (Scale bar: **A**= 30 mm; **B**= 30 μ m; **C** & **H** – **K**= 20 μ m; **D** – **G**= 10 μ m). **A** – **K**: *C. SEGER*.

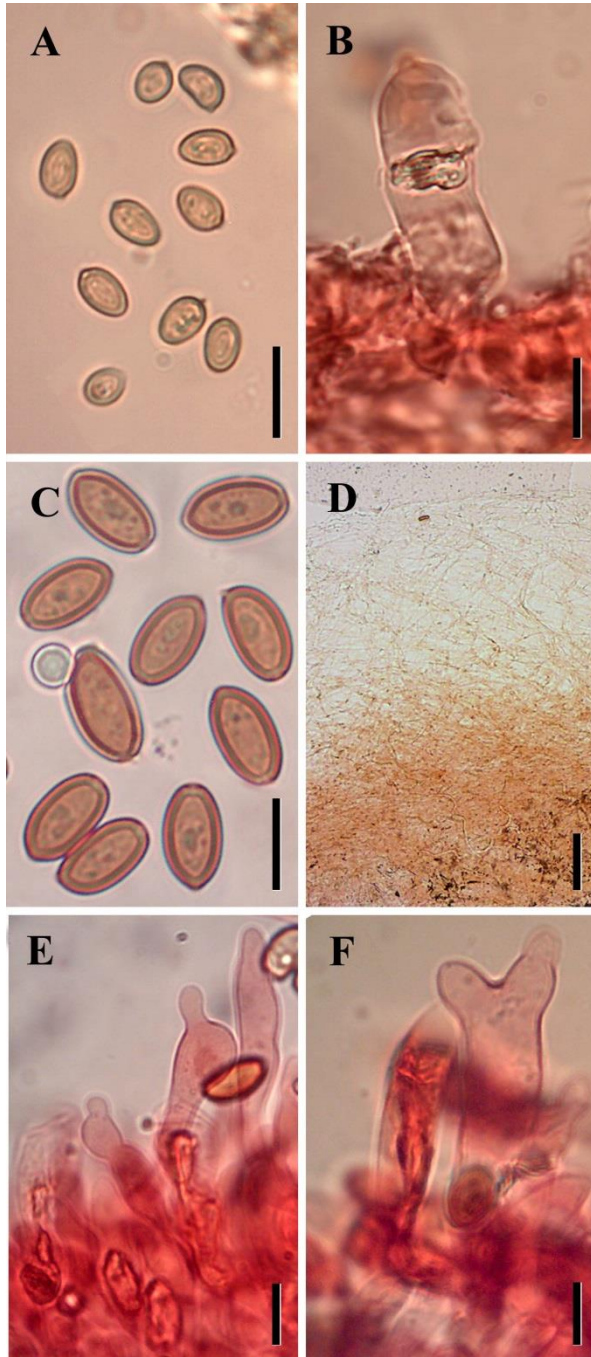


Fig. 6. *S. trinitensis* (MBM 397.337): **A** Basidiospores; **B** Pleurocystidia; *S. venusta* (C – E: HCP 897 and F: HCP 898): **C** Basidiospores; **D** Pileipellis; **E & F** Cheilocystidia. (Scale bar: A – C & E – F= 10 μ m; D= 100 μ m). A – F: C. SEGER.

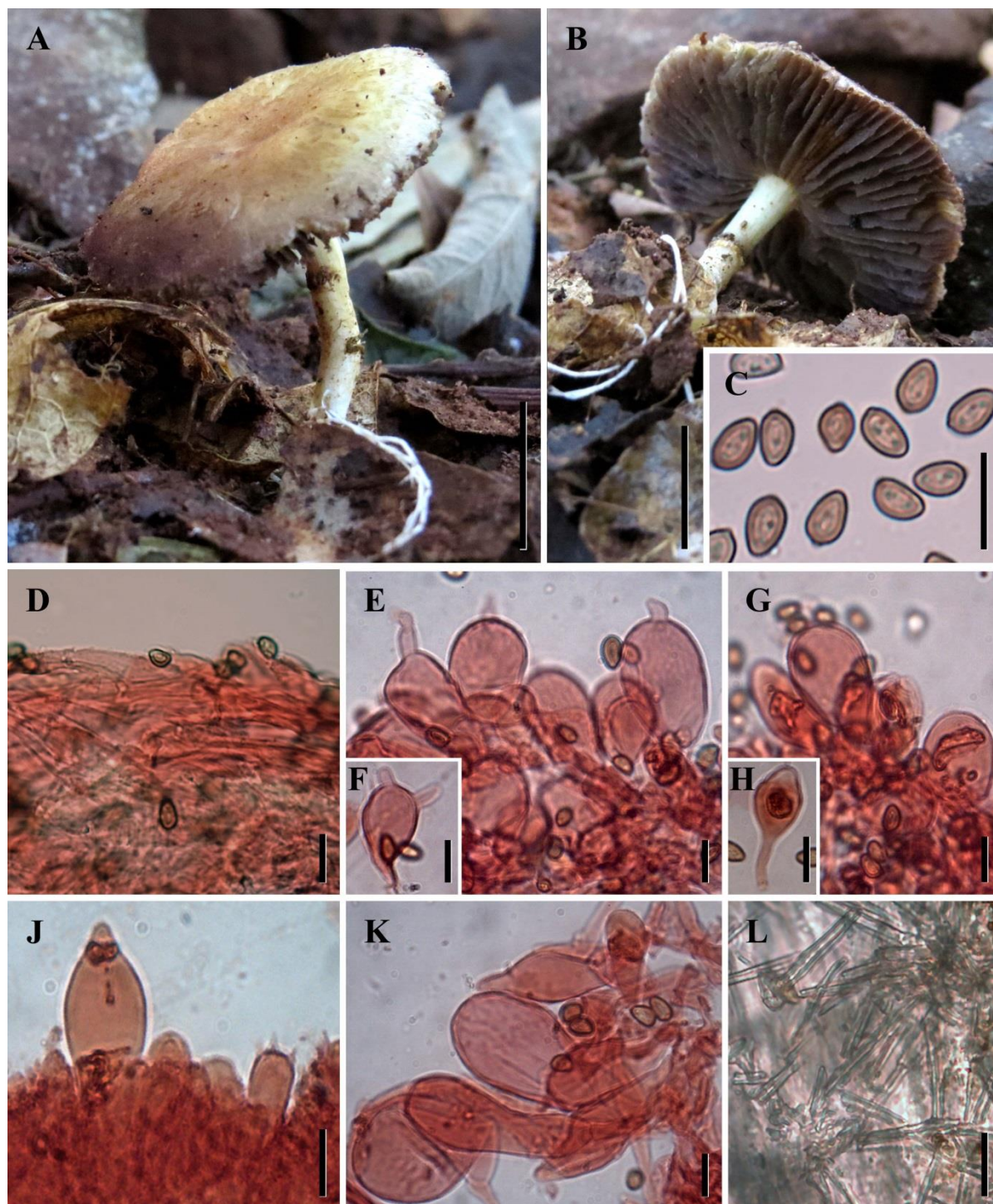


Fig 7. *S. versiformis*. **A – B** Basidiomata; **C** Basidiospores; **D** Pileipellis; **E – H** Cheilocystidia; **J** Pleurocystidia; **K** Caulocystidia; **L** Acanthocytes on rhizomorphs. All from *C. Seger & G. Coelho* 086 (UPCB, holotype) (Scale bar: A & B= 10 mm; C – J= 10 μ m). A – L: C. SEGER.

