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Sistemática filogenética e taxonomia da subtribo Calothecinae (Poaceae, Pooideae, Poeae *s.l.*)

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“Accept all happiness from me
Accept all happiness from me
Can you touch my body
Like it’s something new?

I never thought I'd cry so much
I never thought I would be this cry, baby
Baby, cry

Life is not as easy as I thought it would be
When I was five
Accept all happiness from me

When I was five
When I was five
When I was five
When I had a life

Accept all happiness from me
Accept all happiness from me
Accept all happiness from me
Accept all happiness from me

Eu aceito as minhas outras vidas”

Letícia Novaes (Letrux)

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SUMÁRIO

RESUMO

A subtribo Calothecinae atualmente inclui três gêneros, *Chascolytrum s.l.*, *Laegaardia* e *Paramochloa*, dos quais o primeiro se destaca por incluir o maior número de espécies. O gênero *Chascolytrum s.l.* apresenta um histórico taxonômico e controverso, em virtude das diferentes circunscrições propostas anteriormente. Como atualmente circunscrito, o gênero inclui 22 espécies que já foram anteriormente reconhecidas em nove gêneros distintos (*Chascolytrum*, *Briza*, *Poidium*, *Calotheca*, *Microbriza*, *Gymnachne*, *Rhombolytrum*, *Lombardochloa* e *Erianthecium*). Contudo, as relações entre as espécies de *Chascolytrum s.l.* permanecem pouco resolvidas devido à baixa resolução filogenética encontrada em estudos anteriores. A presente tese utiliza sequências de DNA, dados morfológicos e uma ampla amostragem e táxons, para resolver a circunscrição dos gêneros reconhecidos em Calothecinae, particularmente aqueles incluídos na sinonímia de *Chascolytrum s.l.* As análises filogenéticas suportaram, em um primeiro momento, *Chascolytrum s.l.* como uma linhagem monofilética, permitindo o reconhecimento de oito seções para o gênero, das quais três são novas. Além disso, uma nova espécie de *Chascolytrum* sect.. *Hildaea* foi descrita para a ciência (*C. serranum*). Subsequentemente, análises filogenéticas incluindo uma amostragem mais ampla, tanto em número de táxons quanto marcadores moleculares, revelaram que tanto Calothecinae quanto *Chascolytrum s.l.* são polifiléticos, devido à inclusão de *Trisetum brasiliense* e *T. bulbosum* dentro do clado *Chascolytrum s.l.* Como resultado, o gênero *Chascolytrum s.l.* foi desmembrado em nove gêneros, dos quais dois são novos para a ciência, e os gêneros *Laegaardia* e *Paramochloa* foram transferidos para a nova subtribo Paramochloinae. As espécies *T. brasiliense* e *T. bulbosum* foram formalmente incluídas em Calothecinae, como *incertae sedis*, e seus posicionamentos taxonômicos necessitam estudos mais aprofundados, incluindo novas amostras sul-americanas do gênero *Trisetum*.

Palavras-chave: *Chascolytrum s.l.*, classificação infragenérica, delimitação de gêneros, evidência total, filogenia molecular, gramíneas.

ABSTRACT

Currently, the subtribe Calothecinae includes three genera, *Chascolytrum s.l.*, *Laegaardia* and *Paramochloa*, of which *Chascolytrum* is the most diverse. The genus *Chascolytrum s.l.* has a complex and controversial taxonomic background due to several circumscriptions proposed in the past. As now cirscumscribed, the genus includes 22 species that were previously recognized in nine different genera (*Chascolytrum*, *Briza*, *Poidium*, *Calotheca*, *Microbriza*, *Gymnachne*, *Rhombolytrum*, *Lombardochloa* e *Erianthecium*). However, the internal relationships of *Chascolytrum s.l.* remain poorly resolved due to lack of phylogenetic resolution found in previous studies. The present thesis includes DNA sequences, morphological data and a comprehensive taxon sampling to resolve the generic circumscription of Calothecinae, mainly of those genera included as synonymy of *Chascolytrum s.l.* The phylogenetic analyses supported, at first, *Chascolytrum s.l.* as monophyletic and allowed the recognition of eight sections, of which three are newly described. Besides, a new species of *Chascolytrum* sect. *Hildaea* was first described (*C. serranum*). Thereafter, phylogenetic analyses including a broader sampling and more molecular markers revealed that both Calothecinae and *Chascolytrum s.l.* are polyphyletic, due to the inclusion of *Trisetum brasiliense* and *T. bulbosum* in the *Chascolytrum s.l.* clade. As a result, *Chascolytrum s.l.* was split in nine different genera of which two are newly described, and *Laegaardia* and *Paramochloa* were transferred to the new subtribe Paramochloinae. *Trisetum brasiliense* and *T. bulbosum* were formally included in Calothecinae, although as *incertae sedis*. Further studies to resolve their taxonomic placement are still needed.

Keywords: *Chascolytrum s.l.*, generic circumscription, grasses, infrageneric classification, molecular phylogeny, total evidence.

INTRODUÇÃO GERAL

1. A família Poaceae

Poaceae (Gramineae) é uma das maiores famílias de Angiospermas, incluindo mais de 700 gêneros e 12.000 espécies (Soreng *et al.*, 2017), com distribuição cosmopolita e ocorrendo em uma grande variedade de hábitats (Clayton & Renvoize, 1986; Osborne *et al.,* 2011). As gramíneas constituem o principal componente das formações campestres, e ocupam com menor frequência o interior de formações florestais (Welker & Longhi-Wagner, 2007; Boldrini *et al.*, 2008). As gramíneas apresentam uma grande importância ecológica, pela dominância em diversos ecossistemas terrestres, e econômica, pela sua contribuição direta e indireta na alimentação humana (Boldrini *et al.*, 2008). No Brasil, ocorrem 224 gêneros e 1497 espécies (Flora do Brasil, 2020).

Atualmente a família está dividida em 12 subfamílias (Soreng *et al.* 2015; 2017), dentre as quais Pooideae Benth. [popularmente conhecidas por "*cool-season grasses*" (Saarela *et al.*, 2015; 2017)] se destaca por incluir cerca de um terço das espécies de gramíneas [cerca de 4000 espécies em 202 gêneros (Soreng *et al.* (2017)], distribuídas principalmente nas regiões temperadas e subtropicais ao redor do mundo (Clayton & Renvoize 1986; Saarela *et al.*, 2017). Além disso, espécies de grande importância econômica, como os cereais de inverno (trigo, aveia, centeio e cevada) pertencem a esta subfamília (Davis & Soreng, 2007; Boldrini *et al.*, 2008; Judd *et al.*, 2009), além de inúmeras forrageiras (Tkach *et al.*, 2020). Classificações mais recentes reconhecem de 10 (Kellogg, 2015) a 15 (Soreng *et al.*, 2017) tribos dentro de Pooideae, sendo Poeae R.BR. a mais diversa, incluindo 121 gêneros e 2562 espécies (Soreng *et al.*, 2017).

2. A subfamília Pooideae e a tribo Poeae *s.l.*

A classificação interna de Pooideae tem sido controversa ao longo do tempo. Nas últimas duas décadas, as relações filogenéticas e taxonômicas entre grandes grupos (e.g. tribos) começaram a ser elucidadas concomitantemente ao avanço das técnicas de biologia molecular e análises filogenéticas (Soreng *et al.*, 1990; Davis & Soreng, 2007; Döring *et al.*, 2007; Quintanar *et al.*, 2007; Saarela *et al.*, 2010; 2015; 2017, 2018; Kellogg, 2015; Soreng *et al.*, 2015; 2017). Grupos taxonômicos bem definidos morfologicamente não se sustentaram com base em caracteres moleculares, e novas classificações aos níveis de tribos e subtribos foram propostas. As relações entre as tribos Poeae *s.s.* e Aveneae Dumort., por exemplo, vêm sendo alvo de diversos estudos e suas circunscrições como tradicionalmente aceitas (Clayton & Renvoize, 1986) não são monofiléticas (Soreng & Davis, 2000; Döring *et al.*, 2007; Saarela *et al.*, 2010). Embora existam caracteres morfológicos marcantes para o reconhecimento destas duas tribos, *sensu* Clayton & Renvoize (1986), o posicionamento atual de alguns gêneros anteriormente reconhecidos ou em Poeae *s.s.* ou em Aveneae sugerem uma história evolutiva mais complexa para este grupo, envolvendo múltiplos eventos de hibridação e evolução reticulada (Saarela *et al.*, 2010; Soreng *et al.*, 2015; Tkach *et al.*, 2020).

Os gêneros *Briza* L. e *Chascolytrum* Desv. (*sensu* Essi *et al.*, 2017), por exemplo, foram tradicionalmente incluídos na tribo Poeae *s.s.* com base em morfologia da espigueta (Clayton & Renvoize 1986; Longhi-Wagner, 1987), mas são filogeneticamente próximos a gêneros morfologicamente incluídos na tribo Aveneae (Soreng & Davis, 2000; Döring *et al.*, 2007; Saarela *et al.*, 2010; Soreng *et al.*, 2017). O reconhecimento de duas tribos distintas se tornou morfologicamente impraticável, enquanto a ocorrência de dois grandes clados com genomas plastidiais distintos ("Aveneae *type*" e "Poeae *type*") suportou o reconhecimento de dois grupos informais (Poeae chloroplast group 1 e Poeae chloroplast group 2, respectivamente) (Soreng & Davis, 2000; Davis & Soreng, 2007; Döring *et al.*, 2007; Quintanar *et al.*, 2007; Saarela *et al.*, 2010; 2017, 2018; Kellogg, 2015; Pimentel *et al.,* 2017; Orton *et al.,* 2019). A partir de então, Poeae passou a ser aceita com uma circunscrição mais ampla (Poeae *s.l.*), englobando também gêneros outrora incluídos em Aveneae. Além disso, as relações de parentesco e evolutivas internas do clado Poeae *s.l.* passaram a ser alvo de estudos filogenéticos mais detalhados (Kellogg, 2015; Soreng *et al.*, 2015; 2017; Saarela *et al.*, 2017), e 18 subtribos foram reconhecidas (Soreng *et al.*, 2017). Recentemente, com base em dados moleculares, Tkach *et al.* (2020) sugeriram o reestabelecimento Poeae *s.s.* e Aveneae como tribos distintas, incluindo ambas na supersubtribo Poodae junto com Festuceae Dumort. No entanto, nenhuma circunscrição morfológica foi sido proposta para o reconhecimento de Poeae *s.s.* e Aveneae.

O trabalho de Saarela *et al.* (2017) pode ser considerado como um dos mais importantes e abrangentes estudos acerca das relações filogenéticas do clado "Aveneae *type*" ("*Poeae Chloroplast Group 1*") da tribo Poeae *s.l.* Usando uma vasta amostragem de táxons e marcadores moleculares, Saarela *et al.* (2017) investigaram as relações filogenéticas entre as subtribos Agrostidinae Fr., Anthoxanthinae A. Gray, Aveninae J. Presl, Brizinae Tzvelev, Calothecinae Soreng, Phalaridinae Fr. e Torreyochloinae Soreng & J. I. Davis, e evidenciaram que as subtribos Agrostidinae e Calothecinae, como até então circunscritas, não eram monofiléticas. Além disso, gêneros com expressiva diversidade e amplamente distribuídos, como *Calamagrostis* P.Beauv., *Deyeuxia* Clarion ex P.Beauv. e *Trisetum* Pers. se revelaram polifiléticos, corroborando a necessidade de estudos filogenéticos e revisões taxonômicas mais detalhadas para estes grupos. Alguns destes gêneros foram recentemente reavaliados, e novos gêneros e combinações foram propostos, respeitando o princípio do monofiletismo (Barberá *et al.*, 2019; Peterson *et al.*, 2019). As subtribos Agrostidinae e Calothecinae foram recircunscritas, e a subtribo Echinopogoninae foi descrita para acomodar gêneros anteriormente incluídos em Agrostidinae [(.e., *Dichelachne* Endl. e *Echinopogon* P.Beauv. (Soreng *et al.*, 2017)]. Estas três subtribos correspondem a três linhagens filogeneticamente próximas (Saarela *et al.*, 2017; Soreng *et al.*, 2017; Tkach *et al.*, 2020), embora suas relações de parentesco não tenham sido completamente elucidadas até o momento.

3. A subtribo Calothecinae

A subtribo Calothecinae foi primeiramente descrita por Soreng *et al.* (2015), incluindo dois gêneros americanos: *Chascolytrum s.l.* (22 espécies) e *Relchela* Steud (uma espécie). O estudo de Saarela *et al.* (2017), entretanto, revelou Calothecinae como polifilética devido à inclusão de *Relchela panicoides* Steud. em um clado mais distante, junto com *Calamagrostis coarctata* Steud., *Echinopogon caespitosus* C.E.Hubb., e *Dichelachne* spp., tanto nas filogenias baseadas em marcadores nucleares quanto nas árvores baseadas em marcadores plastidiais. Este clado foi posteriormente reconhecido como a subtribo Echinopogoninae, com cinco gêneros e 20 espécies, incluindo *Relchela panicoides* (Soreng *et al.*, 2017). As espécies de *Chascolytrum s.l.*, no entanto, (i) formaram um clado com *Deyeuxia effusa* Kunth, posicionado como grupo irmão de um segundo clado formado por espécies pertencentes à subtribo Agrostidinae (incluindo *Agrostis* L., *Calamagrostis*, *Deyeuxia* e *Polypogon* Desf.) nas filogenias reconstruídas à luz de marcadores nucleares; e (ii) formaram uma politomia com *Deyeuxia effusa* e parte da subtribo Agrostidinae nas árvores plastidiais (Saarela *et al.*, 2017). Consequentemente, Calothecinae ficou restrita apenas ao gênero *Chascolytrum s.l.* (Soreng *et al.*, 2017).

Recentemente, os gêneros *Paramochloa* P.M.Peterson, Soreng, Romasch. & Barberá (duas espécies, incluindo *Deyeuxia effusa*) e *Laegaardia* P.M.Peterson, Soreng, Romasch. &

Barberá (= *Calamagrostis ecuadoriensis* Laegaard) foram propostos por Peterson *et al.* (2019) e alocados em Calothecinae juntamente com *Chascolytrum s.l.* baseado em filogenias moleculares ainda inéditas, embora nenhuma árvore tenha sido apresentada neste estudo. Desta forma, Peterson *et al.* (2019) sugerem o reconhecimento de três gêneros em Calothecinae. O estudo de Tkach *et al.* (2020), no entanto, e;videnciou Calothecinae não monofilética nas análises baseadas em marcadores plastidiais devido a uma politomia formada entre as espécies de *Chascolytrum s.l.* (não monofilético) e Agrostidinae. Nas análises baseadas em marcadores nucleares, no entanto, Calothecinae e *Chascolytrum s.l.* são monofiléticos, e formaram uma extensa politomia com as subtribos Brizinae (monofilética), Echinopogoninae (não monofilética), Agrostidinae (não monofilética) e Hypseochloinae (representada por uma única espécie). Contudo, as análises filegenéticas de Tkach *et al.* (2020) incluíram apenas quatro espécies de *Chascolytrum s.l.*, e os gêneros *Paramochloa* e *Laegaardia* não foram amostrados. Desta forma, tanto as relações filoegenéticas entre os gêneros de Calothecinae (*sensu* Peterson *et al.*, 2019) quanto suas relações com as subtribos Agrostidinae e Echinopogoninae permanecem pouco compreendidas até o momento.

4. O gênero *Chascolytrum s.l.*

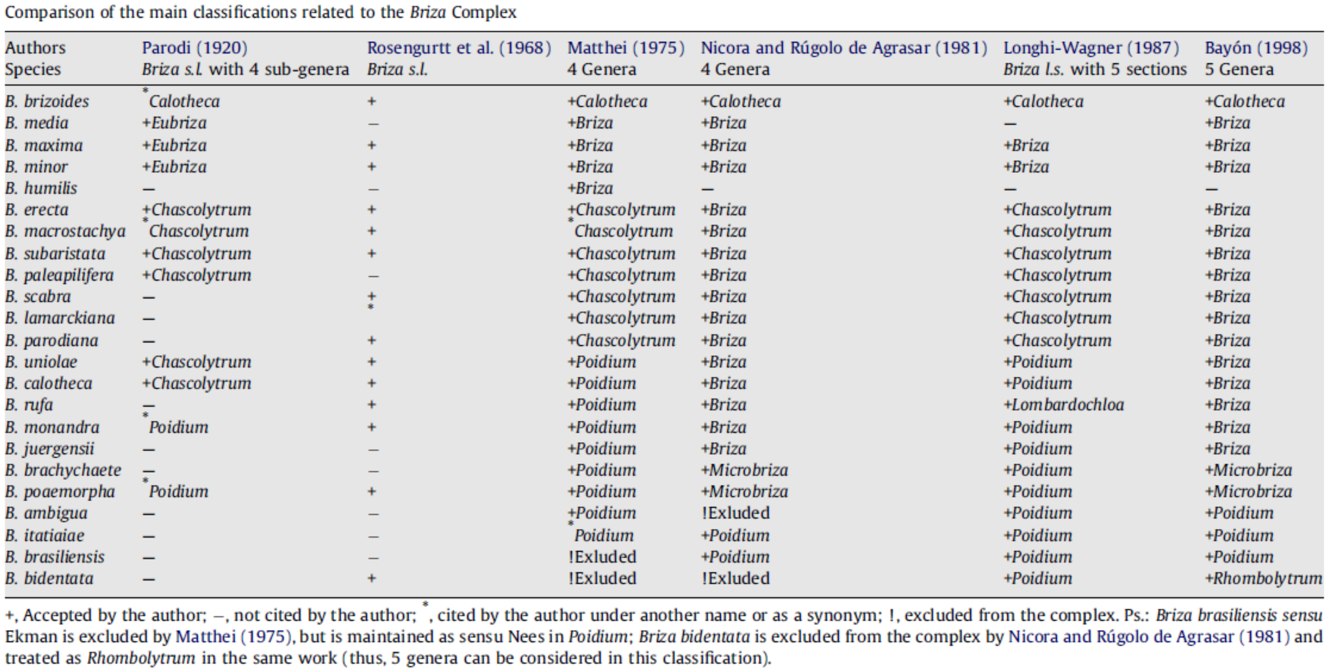
Chascolytrum s.l. é o maior gênero de Calothecinae (22 espécies), com distribuição concentrada na América do Sul, sendo o Sul do Brasil considerado seu centro de diversidade devido à elevada diversidade de espécies e endemismos (Essi *et al.*, 2017; Essi & da Silva, 2018). É um gênero morfologicamente diverso (Figura 1), devido a seu complexo histórico taxonômico. *Chascolytrum subaristatum* Desv. é a espécie mais comum do gênero, apresentando grande variação morfológica e sendo amplamente distribuída nas regiões subtropicais e temperadas da América do Sul, se estendendo pela América Central até o México e ocorrendo como adventícia na Austrália (Essi *et al.*, 2017).

A sistemática e a taxonomia das espécies atualmente reconhecidas em *Chascolytrum s.l.* tem sido controversa e mudou consideravelmente ao longo do tempo. Diferentes circunscrições foram propostas no intuito de melhor organizar este grupo morfologicamente variável de espécies. Devido a semelhanças morfológicas, as espécies americanas de *Chascolytrum s.l.* foram consideradas relacionadas às espécies Euroasiáticas do gênero *Briza* por diversos autores (Parodi, 1920; Rosengurtt *et al.*, 1968; Matthei, 1975; Longhi-Wagner, 1987), e Matthei (1975) denominou este grupo de espécies como "Complexo *Briza*".

As primeiras espécies Sul-Americanas descritas deste grupo foram incluídas no gênero *Briza* (*B. subaristata* e *B. erecta*) por Lamarck (1791). Subsequentemente, no século 19, outros gêneros, como *Chascolytrum*, *Calotheca* Desv. (Desvaux, 1810) e *Poidium* Nees (Nees von Esenbeck, 1836) foram propostos, à medida que novas espécies Sul-Americanas foram sendo descobertas e descritas. Ao longo do tempo, diferentes circunscrições e rearranjos foram propostos. As principais classificações propostas para o "Complexo *Briza*" são apresentadas na Figura 1, extraída de Essi *et al.* (2008).

Autores como Parodi (1920), Rosengurtt *et al.* (1968) e Longhi-Wagner (1987) reuniram parte das espécies Sul-Americanas com as espécies Euroasiáticas em uma circunscrição mais ampla do gênero *Briza*. Rosengurtt *et al.* (1968) reconheceu *Briza s.l.* sem nenhuma categoria infragenérica; enquanto Parodi (1920) dividiu o gênero em quatro subgêneros (*Calotheca*, *Chascolytrum*, *Eubriza* e *Poidium*), e Longhi-Wagner (1987) aceitou cinco seções (*Briza*, *Calotheca*, *Chascolytrum*, *Lombardochloa* e *Poidium*) para as espécies Sul-Riograndenses. Além dos aspectos morfológicos, o trabalho de Longhi-Wagner (1987) baseou-se em dados de cariótipo, quimiotaxonômicos, palinológicos e ontogenéticos (Williams

& Murray, 1972; Murray, 1975; Leyser & Winge, 1979; Schifino & Winge, 1983; Winge *et al.*, 1984), suportando o reconhecimento de um único gênero.



Comparison of the main classifications related to the *Briza* Complex

Authors Species	Parodi (1920) *Briza s.l.* with 4 sub-genera	Rosengurtt et al. (1968) *Briza s.l.*	Matthei (1975) 4 Genera	Nicora and Rúgolo de Agrasar (1981) 4 Genera	Longhi-Wagner (1987) *Briza l.s.* with 5 sections	Bayón (1998) 5 Genera
B. brizoides	*Calotheca*	+	+*Calotheca*	+*Calotheca*	+*Calotheca*	+*Calotheca*
B. media	+*Eubriza*	–	+*Briza*	+*Briza*	–	+*Briza*
B. maxima	+*Eubriza*	+	+*Briza*	+*Briza*	+*Briza*	+*Briza*
B. minor	+*Eubriza*	+	+*Briza*	+*Briza*	+*Briza*	+*Briza*
B. humilis	–	–	+*Briza*	–	–	–
B. erecta	+*Chascolytrum*	+	+*Chascolytrum*	+*Briza*	+*Chascolytrum*	+*Briza*
B. macrostachya	**Chascolytrum*	+	**Chascolytrum*	+*Briza*	+*Chascolytrum*	+*Briza*
B. subaristata	+*Chascolytrum*	+	+*Chascolytrum*	+*Briza*	+*Chascolytrum*	+*Briza*
B. paleapilifera	+*Chascolytrum*	–	+*Chascolytrum*	+*Briza*	+*Chascolytrum*	+*Briza*
B. scabra	–	+	+*Chascolytrum*	+*Briza*	+*Chascolytrum*	+*Briza*
B. lamarckiana	–	*	+*Chascolytrum*	+*Briza*	+*Chascolytrum*	+*Briza*
B. parodiana	–	+	+*Chascolytrum*	+*Briza*	+*Chascolytrum*	+*Briza*
B. uniolae	+*Chascolytrum*	+	+*Poidium*	+*Briza*	+*Poidium*	+*Briza*
B. calotheca	+*Chascolytrum*	+	+*Poidium*	+*Briza*	+*Poidium*	+*Briza*
B. rufa	–	+	+*Poidium*	+*Briza*	+*Lombardochloa*	+*Briza*
B. monandra	**Poidium*	+	+*Poidium*	+*Briza*	+*Poidium*	+*Briza*
B. juergensii	–	–	+*Poidium*	+*Briza*	+*Poidium*	+*Briza*
B. brachychaete	–	–	+*Poidium*	+*Microbriza*	+*Poidium*	+*Microbriza*
B. poaemorpha	**Poidium*	+	+*Poidium*	+*Microbriza*	+*Poidium*	+*Microbriza*
B. ambigua	–	–	+*Poidium*	!Exluded	+*Poidium*	+*Poidium*
B. itatiaiae	–	–	**Poidium*	+*Poidium*	+*Poidium*	+*Poidium*
B. brasiliensis	–	–	!Exluded	+*Poidium*	+*Poidium*	+*Poidium*
B. bidentata	–	+	!Exluded	!Exluded	+*Poidium*	+*Rhombolytrum*

+, Accepted by the author; –, not cited by the author; *, cited by the author under another name or as a synonym; !, excluded from the complex. Ps.: *Briza brasiliensis sensu* Ekman is excluded by Matthei (1975), but is maintained as sensu Nees in *Poidium*; *Briza bidentata* is excluded from the complex by Nicora and Rúgolo de Agrasar (1981) and treated as *Rhombolytrum* in the same work (thus, 5 genera can be considered in this classification).

Figura 1. Tabela comparativa entre as principais classificações propostas para o Complexo *Briza*. Extraída de Essi *et al.* (2008).

Outros autores, como Matthei (1975), Nicora & Rúgolo de Agrasar (1981) e Bayón dividiram o "Complexo *Briza*" em diversos gêneros, considerando as espécies euroasiáticas de *Briza s.s.* (com três espécies) como um grupo distinto das demais espécies americanas. Nicora & Rúgolo de Agrasar (1981) e Bayón (1998) propuseram arranjos semelhantes, diferindo apenas na exclusão de *Briza ambigua* Hack. e *B. bidentata* Roseng., B.R.Arrill. & Izag. do complexo por Nicora & Rúgolo de Agrasar (1981); enquanto que Bayón (1998) incluiu ambas as espécies em suas análises cladísticas (baseadas unicamente em dados morfológicos), transferindo *B. bidentata* para o gênero *Rhombolytrum* Link [= *Rhombolytrum monandrum* (Hack) Nicora & Rúgolo] junto com *R. rhomboideum* (também excluída do complexo por Nicora & Rúgolo de Agrasar, 1981). As demais espécies tratadas por ambos os autores foram distribuídas nos gêneros *Briza s.s.*, *Calotheca*, *Microbriza* Parodi ex Nicora & Rúgolo e *Poidium*.

O trabalho de Matthei (1975) pode ser considerado um dos mais completos estudos envolvendo o "Complexo *Briza*". Além de uma extensiva revisão taxonômica, o autor também levou em consideração dados embriológicos, citológicos e anatômicos para melhor circunscrever os diferentes gêneros aceitos por ele. Matthei (1975) propôs o reconhecimento de quatro gêneros distintos, aceitando *Briza s.s.* restrito às espécies Euroasiáticas, e dividiu as espécies americanas nos gêneros *Chascolytrum s.s.*, *Calotheca* e *Poidium*. No entanto, Matthei excluiu *Briza bidentata* e *B. itatiaiae* Ekman do seu estudo, sugerindo a inclusão da primeira em *Rhombolytrum* e a última na sinonímia de *Poidium brasiliensis* Nees ex Steud.

