UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
INSTITUTO DE BIOCIÊNCIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

Tese de Doutorado

Estudos morfológicos e moleculares de *Phallales* e *Geastrales* (*Phallomycetidae*, *Agaricomycetes*), com ênfase em espécies da Região Sul do Brasil

Larissa Trierveiler Pereira

PORTO ALEGRE, 2014

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Larissa Trierveiler Pereira

Tese apresentada ao **Programa de Pós-Graduação em Botânica**, Área de Concentração em **Taxonomia Vegetal (Micologia e Ficologia)**, da Universidade Federal do Rio Grande do Sul (UFRGS), como requisito parcial para a obtenção do título de **Doutor em Ciências (Botânica)**.

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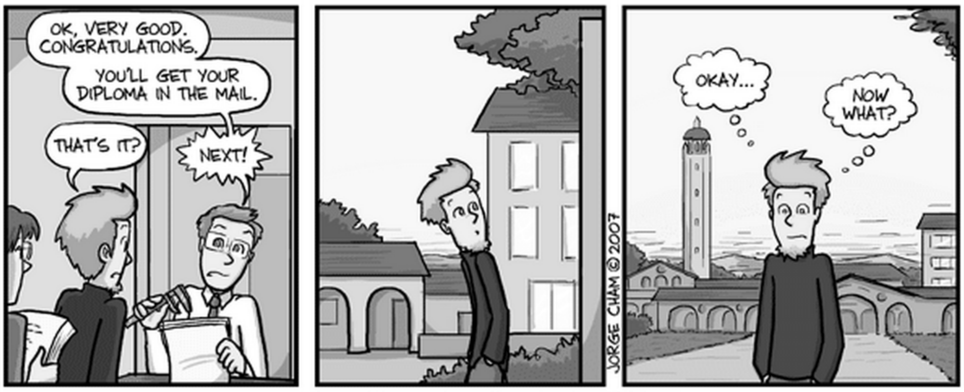

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RESUMO

Estudos morfológicos e moleculares de *Phallales* e *Geastrales* (*Phallomycetidae*, *Agaricomycetes*), com ênfase em espécies da Região Sul do Brasil

Os membros de *Phallales* e *Geastrales* são basidiomicetos de hábito gasteroide comumente encontrados em diversas formações vegetais da Região Sul do Brasil, sendo que muitas espécies exibem formas e cores diferenciadas que estão relacionadas com a dispersão dos esporos (principalmente por entomocoria, hidrocoria e anemocoria). Com o intuito de melhor conhecer a diversidade e distribuição destes organismos na região, além de contribuir para os estudos filogenéticos dos mesmos, expedições a campo foram realizadas durante dois anos (2010–2012) nos três Estados da Região Sul, além da análise de materiais de herbário nacionais e internacionais. No total, foram analisados 593 espécimes, confirmando a ocorrência de 24 espécies de fungos faloides e 20 espécies/morfoespécies de fungos geastroides na área de estudo. Para a realização dos estudos moleculares, 271 novas sequências de quatro marcadores moleculares (ITS, LSU, *rpb2* e *apt6*) foram geradas. Como principais resultados deste estudo, destaca-se a descrição de um novo gênero, *Sphaerophallus*, e de uma nova espécie, *Phallus aureolatus*; a proposição de uma nova combinação, *Lysurus arachnoideus*; a reconsideração de sete espécies; e a proposição de sinonímias para quatro espécies. Ainda, *Geastrum morganii* corresponde a um novo registro para o Brasil, enquanto *Abrachium floriforme*, *Geastrum entomophilum* e *Staheliomyces cinctus* são novas ocorrências para a Região Sul do Brasil. Quanto às contribuições para a filogenia de *Phallales*, é proposta uma nova circunscrição para o gênero *Protubera*; *Gastrosporiaceae* é aceita como clado irmão de *Phallaceae* e uma emenda é formulada para a ordem. O estudo filogenético dos espécimes de *Geastrum* permitiu identificar a ocorrência de táxons crípticos nas morfoespécies: *G. saccatum*, *G. schweinitzii*, *G. trichiferum* e *G. triplex*. Chaves para a identificação de todas as espécies e morfoespécies, assim como fotografias coloridas e ilustrações para a maioria destas, são apresentadas.

Palavras-chave: *Basidiomycota* · *Gasteromycetes* · micota neotropical · sistemática · taxonomia

ABSTRACT

Morphological and molecular studies on *Phallales* and *Geastrales* (*Phallomycetidae*, *Agaricomycetes*) with emphasis on species from the Southern Region of Brazil

Members of *Phallales* and *Geastrales* are basidiomycetes with gasteroid habit commonly found in different vegetation types from Southern Brazil. Many species exhibit different morphologies and colors which are related to spore dispersal (mainly by entomochory, hydrochory and anemochory). With the aim to better understand the diversity and distribution of these organisms, and to contribute to their phylogenetic studies, field expeditions were carried out during two years (2010–2012) in the three States from the Southern Region. Moreover, specimens from national and international herbarium were also analyzed. In total, 593 specimens were analyzed, confirming the occurrence of 24 phalloid species and 20 geastroid species and morphospecies. For the molecular studies, 271 new sequences were generated from four molecular makers (ITS, LSU, *rpb2* and *apt6*). The main results of this study are: the description of a new genus, *Sphaerophallus*, and a new species, *Phallus aureolatus*; the proposal of a new combination, *Lysurus arachnoideus*; reconsideration of seven species and the proposal of synonyms for four species. In addition, *Geastrum morganii* is reported for the first time from Brazil, while *Abrachium floriforme*, *Geastrum entomophilum* and *Staheliomyces cinctus* are new records from Southern Brazil. Concerning the contribution to *Phallales* phylogeny, a new circumscription of *Protubera* is proposed; *Gastrosporiaceae* is accepted as the sister group of *Phallaceae* and an emendation is formulated for the order. The phylogenetic studies with *Geastrum* allowed identifying the occurrence of cryptic taxa in the morphospecies: *G. saccatum*, *G. schweinitzii*, *G. trichiferum* e *G. triplex*. Keys to the identification of all species and morphospecies, as well as color photographs and illustrations for most of them, are presented.

Key words: *Basidiomycota* · *Gasteromycetes* · Neotropical mycota · systematics · taxonomy

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1. INTRODUÇÃO

A classificação dos organismos pertencentes ao Reino *Fungi* sofreu profundas modificações nas últimas décadas. Até então, os fungos vinham sendo classificados principalmente com base na morfologia das estruturas reprodutivas, aliada com características biológicas, bioquímicas e ecológicas destes organismos. Entretanto, os estudos filogenéticos baseados em dados moleculares começaram a ficar mais frequentes no início dos anos 90 (séc. XX) e revolucionaram os sistemas de classificação que conhecíamos até então.

A divisão *Basidiomycota* R.T. Moore – que compreende organismos que produzem esporos sexuais em hifas modificadas chamadas de basídios – era tradicionalmente dividida em classes baseadas em características morfológicas, e.g., formação ou não de basidioma, tipo de basidioma (angiocárpico × gimnocárpico), septação do basídio, forma de liberação dos esporos (ativa × passiva) e ultramorfologia do poro septal (Ainsworth et al. 1973, Kendrick 1992, Alexopoulos et al. 1996). Em algumas dessas classificações, era reconhecida a classe *Gasteromycetes* Fr., que agrupava homobasidiomicetos com características exclusivas, como o basidioma angiocárpio, a liberação dos esporos de forma passiva (estatimosporos) e o posicionamento dos esporos de forma simetricamente radial nos esterigmas (Dring 1973, Miller & Miller 1988, Reijnders 2000).

O hábito gasteroide foi durante muito tempo utilizado como principal característica para agrupar os fungos em uma única classe. Persoon (1801), em seu livro *Synopsis Methodica Fungorum*, agrupou estes organismos na classe Angiocarpi, sendo este trabalho considerado o ponto de partida para nomenclatura de fungos gasteroides (McNeill et al 2012). Já o termo '*Gasteromycetes*' foi cunhado por Fries (1821) em sua obra *Systema mycologicum*. Até aquele momento, estes dois autores tratavam a classe de forma bastante artificial, incluindo espécies de ascomicetos e mixomicetos. Berkeley (1860) promoveu profundas mudanças no conceito friesiano da classe, porém ainda manteve alguns mixomicetos. Foi somente a classificação apresentada por G. Winter, no final do século XIX, que apresentou *Gasteromycetes* (como ordem) contendo apenas espécies de basidiomicetos (Cunningham 1944), sendo este o conceito tradicional da classe adotado por muitos micólogos até recentemente (Lloyd 1902, Coker & Couch

1928, Cunningham 1944, Bottomley 1948, Demoulin 1969, Dring 1964, Liu 1984, Miller & Miller 1988, Calonge 1998, Sarasini 2005).

Entretanto, desde o início do século XX a naturalidade da classe *Gasteromycetes* já vinha sendo questionada devido à semelhança morfológica com outras espécies de himenomicetos (Thiers 1984, Reijnders 2000). O trabalho de Hibbett et al. (1997) foi um dos primeiros a demonstrar, com base em dados moleculares, que alguns fungos gasteroides estavam relacionados com outros grupos de fungos, deixando claro que o hábito gasteroide evoluiu diversas vezes em diferentes linhagens de himenomicetos.

Atualmente, a polifilia de *Gasteromycetes* é amplamente aceita e está embasada tanto em características morfológicas como moleculares. Segundo a classificação apresentada por Hibbett et al. (2007), as espécies de fungos gasteroides se encontram distribuídas em diferentes grupos taxonômicos, estando presentes em oito ordens de homobasidiomicetos: *Agaricales* Underw., *Boletales* E.-J. Gilbert, *Polyporales* Gäum., *Russulales* Kreisel ex P.M. Kirk et al., *Geastrales* K. Hosaka & Castellano, *Gomphales* Jülich, *Hysterangiales* K. Hosaka & Castellano e *Phallales* E. Fisch. (Fig. 1).

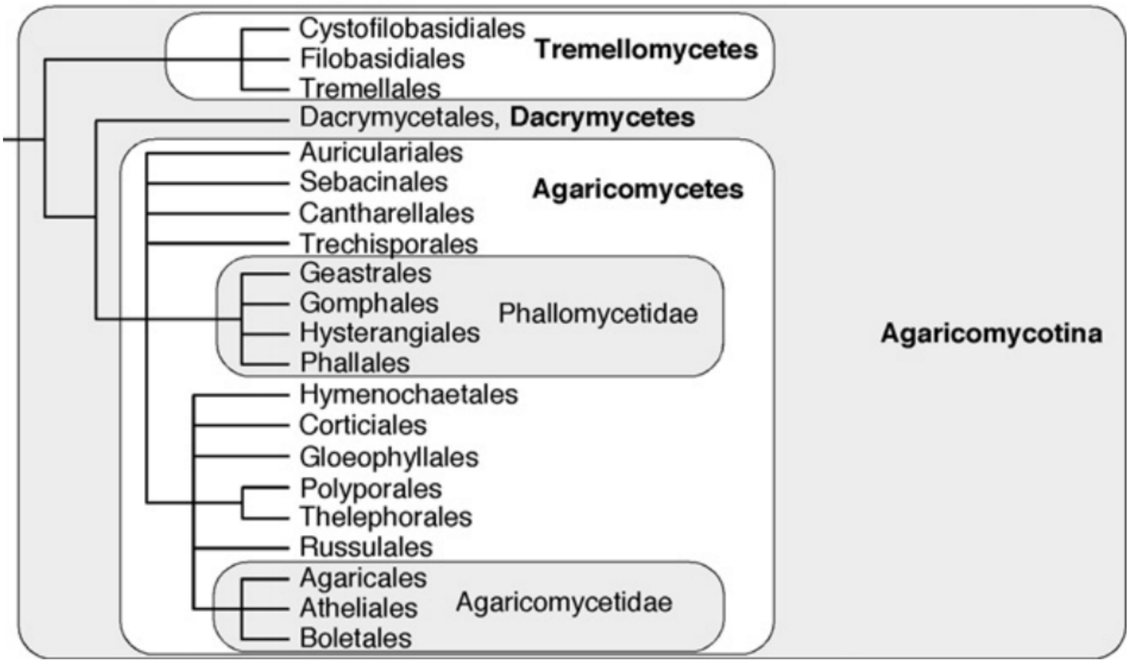

Fig. 1. Filogenia e classificação de *Agaricomycotina* (*Basidiomycota*, *Fungi*) sensu Hibbett et al. (2007).

1.1. A SUBLCASSE *PHALLOMYCETIDAE*

Hibbett et al. (1997) demonstraram como espécies com hábito gasteroide podem estar relacionadas com espécies de fungos que apresentam diferentes morfologias de basidioma. Segundo a análise filogenética apresentada por esses autores, três espécies gasteroides, viz. *Sphaerobolus stellatus* Tode, *Geastrum saccatum* Fr. e *Pseudocolus fusiformis* (E. Fisch.) Lloyd, foram recuperadas com suporte máximo relacionadas à *Gomphus floccosus* (Schwein.) Singer (gonfóide), *Ramaria stricta* (Pers.) Quél. (coralóide) e *Clavariadelphus pistillaris* (L.) Donk (clavarióide).

Posteriormente, os trabalhos de Bruns et al. (1998), Colgan et al. (1997), Pine et al. (1999) e Thorn et al. (2000) incluíram neste clado táxons adicionais pertencentes aos gêneros *Aseroë* Labill., *Chondrogaster* Maire, *Clathrus* P. Micheli ex L., *Gautieria* Vittad., *Gloeocantharellus* Singer, *Hysterangium* Vittad., *Kavinia* Pilát, *Kjeldsenia* W. Colgan et al., *Lentaria* Corner, *Lysurus* Fr., *Phallus* Junius ex L., *Protubera* Möller e *Trappea* Castellano. Este clado contendo táxons morfologicamente tão diversos foi mais tarde nomeado "gonfóide-faloide" por Hibbett & Thorn (2001).

Uma primeira tentativa de incorporar estes resultados em um esquema formal de classificação foi apresentada por Kirk et al. (2001), agrupando todos os membros do clado gonfóide-faloide em uma única ordem, *Phallales*. Posteriormente, Hosaka et al. (2006) apresentaram uma análise filogenética multigênica com uma amostragem muito ampla de táxons pertencentes ao clado gonfóide-faloide e demostraram que este poderia ser subdividido em quatro grandes clados com alto suporte. A ordem *Phallales* sensu Kirk foi então elevada ao status de subclasse e formalmente nomeada *Phallomycetidae* K. Hosaka et al. Os quatro clados dessa subclasse corresponderam a quatro ordens: *Geastrales*, *Gomphales*, *Hysterangiales* e *Phallales* (Fig. 2).

Os membros de *Phallomycetidae* podem ser sapróbios, i.e. decompositores de matéria orgânica, ou ectomicorrízicos, que estabelecem relações simbiônticas com plantas vasculares. Devido à grande diversidade de organismos incluídos nesta subclasse, é difícil apontar quais seriam as sinapomorfias deste grupo, porém Hosaka et al. (2006) apresentam como possíveis características sinapomórficas a morfologia dos rizomorfos (presença de hifas ampuliformes e acantohifas), conteúdo de pistilarina (metabólito produzido por alguns fungos) e a ultraestrutura do poro septal.

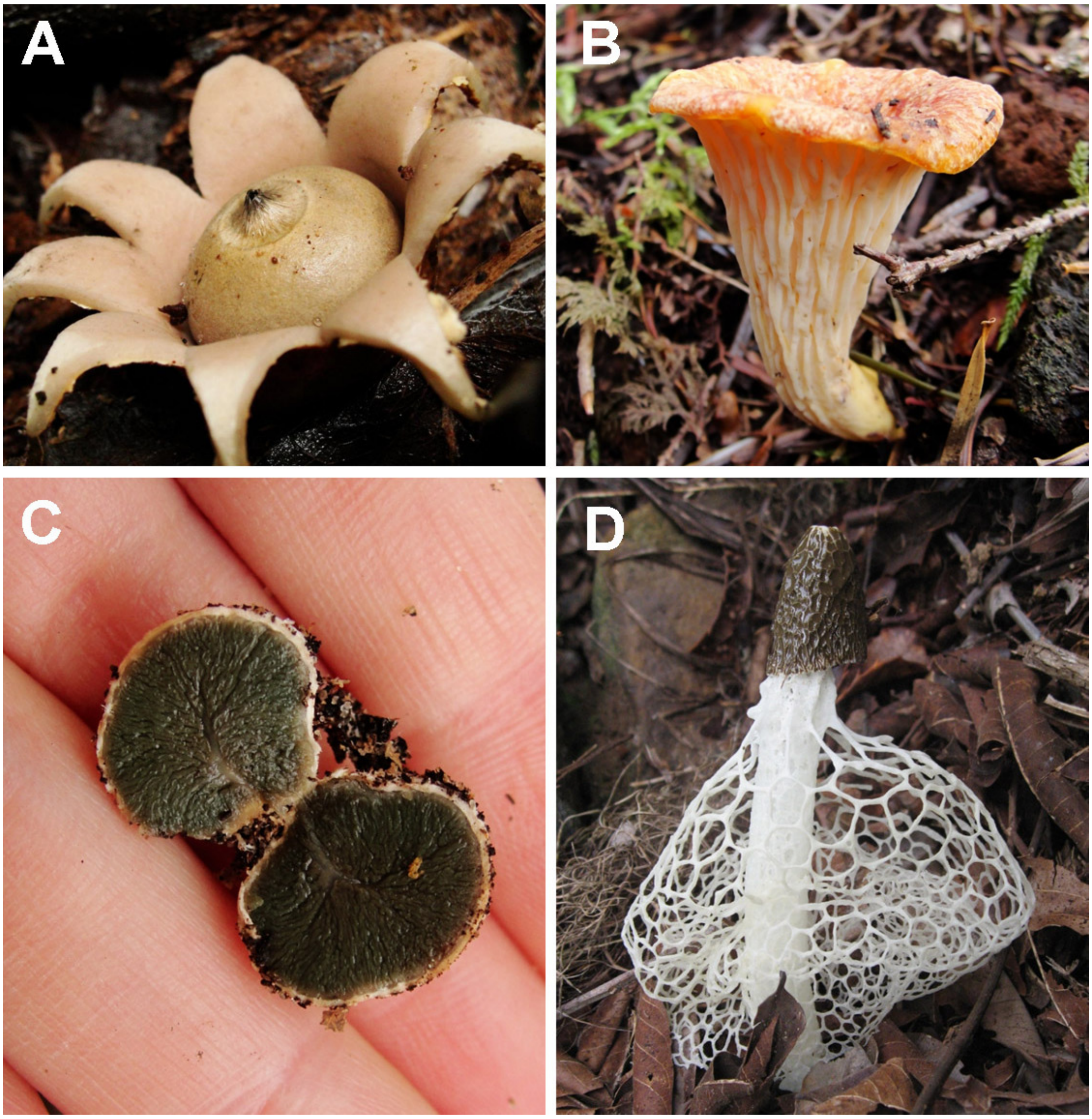

Fig. 2. Exemplos de membros da subclasse *Phallomycetidae*: **A**. *Geastrum saccatum* (*Geastrales*). **B**. *Turbinellus floccosus* (*Gomphales*). **C**. *Hysterangium inflatum* (*Hysterangiales*). **D**. *Phallus indusiatus* (*Phallales*). Fotografias: L. Trierveiler-Pereira.

1.2. A ORDEM *PHALLALES*

Phallales foi proposta como ordem para acomodar as famílias *Phallaceae* Corda e *Clathraceae* Chevall. (Fischer 1898-99). Este autor não reconheceu *Lysuraceae* Corda proposta por Corda (1842) como uma família independente, e ao invés, incluiu as espécies de *Lysurus* em *Clathraceae*. *Phallales* sensu Fischer incluía apenas espécies epígeas, com receptáculo expandido (e.g. *Aseroë*, *Clathrus*, *Phallus*).

Cunningham (1931), ao criar a família monogenérica *Claustulaceae* G. Cunn., incluiu esta em *Phalalles*. Este autor discute que apesar do receptáculo indeiscente, *Claustula* K.M. Curtis possuía muitas características em comum com outros faloides, como o perídio gelatinoso típico, receptáculo imaturo dividido em câmaras e esporos lisos e elípticos. Entretanto, muitos trabalhos posteriores seguiram adotando o conceito de *Phallales* sensu Fischer (e.g. Fischer 1933, Long & Stouffer 1948, Pilát 1958, Dennis 1970, Calonge 1998).

A família *Hysterangiaceae* E. Fisch. também foi considerada integrante de *Phallales* por alguns autores (e.g., Dring 1973, Miller & Miller 1988), apesar da maioria dos especialistas a aceitarem em uma ordem distinta, *Hysterangiales* (Fischer 1933, Zeller 1939, 1949, Pilát 1958, Jülich 1981). *Protophallaceae* Zeller, uma família criada para acomodar os gêneros *Calvarula* Zeller, *Protophallus* Murril e *Protubera* (Zeller 1939) vinha sendo tratada até então em *Hysterangiales*.

A organização das famílias de *Phallales* ficou mais clara com os resultados de estudos filogenéticos moleculares publicados recentemente. Hosaka et al. (2006) demonstraram que *Hysterangiales* e *Phallales* formam clados independentes na subclasse *Phallomycetidae*, suportando a hipótese de serem ordens distintas. Este estudo também sugeriu que *Phallales* pode ser subdivida em seis famílias: *Clathraceae*, *Phallaceae*, *Lysuraceae*, *Protophallaceae*, *Claustulaceae* e '*Trappeaceae*'; a última sendo um nome provisório na obra supracitada para um clado basal contendo *Trappea darkeri* (Zeller) Castellano e *Phallobata alba* G. Cunn. Posteriormente, Kirk et al. (2008) propuseram o nome formalmente: Trappeaceae P.M. Kirk.

O gênero *Gastrosporium* Mattir. apareceu relacionado a gêneros de faloides (*Anthurus* Kalchbr. & MacOwan e *Pseudocolus* Lloyd) em um estudo filogenético realizado por Hibbett & Binder (2002). É possível que esse gênero também pertença a alguma família de *Phallales*, mas ainda não foi incluído em nenhuma análise filogenética. Hosaka et al. (2006) mencionam que excluíram o gênero de seu *dataset* pela falta de sequência de algum gene codificador de proteína (e.g. *atp6*).

Segundo o conceito mais atual da ordem, os *Phallales* podem ser caracterizados pelo perídio com duas a três camadas gelatinosas, receptáculo expandido ou sequestrado, pseudoestipe quando presente pseudoparenquimatoso, gleba verde a olivácea e mucilaginosa quando madura, esporos pequenos (até aproximadamente 8 µm de comprimento), elipsóides a bacilarióides, lisos, hialinos a esverdeados (Fig. 3).

Os basidiomas sequestrados são na sua maioria globosos a cerebriformes. Já os basidiomas com receptáculo expandido podem exibir uma grande variedade de formas, incluindo morfologias bizarras. Os resultados de reconstrução de caractere ancestral demonstraram que a forma de basidioma expandida evoluiu das formas sequestradas, que ocupam um lugar basal na filogenia dos *Phallales* (Hosaka et al. 2006).

Os membros de *Phallales* são de ampla distribuição, porém são encontrados em maior diversidade e com maior frequência em regiões tropicais e subtropicais do planeta.

Fig. 3. Exemplos de diferentes formas de basidiomas exibidas por membros de *Phalalles*: **A**. *Pseudocolus fusiforme* (*Clathraceae*). **B**. *Laternea pusilla* (*Clathraceae*). **C**. *Phallus glutinolens* (*Phallaceae*). **D**. *Blumenavia angolensis* (*Clathraceae*). **E**. *Protubera maracuja* (*Protophallaceae*) Fotografias: L. Trierveiler-Pereira (A-C, E) e A.C. Magnago (D).

1.3. A ORDEM *GEASTRALES*

Para compreender a origem do nome *Geastrales*, é preciso primeiramente retroceder na história da micologia e clarificar a história do gênero-tipo da ordem, *Geastrum* Pers. : Pers. O gênero foi primeiramente descrito como *Geaster* P. Micheli (Micheli 1729), mas teve sua variante ortográfica *Geastrum* Pers. publicada por Persoon (1794), sendo esta ortografia posteriormente sancionada pelo mesmo autor (Persoon

1801). Entretanto, Fries (1829) reintroduziu o nome *Geaster* e considerou *Geastrum* um erro ortográfico. Entretanto, como a obra de Persoon (1801) é considerada o ponto de partida para nomenclatura dos gasteromicetos (McNeill et al. 2012), o nome *Geastrum* deve ser adotado com essa ortografia por ser um nome sancionado. Ainda, *Geastrum* foi sugerido por Kirk et al. (2013) para compor a lista de nomes genéricos protegidos.

Corda (1842) foi quem propôs a família *Geastrideae* Corda (variante ortográfica de *Geastraceae*) e além de *Geastrum*, incluiu também nessa família os gêneros *Plecostoma* Desv. e *Myriostoma* Desv. *Plecostoma* é atualmente aceito em *Geastrum* e *Myriostoma* é tratado como um gênero independente. Fischer (1933) reconheceu a família com a grafia *Geastraceae*.

A família *Geastraceae* foi então aceita por diversos autores (Fischer 1933, Zeller 1949, Pilát 1958, Miller & Miller 1988, Dring 1973, Sunhede 1989, Moyersoen & Demoulin 1996) desde o início do século XX e tradicionalmente era incluída em *Lycoperdales* Clem.. Alguns autores não aceitaram *Geastraceae* como uma família independente, e dessa maneira incluíam *Geastrum* e gêneros afins em *Lycoperdaceae* Chevall. (Coker & Couch 1928, Cunningham 1944, Wright 1956, Dring 1964).

O número de gêneros em *Geastraceae* varia segundo os autores, sendo que o maior número foi incluído por Sunhede (1989), em sua monografia da família para o norte da Europa: *Geasteropsis* Hollós, *Geastrum*, *Myriostoma*, *Phialastrum* Sunhede, *Pyrenogaster* Malençon & Riousset, *Radiigera* Zeller, *Terrostella* Long e *Trichaster* Czern.

Já na década de 90, os trabalhos com filogenia molecular levaram a uma forte mudança no conceito da classificação dos fungos geastroides. Hibbett et al. (1997) demonstraram que *Geastrum* estava filogeneticamente distante de gêneros licoperdóides (*Lycoperdon* P. Micheli, *Calvatia* Fr. e *Scleroderma* Pers.) e próximo do gênero faloide *Pseudocolus*. Ainda mais surpreendente, os resultados desses autores indicaram que *Geastrum* estaria próximo de *Sphaerobolus* Tode, um gênero que até o momento não havia sido relacionado à família *Geastraceae*. Na antiga classe *Gasteromycetes*, *Sphaerobolus* representava a família *Sphaerobolaceae* J. Schröt., sendo que esta foi classificada em diferentes ordens dependendo dos autores: *Sphaerobolales*, *Nidulariales* G. Cunn. ou *Sclerodermatales* G. Cunn. (Geml et al. 2005).

Kirk et al. (2001) consideraram *Geastraceae* (incluindo *Sphaerobolus*) na ordem *Phallales*. Estudos filogenéticos posteriores confirmaram a posição de *Geastrum* próxima aos faloides (Krüger et al. 2001, Binder & Bresinsky 2002).

Hosaka et al. (2006) consideram a família *Geastraceae* em uma ordem própria, *Geastrales*. O nome *Geastrales* já havia sido proposto anteriormente por Kreisel (1969), porém invalidamente (sem uma diagnose em latim). A ordem *Geastrales* sensu Hosaka et al. (2006) incluiu quatro famílias e os seguintes gêneros: *Geastrum*, *Radiigera*, *Myriostoma* (*Geastraceae*), *Pyrenogaster* (*Pyrenogastraceae* Jülich), *Sclerogaster* R. Hesse (‘*Sclerogastraceae*’) e *Sphaerobolus* (*Sphaerobolaceae*). *Sclerogastraceae* Locq. ex P.M. Kirk foi um nome provisório adotado por Hosaka et al. (2006), porém formalmente proposto por Kirk et al. (2008). Hosaka & Castellano (2008) corrigiram o nome da família *Pyrenogastraceae* para *Schenellaceae* Nann.-Bremek., já que *Schenella* Nann.-Bremek. é o nome correto para o gênero, devido à lei de prioridade (Estrada-Torres et al. 2005).

Segundo o conceito mais atual de *Geastrales*, os basidiomas podem apresentar diferentes morfologias, desde os basidiomas clássicos de forma estrelada (*Geastrum*, *Myriostoma*), até espécies com basidiomas hipógeos e sequestrados (*Radiigera*, *Sclerogaster*, *Schenella*).

A gleba (região fértil) pode ser pulverulenta (*Geastrum*, *Myriostoma, Radiigera*), compacta e com pequenos lóculos (*Sclerogaster*), com inúmeros peridíolos alongados que colapsam na maturidade (*Schenella*) ou com um único peridíolo arredondado (*Sphaerobolus*) (Fig. 4). A gleba geralmente é de coloração escura, amarronzada, mas em *Sclerogaster* esta pode ser amarelada ou esverdeada. Os esporos geralmente são ornamentados e pigmentados, com exceção de *Sphaerobolus*, cujos esporos são lisos e hialinos.

O basidioma sequestrado não aparece somente como caractere basal na ordem (Hosaka et al. 2006), e de acordo com Jeppson et al. (2013), as espécies de *Radiigera* correspondem à formas sequestradas de *Geastrum.*

Os membros de *Geastrales* são cosmopolitas, porém alguns gêneros são conhecidos somente para algumas regiões, e.g. *Geasteropsis*, *Phialastraum* (ambos da África) e *Terrostella* (U.S.A). A maioria das espécies geastroides são sapróbias (terrícolas ou lignícolas), e poucas estão associadas a ninhos de cupins (Sunhede 1989). Agerer & Beenken (1998) afirmam que *Geastrum fimbriatum* Fr. forma associações micorrízicas atípicas com *Fagus sylvatica* L., entretanto, ainda são necessários mais estudos para investigar quantas espécies do gênero estabelecem esse tipo de relação.

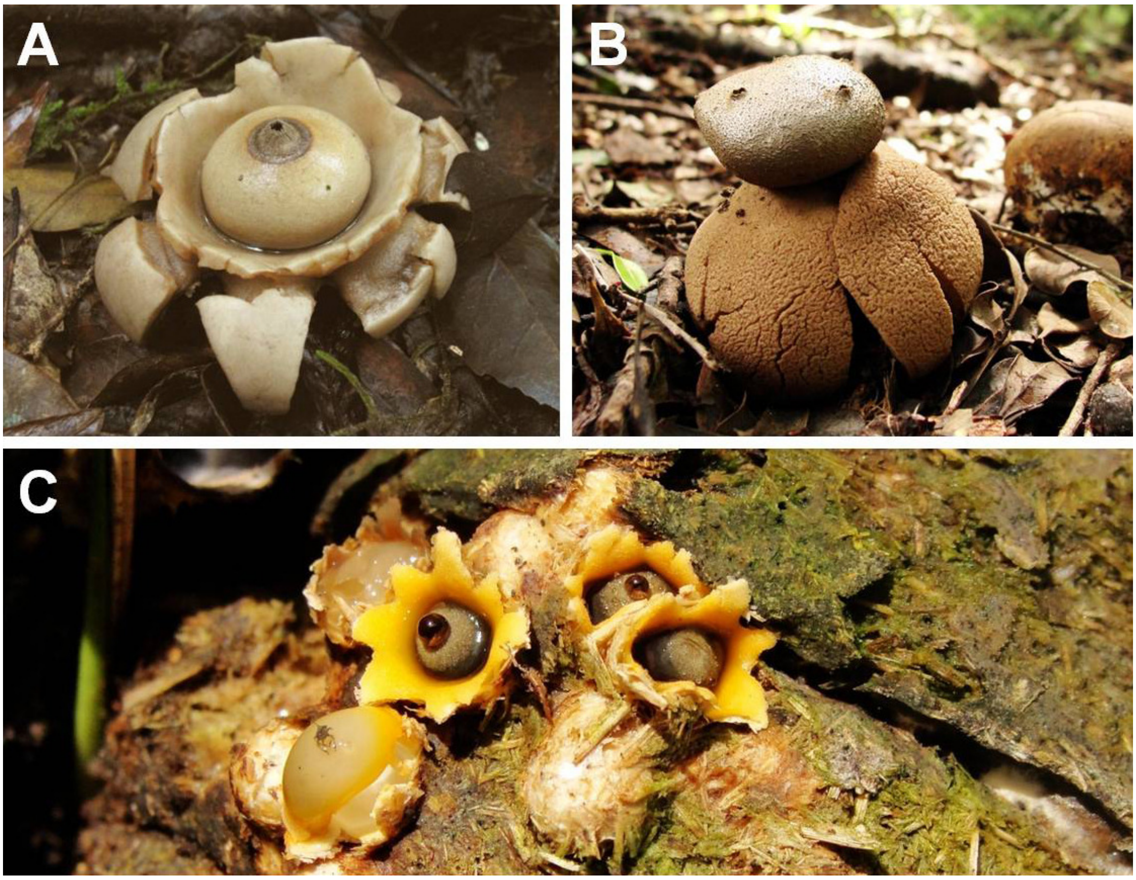

Fig. 4. Exemplos de membros da ordem *Geastales*: **A**. *Geastrum triplex* (*Geastraceae*). **B**. *Myriostoma coliforme* (*Geastraceae*). **C**. *Sphaerobolus stellatus* (*Sphaerobolaceae*). Fotografias: L. Trierveiler-Pereira.

1.4. *PHALLALES* E *GEASTRALES* NO SUL DO BRASIL

Os primeiros registros de *Phallales* na Região Sul do Brasil foram publicados por Alfred Möller, um naturalista alemão que viveu na cidade de Blumenau, Santa Catarina, entre 1890 e 1893 (Stafleu & Cowan 1983). Apesar de enviar alguns materiais ao exterior para serem identificados por outros micólogos (e.g. Bresadola 1896), Möller se dedicou à taxonomia de alguns grupos de fungos, como os faloides (Möller 1895) e os ficomicetos e ascomicetos (Möller 1901). O trabalho de Möller sobre os faloides foi intitulado de "Brasilische Pilzblumen" (do alemão: *Pilz* = fungo; *Blumen* = flores), porque segundo o autor, o título de "fungos-flores" chamaria a atenção para o valor estético dos faloides, devido às suas cores e formas atrativas, além do forte cheiro que atraía insetos.

Em seu trabalho, Möller (1895) descreve quatro novos gêneros para a ciência, *Aporophallus* Möller, *Blumenavia* Möller, *Itajahya* Möller e *Protubera* Möller, e oito novas espécies: *Aporophallus subtilis* Möller, *Blumenavia rhacodes* Möller, *Clathrus chrysomycelinus* Möller, *Colus garciae* Möller, *Dictyophora callichroa* Möller, *Itajahya galericulata* Möller, *Ithyphallus glutinolens* Möller e *Protubera maracuja* Möller. Ainda, descreve com detalhes *Laternea columnata* Nees, *Mutinus bambusinus* (Zoll.) E. Fisch. e *Dictyophora phalloidea* Lév.

A obra de Möller é considerada um marco no conhecimento dos faloides sul-americanos. Algumas espécies foram descritas e registradas para a Argentina por Spegazzini (1887) na mesma época, mas Lloyd (1907b, d) comenta que os registros de Spegazzini eram acompanhados de descrições vagas e ilustrações, quando presentes, pobres.

Em seus trabalhos, o micólogo americano Curtis Gates Lloyd cita muitas espécies de faloides ocorrentes no sul do Brasil (Lloyd 1906, 1907c, 1908, 1909). Além de o autor citar repetidas vezes as espécies bem descritas por Möller, ele também faz relatos de espécimes, cartas e fotografias enviadas a ele por Pe. Rick (que naquele momento residia em Salvador do Sul, RS) e Pe. Schupp (Pelotas, RS). Rick era um correspondente assíduo – enviava uma grande quantidade de espécimes para Lloyd e escrevia cartas extensas, em muitas delas relatando detalhadamente características dos espécimes fúngicos quando frescos e notas sobre a abundância das espécies.

Johannes Rick, um jesuíta austríaco que se estabeleceu no Rio Grande do Sul em 1903 e aqui residiu até sua morte em 1946, era um micólogo amador apaixonado e um coletor entusiasta. Publicou muitos trabalhos no início do século XX e em alguns destes relata a ocorrência de faloides: *Clathrus columnatus* Bosc, *B. rhacodes*, *Simblum sphaerocephalum* Schltdl., *I. galericulata*, *D. phalloidea* e *P. maracuja* (Rick 1905, 1906a, b, 1911). Rick (1924) cita a ocorrência de onze espécies de faloides no seu trabalho sobre fungos gelatinosos e cartilaginosos do Rio Grande do Sul, e posteriormente dedica um trabalho exclusivamente a este grupo de fungos (Rick 1929), onde descreve uma nova espécie, *Mutinus rugulosus* Rick.

Bertoldo Braun, também um jesuíta e aprendiz de Rick, publicou um trabalho extenso a respeito das espécies de faloides do Rio Grande do Sul e comparou-as com outras espécies já conhecidas (Braun 1932). Nessa obra, uma nova espécie é descrita para a ciência: *Phallus granulosodenticulatus* B. Braun.

Finalmente, o trabalho póstumo de Rick (1961), compilado por Pe. Balduíno Rambo, traz uma listagem completa de todos os faloides identificados por Rick e colaboradores durante seu trabalho micológico no sul do Brasil.

Zeller & Dodge (1929) descreveram para o Rio Grande do Sul *Gelopellis thaxteri* (Zeller & C.W. Dodge) Zeller, uma espécie sequestrada e hipógea. Posteriormente, essa espécie também foi tratada em detalhes por Homrich (1969). Em um livro didático publicado por Guerrero & Homrich (1999) algumas espécies de faloides comuns no estado do Rio Grande do Sul foram ilustradas.

No Estado do Paraná, muitas espécies foram citadas no levantamento micológico realizado pelo micólogo amador holandês André de Meijer, ao longo de 30 anos de investigação: *Aseroë rubra* La Bill., *Blumenavia angolensis* (Welw. & Curr.) Dring, *C. chrysomycelinus*, *Clathrus crispus* Turpin, *Laternea pusilla* Berk. & M.A.Curtis, *Mutinus argentinus* Speg., *Phallus callichrous* (Möller) Lloyd, *Phallus indusiatus* Vent.: Pers., *Phallus* cf. *merulinus* (Berk.) Lloyd e *P. maracuja* (Meijer 2006).

No Estado de Santa Catarina, Trierveiler-Pereira et al. (2009) redescobriram *Phallus glutinolens* e, baseando-se em novas evidências morfológicas observadas em materiais frescos, publicaram uma emenda para a espécie.

Mais recentemente, Cortez e colaboradores citaram algumas espécies para o Rio Grande do Sul: *Mutinus elegans* (Mont.) E. Fisch., *Lysurus cruciatus* (Lepr. & Mont.) Henn., *L. periphragmoides* (Klotzsch) Dring, *Phallus duplicatus* Bosc e *P. granulosodenticulatus* (Cortez et al. 2008a, 2011a, b). A contribuição mais recente para o conhecimento do grupo foi a de Sulzbacher et al. (2013), onde apresentam uma descrição moderna e fotos coloridas de *Pseudocolus garciae*, espécie citada poucas vezes na literatura micológica.

Os primeiros registros de *Geastrales* na Região Sul do Brasil datam do final do séc. XIX. Esses registros foram feitos por naturalistas europeus que recebiam espécimes de coletores viajantes que passavam pela América do Sul. Bresadola (1896) registrou para Santa Catarina a ocorrência de *Geastrum mirabile* Mont. Hennings (1897), entre outras espécies de gasteromicetos, também citou para o mesmo Estado *G. mirabile* e *G.* cf. *fimbriatum*.

Rick relatou em seus trabalhos a ocorrência de muitas espécies de *Geastrum*: *G. mirabile*, *G. triplex* Jungh., *G. asper* Lloyd, *G. pectinatum* Pers., *G. saccatum*, *G. lageniforme* Vittad., *G. radicans* Berk. & M.A. Curtis, *G. lignicola* Berk., *G. englerianum* Henn., *G. velutinum* Morgan (Rick 1904, 1906a, 1911), além da descrição de duas novas espécies para a ciência: *G. violaceum* Rick e *G. lloydianum* Rick (Rick 1906a). Uma nova espécie de *Geastrum* enviada por Rick para Lloyd foi descrita por este como *G. trichiferum* Rick, in Lloyd (Lloyd 1907a, Rick 1928).

Rick publicou um manuscrito onde discute as espécies de *Geastrum* e suas possíveis relações naturais de acordo com características morfológicas (Rick 1910). Um compilado das espécies de *Geastrales* (30 no total, incluindo os gêneros *Geastrum*, *Myriostoma* e *Sphaerobolus*) identificadas em seus trabalhos (incluindo Rick 1930) é apresentado em Rick (1961).

Após a contribuição de Rick, poucos estudos focaram na taxonomia de fungos geastroides no sul do Brasil. Homrich (1973) publicou um trabalho com dados adicionais sobre a morfologia de *Myriostoma coliforme* e Cortez et al. (2008b) registraram a ocorrência de *Geastrum ovalisporum* Calonge & Mor.-Arr. pela primeira vez no sul do Brasil.

Assim como ocorreu com os *Phallales*, algumas espécies de *Geastrum* foram citadas em livros didáticos ou levantamentos micológicos, a saber: Guerrero & Homrich (1999), Sobestiansky (2005), Meijer (2006, 2009, 2010) e Cortez et al. (2008a).

2. OBJETIVOS

Este trabalho tem como objetivo geral ampliar o conhecimento taxonômico e contribuir para a filogenia dos fungos pertencentes às ordens *Phallales* e *Geastrales* (subclasse *Phallomycetidae*), fornecendo dados morfológicos e moleculares especialmente de espécies coletadas na Região Sul do Brasil, porém inclui também dados de táxons encontrados em outras regiões.

Ainda, são apresentados os seguintes objetivos específicos:

* fornecer meios para a identificação das espécies encontradas, através de chaves de identificação, descrições e ilustrações;

* atualizar os registros dos herbários consultados e ampliar o acervo dos herbários ICN e FLOR com o depósito dos materiais coletados e identificados;

* contribuir para a filogenia e sistemática dos fungos, adicionando as sequências obtidas em bancos de dados públicos, como o GenBank.