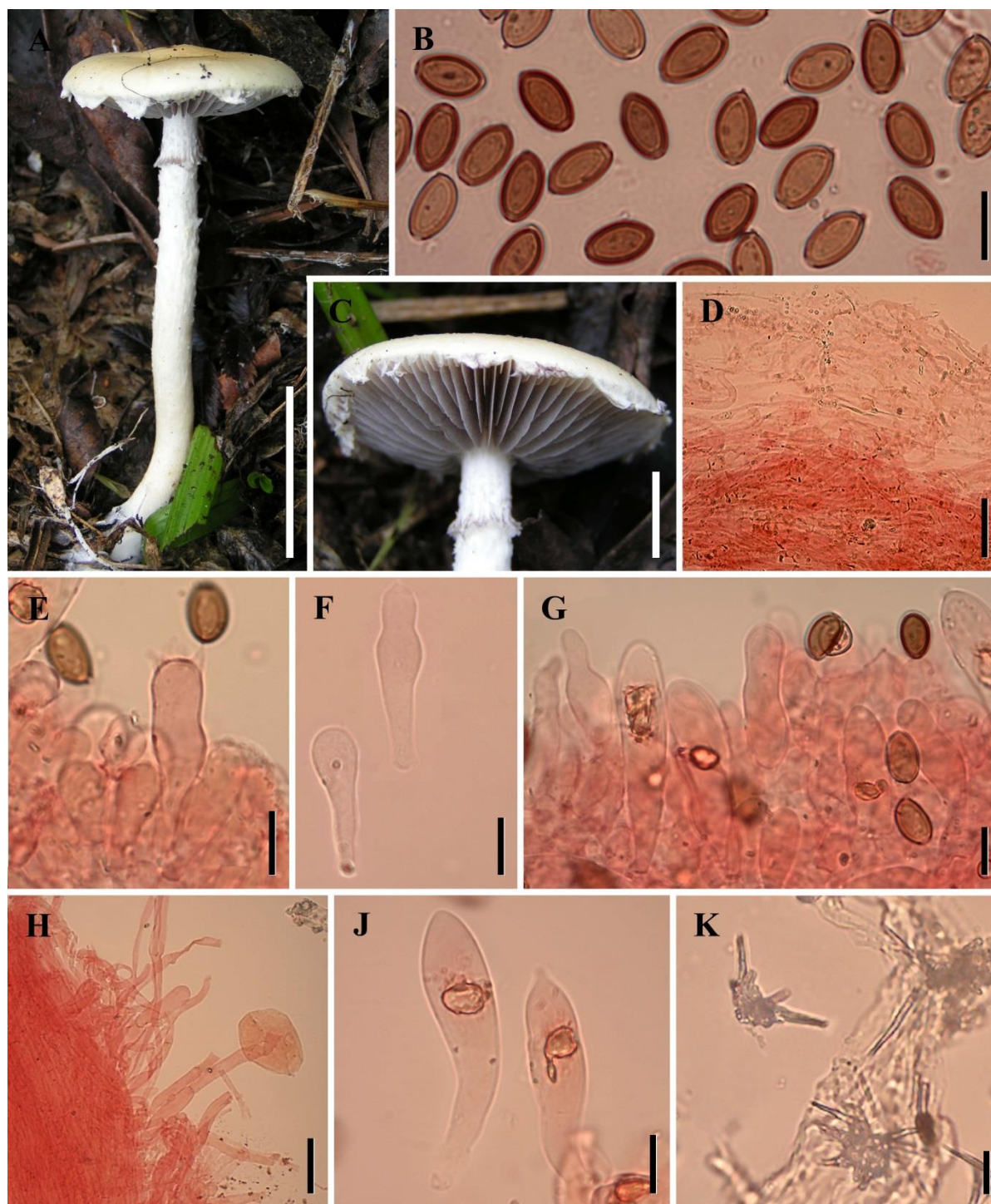


Fig. 8. *S. watlingii*. **A** Basidiomata; **B** Basidiospores; **C** Lamellae; **D** Pileipellis; **E** Basidia; **F** Basidioles; **G** Cheilocystidia; **H** Caulocystidia; **J** Pleurocystidia; **K** Acanthocytes on rhizomorphs. All from ICN 139.157 (holotype). (Scale bar: A= 30 mm; B, E – G & J – K= 10 µm; C= 10 mm; D & H= 30 µm). A – K: C. SEGER.

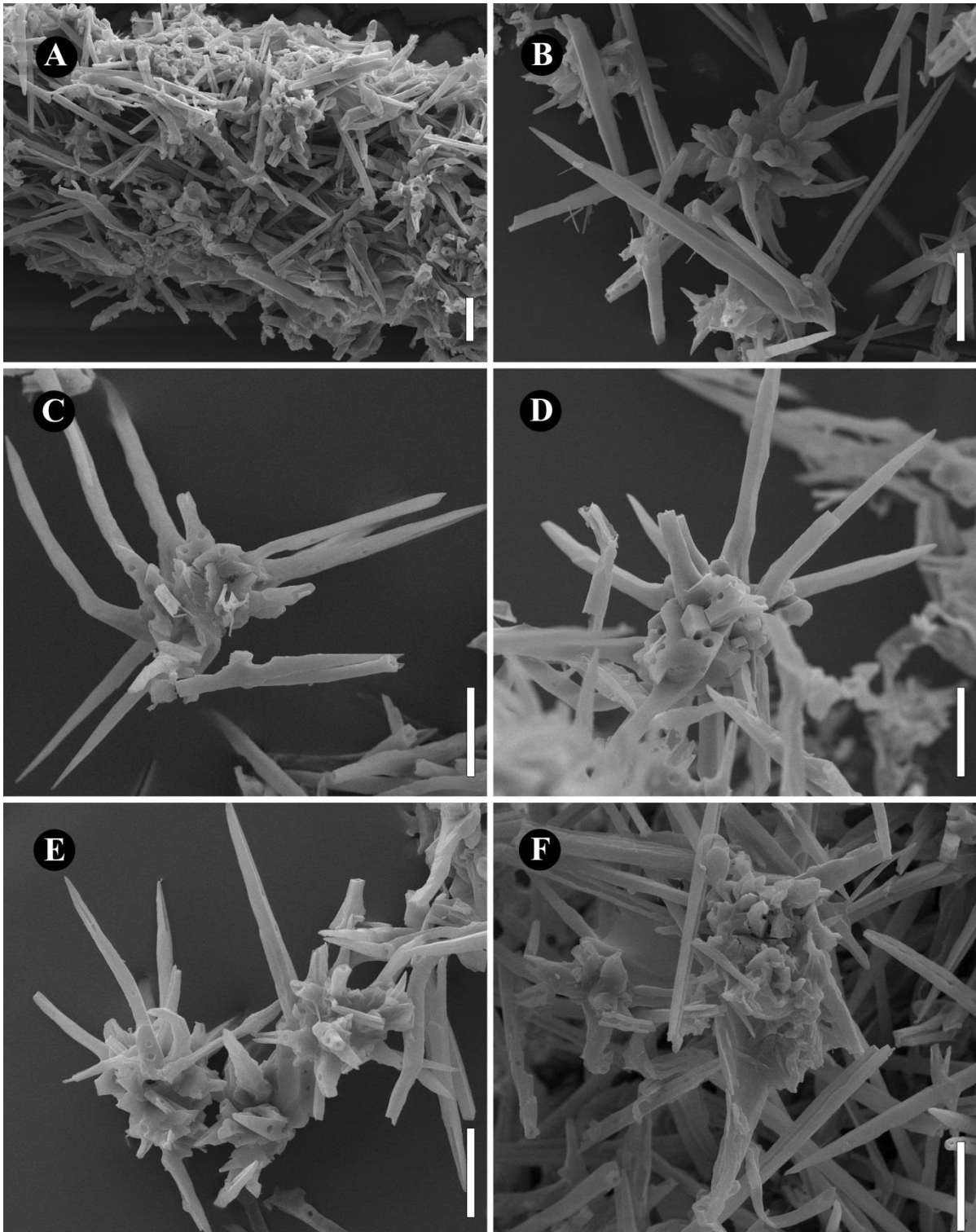


Fig. 9. Acanthocytes on rhizomorphs under scanning electron microscopy. **A & B** *S. apiahyna* (HCP 901); **C** *S. alvesiana* (C. Seger & C.R. Alves 133 - UPCB); **D** *S. ombrophila* (HCP 896); **E** *S. venusta* (HCP 897); **F** *S. versiformis* (C. Seger & G. Coelho 086 - UPCB). (Scale bar: A – F= 10 μ m). A – F: C. SEGER.