Devido à importância do trabalho de Longhi-Wagner (1987) para as espécies ocorrentes no Rio Grande do Sul e países limítrofes, por muito as espécies brasileiras foram tratadas como *Briza*. Devido à divergência entre as circunscrições propostas pelos autores anteriormente citados, a classificação das espécies americanas do "Complexo *Briza*" permaneceu controversa. Um dos primeiros estudos abordando as relações filogenéticas em Pooideae, baseado em marcadores moleculares, evidenciou que o "Complexo *Briza*" é parafilético, devido à separação de *Briza* e *Chascolytrum* como duas linhagens distintas, dentro do clado "Aveneae *type*" (Soreng *et al.*, 1990). Filogenias moleculares subsequentes, incluindo diferentes amostragens e marcadores moleculares, corroboraram esta separação. No estudo de Soreng *et al.* (2007), por exemplo, as amostras de *Chascolytrum, Calotheca* e *Gymnachne* Parodi formaram um clado à parte de *Briza s.s.*, posicionado como irmão de um clado formado por espécies de Agrostidinae; enquanto que no estudo de Döring *et al.* (2007) as duas amostras de *Chascolytrum* [*C. subaristatum* e *C. erectum* (Lam.) Desv.] formaram um clado posicionado em uma politomia com espécies de Agrostidinae. Tais estudos não foram conclusivos acerca da circunscrição das espécies americanas devido à amostragem taxonômica insuficiente deste grupo nas análises.

O primeiro estudo investigando as relações filogenéticas do "Complexo *Briza*" utilizando uma ampla amostragem taxonômica, tanto de espécies americanas quanto euroasiáticas, foi publicado por Essi *et al.* (2008). Este estudo utilizou marcadores plastidiais (*trnL-trnF*) e nucleares [*Granule-bound Starch Synthase I* (GBSSI) e *Internal Transcribed Spacer* (ITS)], e evidenciou uma clara separação entre as espécies americanas e as euroasiática *(Briza s.s.*). Contudo, as relações de parentesco entre as espécies do clado americano não ficaram resolvidas devido à presença de politomias, sugerindo que todas as espécies deste clado (incluindo *Gymnachne*, *Rhombolytrum* e *Erianthecium* Parodi) deveriam ser reconhecidas em um único gênero, *Chascolytrum s.l.* (Essi *et al.*, 2008). Subsequentemente, novos táxons americanos foram descritos para este gênero (Essi *et al.*, 2010), e as espécies de *Calotheca Erianthecium*, *Gymnachne*, *Poidium* e *Rhombolytrum* foram formalmente sinonimizadas em *Chascolytrum s.l.* (Essi *et al.*, 2011). Mais tarde, Persson & Rydin (2016) testaram o monofiletismo do "Complexo *Briza*" e suas relações com outras linhagens de Pooideae usando uma amostragem mais ampla que em Essi *et al.* (2008), corroborando a separação de *Briza s.s.* e *Chascolytrum s.l.* como duas linhagens distintas, e ainda evidenciando *Briza humilis* M.Bieb. como uma terceira linhagem, ainda mais distante, que foi reconhecida como o gênero monoespecífico *Brizochloa* V.Jirásek & Chrtek. Além disso, foi confirmado que *Chascolytrum s.l.* representa uma linhagem monofilética, contudo com relações internas ainda pouco resolvidas. No estudo de Saarela *et al.* (2017) as espécies de *Chascolytrum s.l.* formaram um clado irmão de *Deyeuxia effusa* [= *Paramochloa effusa* (Kunth) P.M.Peterson, Soreng, Romasch. & Barberá] nas árvores plastidiais, mas não foram recuperadas como monofiléticas nas análises nucleares. Em seguida, uma sinopse taxonômica para os gêneros *Chascolytrum s.l.*, incluindo as espécies anteriormente incluídas em nove gêneros distintos (*Chascolytrum*, *Briza*, *Poidium, Calotheca, Microbriza*, *Gymnachne*, *Rhombolytrum*, *Lombardochloa* e *Erianthecium*), *Briza s.s.* (três espécies euroasiáticas) e *Brizochloa* (uma espécie) foi proposta (Essi *et al.*, 2017). Recentemente, no estudo de Tkach *et al.* (2020) *Chascolytrum s.l.* foi recuperado como monofilético com base em marcadores nucleares, mas polifilético nas análises plastidiais, embora apenas quatro espécies foram incluídas neste estudo.

5. Importância de estudos filogenéticos moleculares em Calothecinae

Um dos principais desafios em reconstruções filogenéticas moleculares diz respeito à baixa variabilidade dos marcadores que são comumente utilizados, principalmente para acessar relações de parentesco aos níveis inter e intraespecíficos (Sang 2002, Després *et al.*, 2003, Shaw *et al.*, 2007). Além disso, uma amostragem taxonômica extensa e apropriada é determinante para melhorar a precisão filogenética (Heath *et al.*, 2008). Embora diversas filogenias moleculares envolvendo representantes de Calothecinae, principalmente do gênero *Chascolytrum s.l.*, tenham sido publicadas, até o momento nenhum estudo combinou uma amostragem taxonômica densa com um número expressivo de marcadores moleculares. Além disso, o monofiletismo de Calothecinae, incluindo representantes dos gêneros *Paramochloa* e *Laergaardia*, ainda não foi propriamente testado, e suas relações filogenéticas com as subtribos Agrostidinae e Echinopogoninae permanecem pouco resolvidas. O estudo de Saarela *et al.* (2017), apesar de abrangente em termos taxonômicos e de marcadores moleculares, não teve como objetivo principal resolver as relações internas em *Chascolytrum s.l.*, e o gênero não foi completamente amostrado. Por outro lado, estudos com amostragens mais expressivas para as espécies de *Chascolytrum s.l.*, como o de Essi *et al.* (2008) e Persson & Rydin (2016), foram baseados em poucos marcadores moleculares. Desta forma, novos estudos incluindo táxons ainda não amostrados e marcadores moleculares variáveis e informativos podem ajudar a prover resolução nas relações internas de *Chascolytrum s.l.*, evidenciar as relações intergenéricas em Calothecinae e suas relações com as subtribos Agrostidinae e Echinopogoninae.

6. Objetivos

A presente tese teve como objetivos:

- reconstruir as relações de parentesco entre as subtribos Calothecinae, Agrostidinae e Echinopogoninae, através de filogenias moleculares;

- testar o monofiletismo de Calothecinae incluindo representantes dos três gêneros (*Chascolytrum*, *Laegaardia* e *Paramochloa*) atualmente reconhecidos na subtribo, através de filogenias moleculares;

- elucidar as relações filogenéticas e morfológicas entre as espécies de *Chascolytrum s.l.* e analisar suas circunscrições taxonômicas;

- descrever e circunscrever novos táxons, morfologicamente diagnosticáveis, com base no princípio do monofiletismo.

7. Organização geral da tese

A presente tese é composta por três capítulos:

(1) o primeiro deles, corresponde a um artigo publicado no periódico *Botanical Journal of the Linnean Society* em 2020, o qual consiste numa reconstrução filogenética das relações internas de *Chascolytrum s.l.*, utilizando dados moleculares e morfológicos, e propondo o reconhecimento de oito seções para o gênero;

(2) o segundo corresponde a um artigo publicado no periódico *Phytotaxa* em 2020, o qual descreve e ilustra uma nova espécie de *Chascolytrum*, *C. serranum* L.N.Silva, restrita aos campos de altitude em Cambará do Sul, Rio Grande do Sul, Brasil.

(3) o terceiro e último, trata de um manuscrito a ser submetido ao periódico *Journal of Sistematics and Evolution*, e consiste na análise filogenética da subtribo Calothecinae, com ênfase nas suas relações internas, baseada em marcadores moleculares nucleares e plastidiais.

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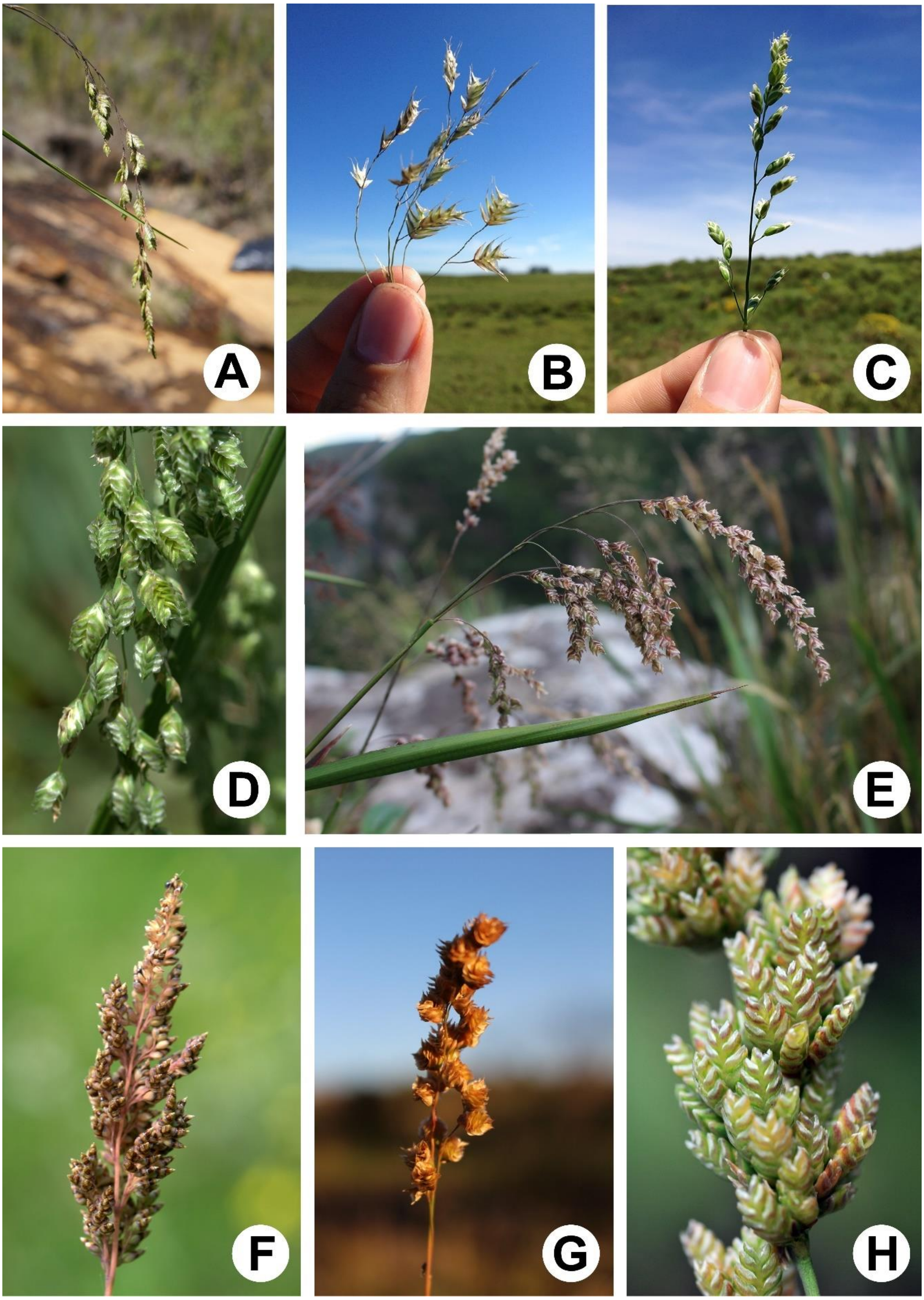

FIGURA 1. Fotos de espécies de *Chascolytrum s.l.* em campo. **A.** Inflorescência de *C. ambiguum*. **B.** Inflorescência de *C. brizoides*. **C.** Inflorescência de *C. bulbosum*. **D.** Detalhe da inflorescência e espiguetas de *C. juergensii* var. *juergensii*. **E.** Inflorescência de *C. latifolium*. **F.** Inflorescência de *C. rufum* var. *rufum*. **G.** Inflorescência de *C. subaristatum*. **H.** Inflorescência de *C. uniolae*. Fotos: L.N. da Silva (A-C, E, G) e S.A. Bordignon (D, F, H).

CAPÍTULO I:

Advances in the phylogeny of the South American cool-season grass genus *Chascolytrum* (Poaceae, Pooideae): a new infrageneric classification

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Advances in the phylogeny of the South American cool-season grass genus *Chascolytrum* (Poaceae, Pooideae): a new infrageneric classification

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Chascolytrum, as currently circumscribed, includes 22–23 South American species that were previously included in nine different genera (*Chascolytrum*, *Briza*, *Poidium*, *Calotheca*, *Microbriza*, *Gymnachne*, *Rhombolytrum*, *Lombardochloa* and *Erianthecium*). Due to the remarkable morphological diversity, the relationships in *Chascolytrum s.l.* have remained poorly understood, and no infrageneric classification could be proposed based on the latest molecular phylogenetic studies. In this study, we combined molecular (GBSSI, *trnL-trnL-trnF* and *rps16* intron) and morphological characters to investigate the phylogenetic relationships in *Chascolytrum s.l.* Based on this, morphologically diagnosable clades were recognized as eight sections (*Calotheca*, *Chascolytrum*, *Hildaea*, *Lombardochloa*, *Microbriza*, *Obovatae*, *Poidium* and *Tricholemma*), of which three are new and three are monospecific. We describe each section and discuss the new infrageneric classification in comparison with the previous infrageneric classification proposed for the group under the genus *Briza*. A taxonomic key and images for most of the species in each section are provided. Last, the use of single-copy nuclear genes and morphological data for future phylogenetic reconstructions encompassing *Chascolytrum* is highlighted.

ADDITIONAL KEYWORDS: integrative taxonomy – molecular phylogeny – morphological data – neotropical grasses – Poeae – total evidence – waxy.

INTRODUCTION

The cool-season grasses belong to Poaceae subfamily Pooideae, the most diverse lineage of grasses (3968 species in 208 genera; Soreng *et al.*, 2017), which diversified in open habitats in temperate regions around the world (Saarela *et al.*, 2017). Pooideae also include species of major economic importance, including the temperate cereals (e.g. wheat, barley and oats) (Saarela *et al.*, 2015). The earliest molecular phylogenetic analysis investigating relationships in Pooideae, published by Soreng, Davis & Doyle (1990), provided the first evidence for the separation of the South American *Chascolytrum* Desv. from the morphologically similar Eurasian genus *Briza* L., and revealed its unexpected placement in Aveneae instead of Poeae, as traditionally proposed (Clayton & Renvoize, 1986).

The systematic relationships between the South American and Eurasian species (the '*Briza* complex' *sensu* Matthei, 1975) have been studied by many authors (Bentham & Hooker, 1883; Ekman, 1913; Parodi, 1920; Rosengurtt, Arrillaga de Maffei & Izaguirre de Artucio, 1968; Matthei, 1975; Nicora & Rúgolo de Agrasar, 1981; Longhi-Wagner, 1987; Bayón, 1998; Essi, Longhi-Wagner & de Souza-Chies, 2008, 2017; Persson & Rydin, 2016), and different taxonomic treatments have been proposed, splitting the South

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American species into several smaller genera (*Calotheca* Desv., *Chascolytrum*, *Erianthecium* Parodi, *Gymnachne* Parodi, *Lombardochloa* B.Rosengurtt & B.R.Arrill., *Microbriza* Parodi ex Nicora & Rúgolo, *Poidium* Nees and *Rhombolytrum* Link) or considering most of the species in a broader circumscription of *Briza s.l.*, with or without infrageneric categories (Table 1) [for more detailed information see Table 1 in Essi *et al.* (2008)].

One of the most complete studies encompassing the *Briza* complex was published by Matthei (1975), consisting of an extensive taxonomic revision for the South American species. The author included morphological, embryological, cytological and anatomical data that supported the recognition of four distinct genera. Matthei considered *Briza* to be restricted to the Eurasian species (three of which are widespread exotics in temperate regions of the Western Hemisphere), and *Calotheca*, *Chascolytrum* and *Poidium* were accepted for the South American species, with the exception of *Briza bidentata* Roseng., B.R.Arrill & Izag. and *B. itatiaiae* Ekman, which were included in *Rhombolytrum* Link and as a synonym of *Poidium brasilensis* Nees, respectively.

Longhi-Wagner (1987) published a taxonomic revision for *Briza s.l.* in Rio Grande do Sul, southern Brazil, a region considered as a centre of diversity for the South American species (=*Chascolytrum*). Her work included all species previously treated by Matthei (1975), including *B. bidentata* and *B. itatiaiae*, and was based on several sources of data, including karyological, chemotaxonomic, palynological and ontogenetic characters (Williams & Murray, 1972; Murray, 1975; Leyser & Winge, 1979; Schifino & Winge, 1983; Winge *et al.*, 1984). Longhi-Wagner divided *Briza s.l.* into five sections (Table 1), including section *Briza* for the Eurasian species, with similar circumscriptions to those of the genera accepted by Matthei (1975). In addition, the monospecific section *Lombardochloa* (Roseng. & B.R. Arrill.) Longhi-Wagner was described to include the distinct *B. rufa* (J.Presl) Steud.

The first molecular phylogenetic study focusing on the *Briza* complex was published by Essi *et al.* (2008). In this study, using a plastid (*trnL-trnL-trnF*) and

Table 1. Comparison of the main classifications for the species currently accepted under *Chascolytrum s.l.*, excluding *Briza s.s.*, and the new classification proposed in this study. A dash indicates species not treated by the author

Author	Matthei (1975)	Longhi-Wagner (1987)	Bayón (1998)	Essi *et al.* (2017)	This study
Species	Four genera (including *Briza s.s.*)	*Briza s.l.* (five sections, including section *Briza*)	Five genera	One genus	*Chascolytrum s.l.* (eight sections)
C. altimontanum	—	—	—	*Chascolytrum s.l.*	*C.* section *Chascolytrum*
C. ambiguum	*Poidium*	*B.* section *Poidium*	*Poidium*	*Chascolytrum s.l.*	*C.* section *Hildaea*
C. bidentatum	—	*B.* section *Poidium*	*Rhombolytrum*	*Chascolytrum s.l.*	*C.* section *Tricholemma*
C. brachychaetum	*Poidium*	*B.* section *Poidium*	*Microbriza*	*Chascolytrum s.l.*	*C.* section *Poidium*
C. brasiliense	*Poidium*	*B.* section *Poidium*	*Poidium*	*Chascolytrum s.l.*	*C.* section *Poidium*
C. brizoides	*Calotheca*	*B.* section *Calotheca*	*Calotheca*	*Chascolytrum s.l.*	*C.* section *Calotheca*
C. bulbosum	—	under *Erianthecium*	—	*Chascolytrum s.l.*	*C.* section *Calotheca*
C. calotheca	*Poidium*	*B.* section *Poidium*	*Briza*	*Chascolytrum s.l.*	*C.* section *Hildaea*
C. erectum	*Chascolytrum s.s.*	*B.* section *Chascolytrum*	*Briza*	*Chascolytrum s.l.*	*C.* section *Chascolytrum*
C. itatiaiae	*Poidium*	*B.* section *Poidium*	*Poidium*	*Chascolytrum s.l.*	*C.* section *Poidium*
C. juergensii	*Poidium*	*B.* section *Poidium*	*Briza*	*Chascolytrum s.l.*	*C.* section *Hildaea*
C. koelerioides	—	—	*Rhombolytrum*	*Chascolytrum s.l.*	*C.* section *Tricholemma*
C. lamarckianum	*Chascolytrum s.s.*	*B.* section *Chascolytrum*	*Briza*	*Chascolytrum s.l.*	*C.* section *Chascolytrum*
C. latifolium	—	—	—	*Chascolytrum s.l.*	*C.* section *Hildaea*
C. monandrum	*Poidium*	*B.* section *Poidium*	*Briza*	*Chascolytrum s.l.*	*C.* section *Tricholemma*
C. paleapiliferum	*Chascolytrum s.s.*	*B.* section *Chascolytrum*	*Briza*	*Chascolytrum s.l.*	*C.* section *Chascolytrum*
C. parodianum	*Chascolytrum s.s.*	*B.* section *Chascolytrum*	*Briza*	*Chascolytrum s.l.*	*C.* section *Obovatae*
C. poomorphum	*Poidium*	*B.* section *Poidium*	*Microbriza*	*Chascolytrum s.l.*	*C.* section *Microbriza*
C. rhomboideum	—	—	*Rhombolytrum*	*Chascolytrum s.l.*	*C.* section *Tricholemma*
C. rufum	*Poidium*	*B.* section *Lombardochloa*	*Briza*	*Chascolytrum s.l.*	*C.* section *Lombardochloa*
C. scabrum	*Chascolytrum s.s.*	*B.* section *Chascolytrum*	*Briza*	*Chascolytrum s.l.*	*C.* section *Chascolytrum*
C. subaristatum	*Chascolytrum s.s.*	*B.* section *Chascolytrum*	*Briza*	*Chascolytrum s.l.*	*C.* section *Chascolytrum*
C. uniolae	*Poidium*	*B.* section *Poidium*	*Briza*	*Chascolytrum s.l.*	*C.* section *Hildaea*

the nuclear *granule bound starch synthase* (GBSSI) and internal transcribed spacer (ITS), a clearly well-supported separation between the South American clade (=*Chascolytrum s.l.*) and the Eurasian clade (=*Briza s.s.*) was recovered. *Gymnachne*, *Rhombolytrum* and *Erianthecium* were recovered in the South American clade and included as synonyms in a wider circumscription of *Chascolytrum*. Later, Persson & Rydin (2016) used the same nuclear regions plus the plastid *matK* to test the monophyly of the *Briza* complex. They included a more extensive sampling of other genera of Pooideae, but used mostly the same sequences produced earlier by Essi *et al.* (2008) for species of the South American *Briza* complex, adding only the *matK* region. Their phylogenetic analyses revealed three lineages, thereafter recognized as three independent genera accepted in the current circumscription of the *Briza* complex: *Chascolytrum s.l.*; *Briza s.s.*; and *Brizochloa* V.Jirásek & Chrtek. *Brizochloa* is a monospecific genus [only *Brizochloa humilis* (M.Bieb.) Chrtek & Hadač], segregated from *Briza*. This study also included divergence time estimations, and the most recent common ancestor among the three lineages was estimated to have originated *c.* 26.1 Mya [standard deviation (SD) 18.1–34.4 Mya] (Persson & Rydin, 2016)].

More recently, Essi *et al.* (2017) presented a new revision for the group, accepting *Briza*, *Brizochloa* and *Chascolytrum* as independent genera, and included all South American species previously included in the *Briza* complex in *Chascolytrum s.l.* However, the morphological and phylogenetic relationships in the *Chascolytrum* clade remain poorly understood. At present, *Chascolytrum* is placed in Poeae subtribe Calothecinae (Soreng *et al.*, 2015, 2017) in the 'Poeae chloroplast group I' clade, nested with other 'Aveneae-type' species belonging to *Agrostis* L., *Calamagrostis* Adans., *Deyeuxia* P.Beauv. and *Polypogon* Desf. (Saarela *et al.*, 2017), and the morphological relationships between *Chascolytrum* and these genera are also still unclear. Moreover, *Brizochloa* is now considered to be separate from *Briza* and *Chascolytrum*, falling in Poinae *s.l.*

Chascolytrum currently comprises 22 or 23 species (Essi *et al.* 2017; Clayton *et al.*, 2019); as a genus, it is not easy to circumscribe and requires a combination of multiple morphological characters to distinguish it from morphologically similar genera (Essi *et al.*, 2017). It may be characterized by perennial plants (vs. annual in *Briza* and *Brizochloa*), with the basal leaf sheaths fibrous (vs. not fibrous in *Briza* and *Brizochloa*), more than three- to many-flowered spikelets (vs. uni- or biflorous spikelets in other Aveneae-type genera of supersubtribe Agrostidodinae (Soreng *et al.*, 2017); two- to many-flowered in *Briza* and *Brizochloa*) and straight pedicels (vs. recurved pedicels in *Briza*; straight pedicels in *Brizochloa*). Additionally, identification of *Chascolytrum* spp. is hindered due to overlapping morphology, although major morphological groups are relatively easier to identify.

Extensive sampling of *Chascolytrum s.l.* was included in previous phylogenetic studies (Essi *et al.*, 2008; Persson & Rydin, 2016), but no infrageneric classification was proposed. The molecular markers used to assess the phylogenetic relationships in *Chascolytrum* are few and most sequences found in GenBank (e.g. of the GBSSI gene) are incomplete, resulting in low or weak support for internal taxonomic structure.

Cladistic analyses based on morphological data were used to improve the taxonomy of this group by Bayón (1998), but no one has published a total-evidence approach combining molecular and morphological sources in *Chascolytrum*. The use of morphological and molecular data in combination in phylogenetic analyses could be useful particularly for groups exhibiting higher morphological variation than genetic divergence, providing support for either taxa or identification of synapomorphies (Assis, 2009; Gomes-da-Silva & Souza-Chies, 2018). However, combining morphological and molecular data must be done cautiously, since morphological traits can be highly homoplastic (Patterson, 1982). The ambiguity and the lack of accuracy are the major criticisms for phylogenetic reconstructions based on morphological characters rather than molecular markers (Scotland, Olmstead & Bennett, 2003), which culminated in a decrease in the use of these markers (Assis, 2009). However, morphological data have several advantages for phylogenetic reconstructions when addressing taxonomic issues, and they can be applied as an informative source of phylogenetic information into the analysis (Wiens, 2004).

In this study, we attempt to reconstruct the phylogenetic structure of *Chascolytrum* using a total-evidence approach. We use three molecular markers, the plastid intergenic spacer *trnL-trnL-trnF*, the *rps16* intron and the nuclear single-copy gene GBSSI (or *waxy*), plus 22 morphological characters. An infrageneric classification for *Chascolytrum s.l.* is proposed, including descriptions, images of the reproductive characters and an identification key for the sections. We discuss the new infrageneric classification and compare it with the taxonomic treatments of Matthei (1975) and Longhi-Wagner (1987).

MATERIAL AND METHODS

SAMPLING

We sampled 47 accessions belonging to 20 species and two varieties of *Chascolytrum s.l.*, representing *c.* 88% of the species diversity, plus two specimens with unplaced morphologies [*C*. sp. and *C.* aff. *parodianum* (Roseng., B.R.Arrill. & Izag.) Matthei]. We sampled representatives of the *Briza* complex according to previous classifications, except for three species, *C. altimontanum* Essi, Souza-Chies & Longhi-Wagner, *C. koelerioides* (Trin.) Essi, Longhi-Wagner & Souza-Chies and *C. rhomboideum* (Link) Essi, Longhi-Wagner & Souza-Chies. Species with high morphological variation or wide geographical distribution were sampled more than once whenever possible. Nine species belonging to subtribes Agrostidinae, Airinae, Brizinae, Holcinae and Koeleriinae were used as outgroups.

Plant material was obtained mostly through field work in Brazil and Uruguay. Additional sequences were obtained from GenBank. All samples collected for this study were deposited in the herbarium ICN at Universidade Federal do Rio Grande do Sul. The complete list of taxa, DNA regions sampled and voucher information is presented in the Supporting Information (Table S1).