3. MATERIAIS E MÉTODOS

3.1. ÁREAS DE COLETA

As coletas em campo foram realizadas nos três estados da Região Sul do Brasil: Rio Grande do Sul, Santa Catarina e Paraná. Estes três estados abrangem uma área de 576.409,6 km^2 e contam com diferentes formações vegetais em seus territórios, a saber: Floresta Ombrófila Densa, Floresta Ombrófila Mista, Floresta Estacional Decidual, Floresta Estacional Semidecidual, Estepe, Savana, Savana Estépica e áreas de formações pioneiras (IBGE 2014).

As excursões de coleta foram realizadas entre março de 2010 e abril de 2012, durante todas as estações do ano, em diferentes formações florestais que podem ser encontradas na região, percorrendo preferencialmente áreas bem conservadas ou Unidades de Conservação (Tabela 1). As licenças de coletas para as áreas, quando exigidas, foram obtidas junto aos órgãos competentes.

Tabela 1: Unidades de Conservação ou regiões representativas das áreas visitadas e suas formações vegetais. FOD: Foresta Ombrófila Densa; FOM: Floresta Ombrófila Mista; FES: Floresta Estacional Semidecidual; FED: Floresta Estacional Decidual; S: Savana; E: Estepe; MR: Mata de Restinga (IBGE 2014).

Área de coleta	**Estado**	**Formação vegetal**
Floresta Nacional de São Francisco de Paula	RS	FOM
CPCN PRÓ-MATA, São Francisco de Paula	RS	FOM
Veraneio Hampel, São Francisco de Paula	RS	FOM
Região do município de Riozinho	RS	FOD
Serra do Sudeste	RS	E
Parque Municipal da Pedra do Segredo	RS	E
Região do município de Santa Maria	RS	E
RPPN do Prof. Luís Baptista, Dom Pedro de Alcântara	RS	FOD
Morro Santana, Porto Alegre	RS	FES
Parque Municipal de Saint'Hillaire, Viamão	RS	FES
Fazenda São Maximiano, Guaíba	RS	FES
Parque Estadual de Itapuã	RS	FES e MR
Parque Estadual do Turvo	RS	FED
Parque Nacional da Serra Geral	RS	FOD e FOM
Parque Nacional de Aparados da Serra	RS	FOD e FOM

Tabela 1: continuação.

Área de coleta	**Estado**	**Formação vegetal**
Parque Estadual Serra do Tabuleiro	SC	FOD e FOM
Unidade de Conservação Ambiental Desterro	SC	FOD
Ilha de Santa Catarina	SC	FOD e MR
Região do Município de Joinville	SC	FOD
Região do Município de Mondaí	SC	FED
Parque Estadual do Cerrado	PR	S
APA de Guaratuba	PR	FOD
RPPN de Salto Morato, Guaraqueçaba	PR	FOD
Estrada da Graciosa, Morretes	PR	FOD
Parque Nacional do Iguaçu	PR	FES

3.2. COLETA E CONSERVAÇÃO DE ESPÉCIMES

Foram investigados os substratos propícios ao surgimento de basidiomas de fungos gasteroides, como por exemplo: solo, serrapilheira, madeira em decomposição e esterco. Os espécimes encontrados foram cuidadosamente coletados manualmente ou com auxílio de um canivete e logo após acondicionados em caixas plásticas com compartimentos individualizados, conforme metodologia adotada por Lodge et al. (2004). No caso de alguns basidiomas mais frágeis, como ocorre com algumas espécies faloides, os basidiomas foram envoltos em papel alumínio antes de serem guardados.

Para cada espécime coletado, foi anotado em um caderno de campo o local e a data de coleta, substrato e hábito. Sempre que possível, os espécimes coletados foram fotografadas *in situ* com câmera digital Sony Cyber-shot DSC-H20.

Após coleta, os basidiomas foram transportados o mais rápido possível para o laboratório ou alojamento, onde foram lentamente desidratados com o auxílio de um desidratador elétrico (Fun Kitchen, Brasil), durante 12 a 24 horas, em baixa temperatura (± 40ºC). Após o processo de desidratação, os espécimes foram acondicionados em pequenas bolsas plásticas com fecho do tipo Ziplock®, identificados com seu número de coleta e armazenados em congelador (aproximadamente a –6ºC) por pelo menos sete dias antes de serem depositados nos herbários.

Os materiais coletados foram incorporados ao Herbário ICN do Instituto de Biociências da Universidade Federal do Rio Grande do Sul, com a exceção de espécimes coletados por outros pesquisadores, que foram depositados no Herbário FLOR do Departamento de Botânica da Universidade Federal de Santa Catarina.

3.3. REVISÃO DE MATERIAIS DE HERBÁRIO

Além dos materiais coletados, foi também consultado o acervo dos principais herbários da Região Sul com a finalidade de aumentar o conhecimento acerca da ocorrência e distribuição das espécies, além da atualização das identificações dos materiais preservados. Os herbários visitados foram os seguintes:

* Departamento de Botânica, Universidade Federal do Rio Grande do Sul (ICN);
* Instituto Anchietano de Pesquisas/UNISINOS (PACA);
* Universidade de Caxias do Sul/UCS (HUCS);
* Departamento de Botânica, Universidade Federal de Santa Catarina (FLOR);
* Museu Botânico Municipal, Curitiba (MBM).

Ainda, coleções provenientes da Região Sul e outras regiões do Brasil depositadas em herbários nacionais ou internacionais também foram analisadas, assim como materiais adicionais para confirmação e comparação das espécies. Espécimes dos seguintes herbários foram consultados:

* Instituto de Botânica, São Paulo (SP);
* Departamento de Micologia, Universidade Federal de Pernambuco (URM);
* Departamento de Ciencias Biológicas, Universidad de Buenos Aires (BAFC);
* Instituto de Botánica Carlos Spegazzini, La Plata (LPS);
* Museo Botánico, Universidad Nacional de Córdoba (CORD);
* Botany Department, Field Museum of Natural History (F);
* U.S. National Fungus Collections, Beltsville (BPI);
* Royal Botanic Gardens, Kew (K);
* Département Systématique et Evolution, Muséum National d'Histoire Naturelle (P);
* Swedish Museum of Natural History (S);
* University of Gothenburg (GB);
* Real Jardín Botánico de Madrid (MA-Fungi);
* Department of Botany, National Museum of Nature and Science, Tsukuba (TNS).

Os acrônimos dos herbários estão de acordo com o Index Herbariorum (http://sweetgum.nybg.org/ih/).

3.4. CARACTERIZAÇÃO MORFOLÓGICA

A caracterização morfológica dos espécimes foi iniciada em campo, quando as principais características dos materiais foram anotadas em um caderno de campo. Posteriormente, fotos digitais dos espécimes também serviram como ferramenta de análise, contribuindo para a descrição de características e cores dos materiais quando frescos. No caso dos fungos faloides, o registro fotográfico dos espécimes frescos é praticamente indispensável para uma posterior descrição completa dos materiais.

Em laboratório, os fungos foram examinados sob microscópio estereoscópico para obtenção dos principais dados macromorfológicos dos espécimes, de acordo com metodologia adotada para o grupo (Miller & Miller 1988, Sunhede1989). Para a determinação de coloração de diferentes estruturas dos basidiomas, foi utilizada a carta de cores de Kornerup & Wanscher (1978). As principais alterações quanto às características macroscópicas após a desidratação do material também foram anotadas.

Para a análise microscópica do material, pequenas porções da gleba ou do perídio foram retiradas dos basidiomas e montadas entre lâmina e lamínula com KOH 5% ou corante floxina 1% e KOH 5%. A partir destas preparações, e com o auxílio de microscópio óptico, diversas microestruturas (hifas do perídio, hifas do capilício, basídios, basidiosporos, etc.) foram analisadas quanto à forma, dimensões, tipo de ornamentação e coloração. As medidas foram efetuadas, sempre que possível, a partir de 20 unidades de cada microestrutura, através de uma ocular com régua micrometrada e objetivas com aumento de 40X e 100X, esta última com óleo de imersão. Ilustrações das microestruturas foram feitas com o auxílio de uma câmera clara acoplada ao microscópio.

As análises de microestruturas feitas com microscópio eletrônico de varredura (JEOL-6060, JEOL-5800) foram realizadas no Centro de Microscopia Eletrônica da Universidade Federal do Rio Grande do Sul. Para a realização das mesmas, pedaços de filme fotográfico preto e branco (aproximadamente 0,7 × 0,7 cm) foram colados sobre pequenos cilindros de alumínio (*stubs*) próprios para o uso nestes microscópios, seguindo a metodologia modificada de Claugher (1986). Na superfície deste filme fotográfico (previamente exposto à luz e revelado), sobre o lado da emulsão, foi depositada uma pequena amostra fúngica (porção da gleba, capilício, esporos ou porções do perídio) imersa em uma gota de água destilada. Anteriormente às análises microscópicas, os *stubs* passaram por um processo de metalização onde uma fina camada (de 10 a 20 nm) de ouro ou platina foi depositada sobre a amostra com o auxílio do aparelho metalizador Bal-Tec Sputter Coater SCD050.

A partir de análises macro e microscópicas, os espécimes examinados foram identificados, quando possível, ao nível específico por comparação com materiais de herbário e com a utilização de trabalhos com chaves dicotômicas e descrições detalhadas, tais como: Möller (1895), Ponce de Leon (1968), Dring (1980), Miller & Miller (1988), Sunhede (1989), Calonge (1998, 2005), Soto & Wright (2000), Kreisel (1996), Baseia et al. (2006), Cheype (2010) e Jeppson et al. (2013).

3.5. METODOLOGIA PARA AS ANÁLISES FILOGENÉTICAS

Os procedimentos de extração, amplificação e sequenciamento de DNA foram realizados no Laboratório de Biologia Molecular do Departamento de Botânica, Museu Nacional da Natureza e Ciência (TNS), Tsukuba, Japão. Para a extração do material genético dos espécimes, duas distintas metodologias foram adotadas de acordo com a natureza da gleba: compacta (*Phallales* e basidiomas jovens de *Geastrales*) ou pulverulenta (basidiomas maduros de *Geastrales*).

No primeiro caso, pequenos fragmentos (5 a 8 cubos de aproximadamente 8 mm^3) foram retirados da gleba desidratada e transferidos para tubos de 2mL contendo tampão DMSO (Seutin et al. 1991) com adição de Tris-HCl 1M (pH 8.0) e sulfito de sódio 0.1M (NA_2SO_3). Estas porções de gleba permaneceram imersas na solução tampão por pelo menos 12 horas. Posteriormente, dois ou três destes cubos que estavam imersos no DMSO foram macerados utilizando-se almofariz, pistilo e nitrogênio liquido. Após maceração, o material pulverizado foi imediatamente transferido para tubos do tipo Eppendorf® (2mL) contendo 1mL de tampão 2X CTAB (Doyle & Doyle 1987) com adição de NA_2SO_3 0.1M (Hosaka & Castellano 2008).

No caso das amostras pulverulentas, pequenas porções da gleba foram retiradas dos basidiomas com auxílio de pinça e/ou agulha esterilizadas e colocadas em um tubo do tipo Eppendorf® (2mL) contendo 1,5g de pequenas esferas de vidro (1mm diam., 800mg, BioSpec). Estas amostras foram submetidas a uma vigorosa agitação mecânica utilizando um equipamento especifico para lise de tecidos (TissueLyser QIAGEN) durante 60 segundos e frequência de 27 Hertz. Após esse procedimento, completou-se o conteúdo do tubo até aproximadamente 2mL com tampão 2X CTAB.

Todas as amostras, independente de qual processo inicial de extração foram submetidas, foram mantidas em freezer -20ºC até a próxima etapa. A purificação do DNA foi realizada de acordo com o protocolo de Doyle & Doyle (1987), com algumas modificações (Hosaka 2009, Hosaka & Castellano 2008).

A amplificação do DNA foi realizada por meio da reação em cadeia da polimerase (PCR) utilizando um termociclador (Applied Byosystems GeneAmp* PCR System 9700). Para isso, 1 µL da solução contendo DNA purificado foi colocado em um microtubo contendo 9 µL de pré-mix (3,9 µL de TDW; 1µL de GoldBuffer; 1µL de BSA; 1 µL de 25mM MgCl^2; 1 µL de 4nM dNTP; 0,1 µL de Taq polimerase e 0,5 µL de cada primer 10µM).

Os primers utilizados nesse estudo tinham como finalidade amplificar os seguintes marcadores nucleares: espaçador transcrito interno do DNA nuclear ribossomal (ITS1 parcial, 5.8S completa e ITS2 parcial), região que codifica a maior subunidade do DNA nuclear ribossomal (LSU), gene do DNA nuclear codificante da segunda maior subunidade da RNA polimerase (*rpb2*) e o gene do DNA mitocondrial codificante da proteína *atp6*. Os primers utilizados estão listados na Tabela 2.

Tabela 2: Dados sobre os primers utilizados.

Nome do primer	sentido	sequência de nucleotideos (5'–3')	Referência
ITS1	*forward*	TCCGTAGGTGAACCTGCGG	White et al. (1990)
ITS4	*reverse*	TCCTCCGCTTATTGATATGC	White et al. (1990)
ITS8-F	*forward*	AGTCGTAACAAGGTTTCCGTAGGTG	Dentinger et al. (2010)
ITS6-R	*reverse*	TTCCCGCTTCACTCGCAGT	Dentinger et al. (2010)
LR0R	*forward*	ACCCGCTGAACTTAAGC	Vilgalys & Hester (1990)
LR5	*reverse*	TCCTGAGGGAAACTTCG	Vilgalys & Hester (1990)
LR3	*reverse*	CCGTGTTTCAAGACGGG	Vilgalys & Hester (1990)
atp6-3	*forward*	TCTCCTTTAGAACAATTTGA	Kretzer & Bruns (1999)
atp6-2	*reverse*	TAATTCTANWGCATCTTTAATRTA	Kretzer & Bruns (1999)
b*RPB2*–6F	*forward*	TGGGGYATGGTNTGYCCYGC	Matheny (2005)
b*RPB2*-7R	*reverse*	GAYTGRTTRTGRTCRGGGAAVGG	Matheny (2005)

As amplificações foram realizadas para um volume final de 10 µL com diferentes programas dependendo da região alvo a ser amplificada:

* ITS e LSU: ciclo inicial de desnaturação a 94ºC por 3 min; 35 ciclos de 94ºC por 35s, 51ºC por 30s e 72ºC por 1 min; extensão final a 72ºC por 10 min.

* *atp6*: ciclo inicial de desnaturação a 94ºC por 3 min; 4 ciclos de 94 ºC por 35s, 37°C por 55s e 72ºC por 1 min, seguido por 30 ciclos de 94 ºC por 35s, 45°C por 55s e 72ºC por 1 min; extensão final a 72ºC por 10 min.

* *rpb2*: ciclo inicial de desnaturação a 94º por 2 min; 34 ciclos de 93ºC por 1min, 50ºC por 1min e 72ºC por 1 min; extensão final a 72ºC por 10 min.

Para verificar o sucesso da reação de amplificação das amostras de DNA, alíquotas de 2 µL de cada amostra foram misturadas a 1 µL de corante e depositadas em poços de um gel de agarose 1,5%. Foi realizada a eletroforese desse gel a 110V por 15 minutos. Posteriormente, este foi corado em solução de brometo de etídio e visualizado em transiluminador sob luz ultravioleta (Fig. 5).

Fig. 5. Fotoregistro de um gel de agarose 1,5 % contendo amostras de DNA amplificado (1–14). (-): controle negativo; (+): controle positivo. Fotografia: L. Trierveiler-Pereira.

Quando a amplificação das bandas foi confirmada, os produtos de PCR foram enzimaticamente purificados utilizando o kit ExoSap-IT (Millipore, Molsheim, França) seguindo as especificações do fabricante. O sequenciamento nucleotídico foi realizado utilizando o kit BigDye Terminator Cycle Sequencing v. 3.1 (Applied Biosystems, Norwalk, USA) e sequenciador automático (AB3130xl Genetic Analyzer, Applied Biosystems) seguindo as especificações dos fabricantes.

Após esse processo, as sequências *forward* e *reverse* de cada amostra foram combinadas (*assembly*) para gerar uma única sequência consenso (*contig*), utilizando-se o programa ATGC v.6 (Genetyx, Japão).

Para verificar se as sequências obtidas correspondiam às regiões desejadas, estas foram primeiramente submetidas a buscas online BLAST (Basic Local Alignment Search Tool) através do National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/blast/). As sequências duvidosas foram eliminadas da análise. Sequências de interesse, depositadas do banco de dados do GenBank (http://www.ncbi.nlm.nih.gov/genbank/), foram baixadas para também serem utilizadas nas análises filogenéticas.

As sequências que compunham o *dataset* desejado foram alinhadas utilizando o programa Muscle v.3.6 (Edgar 2004) e manualmente editadas no BioEdit v.7.0.1 (Hall 1999). As análises filogenéticas foram feitas utilizando-se critérios de máxima parcimônia, inferência bayesiana e/ou máxima verossimilhança, utilizando os seguintes programas, respectivamente: PAUP* 4.0b10 (Swofford 2002), MrBayes v.3.2.1 (Huelsenbeck & Ronquist 2001) e PhyML 3.0 online web server (Guindon et al. 2010).

O programa ModelTest (Posada & Crandall 1998) foi utilizado para determinar qual modelo evolutivo melhor se ajustava aos dados obtidos. As regiões de DNA foram primeiramente analisadas separadamente, e quando não foram observados grandes conflitos entre as topologias obtidas, os dados foram combinados em uma única matriz para as análises subsequentes. A visualização e edição das árvores geradas foram feitas nos programas TreeView v.1.6.6 (Page 2001) e TreeGraph 2 (Stöver & Müller 2010).

As sequências que foram obtidas durante este estudo foram ou ainda serão enviadas ao GenBank e as árvores geradas serão depositados no banco de dados do TreeBASE (http://www.treebase.org).

4. RESULTADOS E DISCUSSÃO

4.1. ESPÉCIMES COLETADOS E REVISÕES DE HERBÁRIOS

Durante as expedições realizadas para este estudo, 311 espécimes de fungos gasteroides foram coletados, sendo 17 pertencentes à ordem *Phallales* e 113 à ordem *Geastrales*. Os demais espécimes coletados correspondem às ordens *Agaricales*, *Boletales* e *Hysterangiales*.

Foi constatado que a observação de basidiomas de fungos faloides é mais rara, podendo esse fato ser atribuído à efemeridade e fragilidade dos basidiomas (Dring 1980). Espécimes do gênero *Geastrum* foram os mais abundantes entre os fungos coletados, seguido de espécies licoperdóides (*Morganella* Zeller, *Lycoperdon* e *Calvatia*). Resultados similares foram encontrados por Cortez (2009) durante seu estudo de fungos gasteroides no Rio Grande do Sul e Trierveiler-Pereira et al. (2013) durante um ano de inventário em florestas de Mata Atlântica de Pernambuco.

Quanto à representatividade das ordens incluídas neste trabalho, *Phallales* está representada pelos gêneros *Clathrus* (1 sp.), *Blumenavia* (1 sp.), *Mutinus* Fr. (1 sp.), *Phallus* (3 spp.) e *Protubera* (1 sp.). Os *Geastrales* estão representados pelos gêneros *Geastrum* (26 táxons), *Myriostoma* (1 sp.) e *Sphaerobolus* (1 sp.).

Quanto aos materiais de herbários, 463 espécimes de espécies faloides e geastroides foram examinados, sendo que o maior número de materiais revisados pertence aos acervos dos herbários PACA (153 exsicatas) e BPI (134 exsicatas).

Analisando conjuntamente os dados resultantes das análises de materiais coletados e as exsicatas de herbário, foi possível constatar que 24 espécies de fungos faloides (representando 15 gêneros), 16 espécies e quatro morfoespécies de fungos geastroides (representando três gêneros) ocorrem na Região Sul do Brasil.

4.2. ÉPOCA DE COLETA E VEGETAÇÃO

A relação entre a abundância de espécimes coletados e a época do ano foi fortemente influenciada pelo período de chuvas nas áreas de coleta, sendo que na região litoral e central do Rio Grande do Sul este período corresponde ao outono e inverno. Já no noroeste do Estado, assim como também no oeste e litoral dos estados do Paraná e de

Santa Catarina, a melhor época de coleta foi entre os meses de novembro e março (primavera e verão), sendo o período de dezembro a fevereiro o mais propício para a coleta de fungos gasteroides.

Entretanto, é importante também mencionar que algumas espécies podem ser encontradas durante todas as estações (explorando diferentes regiões e formações vegetais), como registrado por Meijer (2006) ao longo de 30 anos de inventário com fungos macroscópicos.

Os *Geastrales* foram coletados na maioria das vezes quando o período de coleta era propício, mesmo que em algumas expedições apenas poucos espécimes foram encontrados. Já os espécimes de *Phallales* foram raramente encontrados, mesmo quando em época propícia para a coleta. Ainda, alguns basidiomas faloides foram encontrados já em processo avançado de deterioração.

Os espécimes de *Geastrum* foram sempre abundantes em florestas, como nas Florestas Ombrófilas Densa e Mista, Estacionais Decidual e Semidecidual. Nas regiões de Pampa, espécimes de *Geastrum* e *Myriostoma* foram encontrados em ambiente úmidos, onde havia agrupamentos de árvores (pequenos bosques) e nunca em campo aberto. Espécimes de *Sphaerobolus* foram coletados em campo aberto sobre esterco.

Espécimes de *Phallus* foram encontrados em áreas de Floresta Ombrófila Densa, Floresta Estacional Semidecidual e em Mata de Restinga. Também em Mata de Restinga, foram coletados espécimes de *Clathrus*. Em Floresta Estacional Semidecidual foram coletados *Blumenavia*, *Mutinus* e *Protubera*, porém espécimes de *Protubera* também foram encontrados em Floresta Ombrófila Densa. Na região do Pampa, em área de pastagens com grama, foi coletado um espécime de *Clathrus columnatus*. Apesar das poucas coletas de faloides em Floresta Ombrófila Densa durante este estudo, os registros de literatura, herbário e coletas de colaborados mostram que várias espécies ocorrem nesse tipo de formação vegetal. Möller (1895), durante os três anos que inventariou neste tipo de vegetação, encontrou 13 espécies de faloides.

Não houve nenhuma coleta de *Phallales* ou *Geastrales* no Parque do Cerrado (estado do Paraná), apesar da região ter sido explorada durante quatro dias no período de maior precipitação anual (mês de dezembro).

4.3. RESULTADOS DE SEQUENCIAMENTO

O sucesso dos processos de extração, amplificação e sequenciamento de DNA foi determinante para os trabalhos que compõe essa tese e envolvem análises filogenéticas com dados moleculares.

Foram realizadas tentativas de sequenciamento de quatro marcadores moleculares para 16 espécimes de *Phallales*, sendo destes a maioria coletada durante a execução deste trabalho, além de três espécimes enviados por um colaborador, um material de acervo do herbário MBM e um do TNS. O sucesso de obtenção de sequências para estes materiais foi de 81,25% para ITS (13/16) e 93,75% para LSU, *rpb2* e *atp6* (15/16). Outros dados adicionais utilizados nos estudos moleculares foram fornecidos pelo coorientador deste trabalho.

No caso dos *Geastrales*, foram realizadas tentativas de sequenciamento de duas regiões nucleares, ITS e LSU, para 130 espécimes. A maioria destes materiais foi coletada durante a execução deste trabalho, mas também foram incluídos 11 espécimes enviados por colaboradores e cinco materiais de acervo dos herbários MBM (2) e S (3). O sucesso de obtenção de sequências para estes materiais foi de 72,31% para ITS (94/130) e 91,54% para LSU (119/130).

Ao todo, 271 novas sequências de quatro marcadores moleculares (ITS, LSU, *rpb2* e *apt6*) foram geradas.

4.4. CONTRIBUIÇÕES RELEVANTES

Os resultados mais relevantes deste trabalho estão apresentados a seguir com itens da seção “Resultados e Discussão” (Manuscritos I–X). Os manuscritos foram redigidos em inglês seguindo as normas dos periódicos. Quatro manuscritos (I, III, VI, VII) já foram publicados antes da redação desta tese, e pelo fato das editoras deterem os direitos autorais, estes não serão disponibilizados integralmente nesta tese.

As principais contribuições para o estudo dos *Phallales* são: a proposição de um novo gênero, *Sphaerophallus*, para acomodar *P. glutinolens* (Manuscrito V); a descrição de uma nova espécie para a ciência, *P. aureolatus* (Manuscrito II); a nova combinação de *Lysurus arachnoideus* (Manuscrito IV); as sinonímias de *P. pygmaeus* Baseia com *Xylophallus xylogenus* (Mont.) E. Fisch. (Manuscrito I) e *P. granulosodenticulatus* com *P. campanulatus* Berk. (Manuscrito V); dados adicionais sobre a morfologia e ecologia de *Protubera maracuja*, assim como uma nova circunscrição para o gênero (Manuscrito III); os primeiros registros de *Abrachium floriforme* (Baseia & Calonge) Baseia & T.S. Cabral e *Staheliomyces cinctus* E. Fisch. para a região Sul do Brasil e o primeiro registro de *Laternea pusilla* Berk. & M.A. Curtis para o Estado de Santa Catarina (Manuscrito V).

Ainda, são apresentadas contribuições à filogenia dos *Phallales*, com a adição de uma nova família, *Gastrosporiaceae* Pilát, e uma emenda à ordem (Manuscrito IV). Finalmente, também são apresentados dados que contribuem para a ampliação da distribuição de fungos faloides (Manuscrito VI).

Os principais resultados sobre os fungos da ordem *Geastrales* são: a reconsideração das espécies *G. trichiferum*, *G. violaceum*, *G. lloydianum*, *G. hariotii* Lloyd e *G. reinkingii* Lloyd (Manuscrito VII–IX); a proposição das sinonímias de *G. episcopale* Kuhar & Papin. com *G. violaceum* e *G. hirsutum* Baseia & Calonge com *G. trichiferum* (Manuscrito VII); o primeiro registro de *G. morganii* Lloyd para o Brasil e o primeiro registro de *G. entomophilum* Fazolino et al. para a Região Sul (Manuscrito IX). O estudo filogenético dos espécimes de *Geastrum* permitiu identificar a ocorrência de táxons crípticos nas morfoespécies: *G. schweinitzii* (Berk. & M.A. Curtis) Zeller, *G. trichiferum*, *G. saccatum* e *G. triplex* (Manuscrito IX).

Finalmente, espécies de *Geastrales* e *Phallales* coletados na região do Pampa estão listadas, juntamente com outras espécies de fungos gasteroides, no Manuscrito X. Neste manuscrito, é também apresentada a descrição de duas novas espécies licoperdóides: *Arachnion setosum* e *Bovista kreiselii*.

4.5. MANUSCRITO I

Trierveiler-Pereira L, Silveira RMB. 2012. Notes on *Xylophallus xylogenus* (*Phallaceae*, *Agaricomycetes*) based on Brazilian specimens. **Mycotaxon** 120: 309–316.

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Notes on *Xylophallus xylogenus* (*Phallaceae, Agaricomycetes*) based on Brazilian specimens

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Abstract — From morphological analysis of *Phallus pygmaeus* specimens collected in Brazil and reexamination of the holotype, we conclude that this species is a synonym of the previously described *Xylophallus xylogenus* and agree with the recently proposed synonymy. We describe new specimens of *X. xylogenus* from Northeastern Brazil and provide color photos and a key for the species of the small xylophilous members of *Phallus,* including *Xylophallus.*

Key words — gasteromycetes, herbarium revision, *Mutinus*, stinkhorns, tropical fungi

Introduction

Phallus pygmaeus was described from the tropical rainforest in Northeastern Brazil, growing on decaying wood (Baseia et al. 2003). The species is characterized by small basidiomata (≤ 15 mm high), a smooth receptacle with minute perforate apex, and lignicolous habitat. The remarkably small size of this phalloid is not exclusive to *P. pygmaeus*, as other small species are known from the tropics, e.g., *Phallus tenuis* (E. Fisch.) Kuntze, *P. minusculus* Kreisel & Calonge, and *P. drewesii* Desjardin & B.A. Perry (Calonge & Kreisel 2002, Calonge 2005, Desjardin & Perry 2009).

Xylophallus xylogenus is a taxon with a problematic generic position. Originally described as *Phallus xylogenus* from Cayenne (French Guiana), its author (Montagne 1855) placed the species in sect. *Mutinus,* while some years later Schechtendal (1861) classified it in *Phallus* sect. *Xylophallus* Schltdl. Fischer (1898-99), who first proposed the combination *Mutinus xylogenus,* later erected the genus *Xylophallus* (Schltdl.) E. Fisch. for the species (Fischer 1933a). Sáenz et al. (1972) also contributed to the morphology and ontogeny of the species based on collections from Costa Rica. Since its original description, *X. xylogenus* has been ambiguously illustrated by different authors (Fig. 1).

4.6. MANUSCRITO II

Trierveiler-Pereira L, Meijer AAR, Hosaka K, Silveira RMB. 2014. *Phallus aureolatus* (*Phallaceae*, *Agaricomycetes*), a new species from the Brazilian Atlantic Forest. **Mycological Progress**: a ser submetido.

Phallus aureolatus (Phallaceae, Agaricomycetes)*,* a new species from the Brazilian Atlantic Forest

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Abstract *Phallus aureolatus,* a new gasteroid species found in the Atlantic Rainforest in Southern Brazil, is herein described, illustrated and its phylogenetic affinities are discussed. This new species is characterized by a pinkish volva, rhizomorphs with purplish pigments, a long white indusium, and a merulioid receptacle with a prominent pseudoparenchimatous cap on the top. A comparison with the morphologically similar taxa is presented. A key to identify species of *Phallus s.l.* known to occur in Brazil is also provided.

Keywords Basidiomycota; Gasteromycetes; Neotropics; stinkhorns

Introduction

Phallus Junius ex L. is one of the most easily recognizable genera of gasteromycetes in the field. Many species have been described in the genus (about 170 according to the Index Fungorum database, http://www.indexfungorum.org/) but currently only circa 35 are considered good species. Other taxa accepted in the genus by different authors (Montagne 1855; Kreisel 1996; Calonge 2005), e.g *Itajahya galericulata* Möller and *Xylophallus xylogenus* (Mont.) E. Fisch., have recently been segregated from *Phallus* based on morphological and molecular data (Cabral et al. 2012; Trierveiler-Pereira et al. 2014).

During the last decade several new species have been added to the genus, most of them from tropical and subtropical regions of the Paleotropics: *P. anamudii* C. Mohanan, *P. calongei* G. Moreno & Khalid, *P. drewesii* Desjardin & B.A. Perry, *P. flavidus* Kreisel & Hauskn., *P. maderensis* Calonge, *P. minusculus* Kreisel & Calonge, *P. tenuissimus* T.H. Li, W.Q. Deng & B. Liu (Calonge and Kreisel 2002; Li et al. 2005; Calonge et al. 2008; Desjardin and Perry 2009; Kreisel and Hausknecht 2009; Moreno et al. 2009; Mohanan 2011); and *P. atrovolvatus* Kreisel & Calonge from the Neotropics (Calonge et al. 2005). *Phallus pygmaeus* Baseia, described from Brazil (Baseia et al. 2003), was reduced to a synonym of *X. xylogenus* (Cheype 2010).

Although some *Phallus* species occur in temperate regions, most members of the genus are found in tropical and subtropical areas, South East Asia apparently being the centre of its diversity, with numerous endemic taxa (Kreisel 1996). While some morphospecies have a pantropical distribution, e.g. *P. indusiatus* Schltdl., *P. rubicundus* (Bosc) Fr. and *P. callichrous* (Möller) Lloyd, others seem to be strongly endemic. In the Neotropics *Phallus* basidiomata are frequently encountered during field excursions, and a few species seem to be endemic, viz. *P. campanulatus* Berk., *P. glutinolens* (Möller) Kuntze, and *P. subtilis* (Möller) Lloyd.

During our studies on the diversity of phalloid species in Brazil, the taxonomic status of three recent *Phallus* collections from the Atlantic Rainforest in the State of Paraná was brought into question. These specimens were morphologically similar to *P. merulinus* and *P. atrovolvatus*, and were tentatively identified as *P.* aff. *merulinus* by Meijer (2006). Additional morphological studies combined with phylogenetic analyses based on nuclear ribosomal internal transcribed spacer (ITS) and nuclear ribosomal large subunit (LSU) sequences data, do support the hypothesis that they belong to an undescribed taxon, here proposed as *Phallus aureolatus*. A key to identify species of *Phallus s.l.* (also including the genera *Aporophallus*, *Itajahya* and *Xylophallus*) reported from Brazil is presented.

Materials and methods

Collection localities and specimens

Brazilian specimens were collected in two municipalities located in the State of Paraná: Antonina (25°25′44″S 48°42′43″W) and Matinhos (25°49′04″S 48°32′34″W), near the Atlantic coast. This area is covered with Atlantic Rainforest, ranging from lowland to upper-montane forests, and the climate is humid subtropical (Cfa in the Köppen classification).

Additional collections used to generate original sequences for the phylogenetic analyses proceed from French Guiana, Taiwan and Thailand. Vouchers are preserved at ICN, MBM and TNS (herbarium acronyms are according to Thiers 1997).

Morphology and anatomy

Collections of the new species were examined macro and microscopically following traditional techniques used in the taxonomy of gasteromycetes (Miller & Miller 1988). For microscopical analyses, sections from basidiomata were mounted in 3% KOH with 1% aqueous phloxine solution to observe and measure the structures. Colors are coded according to Kornerup & Wanscher (1978) and refer to the fresh materials.

Sequencing

Before the DNA extraction, sections of the basidiomata (from the receptacle, when possible) were soaked overnight in DMSO buffer (Seutin et al. 1991) with an addition of 100mM Tris-HCl (pH 8.0) and 0.1M sodium sulphite (Na_2SO_3). DNA extraction followed the protocol of Hosaka and Castellano (2008). PCR and sequencing protocols followed Kasuya et al. (2012). Raw forward and reverse sequences for each sample were assembled into contigs and were manually edited using the program ATGC v.6 (Genetyx). After assembling, the sequences were used to query GenBank using a BLAST search to verify their identity.

Sequence data were obtained from two independent loci: nuclear ribosomal internal transcribed spacer (ITS1 partial, 5.8S complete, ITS2 partial) and nuclear ribosomal large subunit (LSU). Primers used to amplify each DNA region and for sequencing were: ITS1, ITS4, ITS5, LR0R, LR3 and LR5 (http://biology.duke.edu/fungi/mycolab/).

Phylogenetic analysis

The ingroup is represented by eighteen taxa of Phallaceae, and outgroups were selected from the genera *Gautieria* Vittad. and *Ramaria* Fr. ex Bonord. (Gomphales), which are closely related to *Phallales* (Hosaka et al. 2006). Taxa sampled, voucher information and GenBank (www.ncbi.nlm.nih.gov/genbank/) accession numbers are listed in Table 1.

Molecular datasets consisting of original and GenBank sequences were automatically aligned on MUSCLE v.3.6 (Edgar 2004), and manually edited in BioEdit v.7.0.1 (Hall 1999). Final alignments are available from TreeBase as a NEXUS file.

Phylogenetic analyses were conducted separately for ITS and LSU datasets, but also for ITS combined with LSU sequences, under Maximum Parsimony (MP) and Bayesian inference (BI).

MP analyses was conducted under the equally weighted parsimony criterion using *PAUP v.4.0b10 (Swofford 2002). Gaps were treated as missing data. All the analyses were performed under the heuristic search option (TBR and Multrees options on) and 1,000 replicates of random addition sequence were conducted. Nodal supports were tested by bootstrapping (BS) of 1,000 replicates using the heuristic search option (TBR and Multrees options on) with 10 random addition sequences.

Models of evolution for Bayesian inference were estimated with the Akaike information criterion (AIC) as implemented in jModelTest v2.1.2 (Darriba et al. 2012), resulting in the model TPM3uf+I+G for ITS and TrN+I+G for LSU.

BI was conducted with MrBayes v.3.2.1 (Ronquist et al. 2012), with 1.5×10^6 generations of MCMC by sampling every 100th tree. All trees sampled after convergence (av. standard deviation of split frequencies < 0.01 and confirmed with Tracer v1.5, http://tree.bio.ed.ac.uk/software/tracer/) were used to reconstruct a 50% majority rule consensus tree (BC) and to calculate Bayesian posterior probabilities (PP). The determined burn in was 15% trees of each run. PP of each node was estimated based on the frequency at which the node was resolved among the sampled trees with the consensus option of 50% majority rule (Simmons et al. 2004). A probability of 0.95 was considered significant.

Results and Discussion

For the ITS individual dataset, the final DNA sequence alignments resulted in 654 characters, including gaps (115 variable parsimony uninformative and 256 parsimony informative positions). Heuristic search under maximum parsimony criterion yielded only two equally parsimonious trees (966 steps long; CI = 0.6480, RI = 0.5755, RC = 0.3730), which were very similar to BC tree (Fig. 1A).

For the combined analyses (ITS dataset + LSU gene), the final DNA sequence alignments resulted in 679 characters, including gaps, for the ITS dataset, and 922 for the LSU gene. By comparing MP bootstrap tree topologies obtained for the individual datasets, no conflict involving significantly supported nodes was found; the datasets therefore were combined into a single matrix. A total of 1601 characters were included in the analysis of which 171 were variable but parsimony uninformative and 406 parsimony informative. The heuristic search produced eight equally most parsimonious trees (1306 steps long; CI = 0.6884, RI = 0.6642, RC = 0.4572), representing one main topology. The strict consensus of the six equally most parsimonious trees was mostly identical to the BC tree (Fig 1B).

The results show that in both analyses, sequences of *P. aureolatus* did not cluster with other sequences and its branch collapsed in the combined analyses or had low support in the ITS analysis. The only exception was for the BI analysis in the ITS individual dataset, where *P. aureolatus* had a significant support (PP 96) to group with *P. indusiatus*, *P. echinovolvatus* and *P.* cf. *multicolor*. Other taxa which had their branches collapsed were *P. merulinus*, *P. impudicus*, and *P. hadriani*. To better understand the phylogenetic relationships of *P. aureolatus*, more ITS sequences of *Phallus* species must be added in future analysis.

According to the infrageneric classification proposed by Kreisel (1996), *P. aureolatus* belongs to subgenus *Phallus*, section *Clautriavia*, due to its rugulose, merulioid surface and whitish pseudostipe, together with *P. merulinus, P. echinovolvatus* (M. Zang, D.R. Zheng & Z.X. Hu) Kreisel and *P. lauterbachii* (Henn.) Kreisel. In our opinion, these last two, which are paleotropical species with an echinate volva (synonymous taxa?), should not have been included in this section, since they have a reticulate receptacle (Hennings 1898; Zang et al. 1988).

In the present study, we have included sequences of four taxa with rugulose/merulioid receptacle: *P. aureolatus* sp. nov., *P. campanulatus*, *P. rugulosus*, and *P. merulinus*. These four do not appear clustered, which indicates that receptacle surface morphology is not a decisive character to define groups within the Phallaceae phylogeny. It is also noteworthy that *P. rugulosus*, a species with reddish basidiomata, highly supported clustered with *P. campanulatus*, a species with whitish receptacle. This result indicates that the presence of pigments in the receptacle or pseudostipe may not be important to define infrageneric categories in natural groups within *Phallus*. *Phallus* cf. *multicolor* (CJL 120318-01) had been tentatively identified with this name due to the presence of orange pigments in the pseudostipe, veil and receptacle (Cheype 2010), but our molecular results indicate that it should be considered a colored form of *P. indusiatus*.

Phallus indusiatus, which has been reported from several regions of the Paleotropics, appears in different clades in our analysis, indicating that this morphological species probably corresponds to a complex of phylogenetic taxa.

Taxonomy

Phallus aureolatus Trierv.-Per. & de Meijer, sp. nov. (Figs. 2–4)

Mycobank MB xxxxxx

Holotypus. BRAZIL. State of Paraná, Matinhos, 13.XI.2010, leg. J.M. Baltazar, LTP109, in herbario ICN conservatur.

Etymology. Named for its white cap on the top of the receptacle.

Immature basidiomata not observed. *Mature basidiomata* usually solitary, up to 12 cm high; *volva* globose to subglobose, 2.0–2.5 cm high × 2.5–3.0 cm diam., externally smooth, reddish grey (7B2) to brownish orange (6C3, 7C3), context < 0.7 cm thick, light orange (6A4) to greyish orange (5B4), gelatinized;

rhizomorphic strands few to numerous, single or branched, purplish pink (14A3) to reddish brown (8E4); *pseudostipe* cylindrical, spongy, hollow, cylindrical or slightly attenuated towards apex, 8.0–10 cm high × 0.8–1.8 cm diam., white (1A1) when fresh; *indusium* pendant, rigid, inserted at the apical collar, not touching the receptacle margins, up to 7.0 cm long, white (1A1), lattices rather regular, 0.3–1.7 cm in diam.; *receptacle* cylindrical, campanulate to truncately conical, 1.5–2.0 cm high × 2.0–2.5 cm diam., surface rugulose to merulioid, whitish under the gleba, with a white tissue expansion (‘cap’) on the top when recently exposed, up to 1.0 cm high × 1.6 cm diam., later with a large apical pore; *gleba* mucilaginous, olive brown (4F4, 4F5), yellowish brown (5E5) to dark brown (6F5); *odour* strong, fetid.

Basidiospores cylindrical, usually attenuated to base, 3.0–4.1 × 1.5–2.0 µm, slightly thick-walled, smooth, pale green, in mass olive brown. *Pseudostipe*, *receptacle* and *indusium* pseudoparenchymatous, consisting of globose, subglobose to irregularly shaped cells, up to 60 µm in diam., and with up to 1.0 µm thick wall. *External hyphae of volva* filamentous, interwoven, hyaline, clamped, up to 9.0 µm wide. *Rhizomorphs hyphae* hyaline, clamped, some inflated near the septa, 2.0–8.0 µm wide, encrusted or not; large crystals forming rosette patterns are abundant, 18–40 µm wide.