7 CAPÍTULO 3: Artigo a ser submetido à revista Rodriguésia.

Taxonomy of the Southern Brazilian species of *Protostropharia* (Strophariaceae, Agaricales)

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Taxonomy of Southern Brazilian *Protostropharia*

Abstract (Taxonomy of Southern Brazilian species of *Protostropharia* (Strophariaceae, Agaricales))

Three species of *Protostropharia* are reported in Brazil: *P. alcis* ssp. *austrobrasiliensis*, *P. dorsipora* and *P. semiglobata*. Morphological variations are described and discussed for *P. alcis* ssp. *austrobrasiliensis* and *P. dorsipora*, as well sequences of the ITS region of rDNA, which were obtained and compared to other members of the genus. The occurrence of *P. semiglobata* is considered based on previous records. A key for the Southern Brazilian species and photographs of macro- and microscopic features are furnished.

Keywords: coprophilous agarics, fungal barcode, mycobiota, taxonomy.

Resumo (Taxonomia das espécies sulbrasileiras de *Protostropharia* (Strophariaceae, Agaricales))

Três espécies de *Protostropharia* são registradas para o Brasil: *P. alcis* ssp. *austrobrasiliensis*, *P. dorsipora* e *P. semiglobata*. Variações morfológicas são descritas e discutidas em *P. alcis* ssp. *austrobrasiliensis* e *P. dorsipora*, bem como a região ITS do rDNA foi sequenciada e comparada com outras espécies do gênero. A ocorrência de *P. semiglobata* é considerada baseada em trabalhos anteriores. Uma chave de identificação das espécies sul brasileiras e imagens das suas características macro e microscópicas são disponibilizadas.

Palavras-chave: cogumelos coprófilos, “código de barras” de fungos, micobiota, taxonomia.

Introduction

Protostropharia Redhead, Moncalvo & Vilgalys is a recently proposed genus in the *Strophariaceae* Singer & A.H. Sm. (Redhead 2013). For many years, the species now placed in this genus were classified in subgenus or section *Stercophila* Romagn. ex Noordel. of *Stropharia* (Fr.) Quél., since they are coprophilous, present glutinous basidiomata, a poorly developed and glutinous annulus, dark purplish spore print, large basidiospores and presence of chrysocystidia (Singer 1986; Noordeloos 2011).

However, members of *Protostropharia* are distinguished from *Stropharia* due to lack of true acanthocytes, which are crystalline spiny cells, which cover the rhizomorphs or grows in cultured mycelium (Farr 1980). The presence of acanthocytes is considered a synapomorphic feature for *Stropharia* and has been documented by many researchers (Cortez & Silveira 2008; Luo *et al.* 2006).

Recently, Redhead (2013) proposed *Protostropharia* as autonomous genus based on the presence of astrocystidia on basal mycelium (instead of true acanthocytes), which are short hyphae with globose and sharp apex with thick hyaline crystals, and also the glutinous basidiomata, smooth and thick-walled basidiospores with wide germ-pore.

Previous molecular studies just had indicated that coprophilous taxa were in fact distinct from *Stropharia* s.str. (Moncalvo *et al.* 2002) and the recently proposed genus has received good acceptance within mycologists (Kaur *et al.* 2013; Hahn 2014; Halama & Kudławiec 2014; Wang & Tzean 2015).

The present paper presents a review of the Southern Brazilian species of *Protostropharia*, as well a discussion on the taxonomy, distribution and phylogenetic relationships.

Material and Methods

Collecting and morphological analysis

Specimens were collected in areas of Atlantic Rainforest in the Southern Brazilian States of Rio Grande do Sul (RS), Santa Catarina (SC) and Paraná (PR). Herbarium specimens from ICN, MBM and SMDB (Thiers 2016) were also examined. Color names and codes in the description of macroscopic features follow Kornerup & Wanscher (1978). For microscopic examination, slides were mounted with 3% KOH and 1% Congo Red and dimensions were taken according to Halama & Kudławiec (2014), with a Moticam[®] 2500 camera in a Motic[®] trinocular microscope, by means of software Motic Image Plus 2.0[®]. All collected specimens are preserved at the Herbarium of Campus Palotina, Universidade Federal do Paraná (HCP).

DNA extraction, amplification and nucleotide sequencing

DNA was obtained from five dried specimens of *Protostrophia* collected in Southern Brazil, for molecular analysis. The DNA extraction protocol described by De Hoog *et al.* (2005), modified by Balini *et al.* (2015) was followed. The rDNA region ITS1-5.8S-ITS2 was amplified with primers ITS1-*forward* (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4-*reverse* (5'-TCCTCCGCTTATTGATATGC-3'), according to White *et al.* (1990). Polymerase chain reaction (PCR) were done in a volume of 25 µL containing 1x PCR reaction buffer (20 mM Tris-HCl pH 8.4 and 50 mM KCl), MgCl₂ 3mM, dNTP mix 0,4 mM, 0,2µM of each *primer* and 1U Platinum Taq DNA polymerase (Invitrogen[®]). Thermal cycles started at 94°C for 5 min, followed by 25 cycles at 94°C of 30 sec, 56°C of 45 sec and 72°C of 1 min, and a final extension at 72°C for 7 min.

PCR products (≈ 630 bp) were analysed by 2% agarose gel electrophoresis in TBE buffer (89Mm Tris, 89 mM boric acid, 2mM EDTA), pH 8.4, stained with ethidium bromide

and visualized under UV light. The amplicons were quantified by NanoDrop and sequenced on ABI-Prism 3500 Genetic Analyzer (Applied Biosystems) in both directions using the forward and reverse primers.

Phylogenetic analysis

All generated sequences were initially evaluated in the program Phred/Phrap (<http://asparagin.cenargen.embrapa.br/phph/>) and edited manually using BioEdit sequence alignment editor version 7.2.5 (HALL 1999) simultaneously with FinchTV version 1.4 (<http://www.geospiza.com/Products/finchtv.shtml>), and will be deposited in GenBank. The homology of the sequences were compared with others sequences from database at GenBank by using BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Our sequences and more six species of *Strophariaceae* at GenBank were aligned with ClustalW on BioEdit, which was also used to create a DNA identity matrix. Maximum likelihood (ML) phylogenetic tree were construct in MEGA version 6.0 (Tamura *et al.* 2013), employing the GTR+I+G model and bootstrap of 1000 replicates.

Results

Phylogenetic analysis

The maximum likelihood phylogenetic tree show a clade formed by *Protostropharia* spp. is well-supported with high bootstrap values (86%) (Fig. 1). These results justify the placement of the Southern Brazilian specimens in the genus *Protostropharia*, as well support the recognition of three distinct species in this study: *P. alcis* ssp. *austrobrasiliensis*, *P. dorsipora*, and *P. ovalispora*. All these species presented identity values between 0.93–0.94 and smaller than 0.92 when compared to species belonging to other closely related genera, as

Hypholoma and *Stropharia* s.str. In spite of the wide morphological variety, which will be discussed further, the specimens of *P. alcis* ssp. *austrobrasiliensis* C. Seger 101, 170, 181 and C.R. Alves 467 exhibited high values (>0.99) in the identity matrix, showing that all these are conspecific.