DNA EXTRACTION, PCR AMPLIFICATION AND SEQUENCING

Total DNA was extracted from 0.3–0.5 mg of silica gel-dried leaves using a modified CTAB method (Doyle & Doyle, 1987). We analysed the nuclear single-copy gene GBSSI (Mason-Gamer, Weil & Kellogg, 1998) and two plastid DNA regions, the *trnL-trnL-trnF* region (Taberlet *et al.*, 1991) and the *rps16* intron (Oxelman, Liden & Berglund, 1997). PCR amplifications were conducted with a total reaction volume of 25 µL containing 20–50 ng genomic DNA, 1× Taq DNA polymerase buffer, 0.5 µM each primer, 100 µM dNTPs, 1.5 mM $MgCl_2$, 2% dimethylsulphoxide (DMSO) and 1.5 U of *Platinum* Taq DNA polymerase (Invitrogen). The GBSSI gene was amplified using a set of three primers, F-for, K-bac and M-bac, following Mason-Gamer *et al.* (1998). The GBSSI and *trnL-trnL-trnF* regions were amplified following Essi *et al.* (2008) and the *rps16* intron region was amplified following Shaw *et al.* (2005). PCR products were purified with 20% polyethylene glycol (PEG) (Dunn & Blattner, 1987) and sequenced using an Applied Biosystems Genetic Analyzer 3500xL. Forward and reverse sequences were assembled with CodonCode Aligner 3.5.3 (CodonCode Corp., Dedham, MA, USA). Ambiguous bases were corrected after examination of chromatograms.

MORPHOLOGICAL DATA

Twenty-two morphological traits, four vegetative and 18 reproductive, were analysed for all taxa sampled in the phylogenetic analysis (Table 2). Characters and character states were selected and defined following the most recent taxonomic revision published for *Chascolytrum* (Essi *et al.*, 2017) and on observations made on the specimens sampled for the phylogenetic analyses. Additional materials deposited in the ICN Herbarium were examined to confirm character states for each species. Binary and multistate morphological traits were scored for all taxa of the ingroup and for the outgroup when data were available or applicable.

PHYLOGENETIC ANALYSES

Sequences were aligned with MAFFT v.7 (Katoh & Standley, 2013) and then manually checked using MEGA6 (Tamura *et al.*, 2013). Unambiguous aligned gaps were coded using the modified complex indel coding approach (Simmons, Müller & Norton, 2007) with SeqState v.1.4.1 (Müller, 2005). Phylogenetic analyses were first conducted on each DNA region (single-region analysis) and on the combined plastid DNA dataset (*trnL-trnL-trnF* + *rps16* intron). Incongruences between the different DNA datasets were explored through visual comparison of topologies and support values between conflicting branches. Incongruent topologies were considered strongly supported with a minimum of 85% bootstrap support (Baker *et al.*, 2011; Tkach *et al.*, 2015) or 0.99 posterior probability (Inácio *et al.*, 2017). Then, combined analyses of plastid + nuclear DNA were performed. Last, to investigate the influence of morphological characters on the phylogenetic relationships among the taxa and clade supports, the concatenated DNA matrix was combined with the morphological matrix for a total-evidence analysis.

Bayesian inference (BI) analyses were run using MrBayes v.3.2.6 (Ronquist *et al.*, 2012). The most appropriate evolutionary model for each dataset was defined in MrModeltest v.2.3 (Nylander, 2004) based on the Akaike information criterion. The GBSSI gene was divided in two different partitions corresponding to the exon and intron regions. The best-fitting models selected were F81 + I and HKY for the exon and intron partitions for GBSSI, respectively, GTR for each partition for *trnL-trnL-trnF* and GTR + G for *rps16* intron. Two parallel runs with four Markov chain Monte Carlo were run for 10^7 generations, sampling every 1000th generation. Average standard deviation of diagnostic frequencies was calculated every 10^3 generation and the critical value for automatically stopping the analysis was defined by the 'stopval' command (=0.01). The first 25% of the trees of each run were discarded as burn-in, and the remaining trees were used to build a majority-rule consensus including the posterior probabilities as branch support estimates.

Maximum likelihood (ML) analyses were performed using RAxML v.8.2.1 (Stamatakis, 2014), with the GTRGAMMA model for each coding DNA partition and the BINGAMMA model for indels. Partitions were defined for each region (gene exon, intron and spacers) separately. The best-scoring ML tree was found after 100 independent ML searches and the reliability of ML

Table 2. Morphological characters and their states for *Chascolytrum s.l.*

1. Rhizomes (0 = absent; 1 = present)
2. Basal nodes thickened (0 = absent; 1 = present)
3. Leaf blades (0 = flat; 1 = involute or convolute)
4. Leaf apex (0 = acute or obtuse; 1 = truncate)
5. Number of florets (0 = up to 4, rarely; 1 = 5 or more, rarely 4)
6. Spikelets compression (0 = lateral; 1 = cylindrical or subcylindrical (not compressed); 2 = dorsiventral)
7. Spikelets shape (0 = lanceolate; 1 = elliptic; 2 = ovate; 3 = oblong; 4 = orbicular)
8. Gibbous back (0 = absent; 1 = present)
9. Gibbous back colour (0 = pale or greenish; 1 = yellow, orange, brown or rufous)
10. Awns on lemmas (more than 1 mm) (0 = absent; 1 = present)
11. Lemma surface (0 = glabrous; 1 = strigose; 2 = pilose or pubescent; 3 = scabrous; 4 = papillate; 5 = echinulate)
12. Margins of the lemma at the lower third (0 = glabrous; 1 = ciliate)
13. Lemma base (0 = not cordate cordate at the base; 1 = cordate at the base)
14. Lemma consistency (0 = herbaceous; 1 = chartaceous; 2 = coriaceous)
15. Lemma compression (0 = not compressed to slightly compressed; 1 = lateral; 2 = dorsiventral)
16. Pair of oil glands at the base of the lemma (0 = absent; 1 = present)
17. Palea consistency (0 = membranous; 1 = chartaceous; 2 = coriaceous)
18. Palea shape (0 = orbicular to elliptic; 2 = lanceolate to linear-lanceolate; 3 = obovate to obovate-truncate)
19. Palea between the keels (0 = glabrous; 1 = strigose; 2 = pilose or pubescent; 3 = echinulate; 4 = with sparse capitate trichomes)
20. Palea keels (0 = glabrous; 1 = ciliate or ciliolate)
21. Glumes consistency (0 = herbaceous; 1 = chartaceous)
22. Glumes shape (0 = naviculate; 1 = convex)

topology was evaluated by non-parametric standard bootstrap using 1000 pseudo-replicates. Maximum parsimony (MP) analyses were only performed for the total evidence (DNA + morphology) dataset. MP analyses were performed in PAUP* v.40b10, with the heuristic searches using 1000 random replications, stepwise data addition, TBR branch swapping and equal and unordered weight for all characters. Most-parsimonious trees were limited to 100 per replicate and were used to calculate the strict consensus trees. Branch support values were estimated using non-parametric bootstrapping, with 1000 replicates, random taxon-addition, TBR branch swapping and multitrees on. Only nodes scoring ≥ 0.95 in Bayesian posterior probabilities (PP) or $\geq 70\%$ in the ML bootstrap support (LB) and MP bootstrap support (PB) were considered significant.

For the morphological character evaluation, the BI tree was manually reduced by the removal of taxa sampled more than once. Therefore, eight of the 22 morphological characters were selected based on their use in past taxonomic studies for taxa delimitations and were plotted onto the reduced cladogram using MP implemented in MESQUITE v.3.5.1 (Maddison & Maddison, 2018).

TAXONOMIC TREATMENT

The taxonomic treatment was based on revision of herbarium specimens and literature, including protologues. Field collections and observations were concentrated in south-eastern and southern Brazil and Uruguay. The herbaria B, BLA, BM, BR, CEN, CNPO, CONC, CORD, FLOR, G, GUA, HAS, HB, HBR, ICN, K, L, LINN, LPB, M, MBM, MVFA, MO, MSB, NY, P, PACA, RB, S, SGO, SMDB, UEC, VIC and W were consulted [acronyms following Thiers (2018) onwards]. Digital images provided by BAA, GOET, LIL, PR, PRC, R and US were also examined (acronyms following Thiers, 2018 and ongoing). Photographs of the reproductive structures were taken using a digital stereoscopic microscope and plant habits and inflorescences were taken in the field. The taxonomic key for identification of the sections was constructed based on the morphological characters listed as diagnostic.

RESULTS

PHYLOGENETIC ANALYSES

Nuclear GBSSI

The length of the GBSSI gene ranged from 978 bp in *Chascolytrum bidentatum* (Roseng., B.R.Arrill. & Izag.) Essi, Longhi-Wagner & Souza-Chies to 1112 bp in *Deschampsia cespitosa* (L.) P.Beauv. The GBSSI matrix included 53 accessions and 1252 characters (1242 bp and ten indels), of which 429 were variable (34.26%) and 167 (13.34%) were potentially parsimony informative. The BI tree was better resolved than the ML tree, and the cladogram is presented in the Supporting Information (Fig. S1) with the bootstrap support values from the ML analysis.

Chascolytrum s.l. was recovered as monophyletic with full support in both analyses (PP = 1, LB = 100%); it was divided into six major clades, significantly supported at least in BI analysis. Four species [*C. brizoides* (Lam.) Essi, Longhi-Wagner & Souza-Chies, *C. bulbosum* (Parodi) Essi, Longhi-Wagner & Souza-Chies, *C. poomorphum* (J.Presl) Essi, Longhi-Wagner & Souza-Chies and *C.* aff. *parodianum*)] were placed in a basal polytomy. The *Chascolytrum s.l.* clade was placed in a polytomy with *Agrostis capillaris* L., *Briza minor* L. and a clade consisting of *Bromidium tandilense* (Kuntze) Rúgolo, *Briza maxima* L. and *Calamagrostis varia* Host.

Plastid DNA dataset

The length of the *trnL-trnL-trnF* region ranged from 735 bp in *Calamagrostis rupestris* Trin. ex Steud. and *Briza minor* to 955 bp in *Avenella flexuosa* (L.) Drejer. The *trnL-trnL-trnF* matrix consisted of 1009 characters (1001 bp and eight indels), of which 105 (10.41%) were variable and 59 (5.85%) were potentially parsimony informative. The length of the *rps16* intron ranged from 792 bp in *Deschampsia cespitosa* to 818 bp in *C. brachychaetum* (Ekman) Essi, Longhi-Wagner & Souza-Chies. The *rps16* intron alignment consisted of 832 characters (828 bp and four indels) of which 65 (7.81%) were variable and 42 (5.05%) were potentially parsimony informative. The concatenated plastid DNA matrix included 57 accessions and 1841 characters (1829 bp and 12 indels), of which 170 (9.23%) were variable and 101 (5.49%) were potentially parsimony informative.

The topologies obtained in the plastid DNA analyses were less resolved than those recovered in the GBSSI analyses. The BI tree was better resolved than the ML tree, and the cladogram is presented in the Supporting Information (Fig. S2) with the bootstrap support values from the ML analysis. In the plastid DNA analyses, *Chascolytrum s.l.* was not recovered as monophyletic, and its species were placed into a large clade also including *Agrostis capillaris, Calamagrostis varia* and the two species of *Bromidium* Nees & Meyen (PP = 1, LB = 92%). Most *Chascolytrum* spp. were placed into five clades, and the remaining species were placed in a basal polytomy with the other genera.

Combined DNA dataset (plastid DNA + GBSSI)

There were no supported topological conflicts among the DNA datasets. Thus, we concatenated the GBSSI and plastid DNA regions, including their coded indels, into a combined DNA dataset. The final DNA matrix with the three regions combined totalled 3093 characters (3071 bp and 22 indels), of which 599 (19.37%) were variable and 268 (8.66%) were potentially parsimony informative. The ML and BI analyses produced similar topologies, but the BI tree was well resolved and well supported. The BI cladogram, along with the bootstrap support values from the ML analyses, is presented in the Supporting Information (Fig. S3).

The combined DNA analyses recovered *Chascolytrum s.l.* as monophyletic (PP = 1, LB = 98%), but did not resolve the basal polytomy observed in the individual analyses of the cpDNA and GBSSI datasets (Supporting Information, S1, S2). However, support values for the major clades increased and helped to resolve the phylogenetic relationships of *Chascolytrum brizoides*, *C. bulbosum* and *C.* aff. *parodianum*. The major clades are hereafter treated by the names of the taxonomic sections that correspond to the new classification proposed for the genus.

The *Chascolytrum* (PP = 0.95), *Microbriza* (PP = 1, LB = 98%) *Tricholemma* (PP = 1, LB = 95%), *Obovatae* (PP = 1, LB = 84%) and *Calotheca* (PP = 1, LB = 75%) clades, and the major clade including the *Lombardochloa* (PP = 1, LB = 100%), *Poidium* (PP = 1, LB = 99%) and *Hildaea* (PP = 1, LB = 77%) clades, were all placed in a basal polytomy (S3). Many of the taxa sampled more than once were grouped together, but some of them were not fully resolved, especially in the *Hildaea* clade [e.g. *C. ambiguum* (Hack.) Essi, Longhi-Wagner & Souza-Chies, *C. calotheca* (Trin.) Essi, Longhi-Wagner & Souza-Chies and *C. juergensii* (Hack.) Essi, Souza-Chies & Longhi-Wagner var. *juergensii*].

Total evidence (DNA + morphology)

The three DNA regions and the morphological data combined matrix totalled 3115 characters, including 3071 bp, 22 indels and 22 binary or multistate morphological characters. Of these, 621 (19.94%) were variable and 289 (9.28%) were potentially parsimony informative. The MP analysis yielded 424 most-parsimonious trees of 999 steps with CI = 0.73 and RI = 0.76 (excluding uninformative characters). The inclusion of the morphological data in the phylogenetic analyses generated similar results among BI, ML and MP analyses. The total-evidence analyses were still unable to resolve the basal polytomies found in the previous analyses. However, the support of the major clades was higher compared to the analyses of the combined and separate DNA datasets. The BI tree including the support values from the ML and MP analyses is given in Fig. 1.

All phylogenetic analyses based on the total-evidence dataset recovered *Chascolytrum s.l.* as monophyletic (PP = 1, LB = 99%, PB = 99.9%), and six major clades were recovered in the BI analysis (Fig. 1). Unlike the previous combined DNA analyses, the *Obovatae* clade (PP = 1, LB = 98%, PB = 99.1%) was recovered

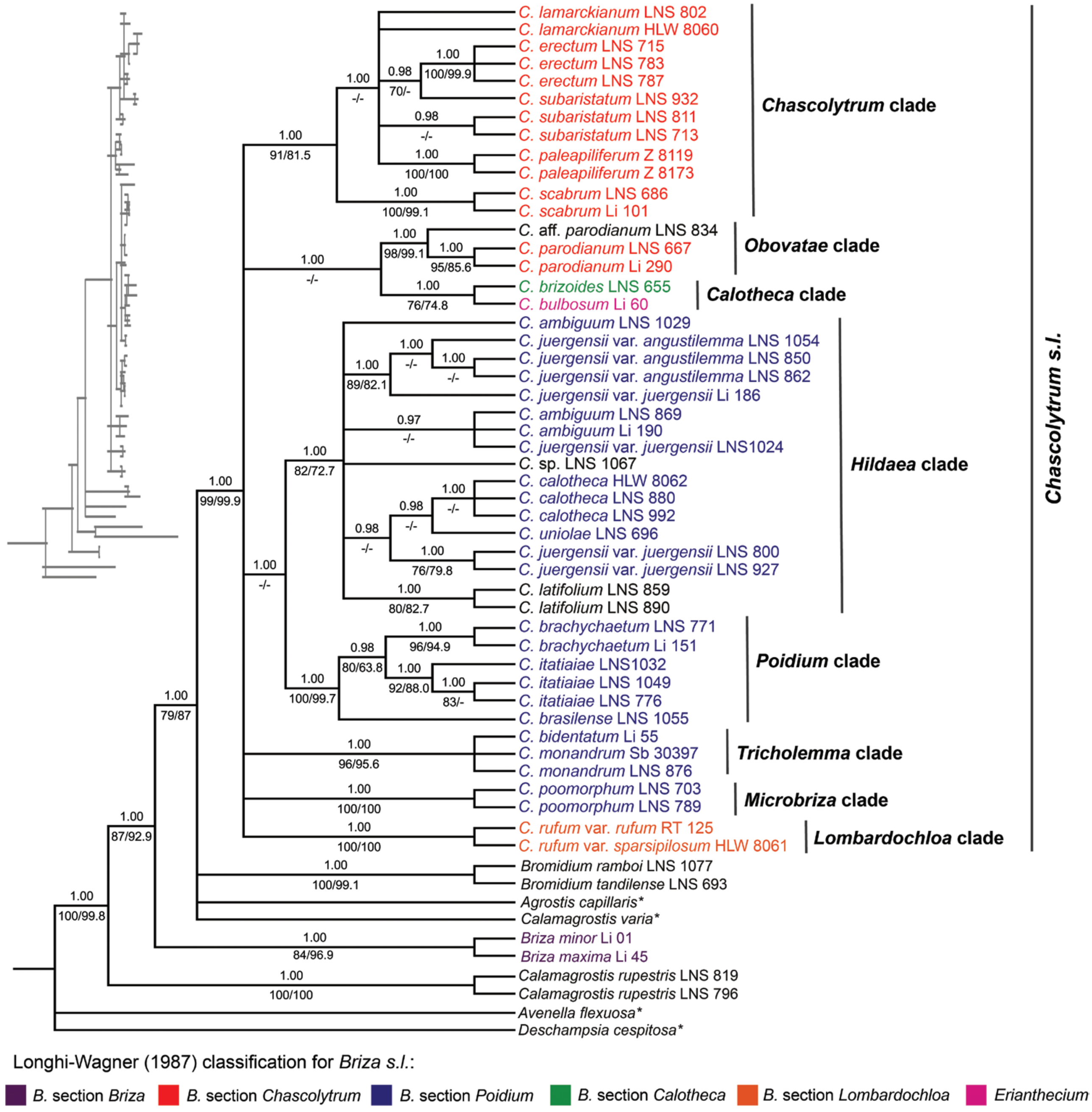

Figure 1. Bayesian inference (BI) consensus cladogram and respective phylogram from the analysis of the total-evidence dataset (*trnL-trnL-trnF*, *rps16* intron, GBSSI and morphological data) for *Chascolytrum s.l.* Numbers above the branches indicate the BI posterior probabilities (PP; values > 0.95), and below the branches indicate maximum likelihood (LB; left; values > 50%) and maximum parsimony (PB; right; values > 50%) bootstrap support, respectively. Asterisk indicates species with sequences sampled from GenBank.

here as sister (PP = 1) to the *Calotheca* clade (PP = 1, LB = 76%, PB = 74.8%), whereas the *Lombardochloa* clade (PP = 1, LB = 100%, PB = 100%) was placed in the basal polytomy. In addition, as in the previous analyses, the *Poidium* clade (PP = 1, LB = 100%, PB = 99.7%) was recovered as sister (PP = 1) to the *Hildaea* clade (PP = 1, LB = 82%, PB = 72.7%). The *Chascolytrum* (PP = 1, LB = 95%, PB = 96%), *Tricholemma* (PP = 1, LB = 95%, PB = 96%), *Calotheca* (PP = 1, LB = 74%, PB = 57.9%) and *Microbriza* (PP = 1, LB = 93%, PB = 88.6%) clades were place in the basal polytomy with the major clades.

At the species level, the relationships obtained in the total-evidence analyses were more resolved than those obtained in the combined DNA analyses. Most of the taxa sampled more than once formed well-supported clades at least in the BI analysis, except for *Chascolytrum lamarckianum* (Nees) Matthei, *C. subaristatum* (Lam.) Desv., *C. juergensii* var. *juergensii*, *C. ambiguum* and *C. monandrum* (Hack.) Essi, Longhi-Wagner & Souza-Chies.

The reduced BI tree with the eight selected morphological characters plotted onto the cladogram for each taxon is shown in Fig. 2. The combination of the character states for each clade, represented by circles and colours, was used to identify the diagnostic characters to identify and circumscribe each section proposed for *Chascolytrum s.l.*

DISCUSSION

THE GENUS *CHASCOLYTRUM*

Chascolytrum s.l. was recovered as monophyletic in most of the analyses carried out in this study, except for that based on the plastid DNA dataset. Thus, the acceptance of a single large genus for the South American species of the *Briza* complex is supported in accordance with previous phylogenetic studies (Essi *et al.*, 2008; Persson & Rydin, 2016). However, the relationships among the major clades in *Chascolytrum s.l.* are still not fully resolved due to basal polytomies. Polytomies have been currently reported in the phylogenetic studies that included the largest sampling of *Chascolytrum* (Essi *et al.*, 2008; Persson & Rydin, 2016) and other cool-season grasses (Saarela *et al.*, 2017), especially at deeper nodes, representing the major challenge in the comprehension of the evolution of this group.

The morphological diversity found in *Chascolytrum* (Figs 3–6) is remarkable considering its species diversity (23 recognized in this study), which becomes more noteworthy, since most of its diversity and endemisms is found in subtropical and temperate grasslands of southern South America, mainly in

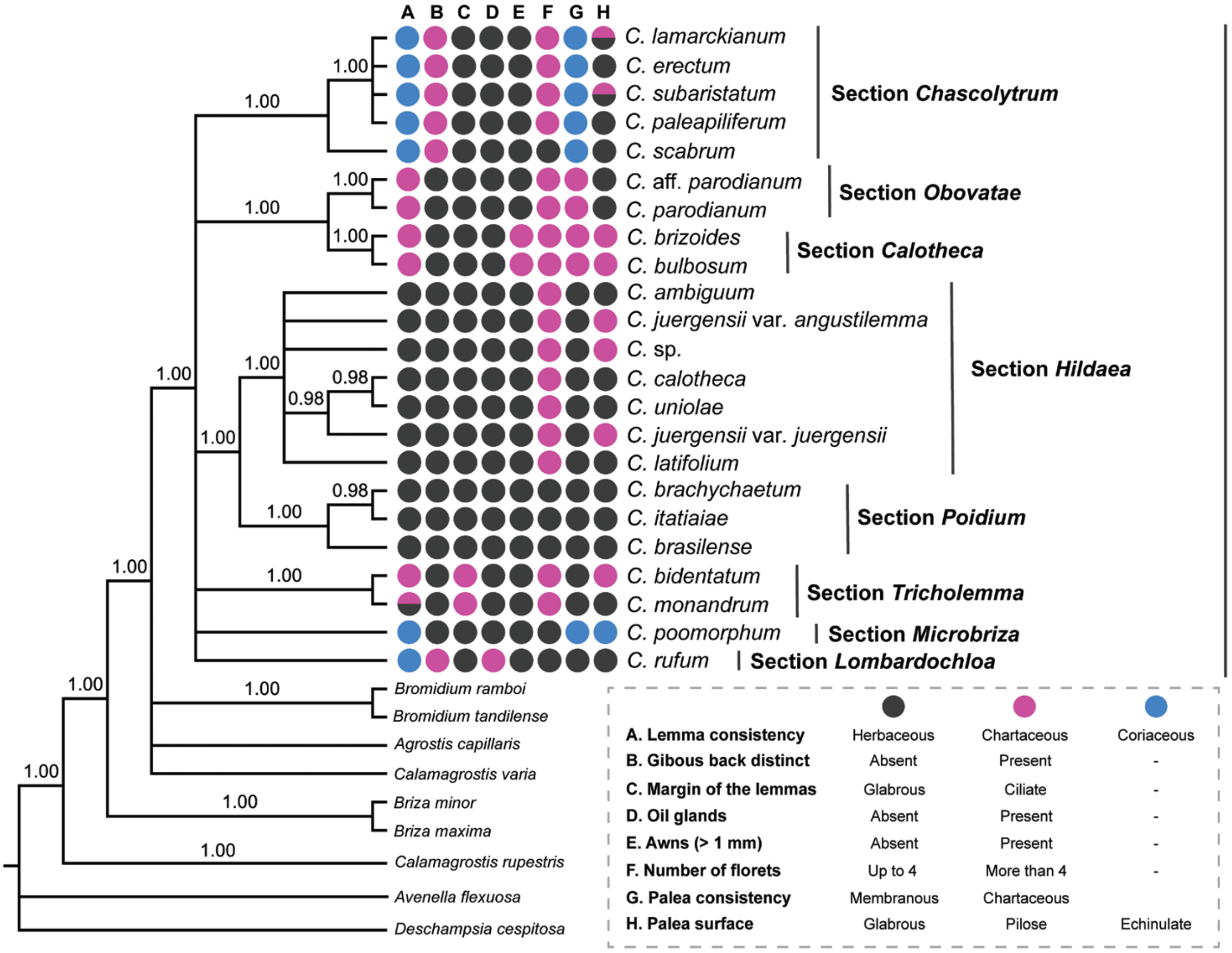

Figure 2. BI reduced consensus cladogram from the analysis of the combined DNA dataset (*trnLtrnL-trnF*, *rps16* intron and GBSSI regions) for *Chascolytrum s.l.,* including the combination of selected morphological characters for sections recognized in this study. Numbers above the branches indicate the BI PP values > 0.95.