Substrate. Forest soil.

Known distribution. Brazilian Atlantic rainforest (dense ombrophilous forest) in the States of Santa Catarina, Paraná and São Paulo.

Examined specimens. BRAZIL. State of Paraná, Antonina, Reserva Natural Rio Cachoeira, 08.II.2005, A.A.R. de Meijer 4332 (MBM); ibid., 05.XI.2007, A.A.R. de Meijer 4376 (MBM); ibid., Matinhos, 13.XI.2010, leg. J.M. Baltazar, LTP109 (ICN, holotype).

Additional specimen examined. *Phallus merulinus*. FRENCH GUIANA. Tonnégrande-Montsinery, Pont des Cascades, 14.II.2012, in garden, J.L. Cheype 120214-03 (ICN).

Remarks. Main features that characterize *P. aureolatus* are the whitish pseudostipe, receptacle and indusium; pinkish volva, rhizomorphs with purplish pigments, merulioid to rugulose receptacle and the long indusium. The white pseudoparenchymatous cap on the top of the receptacle (Fig. 2) also seems to be a constant feature from the recently exposed mature basidiome. This cap later disappears to reveal the receptacle's large apical pore.

The species has so far been only observed in areas of dense ombrophilous lowland forest near the coast. In the State of Santa Catarina it was photographed in 2007 in Florianópolis, Island of Santa Catarina (Fig. 4). We also came across a published photograph of two specimens from Juréia, at the coast of São Paulo State (Colombini & Miranda 2007, p. 125, named as '*Phallus indusiata*').

Phallus aureolatus usually has a solitary habit, being the group of two photographed in São Paulo the only known exception.

Species sharing with *P. aureolatus* the merulioid surface of the receptacle are *P. merulinus* and *P. atrovolvatus*. *Phallus merulinus*, best known from the Paleotropics, has also been reported from the Neotropics (Reid 1977; Cheype 2010), where it may have been introduced (Kreisel 1996). It can be distinguished from *P. aureolatus* by its shorter indusium, descending half the length of the pseudostipe at most, but mostly much shorter (Boedijn 1932); and the whitish volva and rhizomorphs (Calonge 2005; Sridhar & Karun 2013). *Phallus atrovolvatus* is known from Central and South America and it can be separated from *P. merulinus* by the blackish volva (Calonge et al. 2005; Cheype 2010). Specimens of *P. atrovolvatus* were found on wood chips and plant debris and the colour of the rhizomorphs was not described. An important detail is that both P. *merulinus* and *P. atrovolvatus* have been described as lacking a strong, repellent odour (Boedijn 1931; Calonge et al. 2005; Sridhar & Karun 2013), while the odour of *P. aureolatus* is clearly fetid.

In Brazil, two species with rugulose to granulose receptacle have been previously reported from Brazil, namely *P. campanulatus* (Rick 1961) and *P. rubicundus* (Bononi et al. 1984), but both of them lack an indusium and *P. rubicundus* has a reddish to orange pseudostipe and receptacle.

Key to identify species of *Phallus s.l.* known to occur in Brazil

1. Basidiomata minute, up to 1.5 cm high, gregarious on dead wood; receptacle surface smooth *Xylophallus xylogenus*

1*. Basidiomata higher than 1.5 cm, growing on wood debris or soil, receptacle surface lamellate, merulioid to reticulate 2

2. Receptacle surface lamellate, with a white calyptra on the top 3

2*. Receptacle surface merulioid to reticulate, calyptra absent 4

3. Pseudostipe white *Itajahya galericulata*

3*. Pseudostipe pinkish *I. rosea*

4. Basidiomata small, < 5 cm high, receptacle slenderly campanulate, not perforate *Aporophallus subtilis*

4*. Basidiomata larger, receptacle with a visible pore at apex when mature 5

5. Receptacle spherical at first, then campanulate; outer volva light brown, tuberculate; inner volva yolk-yellow *Phallus glutinolens*

5*. Receptacle campanulate, outer volva smooth, inner volva whitish or pale 6

6. Indusium present 7

6*. Indusium absent 10

7. Indusium short, not covering the pseudostipe entirely *P. duplicatus*

7*. Indusium long, covering the pseudostipe entirely 8

8. Receptacle surface rugulose to merulioid *P. aureolatus*

8*. Receptacle surface honeycomb-like reticulate 9

9. Receptacle white *P. indusiatus*

9*. Receptacle orange to pink *P. callichrous*

10. Pseudostipe and receptacle reddish to orange *P. rubicundus*

10*. Pseudostipe white *P. campanulatus*

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Table 1. GenBank accession numbers of DNA sequences used in the phylogenetic analyses. Sequences generated for this study are indicated with an asterisk (*).

Taxon	Voucher	ITS	LSU
Outgroup			
Gautieria monticola	ORHF20	AF377094	- - -
Gautieria monticola	OSC65121	- - -	AY574651
Ramaria conjunctipes	OSC110613	KC346861	KC345748
Ingroup			
Dictyophora phalloidea	ASI32012	AF324162	- - -
Dictyophora echinovolvata	ASI32002	AF324164	- - -
Dictyophora echinovolvata	ASI32007	AF324165	- - -
Phallus aureolatus	ICN-LTP109	new*	new*
Phallus echinovolvatus	KH-TH09-070	new*	new*
Phallus campanulatus	ICN-LTP255	new*	new*
Phallus campanulatus	ICN-LTP256	new*	new*
Phallus hadriani	AFTOL-ID683	DQ404385	AY885165
Phallus impudicus	ASI25008	AF324171	- - -
Phallus indusiatus	- - -	JN182874	- - -
Phallus indusiatus	ICN-LTP81	new*	new*
Phallus indusiatus	ASI32001	AF324172	- - -
Phallus indusiatus	ASI32013	AF324161	- - -
Phallus merulinus	CJL120214-03	new*	new*
Phallus cf. *multicolor*	CJL120318-01	new*	new*
Phallus rugulosus	KH-TW11-061	new*	new*
Phallus rugulosus	ASI32004	AF324169	- - -
Phallus rugulosus	ASI25007	AF324170	- - -

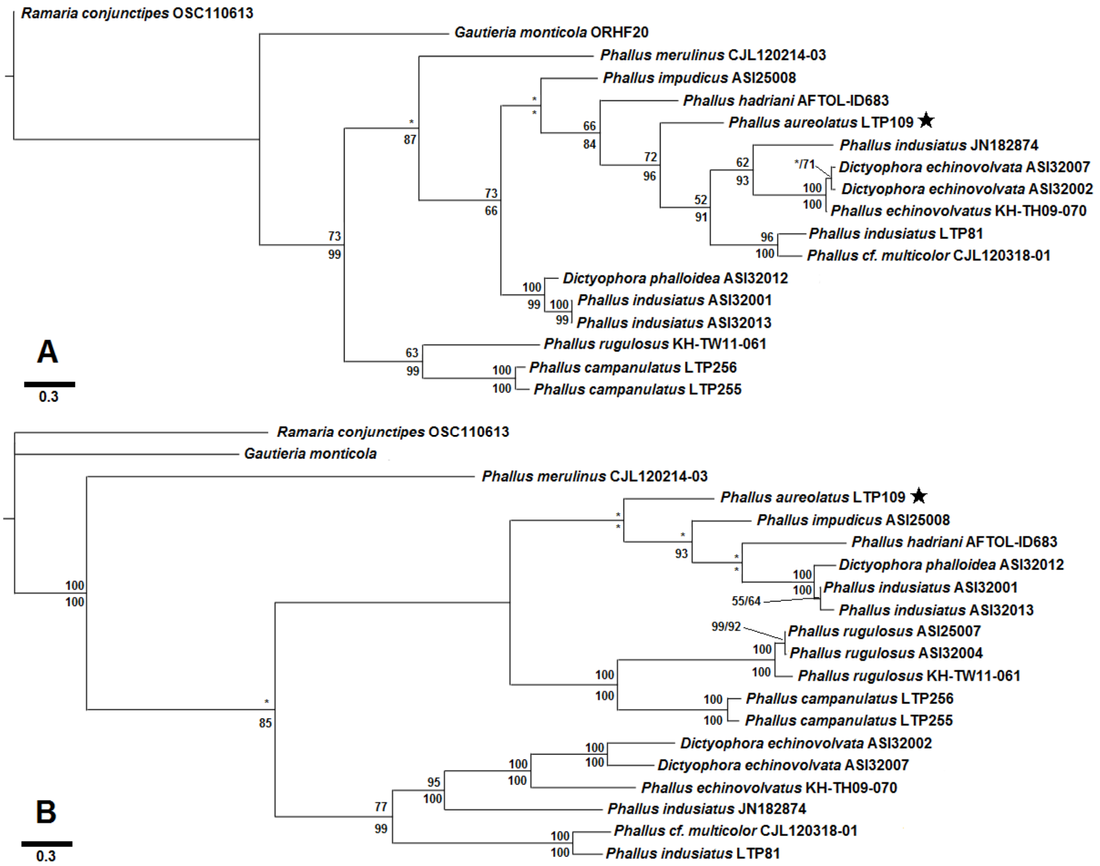

Fig. 1 Phylogenetic relationships among the Phallaceae. **A**. Single gene analyses using ITS. **B**. Combined analyses using ITS and LSU. Represented trees are one of the most parsimonious trees retrieved from the analysis. Support values for internal nodes are given as bootstrap values (above branches) and posterior probability (below branches). The new *Phallus* species is indicated with a star.

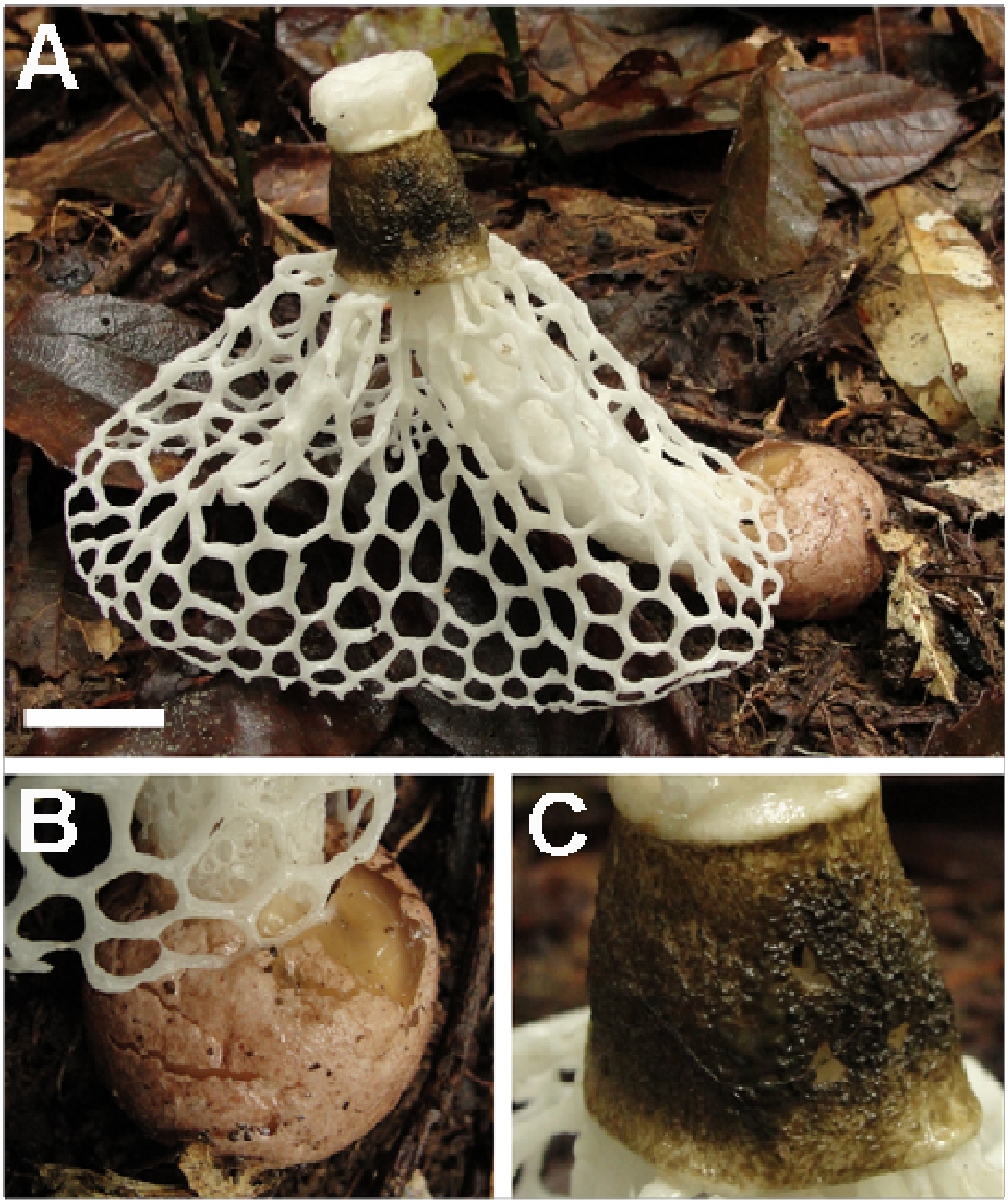

Fig. 2 Macroscopical features of *Phallus aureolatus*. **A**. Basidiome in situ, State of Paraná, Brazil. B. Detail of the volva. C. Detail of the merulioid receptacle. Photographs are copyright of J.M. Baltazar, all from the holotype (LTP109, ICN). Scale bar = 1 cm.

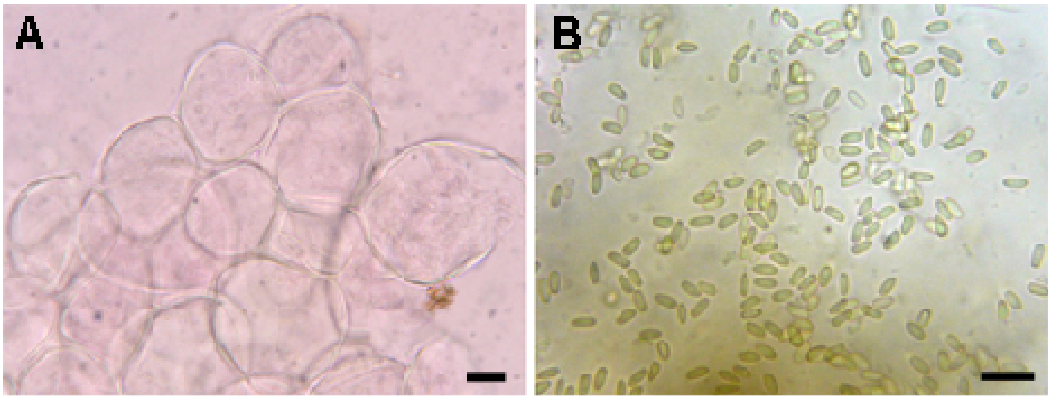

Fig. 3 Microscopical features of *Phallus aureolatus.* A. Pseudoparenchymatous structure from the indusium. B. Basidiospores. Scale bars = 10 µm.

Fig. 4 *Phallus aureolatus* photographed in 2007 in the State of Santa Catarina, Brazil. Photograph is copyright of L. Trierveiler-Pereira, voucher not preserved.

4.7. MANUSCRITO III

Trierveiler-Pereira L, Meijer AAR, Hosaka K, Silveira RMB. 2014. Updates on *Protubera* (*Protophallaceae*, *Phallales*) and additional notes on *P. maracuja*. **Mycoscience** 55: 35–42.

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Full paper

Updates on *Protubera* (Protophallaceae, Phallales) and additional notes on *P. maracuja*

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ABSTRACT

Since the genus *Protubera* was erected in 1895 by Alfred Möller, fourteen species have been described in the genus, from tropical, subtropical and temperate regions of the globe. Based on morphological, ecological and molecular data, we concluded that only six species (*P. borealis*, *P. jamaicensis*, *P. maracuja*, *P. nipponica*, *P. parvispora* and *P. sabulonensis*) should be accepted in Protophallaceae. In this article we present an update of the genus from a morphological and phylogenetic perspective, including comments on the excluded and doubtful taxa, and a key for the accepted species. Additional morphological and ecological notes of *P. maracuja*, especially regarding features of peridium and substrata, as well as color photos, are presented.

1. Introduction

Möller (1895) erected the curious genus *Protubera* based on specimens collected in coastal rainforests of southern Brazil. Basidiomata resembled immature phalloids but Möller was cautious enough to follow up the ontogeny of the basidiomata and noticed that they never produce an expanded receptacle. When completely mature, basidiomata open in an irregular way and expose the brownish green mass of spores. During his stay in Brazil from 1890 to 1893, Möller found the type species, *P. maracuja* Möller, to be abundant in forests of Itajaí Valley (state of Santa Catarina) and basidiomata were found throughout the year. Up to date, 14 species have been described in the genus (Heim 1977; Kirk et al. 2008).

A revision of the genus was published by Malloch (1989) who considered all the species described until then (seven in total) as belonging to the genus, and synonymized *Kobayasia* and *Protuberella* – both proposed by Imai and Kawamura (1958) – with *Protubera* and described *P. sabulonensis* Malloch as a new species.

Since Malloch's review, six new species were described or combined to *Protubera*, *viz*. *P. burburiana* (Rodway) Castellano & Trappe, *P. canescens* G.W. Beaton & Malajczuk, *P. hautuensis*

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4.8. MANUSCRITO IV

Trierveiler-Pereira L, Silveira RMB, Hosaka K. 2014. Multigene phylogeny of the *Phallales* (*Phallomycetidae*, *Agaricomycetes*) focusing on some previously unrepresented genera. **Mycologia**: aceito para publicação.

Short title: Trierveiler-Pereira et al.: Phylogeny of the *Phallales*

Multigene phylogeny of the *Phallales* (*Phallomycetidae*, *Agaricomycetes*) focusing on some previously unrepresented genera

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Abstract: Phylogenetic relationships within the *Phallales* were estimated via combined sequences: nuclear ribosomal large subunit (LSU), second largest subunit of RNA polymerase (*rpb2*), and mitochondrial ATPase subunit 6 (*atp6*). The ingroup is represented by sixty-two taxa comprising 18 genera and 44 species, including members of the *Clathraceae*, *Claustulaceae*, *Gastrosporiaceae*, *Lysuraceae*, *Phallaceae*, and *Protophallaceae*. Sixty-one new sequences were generated for this study, including tropical and subtropical taxa. This is one of the first studies discussing the phylogenetic placement of *Abrachium*, *Aseroë*, *Blumenavia*, *Gastrosporium*, *Jansia*, and *Xylophallus*. *Gastrosporiaceae* was demonstrated to be sister to *Phallaceae* and an emended description of the order is presented. *Aseroë* was demonstrated to be polyphyletic, and as a result, *A. arachnoidea* is transferred to *Lysurus*.

Keywords: *atp6*, Gasteromycetes, LSU, molecular phylogeny, *rpb2*, stinkhorns

INTRODUCTION

Phallales E. Fisch. is a well-supported clade within *Phallomycetidae* Hosaka, Castellano & Spatafora (Hosaka et al. 2006). Members of this order usually produce expanded basidiomata, commonly known as stinkhorns, but some sequestrate taxa (truffle-like fungi), such as *Claustula* K.M. Curtis, *Protubera* Möller, and *Gelopellis* Zeller, also occur. Most species are saprotrophic, although some species of *Protubera* have been reported to be ectomycorrhizal (Comandini et al. 2012, Giachini et al. 2010, Tedersoo et al. 2010). A recent study, however, confirmed that *Protubera* species are saprophytic (Trierveiler-Pereira et al. 2014).

The order suffered many changes through the decades and the family-level organization still needs to be resolved. Fischer (1898-99) originally erected *Phallales* to accommodate *Phallaceae* Corda and *Clathraceae* Chevall. He did not recognize *Lysuraceae* Corda (Corda 1842) as an independent family, and instead included it within *Clathraceae*. Later, Cunningham (1931) added the monogeneric family *Claustulaceae* G. Cunn., although many authors continued to follow Fischer's two-family concept for the order (e.g., Calonge 1998, Dennis 1970, Fischer 1933, Long and Stouffer 1948, Pilát 1958). *Hysterangiaceae* E. Fisch. was included within the *Phallales* (e.g., Dring 1973, Miller and Miller 1988), although it is generally accepted as a distinct order, *Hysterangiales* Hosaka & Castellano (Fischer 1933, Jülich 1981, Pilát 1958, Zeller 1939, 1949). *Protophallaceae* Zeller was erected to accommodate species of *Calvarula* Zeller, *Protophallus* Murril and *Protubera* (Zeller 1939), and it was included in *Hysterangiales* until recently.

Modern molecular methods clarified the phylogenetic structure of the *Phallales*. Hosaka et al. (2006) showed, for example, that members of the *Hysterangiales* and *Phallales* form independent clades within the *Phallomycetidae*, supporting the division of these taxa as distinct orders. The result of that study also suggested that *Phallales* can be divided into six families: *Clathraceae*, *Phallaceae*, *Lysuraceae*, *Protophallaceae*, *Claustulaceae* and *Trappeaceae* P.M. Kirk. *Trappeaceae* was a provisional name suggested for the basal clade formed by two species, *Trappea darkeri* (Zeller) Castellano and *Phallobata alba* G. Cunn., and it was later formally proposed (Kirk et al. 2008).

Another intriguing question about the *Phallales* classification is the phylogenetic relationship of *Gastrosporium* Mattir. Hibbett and Binder (2002) showed that the genus is phylogenetically related to *Anthurus* Kalchbr. & MacOwan and *Pseudocolus* Lloyd, both belonging to *Clathraceae*, in the gomphoid-phalloid clade (= *Phallomycetidae*). Iosifidou and Agerer (2002) suggested that morphological features of the rhizomorphs may justify a separate order for the genus. However, *Gastrosporium* was not included in the phylogenetic analyses of Hosaka et al. (2006) due to the lack of sequence data for a protein coding gene, and its phylogenetic placement within the *Phallales* still needs to be investigated.

Besides *Gastrosporium*, many other phalloid genera such as *Abrachium* Baseia & T.S. Cabral (1 species), *Aseroë* Labill. (3 species), *Blumenavia* Möller (1 species), *Jansia* Penz. (3 species) and *Xylophallus* (Schltdl.) E. Fisch. (1 species) (Fig. 1), have not been included in any previous phylogenetic study or were poorly represented. Phylogenetic analyses based on combined molecular sequence data from three independent loci obtained from 18 genera and 44 species were conducted to produce the first extensive phylogeny of the *Phallales*. The resulting phylogenetic hypothesis was used to review the systematic relationships of the order.

MATERIAL AND METHODS

Taxa sampled, voucher information and GenBank (www.ncbi.nlm.nih.gov/genbank/) accession numbers are listed in Table 1. The ingroup is represented by sixty-two taxa of

Phallales. Members of all known families in the order, except *Trappeaceae*, which is the most basal clade of the order, were included in the analyses. Outgroups were selected from the genera *Gomphus* Pers. and *Ramaria* Fr. ex Bonord. (*Gomphales*), which are closely related to *Phallales* (Hosaka et al. 2006).

Sequence data were obtained from three independent loci: nuclear ribosomal large subunit DNA (LSU), the gene region for the second largest subunit of RNA polymerase (*rpb2*), and mitochondrial ATPase subunit 6 (*atp6*). Primer combinations used to amplify each DNA region and for sequencing were: LR0R/LR5 (Vilgalys and Hester 1990), bRPB2–6F/bRPB2-7R (Matheny 2005), and atp6-3/atp6-2 (Kretzer and Bruns 1999), respectively.

DNA was extracted from glebal tissues of dried basidiomata following the protocol of Hosaka and Castellano (2008). PCR and sequencing protocols followed Kasuya et al. (2012). Raw forward and reverse sequences for each sample were assembled into contigs and manually edited using the program ATGC v.6 (Genetyx). After assembling, the sequences were used to query GenBank using a BLAST search to verify their identity.

Molecular datasets consisting of original and GenBank sequences were aligned using Muscle v.3.6 (Edgar 2004), and manually edited in BioEdit v.7.0.1 (Hall 1999). The final alignment is available from TreeBase as a NEXUS file (#14925). Hypervariable, indel-rich and ambiguously aligned regions were removed from the analyses. A few remaining single-gap regions occurring in only one or few sequences were treated as missing data. All three DNA regions were first analyzed independently (results not shown) and since the comparison of the resulting topologies revealed no major topological conflict (based on 70% or higher bootstrap values), all data were combined into a single matrix for subsequent analysis. Phylogenetic analyses were conducted for the three loci dataset under Maximum Parsimony (MP), Bayesian inference (BI) and Maximum Likelihood (ML).

MP analysis was conducted under the equally weighted parsimony criterion using PAUP* v.4.0b10 (Swofford 2002). The analysis was performed under the heuristic search option (TBR and Multrees options on) and 1,000 replicates of random addition sequence were conducted. Nodal supports were tested by bootstrapping (BS) of 1,000 replicates using the heuristic search option (TBR and Multrees options on) with 10 random addition sequences.

BI was conducted with MrBayes v.3.2.1 (Ronquist et al. 2012), with 5×106 generations of MCMC by sampling every 100th tree. The GTR+I+G model was used for the nucLSU, and each codon position for the remaining protein coding genes. The support of nodes was tested by posterior probabilities (PP) reported on the majority rule consensus tree obtained after deletion of the first 15% of trees sampled from each run. The burn in was determined using Tracer v1.5 (http://tree.bio.ed.ac.uk/software/tracer/) to analyze MrBayes output files.

ML analyses were performed using PhyML 3.0 online web server (Guindon et al. 2010) using the GTR model of nucleotide substitutions. The support of nodes (MLB) was determined using 500 bootstrap pseudo-replicates utilizing nearest neighbor interchange (NNI) branch-swapping.

RESULTS AND DISCUSSION

The three DNA regions were combined into a single supermatrix totaling 1979 characters (LSU= 647; atp6= 662; rpb2= 670; among which 51 characters from LSU were excluded from the analyses due to ambiguous alignment), of which 682 were phylogenetically informative.

MP analysis yielded 5475 equally parsimonious trees (tree length = 3204, CI= 0.4092, RI= 0.7591, RC= 0.3106). Trees generated from MP analysis show similar topology to the ones from BI and ML, with some differences in terminal clades weakly supported.

MP, and BI and ML analyses fully support the monophyly of the *Phallales*, and six major clades (viz. *Clathraceae*, *Claustulaceae*, *Gastroporiaceae*, *Lysuraceae*, *Phallaceae*, and *Protophallaceae*) received full or strong support (Fig. 2).

Gastrosporiaceae was demonstrated to be sister to *Phallaceae* with full support, although the morphology of *Gastrosporium* species is rather different from species included in *Phallaceae*. *Gastrosporium* species have sequestrate basidiomata, powdery gleba at maturity, and ornamented spores (Domínguez de Toledo and Castellano 1997). On the other hand, basidiomata of *Gastrosporium* have gelatinous peridia like other phalloid species. Moreover, *Gastrosporium* rhizomorphs are ramarioid-type (which is characterized by ampullate inflations below hyphal septa), a feature that also occurs in *Phallales* (Agerer and Iosifidou 2002, Agerer 2006). The monophyly of the genus still needs to be better investigated by including sequences of *G. asiaticum* Dörfelt & Bumžaa (Dörfelt and Bumžaa 1986), the second species of the genus, in further phylogenetic studies.

Phallaceae (fully supported) included the genera *Itajayha* Möller, *Phallus*, *Xylophallus*, *Mutinus* Fr. and *Jansia*. *Itajayha* has been treated by different authors in *Phallus* (e.g. Kreisel 1996, Calonge 2005, Ottoni et al. 2010) and a recent study reconsiders the genus independent from *Phallus* based on molecular data (Cabral et al. 2012). *Itajayha* is a genus morphologically different from *Phallus* due to the nature of the receptacle surface (lamellate) and the presence of a calyptra (membranous tissue on the top of the receptacle). In our analysis, *Itajayha* appears separated from other *Phallus* species, indicating that it could be a good genus. However, more sequences from different specimens would be necessary to confirm its independence. *Phallus merulinus* (Berk.) Cooke (≡ *Dictyophora merulina* Berk.) was combined to *Clautriavia* (Pat.) Lloyd due to the merulioid aspect of the receptaculum (Lloyd 1909). *Clautriavia* was firstly considered a section in *Dictyophora* Desv. but later it was elevated to generic status by Lloyd (1909). In our study, *P. merulinus* is separated from other *Phallus* species, which is consistent with Lloyd's view. However, we tentatively keep this species in *Phallus* until more species are included in molecular phylogenetic analyses. *Xylophallus xylogenus* (Mont.) E. Fisch. is an interesting species from the Neotropics with remarkably small-sized basidiomata (Trierveiler-Pereira and Silveira 2012). Originally described as *Phallus xylogenus* in sect. *Mutinus* (Montagne 1855), it was combined to *Mutinus* by Fischer (1898-99) and later erected to the genus *Xylophallus* (Fischer 1933). According to our studies, *Xylophallus* is more closely related to *Mutinus* than *Phallus*. Since *Mutinus* species are clustered with full support, we prefer to maintain *Xylophallus* independent from *Mutinus*. *Jansia boninensis* E. Fisch. (Lloyd) was originally described in *Mutinus* (Lloyd 1908) and later combined to *Jansia* (Lloyd 1909). Lloyd (1909) stated that the receptaculum of *J. boninensis* was slightly rugulose and therefore, represented an intermediate form between *Mutinus* and *Jansia*. In our analyses, the species is nested within *Mutinus* with full support and therefore, should be treated in *Mutinus*.

Clathraceae (fully supported) included the genera *Clathrus* P. Micheli ex L., *Abrachium*, *Aseroë*, *Anthurus* Kalchbr. & MacOwan, *Pseudocolus* Lloyd, *Blumenavia*, *Laternea* Turpin, and *Ileodictyon* Tul. ex M. Raoul. The genus *Clathrus* is polyphyletic in our analyses due to one sequence of *C. chrysomycelinus* Möller from New Zealand, which is more related to sequences of the Australasian species (*Ileodyction* spp).

Unfortunately, sequences of *C. chrysomycelinus* from the type locality (Brazil) are missing in our analyses preventing us to elucidate this issue. Other *Clathrus* species, either from tropical or temperate areas, are resolved with weak support separately from other clathroid genera. *Abrachium*, a genus proposed by Cabral et al. (2012) to accommodate the previously described species *Aseroë floriformis* Baseia & Calonge, is nested within *Clathrus* in our analyses. More sequences of *Abrachium* and *Clathrus* should be included in the *Clathraceae* phylogeny to ascertain the phylogenetic placement of *Abrachium*. The relationship among other clathroid genera is still obscure, since many clades are only weakly or not supported. However, it is noteworthy that *Aseroë* appears polyphyletic, with one species, *A. arachnoidea*, nested within *Lysuraceae*. *Blumenavia* is closely related to *Laternea* with strong support. *Blumenavia rhacodes* Möller was transferred to *Laternea* by Lloyd (1909) but the combination was not accepted by many authors. The inclusion of more sequences of *Laternea* species in the phylogeny is necessary to elucidate if *Blumenavia* should be synonymized with *Laternea*.

Lysuraceae (fully supported) included only the genus *Lysurus* Fr. *Aseroë arachnoidea*, which was nested within the family with full support, should be treated in *Lysurus*. Traditionally, the two genera were separated based on gleba position, and the arms' length, attachment and tendency to separate at maturity (Dring 1980, Miller and Miller 1988), but classification based on these features is not supported by the phylogeny. *Simblum* Klotzsch ex Hook. is usually considered separated from *Lysurus* (Fischer 1933, Lloyd 1909, Miller and Miller 1988, Pilát 1958) due to the subglobose clathroid receptaculum. In our analyses, the genus is represented by *S. sphaerocephalum* Schltdl. [= *Lysurus periphragmoides* (Klotzsch) Dring], which is nested within *Lysurus* with full support. This study confirms the synonymy of *Simblum* with *Lysurus* as proposed by Dring (1980).

Protophallaceae (PP 100/ MLB87) and *Claustulaceae* (fully supported) included exclusively sequestrate genera. *Protophallaceae* is only represented by *Protubera*, since no sequences of *Calvarula* are available. *Protophallus* is considered a synonym of *Protubera* (Malloch 1984, Trierveiler-Pereira et al. 2014). *Claustulaceae* includes the genera *Gelopellis* and *Claustula*. Hosaka et al. (2006) showed that *Kjeldsenia* W. Colgan, Castellano & Bougher and *Phlebogaster* Fogel also belong to the family. As in *Gastrosporium*, species of *Kjeldsenia* and *Phlebogaster* have ellipsoid to oblong, verrucose basidiospores (Fogel 1980, Colgan et al. 2005), an unusual feature among the *Phallales*.

According to our results, most families in the order are represented by expanded forms, while sequestrate forms occur in three families: *Claustulaceae*, *Protophallaceae* (basal families), and *Gastrosporiaceae*. Hosaka et al. (2006) discussed that stinkhorn's expanded basidiomata were restricted to derived clades, but our results indicate that reversion from expanded to sequestrate forms occurred at least once in the order.

In this study, molecular and morphological evidence was used to clarify phylogenetic relationships in the *Phallales*. The results confirm the monophyly of six families, and the position of *Gastrosporium* within the order is identified. The phylogenetic placement of interesting tropical and subtropical taxa was also discussed. *Xylophallus* was retrieved as an independent genus closely related to *Mutinus*, and *Jansia boninensis* was showed to be a *Mutinus* species. Further investigations are still necessary to test the authenticity of *Abrachium* and *Blumenavia*. In the same way, more phalloid taxa should be included in further analyses to better understand the relationship among genera. Many tropical monotypic genera, such as *Aporophallus* Möller, *Floccomutinus* Henn., *Kalchbrennera* Berk., *Ligiella* J.A. Sáenz, *Neolysurus* O.K. Mill., Ovrebo & Burk, and *Staheliomyces* E. Fisch., still need to be included in phylogenetic studies.

TAXONOMY

Based on the results of our phylogenetic analyses, we propose to transfer *Aseroë arachnoidea* to *Lysurus*. An emendation for *Phallales* is also presented.

Lysurus arachnoideus (E. Fisch.) Trierv.-Per. & Hosaka, comb. nov.

MycoBank MB 803901

≡ *Aseroë arachnoidea* E. Fisch., Denkschr. Schweiz. Naturf. Ges. 32:76, 1890 (basionym).

Remarks. The species is characterized by whitish pseudostipe and receptacle. Arms (8–12) are long, slender, and formed by a hollow tube, as occurs in *Lysurus cruciatus* (authors' personal observations). The gleba is situated at the lower part of the inner surface of each arm, and as noticed by Dring (1980), the species has no fertile disc as occur in other *Aseroë* species. When the receptacle expands from the egg, the arms are united at the apex, but soon they become detached and expand. This feature might also be observed in other *Lysurus* species at maturity. According to Dring (1980), the glebal mass of *Lysuraceae* tends to migrate to the exterior face of the arms, while in *Clathraceae* it remains restricted to the interior face of the arms/receptacle. In *A. arachnoidea*, the glebal mass is not restricted to the internal face of the arms, but it also migrates to the laterals.

Phallales E. Fisch. emend. Trierv.-Per. & Hosaka

Emended description. Immature basidiomata hypogeous or epigeous, consisting of 2–3 layered peridium, usually with thick, white rhizomorphs at base; mature basidiomata usually epigeous or partially hypogeous, expanded or indehiscent, receptaculum pseudostipitate or sessile, pseudostipe peseudoparenchymatous, receptacle bearing the gleba; gleba green, olivaceous to brown, mucilaginous when immature, mucilaginous or pulverulent when mature; basidiospores hyaline, green, golden to brown, bacillarioid, cylindrical to oblong, smooth to ornamented.

Type genus. *Phallus* Junius ex L.

Remarks. The emended description of *Phallales* was elaborated to include sequestrate species with powdery gleba at maturity, and golden to brown, ellipsoid to oblong basidiospores ornamented with warts. The order includes the families: *Clathraceae*, *Claustulaceae*, *Gastrosporiaceae*, *Lysuraceae*, *Phallaceae*, *Protophallaceae* and *Trappeaceae*.

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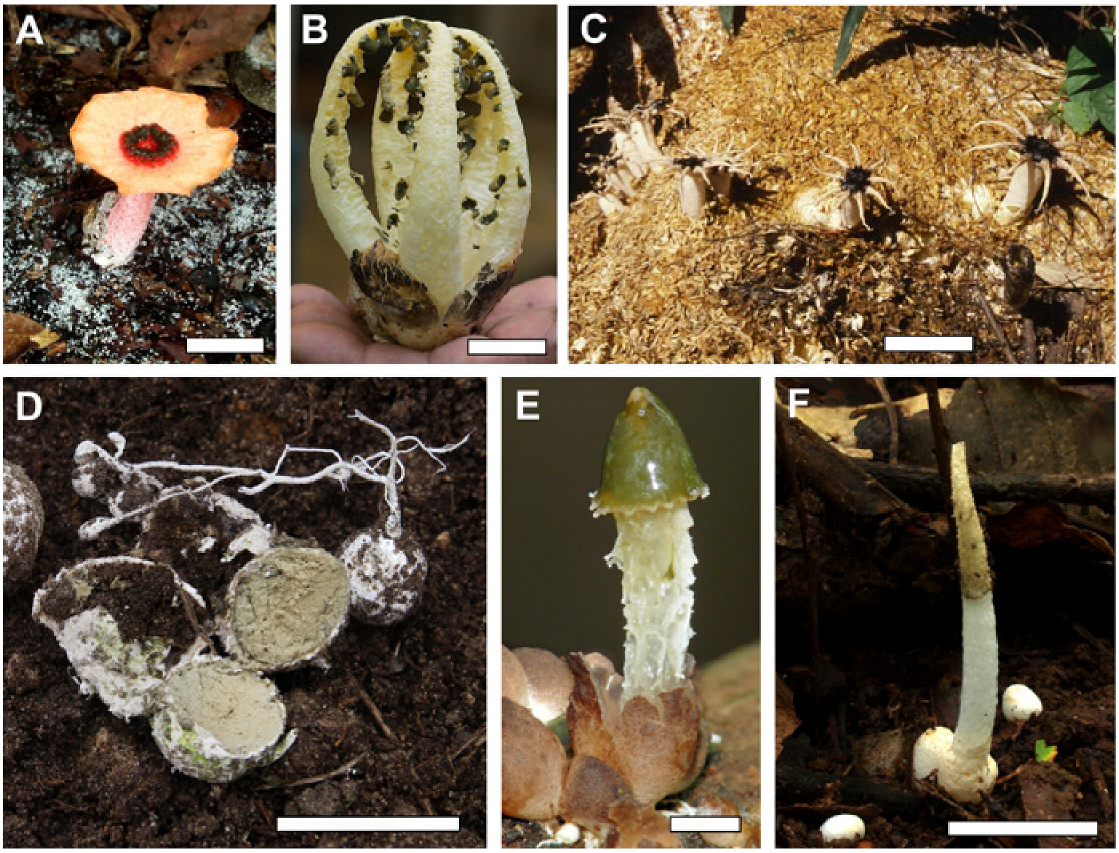

FIG. 1. Phalloid species. A. *Abrachium floriforme* (Brazil). B. *Blumenavia rhacodes* (Mexico). C. *Aseroë arachnoidea* (Japan). D. *Gastroporium simplex* (Slovakia). E. *Xylophallus xylogenus* (French Guiana). F. *Jansia boninensis* (Bonin Islands, Japan). Scale bars: A, B, D = 2.5 cm; C = 6.0 cm; E = 0.25 cm; F = 2.0 cm. Photographs courtesy: A. L. Trierveiler-Pereira; B. M. Armando López R.; C. S. Kurogi; D. R. Bednár; E. J.-L. Cheype; F. K. Hosaka.

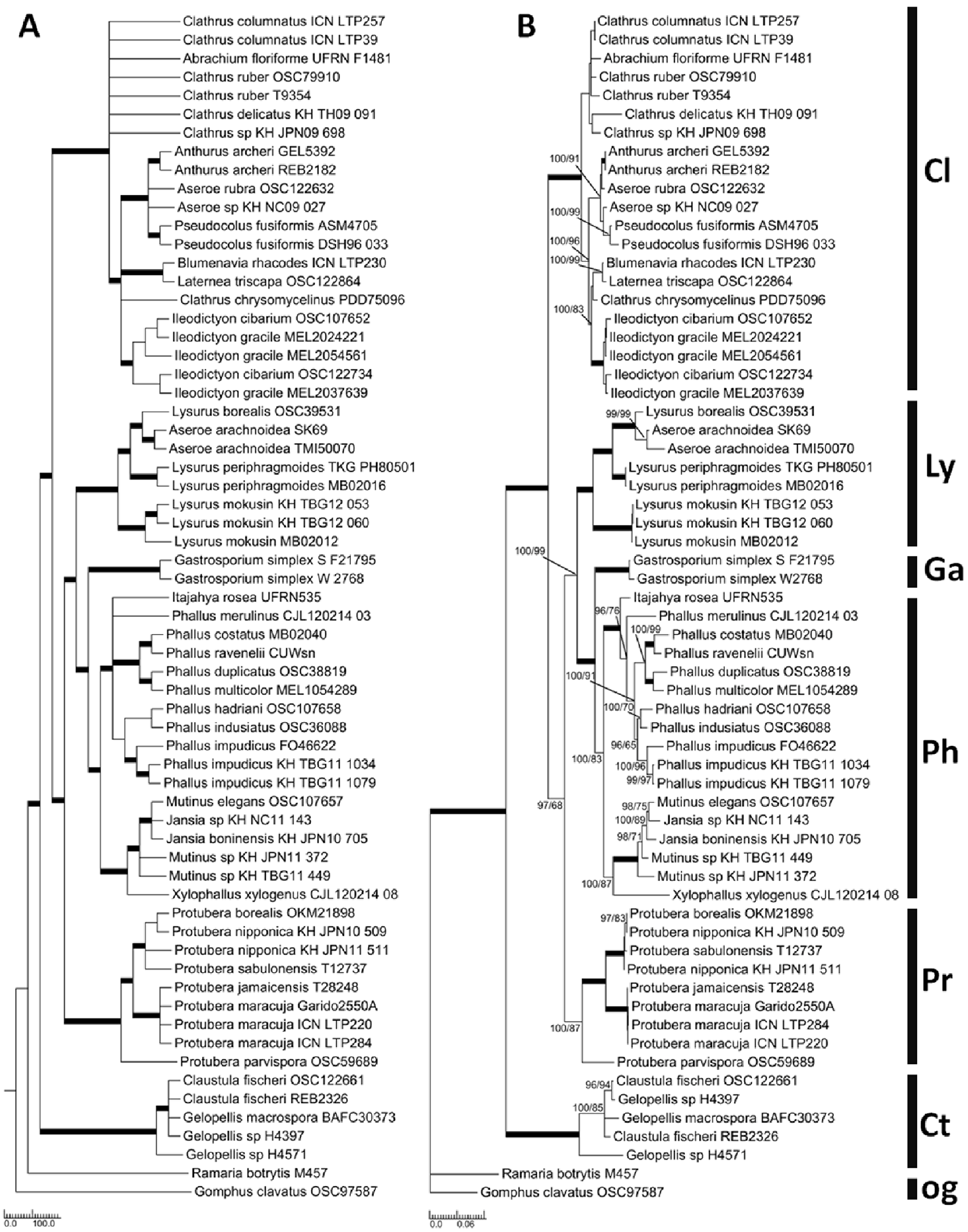

FIG. 2. Phylogenetic relationships among the *Phallales*. A. Majority-rule consensus tree of the 5475 most parsimonious trees obtained after 1000 heuristic search replicates. Thickened branches in boldface indicate nodes fully or strongly supported (≥ 80). B. Bayesian consensus tree. Support values for internal nodes are given on the branches as posterior probability/maximum likelihood bootstrap. Thickened branches in boldface

indicate nodes fully supported. Cl = *Clathraceae*; Ly = *Lysuraceae*; Ga = *Gastrosporiaceae*; Ph = *Phallaceae*; Pr = *Protophallaceae*; Ct = *Claustulaceae*; og = outgroups.