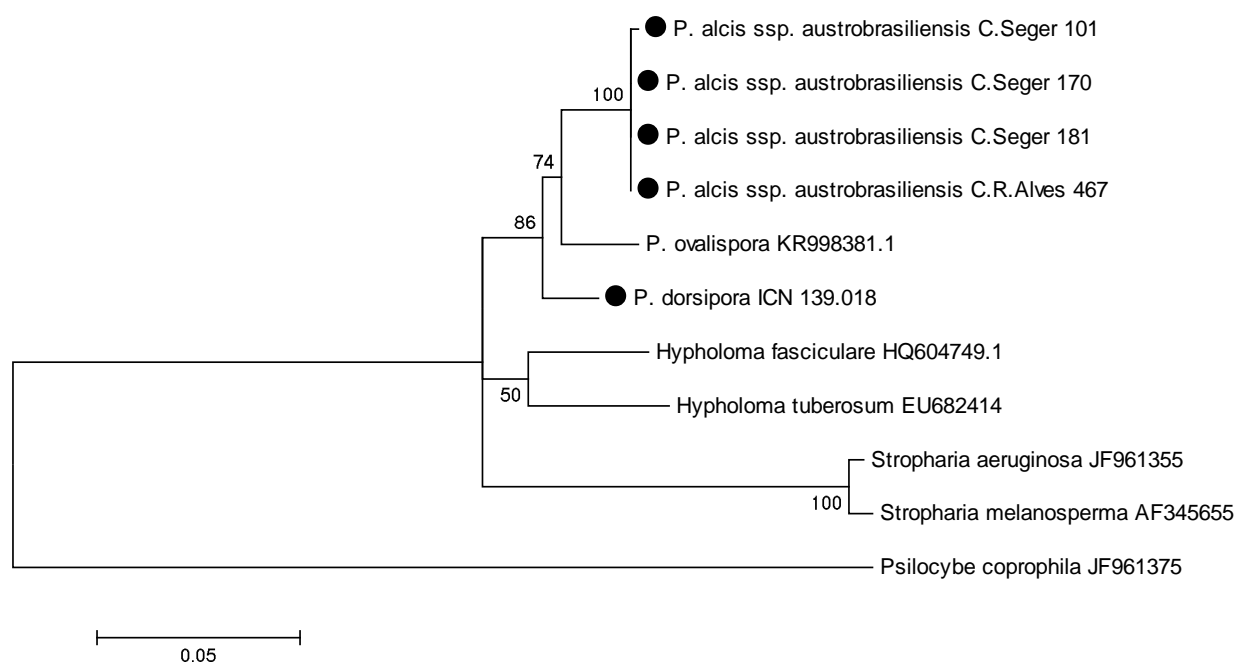


Fig. 1 – Maximum likelihood phylogenetic tree of the ITS region of Southern Brazilian *Protostropharia* (●) and correlated species with their *GenBank* accession number. Bootstrap values are shown on branches.

Taxonomy

Protostropharia alcis* ssp. *austrobrasiliensis (Cortez & R.M. Silveira) C. Hahn, *Mycol. Bav.* 15: 28, 2014. Type: BRAZIL. RIO GRANDE DO SUL: Santa Maria, 24.IV.2004, V.G. Cortez 006/04 (ICN 139.000).

Fig. 2a–e; 3a–j

Pileus 8–29 mm, globose in young basidiomata becoming hemispheric to convex in age, sometimes with slight umbo, surface smooth and viscid-glutinous, margin smooth or translucent-striate, dark yellow (4C8) to olive yellow (3D8) in the centre, greyish yellow (2B5) toward the margin, and yellowish green (30A7-30B7) to greyish yellow (2C3) at margin. *Context* 1–2.2 mm, concolorous with the pileus, translucent appearance when wet, centrally thick and gradually thinner to the margin. *Lamellae* 2–5 mm, adnate, olive brown (4E3) to brownish grey (4E2), near, with white and serrate edge. *Stipe* 50–125 × 1–2.5 mm, central, cylindrical, smooth, viscid to glutinous surface, greyish yellow (4B3) to pale yellow (4A3). *Veil* forming an apical annulus, or often only an ephemeral and glutinous annular zone. *Mycelium* present on stipe base, without rhizomorphs. *Pseudoesclerotia* can be present on stipe base of some collections, but not in all examined specimens. *Spore print* brownish grey (7E2).

Basidiospores (10.5–) 11–14.5 (–15) × (6–) 6.5–9 (–9.5) μm , $Q=1.31\text{--}2.15$, $Q_m=1.65$, $n=262$, ellipsoid to oblong in face and side-view, apically truncate by conspicuous germ pore, brown in KOH, smooth and thick-walled. *Basidia* 23–39 × 7.5–14.5 μm , clavate, some with slight constriction near at middle part, tetrasporic (rarely bisporic), sterigmata ≤ 8 μm , thin-walled, hyaline. *Pleurocystidia* 22.5–60.5 × 8.5–18 μm , as chrysocystidia, fusiform, mucronate to rostrate apex (≤ 10.5 μm), rounded apex present but are rare, thin-walled. *Lamellae edge* sterile. *Cheilocystidia* 17–42.5 × 3.5–10.5 μm , as leptocystidia, sublageniform to flexuous-cylindrical, apex rounded, subcapitate or capitate, thin-walled, numerous on gill edge. *Suprapellis* an ixotrichoderm, composed of cylindrical, anticlinally arranged, hyaline to yellowish, smooth or incrustated by yellowish spiraled pigment, thin-walled hyphae, 1–6 (–9) μm diam, immersed in a strongly gelatinized layer. *Subpellis* an ixocutis, composed of cylindrical, prostrate, yellowish hyphae, 2.5–22.5 μm diam. *Pileal context* formed by interwoven, hyaline to yellow in mass, smooth and thin-walled hyphae, 2.5–33 μm diam. *Hymenophoral trama* subregular to regular with thin-walled and hyaline hyphae, 3.5–22 (–29.5) μm diam.

Subhymenium slightly gelatinized with inflated to subcellular hyphae. *Caulocystidia* 13–59.5 × 3.5–12 (–15.5) µm, as leptocystidia flexuous-cylindrical, apex rounded, subcapitate or capitate, thin-walled, similar to cheilocystidia. *Stipitipellis* gelatinized, with thin, loose, hyaline and thin-walled hyphae, 1.5–4 µm diam. *Stipe trama* with parallel, hyaline and thin-walled hyphae, 1.5–24 µm diam. *Chrysovessels* present. *Acanthocytes* absent. *Astrocystidia* not found. *Clamp connections* present.