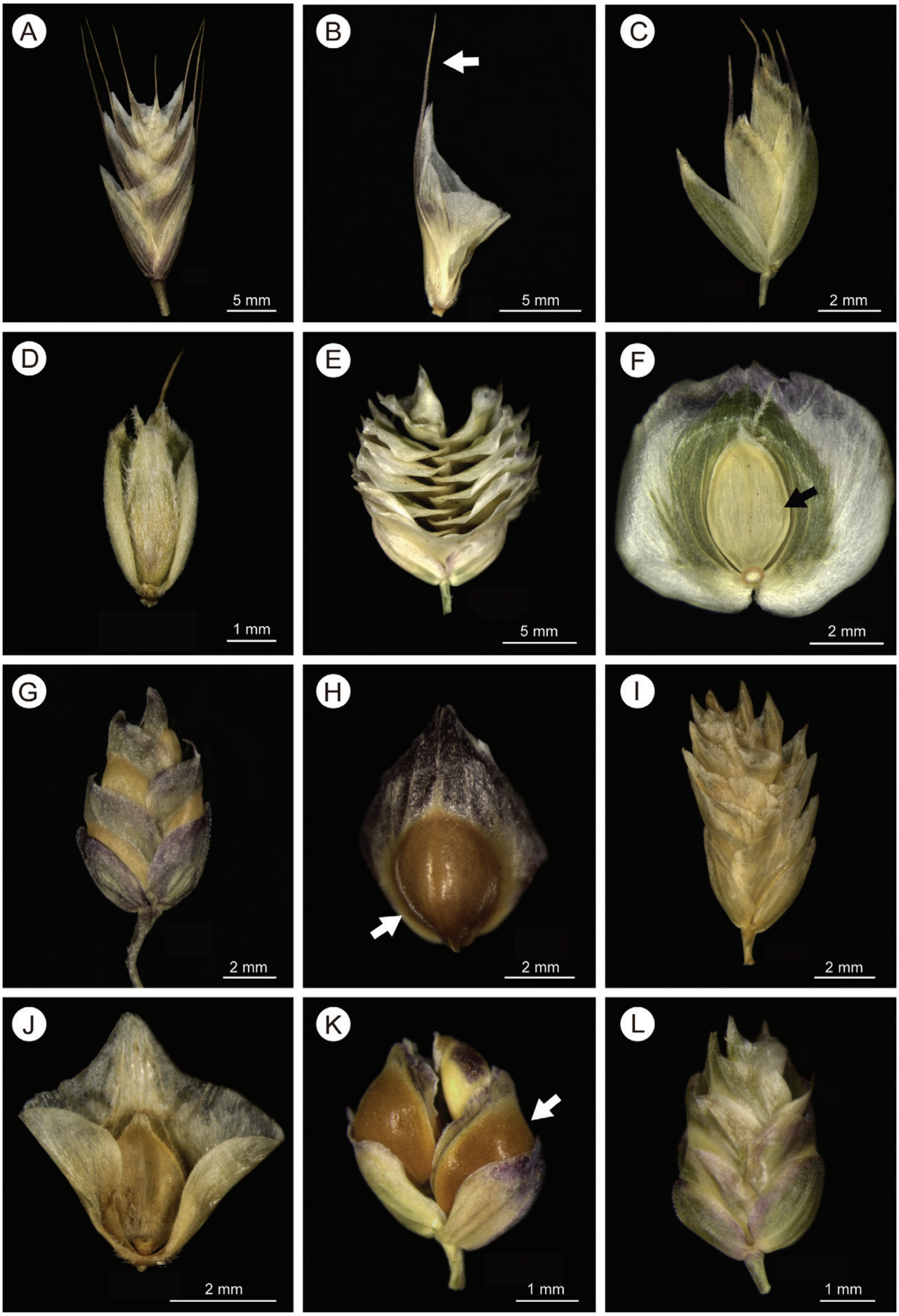

Figure 3. Spikelet morphology in *Chascolytrum*. *Chascolytrum* section *Calotheca*: *C. brizoides*. A, spikelet. B, lemma in lateral view, arrow indicates the awn. *C. bulbosum*. C, spikelet. D, floret in ventral view, densely pilose palea visible.

southern Brazil, in a narrow geographical range (Essi *et al.*, 2017; Essi & da Silva, 2018). This striking diversity associated with the lack of internal phylogenetic structure could result from rapid and adaptive radiation (Whittall *et al.*, 2006). Although the estimated median age for the origin of *Chascolytrum c.* 10.9 Mya (SD 6.6–16.0 Mya) is in the mid-Miocene, the estimated ages for the crown nodes of the major clades in *Chascolytrum* are *c.* 5–10 Mya (Persson & Rydin, 2016). This somewhat coincides with the diversification ages estimated for other lineages of South American plants (e.g. Fabaceae and Asteraceae) concurrent with the expansion of the South American subtropical grasslands (Eastwood *et al.*, 2008; Iganci *et al.*, 2013; Pasini *et al.*, 2016). Furthermore, hybridization events are widely reported among numerous lineages in the 'Poeae chloroplast group 1' (Döring *et al.*, 2007; Saarela *et al.*, 2017) and may be related to the diversification and evolution of brizoid spikelets of *Briza*, *Brizochloa* and *Chascolytrum*, although this scenario still needs to be tested.

Infrageneric classification

The increase in taxon sampling and DNA markers used in this study provided a more resolved and well-supported and morphological informative phylogenetic framework for *Chascolytrum s.l.* than detected in previous studies. Considering this, we propose an infrageneric classification for the genus. The most similar circumscription to our new proposal was made by Longhi-Wagner (1987), under *Briza*, who accepted four sections for the South American species (*Calotheca*, *Chascolytrum*, *Lombardochloa* and *Poidium*). However, the sections *Chascolytrum* and *Poidium*, as circumscribed by Longhi-Wagner (1987), were here divided into different sections, due to the lack of resolution in the basal polytomy.

In total, eight sections are recognized here for *Chascolytrum s.l.*, three of them monospecific. Monospecific sections were only recognized when the combination of morphological characters was considered unique compared to other sections (Fig. 2). In most cases, taxa assigned to monospecific sections were sampled twice and were recovered as monophyletic (*Lombardochloa*, *Microbriza* and *Obovatae*). In the case of the *Microbriza* clade, *Chascolytrum poomorphum* was previously placed in the *Poidium* or *Briza* section *Poidium* by Matthei (1975) and Longhi-Wagner (1987), respectively. However, in our phylogenetic analyses, the *Microbriza* clade was not considered related to other species and belonged to the *Briza* section *Poidium* (Fig. 1); instead, it was placed in the basal polytomy in most of the phylogenetic analyses. *Chascolytrum poomorphum* presents several morphological differences compared from the other sections (Fig. 2), which supported the recognition of the monospecific section *Microbriza* to accommodate this species.

Traditionally, *Chascolytrum parodianum* was included in *Chascolytrum s.s.* or *Briza* section *Chascolytrum* by Matthei (1975) and Longhi-Wagner (1987), respectively. In most of our phylogenetic analyses, the accessions of *C. parodianum* formed a well-supported clade apart from the *Chascolytrum* clade, often placed in the basal polytomy (Supporting Information Figs S1, S3). However, in the total-evidence analyses, the *Obovatae* clade was recovered as sister to the *Calotheca* clade (Fig. 1), whereas in the plastid DNA analyses it was recovered as sister to the *Microbriza* clade (=*Chascolytrum poomorphum*; Supporting Information Fig. S2). *Chascolytrum parodianum* does not share any morphological similarity with *C. poomorphum* or the species in *Calotheca*. *Chascolytrum parodianum* can be easily distinguished from those by the presence of chartaceous and obovate paleas, which supported the proposition of the monospecific section *Obovatae* to accommodate *C. parodianum*.

The *Calotheca* clade, consisting of *Chascolytrum brizoides* and *C. bulbosum*, was also recovered in the previous plastid analysis of Essi *et al.* (2008). This relationship was supported in our analyses only when the different datasets were combined (Fig. 1; Supporting Information Fig. S3). The overall morphology of *C. brizoides* and *C. bulbosum* can be considered diverse, especially in vegetative features. However, section *Calotheca* including these two species can be easily defined by the presence of awned lemmas (Fig. 4A–D), a character unique to these two species in *Chascolytrum* (Fig. 2).

The type section *Chascolytrum* is here accepted with a similar circumscription compared to those proposed by Matthei (1975) for *Chascolytrum s.s.* or Longhi-Wagner (1987) for *Briza* section *Chascolytrum*, except with the removal of

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Chascolytrum section *Chascolytrum*: *C. erectum*. E, spikelet. F, floret in ventral view, arrow indicates the coriaceous palea. *C. lamarckianum*. G, spikelet. H, lemma in dorsal view, arrow indicates the gibbous back strongly distinct from the margins. *C. paleapiliferum*. I, spikelet. J, floret in ventral view, coriaceous palea visible. *C. scabrum*. K, spikelet, arrow indicates the gibbous back strongly distinct from the margins. *C. subaristatum*. L, spikelet. Photographs: L.N. da Silva.

C. parodianum. The *Chascolytrum* clade was strongly supported in the total-evidence analyses (Fig. 1), although the internal relationships were not well resolved. The polymorphic species *C. subaristatum* was not considered monophyletic in our analyses; whereas *C. erectum* (Lam.) Desv., which was treated as a synonym of *C. subaristatum* by Essi *et al.* (2017), formed a well-supported clade in all our phylogenetic analyses (Fig. 1; Supporting Information Figs S1–S3). Although one accession of *C. subaristatum* (LNS 932) appeared as sister to the *C. erectum* clade in the total-evidence analyses (Fig. 1), the two species can be morphologically distinguished (Longhi-Wagner, 1987). Thus, *C. erectum* is accepted here as a species distinct from *C. subaristatum*.

The proposition of Bayón (1998) including *Chascolytrum brachychaetum* (Ekman) Essi, Longhi-Wagner & Souza-Chies in *Microbriza* with *C. poomorphum* is rejected here. Our analyses confirmed the close phylogenetic and morphological relationship of *C. brachychaetum* with *C. itatiaiae* (Ekman) Essi, Longhi-Wagner & Souza-Chies and *C. brasilense* (Nees) Essi, Longhi-Wagner & Souza-Chies in the *Poidium* clade, as suggested by Essi *et al*. (2008, 2017). These three species formed a well-supported clade in most of our analyses, often placed as sister to the *Hildaea* clade (Fig. 1; Supporting Information Figs S1, S3). Although the recognition of a single large section including both *Poidium* and *Hildaea* clades would be possible, given their strong support in the phylogenetic analyses and morphological differentiation, we decided to recognize these clades as distinct taxonomic sections.

The two varieties of *Chascolytrum rufum* J.Presl. formed a well-supported clade in all phylogenetic analyses. The *Lombardochloa* clade was often placed as sister to *Hildaea* and *Poidium* clades (Supporting Information Figs S1, S3). However, their relationship was not recovered in the total-evidence analyses, and the *Lombardochloa* clade was placed in the basal polytomy (Fig. 1). Based on morphology, Matthei (1975) accepted *C. rufum* in the genus *Poidium* [=*P. rufum* (J.Presl) Matthei], whereas Longhi-Wagner (1987) accepted it in the monospecific *Briza* section *Lombardochloa* (Roseng. & B.R.Arrill.) Longhi-Wagner. *Chascolytum rufum* presents the unique combination of membranous paleas and coriaceous lemmas, with a pair of oil glands at the base (Figs 2, 6B), characters used to recognize the genus *Lombardochloa* (Rosengurtt & Arrillaga de Maffei, 1979). Thus, we propose the resurrection of the monospecific section *Lombardochloa* in *Chascolytrum s.l.* to accommodate the unusual morphology of *C. rufum*.

The *Tricholemma* clade, comprising *Chascolytrum bidentatum* and *C. monandrum*, was recovered with strong support in most of the phylogenetic analyses (Fig. 1; Supporting Information Figs S1, S3). Although we did not sample *C. koelerioides*, both Essi *et al.* (2008) and Persson & Rydin (2016) included one accession in their phylogenetic analyses, which resulted in the grouping of *C. koelerioides* with *C. monandrum* in their studies. We performed preliminary analyses for the *trnL-trnL-trnF* region (data not shown), including *C. koelerioides* in our matrix, but no phylogenetic resolution regarding this species was obtained. Since there is little variation for the *trnL-trnL-trnF* region and no data for GBSSI, we excluded *C. koelerioides* from the combined dataset analyses.

Polytomies occur in some clades, and some species with high levels of morphological variation are still not fully resolved, particularly in the section *Hildaea* clade. The circumscription of several taxa, including *Chascolytrum ambiguum*, *C. calotheca* and both varieties of *C. juergensii*, is difficult due to highly variable and overlapping morphologies (Longhi-Wagner, 1987; Essi *et al.*, 2017). This taxonomic complex will need further investigations to better understand the evolutionary processes linked to species diversification, especially in the south-eastern and southern Brazilian highland grasslands where most of the taxa are endemic and occur in sympatry (Iganci *et al.*, 2011; Essi & da Silva, 2018).

The use of molecular and morphological data

More molecular data are needed to elucidate the phylogenetic relationships in sections of *Chascolytrum s.l.* The use of integrative approaches including molecular, morphological and ecological data was heuristic, since better resolution was obtained when the different datasets were combined in our analyses. The GBSSI gene provided the most infrageneric resolution obtained in our analyses. It has been consistently used for phylogenetic reconstructions in *Chascolytrum* (Essi *et al.*, 2008; Persson & Rydin, 2016) and grasses in general (e.g. Mathews *et al.*, 2002; Guo & Li, 2004; Davis & Soreng, 2008; Yang *et al.*, 2010). The advantage of using the GBSSI gene relies on its single-copy presence in grass genomes (Shure, Wessler & Fedoroff, 1983; Mason-Gamer *et al.*, 1998; and references therein), providing high phylogenetic signal to reconstruct robust phylogenetic trees when compared to other nuclear regions such as ITS (Mason-Gamer *et al.*, 1998). However, most of the GBSSI sequences generated for *Chascolytrum* spp. in previous studies (Essi *et al.*, 2008; Persson & Rydin, 2016) are incomplete, emphasizing the difficulty for obtaining complete and good quality sequences for this region.

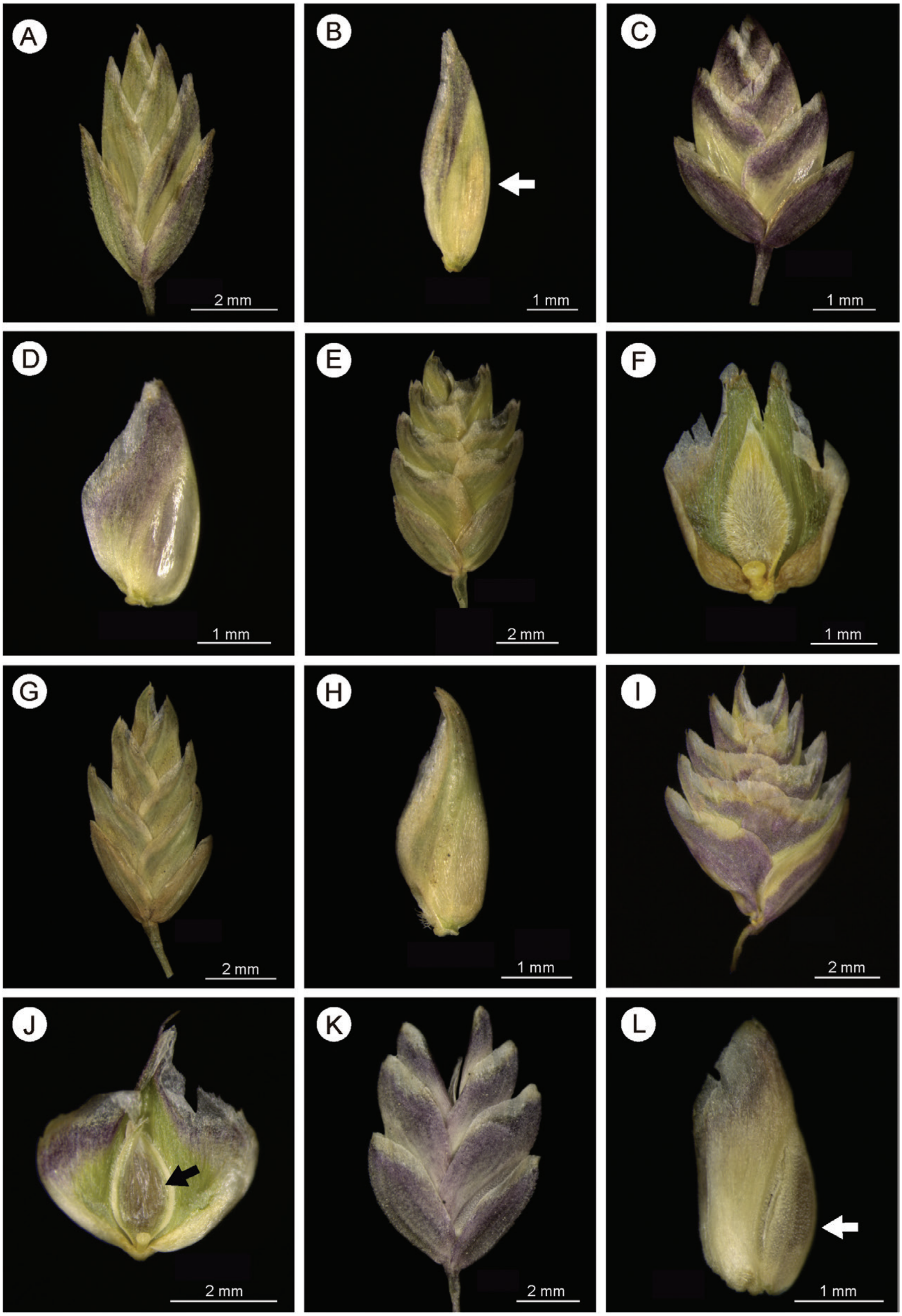

Figure 4. Spikelet morphology in *Chascolytrum*. *Chascolytrum* section *Hildae*: *C. ambiguum*. A, spikelet. B, lemma in lateral view, arrow indicates the back not gibbous. *C. calotheca*. C, spikelet. D, lemma in lateral view. *C. juergensii* var.

The use of internal primers, as proposed by Mason-Gamer *et al.* (1998), enhances sequencing costs, but is worthwhile considering the amount of the phylogenetic information found in this gene.

In contrast, the phylogenetic trees obtained based on the plastid DNA datasets (*trnL-trnL-trnF* and *rps16* intron) were less resolved than the GBSSI-based trees (Supporting Information Fig. S2), as seen in previous studies (Zhang, Zeng & Li 2012; Drew & Sytsma, 2013). Plastid molecular markers are often less variable and phylogenetic informative than nuclear regions (Simeone *et al.*, 2013), although they are valuable sources for investigate some evolutionary processes, including hybridization and incomplete lineage sorting (Jakob & Blattner, 2006; Willyard, Cronn & Liston, 2009).

The total-evidence approach combining both molecular and morphological data has been widely applied, particularly in cases in which phylogenetic trees based only on molecular markers cannot resolve taxonomic issues, encompassing different hierarchical categories such as order (Renner, 1999; Caddick *et al.*, 2002), family (Olson, 2002), tribe (Penneys & Judd, 2013) and genus (Clennett *et al.*, 2012; Gomes-da-Silva & Souza-Chies, 2018). This approach has proven to provide more resolution and support in phylogenetic reconstructions (Catalán *et al.*, 2007; Clennett *et al.*, 2012; Gutiérrez *et al.*, 2017). In the present study, it helped to resolve the placement of some species (e.g. *C. brizoides*, *C. bulbosum* and *C. parodianum*) and improved support for most clades. The addition of morphological characters to the molecular phylogenetic matrix is feasible for *Chascolytrum s.l.* due to the easiness of obtaining morphological data, even from old herbarium material, which is one of its major advantages (Hillis, 1987). Hence, the combination of molecular and morphological data is regarded as highly desirable in future studies regarding species complexes in *Chascolytrum s.l.*, e.g. for elucidating the taxonomic boundaries among the species in section *Hildaea*.

CONCLUSIONS AND PERSPECTIVES

The use of plastid and nuclear markers, combined with morphological data and a comprehensive taxon sampling, allowed reconstruction of the most resolved and well-supported phylogenetic hypothesis for *Chascolytrum* to date. It was helpful for reevaluating morphological diversity in the genus, and we propose an infrageneric classification for the genus. In addition, we produced the first DNA data for *Bromidium*. The inclusion of additional nuclear regions or next-generation sequencing data, even with smaller sampling, is still needed to resolve the basal polytomies, allowing an accurate reconstruction of the biogeographic history of this group. Studies in this context can clarify the events that shaped the current morphological diversity in *Chascolytrum* and the role of the southern Brazilian highlands in its diversification (Iganci *et al.*, 2011). More data on *Chascolytrum koelerioides* and *C. rhomboideum* will be essential for resolving the complete phylogeny of *Chascolytrum s.l.*, and more extensive sampling of the South American genera of supersubtribe Agrostidodinae will be needed to clarify the phylogenetic classification of this unusual genus.

TAXONOMIC TREATMENT OF SECTIONS OF *CHASCOLYTRUM*

According to the phylogenetic results obtained in this work, we propose the recognition of eight sections in *Chascolytrum*. A taxonomic key for their identification and descriptions, distribution data, taxonomic notes and images of taxonomically relevant characters are presented here.

Chascolytrum *section* **Calotheca** *(Desv.) L.N.Silva,* ***comb. nov.***

Basionym: Calotheca Desv., Nouv. Bull. Sci. Soc. Philom. Paris 2: 190. 1810. ≡ *Briza* section *Calotheca* (Desv.) Benth. & Hook., Gen. Pl. 3(2): 1195. 1883. ≡ *Briza* subgenus *Calotheca* (Desv.) Parodi, Revista Fac. Agron. Veterin. (Buenos Aires) 3: 130. 1920. = *Erianthecium* Parodi, Notas Mus. La Plata, Bot. 8: 75, fig. 1. 1943. Type: *Erianthecium bulbosum* Parodi.

Type: Chascolytrum brizoides (Lam.) Essi, Longhi-Wagner & Souza-Chies.

Plants caespitose, not rhizomatous, basal internodes of the culms thickened or not. Leaf sheaths glabrous or pilose. Leaf blades firm or soft, erect or pendulous, flat or convolute, apex acute or obtuse, glabrous or pilose in both surfaces. Panicle open to contracted, erect. Spikelets four- to 11-flowered, subcylindrical

juergensii. E, spikelet. F, floret in ventral view, palea conspicuously pilose. *C. juergensii* var. *angustilemma*. G, spikelet. H, lemma in lateral view. *C. latifolium*. I, spikelet. J, floret in ventral view, arrow indicates the membranous palea. *C. uniolae*. K, spikelet. L, lemma in lateral view, arrow indicates papillate gibbous back. Photographs: L.N. da Silva.

TAXONOMIC KEY TO SECTIONS OF *CHASCOLYTRUM*

1. Lemmas awned (Fig. 3A–D), awns 1–4.4 mm .... *C.* section *Calotheca*
1. Lemmas muticous or mucronate, mucron up to 0.5 mm.
  2. Paleas rigid, chartaceous (Fig. 5E) to coriaceous (Fig 3F).
    3. Paleas chartaceous, obovate to obovate-truncate (Fig. 5E) .... *C.* section *Obovatae*
  3. Paleas coriaceous, orbiculate to elliptic (Fig. 3F, J)
    4. Lemmas with a gibbous back clearly distinct from the broad or narrow margins (Fig. 3G, H, K), surface glabrous or strigose. Paleas glabrous, smooth, strigose or with sparse capitate trichomes ....*C.* section *Chascolytrum*
    4. Lemmas without a gibbous back distinct from the margins, surface echinulate (Fig. 5C). Paleas glabrous, echinulate .... *C.* section *Microbriza*
2. Paleas soft, membranous to hyaline (Fig. 4J).
  5. Lemmas coriaceous, rufous on the back, with a pair of oil glands or its scars at the base (Fig. 5B) .... *C.* section *Lombardochloa*
  5. Lemmas herbaceous to chartaceous, pale, greenish or purplish on the back, without a pair of oil glands or its scars at the base.
    6.Margins of the lemma ciliate at the lower third (Fig. 5M) .... *C.* section *Tricholemma*
    6. Margins of the lemma glabrous.
      7. Spikelets ovate, three- to four- (five-)flowered (Fig. 4F, H, I) .... *C.* section *Poidium*
      7. Spikelets oblong, elliptic or lanceolate, (four-) five- to 11-flowered (Fig. 4) .... *C.* section *Hildaea*

or laterally compressed, oblong or lanceolate. Glumes chartaceous, convex to naviculate. Lemmas chartaceous, glabrous or pilose, laterally to slightly dorsiventrally compressed, pale, greenish to purplish, without a gibbous back, back not distinct from the margins, margins not ciliate at the lower third, without a pair of oil glands or their scars at the base, awned, awns 1.0–4.4 mm. Paleas chartaceous, elliptic to lanceolate, pilose between the keels, keels ciliate or ciliolate.

Species included: Chascolytrum brizoides and *C. bulbosum*.

Distribution: Argentina, Brazil, Chile and Uruguay (Essi *et al.*, 2017).

Etymology: The name *Calotheca* refers to the beauty of the lemmas, with the margins expanded (from Greek; *kalos* = beautiful; *theke* = box) (Clifford & Bostock, 2007).

Taxonomic notes: The circumscription of *Chascolytrum* section *Calotheca* is expanded from that of *Briza* section *Calotheca* (Longhi-Wagner, 1987), here now including *Chascolytrum bulbosum*, which was previously included in *Erianthecium* (Parodi, 1920; Longhi-Wagner, 1987). This section is easily distinguished from the others by the presence of well-developed (more than 1 mm) awns in the lemmas in both species.

Images: Figs 4A–D, 6A.

Chascolytrum ***Desv.*** *section* **Chascolytrum**

Basionym: Chascolytrum Desv., Nouv. Bull. Sci. Soc. Philom. Paris 2: 190. 1810. ≡ *Briza* section *Chascolytrum* (Desv.) Benth. & Hook., Gen. Pl. 3(2): 1195. 1883. ≡ *Briza* subgenus *Chascolytrum* (Desv.) Parodi, Revista Fac. Agron. Veterin. (Buenos Aires) 3: 120. 1920.