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4.9. MANUSCRITO V

Trierveiler-Pereira L, Meijer AAR, Silveira RMB. 2014. *Phallales* (*Agaricomycetes*, *Fungi*) from Southern Brazil. **Journal of the Torrey Botanical Society**: a ser submetido.

Short title: **Phallales from Southern Brazil**

Phallales (Agaricomycetes, Fungi) from Southern Brazil

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Abstract: TRIERVEILER-PEREIRA, L.[1], MEIJER, A.A.R. [2] & SILVEIRA, R.M.B[1] ([1]Universidade Federal do Rio Grande do Sul, Brazil; [2]Guaraqueçaba, Paraná, Brazil). Phallales (Agaricomycetes, Fungi) from Southern Brazil. J. Torrey Bot. Soc. XXX: 000 000. 20XX. An illustrated, annotated checklist and key to the 24 species of phalloids known to occur in Southern Brazil (States of Paraná, Santa Catarina and Rio Grande do Sul) are presented. *Sphaerophallus* gen. nov. is introduced to accommodate *Phallus glutinolens*, and *P. granulosodenticulatus* is reduced to a synonym of *P. campanulatus*. *Abrachium floriforme* and *Staheliomyces cinctus* are first reports from Southern Brazil and *Laternea pusilla* is new from the Sate of Santa Catarina.

Key words – Brazilian mycota, fungal taxonomy, phalloid fungi, stinkhorns

Introduction

Members of Phallales (Phallomycetidae, Agaricomycetes) show great variability in size, shape and color. Some species are remarkable due to bizarre shape allied to bright colors, which calls the attention not only of mycologists, but also of people in general. Traditionally the order included species with expanded receptacle, commonly known as stinkhorns or lattice stinkhorns, but since the sequestrate genus *Claustula* (Cunningham 1931) was added to the order, many other truffle-like genera have been included, such as *Protubera* Möller, *Gelopellis* Zeller, *Kjeldsenia* W. Colgan et al., *Phlebogaster* Fogel, *Trappea* Castellano, and *Gastrosporium* Mattir. (Hosaka et al. 2006, Kirk et al. 2008, Trierveiler-Pereira et al. 2014b).

Although the Neotropical region has a high diversity of phalloids, with many rare and endemic species, the knowledge of the group in this area is still fragmentary. This fact can be attributed to several reasons, such as: ephemeral nature of the basidiomata, especially in tropical rainforests; lack of researchers familiarized with the species in the field; poor field notes of the collected specimens; difficulties in preserving specimens and therefore, badly preserved vouchers in herbaria; publications with poor descriptions and without accurate illustrations or photographs; usage of paleotropical or temperate species names to identify the neotropical species.

In South America, few surveys dedicated to the group have been published since the 19th century (Spegazzini 1886, Möller 1895, Rick 1929, Braun 1932, Wright 1949, 1960; Domínguez de Toledo 1995, Baseia et al. 2006, Cheype 2010, Magnago et al. 2013, Sandoval et al. 2014), but many records and descriptions of new species have been published on mycological inventories (e.g. Rick 1906, 1961; Fischer 1933, Dennis 1970, Bononi et al. 1984, Lazo 2001, Meijer 2006, Cortez 2008, Trierveiler-Pereira et al. 2009b, Hernández Caffot et al. 2013) or isolated publications (Domínguez de Toledo 1985, Baseia et al. 2003, Baseia & Calonge 2005, Gómez & Gazis 2006, Fazolino et al. 2010, Ottoni et al. 2010, Cortez et al. 2011a, Trierveiler-Pereira et al. 2009a, Trierveiler-Pereira & Silveira 2012).

Studies on the Phallales in southern Brazil are scarce; nevertheless more than 35 names of species have been reported from this region, including several new taxa (Möller 1895, Braun 1932). The aim of this study is to verify which phalloid species occur in the Southern region of Brazil, based on field collections and examination of herbarium specimens. Illustrations of all the species and a key to facilitate further identification are also presented.

Materials and Methods

Southern Brazil includes the States of Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS), and covers 576,410 km^2 (about 7% of the Brazilian territory). The climate is subtropical in almost the entire region, except the northeastern part of the region that is tropical, and the annual average temperatures vary between 12°C (53.6°F) and 22°C (71.6°F).

The list of species presented here was compiled from recent fieldwork, herbaria exsiccates, and literature. Macro and microscopical analysis of the material followed traditional methods used to study gasteroid basidiomycetes (Miller & Miller 1988). Brazilian specimens of *L. pusilla* are fully described, while for other species, one or more references are indicated where description can be found.

The macroscopic illustrations were based on photographs taken in the field or dried specimens kept at herbaria. Illustrations are represented for all species, with the exception of *P. callichrous*, a species morphologically very similar to *P. indusiatus*, except for the color. Color codes (e.g. 8A4) are based on Kornerup & Wanscher (1978). Voucher material was deposited at ICN, HUCS, FLOR, and MBM (Thiers 2013). Species are listed alphabetically inside each family (according to the classification presented by Trierveiler-Pereira et al. 2014b).

Results (Taxonomy)

Key to the Phallales from Southern Brazil

1. Mature basidiome globose to subglobose, not forming expanded pseudostipe or receptacle (truffle-like) - **2**

1'. Mature basidiome with pseudostipe simple, ramified into arms, columns, or clathroid receptacle - **3**

2. Basidiome hypogeous to subhypogeous, yellowish brown; in longitudinal section can be observed a thick gelatinous matrix below the peridium; glebal mass globose surrounding a central columella, gleba not connected to the peridium - ***Gelopellis thaxteri***

2'. Basidiome epigeous, whitish, grayish yellow to light brown; in longitudinal section elongated elliptical glebal plates immersed in a gelatinous matrix can be observed; columella absent; glebal plates connected to the inner part of the peridium by sutures - ***Protubera maracuja***

3. Basidiome with pseudostipe ramified into arms, columns, or receptacle clathroid - **4**

3'. Basidiome with a simple pseudostipe - **9**

4. Pseudostipe ramified into 2–5 columns or arms - **5**

4'. Receptaculum clathroid, forming more or less polygonal meshes - **8**

5. Gleba confined to lateral projections (‘teeth’) of the columns - **6**

5’. Gleba confined to a single glebifer or spread along the inner side of the columns - **7**

6. Basidiomata robust, 9–13 cm high, columns up to 2 cm in wide when fresh, receptacle light yellow - ***Blumenavia rhacodes***

6’. Basidiomata delicate, 4–6 cm high, columns up to 0.5 cm in wide when fresh, receptacle pure white - ***Blumenavia angolensis***

7. Basidiomata < 4cm in high, bright red to pale pink, with crests along the margins; gleba confined to a single glebifer, suspended below the columns’ junction columns - ***Laterna pusilla***

7’. Basidiomata larger, yellowish to orange, without crests along the margins; gleba spread along the inner side of the columns - ***Clathrus columnatus***

8. Receptacle salmon to reddish; meshes surrounded by a corrugated and folded membrane - ***Clathrus crispus***

8’. Receptacle white to beige; no membrane surrounding the meshes - ***Clathrus chrysomycelinus***

9. Pseudostipe laterally perforate, constricted by a ring at the upper part, glebal mass covering the ring - ***Staheliomyces cinctus***

9’. Pseudostipe not laterally perforate; constricted annular region absent - **10**

10. Gleba spread directly over the pseudostipe - **11**

10’. Gleba confined to a receptacle - **12**

11. Fertile area delimited, surface of the pseudostipe rugulose - ***Mutinus argentinus***

11'. Fertile area not delimited, surface of the pseudostipe even - ***Mutinus elegans***

12. Pseudostipe surmounted by arms or with a flower-shaped receptacle - **12**

12'. Not as above - **16**

13. Pseudostipe with a flower-shaped receptacle - ***Abrachium floriforme***

13'. Pseudostipe surmounted by arms - **14**

14. Arms usually in a horizontal position, long and slender, smooth, attached to the margin of a flat disc across which the gleba is spread - ***Aseroë rubra***

14'. Arms upright, short, thick, conical, transversally furrowed, attached to apex of the pseudostipe - **15**

15. Basidiomata < 5 cm in high, pseudostipe reduced, arms concolorous with the pseudostipe (white) - ***Pseudocolus garcie***

15'. Basidiomata larger, pseudostipe long, arms internally whitish, orange to reddish - ***Lysurus cruciatus***

16. Gleba situated on a spherical, clathroid, reddish receptacle at the apex of the pseudostipe - ***Lysurus periphragmoides***

16'. Not as above - **17**

17. Receptacle surface lamellate, with a white calyptra (membranous cap) at the apex - ***Itajahya galericulata***

17'. Not as above - **18**

18. Receptacle spherical at first, then campanulate; outer volva tuberculate, inner volva yolk-yellow - ***Sphaerophallus glutinolens***

18'. Receptacle campanulate, outer volva smooth, inner volva whitish or very pale - **19**

19. Basidiomata < 5 cm in high, receptacle slenderly campanulate, not perforate - ***Aporophallus subtilis***

19'. Basidiomata larger, receptacle with a visible pore at apex when mature - **20**

20. Indusium absent - ***Phallus campanulatus***

20'. Indusium present - **21**

21. Indusium short, not covering the pseudostipe entirely - ***Phallus duplicatus***

21'. Indusium long, covering the pseudostipe entirely - **22**

22. Receptacle surface rugulose - ***Phallus areolatus***

22'. Receptacle surface reticulate-alveolate - **23**

23. Receptacle white - ***Phallus indusiatus***

23'. Receptacle orange to pink - ***Phallus callichrous***

1. Clathraceae

Abrachium floriforme (Baseia & Calonge) Baseia & T.S.Cabral, Mycotaxon 119: 424, 2012. **Fig. 1A**

Description: Baseia & Calonge (2005), Trierveiler-Pereira & Baseia (2011), Magnago et al. (2013).

Examined material: BRAZIL. SANTA CATARINA: Santo Amaro da Imperatriz, Plaza Hotel Caldas da Imperatriz, 30/X/2012, *A.C. Magnago* 452 (FLOR 47631).

Distribution in Southern Brazil: SC (present study).

Remarks: the species is easily recognized in the field due to its characteristic sunflower-shaped receptacle. Pseudostipe and receptacle may show different shades of pink, yellow and orange. The glebal mass is spread over a reddish perforate disc in the center of the receptacle. The species is known from the Atlantic rainforest in Northeastern and Southeastern Brazil (Baseia & Calonge 2005, Trierveiler-Pereira & Baseia 2011, Magnago et al. 2013) and its occurrence in Southern Brazil is here reported for the first time.

Aseroë rubra Labill, Bull. Murith. Soc. Valais. Sci. Nat. 1: 145, 1800. **Fig. 1B**

Description: Dring (1980).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Salvador [= Salvador do Sul], 1943, *P. Hansen* (PACA-FR 12206); São Francisco de Paula, Fazenda dos Novilhos, near *Pinus* plantation, 28/I/2011, *L.O. Rosa* 513 (MIUCS 830).

Distribution in Southern Brazil: RS (Rick 1961, Guerrero & Homrich 1999), PR (Meijer 2006).

Remarks: In Southern Brazil the species is characterized by bright red color and receptacle formed by numerous long arms (up to 22). The glebal mass is spread over a central disc. *Aseroë rubra* is recorded from different parts of the globe, including tropical and temperate areas, and much variability has been observed in the species, which justify its long list of synonyms. Nevertheless, many names treated as synonym may correspond to independent taxa. In Southern Brazil, the species has been observed several times near *Pinus* plantation, so it might indicate that it is introduced.

Blumenavia angolensis (Welw. & Curr.) Dring, Kew Bull. 35(1): 53, 1980. **Fig. 1C**

Description: Welwitsch & Currey (1870), Dring (1980).

Examined material: BRAZIL. PARANÁ: Antonina, Reserva Natural do Rio Cachoeira, 17/VIII/2005, *A.A.R. de Meijer* 4339 (MBM).

Distribution in Southern Brazil: PR (Meijer 2006)

Remarks: the species is characterized by a pure white receptacle formed by 3–5 columns and a gleba confined to delicate glebifers situated on lateral projections ('teeth') of the columns' margins. *Blumenavia angolensis* has narrower columns than *B. rhacodes*, and according to Dring (1980), the tubular arrangement of the columns in transversal section is also different. In Brazil the species is known from the Southern and Southeastern regions (Meijer 2006, Dring 1980). *Ligiella*, a new genus proposed from Costa Rica (Sáenz 1980), also has whitish receptacle and gleba restricted to the upper part of the basidiomata. According to Sáenz (1980) *Ligiella* differs from *Blumenavia* in having the gleba borne directly on the underside of the columns, instead of confined to glebifers; and having a clathroid receptacle instead of columns.

Blumenavia rhacodes Möller, Bot. Mitt. Trop. 7: 57, 1895. **Fig. 1D**

Description: Möller (1895), Dring (1980), López et al. (1981).

Examined material: BRAZIL. RIO GRANDE DO SUL: Porto Alegre, Morro Santana, on rotten wood, 17/V/2011, *L. Trierveiler-Pereira* 230 (ICN); ibid, on rotten wood, 31/V/2011, *L. Trierveiler-Pereira* 247 (ICN); São Leopoldo, *J. Rick* (PACA-FR 12549); ibid., 1906, *J. Rick* (PACA-FR 12550); ibid., 1907, *J. Rick* (PACA-FR 12552); ibid., 1932, *J. Rick* (PACA-FR 12551); ibid., *J. Rick* (PACA-FR 12553); ibid., 1929, *B. Braun* (PACA-FR 13857 – as *Laternea pseudocrispa*); ibid., 1930, *J. Rick* (PACA-FR 21358 – as *Pseudocolus rugulosus*).

Distribution in Southern Brazil: RS (Rick 1961), SC (Möller 1895, Lloyd 1907a).

Remarks: the species is characterized by a pale orange to cream receptacle consisting of 3–5 robust columns, and glebifers distributed on lateral expansions ('wings') distributed along the column's margins. The species is found on forest soil and rotten wood and immature forms are usually dark, as in *B. angolensis*. The color of the receptacle has been reported as orange fading to yellow or white (Dring 1980), but to our own experience they are usually pale yellow. The species is referred to *Laternea rhacodes* (Möller) Lloyd by some authors (Lloyd 1909, Rick 1929, Rick 1961). Recent molecular studies have shown that *B. rhacodes* is phylogenetically very close to *Laternea triscapa* (Trierveiler-Pereira et al. 2014b). *Blumenavia toribiotalpaensis* Vergas-Rodríguez, described from Mexico, has been reduced to a synonym of *B. rhacodes* (Calonge et al. 2007).

Clathrus chrysomycelinus Möller, Bot. Mitt. Trop. 8: 22, 146, 1895. **Fig. 1E**

Description: Möller (1895), Fazolino et al. (2010).

Examined material: BRAZIL. RIO GRANDE DO SUL: Pelotas, 1907, *J. Rick* (PACA-FR 12620, not well preserved); Dom Pedro de Alcântara, 15/V/2012, *M.A. Reck* 733/12 (ICN 175592); PARANÁ: Antonina, Reserva Natural Rio Cachoeira, 06/V/2006, *C.L. Bandeira & A.A.R. de Meijer* 4345 (MBM); Paranaguá, Ilha do Mel, 03/II/1990, *A.A.R. de Meijer* 1472 (MBM); ibid., 03/VI/1990, *A.A.R. de Meijer* 1797 (MBM).

Distribution in Southern Brazil: RS (Rick 1961), SC (Möller 1895), PR (Meijer 2006).

Remarks: the species is characterized by whitish, clathroid receptacle and gleba arranged in glebifers on the inner part of the receptacle. The lower meshes are usually more elongated them those at the apex. The branches of the receptacle are typically dorsiventrally compressed, wrinkled, and with a median longitudinal groove. Three collections as registered as *C. chrysomycelinus* at PACA, but two of them are badly damaged (FR 12631 and 12633), and the other one doesn't seems to correspond to the species, since the gleba is not confined to glebifers and meshes are robust.

A photograph of *Ileodictyon cibarium* from Northeastern Brazil (Baseia et al. 2006) depicts a young basidiome of *C. chrysomycelinus* (note the internally situated glebifers, which do not occur in *Ileodictyon*, and dorsiventrally compressed meshes with a median longitudinal groove). Other whitish *Clathrus* species known to occur in tropical America (not considering albino forms of usually colored species) are *C. oahuensis* and *C. roseovolvatus* (Lécuru et al. 2013); *C. oahuensis* presents setae on the lower meshes, and *C. roseovolvatus* has a pinkish to purple volva with the gleba not confined to glebifers.

Clathrus columnatus Bosc, Mag. Gesell. naturf. Freunde, Berlin 5: 85, 1811. **Fig. 1F**

Description: Magnago et al. (2013).

Examined material: BRAZIL. RIO GRANDE DO SUL: Santa Maria, Três Barras, 14/V/2010, *L. Trierveiler-Pereira* 39 (ICN); Viamão, Parque Estadual Itapuã, 18/VI/2011, *L. Trierveiler-Pereira* 257 (ICN); São Leopoldo, 1930, *J. Rick* (PACA-FR 13858); ibid., 1929, *B. Braun* (PACA-FR 13859); ibid., *J. Rick* (PACA-FR 13860); ibid., 1931, *J. Rick* (PACA-FR 13861); ibid., 1929, *J. Rick* (PACA-FR 13862); ibid., 1930, *J. Rick* (PACA-FR 13864); ibid., 1930, *J. Rick* (PACA-FR 13865); ibid., 1930, *J. Rick* (PACA-FR 13866); ibid., *B. Braun* (PACA-FR 13868); ibid., *J. Rick* (PACA-FR 13867 – as *Laternea pusilla*); PARANÁ: São José dos Pinhais, 18/VI/2002, *A.A.R. de Meijer* s/n (MBM).

Distribution in Southern Brazil: RS (Guerrero & Homrich 1999, Rick 1961, Sobestiansky 2005), PR (Meijer 2006).

Remarks: the species is characterized by its pale red to orange receptacle formed by 2–4 robust columns and glebal mass spread over the inner surface of the columns. The columns have a spongy consistence and usually they are dilacerate in the inner part. According to Rick (1929), it is a common species never found inside forests. The species occurs in lawns and pastures, as well as on sandy soil, sometimes near lagoons and along the ocean. Although there is no voucher of the species from Santa Catarina, the first author had already seen specimens on sandy soil near the beach. *Clathrus columnatus* has been reported also as *Linderiella* and *Laternea* (Sobestiansky 2005, Meijer 2006, Rick 1929).

Clathrus crispus Turpin, Dict. Sci. Nat. Atlas Acotyl., Tab. 29, 1820. **Fig. 1G**

Description: Lloyd (1909), Wright (1949), Dring (1980).

Examined material: BRAZIL. RIO GRANDE DO SUL: Pelotas, 1907, *A. Schupp* (PACA-FR 12623; PACA-FR 12624; PACA-FR 12627); São Leopoldo, 1904, *J. Rick* (PACA-FR 12625); ibid, 1930, *J. Rick* (PACA-FR 12626).

Distribution in Southern Brazil: RS (Rick 1961).

Remarks: the species is characterized by the globose to sugblobose, salmon to reddish receptacle with more or less regular meshes, and has a membrane corrugated and folded membrane surrounding the meshes. According to Lloyd (1909) it seems to be a common species in the West Indies, from Mexico southward to Uruguay. Rick (1929) stated that one collection from A. Schupp (PACA-FR 12624) had a yellowish white receptacle, but the material that we examined had the typical folded membrane characteristic of *C. crispus*. This albino form, which was described as *C. americanus* Lloyd (Lloyd 1909) and later reduced to *C. crispus* var. *americanus* (Lloyd) J.E. Wright (Wright 1949), is treated as *C. crispus* by

Dring (1980). The second author has reported the species from Paraná (Meijer 2006), but he now believes that his material could be to a *Colus* species, close to *C. pusillus* (Berk.) Reichert. The voucher specimens (*A.A.R.M. 1183* and *A.A.R.M. 1899*) are unavailable for us to study, since both collections were lent out to herbarium LG (Liège, Belgium).

The species was also reported by Meijer (2006), but voucher specimen (A.A.R.M. 4345, MBM) seems to correspond to a *Colus* species.

Laternea pusilla Berk. & M.A. Curtis, in Berk., J. Linn. Soc., Bot. 10: 343, 1868. **Fig. 1H**

Description: Unexpanded basidiome globose to sugblobose, 0.9-1.4 cm in diam., white (1A1), with long mycelial strands attached at the base, growing on rotten wood. Expanded basidiome epigeous, up to 3.9 cm high. Volva 1.5 cm high × 1.4 cm in diam., sacculiform, externally white (1A1), internally with hyaline gelatinous content. Receptacle 2.7 cm high × 1.5 cm in diam., formed by 2–3 arched columns, columns spongy, orange-red (8B6), pastel red (8A4) to dull red (8B3), with prominent crests at the column's margins (arising from the inner margins and projecting to the outer margins). Gleba mucilaginous, olive brown (4F8), confined to a single glebifer located under the connection point of the columns' apices; glebifer cordiform to star-shaped, hanging from the receptacle, 0.4 cm × 0.35 cm, orange red (8A8). Basidiospores 3–4 × 1–1.5 µm, cylindrical to ellipsoid, hyaline to greenish, smooth.

Examined material: BRAZIL. PARANÁ: Campina Grande do Sul, Parque Estadual Pico Paraná, forest trail to Pico Paraná, 12/II/2011, *A.A.R. de Meijer* 4529 (MBM); SANTA CATARINA: Urubici, Morro da Igreja, 16/II/2013, *C.H. Cabrera* 66 (FLOR 47616); ibid., PARNA São Joaquim, Campus de Santa Bárbara, 02/IX/2013, A.N.M. Furtado 318 (FLOR 50692).

Distribution in Southern Brazil: PR (Meijer 2006) and SC (present study).

***Remarks**: L. pusilla* is one of the smallest species among the phalloids. It is characterized by small-sized basidiomata (about 2.0-3.5 cm high) growing on dead wood at high altitudes in the Neotropics; receptacle bright red fading with age, formed by 2–4 columns with prominent crests and bearing a single glebifer hanging from the apex. *Laternea pusilla* is known from forests in Cuba, Jamaica, Guadaloupe, Nicaragua, Costa Rica, Mexico, Guyana and Brazil (Dring 1980, Sáenz 1975, Sáenz & Nassar 1982, López et al. 1982, Meijer 2006). Here describe Brazilian specimens for the first time. In southern Brazil the species only occurs in cloud forests above 1,000 m a.s.l. One specimen kept at PACA (FR 13867) identified as *L. pusilla* actually corresponds to a small specimen of *C. columnatus*.

Pseudocolus garciae (Möller) Lloyd, Mycol. Notes (Cincinnati) 28: 358, 1895. **Fig. 1I**

Description: Möller (1895), Rick (1929), Dring (1980), Sulzbacher et al. (2013).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1905, *J. Rick* (PACA-FR 12816).

Distribution in Southern Brazil: RS (Rick 1961, Sulzbacher et al. 2013), SC (Möller 1895).

Remarks: The species is characterized by whitish, small-basidiomata (about 2 cm high), formed by a short pseudostipe (1 cm high) surmounted by 3–4 arms tapering upwards and united at the tips. The glebal mass is spread over the inner portion of the arms. The second species currently recognized in the genus, *P. fusiformis* (E. Fisch.) Lloyd [which according to Dring (1980) is the correct name for *P. javanicus* (Penz.) Lloyd and *P. schellenbergiae* (Sumstine) Johnson], has a reddish to orange basidiome and its distribution is restricted to Australasia. Records of *P. fusiformis* from U.S.A. probably concern introductions (Dring 1980). *P. fusiformis* was reported from Peru (Gómez & Gazis 2006), but probably corresponds to a species different from the Paleotropical one. One specimen reported as *P.*

javanicus in Argentina (Wright 1960) probably corresponds to *P. garciae*, judging the receptacle color and basidiome size.

2. Claustulaceae

Gelopellis thaxteri (Zeller & C.W. Dodge) Zeller, Mycologia 31(1): 22, 1939. **Fig. 2B**

Description: Zeller & Dodge (1929), Homrich (1969).

Examined material: BRAZIL. RIO GRANDE DO SUL: Porto Alegre, near a small clump of native bamboo, 28/IV/1970, *M.H. Homrich* 381 (ICN 6052).

Distribution in Southern Brazil: RS (Zeller & Dodge 1929, Homrich 1969).

Remarks: the species is characterized by its hypogeous to subhypogeous habit; globose to sugblobose basidiomata (up to 3.5 cm in diam.), and pluristratified, thick peridium (1–3 mm thick). In transversal section it is possible to observe the olive gray glebal mass immersed in a gelatinous matrix and a central cylindrical, unbranched columella. The columella has a spongy consistence that resembles the pseudostipe of a *Phallus* species (Homrich 1969). The gelatinous layer situated under the peridium is continuous, i.e. it is not interrupted by any cavities (as in *Hysterangium*) or sutures (as in *Protubera*). Basidiospores are 3–4 µm long, which differentiates it from *G. macrospora* (basidiospores 7–14 µm long), a macroscopically similar species described from Chile (Zeller 1939). The examined specimen is very poorly preserved and the only structure left is the thick peridium. Microscopically, it was possible to observe basidiospores and the pluristratified peridial layer typically composed of filamentous hyphae with large clamp connections. Other voucher specimens reported by Homrich (1969) could not be traced by us. Currently six species are recognized in the genus (Kirk et al. 2008), of which *G. thaxteri* is the only one reported from Brazil.

3. Lysuraceae

Lysurus cruciatus (Lepr. & Mont.) Henn., Hedwigia 41(Beibl.): (172), 1902. **Fig. 2C**

Description: Dring (1980), Cortez et al. (2011b).

Examined material: *Lysurus cruciatus* var. *cruciatus*: BRAZIL. RIO GRANDE DO SUL: Pinheiro Machado, 13/IX/2008, *M. Molz* (ICN 154342); Porto Alegre, IV/2009, *M.G. Rossoni* (ICN 154345); São Gabriel, Fazenda Cambará, 08/VII/2012, *L.P. Honaizer* 41 (ICN 175590); São Leopoldo, 1929, *B. Braun* (PACA-FR 14003); ibid., *B. Braun* (PACA-FR 14005); ibid., 1929, *J. Rick* (PACA-FR 14017); ibid., *B. Braun* (PACA-FR 19701); São Salvador [= Salvador do Sul], 1943, *J. Rick* (PACA-FR 20227); PARANÁ: Santa Mariana, Fazenda Palmeira, 31/III/1998, *A.A.R. de Meijer* 3560 (MBM). *Lysurus cruciatus* var. *nanus*: BRAZIL. RIO GRANDE DO SUL: Dom Pedro de Alcântara, Cova Funda, 00/XII/2007, leg. M.A. Reck (ICN 154343); Torres, Lagoa Itapeva, 27.IV.2007, leg. M.A. Reck (ICN 154344); São Leopoldo, *B. Braun* (PACA-FR 14004); ibid., *B. Braun* (PACA-FR 14008).

Distribution in Southern Brazil: RS (Rick 1961, Cortez et al. 2011b), PR (Meijer 2006).

Remarks: *L. cruciatus* is a widespread species and it is characterized by a whitish pseudostipe surmounted by 5-8, reddish to white arms. These arms are usually united at the tip, but they can become detached as the basidiome matures. The species used to be confused with the Asian and African species *L. gardneri* Berkeley, but the latter differs in having the arms with a sterile base and villose fertile parts [as illustrated by Dring (1980) and Dissing & Lange (1963)]. *Lysurus cruciatus* var. *nanus* Calonge & B. Marcos, characterized by smaller basidiomata and orange yellow arms, was reported from Southern Brazil by Cortez et al. (2011b). Molecular data showed that there is no significant difference between this variety and the typical one (Martín et al. 2005).

Lysurus periphragmoides (Klotzsch) Dring, Kew Bull. 35(1): 70, 1980. **Fig. 2D**

Description: Dring (1980), Domínguez de Toledo (1995), Cortez et al. (2011b).

Examined material: BRAZIL. RIO GRANDE DO SUL: Porto Alegre, Baia do Cego, 08/V/1971, *A. Schultz* (ICN 6220); Viamão, 13/VII/1965, *F.R. Schoenwald* (ICN 3722); ibid, 27/VII/1965, *F.R. Schoenwald* (ICN 3780); ibid, 19/XI/1965, *F.R. Schoenwald* (ICN 3787); São Leopoldo, 1907, *J. Rick* (PACA-FR 14818); ibid., 1905, J. Rick (PACA-FR 14819); ibid., 1930, *J. Rick* (PACA-FR 14820); ibid., 1933, *J. Rick* (PACA-FR 14821); ibid., 1946, *Steffen* (PACA-FR 20586); Porto Alegre, *B. Braun* (PACA-FR 14822).

Distribution in Southern Brazil: RS (Rick 1961, Cortez et al. 2011).

Remarks: the basidiome of this species is unique: a pseudostipe bearing a clathroid receptacle. Variation in colour may occur, but in Southern Brazil the pseudostipe is pinkish and receptacle dark red. *Simblum sphaerocephalum* Schltdl. is a synonym (Dring 1980). A whitish form, *S. gracile* var. *australe* Speg., was described from Argentina, but according to Dring (1980) it is a synonym of *L. periphragmoides* as well.

4. Phallaceae

Aporophallus subtilis Möller, Bot. Mitt. Trop. 7: 68, 147, 1895. **Fig. 2E**

Description: Möller (1895), Rick (1929).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1929, *B. Braun* (PACA-FR 15053); ibid., 1933, *B. Braun* (PACA-FR 13514).

Distribution in Southern Brazil: RS (Braun 1932, Rick 1929, 1961), SC (Möller 1895).

Remarks: *A. subtilis* is a small and very curious phalloid. The immature form (egg) is ellipsoidal, about 1 cm in diam. When mature, the pseudostipe is 3–5 cm in high × 0.5 cm in diam. The pseudostipe has a rugulose surface, reminiscent of *Mutinus*. The receptacle, however, is campanulate and clearly separated from the pseudostipe. The receptacle is thick, smooth, densely covered by the glebal mass and has no pore at the apex. The only illustration of the species available in literature (Möller 1895, reproduced by Lloyd 1909) depicts a basidiome in longitudinal section. Here we present and illustration of a dried basidiome (Fig. 2E) based on herbarium material (PACA-FR 15053). *Aporophallus* has been included in *Phallus* by many authors (e.g. Lloyd 1909, Kreisel 1996) and considered a doubtful taxon by Calonge (2005), but since it has characteristics so different from other *Phallus* species, we prefer to keep the genus separate.

Itajahya galericulata Möller, Bot. Mitt. Trop. 7: 79, 148, 1895. **Fig. 2F**

= *Alboffiella argentina* Speg., Anal. Mus. nac. Hist. nat. B. Aires 6: 183, 1899 [1898].

Description: Möller (1895), Braun (1932), Domínguez de Toledo (1995).

Examined material: BRAZIL. RIO GRANDE DO SUL: Porto Alegre, under native bamboo (taquara), 06/VI/1970, *F.R. Schoenwald* (ICN 6143); São Leopoldo, 1930, *B. Braun* (PACA-FR 19681).

Distribution in Southern Brazil: RS (Rick 1961), SC (Möller 1895).

Remarks: the species is characterized by massive eggs (3–8 cm in high), a white pseudostipe, a hemispheric, wig-like receptacle, with a flat apex that is covered with a calyptra (membranous cap). Braun (1932) reported the finding of a basidiome that reached 18 cm in high when fully expanded. The species seems to be common in some localities of Southern Brazil and Argentina, but it was also reported from Bolivia and USA (Long & Stouffer 1943). *Itajahya rosea* (Delile) E. Fisch., reported from Northeastern Brazil (Ottoni et al. 2010), is similar but with a pinkish pseudostipe.

Itajahya has been treated as a synonym of *Phallus* by some authors (e.g. Kreisel 1996, Calonge 2005, Ottoni et al. 2010), but recent molecular studies have shown evidence that it should be considered a distinct genus (Cabral et al. 2012).

Mutinus argentinus Speg., An. Soc. Cient. Argent. 24: 62, 1887. **Fig. 2G**

Description: Möller (1895), Reid (1977).

Examined material: BRAZIL. PARANÁ: Fênix, Parque Estadual de Vila Rica do Espírito Santo, 23/II/1996, *A.A.R. de Meijer* 3290 (MBM); Santa Mariana, Fazenda Guaicurus, 01/IV/1998, *A.A.R. de Meijer* 3561 (MBM); Céu Azul, Parque Nacional do Iguaçu, Trilha Manuel Gomes, 14/XII/2010, *L. Trierveiler-Pereira* 154; SANTA CATARINA, Florianópolis, Trilha de Naufragados, 28/X/2012, S. Urrea-Valencia 178 (FLOR 48789).

Distribution in Southern Brazil: PR (Meijer 2006, present study), SC (Möller 1895, present study).

Remarks: the species is characterized by a pinkish pseudostipe that tapers towards the apex, and a granulose, bright red fertile part with a small pore at the tip when mature. Spegazzini (1886) commented that *M. argentinus* is externally similar to the European species, *M. caninus* (Huds.) Fr., but its internal structure is more similar to *M. bambusinus* (Zoll.) E.Fisch., an Asian/African species. Indeed, *Mutinus argentinus* and *M. bambusinus* have been treated as the same species by different authors (Kobayasi 1938, Cunningham 1944, Liu 1984); however, Dring & Rose (1977) distinguished the two species and presented illustrations of both taxa. According to these authors, *M. bambusinus* has a pseudostipe with a marked sterile tip. The sterile tip of *M. bambusinus* was also illustrated by Petch (1926) and Demoulin & Dring (1975). *Mutinus mulleri* Fisch., originally described from Brazil, is a synonym of *M. argentinus* (Kobayasi 1938, Reid 1977). It is possible that many records of *M. bambusinus* from the Neotropics actually correspond to *M. argentinus* (Reid 1977, Gube & Piepenbring 2009). Möller (1895)'s photograph of *M. bambusinus* corresponds to *M. argentinus*.

Mutinus elegans (Mont.) E. Fisch. in Saccardo, Syll. Fung. 7: 13, 1888. **Fig. 2H**

Description: Cortez et al. (2008a).

Examined material: BRAZIL. RIO GRANDE DO SUL: Parque Estadual de Itapuã, 22/V/2004, *V.G. Cortez* 16/04 (ICN 139004); São Salvador [= Salvador do Sul], 27/III/1944, *J. Rick* (PACA-FR 22605, as *M. bambusinus*).

Distribution in Southern Brazil: RS (Cortez et al. 2008).

Remarks: this species is characterized by its orange pseudostipe and not markedly defined fertile part, although the apex of the pseudostipe might be a little darker (reddish). The pseudostipe has the same surface texture all over, while in *M. argentinus*, the fertile part is rugulose. The species is known to occur in North America (supposed to be native there) and in Europe (introduced, according to Szczepcka 1995). Its recent report from Brazil (Cortez et al. 2008) includes a color photograph of a basidiome growing on sandy soil.

Phallus aureolatus Trierv.-Per. & de Meijer**, nom. prov.** **Fig. 3A**

Description: Trierveiler-Pereira et al. (2014c).

Examined material: BRAZIL. PARANÁ: Antonina, Reserva Natural do Rio Cachoeira, 02/II/2005, *A.A.R. de Meijer* 4332 (MBM); Matinhos, 13/XI/2010, *J.M. Baltazar* LTP109 (ICN); SANTA CATARINA, Florianópolis, UCAD, 23/I/2007, *L. Trierveiler-Pereira* (voucher not preserved).

Distribution in Southern Brazil: PR (Meijer 2006, as *P.* cf. *merulinus*; present study), SC (present study), SP (Colombini & Miranda 2006, as *P. indusiatus*)

Remarks: the species is characterized by a pinkish volva, whitish pseudostipe, merulioid receptacle with a large pore at the apex (when fully mature) and long indusium with large meshes. Mycelial strands at the base of the volva are abundant and have violet pigments. The receptacle is usually surmounted by a narrow ring of white, sterile tissue, at first imperforate, and then perforate. The species is morphologically similar to *P. merulinus* (Berk.) Cooke [native from tropical Asia and Oceania, introduced in the Neotropics (Kreisel 1996, Cheype

2010)] and *P. atrovolvatus* Kreisel & Calonge [known from the Neotropics (Calonge et al. 2005, Cheype 2010)], but both have a short and more delicate indusium with narrower mashes. Molecular data proved that *P. areolatus* is phylogenetically distant from *P. merulinus* (Trierveiler-Pereira et al., unpubl. data). A beautiful color photograph of the species is presented by Colombini & Miranda (2006), from the State of São Paulo (Northeastern Brazil).

Phallus callichrous (Möller) Lloyd, Mycol. Writ.(7): 6, 1907.

Description: Möller (1895), Lloyd (1907b).

Examined material: BRAZIL. PARANÁ: Antonina, Reserva Natural do Rio Cachoeira, 14/I/2004, *A.A.R. de Meijer* 4307 (MBM).

Distribution in Southern Brazil: SC (Möller 1895), PR (Meijer 2006).

Remarks: the species is morphologically very similar to *Phallus indusiatus*, with the only difference that it has an orange receptacle (reason why we do not provide illustration of the species). According to Kreisel & Hausknecht (2009), the receptacle color might also be pink. In the examined specimen, the indusium was white when fresh and became pale orange when dried. *P. callichrous* has been considered a synonym of *P. indusiatus* by some authors (Lloyd 1909, Calonge 2005, Cheype 2010), but here we follow Kreisel (1996) and Kreisel & Hausknecht (2009), who have a rather narrower species concept. *Phallus callichorus* is known from tropical and subtropical Africa and Asia, Australia, and South America (Brazil) (Kreisel 1996).

Phallus duplicatus Bosc, Mag. Gesell. naturf. Freunde, Berlin 5: 86, 1811. **Fig. 3B**

Description: Cortez et al. (2011a), Coker & Couch (1928), Smith (1951).

Examined material: BRAZIL. RIO GRANDE DO SUL: Viamão, Parque Estadual de Itapuã, 22/V/2004, *V.G. Cortez* 16/4 (ICN 154389); São Leopoldo, *B. Braun* (PACA-FR 15052).

Distribution in Southern Brazil: RS (Cortez et al. 2011a).

Remarks: the species is characterized by a whitish pseudostipe, reticulate receptacle and short indusium. Its morphology is very similar to *P. indusiatus*, except for the length of the indusium. According to Cortez et al. (2011a), the examined specimen has a yellowish white indusium, while specimens of *P. duplicatus* from the USA have a rosy pink indusium (Coker & Couch 1928). *P. indusiatus* is a common species in the Neotropics, while *P. duplicatus* seems to be restricted to subtropical and temperate areas (Guzmán et al. 1990).

Phallus campanulatus Berk., Ann. Mag. nat. Hist., Ser. 1 9: 446, 1842. **Fig. 3C**

= *Phallus granulosodenticulatus* B. Braun, Relat. Gin. Anch. 1932: 12, 1932, **syn. nov.**

Description: Berkeley (1842), Braun (1932), Cortez et al. (2011a).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1929-1932, *B. Braun* (PACA-FR 15042, 15043, 15044, 15045, 15046, 15047, 15048, 15050); Viamão, Estância Grande, 21/VII/1965, *F.R. Schoenwald* (ICN 3736); Santa Maria, UFSM, 16/VI/2008, *V.G. Cortez* 117/08 (ICN 154390); Viamão, Parque Estadual de Itapuã, on sand dunes, 18/VI/2011, *L. Trierveiler-Pereira* 255 (ICN); ibid., on sandy soil with grass, 18/VI/2011, *L. Trierveiler-Pereira* 256 (ICN).

Distribution in Southern Brazil: RS (Braun 1932, Cortez et al. 2011a – as *P. granulosodenticulatus*).

Remarks: The species is characterized by a whitish pseudostipe with small perforations, an off-white, perforate, narrow campanulate, wrinkled to minutely rugulose receptacle with a toothed margin. The pore at the receptacle apex is so prominent that a ring-like structure can be observed around it. The basidiospores are large, 4–6 × 2.5–3 µm (from LTP 255 and 256). Basidiome morphology is similar to *P. aurantiacus* Mont. and *P. rubicundus* (Bosc) Fr., except for the presence of orange and reddish pigments in these two species.