Material examined: BRAZIL. PARANÁ. Parque Marumbi, 19.III.1988, A.A.R. Meijer 1088 (MBM 325.995). RIO GRANDE DO SUL: Rio Grande, 07.VI.2008, V.G. Cortez 106/08 (HCP). Santa Maria, Distrito de Arroio do Só, Água Boa, 19.VI.2003, V.G. Cortez 032/03 (SMDB 9.629); Morro da Caturrita, 23.IV.2014, C. Seger & G. Coelho 100 (HCP); C. Seger & G. Coelho 101 (HCP); Morro do Elefante, 24.IV.2004, V.G. Cortez 011/04 (ICN 139.002); Parque de Exposições UFSM, 10.IV.2001, V.G. Cortez 017/01 (SMDB 9.541). Caçapava do Sul, Guaritas, 30.IV.2005, V.G. Cortez 020/05 (ICN 139.103). Cambará do Sul, 02.IV.2005, V.G. Cortez 007/05 (ICN 139.094). Capivari do Sul, Fazenda dos Touros, 28.V.2004, F. Caporal (ICN 139.034). Dom Pedro de Alcântara, 16.V.2009, P.S. Silva 216/09 (ICN 154.245). Viamão, Parque Estadual de Itapuã, Praia da Pedreira, 16.IV.2005, M.A. Reck & P.S. Silva 109/05 (ICN 139.083). SANTA CATARINA: Água Doce, Fazenda Quiben, 24.II.2013, C. Seger 016 (HCP 664). Urubici, Parna São Joaquim, 11.VI.2014, C. Seger & C.R. Alves 129 (HCP); C. Seger & C.R. Alves 130 (HCP); 12.VI.2014, C. Seger & C.R. Alves 140 (HCP); 20.II.2015, C.R. Alves 467 (HCP); 21.II.2015, C.R. Alves 473 (HCP); 22.IV.2015, C. Seger & C.R. Alves 170 (HCP); 23.IV.2015, C. Seger & C.R. Alves 181 (HCP); C. Seger & C.R. Alves 183 (HCP). Vargem Bonita, Linha Coração, 18.III.2013, C. Seger 031 (HCP 666).

Additional material examined: *Protostropharia alcis*. SWEDEN, DALARNAS, St. Kopparberg, 20.IX.1980, S. Jacobsson (ICN).

Distribution and habitat: Southern Brazil: States of Paraná, Rio Grande do Sul and Santa Catarina. Solitary to scattered, growing on manure of cattle or horse, inside forest and in pastures.

Notes: *Stropharia alcis* was originally described by Kytövuori (1999) from Boreal Region of Europe, where it grows on elk dung. Cortez & Silveira (2008) identified the fungus as morphologically identical to European material (compared to Swedish material cited above), but considering the type of substrate (cow or horse dung) and the strongly disjunct distribution, they proposed as a new variety of that species. We examined the Swedish specimen of *S. alcis* again, and now we found caulochrysocystidia, which were not reported in the available descriptions of the species (Kytövuori 1999; Noordeloos 2011; Halama & Kudławiec 2014).

Halama & Kudławiec (2014) examined collections of *P. alcis* from Poland and concluded that size and color of the basidiomata can be much variable in this species, depending on the amount of studied collections, climatic factors and developmental stage. In the same way, *P. alcis* ssp. *austrobrasiliensis* is widely represented in Southern Brazil, where we had also noted a wide morphological variation, including occasional production of pseudoesclerotium, scattered bisporic basidia, and pleurocystidia with forked or triple rostrate apex. Before performing molecular analysis, it was presumed that these variations could be taxonomically significant, however ITS data showed all these collections belong to a single species (Fig. 1).

The production of pseudosclerotium is described in *P. tuberosa* (Beardslee) Redhead (Redhead & Kroeger 1987), and this feature is the most significant to distinguish it from *P. alcis* ssp. *austrobrasiliensis* (as well other species in the genus), once both present strong similarities in their macro- and microscopic features. Unfortunately, *P. tuberosa* seems to be a rare and poorly known species, whose neotype from North Carolina, USA is the only preserved specimen to our knowledge; future new collections of this species will allow to improve the

knowledge on its morphology and phylogenetic relationships within the genus. Bisporic basidia are reported in *P. luteonitens* (Fr.) Redhead, which differs from *P. alcis* ssp. *austrobrasiliensis* by the larger basidiospores ($16\text{--}21 \times 9.5\text{--}12 \mu\text{m}$) and absence of pleurocystidia (Kytövuory 1999). On the other hand, the pleurocystidia with 2-3 rostri at the apex is not reported in the checked literature on the group; however, this condition was noted only in a single collection (C. Seger 101).

Some new taxa recently described share some morphological similarities, especially the basidiospores, and are compared as follows. *Protostropharia alcis* ssp. *punjabensis* (A. Kaur, Atri & M. Kaur) C. Hahn was described as a variety of *P. semiglobata* with shorter basidiospores, but Hahn (2014) considered it also as a subspecies of *P. alcis*, growing on cow dung in India, with small differences on cystidia and pileus color. Kaur *et al.* (2013) reported bisporic basidia in Indian specimens, which were also observed in a single Southern Brazilian specimen.

Another recently described species, *P. ovalispora* Wang & Tzean (2015), from Taiwan has same shape and surface (viscid to glutinous) of the pileus and stipe, and similar pleurochrysocystia, cheiloleptocystia and cauloleptocystia. It differs by more robust basidiomata (pileus with 20–60 mm and stipe up to $110 \times 6 \text{ mm}$), slightly bigger ($12.0\text{--}15.5 \times 7.2\text{--}10.7 \mu\text{m}$) and broadly ellipsoid to oval ($Q_m = 1.53$) basidiospores, and also has chrysocystidia on stipe apex and occasionally on gill edges too.

Psilocybe parvula Y.S. Chang & A.K. Mills, from Tasmania is possibly a *Protostropharia*, due to coprophilous substrate, glutinous pileus and stipe, and chrysocystidia (Chang *et al.* 2006). The basidiospores come close to *P. alcis* subs. *austrobrasiliensis* ($12.9\text{--}15.4 \times 7.5\text{--}9.2 \mu\text{m}$ in *Ps. parvula*), cheilocystidia are similar in shape and size, and epicutis with gelatinized layer. Caulocystidia are not described in that species, but in comparison with

Brazilian materials, the pileus is more orange brown and the substrate is wallaby dung, which can suggest a more specific substrate (Chang *et al.* 2006).

Protostropharia alcis ssp. *austrorbrasiliensis* is also similar to *S. silvatica* A.H. Sm., a name used by Meijer (2006; 2010) for specimens from Paraná, Brazil, since they have the same basidiospore size, but cheilocystidia are chrysocystidia and the mushroom is reported to grow solitary on cedar and hemlock (Smith 1941).

Hypholoma ericaeum (Fr.) Kühner, another common species in Southern Brazil, can be sometimes confounded with *P. alcis* ssp. *austrorbrasiliensis*, since it occurs in the same environments, has similar shape and colors of the basidiomata, as well as the basidiospores of similar size and shape. However, *H. ericaeum* is not coprophilous, the basidioma is dry, pileipellis not gelatinized and does not produce acanthocytes or astrocystidia (Cortez & Silveira 2007).

P. alcis ssp. *austrorbrasiliensis* is known from Brazil, in the States of Paraná (Meijer 2006) and Rio Grande do Sul (Silva *et al.* 2012), and now we recorded for the first time from Santa Catarina State.

Protostropharia dorsipora (Esteve-Rav. & Barrasa) Redhead, *Index Fungorum* 158: 1, 2014.
Type: SPAIN. El Barco de Avila, 11.V.1974, G. López (MA-Fungi).