Type: Chascolytrum subaristatum (Lam.) Desv.
Plants caespitose, rhizomatous or not; basal internodes of the culms not thickened. Leaf sheaths glabrous or pilose. Leaf blades firm or soft, erect or pendulous, flat or convolute, apex acute or truncate, glabrous. Panicle open to contracted, erect or pendulous. Spikelets three- to 14-flowered, cylindrical to subcylindrical or slightly laterally compressed, oblong, globose or orbicular. Glumes herbaceous, convex to naviculate. Lemmas coriaceous, glabrous or strigose, laterally to dorsiventrally compressed, with a pale, greenish, purplish, yellow, orange to brown gibbous back, strongly distinct from the broad or narrow margins,

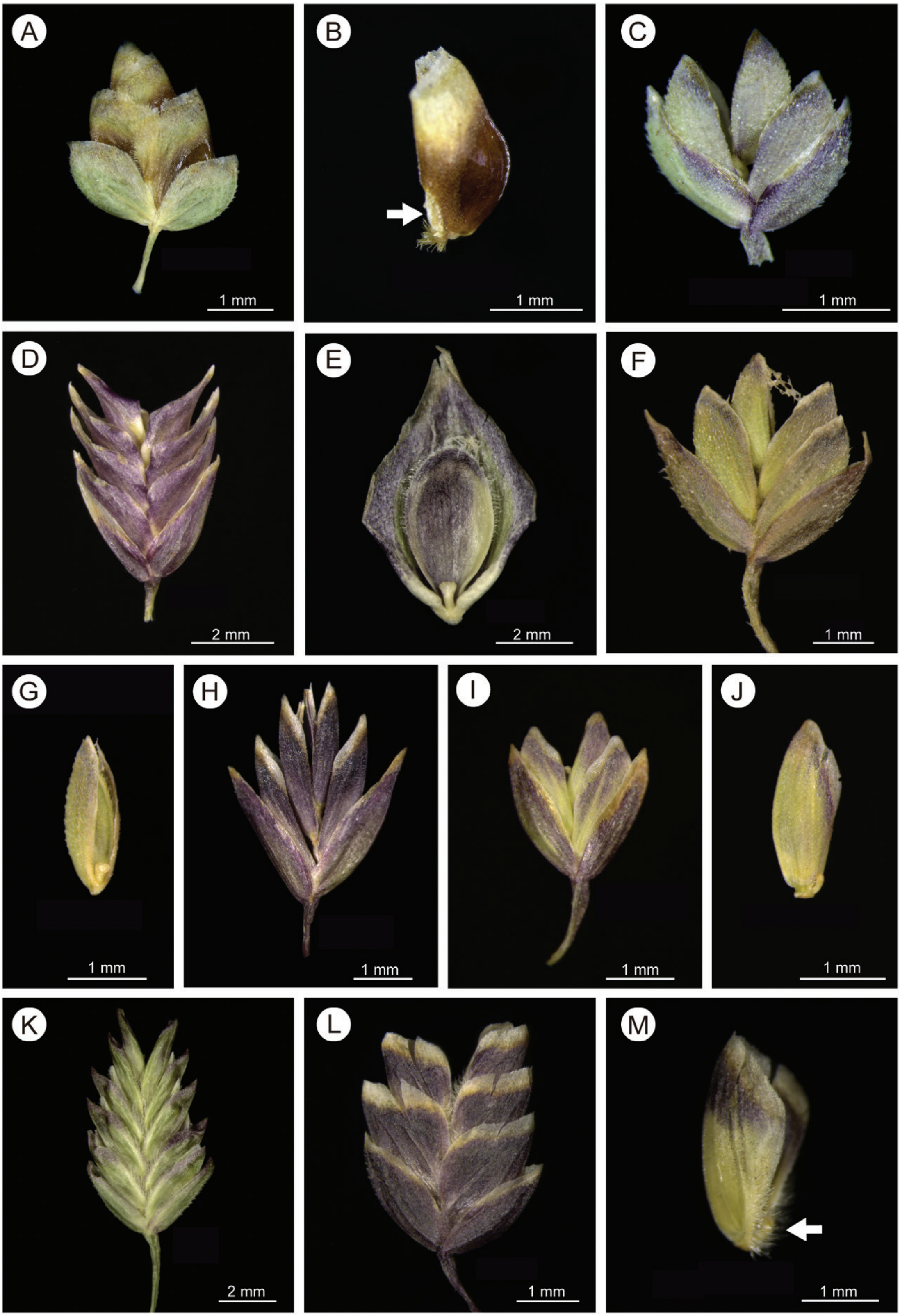

Figure 5. Spikelet morphology in *Chascolytrum*. *Chascolytrum* section *Lombardochloa*: *Chascolytrum rufum* var. *rufum*. A, spikelet. B, lemma in lateral view, arrow indicates the scars of oil gland at the base of the lemma. *Chascolytrum* section

margins not ciliate at the lower third, without a pair of oil glands or their scars at the base, muticous or mucronate (mucron up to 0.5 mm). Paleas coriaceous, elliptic to orbicular, glabrous, strigose or with sparse capitate trichomes between the keels, keels glabrous, ciliate or ciliolate.

Species included: Chascolytrum altimontanum Essi, Souza-Chies & Longhi-Wagner, *C. erectum*, *C. lamarckianum* (Nees) Matthei, *C. paleapiliferum* (Parodi) Matthei, *C. scabrum* (Nees ex Steud.) Matthei and *C. subaristatum*.

Distribution: Argentina, Brazil, Chile, Guatemala, Mexico, Paraguay, Peru and Uruguay (Essi *et al.*, 2017).

Etymology: The name *Chascolytrum* refers to the florets and the grain exposure at maturity (from Greek; *chasko* = gape, *elytron* = cover) (Clifford & Bostock, 2007).

Taxonomic notes: All species assigned to section *Chascolytrum* share coriaceous lemmas with a gibbous back strongly distinct from the margins, and coriaceous paleas (Fig. 2), orbicular to large elliptic in shape (Table 3; Fig. 3E–L). As circumscribed here, section *Chascolytrum* comprises six species, including *C. erectum* and *C. altimontanum*. Although the last species was not sampled in our phylogenetic analyses, it presents the same diagnostic characters as the other species included in this section. Moreover, when *C. altimontanum* was described (Essi, Souza-Chies & Longhi-Wagner, 2010), its morphological resemblance to *C. subaristatum* and *C. paleapiliferum* was emphasized, and thus the inclusion of *C. altimontanum* in section *Chascolytrum* seems logical.

Images: Figs 3E–L, 6B, C.

Chascolytrum *section* **Hildaea** *L.N.Silva, Essi & Souza-Chies,* ***sect. nov.***

Type: Chascolytrum calotheca (Trin.) Essi, Longhi-Wagner & Souza-Chies.

Diagnosis: Chascolytrum section *Hildaea* differs from its sister group, *C.* section *Poidium*, by spikelets being more than four-flowered, oblong, elliptic or lanceolate, whereas section *Poidium* has spikelets with up to four flowers (rarely five), ovate.

Plants caespitose, basal internodes of the culms not thickened. Leaf sheaths glabrous. Leaf blades firm or soft, erect or pendulous, flat, apex acute or obtuse, glabrous or pilose on the abaxial surface. Panicle open to contracted, erect or pendulous. Spikelets four- to 11-flowered, subcylindrical or laterally compressed, oblong, elliptic or lanceolate. Glumes herbaceous, convex to naviculate. Lemmas herbaceous, glabrous, smooth, strigose or papillose, laterally to slightly dorsiventrally compressed, pale, greenish to purplish, with or without a gibbous back, back slightly distinct from the margins, margins not ciliate at the lower third, without a pair of oil glands or their scars at the base, muticous or mucronate (mucron up to 0.5 mm). Paleas membranous, elliptic to lanceolate, glabrous, smooth, punctate, strigose or pilose between the keels, keels ciliate or ciliolate.

Species included: Chascolytrum ambiguum, *C. calotheca*, *C. juergensii* var. *juergensii*, *C. juergensii* var. *angustilemma* Essi, Souza-Chies & Longhi-Wagner, *C. latifolium* Essi, Souza-Chies & Longhi-Wagner and *C. uniolae* (Nees) Essi, Longhi-Wagner & Souza-Chies.

Distribution: Argentina, Bolivia, Brazil, Colombia, Paraguay and Uruguay (Essi *et al.*, 2017).

Etymology: The name *Hildaea* is in honour of Hilda Maria Longhi-Wagner, a renowned agrostologist from Brazil who has dedicated part of her career to study cool-season grasses (Pooideae), especially *Chascolytrum*.

Taxonomic notes: The newly described section *Hildaea* includes the several species recognized in *Poidium* by Matthei (1975) or *Briza* section *Poidium* by Longhi-Wagner (1987). Due to the lack of resolution in the basal polytomy, the monophyly of *Briza* section *Poidium* cannot be proved. Although a larger section *Poidium*, including the species placed in *Hildaea* and *Poidium* clades, could be accepted, given their morphological differentiation we decided to recognize them as distinct sections (Table 3, Fig. 2).

Specimens with intermediate morphology are common in section *Hildae*, possibly due to hybridization events between sympatric taxa, which may explain the lack of

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Microbriza: *C. poomorphum*. C, spikelet. *Chascolytrum* section *Obovatae*: *C. parodianum*. D, spikelet. E, floret in ventral view, obovate palea visible. *Chascolytrum* section *Poidium*: *C. brachychaetum*. F, spikelet; G, floret in lateral-ventral view, membranous palea visible. *C. brasilense*. H, spikelet. *C. itatiaiae*. I, spikelet. J, lemma in the lateral-dorsal view. *Chascolytrum* section *Tricholemma*: *C. bidentatum*. K, spikelet; *C. monandrum*. L, spikelet. M, lemma in lateral view, arrow indicates ciliate margin at the lower third. Photographs: L.N. da Silva.

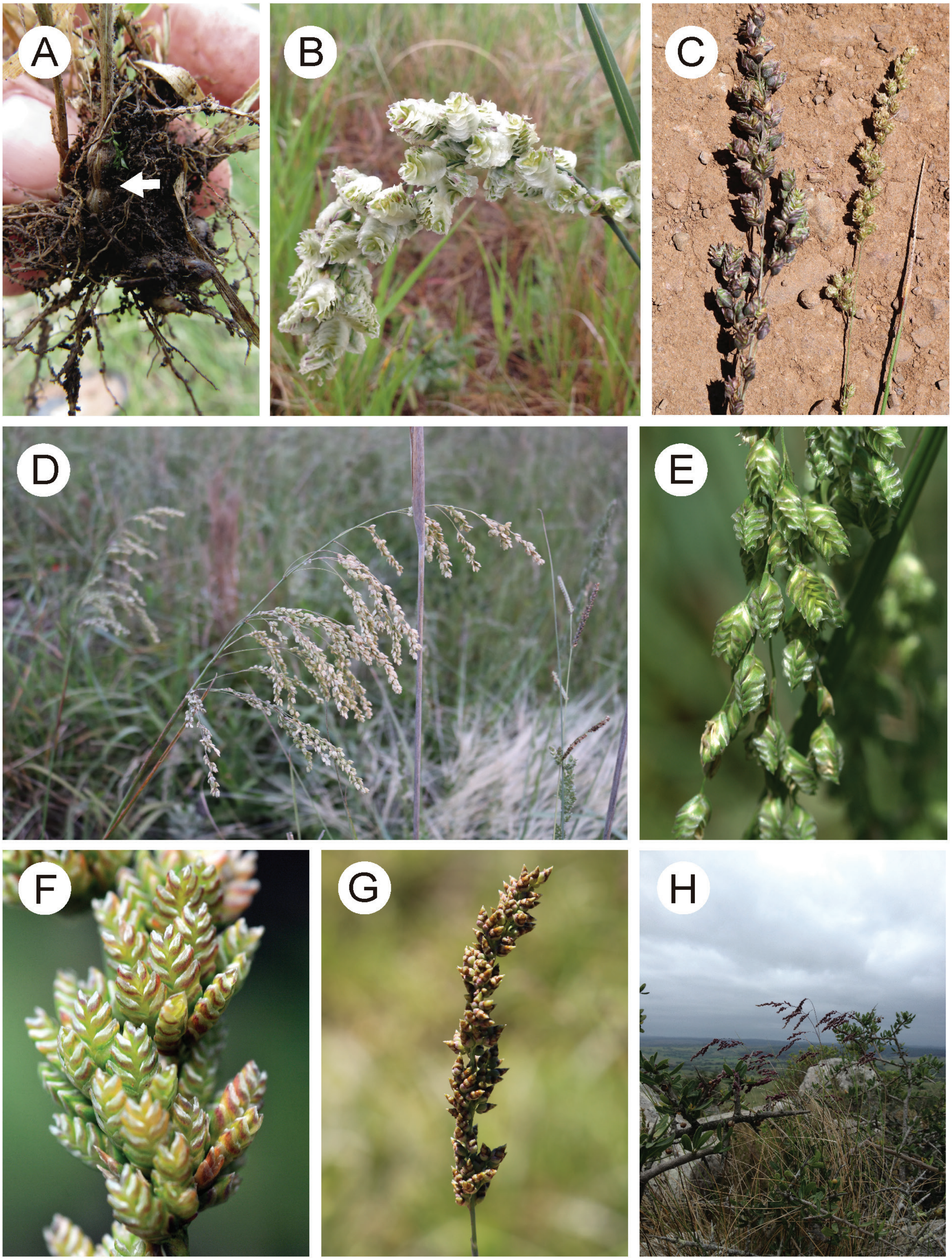

Figure 6. Field photographs of *Chascolytrum*. *Chascolytrum* section *Calotheca*: A, *Chascolytrum bulbosum*, arrow indicates the basal nodes of the culm thickened (Santana da Boa Vista, RS, Brazil). *Chascolytrum* section *Chascolytrum*: B, *C. erectum*

resolution in the internal nodes of the *Hildaea* clade. Thus, further integrative taxonomy studies combining morphometric analysis, additional molecular markers and ecological data would be useful to better understand the evolutionary processes that led to the patterns currently observed in this group

Images: Figs 4A–L, 6D–F.

Chascolytrum *section* **Lombardochloa** *(Roseng. & B.R.Arrill.) L.N.Silva,* ***comb. nov.***

Basionym: Lombardochloa Roseng. & B.R.Arrill., Anales Fac. Quimica Montevideo 9: 260. 1979. ≡ *Briza* section *Lombardochloa* (Roseng. & B.R.Arrill.) Longhi-Wagner, Bol. Inst. Bioci. Univ. Fed. Rio Grande do Sul 41: 22. 1987.

Type: Chascolytrum rufum J.Presl.
Plants caespitose, commonly with short stolons, basal internodes of the culms not thickened. Leaf sheaths glabrous. Leaf blades soft, pendulous, flat, apex acute or obtuse, glabrous. Panicle open to contracted, erect and firm. Spikelets three- or four- (five-) flowered, laterally compressed, ovate. Glumes herbaceous, convex. Lemmas coriaceous, glabrous or pilose on the margins, slightly laterally compressed, pale to greenish, with a rufous gibbous back, strongly distinct from the margins, margins not ciliate at the lower third, with a crown of short hairs next to the callus, and a pair of oil glands or their scars at the base, muticous. Paleas membranous, often hyaline, lanceolate, glabrous and smooth between the keels, keels ciliate or ciliolate.

Species included: Chascolytrum rufum var. *rufum* and *C. rufum* var. *sparsipilosum* (Roseng., B.R.Arrill. & Izag.) Essi, Longhi-Wagner & Souza-Chies.

Distribution: Argentina, Southern Brazil, Paraguay and Uruguay (Essi *et al.*, 2017)

Etymology: The name *Lombardochloa* is in honour of Atilio Lombardo Nolle (1902–1984), a Uruguayan botanist (Clifford & Bostock, 2007).

Taxonomic notes: Chascolytrum rufum was previously placed in *Poidium* (=*P. rufum*) by Matthei (1975) or *Briza* section *Lombardochloa* (=*B. rufa* Steud.) by Longhi-Wagner (1987). In this study, we resurrect the monospecific section *Lombardochloa* to accommodate the unique morphological features present in both varieties of *C. rufum*, which is combined here in *Chascolytrum.*

Images: Figs 5A, B; 6G.

Chascolytrum *section* **Microbriza** *(Parodi ex Nicora & Rúgolo) L.N.Silva,* ***stat. & comb. nov.***

Basionym: Microbriza Parodi ex Nicora & Rúgolo, Darwiniana 23(1): 292. 1981, syn. nov.

Type: Chascolytrum poomorphum (J.Presl) Essi, Longhi-Wagner & Souza-Chies.
Plants caespitose, basal internodes of the culms not thickened. Leaf sheaths glabrous. Leaf blades soft, pendulous, flat, apex acute, glabrous, rarely pilose on the abaxial surface. Panicle open, flaccid to pendulous. Spikelets two- or three-flowered, laterally compressed, ovate to orbicular. Glumes herbaceous, convex. Lemmas coriaceous, glabrous, surface echinulate, slightly laterally compressed, pale to greyish or purplish, without a gibbous back distinct from the margins, margins not ciliate at the lower third, without a pair of oil glands or their scars at the base, muticous. Paleas coriaceous, elliptic, glabrous and echinulate between the keels, keels glabrous or ciliolate.

Species included: Chascolytrum poomorphum.

Distribution: Argentina, Southern Brazil, Paraguay and Uruguay (Essi *et al.*, 2017).

Etymology: The name *Microbriza* is in reference to the spikelets similar to those of genus *Briza*, but smaller (from Greek; *mikros* = small) (Clifford & Bostock, 2007).

Taxonomic notes: Chascolytrum poomorphum was included in *Poidium* [= *P. poomorphum* (Presl) Matthei] by Matthei (1975) or *Briza* section *Poidium* (= *B. poomorpha* Henrard) by Longhi-Wagner (1987), based on the shape of their paleas. However, *C. poomorphum* has coriaceous lemmas and paleas, whereas species in section *Poidium* have herbaceous and membranous lemmas and paleas, respectively (Table 3). The combination of these morphological characters with the echinulate ornamentation of the lemmas and paleas is considered exclusive to *C. poomorphum*. Thus, we propose the monospecific

(Playa de Carrasco, Montevideo, Uruguay). C, *C. subaristatum*, comparison between inflorescence and spikelets size (Jari, RS, Brazil). *Chascolytrum* section *Hildaea*: D, *C. calotheca* (São Francisco de Assis, RS, Brazil). E, *C. juergensii* var. *juergensii*, inflorescence detail (locality unknown). F, *C. uniolae*, inflorescence detail (locality unknown). *Chascolytrum* section *Lombardochloa*: G, *C. rufum* var. *sparsipilosum* (Santiago, RS, Brazil). *Chascolytrum* section Obovatae: H, *C. parodianum*, inhabiting granite hills (Cerro Aguire, Rocha, Uruguay). RS: Rio Grande do Sul. Photographs: A, L. Essi; B–D, G and H, L.N. da Silva; E and F, S.A.L. Bordignon.

Table 3. Main morphological features of the proposed sections for *Chascolytrum*. Text in bold indicates diagnostic characters

Section	Spikelets	Lemmas	Paleas
C. section *Calotheca* (Desv.) L.N.Silva	Four- to 11-flowered; subcylindrical to laterally compressed, oblong or lanceolate.	**Chartaceous**; **awned, awns 1.0–4.4 mm**; glabrous or pilose; without a gibbous back distinct from the margins; margins not ciliate at the lower third; without a pair of oil glands or its scars at the base.	Membranous to chartaceous; elliptic to lanceolate; **pilose between the keels.**
C. section *Chascolytrum*	Three- or four-flowered; cylindrical to subcylindrical or slightly laterally compressed, orbicular to oblong.	**Coriaceous**; muticous or mucronate; glabrous or strigose; **with a gibbous back distinct from the margins**; margins not ciliate at the lower third; without a pair of oil glands or its scars at the base.	**Coriaceous; orbicular to elliptic**; glabrous, strigose or with sparse capitate trichomes between the keels.
C. section *Hildaea* L.N.Silva, Essi & Souza-Chies	**Four- to 11-flowered**; subcylindrical or laterally compressed, **oblong, elliptic or lanceolate**.	**Herbaceous**; muticous or mucronate; **glabrous, papillate or strigose**; with or without a gibbous back slightly distinct from the margins; margins not ciliate at the lower third; without a pair of oil glands or its scars at the base.	**Membranous**; **elliptic to lanceolate**; glabrous, smooth, strigose or pilose between the keels.
C. section *Lombardochloa* (Roseng. & B.R.Arrill.) L.N.Silva	Three- to five-flowered; laterally compressed, ovate.	**Coriaceous**; muticous, glabrous or sparsely pilose; **with a gibbous back distinct from the margins**; margins not ciliate at the lower third; **with a pair of oil glands or its scars at the base**.	**Membranous to hyaline**; lanceolate; glabrous and smooth between the keels.
C. section *Microbriza* (Parodi ex Nicora & Rúgolo) L.N.Silva	**Two- or three-flowered**; laterally compressed, **ovate to orbicular**.	**Coriaceous**; muticous, glabrous; **echinulate**; **without a gibbous back distinct from the margins**; margins not ciliate at the lower third; without a pair of oil glands or its scars at the base.	**Coriaceous**; elliptic; glabrous and **echinulate between the keels**.
C. section *Obovatae* L.N.Silva, Essi & Souza-Chies	Four- to eight-flowered; subcylindrical, elliptic-lanceolate.	**Chartaceous**; muticous or mucronate; glabrous; without a gibbous back distinct from the margins; margins not ciliate at the lower third; without a pair of oil glands or its scars at the base.	**Chartaceous**; **obovate to obovate-trucate**; glabrous between the keels.
C. section *Poidium* (Nees) L.N.Silva	**Three- to five-flowered**; laterally compressed, **ovate**.	**Herbaceous**; muticous or mucronate; glabrous, **smooth or scabrous**; without a gibbous back distinct from the margins; margins not ciliate at the lower third; without a pair of oil glands or its scars at the base.	**Membranous**; elliptic to linear-lanceolate; glabrous, smooth or punctate between the keels.
C. section *Tricholemma* L.N.Silva, Essi & Souza-Chies	Four- to ten-flowered; cylindrical or laterally compressed, oblong, elliptic to lanceolate.	**Herbaceous or chartaceous**; muticous; glabrous or pilose; **without a gibbous back distinct from the margins**; **margins ciliate at the lower third**; without a pair of oil glands or its scars at the base.	**Membranous**; elliptic to lanceolate; glabrous or pilose between the keels.

section *Microbriza*, a new status for *Microbriza*, and a new combination under *Chascolytrum*.

Image: Fig. 5C.

Chascolytrum *section* **Obovatae** *L.N.Silva, Essi & Souza-Chies,* ***sect. nov.***

Type: Chascolytrum parodianum (Roseng., B.R.Arrill. & Izag.) Matthei.

Diagnosis: Chascolytrum section *Obovatae* differs from other sections of the genus by the long and involute leaves, the chartaceous lemmas and paleas, and by the obovate to obovate-truncate paleas.

Plants caespitose, basal internodes of the culms not thickened. Leaf sheaths glabrous. Leaf blades firm, involute, apex acute, glabrous. Panicle open, flaccid to pendulous. Spikelets four-to eight-flowered, subcylindrical, elliptic-lanceolate. Glumes chartaceous, naviculate. Lemmas chartaceous, glabrous, smooth, slightly laterally or dorsiventrally compressed, pale to purplish, without a gibbous back distinct from the margins, margins not ciliate at the lower third, without a pair of oil glands or their scars at the base, muticous or mucronate (mucron up to 0.5 mm). Paleas chartaceous, obovate to obovate-truncate, glabrous and smooth between the keels, keels ciliate or ciliolate.

Species included: Chascolytrum parodianum (Roseng., B.R.Arrill. & Izag.) Matthei.

Distribution: Brazil and Uruguay (Essi *et al.*, 2017).

Etymology: The name *Obovatae* refers to the obovate shape of the paleas (from Latin; *ob-* = contrary; *ovum* = egg, in reference: egg-shaped, with a broad end distal to pedicel) (Clifford & Bostock, 2007), exclusive to this section.

Taxonomic notes: Chascolytrum parodianum was previously included in *Chascolytrum s.s.* by Matthei (1975) or *Briza* section *Chascolytrum* by Longhi-Wagner (1987). However, based on our phylogenetic analyses, we propose the monospecific section *Obovatae* for the species. The new section is easily distinguished from other sections of *Chascolytrum* by the chartaceous and obovate to obovate-truncate paleas (Fig. 5E).
Chascolytrum parodianum has been considered as a narrow endemic to the Pampa Biome in southern Brazil (Rio Grande do Sul) and Uruguay (Longhi-Wagner, 1987; Essi *et al.* 2017), inhabiting open grasslands on granitic outcrops (Fig. 6H). However, doubtful records from the southern Brazilian highlands in the domains of the *Araucaria angustifolia* (Bertol.) Kuntze forests in Rio Grande do Sul (here labelled as *C.* aff. *parodianum*) raised questions about the morphological variation and geographical distribution of *C. parodianum*. In our study, *C.* aff. *parodianum* was recovered as sister of the two typical and pampean accessions of *C. parodianum* in most analyses (Fig. 1; Supporting Information, Figs S2, S3). Specimens of *C.* aff. *parodianum* share the same traditional morphological characters diagnostic of *C. parodianum* (Table 3). However, there are differences in plant size and panicle shapes, which are noteworthy in the field. Further investigations of integrative taxonomy including ecological, morphometric and population genetic approaches are needed to elucidate the taxonomic status of *C.* aff. *parodianum*.

Images: Figs 5D, E; 6H.

Chascolytrum *section* **Poidium** *(Nees) L.N.Silva,* ***comb. nov.***

Basionym: Poidium Nees, Intr. Nat. Syst. Bot. 450. 1836. ≡ *Briza* section *Poidium* (Nees) Ekman, Ark. Bot. 13(10): 70. 1913.

Type: Chascolytrum brasilense (Nees) Essi, Longhi-Wagner & Souza-Chies.
Plants caespitose, basal internodes of the culms not thickened. Leaf sheaths glabrous or pilose. Leaf blades firm or soft, erect or pendulous, involute or flat, apex acute, glabrous or pilose on the abaxial surface. Panicle open, erect or pendulous. Spikelets three- to four- (five-) flowered, laterally compressed, ovate, rarely elliptic when immature. Glumes herbaceous, naviculate. Lemmas herbaceous, glabrous, smooth, tuberculate or scabrous, laterally compressed, pale to purplish, without a gibbous back distinct from the margins, margins not ciliate at the lower third, without a pair of oil glands or their scars at the base, muticous or mucronate (mucron up to 0.5 mm). Paleas membranous, elliptic to linear-lanceolate, glabrous, smooth or punctate between the keels, keels ciliate or ciliolate.

Species included: Chascolytrum brachychaetum, *C. brasilense* and *C. itatiaiae*.

Distribution: Brazil, exclusively in southern and south-eastern highland grasslands (Essi *et al.*, 2017).

Etymology: The name *Poidium* is in reference to the spikelets similar to those of genus *Poa* L., but smaller (the Greek suffix *-idium* means 'diminutive') (Clifford & Bostock, 2007).

Taxonomic notes: Poidium was described by Nees von Esenbeck (1836) based on *P. brasilense* Nees and had its circumscription considerably expanded by Matthei (1975). Longhi-Wagner (1987) recognized *Briza* section *Poidium* with a similar circumscription to that proposed by Matthei, but included *B. bidentata* (=*C. bidentatum*), which was not considered in Matthei's work; Bayón (1998) proposed a narrower circumscription for *Poidium* including only three species [*P. brasilense*, *P. itatiaiae* (Ekman) Nicora & Rúgolo and *P. ambiguum* (Hack.) Matthei)]. Based on our phylogenetic analyses and the morphological cohesiveness between the taxa in the *Poidium* clade, we accept section *Podium* in *Chascolytrum*, excluding *C. ambiguum* [=*Poidium ambiguum*, included by Bayón

(1998)] and including *C. brachychaetum* [=*Microbriza brachychaete* (Ekman) Parodi ex Nicora & Rúgolo, excluded by Bayón (1998)].

Image: Fig. 5F–J.

Chascolytrum *section* **Tricholemma** *L.N.Silva, Essi & Souza-Chies,* ***sect. nov.***

= *Rhombolytrum* Link, Hort. Berol. 2: 296. 1833, syn. nov. Type: *Rhombolytrum rhomboideum* Link.
=*Gymnachne* Parodi, Notas Mus. La Plata, Bot. 3: 29. 1938, syn. nov. Type: *Gymnachne jaffuelii* Parodi.

Type: Chascolytrum bidentatum (Roseng., B.R.Arrill. & Izag.) Essi, Longhi-Wagner & Souza-Chies.