Berkeley's iconotype depicts a very curious cup-shaped remnant of the volva. According to Berkeley (1842), this structure corresponds to the inner volva, whilst the outer volva was badly damaged. Schlechtendal (1861-62), who also analyzed the type specimen, stated that the peridium was too much damaged to describe it, and in his opinion, Berkeley's illustration of the volva was very unusual for a *Phallus* species. Curiously, the same cup-shaped structure is clearly observed in the volva of *P. granulosodenticulatus* (Braun 1932, Pl. XI, Fig. 2g). Interestingly, this cup-shaped inner volva was also observed by Braun (1932) in specimens of *Itajahya galericulata*.

When Braun (1932) described his new species, he already stated that it could be the same as *P. campanulatus*, collected in sand dunes in Uruguay. We conclude that the two species are synonyms based on characteristic morphological features, geographical proximity and species habitat (sand dunes and sandy soil).

Phallus campanulatus was also reported from Argentina (Dominguez de Toledo 1995, Spegazzini 1886, 1908), but there the species was found to have a smooth receptacle. These identifications seems to be correct, since the illustration presented by Spegazzini (1908, Fig. 3d) has the characteristic large pore at the apex, and basidiospores are described with large dimensions (5–6 µm in length) by Dominguez de Toledo (1995) and Spegazzini (1908).

Phallus indusiatus Vent., Mém. Inst. Natl. Sci., Sci. Math. 1: 520, 1798. **Fig. 3D**

Description: Guzmán et al. (1990), Baseia et al. (2006), Magnago et al. (2013).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1905, *J. Rick* (PACA-FR 13139); ibid., *B. Braun* (PACA-FR 13519); São Salvador [= Salvador do Sul], 1945, *J. Rick* (PACA-FR 15040); Guaíba, Balneário Alvorada, II/1969, *I.A. Mensch* (ICN 5815); São Francisco de Paula, FLONA, 21/IV/2007, *V.G. Cortez* 50/7 (ICN 154391); Dom Pedro de Alcântara, 11/VI/2010, *L. Trierveiler-Pereira* 81 (ICN); ibid., 12/VI/2010, *L.*

Trierveiler-Pereira 85 (ICN); Porto Alegre, Campus do Vale, 11/IV/2011, *J.M. Baltazar* LTP201 (ICN); SANTA CATARINA: Florianópolis, Campus da UFSC, 28/II/1985, *C. Loguercio-Leite* (FLOR 10072); ibid., XI/2006, *L. Trierveiler-Pereira* (FLOR 31909); ibid., *A.C. Magnago* (FLOR); ibid., Capoeiras, lawn in garden, 20/III/2013, *L. Trierveiler-Pereira & A.E. Pereira* LTP311 (FLOR 48788); PARANÁ: Antonina, Reserva Natural do Rio Cachoeira, 02/XI/2006, *A.A.R. de Meijer* 4348 (MBM).

Distribution in Southern Brazil: RS (Rick 1961), SC (Magnago et al. 2013), PR (Meijer 2006).

Remarks: the species is characterized by a whitish pseudostipe, a reticulate receptacle and the long indusium. Mycelial strands are often abundant and have purplish pigments. The species is very common in the Neotropics, but is also known from tropical and subtropical Africa and Asia, the Southern Pacific and Australia (Kreisel 1996). *Phallus moelleri* Lloyd (= *Dictyophora phalloidea* sensu A. Möller) is considered a synonym by many authors (e.g. Lloyd 1909, Guzmán et al. 1990, Calonge 2005), but Kreisel (1996) considers it distinct due to insertion of the indusium beneath the apex of the receptacle and the lack of pinkish/purplish pigments in volva and mycelial strands. We have not yet seen a specimen with such characteristics. Meijer (2010) reported from Diamante do Norte, in the northwestern tip of Paraná, a single collection (*A.A.R. de Meijer* 3364) of *P. indusiatus* var. *roseus* Lloyd. His material differs only from the type variety in the color of the indusium: pale orange instead of white (cf. Kreisel and Hausknecht 2009).

Sphaerophallus Trierv.-Per., **gen. nov.**

Mycobank MB ######

Immature basidiomata depressed-globose, tuberculate, up to 5.5 cm in diam., brownish. Mature basiomata up to 7 cm high, inner volva yolk-yellow, pseudostipe whitish, with elongated pores and dilacerate edges, receptacle spherical to depressed-globose at first, later campanulate, surface cracked, with a distinct pore at the apex when mature. Growing on rotten wood.

Type species: *Sphaerophallus glutinolens* (Möller) Trierv.-Per.

Etymology: sphaero- (Latin), referring to the spherical shape of the receptacle.

Sphaerophallus glutinolens (Möller) Trierv.-Per., **comb. nov.** **Figs. 3E, 4**

Mycobank MB ######

Basionym: *Ithyphallus glutinolens* Möller, Bot. Mitt. Trop. 8: 100, 148, 1895.

Description: Möller (1895), Trierveiler-Pereira et al. (2009a).

Examined material: BRAZIL. SANTA CATARINA: Santo Amaro da Imperatriz, 12/V/2007, *L. Trierveiler-Pereira* (FLOR 77079, UFRN 834); Itapoá, Reserva Volta Velha, 18/XI/2012, *Urrea-Valencia* 205 (FLOR 47632); RIO GRANDE DO SUL: São Leopoldo, 1931, *Flach* (PACA-FR 15041).

Distribution in Southern Brazil: RS (Braun 1932, Rick 1961), SC (Möller 1895, Trierveiler-Pereira et al. 2009a).

Remarks: *S. glutinolens* has peculiar morphological features that differentiate it from other *Phallus* species. The immature form is depressed-globose and tuberculate, resembling immature forms of *Clathrus* species. The conspicuous reticulations on the tuberculate surface mark the places where peridial sutures are inserted. The immature form has a membranous external layer with a light brown surface covered by small dark scales. This external layer is easily detached from the inner layer, which is yolk-yellow and has a gelatinous surface.

When expanding from the egg, the receptacle becomes depressed-globose to spherical. The glebal mass is not uniformly spread over the receptacle surface, but confined to juxtaposed irregular plates, which create the view of a cracked surface. In this stage, a depression appears at the top of the receptacle, which will then open into a pore. Next, the receptacle becomes campanulate and the apical pore becomes dilacerate along the margin. The pseudostipe has elongated pores with a dilacerate margin, and in young specimens, it shows a ring marking the point where the receptacle had been connected to the immature pseudostipe. The spores are ellipsoid to cylindrical, 3.5–4.5 × 1.5–2 µm, olive brown. Wright (1960) reported the species from Argentina and described the spores as much larger, viz. 5.1–6.9 µm long.

Staheliomyces cinctus E. Fisch., Mitt. naturf. Ges. Bern: 142, 1921 [1920]. **Fig. 2I**

Description: Sáenz & Nassar (1982), Baseia et al. (2006), Magnago et al. (2013).

Examined material: BRAZIL. PARANÁ: Antonina, Morro do Bom Brinquedo, 22/I/2008, *T. Lockwood & A.A.R. de Meijer* 4388 (MBM).

Distribution in Southern Brazil: PR (present study)

Remarks: this species, the only one described for the genus, is characterized by a white, perforate pseudostipe, with an annular constriction over which the glebal mass is spread. It is a common species in the Neotropics. One specimen kept at FLOR (10402) identified as *Staheliomyces* sp. corresponds to a *Phallus* species, probably *P. campanulatus*. This is the first report of *S. cinctus* from Southern Brazil.

5. Protophallaceae

Protubera maracuja Möller, Bot. Mitt. Trop. 7: 10, 145, 1895. **Fig. 2A**

Description: Furtado & Dring (1967), Trierveiler-Pereira et al. (2014a).

Examined material: BRAZIL. PARANÁ: Curitiba, Parque Bariguí, on humus, 28/VIII/1996, *A.A.R. de Meijer* 3442 (MBM); Parque Barreirinha, on soil, 13/III/1992, *A.A.R. de Meijer* 2183 (MBM); Convento Solitude, on humus, 04/III/1995, *A.A.R. de Meijer* 3032 (MBM);

Parque Regional do Iguaçu, on humus, 23/IV/1995, *A.A.R. de Meijer* 3055 (MBM); Morretes, Parque Marumbi, on humus, 07/IX/1997, *A.A.R. de Meijer* 3461 (MBM); ibid., on decaying wood, 11/IV/1992, *A.A.R. de Meijer* 2237 (MBM); Guaraqueçaba, Tagaçaba Porto da Linha, on humus, 06/VIII/2009, *D. Schause & A.A.R. de Meijer* 4466 (MBM); Reserva Natural Salto Morato, on decaying wood, 14/XII/2011, *L. Trierveiler-Pereira* 284 (ICN); SANTA CATARINA: Florianópolis, Morro da Lagoa da Conceição, on soil, 10/V/1986, J. Furlani (FLOR 10312); ibid., on soil, 19/XII/2011, *M. Jaeger* 94 (FLOR); ibid., on soil, 16/II/2012, *M. Jaeger* 133 (FLOR); RIO GRANDE DO SUL: Porto Alegre, Morro Santana, on decaying wood, 17/V/2011, *L. Trierveiler-Pereira* 219 (ICN); ibid., *L. Trierveiler-Pereira* 220 (ICN 168980).

Distribution in Southern Brazil: RS (Rick 1961), SC (Möller 1895), PR (de Meijer 2006, Trierveiler-Pereira et al. 2013, 2014a).

Remarks: the species is characterized by an epigeous, sequestrate habit; whitish to light brownish, grooved to smooth peridium and the presence of robust mycelial strands at the base. In transversal section are seen the elongated, olive to greenish brown glebal plates immersed in a gelatinous matrix. A columella is absent and the gelatinous layer is interrupted by sutures (sterile plates). Basidiomata can develop either on soil or on decaying wood. *Protubera maracuja* is separated from the other species of the genus by the combination of a pseudoparenchymatous outer peridium and the presence of large crystals forming rosette patterns on the inner peridium. Currently six species are accepted in the genus (Trierveiler-Pereira et al. 2014a) and *P. maracuja* is the only one reported from Brazil. *Protubera jamaicensis* (Murrill) Zeller, originally described from Jamaica and also known from Costa Rica and Argentina (Trierveiler-Pereira et al. 2013, Wright 1960), is expected to occur in Brazil.

Excluded species and doubtful records

Clathrus preussii Henn., Fungi camerun. 1: 108, 1897.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1930, J. Rick (PACA-FR 12628).

Remarks: *C. preussii* is a rare African species characterized by a whitish receptaculum with short teeth surrounding the margins of the meshes (Dring 1964). The examined collection doesn't have the characteristic toothed margin and could correspond to *C. chrysomycelinus* or the albino form of *C. crispus*. The species has been reported from Brazil only once, but we believe that it does not occur in Brazil, since most members of *Clathrus* are known to have a strong continental endemism (Dring 1980, Lécuru et al. 2013).

Clathrus pseudocrispus Lloyd, Mycol. Notes (Cincinnati) (3): 59, 1909.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1929, *B. Braun* (PACA-FR 12629); ibid., 1930, *B. Braun* (PACA FR- 12621); ibid., 1930, *J. Rick* (PACA-FR 12622).

Remarks: According to Dring (1980), *C. pseudocrispus* it is synonymous with *C. crispus*. Two examined collections at PACA seems to correspond to the albino form of *C. crispus* (FR 12629 and 12622), and the other one, the typical form (FR 12621).

Clathrus pusillus Berk., London J. Bot. 4: 67, 1845.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, *B. Braun* (PACA-FR 19683).

Remarks: according to Lloyd (1909), the species was described from Australia and is characterized by a bright ruby red receptacle with subequal meshes above, elongated meshes below, and wrinkled branches (Lloyd 1909). Dring (1980) treated the species as *Colus pusillus* (Berk.) Reichert and reports it from the Australian continent only. Examined collection from PACA is badly damaged and it cannot be identified.

Lysurus borealis (Burt) Henn., Hedwigia 41(Beibl.): (172), 1902.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, *B. Braun* (PACA-FR 1907).

Remarks: Six specimens of. *L. borealis* were mentioned by Rick (1961; PACA-FR 14015, 14002, 14018, 14019, 19691, 16692) but from these we could find at PACA only one specimen, which corresponds to *L. cruciatus*. The synonymy of *L. borealis* with *L. cruciatus* was proposed by Dring (1980).

Lysurus mokusin (L.) Fr., Syst. mycol. (Lundae) 2(2): 288, 1823.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, *B. Braun* (PACA-FR 14004, 14005, 14009).

Remarks: the examined collection identified as *L. mokusin* contains small specimens of a *Lysurus* species. None of the specimens shows the main characteristic that defines *L. mokusin*: a pseudostipe with deeply grooved sides divided lengthwise by ribs. Other collection of *L. mokusin* reported by Rick (1961, PACA-FR 14010, 14011) were not found at PACA. *Lysurus mokusin* is a common species in Asia, Australia and USA (Dring 1980).

Lysurus sanctae-catharinae (E. Fisch.) Henn., Hedwigia 41(Beibl.): (172), 1902.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1929, *J. Rick* (PACA-FR 14017).

Remarks: the examined material corresponds to *L. cruciatus*. The synonym of *L. sanctae-catharinae* with *L. cruciatus* was already proposed by Dring (1980). The other collection reported by Rick (1961, PACA-FR 14001) was not found at PACA.

Lysurus woodii (MacOwan) Henn., Hedwigia 41(Beibl.): (172), 1902.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, *B. Braun* (PACA-FR 14008).

Remarks: the examined material corresponds to *L. cruciatus* var. *nanus*. *Lysurus woodii* was originally described from Africa and is characterized by small basidiomata (Van der Bijl 1921). The synonym of *L. woodii* with *L. cruciatus* was proposed by Dring (1980).

Mutinus bambusinus (Zoll.) E. Fisch., Ann. Jard. Bot. Buitenzorg 6: 30, 1886.

Reported from: RS (Rick 1961), SC (Möller 1895).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Salvador [= Salvador do Sul], 27/III/1944, *J. Rick* (PACA-FR 22605).

Remarks: *Mutinus bambusinus* was originally described from Java and was distinguished from *M. caninus* by the spore-bearing portion of the pseudostipe being long and tapering, and reddish to dirty purplish (Fischer 1898-99). It is possible that some of the records from Central and South America under this name actually correspond to *M. argentinus* (Gube & Piepenbring 2009), a species described from Argentina by Spegazzinii (1886) and also found in Southern Brazil (see above). Illustrations of both species were presented by Dring & Rose (1976), and a color photo of *M. bambusinus* was presented by Demoulin & Dring (1975). The photograph of *M. bambusinus* presented by Möller (1895) depicts *M. argentinus*. Examined collection at PACA (FR 22605) registered as *M. bambusinus* corresponds to *M. elegans*.

Mutinus borneensis Ces., Atti Accad. Sci. fis. mat. Napoli 8: 13, 1879.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1929, *J. Rick* (PACA-FR 13513); ibid., 1933, B. Braun (PACA-FR 13514).

Remarks: the two collections at PACA cited by Rick (1961) are noted as *Jansia boninensis*. Collection nº 13513 corresponds to *Lysurus cruciatus*, and nº 13514 corresponds to *Aporophallus subtilis*.

Mutinus rugulosus (E. Fisch.) Rick, Broteria, Sér. Biol. 21: 141, 1924.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, *B. Braun* (PACA-FR 13512).

Remarks: Rick (1924) combined *Phallus rugulosus* Lloyd in *Mutinus* and stated: "habit similar to *Phallus impudicus*, but smaller and odor less strong; receptacle free and wrinkled" (our translation). It is difficult to understand why Rick transferred to *Mutinus* a species that he knew that had the receptacle separate from the pseudostipe. Later, Rick (1929) referred to *M. rugulosus* as being a species similar to *M. bambusinus* but with larger and whitish pseudostipe. It is therefore impossible to make a conclusion about Rick's concept of the species. The voucher specimen kept at PACA is noted as "*Jansia rugosa?*" and corresponds to a small *Lysurus* specimen.

Laternea triscapa Turpin, Dict. Sci. Nat. 25: 248, 1822.

Reported from: RS (Rick 1961)

Remarks: We couldn't find specimens of *L. triscapa* on Rick's collection at PACA and no voucher is mentioned in his paper (Rick 1961). Judging the description presented by the author, the specimen could indeed be a *Laternea* species, since the gleba is described as separate from the receptacle and hanging from the apex. *Laternea triscapa* is known from the Neotropics, so it might occur in Southern Brazil.

Pseudocolus rugulosus (Kurz) Lloyd, Synopsis of the known phalloids(7): 52, 1909.

Reported from: RS (Rick 1961).

Examined material: BRAZIL. RIO GRANDE DO SUL: São Leopoldo, 1930, *J. Rick* (PACA-FR 21358).

Remarks: The collection reported by Rick (1961, PACA-FR 14978) was not found at PACA. One specimen identified as *Pseudocolus rugulosus* (PACA-FR 21358) actually corresponds to *Blumenavia rhacodes*.

Discussion

To date, we accept 29 phalloid species as occurring in Brazil: *Abrachium floriforme*, *Aseroë rubra*, *Blumenavia angolensis*, *B. rhacodes*, *Clathrus chrysomycelinus*, *C. columnatus*, *C. crispus*, *C. cristatus*, *Laternea dringii*, *L. pusilla*, *Pseudocolus garciae*, *Gelopellis thaxteri*, *Lysurus cruciatus*, *L. periphragmoides*, *Aporophallus subtilis*, *Itajahya galericulata*, *I. rosea*, *Mutinus argeninus*, *M. caninus*, *M. elegans*, *Xylophallus xylogenus*, *Phallus areolatus*, *P. callichrous*, *P. duplicatus*, *P. campanulatus*, *P. indusiatus*, *Sphaerophallus glutinolens*, *Staheliomyces cinctus*, and *Protubera maracuja*.

Phallus rubicundus (Bosc.) Fr., reported from Southeastern Brazil (Bononi et al. 1984), is a common species in tropical areas but since its voucher specimen is completely degraded and there are no photographs are available, we cannot ascertain its occurrence in Brazil. *Phallus impudicus* L., reported from the same region (Averna-Saccá 1923), probably concerns a misidentification, as it is basically a temperate species (Kreisel 1996) and no voucher is available. The genus *Ileodictyon* is apparently absent from the country and the single record from Northeastern Brazil (Baseia et al. 2006) corresponds to *C. chrysomycelinus*. The occurrence in Brazil of the Neotropical *Laternea triscapa* still needs confirmation; in Southern Brazil no specimens were found in the field or in the herbaria, and the collection described from Northeastern Brazil (Baseia et al. 2006) seems to concern *L. dringii*, due to its basidiome color, size and habitat.

Species currently only known from Northeastern Brazil (region with a real tropical climate) are: *C. cristatus*, *I. rosea*, *L. dringii*, and *X. xylogenus*. Examples of species so far only known from the Southern and Southeastern regions are: *Blumenavia angolensis*, *I. galericulata*, *P. garciae*, *M. elegans*, *G. thaxteri*, *S. glutinolens* and *A. subtilis. Blumenavia angolensis* was reported from Northeastern Brazil (Rodrigues & Baseia 2013), but illustrations indicate that it corresponds to *B. rhacodes*.

Flocomutinus sp., reported by Wright (1960) from Bolivia (Cerro Uchumachi, circa 16º S latitude), probably corresponds to *X. xylogenus*. If so, it is likely that the species also occurs in the Central-Western region from Brazil. *Laternea pusilla,* which in this study is described for the first time from Brazil, may occur in cloud forests all over subtropical-tropical Brazil.

We believe that still more phalloid species will be discovered Southern Brazil. Through studies on this group – providing good descriptions and illustrations or photographs – will eventually throw a better light on the pattern of species distribution in the Neotropics.

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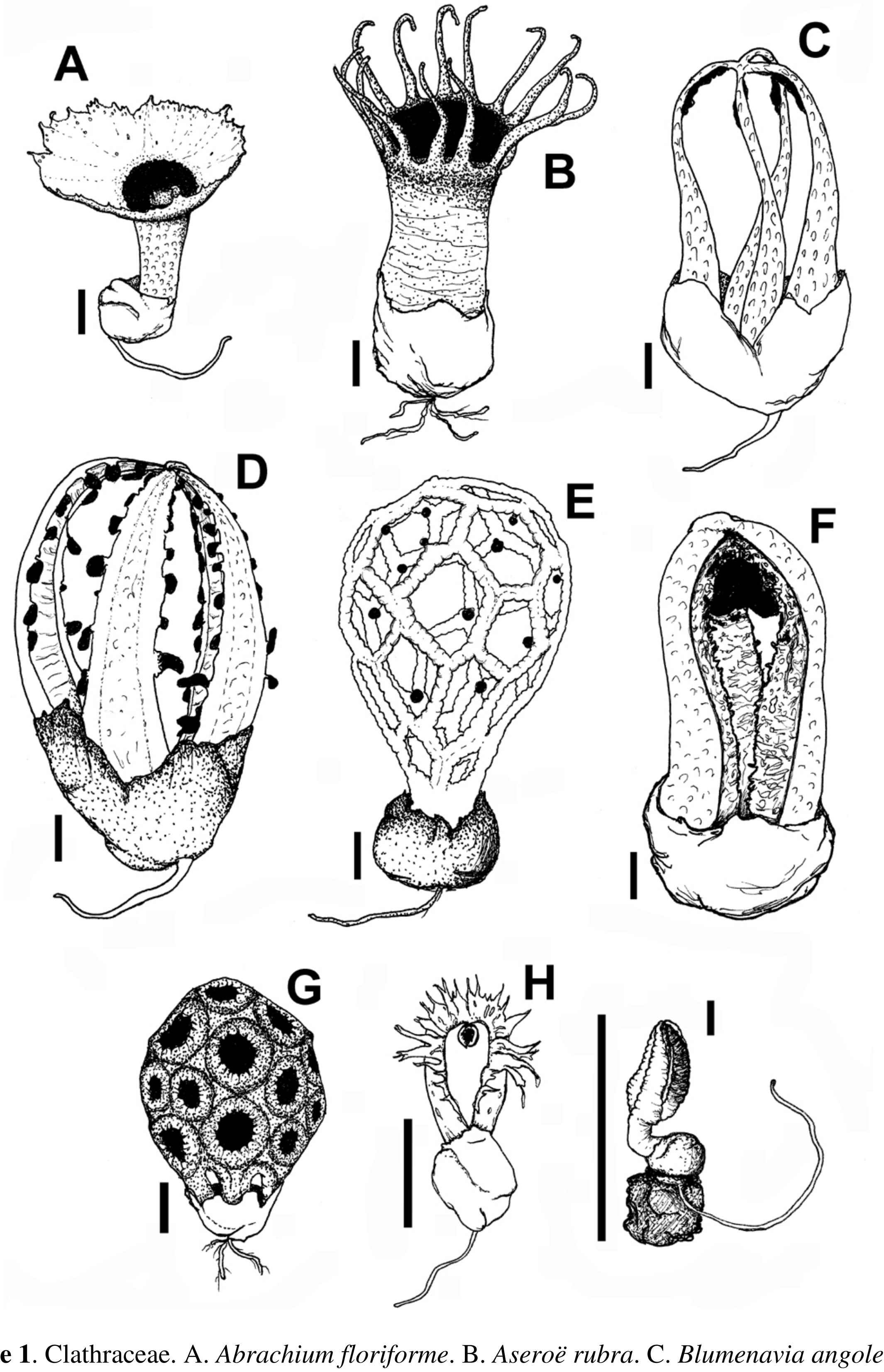

Figure 1. Clathraceae. A. *Abrachium floriforme*. B. *Aseroë rubra*. C. *Blumenavia angolensis*. D. *B. rhacodes*. E. *Clathrus chrysomycelinus*. F. *C. columnatus*. G. *C. crispus*. H. *Laternea pusilla*. I. *Pseudocolus garciae*. Bars = 1 cm.

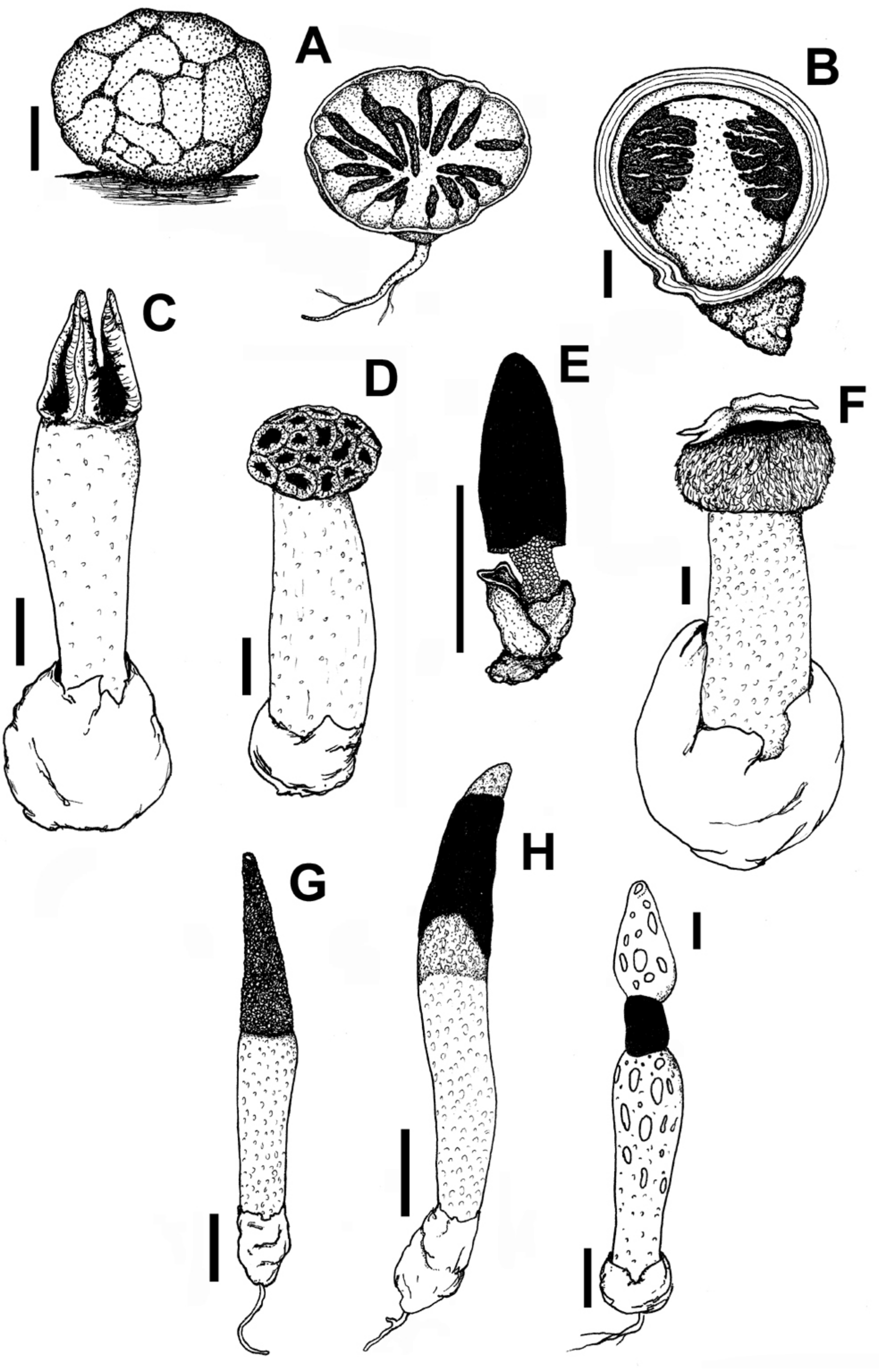

Figure 2. Protophallaceae, Claustulaceae, Lysuraceae and Phallaceae. A. *Protubera maracuja*. B. *Gelopellis thaxteri*. C. *Lysurus cruciatus*. D. *L. periphragmoides*. E. *Aporophallus subtilis*. F. *Itajahya galericulata*. G. *Mutinus argentinus.* H. *M. elegans.* I. *Staheliomyces cinctus*. Bars = 1 cm.

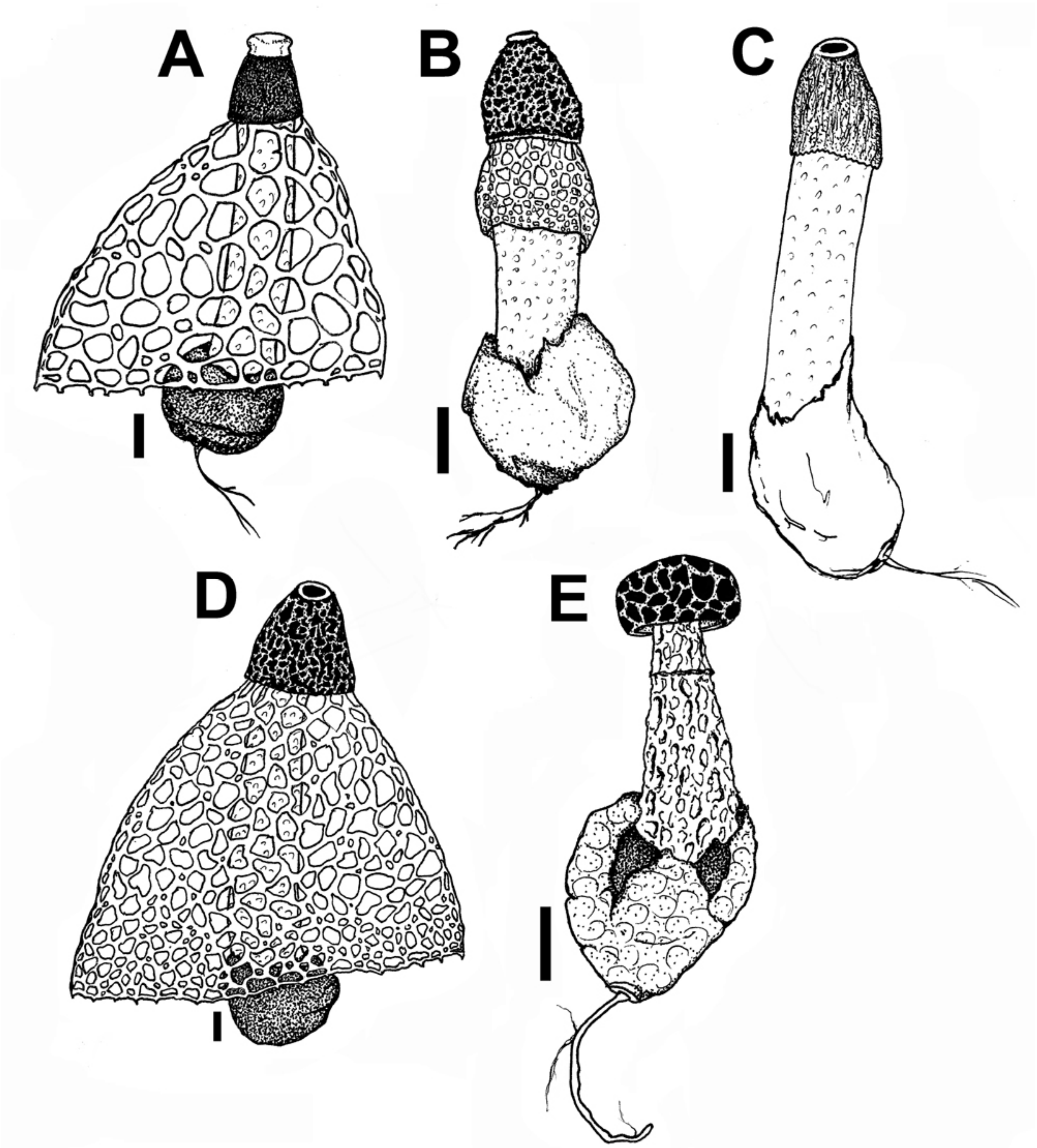

Figure 3. Phallaceae. A. *Phallus aureolatus*. B. *P. duplicatus*. C. *P. campanulatus*. D. *P. indusiatus*. E. *Sphaerophallus glutinolens*. Bars = 1 cm.

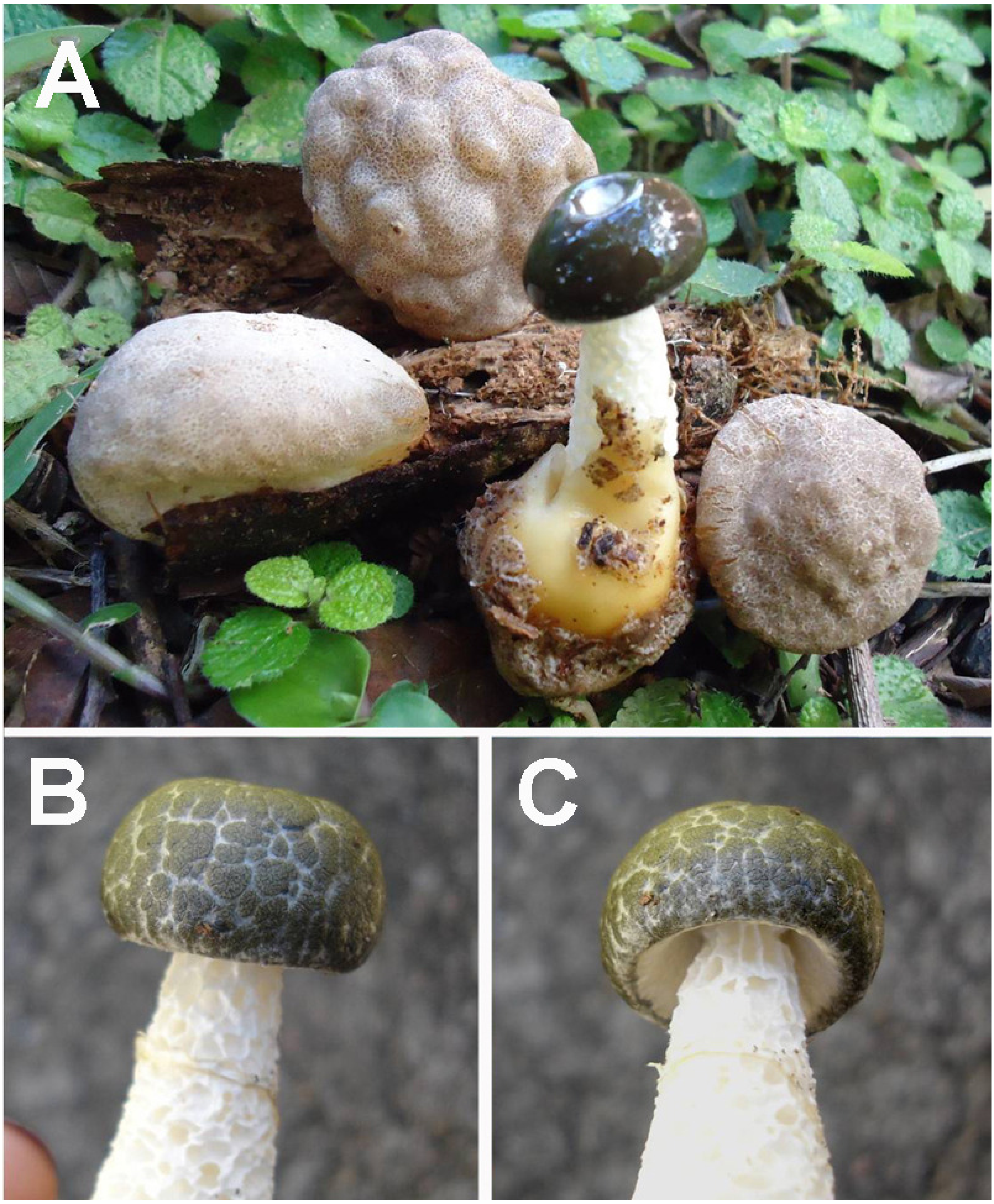

Figure 4. *Sphaerophallus glutinolens*. A. Young basidiome and immature forms (FLOR 47632). B, C. Detail of the receptacle (FLOR 77079). Photograph's credits: A by S. Urrea-Valencia; B and C by L. Trierveiler-Pereira.

4.10. MANUSCRITO VI

Trierveiler-Pereira L, Wilson AW, Silveira RMB, Domínguez LS. 2013. Costa Rican gasteromycetes (*Basidiomycota*, *Fungi*): *Calostomataceae*, *Phallaceae* and *Protophallaceae*. **Nova Hedwigia** 96(3–4): 533–544.

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Nova Hedwigia 2013 Vol. 96 issue 3–4, 533–544 **Article**

Costa Rican gasteromycetes (Basidiomycota, Fungi): Calostomataceae, Phallaceae and Protophallaceae

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With 5 figures

Abstract: Costa Rican specimens of Calostomataceae, Phallaceae and Protophallaceae (gasteroid basidiomycetes) kept at Herbarium F were examined and identified. Eleven species belonging to seven genera were recognized: *Calostoma cinnabarinum*, *C. lutescens* (Calostomataceae), *Aseroë rubra*, *Clathrus columnatus*, *Laternea pusilla*, *L. triscapa*, *Ligiella rodrigueziana*, *Phallus indusiatus*, *P. duplicatus* (Phallaceae), *Protubera maracuja* and *P. jamaicensis* (Protophallaceae). *Calostoma lutescens* is described with larger spores than those reported for the species, but molecular data confirmed the species identification. The occurrence of *Protubera* species in Costa Rica is reported for the first time.

Key words: Boletales, Central America, fungal taxonomy, Neotropical mycota, Phallales.

Introduction

Although the first fungal collections from Costa Rica are from the end of the nineteenth century (Rossman et al. 1998), little attention was given to gasteroid basidiomycetes until the middle of the twentieth century. Garner (1956) believed that his reports of gasteromycetes [*Lycoperdon subincarnatum* Peck and *Cyathus stercoreus* (Schwein.)

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4.11. MANUSCRITO VII

Trierveiler-Pereira L, Silveira RMB. 2012. On the *Geastrum* species (*Geastraceae*, *Basidiomycota*) described by Rick. **Phytotaxa** 61: 37–46.

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Article

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On the *Geastrum* species (Geastraceae, Basidiomycota) described by Rick

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Abstract

Geastrum lloydianum, *G. trichiferum* and *G. violaceum*, described from Brazil by Johannes Rick at the beginning of the 20th century, are redescribed, lectotypified and compared with recent collections. Two recently described species, *viz*. *G. hirsutum* from Brazil and *G. episcopale* from Argentina, are regarded as synonyms to *G. trichiferum* and *G. violaceum*, respectively. Basidiomata are illustrated in color. SEM images show basidiospores, and line drawings show hyphae and basidia.

Key words: earth-star fungi, Gasteromycetes, fungal taxonomy, lectotypification, morphology, South American mycota

Introduction

Johannes Rick (1869–1946) was an Austrian Jesuit who lived in southern Brazil (State of Rio Grande do Sul) since the beginning of the 20th century until his death (Fidalgo 1962). Rick came to Brazil in 1903 (Rabuske & Rambo 2004), although other dates are also presented in literature, e.g. 1902 (Fidalgo 1962) and 1904 (Torrend 1918). According to Rick himself, he came to Brazil as a naturalist to study the local fungal flora (Rabuske & Rambo 2004).

While in Brazil, Rick was an enthusiastic collector and a passionate mycologist. His fungal collection kept at Herbarium PACA is one of the most important in South America and currently compromises more than 4,500 specimens (Mauhs 2000). Moreover, many more Brazilian specimens collected by Rick are distributed among herbaria across the world, such as B, BPI, CUP, FH, IAC, IACM, K, MICH, R, RB, S, SFPA and SI (Rick 1928, Stafleu & Cowan 1983).

Geastrum Pers., in the family Geastraceae, is one of several genera of Gasteromycetes where the exoperidium opens in a stellate way (cf. Sunhede 1989) and species of the genus are frequently found in tropical and subtropical forests in South America. During his studies in Brazil, Rick described three new species of *Geastrum*, *viz*. *G. lloydianum*, *G. trichiferum*, and *G. violaceum*. Since the review of types is urgent in our times (Trierveiler-Pereira & Romero 2011), the main goal of this work was to revisit, redescribe, lectotypify and illustrate the *Geastrum* species described by Rick.

Material and Methods

Macro- and micromorphological analyses of fungal specimens were conducted at Herbarium PACA and the Mycological Laboratory, Depto. Botânica, UFRGS. Colors were coded according to Kornerup & Wanscher (1978) and referred to dry material, unless otherwise stated. For microscopical analysis, portions of the peridium and the gleba were mounted in 5% KOH solution and spore measurements included surface ornamentation. Morphological descriptions and line drawings were based on types and other additional material. Line drawings were made with the aid of a camera lucida.

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4.12. MANUSCRITO VIII

Trierveiler-Pereira L, Silveira RMB. 2014. *Geastrum reinkingii* (*Geastraceae*, *Agaricomycetes*) reconsidered. **Mycotaxon**: a ser submetido.

Geastrum reinkingii (Geastraceae, Agaricomycetes) reconsidered

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ABSTRACT — *Geastrum reinkingii*, originally described based on specimens from Honduras, is a species name that rarely appeared in mycological literature. A few decades after its publication, the species was reduced to a synonym of *G. lloydianum* and it was since been forgotten. New morphological studies based on type specimens revealed that *G. reinkingii* is a good species and it is probably well distributed in tropical and subtropical forests in America. Here we present a modern description and color photographs of the species, and a comparison with the closest morphological South American species: *G. glaucescens*, *G. harriotii*, *G. lloydianum*, and *G. setiferum*.

KEY WORDS — earthstar fungi, *Geastrales*, *Phallales*, Neotropical mycobiota.

Introduction

Geastrum is a widespread, huge genus with numerous taxonomical problems. Many authentic species have been treated as synonyms (Trierveiler-Pereira & Silveira 2012, Zamora et al. 2013), while some "famous" species reported worldwide (e.g. *G. fimbriatum* Fr., *G. triplex* Jungh., *G. saccatum* Fr.) probably correspond to complexes of species, as demonstrated by Kasuya et al. 2012 for *G. triplex*.

Several *Geastrum* species collected in tropical and subtropical areas of America were described by C.G. Lloyd, who during many years received specimens from contributors. The type specimen of *G. reinkingii* Lloyd was collected at the beginning of the 20th century in Honduras and its original publication has no full description of the specimen (Lloyd 1924), but instead, briefly differentiate it from *G. hariotii* Lloyd. Later, the name also appeared in Rick's posthumous list on the gasteroid species found in southern Brazil (Rick 1961) with no additional information. Finally, the species was considered a synonym of *G. lloydianum* Rick by Ponce de Leon (1968) on his revision of the family Geastraceae.