Fig. 4a–i

Pileus 10–17 mm, globose in young basidiomata becoming hemispheric to convex in age, smooth, brownish orange (5C5) to light brown (6D8), can be light orange (5C5) at margin. *Context* 2 mm, greyish yellow (4B4), hick centrally and gradually thinner to the margin. *Lamellae* 3–4 mm, adnate, brown (6E6) to dark brown (6F4), near, whitish edge. *Stipe* 40–48 × 1–2.5 mm, central, cylindrical, smooth, evidence of viscid to glutinous surface by stuff glued

on this, greyish yellow (4B4). *Veil* forming an apical annulus, that may be absent on dry materials. *Rhizomorphs* absent. *Pseudoesclerotia* absent. *Spore print* not seen. &

Basidiospores (14–) 15–20 (–21.5) × 8–11 μm, Q=1.58–2.13, Q_m=1.80, n=62, oblong in face and side-view, apically truncate by broadly (≤2.5 μm) and eccentric germ pore, brown in KOH, smooth and thick-walled (≤1 μm). *Basidia* 24–38 × 9–13.5 μm, broadly clavate or subcylindrical with constriction near at middle part, tetrasporic, sterigmata ≤6.5 μm, thin-walled, hyaline. *Pleurocystidia* 33–54 × 10–15.5 μm, as chrysocystidia, fusiform, with acute, mucronate or rostrate apex, rostrum ≤8 μm, thin-walled, scattered on gill side. *Lamellae edge* sterile. *Cheilocystidia* 25.5–57.5 × 4–8 μm, as leptocystidia, sublageniform to flexuous-cylindrical, rounded apex, thin-walled, abundant on gill edge. *Suprapellis* an ixocutis, composed of prostrate to interwoven, cylindrical, hyaline, smooth and thin-walled hyphae, 1–2 μm diam, immersed in gelatinized layer up to 85 μm. *Subpellis* with prostrate to interwoven, cylindrical, hyaline to yellow in mass hyphae, wider than the suprapellis hyphae (2.5–5 μm diam). *Pileal context* formed by interwoven, hyaline smooth and thin-walled hyphae, some inflated, 5–13 μm diam. *Hymenophoral trama* subregular to regular with thin-walled and hyaline hyphae, 5–11.5 μm diam. *Subhymenium* slightly gelatinized with subcellular hyphae. *Caulocystidia* 15–72.5 × 4–7.5 (–11.5) μm, as leptocystidia flexuous-cylindrical, rounded to subcapitate apex, thin-walled. *Stipitipellis* with prostrate, little gelatinized, hyaline, thin-walled and fine hyphae (1.5–5 μm diam). *Stipe trama* with parallel, hyaline and thin-walled hyphae, 4.5–20 μm diam. *Chryso-vessels* not found. *Acanthocytes* absent. *Astrocystidia* not found. *Clamp connections* abundant.

Material examined: BRAZIL. PARANA: Curitiba/São José dos Pinhais, Reserva Biológica Cambui, 02.X.1979, A.A.R Meijer 153 (MBM 324.078, as *S. semiglobata*). RIO GRANDE DO SUL: Encruzilhada do Sul, Fazenda Xafri, 10.VII.2004, V. G. Cortez 039/04

(ICN 139.018); V. G. Cortez 041/04 (ICN 139.020). Santa Maria, Colégio Politécnico UFSM, 03.III. 2009, G. Coelho (HCP, specimen lost, only microscopic slide preserved).

Additional material examined: SWEDEN. SMÅLAND: Femsjö, 25.IX.1959, F. Karlvall (ICN).

Distribution and habitat: Widespread in Europe (Noordeloos 2011); reported in Asia (Bau & Meng 2008), North (Noordeloos 2011) and Southern America (only in Brazil). Solitary to scattered, growing on manure of cattle or horse, both inside forest and in pastures.

Notes: This is the only *Protostropharia* with basidiospores presenting an eccentric germ-pore, being the diagnostic feature of *P. dorsipora* (Esteve-Raventós & Barrasa 1995; Noordeloos 2011). However, this feature may easily to be overlooked, and for this reason the mushroom has been misidentified, especially as *P. semiglobata* by several authors. When analyzing Bavarian collection, Hahn (2014) described some astrocystidia in basal mycelium that involved the substrate, but this structure was not found in our materials.

This mushroom seems to have some preference for horse dung (Noordeloos 2011; Hahn 2014), but Brazilian specimens also grows on cow dung. In Brazil, it is reported in Rio Grande do Sul State and is new record from Paraná State.

Other recorded species:

Protostropharia semiglobata (Fr.) Redhead, Moncalvo & Vilgalys, *Index Fungorum* 15: 2, 2013.

Description: Cortez & Silveira (2008).

Notes: *Protostropharia semiglobata* has pale yellow basidiomata, small pileus (<30 mm), long stipe (<120 mm), large basidiospores (16–21 × 8.5–11 µm), and, probably, has a worldwide distribution, growing on herbivorous dung (Noordeloos 2011). Misidentifications as *P. semiglobata* are due to similarities with *P. dorsipora*, whose main difference is with the

germ-spore central rather than eccentric of the latter, as previously discussed. In Brazil, *P. semiglobata* was reported from the States of Paraná (Meijer 2006) and Rio Grande do Sul (Cortez & Silveira 2008). Examination of the material from Paraná resulted in re-determination as *P. dorsipora* (MBM 324.078); on the other hand, Cortez & Silveira (2008) reported two collections from Rio Grande do Sul, one was lost and another from Rick's herbarium, which was not checked again. Thus, new fieldwork is necessary to provide new collections of this species and assure its occurrence in Southern Brazil.

Key to species of *Protostrophia* from Southern Brazil

1. Basidiospores up to $15 \times 9 \mu\text{m}$, occasionally forming pseudoesclerotia.....
*P. alcis* ssp. *austrobrasiliensis*
- 1'. Basidiospores larger than $15 \times 8 \mu\text{m}$, never forming pseudoesclerotia
2. Germ-pore central in face and side-view.....**3. *P. semiglobata***
- 2'. Germ-pore distinctly eccentric in side-view.....**2. *P. dorsipora***

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References

- Bau, T. & Meng, T. 2008. Strophariaceae of China (II): *Stropharia*. *Journal of Fungal Research* 6: 7–34.
- Balini, L.C.; Luis, A.S.; Soares, L.C.; Vendruscolo, E.C.G. & Fiorini A. 2015. Identificação pela técnica de PCR-RFLP de *Aspergillus* spp. isolados de grãos de soja e milho. *Revista Brasileira de Energias Renováveis* 4: 83–99.
- Chang, Y.S.; Gates, G.M. & Ratkowsky, D. A. 2006. Some new species of the Strophariaceae (Agaricales) in Tasmania. *Australasian Mycologist* 24: 53–68.
- Cortez, V.G. & Silveira, R.M.B. 2007. Species of *Hypholoma* (Fr.) P. Kumm. (Strophariaceae, Agaricales) from Rio Grande do Sul State, Brazil. *Acta Botanica Brasilica* 21: 609–621.
- Cortez, V.G. & Silveira, R.M.B. 2008. The agaric genus *Stropharia* (Strophariaceae, Agaricales) in Rio Grande do Sul State, Brazil. *Fungal Diversity* 32: 31–57.
- De Hoog, G.S.; Göttlich, E.; Platas, G.; Genilloud, O.; Leotta, G. & Brummelen, J.V. 2005. Evolution, taxonomy and ecology of the genus *Thelebolus* in Antarctica. *Studies in Mycology* 51: 33–76.
- Esteve-Raventós, F. & Barrasa, J.M. 1995. Coprophilous Agaricales from Spain II. The genus *Stropharia* and *Stropharia dorsipora* sp. nov. *Revista Iberoamericana de Micología* 12(3): 70–72.
- Farr, D.F. 1980. The acanthocyte, a unique cell type in *Stropharia* (Agaricales). *Mycotaxon* 11: 241–249.
- Hahn, C. 2014. Ein bayerischer Nachweis von *Protostropharia dorsipora* mit Anmerkungen zur Gattung *Protostropharia* und kommentiertem Bestimmungsschlüssel. *Mycologia Bavarica* 15: 19–32.

- Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Halama, M. & Kudławiec, B. 2014. New localities of *Protostropharia alcis* (Basidiomycota, Agaricales) in Poland. *Acta Mycologica* 49: 47–57.
- Kaur, A; Kaur, M. & Atri, N.S. 2013. *Protostropharia alcis* subsp. *punjabensis*: a new coprophilous agaric from India. *Kavaka* 41: 11–14.
- Kornerup, A. & K. H. Wanscher. 1978. Methuen Handbook of Colour. 3rd ed. London: Eyre Methuen.
- Kytövuory, I. 1999. The *Stropharia semiglobata* group in NW Europe. *Karstenia* 39: 11–32.
- Luo, H.; Li, X.; Li, G.; Pan, Y., & Zhang, K. 2006. Acanthocytes of *Stropharia rugosoannulata* Function as a Nematode-Attacking Device. *Applied and Environmental Microbiology* 72: 2982–2987.
- Meijer, A.A.R. 2006. Preliminary list of the macromycetes from the Brazilian state of Paraná. *Boletim do Museu Botânico Municipal* 68: 1–55.
- Meijer, A.A.R. 2010. Preliminary list of the macromycetes from the Brazilian state of Paraná: corrections and updating. *Boletim do Museu Botânico Municipal* 72: 1–9.
- Moncalvo, J.M; Vilgalys, R.; Redhead, S.A.; Johnson, J.E.; James, T.Y.; Aime, M.C.; Hofstetter, V.; Verduin, S.J.W.; Larsson, E.; Baroni, T.J.; Thorn, R.G.; Jacobsson, S.; Cléménçon, H. & Miller Jr, O.K. 2002. One hundred and seventeen clades of euagarics. *Molecular Phylogenetics and Evolution* 23: 357–400.
- Noordeloos, M.E. 2011. Strophariaceae s.l. *Fungi Europaei* 13. Alassio: Edizioni Candusso.
- Redhead, S.A. 2013. Nomenclatural novelties. *Index Fungorum* 15: 1–2.

Redhead, S.A. & Kroeger, P. 1987. A sclerotium-producing *Hypholoma* from British Columbia. *Mycotaxon* 29: 457–465.

Singer, R. 1986. The Agaricales in Modern Taxonomy. 4th ed. Koeltz, Koenigstein.

Smith, A.H. 1941. Studies of North American Agarics – I. *Contributions from the University of Michigan Herbarium* 5: 1–73.

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

Thiers B. 2016 (continuously updated). Index herbariorum: a global directory of public herbaria and associated staff. New York Botanical Garden's virtual herbarium. Available at: <http://sweetgum.nybg.org/science/ih/>. Accessed on 29 Jan 2016].

Wang, Y-W & Tzean, S-S. 2015. Dung-associated, Potentially Hallucinogenic Mushrooms from Taiwan. *Taiwania* 60:160–168.

White, T.J.; Bruns, T.; Lee, S. & Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenies. In: Innis, M.A.; Gelfand D.H.; Sninsky J.J. & White T.J., eds. 1990. *PCR protocols: a guide to methods and applications*. San Diego: Academic Press. 315–322.

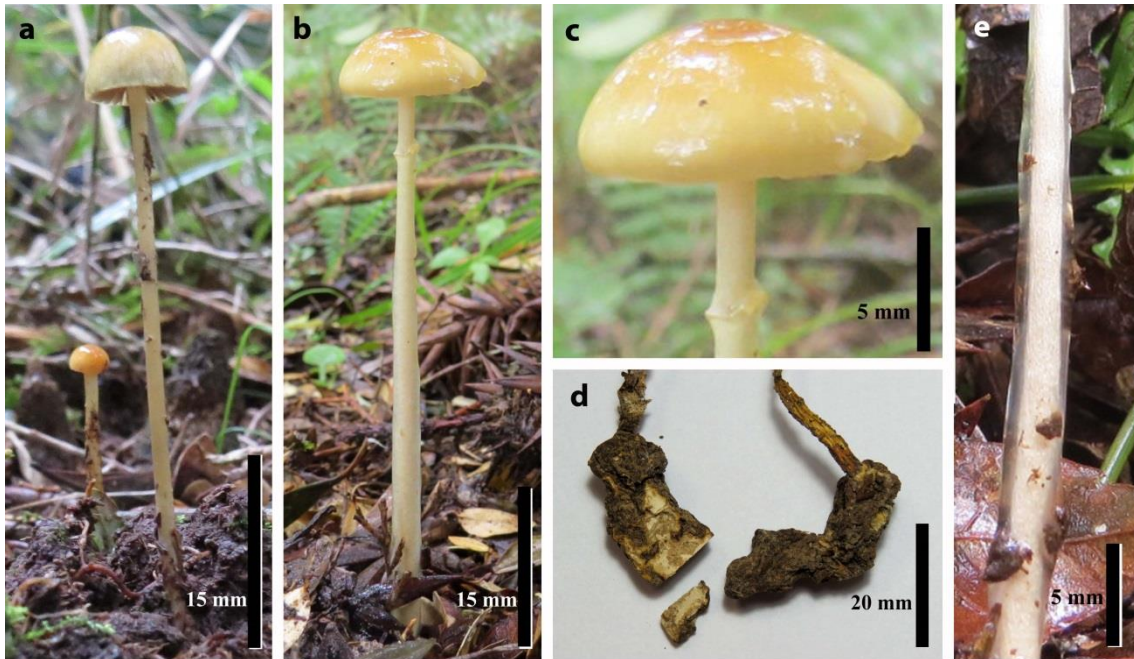


Figure 2 – *Protostropharia alcis* ssp. *austrobrasiliensis* – a-c. basidiomata; d. pseudoesclerotia; e. detail of glutinous stipe. (a: HCP 906; b-c: HCP 909; d: HCP 904; e: HCP 911).