Diagnosis: Chascolytrum section *Tricholemma* is morphologically related to section *Poidium* and section *Hildaea* by the presence of membranous paleas. It differs from the former by the presence of more than four-flowered spikelets (vs. up to four-flowered spikelets in section *Poidium*), and from both by the lemmas with the margins ciliate at the lower third (vs. lemmas with glabrous margins in sections *Poidium* and *Hildaea*).

Plants caespitose, basal internodes of the culms not thickened. Leaf sheaths glabrous or pilose. Leaf blades firm or soft, erect or pendulous, involute or flat, apex acute or obtuse, glabrous or pilose. Panicle open to contracted, erect or pendulous. Spikelets four- to ten-flowered, cylindrical or laterally compressed, oblong, elliptic to lanceolate. Glumes herbaceous to chartaceous, naviculate. Lemmas herbaceous or chartaceous, glabrous or pilose, slightly dorsiventrally to laterally compressed, pale, greenish to purplish, without a gibbous back distinct from the margins, margins ciliate at the lower third, without a pair of oil glands or their scars at the base, muticous. Paleas membranous, elliptic to lanceolate, glabrous or pilose between the keels, keels ciliate or ciliolate.

Species included: Chascolytrum bidentatum, *C. koelerioides*, *C. monandrum* and *C. rhomboideum*.

Distribution: Argentina, Bolivia, Brazil, Chile, Ecuador, Peru and Uruguay (Essi *et al.*, 2017).

Etymology: The name *Tricholemma* refers to the lemmas with margins ciliate at the lower third (from Greek; *thrix* = hair) (Clifford & Bostock, 2007), the diagnostic character of this section.

Taxonomic notes: The new section *Tricholemma* encompasses species with a complex taxonomic background, due to several circumscriptions proposed for the '*Briza* Complex' in the past. Matthei (1975), for example, excluded *Briza bidentata* (=*Chascolytrum bidentatum*) from his taxonomic treatment, suggesting its inclusion in *Rhombolytrum* or *Gymnachne*, while recognizing *C. monandrum* in *Poidium*. Later, Longhi-Wagner (1987) recognized both species in the large *Briza* section *Poidium*. Bayón (1998), however, proposed recognizing *Rhombolytrum*, including *R. monandrum* (Hack.) Nicora & Rúgolo (with *C. bidentatum* as its synonym) and *R. rhomboideum* Link, a circumscription also proposed by Nicora & Rúgolo de Agrasar (1981). More recently, *Rhombolytrum rhomboideum* and *Gymnachne koelerioides* (Trin.) Parodi were placed in *Chascolytrum s.l.* by Essi *et al.* (2017). Although these species were not sampled in our phylogenetic analyses, we place them in section *Tricholemma* based on the morphological characters shared with the other species in this section (Table 3, Fig. 2).

Images: Fig. 5K–M.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Table S1. Voucher information, geographical origin and GenBank accession numbers of the samples used in the phylogenetic analyses of *Chascolytrum*. Circled individuals were analysed together in the combined analyses. N/A = not available

Figure S1. Bayesian inference (BI) consensus cladogram and respective phylogram from the analysis of the nuclear gene GBSSI. Numbers above the branches indicate the BI posterior probabilities (PP; values > 0.95), and below the branches indicate the maximum likelihood (LB; values > 50%) bootstrap supports. Asterisk indicates species with sequences sampled from GenBank.

Figure S2. BI consensus cladogram and respective phylogram from the analysis of the plastid DNA dataset (*trnL-trnL-trnF* and *rps16* intron). Numbers above the branches indicate the BI PP; values > 0.95), and below the branches indicate the ML (LB; values > 50%) bootstrap supports. Asterisk indicates species with sequences sampled from GenBank.

Figure S3. BI consensus cladogram and respective phylogram from the analysis of the combined DNA dataset (*trnL-trnL-trnF*, *rps16* intron and GBSSI regions). Numbers above the branches indicate the BI PP; values > 0.95), and below the branches indicate the ML (LB; values > 50%). Asterisk indicates species with sequences sampled from GenBank.

CAPÍTULO II:

Chascolytrum serranum (Poaceae: Pooideae: Poeae: Calothecinae), a new microendemic species from Campos de Cima da Serra, southern Brazil

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Chascolytrum serranum (Poaceae: Pooideae: Poeae: Calothecinae), a new microendemic species from Campos de Cima da Serra, southern Brazil

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Abstract

Chascolytrum serranum, a new species of *C.* sect. *Hildaea* restricted to Serra Geral National Park and surroundings, in Cambará do Sul, Rio Grande do Sul, Brazil, is described and illustrated. The new species is morphologically similar to *C. ambiguum* and *C. juergensii* var. *angustilemma*, but is distinguished from them mainly by having paleas more than 2/3 the length of the lemmas (up to 2/3 the length of the lemmas in *C. ambiguum* and *C. juergensii* var. *angustilemma*), glabrous and smooth lemmas (strigose in *C. juergensii* var. *angustilemma*), and puberulous paleas (glabrous in *C. ambiguum*). The species can be recognized in the field by the purplish and shiny spikelets. It inhabits river banks and, less frequently, swamps.

Keywords: cool-season grass, grasslands, highlands, taxonomy

Introduction

Chascolytrum Desvaux (1810: 190) belongs to Poaceae, subfamily Pooideae (Poeae *s.l.*, Poeae chloroplast group I, Calothecinae, Soreng *et al.* 2015, 2017), and comprises 23 species distributed in subtropical and temperate areas of South America (da Silva *et al.* 2020). The highest species diversity is concentrated in southernmost South America, and one species, *C. subaristatum* (Lamarck 1791: 187) Desvaux (1810: 190), extends to northern Mexico and is adventitious in Australia (Essi *et al.* 2017). *Chascolytrum* is distinguished from other genera included in Poeae chloroplast group I by having fibrous basal leaf sheaths, 3- to many-flowered spikelets, and straight pedicels (da Silva *et al.* 2020). In recent decades, species now included in *Chascolytrum* have been variously recognized in several genera, including a broadly circumscribed *Briza* Linnaeus (1753: 70) (including the European species; Longhi-Wagner 1987) or several smaller genera: *Chascolytrum s.s.*, *Calotheca* Desvaux (1810: 190), *Erianthecium* Parodi (1943: 75), *Gymnachne* Parodi (1938: 29), *Lombardochloa* Rosengurtt & Arrillaga de Maffei (1982: 260), *Microbriza* Parodi ex Nicora & Rúgolo (1981: 292), *Poidium* Nees von Esenbeck (1836: 450), and *Rhombolytrum* Link (1833: 296) (for more detailed information see Table 1 in Essi *et al.* 2008). Molecular phylogenetic studies focused on the group recovered a single clade including all New World species that have been recognized in the genera listed above, while the European species were divided into two smaller genera, *Briza s.s.* and *Brizochloa* Jirásek & Chrtek (1967: 40) (Essi *et al.* 2008, 2017, Persson & Rydin 2016).

Based on the molecular data, Essi *et al.* (2008) proposed treating all South American species in the single genus *Chascolytrum*. More broadly sampled phylogenetic studies indicate that *Chascolytrum* is not closely related to *Briza* or *Brizochloa*, nor are *Briza* and *Brizochloa* closely related to one another (Saarela *et al.* 2017). A recent, updated classification of *Chascolytrum* based on molecular and morphological data divided the genus into eight sections (da Silva *et al.* 2020).

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At least 18 species of *Chascolytrum* occur in Brazil, with high diversity in the southeastern and southern Brazilian grasslands and highlands, where six species and one variety are endemic (Essi & da Silva 2018). Three of these taxa are classified in the recently described *C.* sect. *Hildaea* Silva, Essi & Souza-Chies in da Silva *et al.* (2020: 112), which includes five species and two varieties, characterized by having spikelets with five or more flowers, herbaceous lemmas and membranous paleas ciliate on the margins (da Silva *et al.* 2020). Here, we describe a new species of *Chascolytrum* sect. *Hildaea* restricted to the Serra Geral National Park and surroundings, in the municipality Cambará do Sul, Rio Grande do Sul, Brazil. We compare its morphology with other species of the section, including micromorphological analysis of the lemmas and paleas, and provide a taxonomic key for identification of the species included in *C.* sect. *Hildaea*.

Material and methods

The new species was initially recorded by the first author in December 2017, during a field trip to Serra Geral National Park in Cambará do Sul, Rio Grande do Sul, Brazil. After a preliminary study of its morphology, a search for additional populations was carried out in December 2018. Types and paratypes are deposited in the following herbaria: CAN, ICN, K, RB, SMDB, and US (acronyms according to Thiers 2020). Additional material of species of *Chascolytrum* sect. *Hildaea* morphologically similar to the new species [*C. ambiguum* (Hackel 1902: 308) Essi, Longhi-Wagner & Souza-Chies (2011: 327) and *C. juergensii* var. *angustilemma* Essi, Souza-Chies & Longhi-Wagner (2010: 154)] was collected and/or analyzed (collections from ICN) for morphological comparison.

Micromorphology of the lemmas and paleas of the new species, and of *C. ambiguum* and *C. juergensii* var. *angustilemma* was analyzed on a FEI Apreo scanning electron microscope (SEM) at the Canadian Museum of Nature (CMN), in Gatineau, Quebec, Canada. One specimen representing the typical morphology from each species was selected for SEM analysis (Table 1), and the lemmas and paleas from the lowermost floret of a mature spikelet from each specimen were imaged using the SEM. Samples were mounted on a SEM stub using a carbon adhesive disc, and then sputter-coated with a gold-palladium alloy.

The map of geographic distribution of the new species was constructed using ArcGis 10.6.1. Conservation status for the new species was evaluated following the criteria of the International Union for Conservation of Nature (IUCN 2017). The extent of occurrence (EOO) and the area of occupancy (AOO) were calculated with GeoCAT (Bachman *et al.* 2011), using 2 × 2 km grid cells (area of 4 km^2).

Taxonomic treatment

Chascolytrum serranum L.N.Silva, *sp. nov.* Type:—BRAZIL. Rio Grande do Sul: Cambará do Sul, Parque Nacional da Serra Geral, road to Cânion Fortaleza, swamp, 29°04’02.70”S, 50°02’28.70”W, 1022 m, 5 December 2017, *L.N. da Silva, R. Oliveira, R. Marchioretto & A. Christ 1067* (holotype ICN!, isotypes CAN!, RB!, US!). (Figs 1, 2, 3I–L).

Diagnosis:—Similar to *Chascolytrum ambiguum* and *C. juergensii* var. *angustilemma* due to the narrow lemmas (up to 1.5 mm wide), but distinguished by having paleas more than 2/3 the length of the lemmas (*vs.* paleas less than 2/3 the length of the lemmas in *C. ambiguum* and *C. juergensii* var. *angustilemma*), puberulous paleas (*vs.* glabrous paleas in *C. ambiguum*), and glabrous and smooth lemmas (*vs.* strigose lemmas in *C. juergensii* var. *angustilemma*).

Description:—Perennial, caespitose, 55–87 cm tall, basal internodes of the culms not thickened, basal innovations extravaginal. Leaf sheaths glabrous, open, margins not overlapping; ligules 2.6–4.8 mm long, membranous, apex obtuse to truncate; leaf blades 9.5–18 × 0.2–0.6 cm, linear-lanceolate, apex acute, glabrous on both surfaces. Panicle open to contracted, erect, 8.5–16 cm long, pedicels scabrous. Spikelets 4–8–flowered, 4.5–7 × 2–4 mm, laterally compressed, elliptic-lanceolate to lanceolate, florets imbricate, loose at maturity. Glumes herbaceous, naviculate, glabrous, 3-nerved, nerves free, midveins scabrous distally, subequal, muticous, apices acute; lower glumes 2.5–3.2 × 0.6–0.8 mm; upper glumes 2.8–3.7 × 0.7–1.2 mm. Lemmas herbaceous, lanceolate to linear-lanceolate, laterally compressed, gibbous back not distinct from the margins, 3–5–nerved, glabrous, smooth, apex acute, muticous, margins glabrous, flat to slightly involute, base attenuate, without oil glands; lower lemma 3.3–4.1 × 1–1.4 mm. Paleas lanceolate or linear-lanceolate, membranous, puberulous between the keels, keels ciliate; lower palea 2.6–3.6 × 0.8–1.1 mm. Lodicules linear-lanceolate, stamens 1. Caryopsis ellipsoid or elliptic-lanceoloid, 1–1.4 mm, hilum linear.

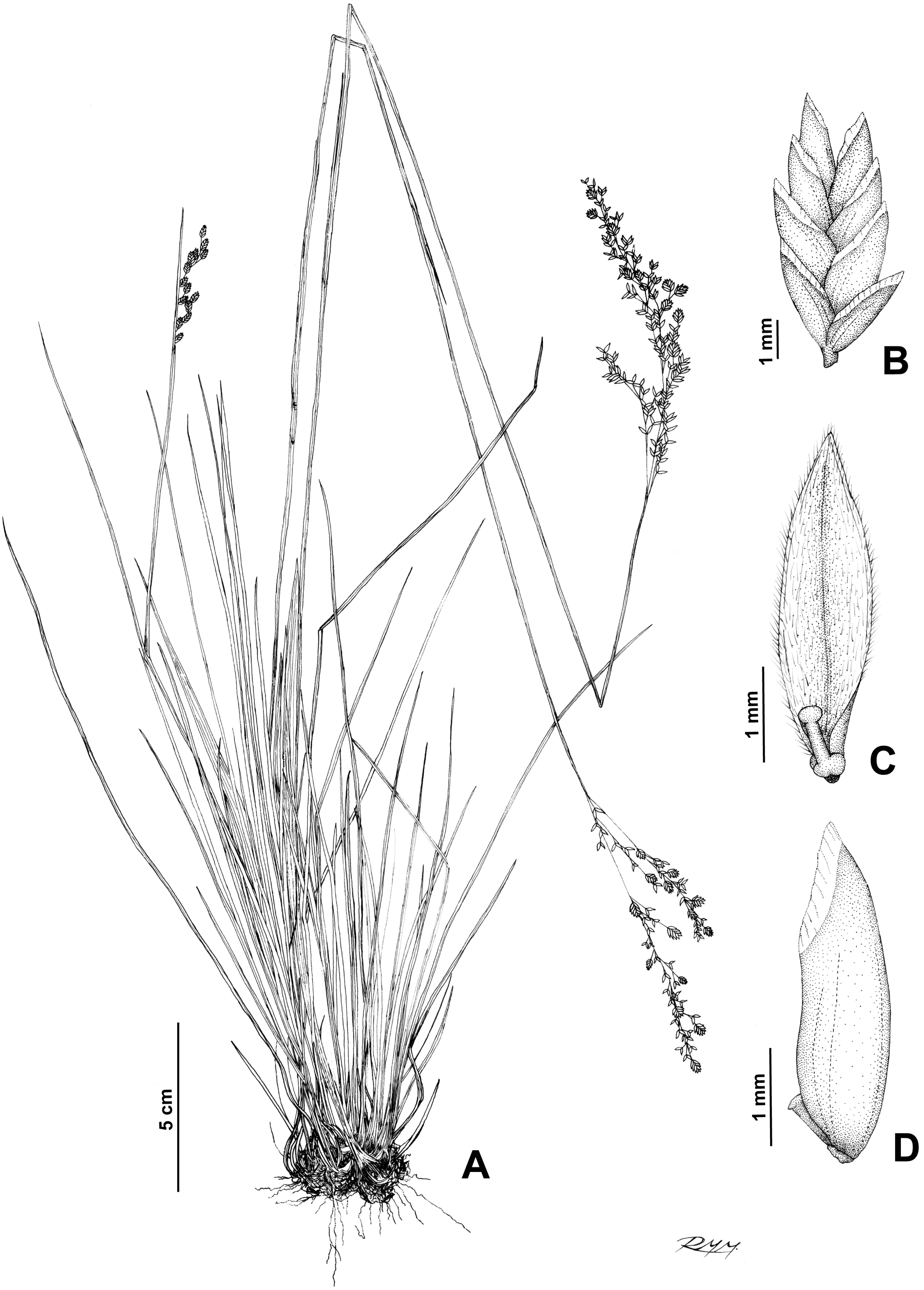

FIGURE 1. *Chascolytrum serranum*. A. Habit. B. Spikelet, lateral view. C. Palea, dorsal view. D. Lemma, lateral view. Drawn from the holotype (*L.N. da Silva et al. 1067*, ICN) by Rafaella M. Marchioretto.

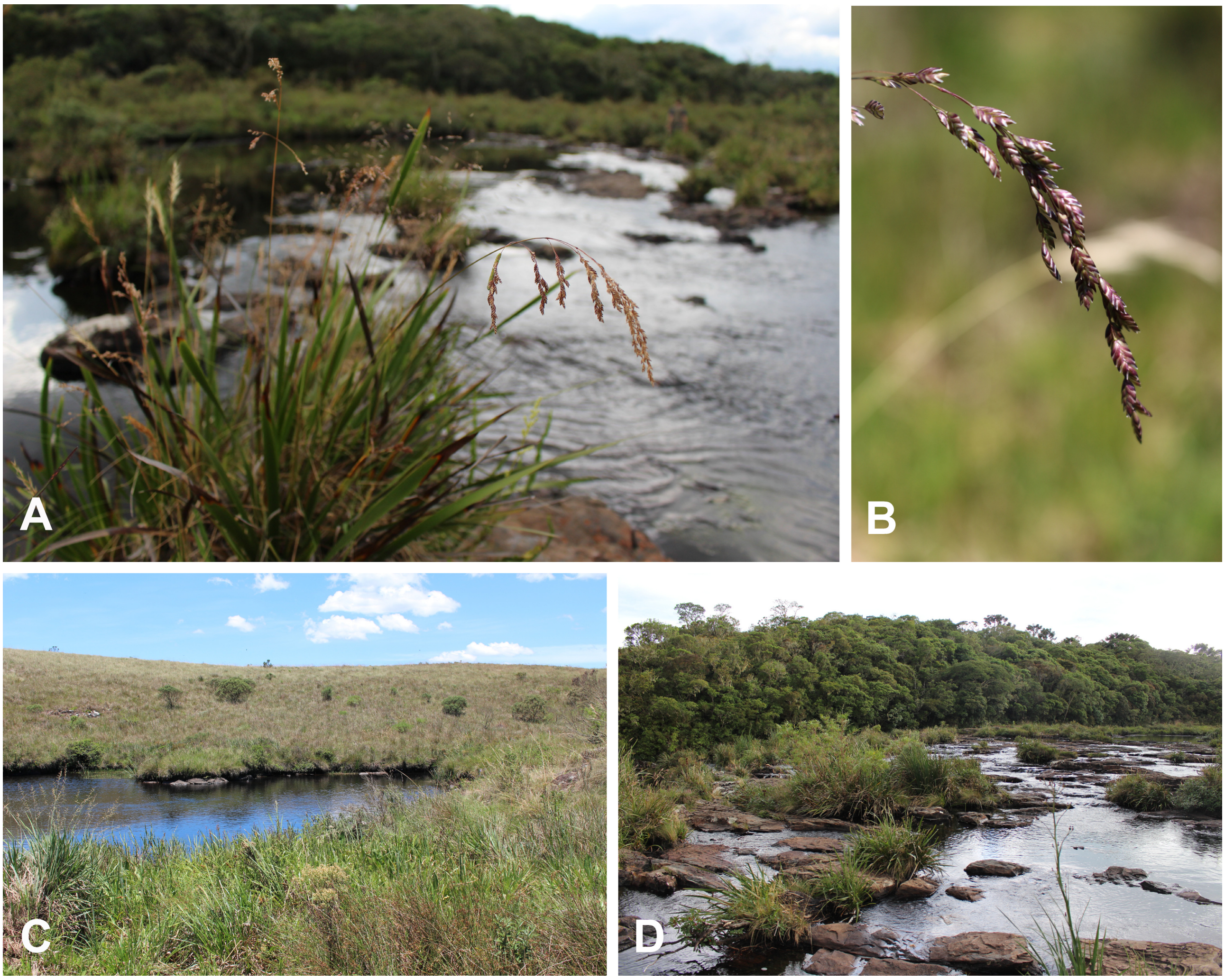

FIGURE 2. *Chascolytrum serranum*. A. Habit of *C. serranum*. B. Detail of the panicle, showing the purplish and shiny spikelets. C. Habitat of *C. serranum*, in the Serra Geral National Park, near to "Arroio Segredo". D. Habitat of *C. serranum*, in the Serra Geral National Park, near to "Cachoeira do Tigre Preto". (Photos by L.N. da Silva)

Etymology:—The specific epithet highlights the area of occurrence of the new species, referring to the locality within Serra Geral National Park where the species is most frequent. The park is located in the Southern Brazilian Highlands where the vegetation is a mosaic of grasslands and *Araucaria angustifolia* (Bertoloni 1819: 411) Kuntze (1898: 375) forest (IBGE 2004, Overbeck *et al.* 2007), a region also called *Campos de Cima da Serra* (Iganci *et al.* 2011). Also, the human population living in this area is colloquially known as "*serranos*" (in Portuguese).

Phenology:—Specimens were collected with flowers and fruits in December.

Distribution and habitat:—The new species is known only from a restricted geographic area: the surroundings of and within Serra Geral National Park, municipality of Cambará do Sul, Rio Grande do Sul, Brazil, at around 1000 m elevation (Fig. 4), inhabiting river banks or swamps (Fig. 2A, C, D). Only five populations are currently known, four of them recorded within the park and located less than 5 km from one another.

Conservation status:—*Chascolytrum serranum* has a restricted geographic distribution, with an extent of occurrence (EOO) and area of occupancy (AOO) of 5.34 km^2 and 12 km^2, respectively. Fewer than five locations are known for the species. Although the species is often recorded within a conservation unit (Serra Geral National Park), populations outside the park may be threatened due to the loss and reduction of habitat quality, especially due to silviculture of pine species. Thus, according to the IUCN Red List guidelines (IUCN 2017), *Chascolytrum serranum* should be considered Endangered (EN) [B2ab(iii)].

Additional specimens examined (paratypes):—BRAZIL. Rio Grande do Sul: Cambará do Sul, Parque Nacional da Serra Geral, road to Cânion Fortaleza, swamp, 29°04'02.70"S, 50°02'28.70"W, 1022 m, 17 December 2018, *L.N. da Silva et al. 1129* (CAN, ICN, RB, K); in wet and peaty grassland, at the riverbank of Arroio Segredo, 29°04'47.10"S, 49°59'17.00"W, 994 m, 17 December 2018, *L.N. da Silva et al. 1132* (CAN, ICN, RB, US); trail to Fortaleza, riverbank, 29°04’01.60”S, 49°57’45.50”W, 1026 m, 17 December 2018, *L.N. da Silva et al. 1137* (CAN, ICN, RB, SMDB); riverbank of Cachoeira do Tigre Preto, 29°04’21.40”S, 49°59’18.70”W, 1001 m, 17 December 2018, *L.N. da Silva et al. 1138* (CAN, ICN, K, US); 29°03’43.90”S, 49°57’23.70”W, 1 December 2003, *L. Essi, A. Guglieri & S. Hefler 135, 136* (ICN).

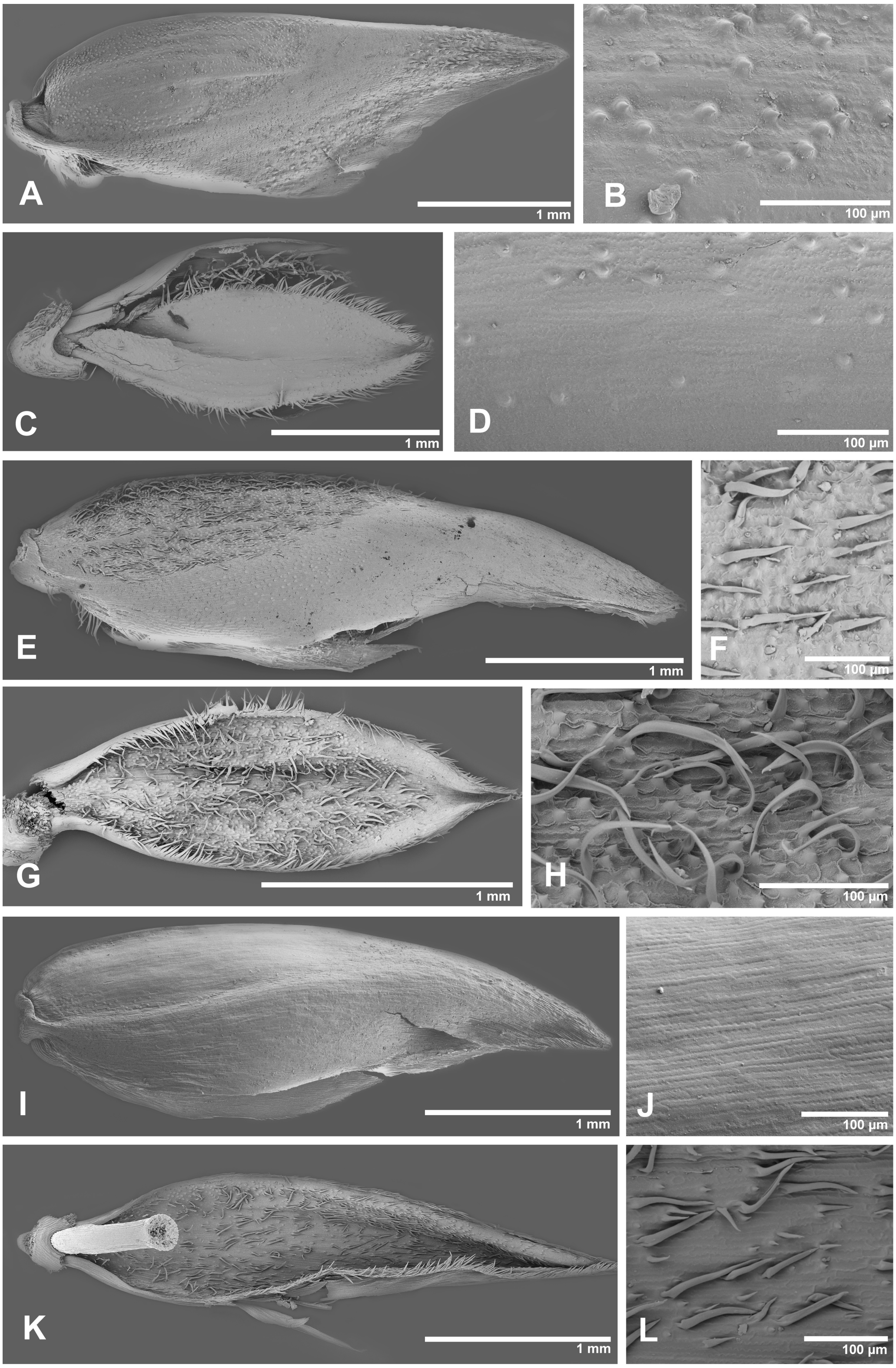

FIGURE 3. Micromorphological features of the floret of *Chascolytrum serranum* and related species. A–D. *C. ambiguum*. A. Lemma, lateral view. B. Detail of the lemma surface. C. Palea, dorsal view. D. Detail of the palea surface. E–H. *C. juergensii* var. *angustilemma*. E. Lemma, lateral view. F. Detail of the lemma surface. G. Palea, dorsal view. H. Detail of the palea surface. I–L. *C. serranum*. I. Lemma, lateral view. J. Detail of the lemma surface. K. Palea, dorsal view. L. Detail of the palea surface. (A–D from *L.N. da Silva 1025*, ICN; E–H from *L.N. da Silva 1054*, ICN; I–L from *L.N. da Silva 1067*, ICN).