Recent morphological studies based on *Geastrum* type specimens revealed that *G. reikingii* is different from *G. lloydianum* and it should be considered an authentic species. Here we present a modern description and color photographs of the species.

Materials & methods

Fungal specimens from Herbaria BPI, ICN, LPS, MBM, PACA, and URM, were examined macro and microscopically. Macroscopic characters were described based on observations of fresh and dried material, according to Sunhede (1989). Colors and measurements refer to dry material unless otherwise stated. Colors of macroscopic structures were determined according to Kornerup & Wanscher (1978); colors of microscopic features were defined in KOH preparation.

Observations of microscopic characters were made under a light microscope on glass slides mounts (in 5% KOH) prepared from dried specimens. Scanning electronic microscopy (SEM) was conducted at 'Centro de Microscopia Eletrônica', UFRGS. Basidiospores were coated with 15 nm of gold using a Bal-Tec SCD050 sputter coater and photographed with a JEOL JSM-5800 scanning electron microscope.

Taxonomy

Geastrum reinkingii Lloyd, Mycol. Writings 7: 1280. 1924. FIG 1

SPECIMENS EXAMINED: **HONDURAS**. **ATLANTIDA**, TELA, 20.III.1923, leg. O.A. Reinkingi (BPI 705664, holotype); **BRAZIL**. **PERNAMBUCO**, SÃO VICENTE FÉRRER, Mata do Estado, 18.IX.2008, leg. L. Trierveiler-Pereira & Baltazar LTP180 (URM 82017, as *G. lloydianum*; ICN XXXXXX); **RIO GRANDE DO SUL**, leg. J. Rick (BPI 705665); SÃO LEOPOLDO, 1933, leg. J. Rick (PACA-FR 15976, as *G. berkeleyi*); CAÇAPAVA DO SUL, Parque Municipal Pedra do Segredo, 06.VI.2011, leg. L. Trierveiler-Pereira LTP277 (ICN 175626); **PARANÁ,** FOZ DO IGUAÇU, Parque Nacional do Iguaçu, Trilha das Bananeiras, 09.II.2011, leg. L. Trierveiler-Pereira LTP179 (ICN XXXXXX).

ADDITIONAL SPECIMENS EXAMINED: *G. glaucescens*: **ARGENTINA**. **BUENOS AIRES**, LA PLATA, IX.1894, leg. C. Spegazzini (LPS 15860, holotype); *G. hariotii*: **BRAZIL**. C.G. Lloyd's Collection cat. no. 52535 (BPI 841469, holotype); *G. lloydianum*: **BRAZIL**. leg. J. Rick, C.G. Lloyd's Collection cat. no. 57279 (BPI 841471, holotype); *G. setiferum*: **BRAZIL**. **PERNAMBUCO**, Serra Negra, 28.V.2002, leg. I.G. Baseia (URM 77077, paratype); **PARAÍBA**, Mataraca, Mineradora Millenium (Cristal), mata controle, 02.IX.2009, leg. L. Trierveiler-Pereira (URM 82118).

IMMATURE BASIDIOMATA not observed. EXPANDED BASIDIOMATA small to medium, 2.0–3.4 cm high. EXOPERIDIUM non-hygroscopic, 1.4–2.7 cm high × 2.8–4.2 cm broad, split into 6–8 rays, some ramifying at the tip, arched; mycelial layer pale yellow (1A3), usually peeling off at maturity, if persistent, encrusted with debris; fleshy usually not persistent, pale orange

(5A3) to brownish orange (5C3) when fresh, then yellowish brown (5D5) to brown (6E5) when dry; fibrous layer brownish orange (5C5) to light brown (5D4). ENDOPERIDIUM subglobose to globose when recently exposed, then depressed globose to ellipsoid, 0.5–1.2 cm high (not including the peristome) × 1.4–2.0 cm broad, yellowish brown (5E4) to grayish brown (5E3, 6E3), when fresh, then yellowish brown (5D4) to dark brown (6F3) when dry, conspicuously stipitate; stipe short, up to 1.0 mm high, cylindrical or flattened, dark blond (5D4) to yellowish brown (5D5), endoperidial surface asperulate to rough due to the presence of conical fascicules of hyphae (most easily observed at the base of the endoperidium, near the apophysis); apophysis whitish to beige; peristome deeply sulcate, usually strongly delimited, up to 0.45 cm high, concolor or slightly darker than endoperidium, almost black in some specimens. GLEBA pulverulent at maturity, dark brown (6F4).

BASIDIA not observed. BASIDIOSPORES globose, 3.5–4.5 µm in diam. (including ornamentation), brown to dark brown, ornamented with short verrucae and columns, some confluent (Fig. 1E). CAPILLITIAL HYPHAE up to 8 µm wide, pale yellow, brown to golden brown, straight, not branched, thick-walled with narrow lumen, tip round, with or without encrusted material (Fig. 1F). Hyphae of the MYCELIAL LAYER up to 3 µm wide, hyaline to pale yellowish, thick-walled; hyphae of the FIBROUS LAYER 3–11 µm wide, yellowish to pale brown, thick-walled to solid; hyphae of the PSEUDOPARENCHYMATOUS LAYER globose to ellipsoid, up to 74 µm in the largest dimension, hyaline to yellowis; hyphae of the ENDOPERIDIUM up to 10 µm wide, yellowish to pale brown, thick-walled to solid, the ones forming the hyphal fascicules of the surface are tortuous, tightly interwoven with other hyphal elements (mycosclerids).

SUBSTRATE: growing solitary or in small groups (four or five basidiomata) on forest soil (litter), without forming subiculum.

DISTRIBUTION: known from Honduras and tropical and subtropical areas from Brazil. It is very likely that the species occurs along Neotropical rainforests and warmer regions of temperate America.

REMARKS: characteristic features of *G. reinkingii* at maturity are: medium-sized basidioma, arched exoperidium, usually not persistent mycelial and fleshy layer, sugblobose to ellipsoid, stipitate endoperidium, dark, asperulate to rough endoperidial surface, and a whitish apophysis.

The endoperidial stipe and the apophysis are not clearly evident when the endoperium is recently exposed. In dried collections, they are remarkable features. Some herbarium specimens exhibit a whitish pruina over the endoperidial surface. The basidiospores are typically dark brown and with discrete ornamentation. Immature forms were not found in herbarium collections or in the field, therefore, basidia morphology remains unknown.

Brazilian specimens show no major differences from the holotype, being the whitish apophysis and the short stipitate, blackish, depressed globose endoperidium the most remarkable features. Basidiospore measurements and morphology are constant among the examined specimens.

One collection of *G. reinkingii* from Southern Brazil, send by Rick to Lloyd, is at BPI (705665), but no collections were deposited at PACA, Rick's most important fungal collection. However, one of Rick's collection identified as *G. berkeleyi* (PACA-FR 15976) corresponds to *G. reikingii*. One specimen of *G. reinkingii* (URM 82017) was reported from Northeastern Brazil as *G. lloydianum* (Trierveiler-Pereira et al. 2011).

Geastrum hariotii and *G. lloydianum* are very closely related species, and their differences have been discussed by Trierveiler-Pereira & Silveira (2012). Microscopically, the differences between *G. reinkingii* and *G. lloydianum* are evident, since the basidiospores of the latter are 5–6 µm in diam., yellowish brown and prominently ornamented. The basidiospores of *G. hariotii* are more similar with those of *G. reinkingii*, however, macroscopically the two species are distinct: the first has smaller basidiomata with a globose, sessile, not asperulate endoperidium. Moreover, no white apophysis is observed in *G. hariotii*.

Geastrum setiferum Baseia has large, arched basidiomata and short stipitate endoperidium (Baseia & Milanez 2002, Trierveiler-Pereira et al. 2011). In many aspects it resembles *G. reinkingii*, but the presence of dark setae in the endoperidium is a unique feature. Moreover, endoperidial surface of *G. setiferum* is lighter (usually leather brown), not remarkably ellipsoidal, without a whitish apophysis, and the peristome morphology is a transition between fibrillose and plicate.

Geastrum glaucescens Speg. was described from Argentina based on a small, arched, dark colored basidiome. The endoperidium is globose, blackish, covered with a whitish gray matter all over it. According to the original publication (Spegazzini 1912), the endoperidium is short stipitate but this feature was difficult to confirm with the analysis of the herbarium specimen, which is sectioned in half. Soto & Wright (2000) illustrated the species clearly stipitate. The main macroscopical features differentiate this species from *G. reinkingii.*

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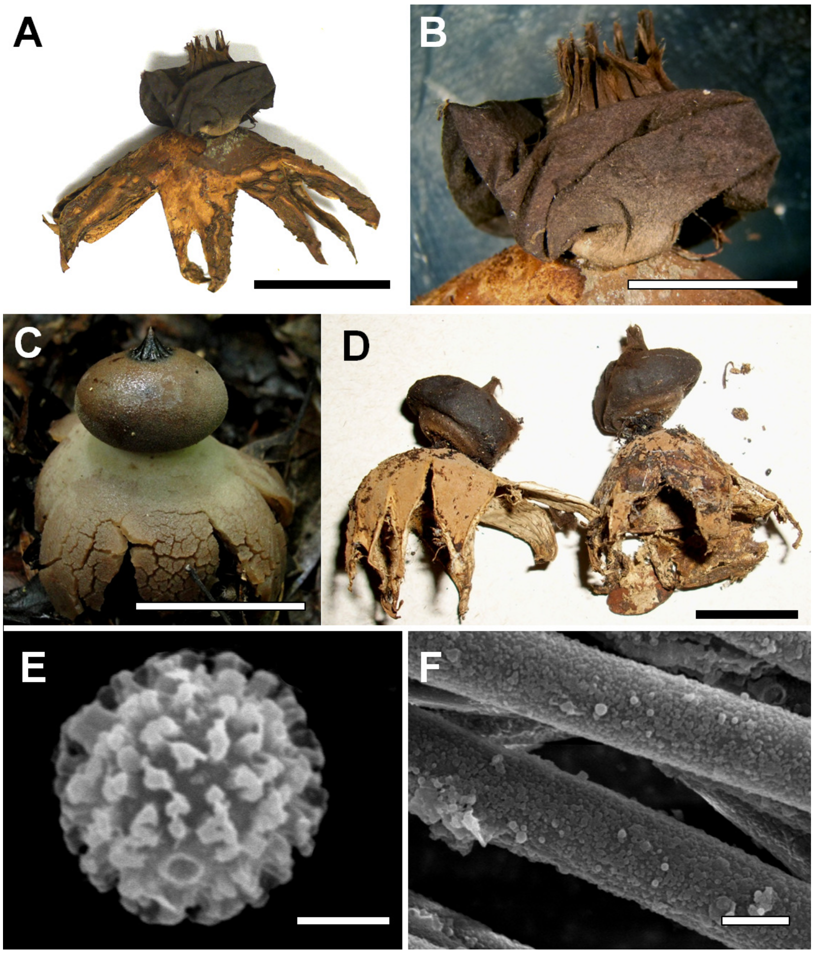

FIGURE 1. *Geastrum reinkingii.*
A. Holotype (BPI 705664). B. Detail of the endoperidium (holotype). C. Fresh basidiome (ICN LTP179). D. Dried specimens (URM 82017). E. Basidiospore (holotype). F. Capillitial hyphae (holotype). Scale bars: A, C, D = 2 cm; B = 1 cm; E = 1 µm; F = 2.5 µm.

4.13. MANUSCRITO IX

Trierveiler-Pereira L, Silveira RMB, Hosaka K. 2014. Phylogenetic relationships of *Geastrum* (*Geastraceae*, *Agaricomycetes*) species from Southern Brazil and a discussion on cryptic species. **Botanical Studies**: a ser submetido.

Phylogenetic relationships of *Geastrum* species (Geastraceae, Agaricomycetes) from Southern Brazil and a discussion on cryptic species

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ABSTRACT

Background: *Geastrum* is a gasteroid fungal genus found worldwide. Although the genus is easily recognized in the field, boundaries among some species are complicated and only few regional monographs for the genus have been published. In order to better understand the diversity and phylogenetic relationships among species from tropical and subtropical areas of America, we present a molecular analysis with 88 *Geastrum* specimens collected in Southern Brazil. In this study, two molecular markers (nuclear ITS and LSU) were analyzed to test phylogenetic relationships and to test species hypothesis.

Results: Phylogenetic inferences support the occurrence of cryptic species within *G. saccatum*, *G. triplex*, *G. trichiferum* and *G. schweinitzii*-complex. *Geastrum entomophilum*, *G. equinulatum*, *G. fimbriatum*, *G. hariotii*, *G. lageniforme*, *G. morganii*, *G. reinkingii*, *G. velutinum*, and *G. violaceum* are considered good species according to molecular and morphological taxa.

Conclusions: This is the first study to use a large dataset to access phylogenetic relationships among American species of *Geastrum*. Despite our efforts, additional molecular and taxonomical (focusing on type specimens) studies are still necessary to improve the knowledge of *Geastrum* in Southern Brazil. A key to species known to occur in this region is provided.

Keywords: earth-star fungi, fungal taxonomy, Geastraceae, molecular phylogenetics, Neotropical mycota

BACKGROUND

Geastrum Pers. is a widespread genus and comprises a large number of species, ca. 50 according to a conservative estimation (Kirk et al. 2008); however, more than 300 species names are available at Index Fungorum database (http://indexfungorum.org). In the field the genus is easily recognized since the gasteroid basidiomata have a characteristic morphology, with the mature outer peridium splitting in a stellate way. On the other hand, boundaries among some species are still complex, especially in tropical and subtropical areas of America, where diversity is high and studies are still insufficient.

A monograph of the genus published by Ponce de León (1968) comprehended American tropical species, but the large list of synonyms proposed by this author is not reliable for many species, since it was not based on accurate analyses of type specimens and/or fresh basidiomata. Recently, several studies have been published regarding the taxonomy of *Geastrum* species in America, including the description of new species (Calonge et al. 2000, Baseia & Milanez 2002, Calonge & Mata 2004, Baseia & Calonge 2006, Fazolino et al. 2008, Kuhar & Papinutti 2009, Silva et al. 2013, Cabral et al. 2014) and the rescue of old names (Trierveiler-Pereira & Silveira 2012, Zamora et al. 2013).

In Brazil, more than 40 *Geastrum* species have been recorded, and 26 were reported from the Southern Region (Trierveiler-Pereira & Baseia 2009). Most of these species were reported by Rick (1961), including the description of new taxa (Rick 1906, Trierveiler-Pereira & Silveira 2012). However, many species reported by Rick are nowadays treated as synonyms or were misidentified under *Geastrum* names from temperate climate regions.

In the present study we have investigated the phylogenetic relationships among *Geastrum* species found in Southern Brazil, including taxonomic notes on the species, a key to species/complexes of species.

METHODS

Geastrum specimens were collected in the Southern Region of Brazil, including the States of Rio Grande do Sul, Santa Catarina and Paraná. The different types of vegetation found in this area the Atlantic Rainforest and Southern grasslands (‘Pampa’). Additional collections used to generate original sequences for the phylogenetic analyses proceed from Northeastern Brazil and Argentina.

Macroscopical and microscopical analyses of the specimens followed traditional techniques used for geastroid fungi (Sunhede 1989). Vouchers are preserved at ICN and FLOR (herbarium acronyms are according to Thiers 2011).

Towards DNA extraction sections of the basidiomata (from the immature or mature gleba) were soaked overnight in DMSO buffer (Seutin et al. 1991) with an addition of 100mM Tris-HCl (pH 8.0) and 0.1M sodium sulphite (Na_2SO_3). DNA extraction followed the protocol of Hosaka and Castellano (2008). PCR and sequencing protocols followed Kasuya et al. (2012). Raw forward and reverse sequences for each sample were assembled into contigs and were manually edited using the program ATGC v.6 (Genetyx). After assembling, the sequences were used to query GenBank using a BLAST search to verify their identity.

Sequence data were obtained from two independent loci: nuclear ribosomal internal transcribed spacer (ITS) and nuclear ribosomal large subunit (LSU). Primers used to amplify each DNA region and for sequencing were: ITS1, ITS4, ITS5, LR0R, LR3 and LR5 (http://biology.duke.edu/fungi/mycolab/).

The ingroup is represented by 88 taxa of *Geastrum*, and outgroup is represent by *Myriostoma coliforme* (Dicks.) Corda (Fig. 5F), which is closely related (Hosaka et al. 2006). Taxa sampled, voucher information and GenBank (www.ncbi.nlm.nih.gov/genbank/) accession numbers are listed in Table 1.

Molecular datasets consisting of original sequences were automatically aligned on MUSCLE v.3.6 (Edgar 2004), and manually edited in BioEdit v.7.0.1 (Hall 1999). Final alignments are available from TreeBase as a NEXUS file.

Phylogenetic analyses were conducted for ITS separately, but also for ITS combined with LSU sequences under Maximum Parsimony (MP). MP analyses was conducted under the equally weighted parsimony criterion using *PAUP v.4.0b10 (Swofford 2002). Gaps were treated as missing data. All the analyses were performed under the heuristic search option (TBR and Multrees options on) and 1,000 replicates of random addition sequence were conducted. Nodal supports were tested by bootstrapping (BS) of 1,000 replicates using the heuristic search option (TBR and Multrees options on) with 10 random addition sequences.

RESULTS AND DISCUSSION

ITS sequences were available for all the specimens (n = 88 + 1 outgroup) included in the analyses, while LSU sequences were missing for seven specimens. The two DNA regions were combined into a single supermatrix totaling 1682 characters (ITS = 779; LSU= 903), of which 473 were phylogenetically informative. MP analysis yielded 11 equally parsimonious trees (tree length = 2808, CI= 0.3789, RI= 0.6842, RC= 0.2593).

One of the equally parsimonious trees is presented as a phylogram in Fig. 1. Nineteen subclades within the ingroup were labelled as A–S in the phylogram of Fig. 1

for discussion. The subclades more or less correspond to morphological species or groups of species; these are further described below.

Subclade A (BS 86) corresponds to *G. hariotii* Lloyd, a good species with arched exoperidium, sessile to pseudostalked endoperidium and plicate peristome (Fig. 2A). It is morphological similar to *G. lloydianum* Rick and *G. reikingii* Lloyd, but the first has larger spores (Trierveiler-Pereira & Silveira 2012) and the latter differs mainly macroscopically (see discussion below). Unfortunately, *G. lloydianum* is only known from the type (BPI) and it has not been included in any molecular analyses.

Subclade B comprises three taxa: *G. reikingii*, *Geastrum* sp. 1 (LTP310) and *G.* aff. *saccatum* (LTP135). *G. reikingii* (fully supported clade) is characterized by its arched exoperidium, distinctly stalked endoperidium, marked white apophysis, and plicate peristome (Trierveiler-Pereira & Silveira 2014) (Fig. 2B). LTP310 is a curious taxon – it was found only once growing on wood, and all specimens (n=5) from this single collection have a whitish, sessile endoperidium without ostiole. Without further collections, it is difficult to ascertain if this morphology corresponds to a malformation. LTP135 is a specimen morphologically similar to *G. saccatum* Fr.: saccate exoperidium, sessile endoperidium and well defined fibrillose peristome; however, microscopically it is possible to observe the presence of mycosclereids, as occur in *G. lilloi* (Domínguez de Toledo 1996).

Subclade C included *Geastrum saccatum* (BS 99) and *Geastrum* aff. *morganii*. Specimens of *G. saccatum* (Fig. 2C) included in this subclade are the ones that better fits into the currently accepted concept of the species. However, *G. saccatum* is polyphyletic according to our analyses, and specimens with similar morphology were included in seven different subclades: B, C, K, N, O, P, and S. Morphologically, it is difficult to separate these cryptic taxa based on subtle differences in basidiomata size, colors, and spores morphology. Further type studies on tropical *Geastrum* species are needed in order to found if there are available names for these taxa. The application of the name *G. saccatum* is uncertain. The type of *G. saccatum* was collected in Brazil (locality not specified), but unfortunately is missing. Since so many *Geastrum* species found in the Brazilian forests have a G. *saccatum*-like morphology, it is very unlike to predict which concept of *G. saccatum* Fries had in mind when he described the species.

Geastrum aff. *morganii* (LTP221) is close to *G. morganii* Lloyd (Subclade J, fully supported), but its basidiome is larger, the endoperidium is globose, light-colored, and the peristome has more folds (Fig. 2D). Microscopically the two species are also similar, except for the more prominently ornamented, larger spores in *G.* aff. *morganii. G. violaceum* Rick (Subclade M, fully supported) has basidiome morphology very similar to *G. morganii* (Fig. 2E), but the exoperidium is violet, pinkish to purplish and the endoperidium is darker (Fig 2F).

Subclade D included two xylophilous species: *G.* aff. *trichiferum* (BS 99) and *G.* cf. *javanicum* (BS 99). The first has smaller-sized basidiomata, browinish outer layer of the exoperidium, and a fibrillose peristome delimited by a whitish circular area (Fig. 3A). Macroscopically, it is similar to *G. trichiferum* Rick (Subclade L, BS 53), but *G.* aff. *trichiferum* has an outer layer with fewer and shorter hairs (< 1 mm), while *G. trichiferum* has a dense hirsute layer with longer hairs or tufts of hairs (< 2 mm) (Fig. 3B). Moreover, spores of the latter are brownish, conspicuously ornamented, ca. 4 µm, while *G.* aff. *trichiferum* has yellowish spores finely ornamented, ca. 3 µm. It is interesting to note that despite the great morphological similarities between these two taxa, they are separated phylogenetically.

Geastrum javanicum Lév. is a name with an uncertain application. Ponce de Leon (1968) included several names as synonyms of this taxon, but they might correspond to independent taxa. The type, which is kept in Herbarium P, is macroscopically similar with specimens from Subclade D, but since we have only seen a photograph of it, and could not analyze it microscopically, we can not ascertain the identification of our specimens. Specimens of *G.* cf. *javanicum* found in Southern Brazil have an orange brown, velvety outer layer of the exoperidium, light gray endoperidium and delimited peristome. The basidiomata arise from a whitish subiculum and the outer mycelial layer has a tendency to detach from the fibrous layer. *Geastrum velutinum* Morgan (Subclade R, BS52) also has similar basidiomata and habit (Fig 3C-D), but the outer layer of the exoperidium easily detaches from the fibrous layer and it is reddish when fresh, drying grayish white. *Geastrum echinulatum* B.D.B. Silva & Baseia (Subclade N, BS 99) resembles both species, but it has a much darker endoperidium when dried (almost black) and a not delimited peristome (Fig 3E-F). Hyphal tufts are very evident on the mycelial layer of unopened basidiomata, but they may be indistinct or scarce on expanded forms. Its type specimen is from the Brazilian Amazon Forest, but it is also found in Northeastern (Trierveiler-Pereira et al. 2011, as *G. javanicum*) and Southern Brazil (present study). It closely resembles *G. argentinum* Speg. (Fig. 4A) and a comparison between the type specimens is necessary to check the authenticity of these two species.

Subclade E (BS 93) corresponds to *G. lageniforme* Vittad., a species morphologically similar to *G. saccatum*, but with longer and narrower rays of the exoperidium (Fig. 4B). Not in all specimens the longitudinal cracks on the outer exoperidium are evident, and we haven’t seen any specimen forming a pseudo-collar around the peristome, as may occur according to literature (Sunhede 1989).

Subclade F included *G.* aff. *fimbriatum* (fully supported). The specimens have small (< 2.5 cm in diam.) basidiomata, sessile endoperidium, and fibrillose peristome. They are macroscopically very similar to *G. fimbriatum* Fr. (Fig. 4C) (Subclade I, fully supported), except by the smaller-sized basidiomata and the spores that are smaller and less evidently ornamented than in *G. fimbriatum*. *Geastrum entomophilum* Fazolino, Calonge & Baseia (Subclade Q, fully supported) is a similar species, except by the arched exoperidium and rugulose endoperidial surface (Fig. 4D-E). Moreover, specimens of *G. entomophilum* may have an endoperidium that is either blackish (LTP27) or whitish (LTP214, 242b) (Trierveiler-Pereira & Baseia 2010). *Geastrum* sp. 3 (LTP127), included in Subclade I, is morphologically similar to *G. fimbriatum*, due to basidiome size and the dark endoperidium; the peristome, however, is indeterminate and plicate with several folds. (ca. 10) (Fig. 5A).

Subclade G (fully supported) included specimens of *G. triplex* Jungh., a species reported worldwide, but that represents a complex of taxa (Kasuya et al. 2012). It can be recognized in the field by its usually large-sized basidiomata (although some Brazilian specimens are only about 3 cm in diam, e.g. LTP161), and the formation of a collar-like structure around the endoperidium (Fig. 5B). The basidiomata are usually light colored, but there are specimens with blackish endoperidium (e.g. LTP242a). The specimen reported by Rick (1961) as *G. englerianum* Henn., actually is a misidentification of *G. triplex* material with blackish endoperidium (PACA, Fungi Rickiani 15966).

Subclade H (fully supported) is represented by *Geastrum* sp. 2 (LTP128, LTP159), a xylophilous species. Basidiomata are externally light-colored, resembling large specimens of *G. schweinitzii* (Berk. & M.A. Curtis) Zeller, but the endoperidium is dark, almost black, and the peristome is fibrillose, not delimited (Fig. 5C). Moreover, basidiospores are small (< 3 µm) and with a very delicate ornamentation, which gives the impression that they are smooth under an ordinary microscope. It might correspond to some species that have been treated as a synonym of *G. schweinitzii* (e.g. *G. mirabile*

Mont., *G. rhizophorum* Dissing & M. Lange, *G. papyraceum* Berk. & M.A. Curtis, *G. lignicola* Berk, *G. subiculosum* Cooke & Massee, *G. tomentosum* Lloyd, *G. caespitosum* Lloyd) so type studies are necessary to ascertain its identity. The typical specimens of *G. schweinitzii* are in Subclade L (fully supported) and they are characterized by a whitish, velvety mycelial layer, pinkish to orange fibrous layer, light gray to brownish gray endoperidium and fibrillose peristome delimited by a white area (Fig. 5D). The basidiospores are brown, verrucose, < 4 µm. *Geastrum* aff. *schweinitzii* (LTP200) from Subclade N appears related to *Geastrum javanicum* specimens (BS 81) and its is similar to *G. schweinitzii*, except for the tomentose, yellowish brown mycelial layer (Fig 5E).

CONCLUSION AND PERSPECTIVES

This study provides the first phylogenetic hypothesis of relationships within the Southern Brazilian species of *Geastrum*. It confirms the authenticity of several morphological species, i.e. *G. entomophilum*, *G. equinulatum*, *G. fimbriatum*, *G. hariotii*, *G. lageniforme*, *G. morganii*, *G. reinkingii*, *G. velutinum*, and *G. violaceum*. Some taxa, viz. *G. triplex*, *G. trichiferum, G. saccatum* and *G. schweinitzii*, correspond to complexes of species and further taxonomic and molecular studies must be undertaken in order to better understand their relationships.

Geastrum aff. *fimbriatum*, *Geastrum* sp. 1, and *Geastrum* sp. 3 probably correspond to taxa that are new to science, but more morphological studies on additional specimens are necessary to confirm this. *Geastrum* sp. 2 most likely has been described before within the *G. javanicum*/*G. schweinitzii*-complex, so a careful morphological analysis on type specimens is necessary to name this species. Moreover, the occurrence of *G. javanicum* still needs to be confirmed.

Other *Geastrum* species known from Southern Brazil (*G. lloydianum*, *G. fornicatum* (Huds.) Hook., *G. ovalisporum* Calonge & Mor.-Arr., *G. pectinatum* Pers., and *G. stipitatum* Solms) have not been included in the present study, either because of lack of fresh specimens or unsuccessful process of extraction/amplification of DNA.

A tentative key is presented to eighteen species/complexes of species of *Geastrum* known to occur in Southern Brazil. To easen the use of this key, many terms used to describe the species are illustrated in Fig. 6. Species recorded in literature that could not be confirmed by us or represent synonyms were not included in this key (i.e. *G. ambiguum* Mont., *G. archeri* Berk., *G. asperum* Lloyd, *G. bryantii* Berk., *G. calcium* Lloyd, *G. coronatum* Pers., *G. elegans* Vittad., *G. fenestratum* (Batsch) Lloyd, *G. englerianum*, *G. lilloi* L.S. Domínguez, *G. limbatum* Fr., *G. minimum* Schwein., *G. peruvianum* Cooke, *G. rufescens* Pers., *G. schmidelii* Vittad., *G. smardae* V.J. Staněk, *G. smithii* Lloyd, *G. striatum* DC., and *G. vittatum* Kalchbr.)

Provisional key to *Geastrum* species/complexes of species known to occur in Southern Brazil

1a. Basidiomata epigeous when immature, usually growing gregarious on rotten wood, usually forming a whitish subiculum (dense hyphal layer) on the substratum 2

1b. Basidiomata hypogeous to epigeous when immature, growing on litter or forest soil, not forming whitish subiculum 6

2a. Basidiomata stipitate .... *G. stipitatum*

2b. Basidiomata sessile 3

3a. Basidiomata small-sized (< 2 cm in diam.), light-colored; mycelial layer not separating from fibrous layer 4

3b. Basidiomata larger, dark-colored; mycelial layer separating from fibrous layer....... 5

4a. Mycelial layer whitish, velvety .... *G. schweinitzii*-complex

4b. Mycelial layer orange to brown, hirsute .... *G. trichiferum*-complex

5a. Mycelial layer reddish brown, whitish gray when dried; endoperidium grayish; peristome delimited by a whitish line or zone .... *G. velutinum*

5b. Mycelial layer orange brown, maintaining the color when dried; endoperidium blackish; peristome not delimited .... *G. echinulatum*

6a. Exoperidium fornicate (mycelial layer remains as a cup firmly attached at the arched exoperidium) at maturity .... *G. fornicatum*

6b. Exoperidium arched, planar to saccate at maturity 7

7a. Exoperidium arched 8

7b. Experidium planar to saccate 13

8a. Endoperidium sessile to pseudostalked 9

8b. Endoperidium clearly stalked 11

9a. Endoperidium sessile; peristome fibrillose, not delimited .... *G. entomophilum*

9b. Endoperidium sessile to pseudostalked; peristome truly sulcate 10

10a. Basidiospores 3–4 µm diam., ornamented with short columns .... *G. hariotii*

10b. Basidiospores 5–6 µm diam., ornamented with high columns (some of them seem to be curved when observed under the light microscope) .... *G. lloydianum*

11a. Basidiospores ovoid to subglobose .... *G. ovalisporum*

11b. Basidiospores globose 12

12a. Apophysis striate .... *G. pectinatum*

12b. Apophysis even .... *G. reinkingii*

13a. Peristome fibrillose 14

13b. Peristome with a few folds 17

14a. Peristome not delimited, endoperidium blackish .... *G. fimbriatum*

14b. Peristome delimited, endoperidium light or dark-colored 15

15a. Pseudoparenchymatous (=fleshy) layer forms a collar-like structure around the endoperidium .... *G. triplex*-complex

15b. Collar-like structure not prominent 16

16a. Exoperidial rays slender and long; outer mycelial layer with thin-walled clamped hyphae .... *G. lageniforme*

16b. Exoperidial rays short; outer mycelial layer with simple-septate hyphae .... *G. saccatum*-complex

17a. Exoperidium purplish to red-violet .... *G. violaceum*

17b. Exoperidium pale orange to beige .... *G. morganii*

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TABLE 1. Taxa information and GenBank accession numbers for the ITS and LSU sequences used in the phylogenetic analysis.

Taxon	Voucher	Origin	ITS	LSU
Geastrum aff. *saccatum*	LTP06 / ICN	Brazil (S)	GenBank	- - -
Geastrum aff. *saccatum*	LTP07 / ICN	Brazil (S)	GenBank	GenBank
Geastrum equinulatum	LTP18 / ICN	Brazil (S)	GenBank	GenBank
Geastrum equinulatum	LTP19 / ICN	Brazil (S)	GenBank	GenBank
Geastrum violaceum	LTP21 / ICN	Brazil (S)	GenBank	- - -
Geastrum lageniforme	LTP22 / ICN	Brazil (S)	GenBank	GenBank
Geastrum lageniforme	LTP23 / ICN	Brazil (S)	GenBank	GenBank
Geastrum equinulatum	LTP24 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP25 / ICN	Brazil (S)	GenBank	GenBank
Geastrum lageniforme	LTP26 / ICN	Brazil (S)	GenBank	GenBank
Geastrum entomophilum	LTP27 / ICN	Brazil (S)	GenBank	- - -
Geastrum aff. *saccatum*	LTP28 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP30 / ICN	Brazil (S)	GenBank	GenBank
Geastrum saccatum	LTP32 / ICN	Brazil (S)	GenBank	GenBank
Geastrum cf. *javanicum*	LTP35 / ICN	Brazil (S)	GenBank	GenBank
Geastrum equinulatum	LTP50 / ICN	Brazil (S)	GenBank	GenBank
Geastrum cf. *javanicum*	LTP87 / ICN	Brazil (S)	GenBank	GenBank
Geastrum schweinitzii	LTP112 / ICN	Brazil (S)	GenBank	GenBank
Geastrum sp. 3	LTP127 / ICN	Brazil (S)	GenBank	GenBank
Geastrum sp. 2	LTP128 / ICN	Brazil (S)	GenBank	GenBank
Geastrum saccatum	LTP131 / ICN	Brazil (S)	GenBank	GenBank

Geastrum aff. *saccatum*	LTP135 / ICN	Brazil (S)	GenBank	GenBank
Geastrum saccatum	LTP136 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP143 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP147 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP150 / ICN	Brazil (S)	GenBank	GenBank
Geastrum saccatum	LTP158 / ICN	Brazil (S)	GenBank	GenBank
Geastrum sp. 2	LTP159 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP160 / ICN	Brazil (S)	GenBank	GenBank
Geastrum triplex	LTP161 / ICN	Brazil (S)	GenBank	GenBank
Geastrum saccatum	LTP164 / ICN	Brazil (S)	GenBank	GenBank
Geastrum morganii	LTP166 / ICN	Brazil (S)	GenBank	GenBank
Geastrum hariotii	LTP169 / ICN	Brazil (S)	GenBank	GenBank
Geastrum reinkingii	LTP179 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP183 / ICN	Brazil (S)	GenBank	GenBank
Geastrum cf. *javanicum*	LTP186 / ICN	Brazil (S)	GenBank	GenBank
Geastrum velutinum	LTP187 / ICN	Brazil (S)	GenBank	GenBank
Geastrum fimbriatum	LTP193 / ICN	Brazil (S)	GenBank	GenBank
Geastrum velutinum	LTP194 / ICN	Brazil (S)	GenBank	GenBank
Geastrum velutinum	LTP195 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *fimbriatum*	LTP196 / ICN	Brazil (S)	GenBank	- - -
Geastrum aff. *fimbriatum*	LTP197 / ICN	Brazil (S)	GenBank	- - -
Geastrum aff. *saccatum*	LTP198 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *schweinitzii*	LTP200 / ICN	Brazil (S)	GenBank	GenBank
Myriostoma coliforme	LTP202 / ICN	Brazil (S)	GenBank	GenBank
Geastrum equinulatum	LTP207 / ICN	Brazil (S)	GenBank	GenBank

Geastrum entomophilum	LTP214 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP217 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *morganii*	LTP221 / ICN	Brazil (S)	GenBank	GenBank
Geastrum lageniforme	LTP223 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP225 / ICN	Brazil (S)	GenBank	GenBank
Geastrum reinkingii	LTP277 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP228 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP232 / ICN	Brazil (S)	GenBank	GenBank
Geastrum lageniforme	LTP241 / ICN	Brazil (S)	GenBank	GenBank
Geastrum triplex	LTP242a / ICN	Brazil (S)	GenBank	GenBank
Geastrum entomophilum	LTP242b / ICN	Brazil (S)	GenBank	GenBank
Geastrum fimbriatum	LTP243 / ICN	Brazil (S)	GenBank	GenBank
Geastrum fimbriatum	LTP245 / ICN	Brazil (S)	GenBank	GenBank
Geastrum morganii	LTP248 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP260 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *trichiferum*	LTP261 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP262 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP263 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP264 / ICN	Brazil (S)	GenBank	GenBank
Geastrum lageniforme	LTP268 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *trichiferum*	LTP270 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP275 / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *trichiferum*	LTP279 / ICN	Brazil (S)	GenBank	GenBank
Geastrum trichiferum	LTP286 / ICN	Brazil (S)	GenBank	GenBank
Geastrum velutinum	LTP293 / ICN	Brazil (S)	GenBank	GenBank

Geastrum aff. *saccatum*	LTP296b / ICN	Brazil (S)	GenBank	GenBank
Geastrum aff. *saccatum*	LTP307 / ICN	Brazil (S)	GenBank	GenBank
Geastrum sp. 1	LTP310 / ICN	Brazil (S)	GenBank	GenBank
Geastrum hariotii	LTP311 / ICN	Brazil (S)	GenBank	GenBank
Geastrum trichiferum	LTP312 / ICN	Brazil (NE)	GenBank	- - -
Geastrum reinkingii	LTP315 / ICN	Brazil (NE)	GenBank	GenBank
Geastrum equinulatum	LTP316 / ICN	Brazil (NE)	GenBank	- - -
Geastrum equinulatum	LTP317 / ICN	Brazil (NE)	GenBank	GenBank
Geastrum triplex	LTP318 / ICN	Brazil (NE)	GenBank	GenBank
Geastrum equinulatum	LTP319 / ICN	Brazil (S)	GenBank	GenBank
Geastrum triplex	NT91 / FLOR	Brazil (S)	GenBank	GenBank
Geastrum trichiferum	MAN672 / FLOR	Brazil (S)	GenBank	GenBank
Geastrum trichiferum	MAN704 / FLOR	Brazil (S)	GenBank	GenBank
Geastrum schweinitzii	MAN731 / FLOR	Brazil (S)	GenBank	GenBank
Geastrum aff. *trichiferum*	MAN766 / FLOR	Brazil (S)	GenBank	GenBank
Geastrum fimbriatum	MAN778 / FLOR	Brazil (S)	GenBank	GenBank
Geastrum hariotii	AM4515 / ICN	Brazil (S)	GenBank	GenBank
Geastrum violaceum	LC187 / ICN	Argentina	GenBank	GenBank

(S) = Southern Region; (NE) = Northeastern Region

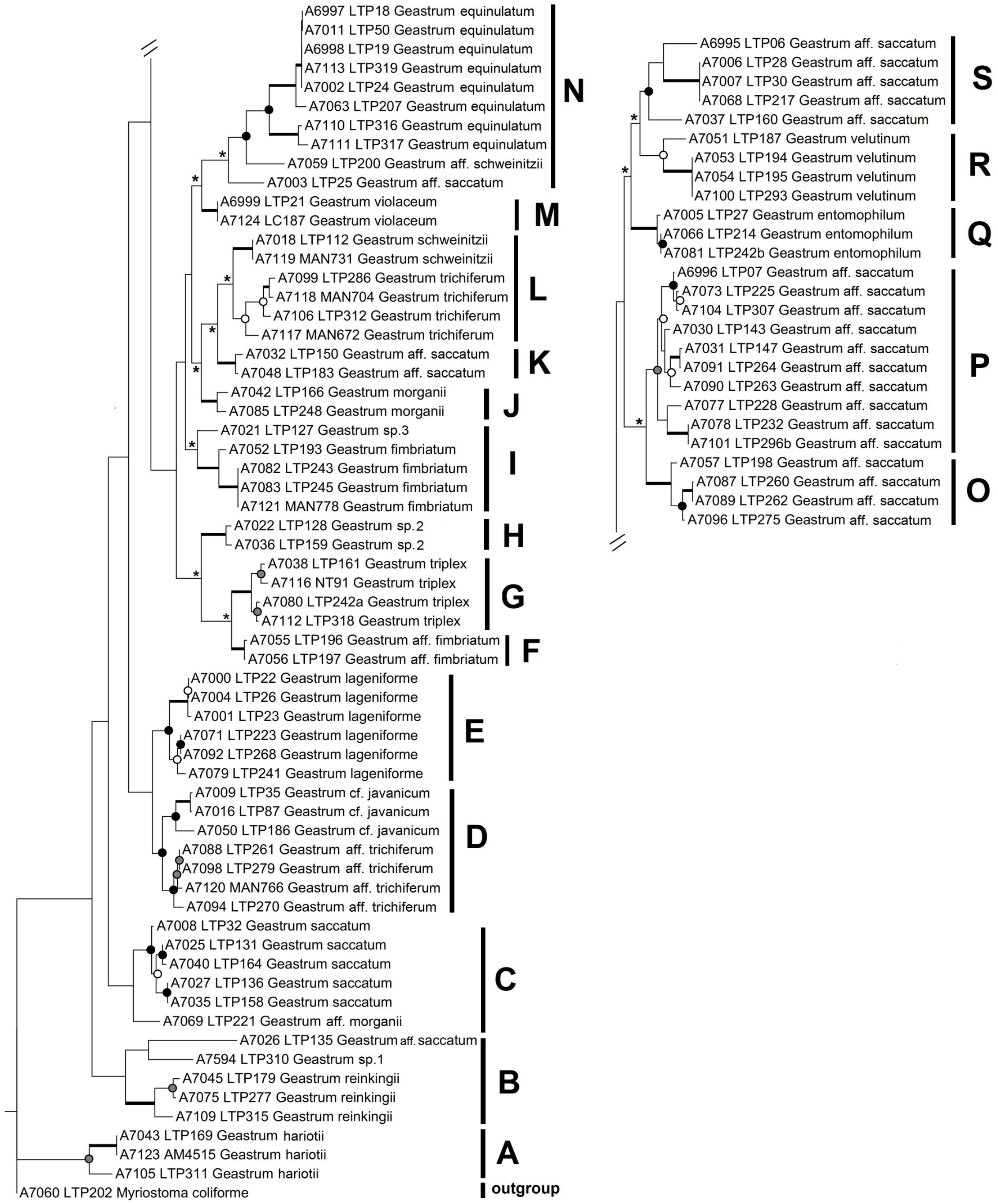

FIG. 1. One of the 11 equally most parsimonious trees derived from parsimony analysis based on ITS and LSU sequence data of *Geastrum* species that occur in Southern Brazil. Parsimony bootstrap values for the nodes support are indicated by white (50-70%), gray (70%-90%) and black circles (> 90%); branches in bold are fully supported. An asterisk (*) indicates branches that collapse in the strict consensus tree. Subclades A–S represent species or groups of species that are discussed in the text.