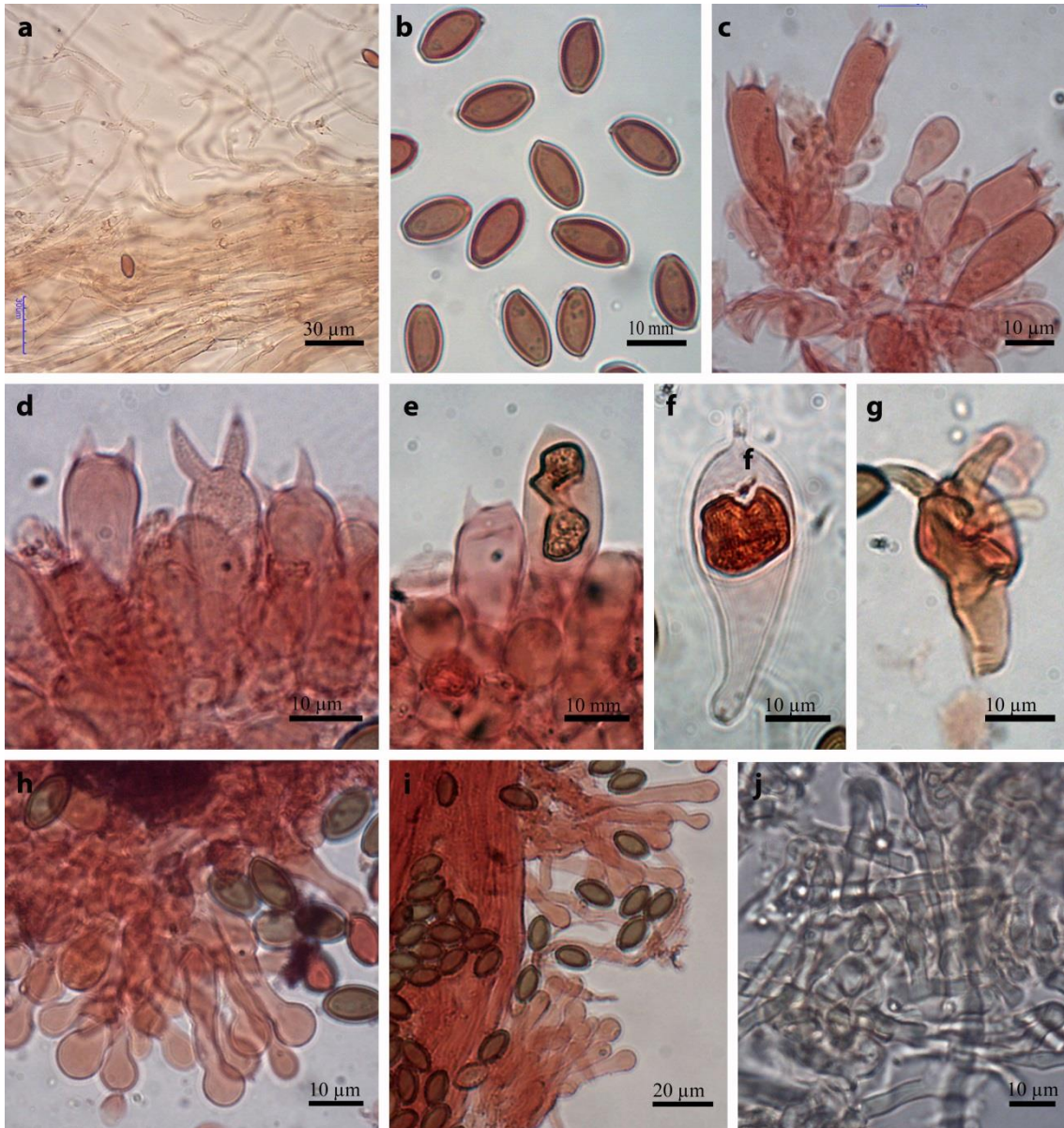


Figure 3 – *Protostropharia alcis* ssp. *austrobrasiliensis* – a. pileal surface; b. basidiospores; c-d. basidia; e-g. pleurocystidia; h. cheilocystidia; i. caulocystidia; j. pseudoesclerotia hyphae. (a-b: HCP 913; c; h-i: HCP 908; d: HCP 912; e: HCP 906; f: HCP 907; g: HCP 905; j: HCP 904)

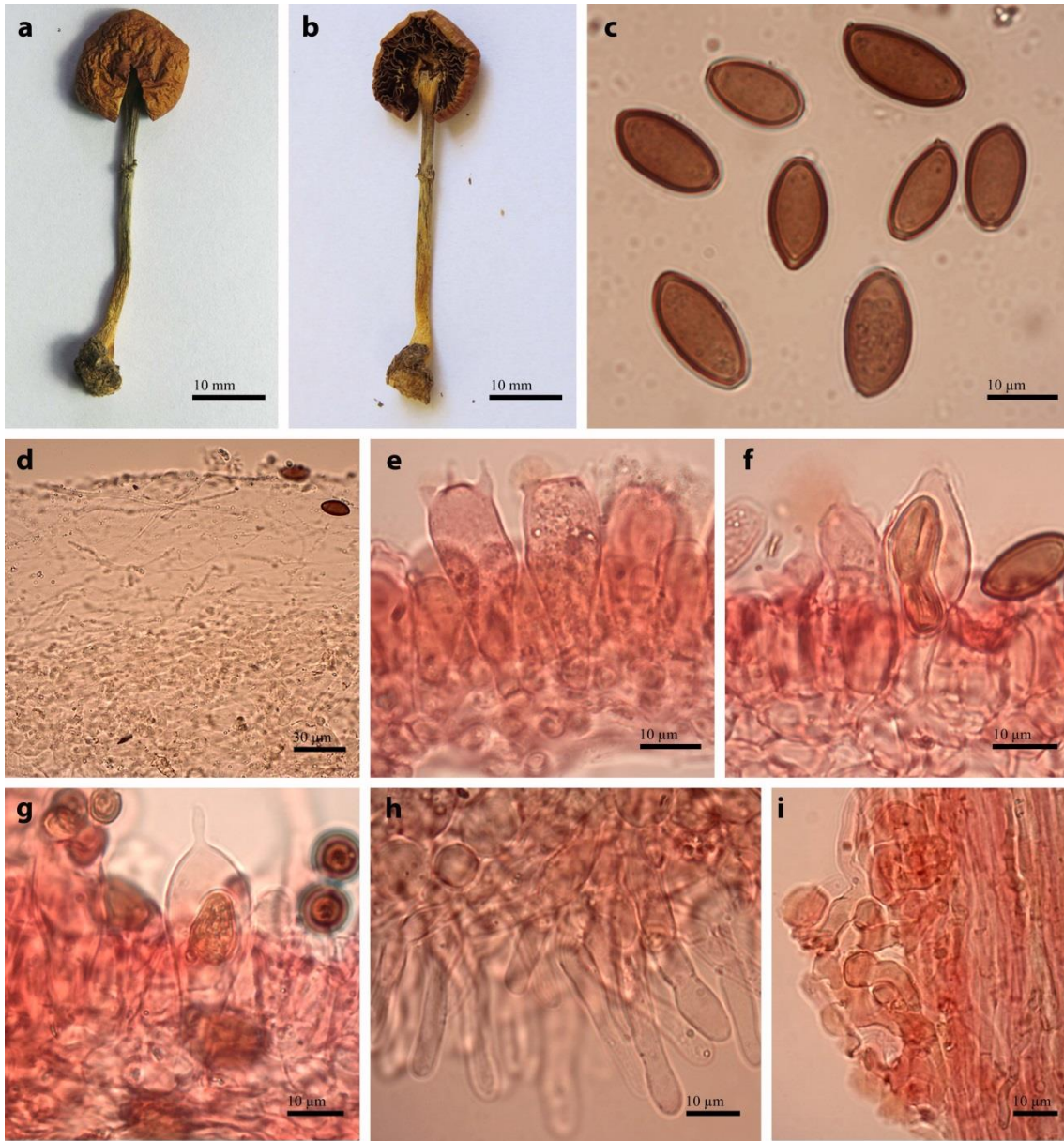


Figure 4 – *Protostropharia dorsipora* – a-b. basidiomata; c. basidiospores; d. pileal surface; e. basidia; f-g. pleurocystidia; h. cheilocystidia; i. caulocystidia. All from ICN 139.020.

CONSIDERAÇÕES FINAIS

O principal objetivo deste estudo foi ampliar o conhecimento acerca de *Stropharia* s.l. no sul do Brasil, especialmente através de coletas em áreas onde o grupo não havia sido pesquisado. Visto que no bioma Mata Atlântica houveram importantes resultados em trabalhos anteriores, era esperado que, ao ampliar a área de pesquisa para outros locais com fitofisionomia semelhante o mesmo viesse a acontecer.

Nossas expectativas estavam corretas e nosso objetivo foi atingido. No Estado de Santa Catarina, onde nenhum registro havia sido feito, foram encontradas cinco espécies, e para o Paraná é citada *S. apiahyna* pela primeira vez. Ao final, dezenove espécies passaram a ser conhecidas para a região sul do Brasil, e destas cinco são novos registros para a ciência.

Com as coletas e analisando os materiais herborizados, ficou aparente a preferência deste gênero em produzir os basidiomas nas épocas de outono e inverno. A Floresta Ombrófila Mista mostrou ser a área com maior número de espécies, quando comparado com as áreas de Floresta Ombrófila Densa e as Florestas Estacionais.

Infelizmente, quase metade das espécies descritas para a região estudada foi analisada apenas a partir de material seco, pois mesmo visitando os mesmos locais e estação do ano em que foram anteriormente encontrados, não conseguimos novas coletas. Isto é frequente quando se trabalha com fungos, já que o basidioma é fugaz e, portanto nem sempre está presente. Ainda, espécies de *Stropharia* s.l. normalmente formam basidiomas solitários ou dispersos, o que dificulta sua visualização em campo.

Das dezenove espécies de *Stropharia* s.l. identificadas com este trabalho, vale salientar que nove foram descritas e até o momento são conhecidas exclusivamente para a Mata Atlântica do sul do Brasil, o que nos mostra a importância deste bioma para o desenvolvimento do grupo.