Comments:—*Chascolytrum serranum* belongs to *C.* sect. *Hildaea* (da Silva *et al.*, 2020), and is morphologically similar to *C. ambiguum* and *C. juergensii* var. *angustilemma* by having narrow lemmas that are at least 2.5 times longer than wide. It differs from *C. ambiguum* by having puberulous paleas (Fig. 1C) (*vs*. glabrous paleas in *C. ambiguum*) and from *C. juergensii* var. *angustilemma* by having glabrous and smooth lemmas (Fig. 1D) (*vs*. strigose lemmas in *C. juergensii* var. *angustilemma*).

Chascolytrum ambiguum is distributed further northeast in Rio Grande do Sul than *C. serranum*, extending to the highlands of Santa Catarina, up to Rio de Janeiro and Minas Gerais, inhabiting rock cliffs and rocky grasslands over 1200 m elevation (Essi *et al.* 2017, Essi & da Silva 2018). Conversely, *C. serranum* is restricted to the Serra Geral National Park and surroundings, in Rio Grande do Sul, inhabiting river banks or swamps, at around 1000 m elevation. *Chascolytrum juergensii* var. *angustilemma* is widespread in Serra Geral National Park, but inhabits open grasslands or edges of *Araucaria angustifolia* forests (Essi *et al.* 2010). Despite their distinct habitats, *C. serranum* and *C. juergensii* var. *angustilemma* are difficult to distinguish in the field. However, the panicles of *C. serranum* are usually more delicate than those of *C. juergensii* var. *angustilemma* and its spikelets are usually purplish and shiny (Fig. 2B), whereas the spikelets of *C. juergensii* var. *angustilemma* are usually greenish and dull.

Essi *et al.* (2010) cited two specimens (*L. Essi, A. Guglieri & S. Hefler 135, 136*, ICN) as paratypes of *C. juergensii* var. *angustilemma*. However, the description of *C. juergensii* var. *angustilemma* (Essi *et al*. 2010) only mentioned pilose lemmas (hereafter treated as strigose) for the taxon, and these two specimens are distinct by having glabrous and smooth lemmas. We include them here in *C. serranum*, as paratypes. Their exclusion from *C. juergensii* var. *angustilemma* does not affect its circumscription.

Micromorphology of the upper anthecium/floret:—Our micromorphological examination of the lemmas and paleas revealed additional characters that distinguish the new species from its congeners. Lemmas of *Chascolytrum serranum* are glabrous and smooth, without prickles (Fig. 3I–J), whereas the lemmas of *C. ambiguum* are glabrous and scabrous, possessing small prickles on the back and larger ones on the margins and apex (Fig. 3A–B). Conversely, the lemmas of *C. juergensii* var. *angustilemma* are strigose and slightly scabrous due to the presence of prickles and hairs densely covering the lemma back, and short prickles distributed towards the margins and apex (Fig. 3E–F). The palea of *C. serranum* has a combination of short and sparse prickles and hairs on the back and margins (Fig. 3K–L), the palea of *C. juergensii* var. *angustilemma* has short and dense prickles and sparse hairs on the back and margins (Fig. 3G–H), and the palea of *C. ambiguum* has only short and sparse prickles on the back and hairs restricted the margins (Fig. 3C–D). The comparison of macro and micromorphological characters among the three taxa is also presented in Table 1.

TABLE 1. Morphological comparison among *Chascolytrum serranum*, *C. ambiguum* and *C. juergensii* var. *angustilemma*.

	C. serranum	*C. ambiguum*	*C. juergensii* var. *angustilemma*
Lemma pilosity	Glabrous	Glabrous	Strigose
Palea pilosity	Puberulous	Glabrous	Puberulent to puberulous
Palea length in relation to the lemma length	More than 2/3 the length	1/2 to 2/3 the length	1/2 to 2/3 the length
Prickles on lemmas	Absent	Present; short and sparse on the back; medium and moderately dense on the margins and apex	Present; short and dense on the back; short and sparse on the margins and apex
Hairs on lemmas	Absent	Absent	Present
Prickles on paleas	Present; short and sparsely distributed over the surface	Present; short and sparsely distributed over the surface	Present; short and densely distributed over the surface
Hairs on paleas	Present; sparsely distributed over the surface	Absent	Present; moderately distributed over the surface

Discovery of this new species raises the number of species in *C.* sect. *Hildaea* to six and increases the number of species in *Chascolytrum* to 24.

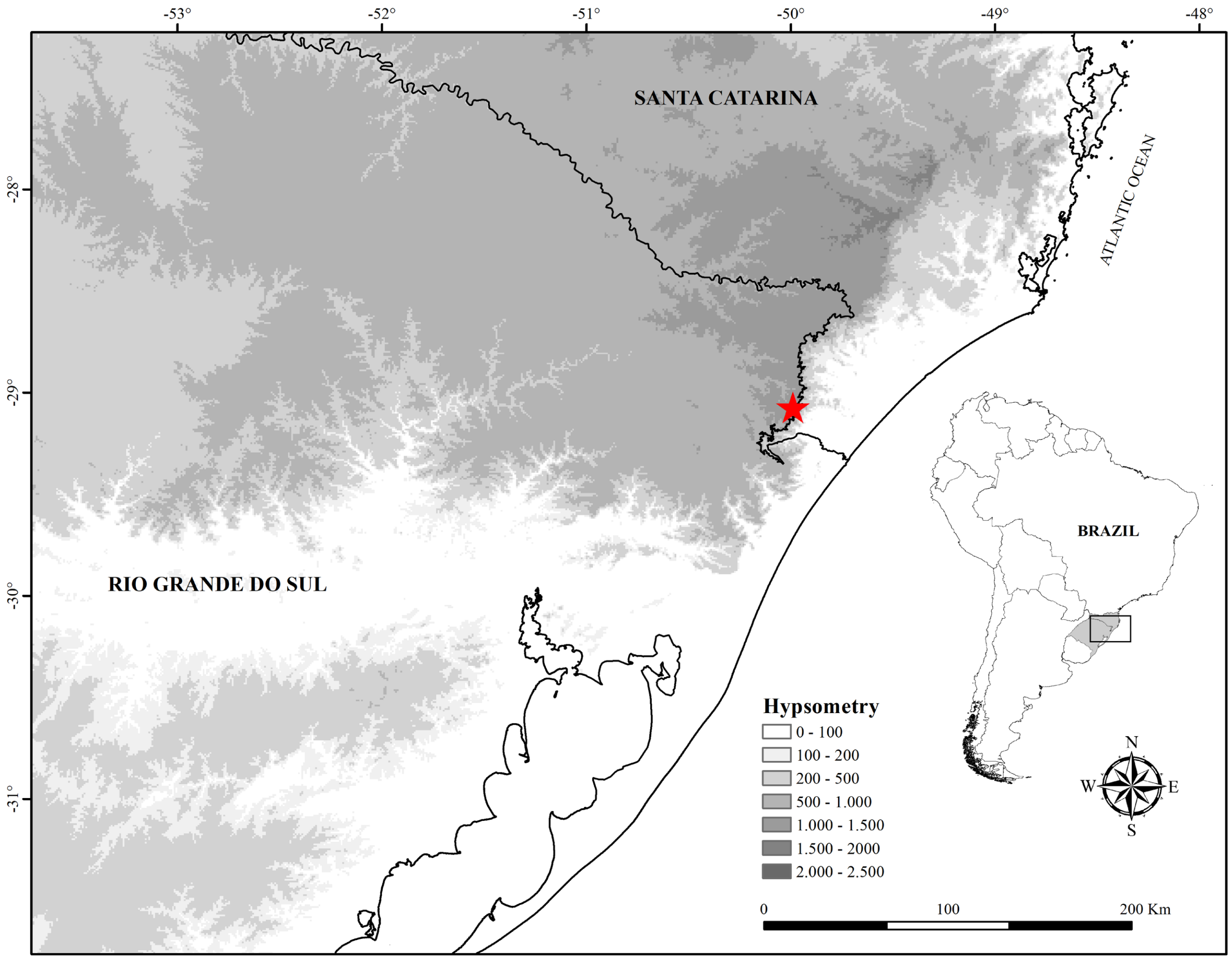

FIGURE 4. Geographic distribution of *Chascolytrum serranum* (star).

Taxonomic key for the species of *Chascolytrum* sect. *Hildaea*

1. Lemmas with a gibbous back distinct from the margins, back papillate .... *C. uniolae* (Nees von Esenbeck 1829: 494) Essi, Longhi-Wagner & Souza-Chies (2011: 329)

\- Lemmas without a gibbous back distinct from the margins, or only slightly distinct, back glabrous or strigose, smooth or rough .... 2.

2. Lemmas with a length/width ratio less than 2.5, cordate at the base .... 3.

\- Lemmas with a length/width ratio more than 2.5, attenuate at the base .... 5.

3. Paleas villose .... *C. juergensii* (Hackel 1915: 76) Essi, Souza-Chies & Longhi-Wagner (2010: 153) var. *juergensii*

\- Paleas glabrous .... 4.

4. Spikelets oblong, subcylindrical; lemmas apices acuminate to caudate .... *C. latifolium* Essi, Souza-Chies & Longhi-Wagner (2010: 151)

\- Spikelets elliptic, laterally compressed; lemmas apices obtuse to acute, occasionally mucronate .... *C. calotheca* (Trinius 1831: 414) Essi, Longhi-Wagner & Souza-Chies (2011: 328)

5. Lemmas strigose .... *C. juergensii* var. *angustilemma*

\- Lemmas glabrous .... 6.

6. Paleas puberulous on the back, more than 3/4 the length of the lemmas .... *C. serranum*

\- Paleas glabrous on the back, less than 3/4 the length of the lemmas .... *C. ambiguum*

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CAPÍTULO III:

A comprehensive sampling of species sheds light on the molecular phylogenetics of Calothecinae (Poaceae, Pooideae): evidence for a new subtribe and multiple genera within the *Chascolytrum s.l.* clade

Artigo a ser submetido ao periódico *Journal of Systematics and Evolution*

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A comprehensive sampling of species sheds light on the molecular phylogenetics of Calothecinae (Poaceae, Pooideae): evidence for a new subtribe and multiple genera within the *Chascolytrum s.l.* clade

Short tittle: Molecular phylogenetics of Calothecinae

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ABSTRACT

The circumscription of subtribe Calothecinae has variously change since its description. Currently, three genera are considered part of the subtribe: *Chascolytrum s.l.*, *Laegaardia* and *Paramochloa*, but no phylogenetic evidence or taxonomic proposition were published so far. In this study we aim to test the monophyly of Calothecinae and the internal classification recently proposed for *Chascolytrum s.l.* using a more comprehensive sampling of taxa and molecular markers. We included species of the three genera of Calothecinae, plus two South American species of *Trisetum s.l.* that have been suggested to be related to *Chascolytrum*, and representatives from the subtribes Agrostidinae, Echinopogoninae, Brizinae, Torreyochloinae, Phalaridinae and Koeleriinae. We performed Bayesian and Maximum Likelihood analyses using a total of six molecular markers, including four plastid DNA regions (a*tpF-atpH*, *matK*, *rps16* intron, and *trnL-trnF*) and two ribosomal nuclear regions (ITS and ETS). Our results revealed that neither Calothecinae nor *Chascolytrum s.l.* are monophyletic considering their last circumscription. *Trisetum brasiliense* and *T. bulbosum* appeared nested with *Chascolytrum s.l.*, and are formally included in Calothecinae as *incertae sedis*. More studies to confirm their taxonomic position are needed. Based on morphological characters, *Laegaardia* and *Paramochloa* are transferred to the new subtribe Paramochloinae, and *Chascolytrum s.l.* is divided in nine different genera, of which two are newly described: *Boldrinia* (gen. nov.), *Calotheca*, *Chascolytrum*, *Erianthecium*, *Lombardochloa*, *Microbriza*, *Poidium*, *Rhombolytrum*, and *Rosengurttia* (gen. nov.).

1 Introduction

The subtribe Calothecinae Soreng (type *Calotheca* Desv.) [Poaceae, Pooideae Benth. (the cool-season grasses), Poeae R.Br. *s.l.*] was described to accommodate two genera, *Chascolytrum* Desv. [*sensu lato*, including *Calotheca* Desv., *Chascolytrum*, *Erianthecium* Parodi, *Gymnachne* Parodi, *Lombardochloa* B.Rosengurtt & B.R.Arrill., *Microbriza* Parodi ex Nicora & Rúgolo, *Poidium* Nees and *Rhombolytrum* Link (Essi *et al.*, 2017)] and the monotypic *Relchela* Steud. (Soreng *et al.*, 2015), although no phylogenetic evidence supporting this circumscription was presented. A subsequent molecular phylogenetic study, conducted by Saarela *et al.* (2017), revealed Calothecinae to be polyphyletic, with *Relchela* recovered in a clade with *Calamagrostis coarctata* Steud., *Echinopogon caespitosus* C.E.Hubb., and *Dichelachne* Endl. spp., in nuclear ribosomal and plastid trees, and *Chascolytrum s.l.* species and *Deyeuxia effusa* Kunth forming a clade sister to Agrostidinae Fr. (type *Agrostis* L) p.p. (including *Agrostis, Calamagrostis* P.Beauv., *Deyeuxia* Clarion ex P.Beauv., and *Polypogon* Desf.) in nuclear ribosomal analyses, and *Chascolytrum s.l., Deyeuxia effusa* and Agrostidinae p.p. forming a polytomy in plastid analyses. In a more recent study, Calothecinae was not monophyletic in plastid trees: three species of *Chascolytrum s.l.* formed a clade that was part of a polytomy with *Chascolytrum rhomboideum* (Link) Essi, Longhi-Wagner & Souza-Chies and Agrostidinae (Tkach *et.al.*, 2020). In the nuclear ribosomal tree in that study, however, Calothecinae is monophyletic.

Relchela was later transferred to the newly described subtribe Echinopogoninae Soreng (type *Echinopogon* P.Beauv.), along with *Ancistragrostis* S.T.Blake, *Dichelachne*, *Echinopogon*, and *Pentapogon* R.Br, and Calothecinae was restricted to *Chascolytrum s.l.* (Soreng *et al.*, 2017). In addition, supersubtribe Agrostidodinae Soreng was described encompassing the subtribes Agrostidinae, Brizinae Tzvelev (type *Briza* L.), Calothecinae and Echinopogoninae (Soreng *et al.*, 2017). Recently, the genera *Paramochloa* P.M.Peterson, Soreng, Romasch. & Barberá (two species, including *Deyeuxia effusa*) and the monotypic *Laegaardia* P.M.Peterson, Soreng, Romasch. & Barberá (= *Calamagrostis ecuadoriensis* Laegaard) were described and placed in Calothecinae (Peterson *et al.*, 2019), increasing to three the number of genera recognized in the subtribe. These revisions to the classification of these grasses were based on unpublished molecular phylogenetic data of these authors (Soreng *et al.* 2017, Peterson *et al.*, 2019), and they are consistent with published molecular results (Saarela *et al.*, 2017, Tkach *et al.*, 2020), although Echinopogoninae was not monophyletic in the nuclear ribosomal tree in Tkach *et al.* (2020).

Chascolytrum s.l. is the largest genus of Calothecinae, currently including 24 species and two varieties (da Silva *et al.*, 2020b), and its highest species diversity is concentrated in the southern Brazilian grasslands (da Silva *et al.*, 2020a; Essi & da Silva, 2018). However, *Chascolytrum subaristatum* Desv., the commonest species, is widely distributed across the subtropical and temperate regions of South America, extending north to Mexico and occurring as adventitious in Australia (Essi *et al.*, 2017). The taxonomic positions of the species currently recognized in *Chascolytrum s.l.* have varied considerably over time, with several generic circumscriptions proposed in the past decades. *Chascolytrum s.l.*, comprising American species, was considered related, based on morphological characteristics, to the Eurasian genus *Briza* by many authors. Matthei (1975), for example, recognized four genera (*Briza*, *Calotheca*, *Chascolytrum*, and *Poidium*) and grouped them together as the "*Briza* Complex". Longhi-Wagner (1987) grouped the species and genera treated by Matthei (1975) into a broadly circumscribed *Briza*, divided into five sections. Rosengurtt *et al.* (1968) accepted the single genus *Briza*, without a subdivisional classification. Other circumscriptions were proposed by Nicora & Rúgolo de Agrasar (1981) and Bayón (1998); both treatments divided the group into five genera (*Briza*, *Calotheca*, *Microbriza*, *Poidium*, and *Rhombolytrum*), with different circumscriptions, in some cases, compared to Matthei's treatment. More detailed summaries of these varying generic classifications are provided in Essi *et al.* (2008) and da Silva *et al.* (2020a).

Early molecular phylogenetics studies of Pooideae found that the Eurasian species of *Briza* are not closely related to the American species that have been included in the genus (Soreng *et al.*, 1990; Davis & Soreng, 2007), and these results were later confirmed by studies focused on the "*Briza* complex" (Essi *et al.*, 2008; Persson & Rydin, 2016; da Silva *et al.*, 2020a) and on tribe Poeae (Saarela *et al.*, 2017; Tkach *et al.*, 2020). In most of these studies, all the American species formed a strongly supported clade. Due to unresolved relationships among lineages in the clade, all species were recognized within the single genus *Chascolytrum s.l.* (Essi *et al.*, 2017). *Briza s.s.* was placed into subtribe Brizinae [along with *Airopsis tenella* Coss. & Durieu (Soreng *et al.*, 2015; Tkach *et al.*, 2020)], and *Briza humilis* M.Bieb., which is not closely related to *Briza* s.s., was recognized in *Brizochloa* V.Jirásek & Chrtek [*Brizochloa humilis* (M.Bieb.) Chrtek & Hadač], where it had earlier been placed (Persson & Rydin, 2016; Essi *et al.*, 2017). *Brizochloa humilis* is part of supersubtribe Poodinae L.J.Gillespie & Soreng (Soreng *et al.*, 2017; Tkach *et al.*, 2020), and was recently recognized in a new monotypic subtribe, Brizochloinae Röser & Tkach (type *Brizochloa*) (Tkach *et al.*, 2020). Recently, both molecular and morphological data confirmed *Chascolytrum s.l.* as monophyletic, as earlier suggested, and a classification for the genus, recognizing eight sections within it, was proposed (da Silva *et al.* 2020a).

Although the New World species currently accepted in *Chascolytrum s.l.* have been studied using multiple approaches, including morphology, molecular phylogenetics, cytology (Schifino & Winge, 1983), anatomy (Pelegrin *et al.*, 2009), and biochemistry (Willians & Murray, 1972), some aspects of relationship within the group remain poorly understood due to internal polytomies (da Silva *et al.*, 2020a), incomplete sampling [e.g., *Chascolytrum rhomboideum* and *C. koelerioides* (Trin.) Essi, Longhi-Wagner & Souza-Chies have not been included in a comprehensive phylogenetic study], sampling of only one or few DNA loci with low interspecific variation, and inappropriate outgroups (Essi *et al.*, 2008). In addition, no published study has tested the relationships among the three genera included in Calothecinae using a wide sampling of species of *Chascolytrum s.l.* and other South American Agrostidinae.

Thus, the aims of this study are to assess phylogenetic relationships among the three genera included in Calothecinae, to reevaluate relationships within *Chascolytrum s.l.* using a more comprehensive taxa sampling in combination with a greater number of DNA regions than in our previous study (da Silva *et al.*, 2020a), and to test the subdivisional classification of *Chascolytrum* we recently proposed (da Silva *et al.*, 2020a) in the context of a broader phylogenetic framework. Based on the new phylogenetic hypothesis presented here, we describe one new subtribe and propose a revised generic classification for the subtribe Calothecinae.

2 Material and Methods

2.1 Taxon sampling

We included 97 samples, representing 25 species, and two varieties, and all the three genera (including their type species) belonging to subtribe Calothecinae: *Chascolytrum s.l.* (23 species and two varieties – only *C. altimontanum* Essi, Souza-Chies & Longhi-Wagner was not sampled), *Paramochloa* (one species), and *Laegaardia* (one species). We also included two South American species of *Trisetum s.l.*: *T. brasiliense* Louis-Marie and *T. bulbosum* Hitchc. that have been found to be closely related to *Chascolytrum s.l.* in unpublished molecular analyses (K. Romaschenko & P.M. Peterson, pers. comm.). For broader phylogenetic context we included representatives of the subtribes Agrostidinae (17 samples/15 species), Echinopogoninae (7/4), Brizinae (1/3), Torreyochloinae Soreng & J.I.Davis (type *Torreyochloa* G.L.Church) (10/9), Phalaridinae Burmeist. (type *Phalaris* L.) (1/1) Koeleriinae Asch. & Graebn. (type *Koeleria* Pers.) (12/9). The trees were rooted on two accessions of *Deschampsia cespitosa* (L.) P.Beauv. (Aristaveninae). Newly studied samples were mostly obtained from fresh leaves collected in the field, and from herbarium material, and new sequences were obtained from GenBank or DNA samples generated by Saarela *et al.* (2017) and da Silva *et al.* (2020a). Previously published sequence data included in our analyses was obtained from GenBank. The complete list of taxa, voucher and sequence information is presented in Table 1.

2.2 DNA extraction, PCR amplification and sequencing

All new sequence data included here was generated in the Laboratory of Molecular Biodiversity, Canadian Museum of Nature (CMN), in Ottawa, Canada. Total DNA was extracted with a modified version of the protocol proposed by Alexander *et al.* (2007).

We sequenced two nuclear ribosomal DNA regions, the Internal Transcribed Spacer (ITS, including ITS1, 5.8S, and ITS2) and a 3'-end portion of External Transcribed Spacer (ETS), plus four plastid regions, (i) the *trnL-trnL-trnF* and (ii) *atpF-atpH* intergenic spacers, (iii) ca. of 850 bp of the central portion of the *matK* gene, and (iv) the *rps16* intron. Primers and PCR conditions followed those outlined by Saarela *et al.* (2017) for ITS, ETS, *trnL-trnL-trnF*, *atpF-atpH* and *matK* regions, and by da Silva *et al.* (2020a) for the *rps16* intron. Sequencing reactions were performed using an Applied Biosystems 3130xl Genetic Analyzer at the CMN.

2.3 Phylogenetic analyses

Sequences were assembled and initially aligned using Geneious v.8.1.8. (Kearse *et al*., 2012), and then manually checked using MEGA6 (Tamura *et al.*, 2013). Unambiguous aligned gaps longer than three base pairs (bp) (single nucleotide repetitions were not considered) and shared among two or more taxa were coded using the modified complex indel coding approach (Simmons *et al.*, 2007) with SeqState v.1.4.1 (Müller, 2005). We checked each DNA matrix for putative instances of contamination, misidentification and/or sequencing errors by examining neighbor-joining (NJ) trees for individuals that clustered apart from congeneric and/or conspecific individuals; their sequences and identification were doubled checked, corrected and/or excluded from the subsequent analyses. The NJ trees were generated using the PAUP* (Swofford, 2002) plugin in Geneious.

Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic analyses were initially conducted on the concatenated nuclear (ITS + ETS) data and the concatenated plastid (*atpF-atpH, matK*, *rps16*, *trnL-trnL-trnF*) data separately. A minimum of 0.95 posterior probability (PP) or 70% bootstrap support (BS) was considered as threshold values for statistically supported relationships. Incongruences between the nuclear and plastid DNA datasets were explored through visual comparison of topologies and support values between conflicting branches. Incongruent topologies were considered strongly supported with a minimum of 85% bootstrap support (Baker *et al.*, 2011; Tkach *et al.*, 2015) or 0.99 posterior probability (Inácio *et al.*, 2017). Then, combined analyses of the nuclear and plastid DNA datasets were performed. We performed both Maximum Likelihood (ML) and Bayesian Inference (BI) analyses to reconstruct the overall phylogeny based on the combined nuclear and plastid DNA dataset. All the analyses were conducted on the CIPRES science Gateway (Miller *et al.*, 2010).

BI analyses were run using MrBayes v.3.2.6 (Ronquist *et al.*, 2012). The models of evolution for each separate DNA region were determined based on the Akaike Information Criterion (AIC) in MrModeltest v.2.3 (Nylander, 2004), and are presented in Table 2. Two parallel runs with four Markov Chain Monte Carlo (MCMC) were run for 10^7 generations, sampling every 1000th generation. Average standard deviation of diagnostic frequencies was calculated every 10^3 generation and the critical value for automatically stopping the analysis was defined by the 'stopval' command (=0.01). The first 25% of the trees of each run were discarded as burn-in, and the remaining trees were used to build a majority-rule consensus including the posterior probabilities as branch support estimates.

ML analyses were performed using RAxML-HPC2 on XSEDE (Stamatakis, 2014), using the GTR+G model (the only one available) and the BINGAMMA model for indels. The best-scoring ML tree was found after 100 independent ML searches, and non-parametric standard bootstrap (BS) was calculated with 1000 pseudo-replicates.

2.4 Taxonomic treatment

Taxonomic treatment was based on revision of herbarium material and review of the relevant literature, including protologues, for the Calothecinae species (*sensu* Peterson *et al.*, 2019). The following herbaria were consulted: B, BLA, BM, BR, CAN, CEN, CNPO, CONC, CORD, FLOR, G, GUA, HAS, HB, HBR, ICN, K, L, LINN, LPB, M, MBM, MVFA, MO, MSB, NY, P, PACA, RB, S, SGO, SMDB, UEC, VIC and W [acronyms following Thiers (2020, onwards)]. We also reviewed numerous type specimens through digital images published in online databases, including JSTOR Global Plants. The taxonomic key for identification of the genera was constructed based on modifications from da Silva *et al.* (2020a).

3 Results

3.1 Phylogenetic analyses

The phylogenetic analyses included a total of 151 accessions and 856 sequences, of which 630 (73.6%) were newly generated in this study (Table. 2). The phylogenetic trees deriving from the separate plastid and nuclear sequences showed no strongly supported topological conflicts among taxa of Calothecinae (Supplementary Material 1 and 2). However, some incongruences were detected regarding the other subtribes: (i) Brizinae is monophyletic in the plastid trees, but paraphyletic in the nuclear trees; (ii) Echinopogoninae is monophyletic in the plastid trees, but polyphyletic in the nuclear trees. We do not discuss these results in detail here because they have been in some way reported by previous studies (Saarela *et al.*, 2010, 2017; Tkach *et al.*, 2020), and do not correspond to the aim of our study.