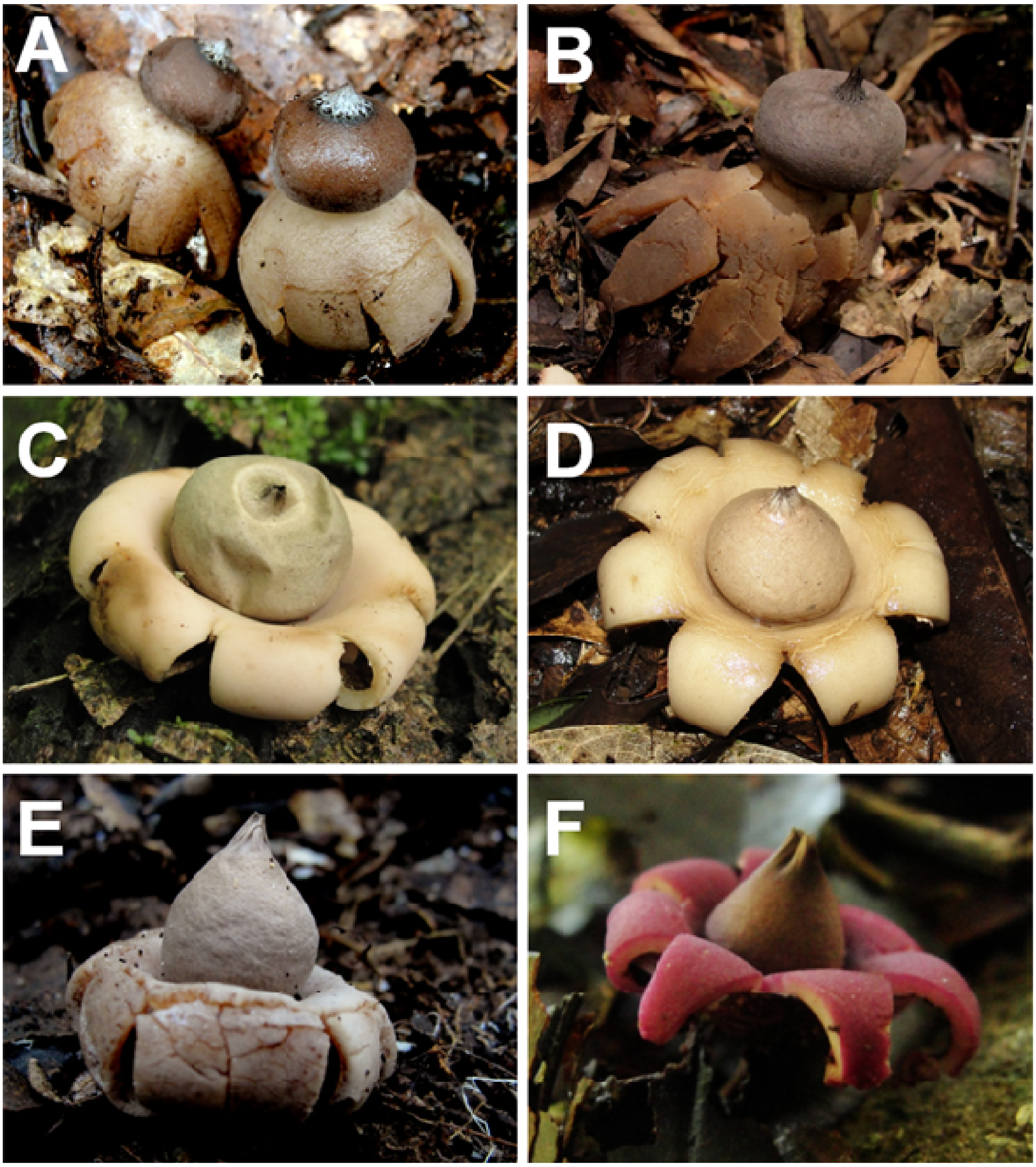

FIG. 2. *Geastrum* species from Southern Brazil. A. *G. hariotii* (LTP169). B. *G. reinkingii* (LTP179). C. *G. saccatum* (LTP131). D. *G.* aff. *morganii* (LTP221). E. *G. morganii* (LTP248). F. *G. violaceum* (LTP21).

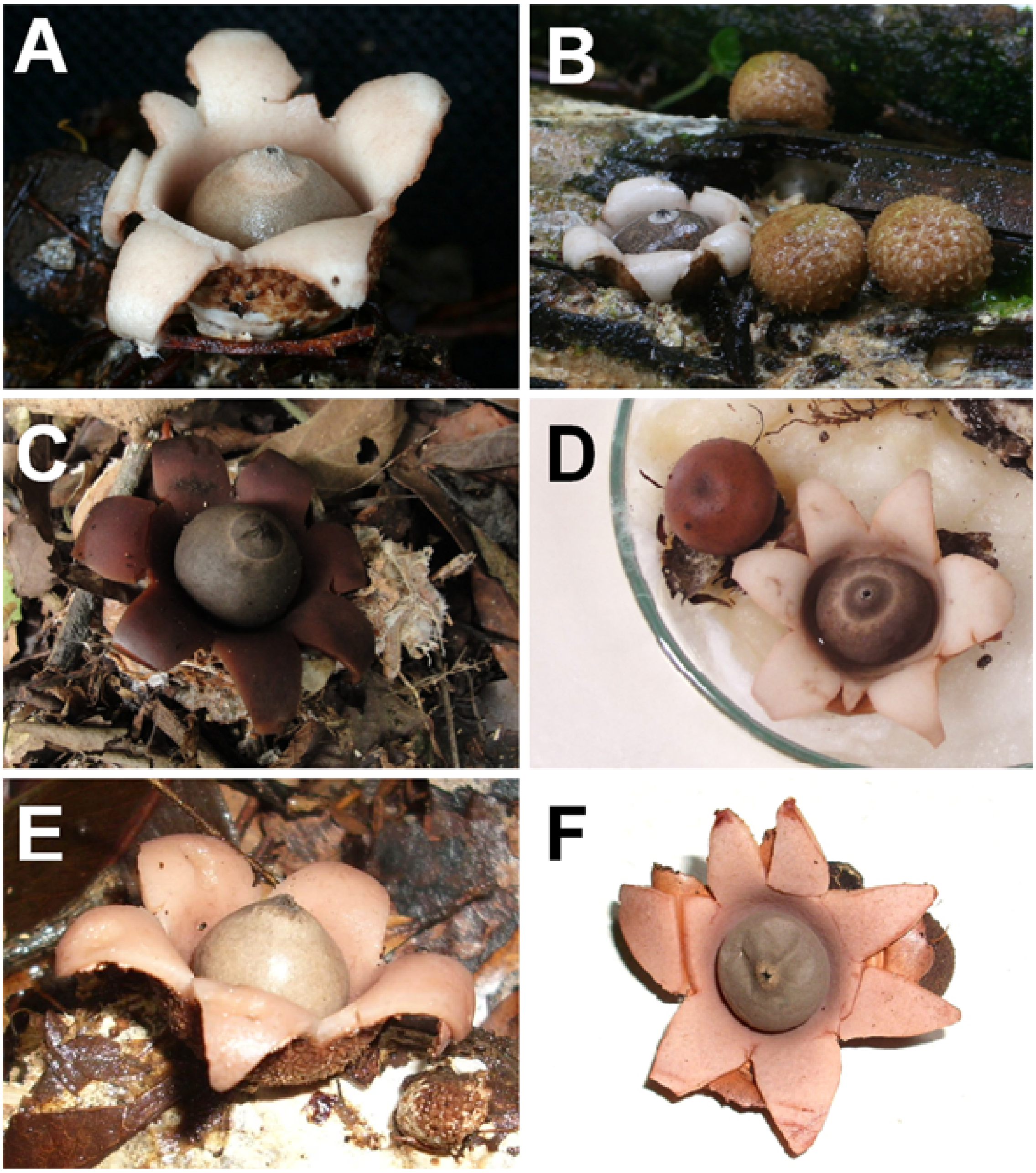

FIG. 3. *Geastrum* species from Southern Brazil. A. *G.* aff. *trichiferum* (MAN766). B. *G. trichiferum* (MAN704). C-D. *G. velutinum* (LTP187, LTP293). E-F. *G. equinulatum* (LTP19, LTP50).

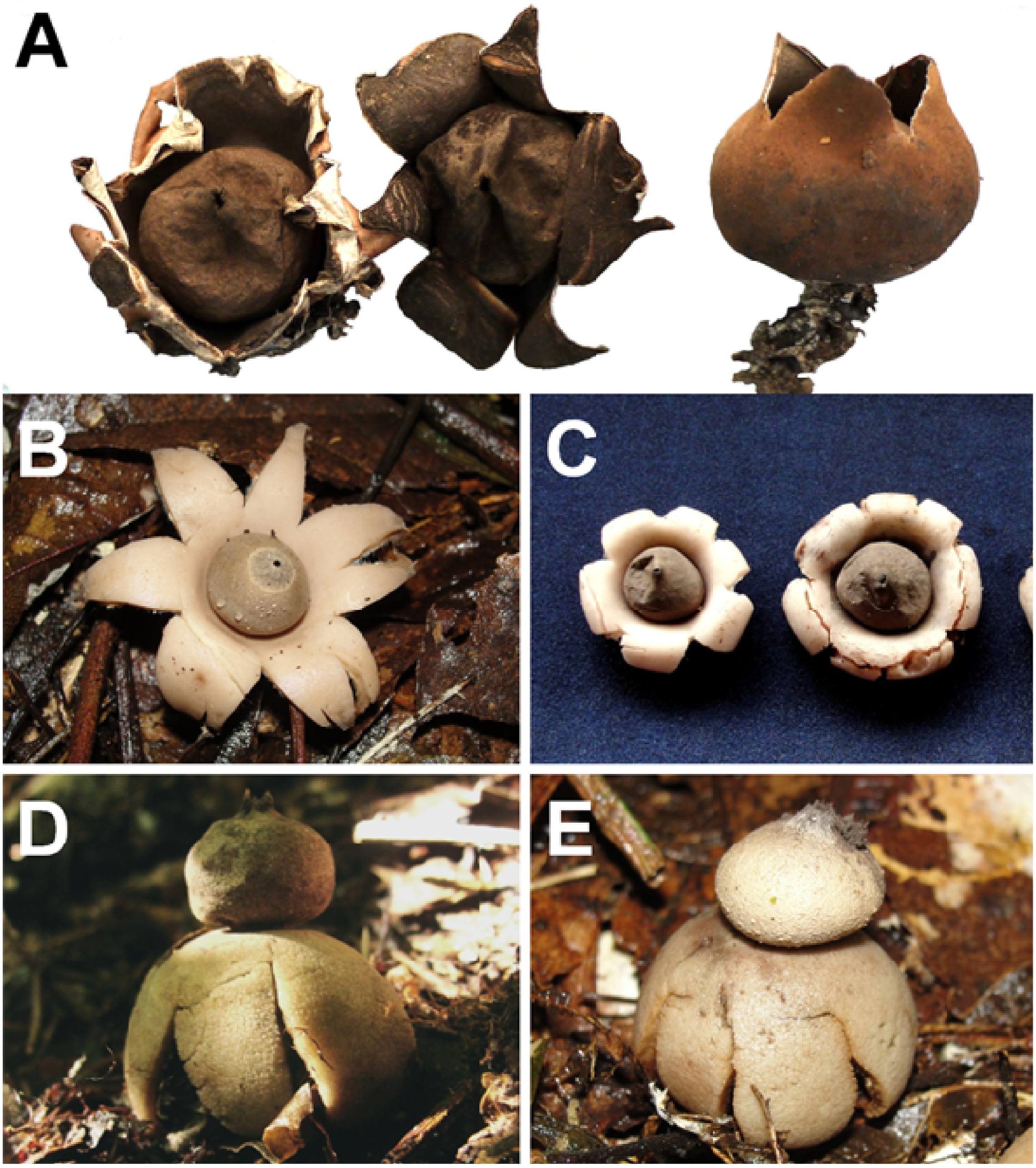

FIG. 4. *Geastrum* species from Southern Brazil. A. *G. argentinum* (epitype, LPS48446). B. *G. lageniforme* (LTP223). C. *G. fimbriatum* (LTP245). D-E. *G. entomophilum* (LTP27, LTP214).

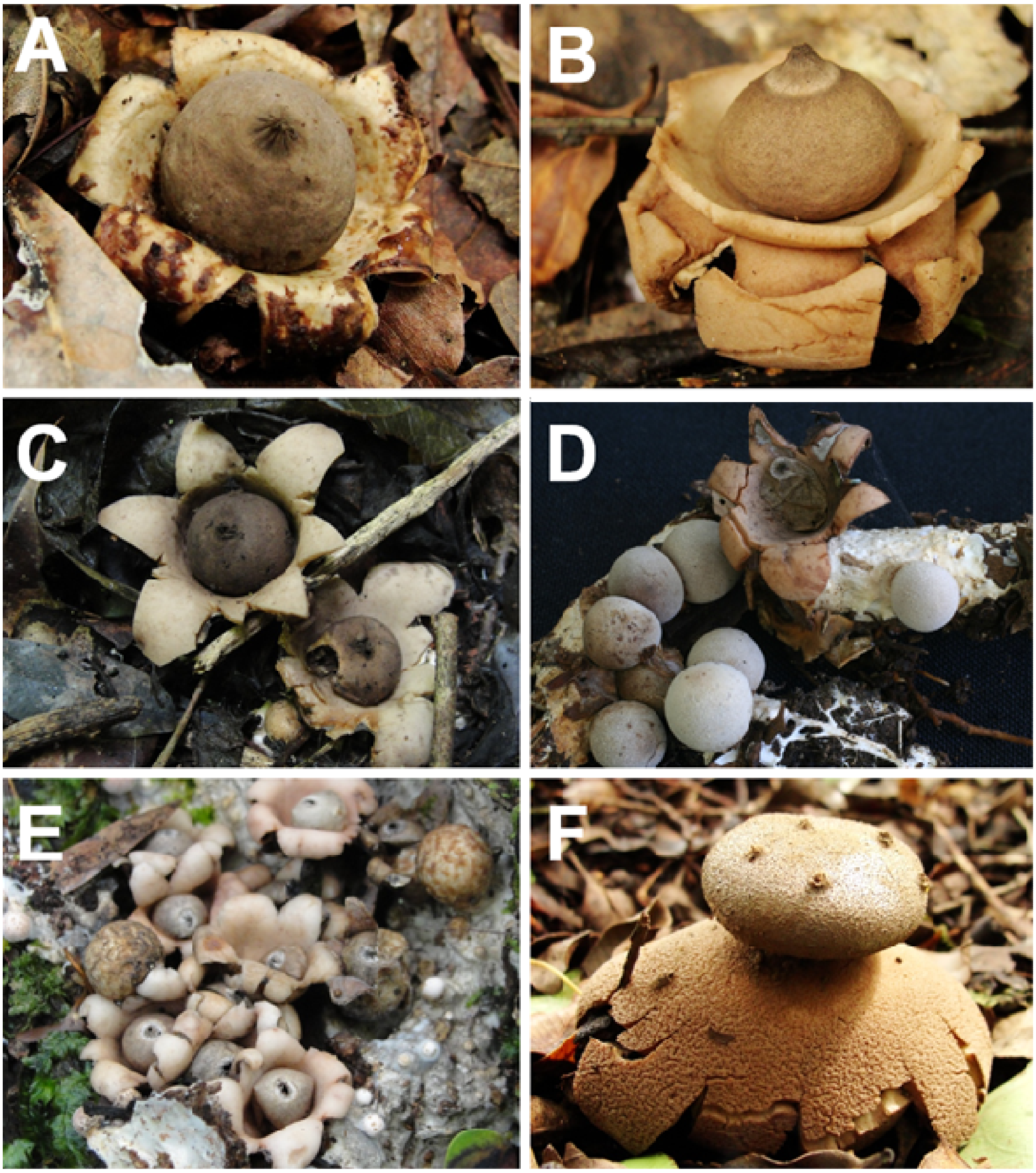

FIG. 5. *Geastrum* species and *Myrisotoma coliforme* from Southern Brazil. A. *Geastrum* sp. 3 (LTP127). B. *G. triplex* (LTP161). C. *Geastrum* sp. 2 (LTP159). D. *G. schweinitzii* (MAN731). E. *G.* aff. *schweinitzii* (LTP200). F. *Myrisotoma coliforme* (LTP202).

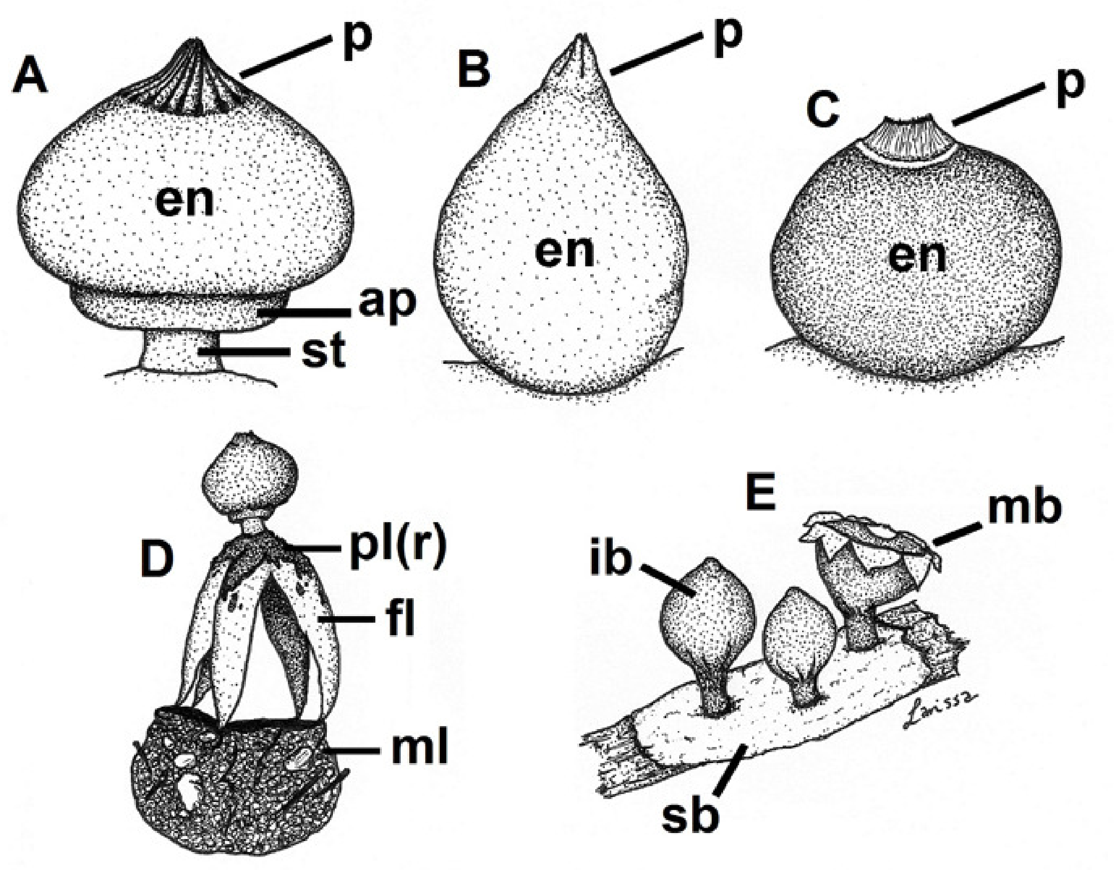

FIG. 6. Morphological features of *Geastrum* specimens. A. Plicate peristome. B. Peristome with a few folds. C. Fibrillose peristome delimited by a white line. D. Fornicate basidiome. E. Stipitate basidiomata on wood. Legend: p = peristome; en = endoperidium; ap = apophysis; st = stalk; pl(r) = pseudoparenchymatous layer (remnants); fl = fibrous layer; ml = mycelial layer; ib = immature basidiome; mb = mature basidiome; sb = subiculum.

4.14. MANUSCRITO X

Trierveiler-Pereira L, Honaiser LP, Silveira RMB. 2014. Diversity of gasteroid fungi (*Agaricomycetes*, *Basidiomycota*) from Brazilian Pampa areas. **Nova Hedwigia**: a ser submetido.

Diversity of gasteroid fungi (Agaricomycetes, Basidiomycota) from Brazilian Pampa areas

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Abstract

The Pampa Biome is characterized by natural grassland vegetation and in Brazil it is found exclusively in the southern half of the State of Rio Grande do Sul (Southern Region). Twenty-six species of gasteroid fungi (Basidiomycota) were identified during a survey in this biome from 2010 to 2012. The species are distributed among eight families and fourteen genera, viz. *Arachnion*, *Bovista*, *Calvatia*, *Clathrus*, *Cyathus*, *Geastrum*, *Lycoperdon*, *Lysurus*, *Morganella*, *Myrisotoma*, *Podaxis*, *Sphaerobolus*, *Tulostoma*, and *Vascellum*. Lycoperdaceae is the better represented family, with six genera e fifteen species. *Arachnion setosum* and *Bovista kreiselii* are described as new. *Morganella afra* is recorded for the first time from South America, and *Bovista cunninghamii* is recorded for the first time from Brazil. *Podaxis pistillaris* was observed for the first time in the Southern Region.

Key words – Gasteromycetes, puffballs, stinkhorns, earth-star fungi, South American mycota

Introduction

Recently the knowledge of Brazilian gasteromycetes has strongly. According to the checklist of the gasteroid fungi from Brazil (Trierveiler-Pereira & Baseia 2009b), it is remarkable that 36 articles (from the total of 49 articles written by Brazilian researchers) were published since the year 2000. After this checklist, more than 35 articles have been published regarding the diversity of gasteromycetes from different Brazilian biomes and ecosystems.

The gasteroid mycobiota of the Atlantic Rainforest is the best known amongst the Brazilian biomes, especially due its proximity with mycological researching centers. More recently, studies have been published from semi-arid regions (Ottoni et al. 2010, Silva et al. 2011) and the Amazonian Rainforest (Trierveiler-Pereira et al. 2009, 2012; Leite et al. 2011, Alfredo et al. 2012a, 2012b; Cruz et al. 2012, Silva et al. 2013a).

However, there is little knowledge about the diversity of gasteroid fungi from the Brazilian Pampa Biome, which is restricted to the southern half of the state of Rio Grande do Sul (Southern Brazil). This biome occupies an area of 176,496 km^2, representing about 2% of the national territory (Roesch et al. 2009), and has a unique kind of vegetation, characterized by C3 and C4 plants adapted to the transition from subtropical to temperate climates (Fonseca et al. 2012). Up to date, no systematic survey of gasteromycetes has been executed in the Brazilian Pampa.

Material and Methods

Field expeditions were carried out in from 2010 to 2012 in municipalities of Rio Grande do Sul included in the Brazilian Pampa Biome: Caçapava do Sul (30S 30' 43", 53W 29' 27"), Manoel Viana (29S 35' 20", 55W 28' 58"), Quaraí (30S 23' 16", 56W 27' 03"), São Gabriel (30S 20' 09", 54W 19' 12"), and Santa Maria (29S 41' 02", 53W 48' 25"). This biome is characterized by grass dominated vegetation mixed with shrubs and treelets. The annual precipitation ranges from 1200–1600 mm and the mean annual temperature is 13–17°C (Overbeck et al. 2007).

Fungal specimens were kept in a plastic container with individualized compartments and transported to laboratory. Later, specimens were dried at low temperature (~ 40ºC) and then analyzed macro- and microscopically. Colors were coded according to Kornerup & Wanscher (1978). Colors and dimensions referred to dry material, unless otherwise stated. Spore measurements included surface ornamentation. Analyzed specimens are kept at Herbarium ICN, and specimens from other herbaria (BPI, K) were also analyzed to confirm some species identification (Thiers 2013). Nomenclature at family level followed Gube et al. (2009) and Hosaka et al. (2006).

Results

During this survey, sixty-three collections of gasteroid fungi were made in the sampled areas. Twenty-six species representing eight families and fourteen genera were identified. The identified species are presented alphabetically within each family.

Collected and identified species associated with exotic trees (*Eucalyptus* spp.) were not included in this list [viz. *Descomyces albus* (Berk.) Bougher & Castellano (ICN 175589), *Scleroderma albidum* Pat. & Trab. (ICN 175567, 175568), *S. bovista* Fr. (ICN 175583), and *Pisolithus arhizus* (Scop.) Rauschert (ICN 175569)].

Taxonomy

1. Agaricaceae

Podaxis pistillaris (L.: Pers.) Fr. emend. Morse, Micologia 25: 27 (1933) Fig 1

DESCRIPTION: Baseia & Galvão (2002).

MATERIAL OBSERVED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, Fazenda Cambará, L.P. Honaiser, 20/IV/2012 (voucher not preserved).

DISTRIBUTION: widespread in dry areas of the globe. In Brazil, it was only known from the Northeastern Region (Baseia & Galvão 2002, Drechsler-Santos et al. 2008).

COMMENTS: this species is characterized by its hard and fibrous pseudostipe that penetrates into the gleba. The gleba is covered by a scaly, subcylindrical peridium, and at maturity, it is dark and pulverulent. A color photograph of a Brazilian specimen is presented by Silva et al. (2013b). According to Jiskani (2009), *P. pistillaris* is used as food by some local communities in India, Afghanistan and Saudi Arabia. During this survey, the species was found growing in an open field among grasses, but since the single basidiome found was in an advanced stage of decomposition it could not be preserved. This is the first time that *P. pistillaris* is observed in Southern Brazil.

2. Clathraceae

Clathrus columnatus Bosc, Mag. Gesell. naturf. Freunde, Berlin 5: 85 (1811) Fig 2

DESCRIPTION: Magnago et al. (2013).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Santa Maria, L. Trierveiler-Pereira 39, 14/V/2010 (ICN 175598).

DISTRIBUTION: America, Africa and Oceania (Dring 1980).

COMMENTS: the species' receptacle is formed by three or four orange, spongy columns. The mucilaginous gleba is spread over the internal portion of the columns. The species is common in Rio Grande do Sul and it is found in sandy soil, gardens and pastures, but never inside forests (Rick 1929).

3. Geastraceae

Geastrum reinkingii Lloyd, Mycol. Writ. 7(Letter 72): 1280 (1924) Fig 3

DESCRIPTION: Lloyd (1924), Trierveiler-Pereira & Silveira (2014).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, P.M. Pedra do Segredo, L. Trierveiler-Pereira 277, 06/VI/2011 (ICN 175626).

ADDITIONAL MATERIAL EXAMINED: HONDURAS. ATLANTIDA: Tela, O.A. Reinkingi, 20/III/1923 (BPI 705664, holotype).

DISTRIBUTION: tropical and subtropical America.

COMMENTS: the species is characterized by arched exoperidium, mycelial layer not persistent at maturity; stipitate, depressed-globose endoperidium with a rugulose surface, white apophysis, and a strongly plicate peristome. It could be easily confused with *G. lloydianum* Rick and *G. hariotii* Lloyd, but features of the endoperidium (shape, surface, white apophysis), stipe (short and compressed) and spores morphology, separate it from these two species. *Geastrum reinkingii* was described from Honduras and Rick (1961) had reported it from Southern Brazil.

Geastrum saccatum Fr., Syst. mycol. (Lundae) 3(1): 16 (1829) Fig 4

DESCRIPTION: Trierveiler-Pereira et al. (2011).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, P.M. Pedra do Segredo, L. Trierveiler-Pereira 260, 261, 262, 263, 264, 268, 270, 273, 275, 279, 06/VI/2011 (ICN 175610, 175611, 175612, 175613, 175614, 175618, 175620, 175622, 175624, 175628).

DISTRIBUTION: worldwide, but probably corresponds to a complex of species.

COMMENTS: the main features to identify this morphospecies are: saccate exoperidium, sessile endoperidium, and peristome surrounded by a white circle. The so delimited peristome separates it from *G. fimbriatum* Fr., another common species in Brazil. Other similar species are *G. triplex* Jungh. and *G. lageniforme* Vittad., but the first has a prominent collar around the endoperidium, and the second usually long and slender rays with longitudinal ridges in the external layer and the hyphae from the external mycelial layer contain clamp connections (Trierveiler-Pereira et al. 2011).

Geastrum trichiferum Rick, in Lloyd, Mycol. Writings 2: 314 (1907) Fig 5

DESCRIPTION: Trierveiler-Pereira & Silveira (2012).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, P.M. Pedra do Segredo, L. Trierveiler-Pereira 269, 06/VI/2011 (ICN 175619).

DISTRIBUTION: pantropical.

COMMENTS: this species is characterized by the gregarious and lignicolous habitat, minute size, saccate exoperidium with brownish hairs on the mycelial layer, sessile endoperidium and delimited peristome. It comes very close to *G. schweinitzii* (Berk. & M.A. Curtis) Zeller, which has whitish mycelial layer without hairs.

Myriostoma coliforme (Dicks.) Corda, Anleit. Stud. Mykol., Prag: 131 (1842) Fig 6

DESCRIPTION: Baseia & Galvão (2002).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, P.M. Pedra do Segredo, L. Trierveiler-Pereira 267, 06/VI/2011 (ICN 175617).

DISTRIBUTION: tropical and subtropical dry areas.

COMMENTS: this monotypic genus is characterized by arched exoperidium, multistipitate endoperium; asperulate, yellowish to silvery endoperidial surface with several ostioles. It is similar to *Geastrum* species but it is easily distinguished by its multistipitate and multi-ostiolate endoperidium.

4. Lycoperdaceae

Arachnion album Schwein., Schr. naturf. Ges. Leipzig 1: no. 14, tab. 1, fig. 2 (1822) Fig 7

DESCRIPTION: Trierveiler-Pereira et al. (2010).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, Fazenda Cambará, L.P. Honaiser 06, 20/IV/2012 (ICN 192187); ibid., L.P. Honaiser 21, 22, 23, 28, 29, 21/IV/2012 (ICN 192188, 192189, 192190, 192191, 192192).

DISTRIBUTION: widely distributed throughout the world. In Brazil, *A. album* is known from the Southern and Northeastern Regions (Trierveiler-Pereira et al. 2010).

COMMENTS: *A. album* is characterized by fragile whitish basidiomata, ash-gray gleba at maturity, and ovoid, hyaline to greenish basidiospores, usually with a short pedicel. The most closely related species is *A. tenerum* (Berk.) Long, described from South Africa, but the latter has glebal granules composed by hyaline hyphae and basidiospores are usually long-pedicellate (Demoulin 1972, Kasuya et al. 2006).

Arachnion setosum Trierv.-Per. & R.M. Silveira**, sp. nov.** Figs 8, 23–24
MYCOBANK #

HOLOTYPE: BRAZIL. RIO GRANDE DO SUL: São Gabriel, Fazenda Cambará, L.P. Honaiser 49, 06/IX/2012 (ICN 192196, holotype).

DIAGNOSIS: basidiomata subglobose to depressed-globose, up to 2.5 cm in diam.; peridium double-layered, exoperidium grayish, endoperidium brownish gray; gleba olive brown; basidiospores ovoid to sugblobose, 4.5–5.5 (–6.0) × 3.5–5 µm, olive yellow, spinulose; browinish setae between the exo- and endoperidial layers.

ETYMOLOGY: due to the presence of setae in the peridium.

DESCRIPTION: immature basidiomata epigeous, sugblobose to ellipsoid, 0.9–1.4 cm high × 1.0–1.5 cm broad, with a small mycelial tuft attached at the base, 2.0 mm in length, mixed with soil particles; peridium 0.25–0.5 mm, glabrous, generally of a wrinkled aspect, yellowish white (2A2); gleba granulose, grayish yellow (1B3) to olive (2D3). Mature basidiomata epigeous, sugblobose, depressed-globose to ellipsoid irregular, 0.9–2.5 cm wide × 1.2–2.3 cm broad × 1.0–1.5 cm high, rhizomorphs not observed; peridium double-layered, with a furfuraceous aspect when fresh; exoperidium ~0.1 mm wide, compact, papyraceous, easily detached from the endoperidium once it is dried, grayish white (1B1), yellowish gray (2C2) to grayish brown (5E3), with a silver shining when dried; endoperidium up to 1.0 mm wide, fibrous to cottony, brownish gray (7D2); gleba granulose, composed by tiny granules, odor not fetid; glebal granules 0.2–1.0 mm in diam., subglobose, ellipsoid to irregularly shaped, greenish brown (5F3); capillitium and subgleba absent.

Basidiospores ovoid to subglobose, 4.5–5.5 (–6.0) × 3.5–5 µm, light yellow to olive yellow, smooth in LM x 1000, spinulose in MEV, slightly thick-walled, uniguttulate, usually with a short pedicel (up to 2 µm) or with a long (up to 40 µm) sterigmal remnant still attached. Exoperidium composed by pseudoparanchymatous hyphae, hyphae sugblobose to ellipsoid, 7–40 µm in diam., hyaline, thin-walled; endoperidium composed by filamentous hyphae, hyphae not-septate, tortuous, some branched, 1.5–5 µm in wide, brownish yellow to olive brown, thick-walled; some yellowish. Unusual spherocysts (setae) brownish, thick-walled, observed between the exo- and endoperidial layer.

ADDITIONAL MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, Fazenda Cambará, L.P. Honaiser 44, 46, 48, 49, 50, 06/IX/2012 (ICN 192193, 192194, 192195, 192196, 192197). ECUADOR, leg. Lagerheim (BPI 711746, as *A. bovista*).

DISTRIBUTION: known from the type locality (Southern Brazil) and Ecuador.

COMMENTS: the species is characterized by sugblobose to ellipsoid basidiomata, double-layered peridium, grayish exoperidium, olive brown glebal granules, and ovoid, olive yellow, basidiospores, usually with a short pedicel. The basidiospores appear smooth under LM, but with MEV it can be seen that they have a spinulose to verruculose surface. *Arachnion* species have always been described with smooth basidiospores (Miller Jr. & Miller 1988), so this is the first report of ornamented basidiospores in the genus.

Between the exoperidium and endoperidium it is possible to observe unusual spherocysts (setae), with brownish thick walls, as occur in some species of *Morganella*, *Bovista* and *Lycoperdon* (Demoulin 1976). The presence of setae might not be exceptional among Lycoperdaceae, especially in the tropics (Demoulin 1976). However, this feature is reported for the first time in *Arachnion*.

Another species with grayish peridium was described from Southern Brazil: *A. irimae* Rick (Rick 1961). However, Rick described this species as being more fragile than *A. album* and with gray glebal granules. These two features separate *A. irimae* from *A. setosum*. *Arachnion irimae* is considered a doubtful species, since no type or voucher specimen were found in Rick's collections (Demoulin 1972, Cortez et al. 2010).

The presence of endoperidium makes *A. setosum* similar to *A. lazoi* Demoulin, *A. lloydianum* Demoulin and *A. bovista* Mont., but the first two have true capillitium, and the last has brownish glebal granules when mature (Demoulin 1972).

Bovista aestivalis (Bonord.) Demoulin, Beih. Sydowia 8: 143 (1979)

DESCRIPTION: Calonge (1998).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, Fazenda Cambará, leg. L.P. Honaiser 15, 24, 25, 21/IV/2012 (ICN 175572, 175578, 175579).

DISTRIBUTION: America, Europe and Asia (Kreisel 2001, Calonge et al. 2005).

COMMENTS: the species is characterized by compact subgleba, brownish olive gleba, capillitial hyphae with pores, and globose, warty basidiospores. It is very similar to *B.*

dermoxantha but can be differentiated by the capillitium, which is of the intermediate type in *B. aestivalis* and of the *Lycoperdon*-type in *B. dermoxantha*. Moreover, *B. dermoxantha* has a darker exoperidium, no subgleba, and basidiospore ornamentation is more distinct (Ortega & Buendía 1989, Calonge 1998, Bautista-Hernández et al. 2011). In America, the species is known from Mexico (Calonge et al. 2004), Costa Rica (Calonge et al. 2005) and Brazil (Baseia 2005).

Bovista capensis (Fr.) J.C. Coetzee & A.E. van Wyk, Bothalia 35(1): 75 (2005)

DESCRIPTION: Coetzee & van Wyk (2005).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, Fazenda Cambará, L.P. Honaiser 17, 18, 19, 20, 21/IV/2012 (ICN 175574, 175575, 175576, 175577); Caçapava do Sul, Parque Municipal Pedra do Segredo, L. Trierveiler-Pereira 271, 06/VI/2011 (ICN 175621).

DISTRIBUTION: Africa and America (Coetzee & van Wyk 2005).

COMMENTS: The species is characterized by yellowish brown gleba, presence of subgleba, and oblong-ellipsoid, brownish yellow, warty basidiospores. *Bovista capensis* is similar to B. *aestivalis*, but the latter has globose basidiospores. Other species with oblong basidiospores, *B. oblongispora*, *B. longispora*, and *B. promontorii*, are mutually very similar (Ortega & Buendía 1989) and were synonymized with *B. capensis* by Coetzee & van Wyk (2005).

Bovista cunninghamii Kreisel, Nova Hedwigia, Beih. 25: 225 (1967) Fig 25

DESCRIPTION: basidiomata depressed-globose to sugblobose, 1.8-3.7 cm in diam., with an apical slit, with a small a tuft of rhizomorphs attached at the base, mixed with soil debris; exoperidium blond (4C4), grayish yellow (4C5) to light orange (5A5), granulose, endoperidium light yellow (4A4) to grayish yellow (3C4), papyraceous. Gleba grayish yellow (3C4, 4C5) to yellowish brown (5D8), pulverulent; subgleba absent.

Capillitium intermediate type, capillitial hyphae olive yellow, 1-5.5 µm wide, slightly thick-walled, pores conspicuous, small, abundant; paracapillitium absent. Basidiospores globose, thick-walled, olive yellow, 4-4.5 um in diam., smooth to punctuate under LM (descrever com MEV), pedicel not always conspicuous, less than 1 µm. Exoperidium formed by inflated, modified hyphae, mixed with filamentous hyphae, up to 12 um in the most inflated cells, thick-walled, yellowish; endoperidium exclusively formed by filamentous hyphae, 1-5 um wide, slightly thick-walled, pale yellow.

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, Fazenda Cambará, L.P. Honaiser 43, 21/IV/2012 (ICN 175591).

DISTRIBUTION: Australia, Spain, Mexico, Costa Rica, and Argentina (Kreisel 1967, Calonge 1998, Calonge et al. 2004, 2005; Hernández Caffot et al. 2013).

COMMENTS: the species is characterized by the absence of subgleba, yellowish brown gleba, intermediate type of capillitium with pores, and globose, puncticulate basidiospores with short pedicel (up to 1 µm) (Grgurinovic 1997, Calonge 1998). The species is similar to *B. aestivalis*, but the latter has a compact gleba (Calonge et al. 2005). This is the first record of the species from Brazil.

Bovista dominicensis (Massee) Kreisel, Feddes Repert. 69: 202 (1964) Fig 9

DESCRIPTION: Trierveiler-Pereira et al. (2010).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, Parque Municipal Pedra do Segredo, L. Trierveiler-Pereira 278, 06/VI/2011 (ICN 175627).

DISTRIBUTION: Neotropical (Trierveiler-Pereira et al. 2010).

COMMENTS: the species is characterized by a *Lycoperdon*-type capillitium without pits, and long-pedicelate, spinose basidiospores. The species is probably not rare in Brazil and it was already reported from Rio Grande do Sul by Homrich (1969).

Bovista kreiselii, Trierv.-Per. & R.M. Silveira**, sp. nov.** Figs 10, 26–28
MYCOBANK #

HOLOTYPE: BRAZIL. RIO GRANDE DO SUL: Quaraí, Cerro do Jarau, M.A. Reck 735/12, 01/VI/2012 (ICN 175594, holotype).

DIAGNOSIS: basidiomata depressed-globose, up to 2.2. cm in diam.; exoperidium light brown, furfuraceous; endoperidium grayish yellow, papyraceous; gleba yellowish brown; sugbleba absent; capillitium heteromorphous, septate, pores not conspicous; paracapillitium absent; basidiospores ovoid to subglobose, 5–6 × 4.5–5 µm, brownish yellowish, warty, with short pedicel.

ETYMOLOGY: named in honor of Dr. Hanns Kreisel, German mycologist who greatly contributed to the taxonomic knowledge of *Bovista* and other gasteroid fungi in general.

DESCRIPTION: mature basidiomata depressed-globose to sugblobose, 1.6-2.2 cm in diam., with or without a tuft of rhizomorphs attached at the base, mixed with soil debris; exoperidium dark blond (5D4), light brown (5D5) to yellowish brown (5E5, 5F5), furfuraceous in appearance, disappearing at maturity and remaining as small granules on the endoperidium, endoperidium grayish yellow (2C3, 3C3) to beige (4C3), with a metallic shining, papyraceous. Gleba yellowish brown (5E4) to grayish brown (5D3), pulverulent; subgleba absent.

Capillitium heteromorphous; intermediate type at the center of the gleba; *Lycoperdon*-type near the endoperidium, capillitial hyphae pale yellow to grayish yellow, usually 1-10 µm wide, however main stems might reach 22 µm at the center of the gleba, thick-walled, septate, septa not frequent, pores not conspicuous with LM x 1000, few observed with MEV; paracapillitium absent. Basidiospores ovoid to sugblobose, 5–6 × 4.5–5 µm, yellow to brownish yellowish, thick-walled, warty, with a short pedicel (about to 1 µm). Exoperidium formed by inflated, modified hyphae, mixed with filamentous hyphae, up to 14 um in the most inflated cells, thick-walled, dark brown; endoperidium exclusively formed by filamentous hyphae, 1-4 um wide, thick-walled, pale yellow, septate.

ADDITIONAL MATERIAL EXAMINED: AUSTRALIA. QUEENSLAND: Brisbane, leg. J.M. Bailey (K 187099, type of *Bovista coprophila*).

DISTRIBUTION: only known from the type locality.

COMMENTS: the species is characterized by grayish brown basidiomata, absence of subgleba, heteromorphous capillitium, and ovoid to sugblobose, warty basidiospores. At the center of the gleba the main hyphae of the capillitium may be so wide (up to 21 um) that they can be confused with the *Bovista*-type of capillitium. According to Kreisel's (1967) classification this new species belongs to subgen. *Globaria*, sect. *Globaria*, ser. *Albosquamosae*, together with *B. heterocapilla* Kreisel, *B. californica* Kreisel, *B. albosquamosa* Kreisel, *B. citrina* (Berk. & Broome) Bottomley, *B. oblongispora* (Lloyd) Bottomley, *B. abyssinica* Mont., *B. colorata* (Peck) Kreisel, and *B. coprophila* (Cooke & Massee) G. Cunn. The closest species is *B. coprophila*, with similar basidiospore size and morphology. *Bovista cophrophila* is known from Australia and Pacific island – Hawaii and Easter Island (Kreisel 1967). However, *B. coprophila* was described with a scanty subgleba indistinctly separated from the gleba (which could not be confirmed by us, since the type specimen is attached to a sheet of paper), and the gleba is reddish brown, while the gleba of *B. kreiselii* is olivaceous brown. Moreover, *B. kreiselli* has a furfuraceous exoperidium, ornamented with dark brown warts, while *B. coprophila* has grayish to yellowish exoperidium, ornamented with conical hyphal tufts ≤ 0.3 mm high.

Calvatia fragilis (Quél.) Morgan, J. Cincinnati Soc. Nat. Hist. 12: 168 (1890) Fig 11

DESCRIPTION: Cortez et al. (2012).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, Pedra do Segredo, L.P. Honaiser 01c, 02c, 29/III/2012 (ICN 175564, 175565); São Gabriel, Fazenda Cambará, L.P. Honaiser 26, 21/IV/2012 (ICN 175580).

DISTRIBUTION: pantropical-subtropical. In Brazil, it is known from the States of Rio Grande do Sul e Rio de Janeiro (Trierveiler-Pereira & Baseia 2009b).

COMMENTS: the species is characterized by the globose basidiome with sterile base, and compact, reduzed subgleba. Basidiomata are very fragile and the peridium easily falls apart at maturity. The species is commonly found in open areas.

Calvatia cyathiformis (Bosc) Morgan, J. Cincinnati Soc. Nat. Hist. 12(4): 168 (1890) Fig 12

DESCRIPTION: Cortez et al. (2012).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, Fazenda Cambará, L.P. Honaiser 07, 20/IV/2012 (ICN 175566); Santa Maria, L. Trierveiler-Pereira 46, 15/V/2010 (ICN 175602); ibid., L. Trierveiler-Pereira 60, 15/V/2010 (ICN 175608).

DISTRIBUTION: widespread, not yet reported from Europe (Cortez et al. 2012).

COMMENTS: the species is characterized by large and globose basidiomata, distinct cellular subgleba and equinulate basidiospores. One of the examined specimens (ICN 175566) correponds to a young basidiome: the gleba is light grayish, becoming purplish. However, its microscopic features (basidiospores and capillitium morphology) agree with *C. cyathiformis*.

Calvatia rugosa (Berk. & M.A. Curtis) D.A. Reid, Kew Bull. 31(3): 671 (1977) Fig 13

DESCRIPTION: Cortez et al. (2012).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Santa Maria, L. Trierveiler-Pereira 42, 43, 14/V/2010 (ICN 175600, 175601); Caçapava do Sul, P.M. Pedra do Segredo, L. Trierveiler-Pereira 266, 276, 06/VI/2011 (ICN 175616, 175625).

DISTRIBUTION: mostly found in America, but also reported from Japan, Australia, Europe and Africa (Reid 1977, Kreisel 2001).

COMMENTS: the species is common in the State of Rio Grande do Sul, and it is found on open areas, but also inside forests. It is characterized by purplish shades in the exoperidium when still young, yellowish gleba, and well-developed sterile base. Microscopically, the species is characterized by globose, echinulate basidiospores and capillitium with large pores. *Calvatia rubroflava* (Cragin) Lloyd is a synonym with *C. rugosa* (Kreisel 2001).

Lycoperdon juruense Henn., Hedwigia 43(3): 184 (1904) Fig 14

DESCRIPTION: Patouillard (1904, as *L. atrum*), Hennings (1904), Cortez et al. (2013).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, P.M. Pedra do Segredo, L. Trierveiler-Pereira 280, 06/VI/2011 (ICN 175629).

DISTRIBUTION: Neotropical (Demoulin 1976).

COMMENTS: this species was described based on Brazilian specimens collected in the Amazon forest (Hennings 1904). It is characterized by pyriform basidiomata with a well-developed sterile base; brownish, minutely spiny to velvety exoperidium; brownish to purplish-gray gleba, and densely verrucose basidiospores. Demoulin (1976) considered the species a synonym of *L. atrum* Pat. (originally described from Martinique and Guadeloupe), but the latter name is illegitimate. *Lycoperdon juruense* and *L. ovoidisporum* are macroscopically similar, but they differ microscopically (see comments below).

Lycoperdon marginatum Vittad., Monogr. Lycoperd.: 41 (1842) Fig 15

DESCRIPTION: Smith (1951), Cortez et al. (2013).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Santa Maria, L. Trierveiler-Pereira 40, 14/V/2010 (ICN 175599).

DISTRIBUTION: widespread, with a preference for continental climate (Demoulin 1976).

COMMENTS: the species is common in the State of Rio Grande do Sul, and it is found in fields and open areas, among grasses. It is characterized by a whitish exoperidium with pyramidal spines that fall away as plates at maturity, exposing the blond yellowish endoperidium. Basidiospores are globose and smooth to minutely punctuate under LM.

Lycoperdon ovoidisporum Cortez, Baseia & R.M.B. Silveira, Sydowia 63(1): 2 (2011)

DESCRIPTION: Cortez et al. (2011).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Santa Maria, L. Trierveiler-Pereira 47, 15/V/2010 (ICN 175603).

DISTRIBUTION: the species is only known from the State of Rio Grande do Sul, Brazil.

COMMENTS: the species is characterized by pyriform basidiome, dark brownish to olive brown, velvety exoperidium; and ovoid basidiospores which appear smooth under LM. Basidiomata morphology of *L. ovoidisporum* (shape and exoperidium color) resembles *L. juruense*, but the latter has globose, ornamented basidiospores.

Morganella afra Kreisel & Dring, Fedd. Repert. 74(1-2): 116 (1967) Fig 16, 29

DESCRIPTION: basidiomata depressed-globose to pyriform, 2.1–2.5 cm in diam., attached at a central point at the base with numerous rhizomorphs; exoperidium yellowish brown (5E4, 5E5), minutely granulose, endoperidium grayish yellow (3C3) to light brown (5D4), papyraceous. Gleba yellowish brown (5D5), pulverulent; subgleba reduced, 1 mm high, grayish yellow (4C4).

Paracapillitium 2.5–4 µm diam., pale yellow to hyaline, regularly septate. Eucapillitium absent. Basidiospores globose, yellowish, 3.2–4.5 × 3–4 µm, echinate, short pedicellate (less than 1 µm). Exoperidium formed by unchained pseudoparenchimatous cells (sphaerocysts), sphaerocysts usually globose to sugblobose, but some with irregular shape, 11–31 × 8–20 µm, with reddish brown content, slightly thick-walled.

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Santa Maria, L. Trierveiler-Pereira 53, 15/V/2010 (ICN 175605). ADDITIONAL MATERIAL EXAMINED: *M. afra*: GHANA. ASHANTI: Yinahin, Dixon 4, VI/1958 (K, holotype); *M. stercoraria*: PUERTO RICO. El Yunque, Fink Bruce 740, 09/XII/1915 (BPI 711047, holotype).

DISTRIBUTION: tropical Africa (Kreisel & Dring 1967) and America (Calonge et al. 2005).

COMMENTS: the species is characterized by depressed-globose basidiomata, olive brown to yellowish brown peridium, granulose exoperidium, olive brown gleba, compact and reduced subgleba, and globose, spinulose basidiospores. Our specimen was found growing on the soil, among cow dung and plant debris, and its main features agree with the type specimen of *M. afra*.

Since our specimen was found near dung, we have also compared our material with the type of *M. stercoraria* P. Ponce de Leon. However, the type of the referred species is poorly preserved, so it could only be analyzed microscopically. Basidiospores are ovoid [not globose, as described by Ponce de Leon (1971)] and spinulose under SEM and have a short pedicel (Fig. 30). Moreover, the sphaerocysts have a yellowish to hyaline content.

According to Calonge et al. (2005), *M. afra* is similar to *M. compacta*, but the latter has a spiny instead of granulose exoperidium.

Vascellum pratense (Pers.) Kreisel, Feddes Repert. 64: 159 (1962) Fig 17

DESCRIPTION: Bononi et al. (1984), Cortez et al. (2013).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Santa Maria, L. Trierveiler-Pereira 57, 15/V/2010 (ICN 175607); Caçapava do Sul, P.M. Pedra do Segredo, L. Trierveiler-Pereira 265, 06/VI/2011 (ICN 175615); ibid, L.P. Honaizer 32c, 19/V/2012 (ICN 175584); São Gabriel, L.P. Honaizer 36, 08/VII/2012 (ICN 175586).

DISTRIBUTION: cosmopolitan (Kreisel 1993).

COMMENTS: this species is characterized by turbinate to pyriform basidiomata, whitish exoperidium with small spines, distinct diaphragm, capillitium without pores and globose basidiospores. *Vascellum hyalinum* Homrich, originally described from Rio Grande do Sul, can be easily separated from *V. pratense*, since the former has an inconspicuous diaphragm and no eucapillitium (Homrich & Wright 1988).

5. Lysuraceae

Lysurus cruciatus (Lepr. & Mont.) Henn., Hedwigia 41(Beibl.): (172) (1902) Fig 18

DESCRIPTION: Dring (1980), Cortez et al. (2011).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, L.P. Honaizer 14, 20/IV/2012 (ICN 175571); ibid., L.P. Honaizer 41, 08/VII/2012 (ICN 175590).

DISTRIBUTION: widespread (Dring 1980).

COMMENTS: this phalloid species is characterized by its whitish pseudostipe surmounted by 5 to 8, reddish to white, short conical arms. The mucilaginous gleba is dark and it is spread in the internal portion of the arms, which are united at the tip at first, and separated afterwards. *Lysurus cruciatus* var. *nanus* Calonge & B. Marcos, characterized by smaller basidiomata and orange yellow arms, was reported from Southern Brazil by Cortez et al. (2011).

6. Nidulariaceae

Cyathus poeppigii Tul. & C. Tul., Annls Sci. Nat., Bot., sér. 3 1: 77 (1844) Fig 19

DESCRIPTION: Trierveiler-Pereira & Baseia (2009a).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, L.P. Honaizer 27, 21/IV/2012 (ICN 175581).

DISTRIBUTION: America, Africa and Asia (Brodie 1975).

COMMENTS: this bird's nest fungus is characterized by darkish, strongly plicate peridium (externally and internally), two-layered cortex of the peridiole, and large, ellipsoid basidiospores (up to 40 µm in length). It seems to be a common species in Brazil.

Cyathus stercoreus (Schwein.) De Toni, in Berlese, De Toni & Fischer, Syll. fung. (Abellini) 7: 40 (1888) Fig 20

DESCRIPTION: Coker & Couch (1928).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Caçapava do Sul, P.M. Pedra do Segredo, L. Trierveiler-Pereira 36, 37, 38, 16/IV/2010 (ICN 175595, 175596, 175597); ibid., L. Trierveiler-Pereira 281, 06/VI/2011 (ICN 175630); São Gabriel, L.P. Honaizer 12, 20/IV/2012 (ICN 175570); ibid., L.P. Honaizer 34, 37, 38, 08/VII/2012 (ICN 175585, 175587, 175588); Quaraí, M.A. Reck 734/12, 01/VI/2012 (ICN 175593).

DISTRIBUTION: cosmopolitan (Brodie 1975).

COMMENTS: the species is characterized by coprophilous habitat, light-colored basidiomata, without plications externally and internally, black peridiole with a two-layered cortex, and large, globose to sugblobose basidiospores (25–40 µm in diam.). This species might be confused with *C. olla* (Batsch) Pers., but the latter has smaller basidiospores and peridioles with a one-layered cortex.

7. Sphaerobolaceae

Sphaerobolus stellatus Tode, Fung. mecklenb. sel. (Lüneburg) 1: 43 (1790) Fig 21

DESCRIPTION: Walker (1927), Bottomley (1948).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: Santa Maria, L. Trierveiler-Pereira 51, 56, 15/V/2010 (ICN 175604, 175606).

DISTRIBUTION: cosmopolitan (Geml et al. 2005).

COMMENTS: this species is characterized by gregarious, small-sized basidiomata (2–4.5 mm in diam. when spread) opening in a stellate manner; beige exoperidium, bright yellow inner layer and a single dark brown peridiole at the center. During our studies, it was found growing on cow dung, although there are also records of the species growing on rotten wood or litter fall.

8. Tulostomataceae

Tulostoma cyclophorum Lloyd, Tylostomeae: 25 (1906) Fig 22

DESCRIPTION: Cortez et al. (2009).

MATERIAL EXAMINED: BRAZIL. RIO GRANDE DO SUL: São Gabriel, L.P. Honaizer 01, 06/IV/2011 (ICN 175563); ibid., L.P. Honaizer 30, 21/IV/2012 (ICN 175582).

DISTRIBUTION: cosmopolitan (Wright 1987).

COMMENTS: the species is characterized by the brownish exoperidium that falls away in plates at maturity; whitish, membranaceous endoperidium with mycosclereids, and mammose, fimbriate peristome. The basidiospores are globose to sugblobose, 3.4–5.1 µm, greenish yellow, and verrucose. It seems to be a common species in Rio Grande do Sul, usually found on sandy soil or pastures.

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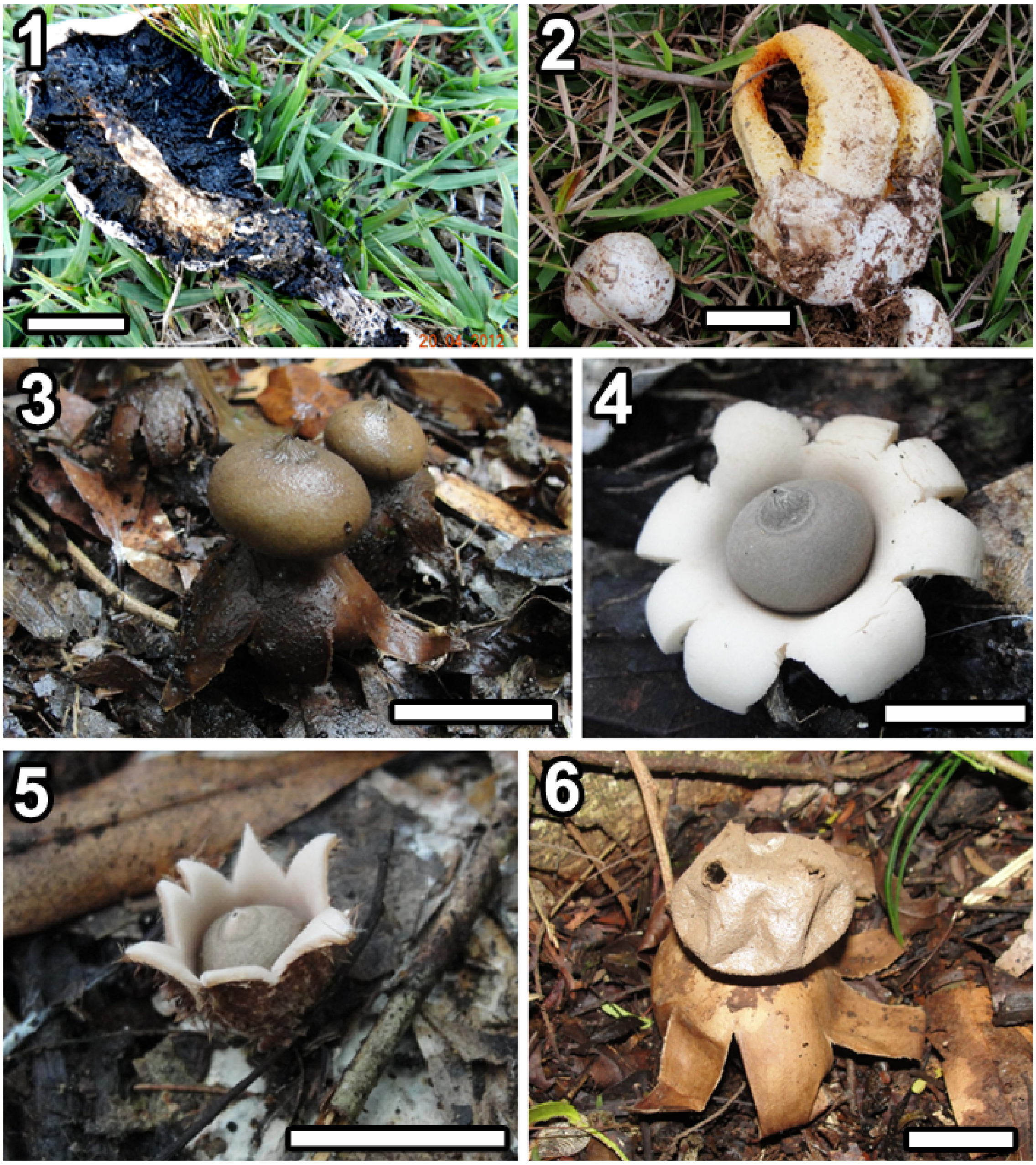
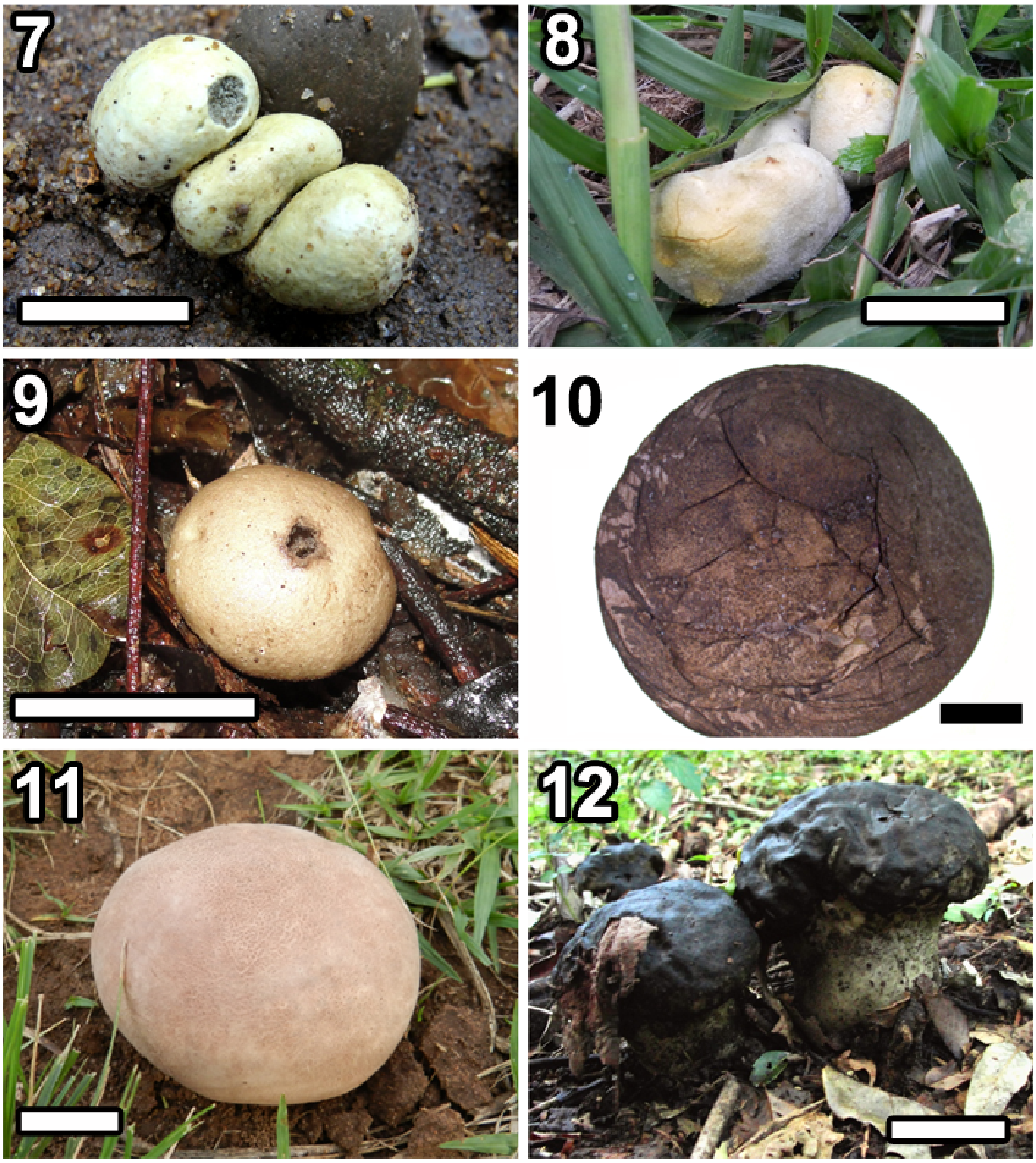
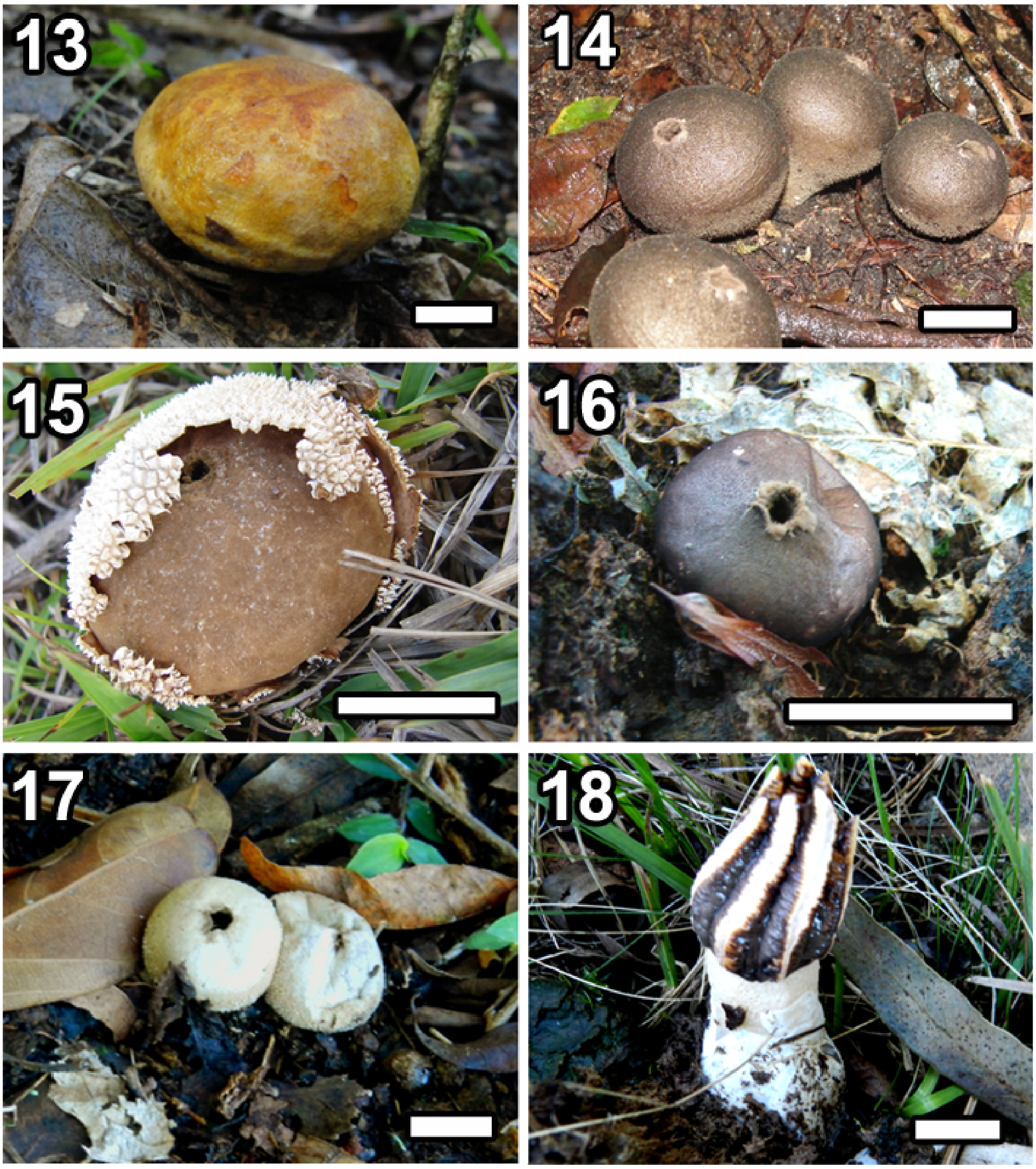


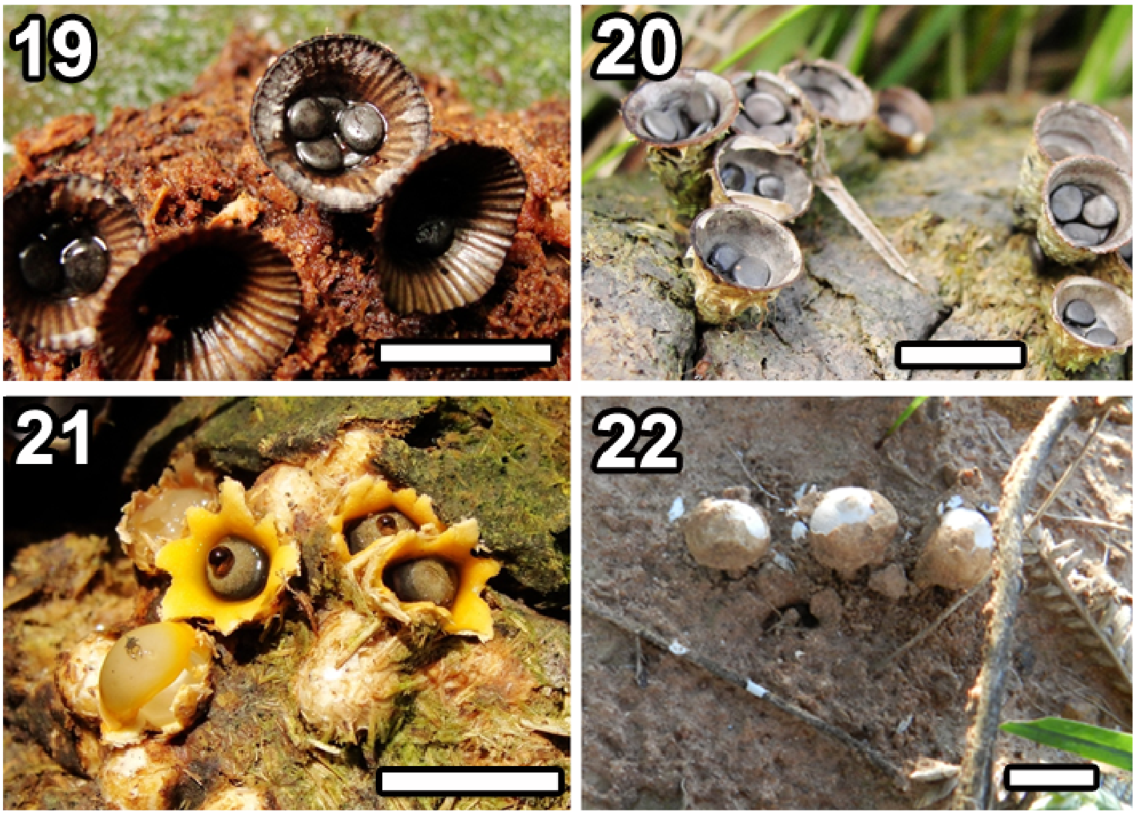
Figs 1–6. Gasteromycetes from the Brazilian Pampa. 1. *Podaxis pistillaris* (voucher not preserved). 2. *Clathrus columnatus* (ICN 175598). 3. *Geastrum* sp. nov. (ICN 175626). 4. *G. saccatum* (ICN 175610). 5. *G. trichiferum* (ICN 175619). 6. *Myriostoma coliforme* (ICN 175617). Bars: 1, 2, 3, 4, 6 = 2 cm; 5 = 1 cm.



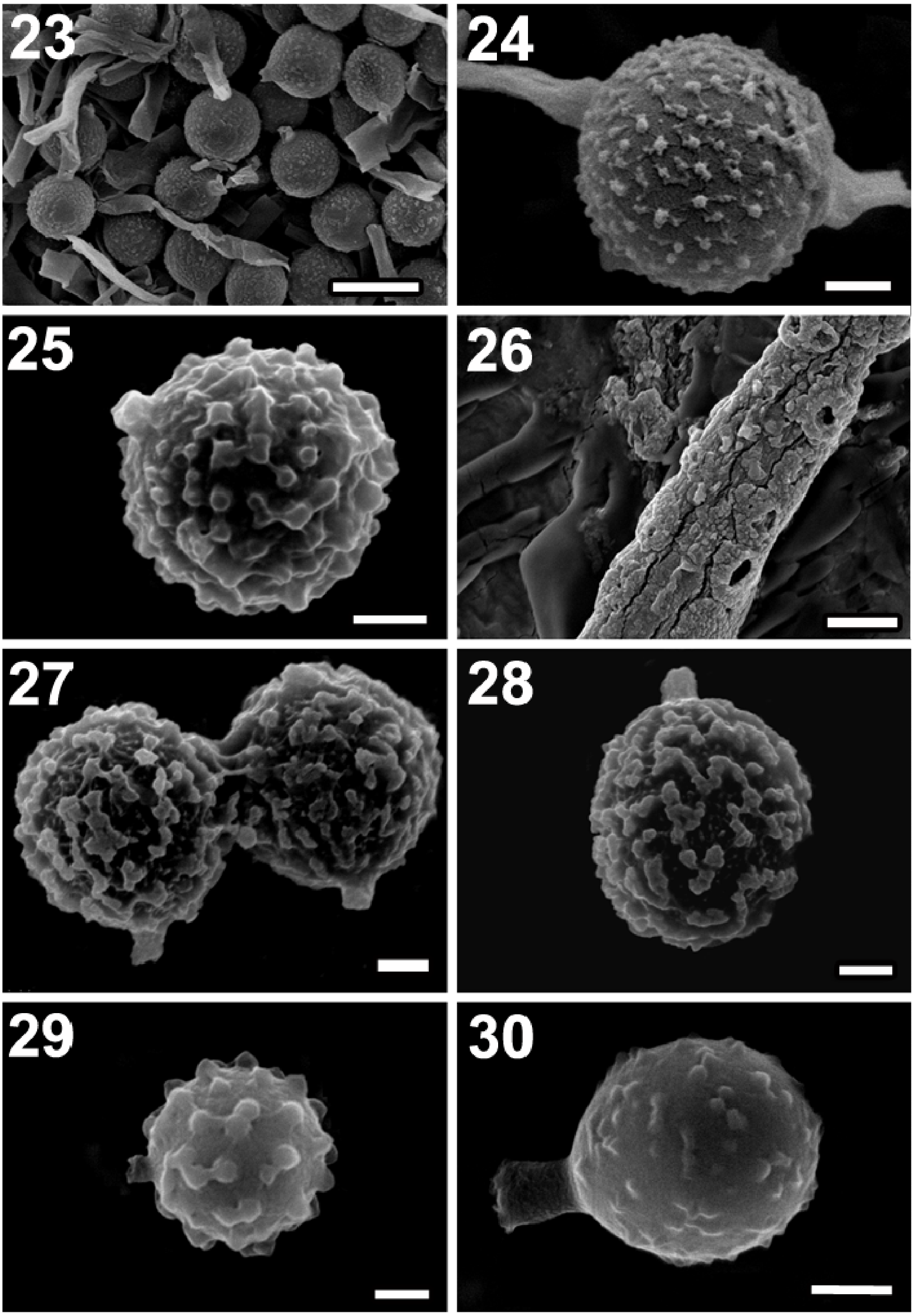
Figs 7–12. Gasteromycetes from the Brazilian Pampa. 7. *Arachnion album* (ICN 192187). 8. *A. setosum* (ICN 192196, holotype). 9. *Bovista dominicensis* (ICN 175627). 10. *B. kreiselii* (ICN 175594, holotype). 11. *Calvatia fragilis* (ICN 175580). 12. *C. cyathiformis* (ICN 175608). Bars: 7, 8, 9, 11 = 2 cm; 10 = 0.5 cm; 12 = 4 cm.



Figs 13–18. Gasteromycetes from the Brazilian Pampa. 13. *Calvatia rugosa* (ICN 175616). 14. *Lycoperdon juruense* (ICN 175629). 15. *L. marginatum* (ICN 175599). 16. *Morganella afra* (ICN 175605). 17. *Vascellum pratense* (ICN 175586). 18. *Lysurus cruciatus* (ICN 175590). Bars = 2 cm.



Figs 19–22. Gasteromycetes from the Brazilian Pampa. 19. *Cyahtus poeppigii* (ICN 175581). 20. *C. stercoreus* (ICN 175595). 21. *Sphaerobolus stellatus* (ICN 175604). 22. *Tulostoma cyclophorum* (ICN 175585). Bars: 19, 20, 21 = 0.5 cm; 22 = 1 cm.



Figs 23–30. SEM photographs of basidiospores and capillitium. 23, 24. *Arachnion setosum* (ICN 192196, holotype). 25. *Bovista cunninghamii* (ICN 175591). 26, 27, 28. *B. kreiselii* (ICN 175594, holotype). 29. *Morganella afra* (ICN 175605). 20. *M. stercoraria* (BPI 711047, holotype). Bars: 23, 26 = 5 µm; 24, 25, 27, 28, 29, 30 = 1 µm.

5. CONSIDERAÇÕES FINAIS

As espécies pertencentes à *Phallales* e *Geastrales* são comumente encontradas em diversas formações vegetais da Região Sul do Brasil, porém, o conhecimento sobre estes grupos ainda é fragmentado, sendo que cada ordem possui diferentes peculiaridades que dificultam o trabalho dos taxonomistas.

Os fungos faloides produzem basidiomas que possuem uma função clara: atrair insetos. A exalação de um forte cheiro desagradável ou adocicado faz com que estes basidiomas recém-expostos sejam atacados por insetos e desapareçam em poucas horas, garantindo assim a dispersão dos esporos. Dessa maneira, encontrar os basidiomas *in situ* é algo esporádico, não pela raridade das espécies, mas sim pela efemeridade dos basidiomas. Dring (1980) constatou que é excepcional um pesquisador conseguir observar em campo mais de meia dúzia de espécies de *Clathraceae* em seu estado fresco.

Ainda, algumas espécies possuem o hábito solitário, o que dificulta a observação da ontogenia dos basidiomas. Por exemplo, quando Möller (1895) descreveu *Phallus glutinolens*, observou que esta possuía um receptáculo hemisférico, característica única dentre as espécies do gênero (Calonge 2005). Achados posteriores mostraram que *P. glutinolens* possui o receptáculo globoso apenas quando recém exposto, e que formas mais maduras possuem o receptáculo campanulado, assim como outras espécies de *Phallus* (Trierveiler-Pereira et al. 2009). Quando apenas um ou dois basidiomas são avistados *in situ*, é recomendável procurar por formas imaturas ('ovos') que possam estar próximas, porém, todavia soterradas.

A fragilidade dos basidiomas faloides também compromete as etapas de coleta, transporte e preservação dos materiais. Basidiomas delicados, como ocorre em espécies de *Clathrus* e *Laternea*, podem ser severamente danificados durante o transporte. O processo de desidratação pode alterar completamente o tamanho, aspecto e coloração do basidioma, e quando não bem controlado, pode destruir o mesmo. Características com alto valor taxonômico podem ser perdidas após a secagem do material, comprometendo o processo de identificação. Segundo minha experiência, a melhor forma de desidratar os basidiomas é com o uso de um desidratador automático, vendido comercialmente para desidratar frutas e alimentos. Esses aparelhos secam lentamente o material e preservam razoavelmente bem a coloração original.

Durante a execução deste trabalho, tive a felicidade de contar com colaboradores que me enviaram fotografias de campo de alta qualidade e materiais bem preservados, mas é importante lembrar que o cheiro desagradável e a dificuldade de preservar os espécimes desestimulam muitos micólogos amadores ou que se dedicam a outros grupos.

Trabalhos mais antigos que descreveram novas espécies de faloides falharam em apresentar descrições acuradas e boas ilustrações ou fotografias dos basidiomas. Dessa maneira, o conceito de muitas espécies descritas é fragmentado e insuficiente para circunscrever o táxon em questão. Atualmente, deve-se tirar proveito da possibilidade de publicar fotografias coloridas em muitos periódicos científicos, sem custo adicional para os autores.

Lloyd (1907b, d) já criticava naquela época a descrição de novos táxons de *Phallales* sem o acompanhamento de ilustrações ou fotografias de boa qualidade. Um caso claro é o de *Xylophallus xylogenus*, que ilustrado de formas muito distintas na literatura, formou um conceito fragmentado sobre a espécie e levou ao limbo o nome de um táxon comum na América tropical (Trierveiler-Pereira & Silveira 2012a). As ótimas ilustrações apresentadas por Dring & Rose (1977) e Demoulin & Dring (1975) mostram a clara diferença que existe entre basidiomas de *Mutinus bambusinus* e *M. argentinus*, duas espécies que até então vinham sendo tratadas como sinônimos por alguns autores.

Em contrapartida, bons desenhos e ilustrações permitem corrigir identificações errôneas, como a ocorrência de *Ileodictyon cibarium* Tul. ex M. Raoul no Nordeste do Brasil (Baseia et al. 2006, Leite et al. 2007), que corresponde à *Clathrus chrysomycelinus*; e *Blumenavia angolensis* (Welw. & Curr.) Dring (Rodrigues & Baseia 2013), que corresponde à *B. rhacodes*.

Na Região Sul do Brasil, o conhecimento do grupo já parece bastante aprofundado, mas ainda são necessárias melhores descrições e fotografias coloridas de alguns táxons pouco tratados na literatura, como *Aporophallus subtilis* e *Gelopellis thaxteri*. Outros gêneros de taxonomia mais complexa, como *Mutinus*, ainda precisam de mais estudos taxonômicos e moleculares para resolver a delimitação de espécies. Além disso, a adição de sequências de fungos faloides em estudos moleculares futuros deve auxiliar a resolução de dúvidas antigas, como por exemplo, a circunscrição de gêneros incluídos em *Clathraceae*.

No caso dos fungos *Geastrales*, os problemas que envolvem questões taxonômicas são outros. Espécimes do gênero *Geastrum* são frequentemente encontradas nas formações florestais brasileiras e americanas e a preservação dos materiais é relativamente simples. Dessa maneira, muitos espécimes foram coletados no passado por estrangeiros e atualmente encontram-se depositados em herbários fora do Brasil, sendo alguns desses espécimes-tipo. Um grande número de espécies de *Geastrum* foi descrita no passado, e hoje o gênero conta com mais de 300 nomes específicos (http://www.indexfungorum.org/).

Entretanto, ainda não há uma monografia confiável para as espécies que ocorrem nas regiões tropicais e subtropicais americanas. A monografia de Ponce de Leon (1968) apresenta uma longa lista de sinonímias que não é confiável, pois não foi baseada na análise de tipos. Assim, muitos nomes continuam no limbo, enquanto novas espécies vêm sendo descritas para a ciência. A meu ver, seria mais prudente no estado atual do conhecimento do gênero, realizar primeiramente estudos aprofundados na história do grupo, consultando publicações antigas e examinando espécimes-tipo. Seguindo esta idéia, nomes antigos foram recentemente resgatados por Trierveiler-Pereira & Silveira (2012b) e Zamora et al. (2014).

Além de todo o problema dos nomes antigos para as espécies de *Geastrum*, é preciso ter-se em conta a dificuldade de trabalhar com o grupo. Apesar de algumas espécies serem circunscritas apenas com base em macromorfologia, as diferenças em características microscópicas precisam ser melhor exploradas, já que espécies com macromorfologia muito similar podem apresentar esporos bastante distintos (e.g. *G. hariotii* e *G. lloydianum*).

As características macroscópicas com valor taxonômico são poucas, já que algumas destas, como coloração e dimensão das estruturas, podem variar conforme as condições ambientais. Análises moleculares apresentadas nessa tese demonstraram que espécies com macromorfologias muito similares podem na verdade representar complexos de espécies. Um estudo publicado recentemente teve como objetivo encontrar outras características confiáveis para a delimitação de espécies, como análises químicas e morfologia dos rizomorfos (Zamora et al. 2013).

Apesar de que até o momento pouquíssimas sequências de espécies de *Geastrum* estão depositadas no GenBank (https://www.ncbi.nlm.nih.gov/genbank), quando comparado com a real diversidade do grupo, estudos recentes têm introduzido novos táxons baseando-se fortemente em dados moleculares. Entretanto, acredito que mais importante que análises moleculares, são as revisões de espécimes-tipos e o resgate de nomes antigos. Sem estas revisões, a proposição de novos táxons pode, na verdade, resultar na introdução de nomes desnecessários.

Estudos futuros ainda são necessários para que se tenha um conhecimento real da diversidade de espécies de *Geastrales* que ocorrem na Região Sul do Brasil. Além disso, até o momento não há registros de espécies hipógeas desta ordem (e.g. *Radiigera*), que provavelmente ocorrem na região devido a sua distribuição. É possível que a falta destes registros seja decorrente da especificidade da metodologia de coleta dessas espécies.

Apesar de todos os problemas e dificuldades expostos acima, acredita-se que o presente trabalho apresenta contribuições relevantes à taxonomia, distribuição, ecologia e filogenia de *Phallales* e *Geastrales*. Espera-se que os resultados aqui apresentados sejam úteis para um melhor entendimento destes fungos, assim como de utilidade para a comunidade científica e micólogos amadores.

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