3.2 Plastid DNA dataset

The plastid matrix included 151 terminals and 563 sequences, of which 396 (70.3%) are newly reported here. Forty-one sequences (6.8%) were missing, mostly of the *rps16* intron. The total plastid alignment length is 3391 bp, plus 15 coded indels. Indels were most common within the intergenic spacers and intron regions (e.g., *matK* showed no indels), and ranged from 3 bp to 32 bp. We also scored a large deletion of 128 bp within the *trnL* intron, shared among all taxa of

Koeleriinae, Brizinae, Echinopogoninae and Calothecinae. The sequence alignment length, substitution model, and number of indels coded for each plastid DNA region is shown in Table 2.

In the phylogenetic tree inferred from the plastid DNA dataset (Supplementary Material 1) Calothecinae was paraphyletic. *Paramochloa* and *Laegaardia* formed a strongly (PP = 1) to moderately (BS = 70%) supported clade sister to a strongly (PP = 1) to moderately (BS = 70%) supported clade including all species of *Chascolytrum s.l.*, *Trisetum bulbosum* and *T. brasiliense.* The relationship between these two clades, however, received no statistical support in the BI analyses (PP = 0.86) and was not recovered by the ML analysis. Agrostidinae (PP = 1, BS = 94%) was maximally supported (PP = 1) as sister to the non-supported (PP = 0.86) group formed by *Chascolytrum s.l.* + *T. bulbosum* + *T. brasiliense* + *Laegaardia* + *Paramochloa* in BI analyses, and it formed a polytomy with *Laegaardia* + *Paramochloa* and *Chascolytrum s.l.* + *T. bulbosum* + *T. brasiliense* in the ML tree (data not shown).

Internal relationships within the *Chascolytrum s.l.* + *T. bulbosum* + *T. brasiliense* clade were not fully resolved due to the backbone polytomy formed by *T. bulbosum* (PP = 1; BS = 90%), a clade consisting of *C. bulbosum*, and two other major clades comprising the remaining taxa of *Chascolytrum s.l.* One clade was strongly (PP = 1) to moderately (BS = 86%) supported, with *C. rufum* sister to a large and poorly resolved clade comprising *T. brasiliense* and several species of *Chascolytrum s.l.* The other clade (PP = 0.99; BS = 57%) consists of several smaller clades, mostly formed by accessions of one or two species, and a larger clade consisting of a polytomy formed by *C. parodianum, C. erectum* p.p. + *C. subaristatum* p.p. and a clade with *C. scabrum, C. lamarckianum*, *C. erectum* p.p. (*LNS782*), *C. paleapiliferum,* and *C. subaristatum* p.p. The plastid DNA analyses do not support most of the classification recently proposed for *Chascolytrum s.l.* (da Silva *et al.*, 2020a).

Subtribe Echinopogoninae is monophyletic with strong support (PP = 1; BS = 95%), and was recovered as sister to Agrostidinae and Calothecinae + *T. bulbosum* + *T. brasiliense* only in the BI analyses (PP = 1). Koeleriinae (PP = 1; BS = 100%) and Brizinae (PP = 1; BS = 99%) were inferred as sister to Echinopogoninae + Agrostidinae + Calothecinae + *T. bulbosum* + *T. brasiliense*, although with no statistical support (PP = 0.90 and PP = 0.94, respectively). Torreyochloinae and Phalaridinae were resolved as sister groups (PP = 1; BS = 84%) and the Torreyochloinae + Phalaridinae clade was sister to all remaining subtribes with strong support (PP = 1; BS = 96%).

3.3 Nuclear DNA dataset

The nuclear matrix included 151 terminals and 293 sequences, of which 234 (79.9%) are newly reported here. Only nine sequences were missing, all for the ETS region (Table 2). The sequence alignment length and the substitution model for each nuclear DNA region is shown in Table 2.

The topology of the nuclear-derived tree differs slightly from the plastid-derived tree, and its support values are generally lower (Supplementary Material 2). Calothecinae was inferred as paraphyletic due to the inclusion of *Trisetum brasiliense* and *T. bulbosum* in the *Chascolytrum s.l.* clade. *Paramochloa effusa* (Kunth) P.M.Peterson, Soreng, Romasch. & Barberá and *Laegaardia ecuadoriense* (Laegaard) P.M.Peterson, Soreng, Romasch. & Barberá formed a group, with no statistical support, sister to the *Chascolytrum s.l.* + *T. bulbosum* + *T. brasiliense* clade, which was moderately (BS = 83%) to strongly supported (PP = 1). *Trisetum bulbosum* and *T. brasiliense* aligned in a non-supported group, placed in a major polytomy with *Chascolytrum rufum* J.Presl. and a moderately supported clade (PP = 0.97) consisting of the remaining species of *Chascolytrum s.l.*

The *Chascolytrum s.l.* clade, excluding *C. rufum*, consists of a polytomy formed by several small clades, sometimes composed of multiple accessions of a single species [*C. parodianum* clade (PP = 1; BS = 97%), *C. bidentatum* + *C. koelerioides* + *C. rhomboideum* clade (PP = 0.94; BS = 77%), *C. brachychaetum* clade (PP = 1; BS = 100%), *C. brasilense* clade (PP = 1; BS = 100%), *C. bulbosum* clade (PP = 1; BS = 100%), *C. itatiaiae* clade (PP = 1; BS = 99%), *C. monandrum* +

C. poomorphum clade (PP = 0.88; BS = 66%), and two accessions of *C. brizoides*), along with a larger, but weakly supported group (PP = 0.91; BS = 53%). This last group is divided into two main clades: (i) one consists of species belonging to *C.* sect. *Chascolytrum* and is strongly supported by the BI analyses (PP = 1; BS = 77%), with *C. scabrum* (Steud.) Matthei as sister to the remaining species, and *C. lamarckianum* (Nees) Matthei as sister (PP = 0.99; BS = 75%) to the polytomy formed by *C. subaristatum*, *C. erectum* Desv., and *C. paleapiliferum* (Parodi) Matthei (PP = 1; BS = 97%); (ii) the other consists of species belonging to *C.* sect. *Hildaea* L.N.Silva, Essi & Souza-Chies and is strongly supported (PP = 1; BS = 95%), with *C. uniolae* (Nees) Essi, Longhi-Wagner & Souza-Chies + *C. calotheca* (Trin.) Essi, Longhi-Wagner & Souza-Chies (*LNS752*) as sister to a larger clade (PP = 1; BS = 72%) consisting of all remaining species aligned in a polytomy. The nuclear DNA analyses do not support most of the classification recently proposed for *Chascolytrum s.l.* (da Silva *et al.*, 2020a).

Subtribes Agrostidinae, Brizinae and Echinopogoninae were not recovered as monophyletic. Species assigned to Agrostidinae were split in three distinct lineages, including non-supported clade (PP = 0.70) formed by Agrostidinae p.p. and *Dichelachne crinita* Hook.f., which appeared as sister to Calothecinae with moderate (PP = 0.98%) to weak support (BS = 53%). *Briza maxima* L., placed in Brizinae in the plastid tree, was recovered as sister to Koeleriinae, although this relationship was not statistically supported. The other two species of *Briza s.s.* grouped in a strongly supported clade (PP = 1; BS = 99%) placed as sister to *Relchela panicoides* Steud. (Echinopogoninae p.p.) without support (PP = 0.85). Unlike the plastid-derived tree, nuclear tree places Koeleriinae + *Briza maxima* as sister to other subtribes, again with no support. Since most of the conflicts detected between the plastid and nuclear trees exhibited weak or no statistical support, we combined the nuclear and plastid datasets into a single matrix.

3.4 Combined nuclear and plastid DNA dataset

The combined matrix included 151 terminals and 856 sequences for the six DNA regions. Fifty sequences were missing (Table 2). The final matrix included 4666 bp and 15 indels. The Bayesian tree exhibited higher statistical support values than the Maximum Likelihood tree, and only the Bayesian tree is shown, with the ML bootstrap values included on it (Fig. 1A-B).

Our combined analyses recovered Calothecinae as paraphyletic, since *T. bulbosum* and *T. brasiliense* were embedded among the *Chascolytrum s.l.* species in a strongly supported clade (PP = 1; BS = 93%). *Laegaardia ecuadoriense* + *Paramochloa effusa* aligned in the strongly supported (PP = 1; BS = 91%) clade A (= Paramochloinae, subtrib. nov.), which is placed as sister to *Chascolytrum s.l.* + *T. bulbosum* + *T. brasiliense* clade (= Calothecinae *s.s.*) with strong support (PP = 1; BS = 99%) (Fig. 1B). The backbone of Calothecinae *s.s.* consists of a polytomy formed by *Trisetum bulbosum* and two major clades, Clade B and C (Fig. 1A).

In Clade B (PP = 1; BS = 70%), the two varieties of *Chascolytrum rufum* formed a maximally supported clade [=*Lombardochloa* (PP = 1; BS = 100%)] that is sister to *Trisetum brasiliense* + *Poidium* (PP = 1; BS = 72%) with weak (BS = 70%) to full support (PP = 1). *Poidium* was strongly supported by the BI analyses (PP = 1), but showed no support in the ML analysis, and includes species from *Chascolytrum* sect. *Poidium* (Nees) L.N.Silva and *C.* sect. *Hildaea* L.N.Silva, Essi & Souza-Chies. The three species belonging to *C.* sect. *Poidium* (da Silva *et al.*, 2020a) appeared in two different clades, one formed by *C. brasilense* (Nees) Essi, Longhi-Wagner & Souza-Chies (PP = 1) and the other formed by *C. itatiaiae* (Ekman) Essi, Longhi-Wagner & Souza-Chies and *C. brachychaetum* (Ekman) Essi, Longhi-Wagner & Souza-Chies (PP = 0.99). These two clades formed a polytomy along with a strongly supported clade (PP = 1; BS = 100%) consisting of the species currently accepted in *C.* sect *Hildaea*. This major clade comprises seven taxa: *C. ambiguum* (Hack.) Essi, Longhi-Wagner & Souza-Chies, *C. calotheca*, two varieties of *C. juergensii* (Hack.) Essi, Souza-Chies & Longhi-Wagner, *C. latifolium* Essi, Souza-Chies & Longhi-Wagner, *C. serranum* L.N.Silva, and *C. uniolae*. Several accessions in this clade did not group with other conspecific accessions, such as *C. calotheca*, *C. latifolium* and *C. ambiguum*, and the internal relationships were not fully resolved.

Clade C was recovered and supported only by the BI analyses (PP = 1), and *Chascolytrum bulbosum* (Parodi) Essi, Longhi-Wagner & Souza-Chies (= *Erianthecium*) was placed as sister to the remaining species included in a major clade consisting of: (i) a strongly supported *C. brizoides* (Lam.) Essi, Longhi-Wagner & Souza-Chies clade (= *Calotheca*) (PP = 1; BS = 99%); (ii) a strongly supported *C. monandrum* (Hack.) Essi, Longhi-Wagner & Souza-Chies clade (*Rosengurttia*, gen. nov.) (PP = 1. BS = 93%); (iii) a maximally supported *C. poomorphum* (J.Presl) Essi, Longhi-Wagner & Souza-Chies clade (*Microbriza*) (PP = 1. BS = 100%); (iv) a *C. monandrum* + *C. poomorphum* clade, strongly supported by the BI analyses (PP = 1), but moderately supported by the ML analysis (BS = 83%); (v) a weakly (BS = 76%) to strongly (PP = 1) supported clade (= *Rhombolytrum*) consisting of *C. bidentatum* (Roseng., B.R.Arrill. & Izag.) Essi, Longhi-Wagner & Souza-Chies (PP = 1; BS = 100%) as sister to *C. koelerioides* + *C. rhomboideum* (PP = 1; BS = 94%); (vi) a maximally supported *C. parodianum* (Roseng., B.R.Arrill. & Izag.) Matthei clade (= *Boldrinia*, gen. nov.) (PP = 1; BS = 100%); (vii) a strongly supported *Chascolytrum s.s.* clade (PP = 1; BS = 98%); and (viii) and strongly supported *Chascolytrum* s.s + *C. parodianum* clade, strongly supported only in the BI analyses (PP = 0.99).

Agrostidinae was recovered as sister to Paramochloinae + Calothecinae *s.s.* clade (Fig. 1B) with strong support in the BI analyses (PP = 1), but moderately supported by the ML analysis (BS = 81%). Echinopogoninae is sister to Agrostidinae + Calothecinae *s.s.* + Paramachloinae, although this relationship was only recovered in the BI analyses (PP = 1). Subsequently, Brizinae (including *B. maxima*), is sister to the Echinopogoninae + Agrostidinae + Paramachloinae + Calothecinae *s.s.* clade with strong to moderate support (PP = 1; BS = 89%), and the Torreyochloinae + Phalaridinae (PP = 1; BS = 80%) is sister to all clades mentioned above. Koeleriinae was strongly supported as sister to the other subtribes with strong support (PP = 1; BS = 97%).

The overall topology of the BI tree inferred from the combined DNA datasets was similar to the BI plastid-derived tree (Supplementary Material 1), although it was better resolved and supported. Thus, we based our discussion and taxonomic propositions on the results of the combined nuclear + plastid analyses.

4 Discussion

Phylogenetic relationships

The phylogenetic results obtained here, even those inferred by single DNA regions, revealed Calothecinae, as circumscribed by Soreng *et al.* (2017) and Peterson *et al.* (2019), to be polyphyletic given the inclusion of *Trisetum brasiliense* and *T. bulbosum* in this clade, nested with the *Chascolytrum s.l.* species. In the recent study of Tkach *et al.* (2020), Calothecinae was recovered as monophyletic in the nuclear analyses, but paraphyletic in the plastid analyses. However, their sampling was not comprehensive as they only included four species of *Chascolytrum*, they did not include representatives of *Laegaardia*, *Paramochloa*, or *Trisetum brasiliense* and *T. bulbosum*, and their plastid trees were based on only two DNA regions (*matK* and *trnL-trnF*).

In our plastid and combined nuclear + plastid trees, *Laegaardia* and *Paramochloa* formed a strongly supported clade, resolved as sister to the *Chascolytrum* + *T. bulbosum* + *T. brasiliense* (= Calothecinae *s.s.*) clade. The backbone of Calothecinae *s.s.*, however, consists on a polytomy comprising two major clades (clades B and C, Fig. 1A) and *T. bulbosum*. Moreover, Agrostidinae is sister to Calothecinae + Paramochloinae, and Echinopogoninae is sister to them. Compared to the polytomy formed by these three lineages in the plastid tree obtained by Saarela *et al.* (2017), both of our plastid and combined nuclear + plastid trees are better resolved, which may be due to our more comprehensive sampling for the Calothecinae species. Three of the four plastid DNA regions we used are the same ones used by Saarela *et al.* (2017); however, they did not show any combined nuclear + plastid tree.

Our results show that *Chascolytrum s.l.*, as currently circumscribed (Essi *et al.*, 2017), is paraphyletic due to the inclusion of *T. bulbosum* and *T. brasiliense,* the last one being placed within the Clade B as sister (Fig. 1A) to the *Poidium* clade. Previous studies found *Chascolytrum s.l.* to be monophyletic (Essi *et al.*, 2008; Persson & Rydin, 2016; Saarela *et al.*, 2017; da Silva *et al.*, 2020a). All of them, however, included fewer taxa than we have included here (we sampled all but one species of *Chascolytrum s.l.*), and none included *Trisetum brasiliense* and *T. bulbosum*. Our phylogenetic results indicate that *Trisetum brasiliense* and *T. bulbosum* are closely related to *Chascolytrum s.l.* No morphological similarities have been suggested among these species of *Trisetum* and *Chascolytrum sl.* Species of *Chascolytrum s.l.* differ from *T. brasiliense* and *T. bulbosum* by having 3- to many-flowered spikelets (*vs.* 2- to 3-flowered spikelets in *T. brasiliense* and *T. bulbosum*), lemmas usually without well-developed awns, or less frequently with awns > 1 mm long, subapical and straight in *Erianthecium* and *Calotheca* (*vs.* middle-basal and twisted or geniculate awns in *T. brasiliense* and *T. bulbosum*), and a short glabrous rachilla (*vs.* a longer hairy rachilla in *T. brasiliense* and *T. bulbosum*).

Previous molecular phylogenetic studies revealed *Trisetum s.l.* as polyphyletic (Saarela *et al.*, 2017; Barberá *et al.*, 2019), but none of them included these two species. Their taxonomic position has been variously circumscribed by the authors who have studied these species. Parodi (1949), for example, included *T. bulbosum* in *Helictotrichon* Besser [*H. bulbosum* (Hitchc.) Parodi], and Valencia (1941) transferred *T. brasiliense* to *Deschampsia* P.Beauv. [*D. brasiliensis* (Louis-Marie) Valencia]. Based on micromorphological analysis of the epidermis of lemmas, Finot *et al.* (2006), however, found that both species did not align with the generic concept of *Helictotrichon* and *Deschampsia*, respectively, nor with the circumscription of *Trisetum s.l.* Finot *et al.* (2005) mentioned that *Trisetum brasiliense*, along with *T. juergensii* Hack., does not fit well in the genus, and more studies to determinate their taxonomic positions are needed. Chiapella &

Zuloaga (2010) excluded *Deschampsia brasiliensis* from their taxonomic treatment of *Deschampsia* for South America, and also mentioned that its generic placement remains unclear.

Our nuclear-inferred tree recovered both *T. brasiliense* and *T. bulbosum* in a weakly supported clade (Supplementary Material 2). However, our plastid and combined nuclear + plastid trees (Fig. 1A) suggested that *T. brasiliense* and *T. bulbosum* are not phylogenetic related to each other, and should be treated as two distinct genera. *Trisetum bulbosum* differs from *T. brasiliense* [and other species of *Trisetum s.l.* (Hitchcock, 1927)] by the presence of smalls bulbs at the base of the culms (*vs.* bulbs absent in *T. brasiliense*) and by the presence of a long and sharp callus projection at the base of the floret, a remnant of the rachilla after disarticulation of the floret (*vs.* callus projection absent in *T. brasiliense*). Nevertheless, we refrain from proposing taxonomic changes to these two species pending further study to clarify the placement of *T. bulbosum*, which in our combined nuclear + plastid analysis forms a polytomy with Clades B and C, and to sample multiple individuals of each taxon in phylogenetic analyses in order to confirm our current results. The South-American species *T. juergensii* was also included in *Trisetum* subsect. *Deschampsioideum* Louis-Marie along with *T. brasiliense* by Louis-Marie (1928; 1929), and the species *Trisetum andicola* Louis-Marie (= *T. longiglume* Hack.), *T. confertum* Pilg., and *T. juergensii* were also treated under *Deschampsia* along with *T. brasiliense* by Valencia (1941). These species have not yet been included in a published molecular phylogeny, and their inclusion in future studies may reveal new evidence on the circumscription of these taxa.

Moreover, we have not yet carefully reviewed herbarium material of these species. We are aware only of the type collections of both species (from which the current sequences were obtained), housed outside of Brazil, and none of these species have been collected recently. *Trisetum bulbosum* is recorded from southern Argentina and southern Chile (Finot *et al.*, 2006), whereas *T. brasiliense* is considered restricted to the southeast Brazilian highlands of Serra do Itatiaia (Flora do Brasil, 2020).

4.1 Clade A

Clade A (Fig. 1A), formed by *Laeggardia ecuadoriense* and *Paramochloa effusa*, was resolved as sister to the *Chascolytrum s.l.* + *T. bulbosum* + *T. brasiliense* clade by the combined DNA analyses, with strong support. These species were previously recognized in *Calamagrostis* and *Deyeuxia*, respectively, and were transferred to new genera in light of molecular phylogenetic evidence, and placed in Calothecinae by Peterson *et al.* (2019). Our results confirm the placement of *Paramamochloa effusa*, as previously shown by Saarela *et al.* (2017) (under *Deyeuxia*) and the generic circumscription proposed by Peterson *et al.* (2019), although we did not sample *P. crispifolia* (Sylvester) P.M.Peterson, Soreng, Romasch. & Barberá, a species from Colombia. Due to morphological differences among *Laegaardia*, *Paramochloa*, the other species of *Chascolytrum s.l.* and the two species of *Trisetum s.l.*, we propose recognizing *Laegaardia and Paramochloa* in the new subtribe Paramochloinae. Both *Laegaardia* and *Paramochloa* are characterized, and distinct from other genera belonging to the Calothecinae *s.l.* clade, by having 1-flowered spikelets (*vs.* 2- to many- flowered spikelets in Calothecinae), with a hairy rachilla prolongation (Peterson *et al.*, 2019) (*vs.* rachilla prolongation absent in Calothecinae). Monotypic *Laegaardia* can be easily distinguished from *Paramochloa* by its narrower and contracted panicles, up to 2 cm wide (*vs.* wider and open panicles, rarely contracted, more than 3 cm wide in *Paramochloa*) and muticous lemmas (*vs.* awned lemmas in *Paramochloa*). Moreover, Paramochloinae is confined to the northwestern South American Páramos (Peterson *et al.*, 2019; Sylvester *et al.*, 2019, under *Calamagrostis*), whereas Calothecinae has a wider distribution, with higher diversity in Southern South America (Essi *et al.*, 2017; da Silva *et al.*, 2020; under *Chascolytrum*).

4.2 Clade B

Clade B encompasses species from the recently described *Chascolytrum* sects. *Lombardochloa*, *Poidium*, and *Hildaea*, plus *Trisetum brasiliense* (Fig. 1A), and it is strongly supported in the combined nuclear + plastid analyses. The placement of *T. brasiliense* within this clade was unexpected because this species does not share clear morphological similarities with these, nor any other, species of *Chascolytrum s.l.* Within this clade, two genera are segregated from *Chascolytrum s.l.*: the monotypic *Lombardochloa* and the larger genus *Poidium*. *Poidium*, as accepted here, differs from earlier circumscriptions of the genus proposed by Matthei (1975), Nicora & Rúgolo de Agrasar (1981), and Bayón (1998), and from the narrower circumscription of *Chascolytrum* sect. *Poidium* (da Silva *et al.*, 2020a). As circumscribed here, *Poidium* comprises nine species and two varieties, all of which were included in the recently proposed *C.* sect. *Poidium* and sect. *Hildaea.* These two sections were resolved as monophyletic based on morphological and molecular characters by da Silva *et al.* (2020a), and differed from each other by the shape of the spikelets (ovate in *C.* sect. *Poidium vs.* ellipitic do lanceolate in *C.* sect. *Hildaea*) and the number of florets (up 3-flowered in *C.* sect. *Poidium vs.* 4- to many-flowered in *C.* sect. *Hildaea*). The current results do not support a comparable infrageneric classification for *Poidium,* as *C.* sect. *Poidium* is not monophyletic. *Poidium* is divided into three clades, which form a polytomy, one composed of *C. brasilense* (= *Poidium brasilense* Nees, the type species), one composed of *C. brachychaetum* and *C. itatiaiae*, and a major clade composed of all species previously recognized in *C.* sect. *Hildaea* (da Silva *et al.*, 2020a, 2020b).

A clade composed of one sample of *C. calotheca* (*LNS752*) sister to all samples of *C. uniolae* was recovered as sister to the remaining species of *C.* sect. *Hildaea* with full support in the BI analyses. Deeper branches within this latter clade were mostly unresolved, and some individuals did not group with their conspecifics. Similar results were obtained by da Silva *et al.* (2020a) and Essi *et al.* (2008), and here we show that increasing taxa and molecular markers does not improve the resolution in this group. Hybridization may explain why most species were not resolved as monophyletic, along with the presence of different individuals of multiple species recovered in strongly supported clades, at least in BI trees. Examples include a strongly supported clade comprising individuals of *C. ambiguum, C. calotheca, C. juergensii* var. *juergensii*, and a strongly supported clade comprising individuals of *C. ambiguum* and *C. juergensii* var. *juergensii.*

The phylogenetic patterns observed for this group are also compatible with incomplete lineage sorting or adaptive radiation, since this clade is morphologically diverse but exhibits low genetic variation and differentiation. Adaptive radiation is an explanation for high species diversification in areas that went through rapid climate and geological changes (Hughes & Eastwood, 2006). *Poidium* exhibits remarkable diversity in the southern Brazilian highland grasslands, where several species of the genus occur (Essi *et al.*, 2008, 2010; Essi & da Silva, 2018; da Silva *et al.*, 2020a; under *Chascolytrum*)], often in sympatry. This region is currently composed of a mosaic of grasslands and *Araucaria angustifolia* (Bertol.) Kuntze forest, and has been highlighted because of its high plant species diversity and endemism (Iganci *et al.*, 2011). The vegetation has changed drastically in the Late Quaternary, mainly in response to climate changes, resulting in the advance of the forest over the grasslands during more warm and humid periods (Behling & Pillar, 2007). In addition, events of glaciation/interglaciation, during the Pleistocene, have been proposed as an important driver for speciation in this region (Iganci *et al.*, 2013; Barros *et al.*, 2015; Mäder & Freitas, 2019). Thus, it is likely that southern Brazilian highland have also played an important role in the diversification of *Poidium*. This hypothesis, however, still needs to be tested using more appropriate analyses.

Clade C

Clade C is morphologically diverse and composed of species hereafter treated in seven different genera, *Erianthecium*, *Calotheca*, *Microbriza*, *Rosengurttia* (gen. nov.), *Rhombolytrum*, *Boldrinia* (gen. nov.), and *Chascolytrum s.s.* (Fig. 1A). *Erianthecium* was recovered as sister to a clade comprising the remaining genera. The latter clade comprises a polytomy formed by *Calotheca*, a clade formed by *Microbriza*, *Rosengurttia*, and *Rhombolytrum*, and a clade formed by *Boldrinia* and *Chascolytrum*.

We transfer *Chascolytrum bulbosum* and *C. brizoides* back to the monotypic genera *Erianthecium* and *Calotheca*, respectively. These two species were recently accepted within

Chascolytrum sect. *Calotheca* (Desv.) L.N.Silva, which was morphologically circumscribed by the presence of awned lemmas, a feature not found in any other species of *Chascolytrum s.l.* (da Silva *et al.*, 2020a). Our phylogenetic results here suggest that *C.* sect. *Calotheca* is not monophyletic because *C. bulbosum* and *C. brizoides* are successive sisters to the rest of clade C (Fig. 1A). The presence of awns in *Erianthecium* and *Calotheca* may be a plesiomorphic state, present in the ancestor of Clade C or the ancestor of Calothecinae, and lost in other lineages in independent events during the evolution of this group. Alternatively, awned lemmas may have arisen independently in the two lineages. We did not perform any ancestral character reconstruction and further analyses may clarify the morphological evolution and diversification of Calothecinae. *Erianthecium*, as circumscribed here, differs from *Calotheca* by having the basal internodes of culms thickened, bulb like (*vs.* not bulb like in *Calotheca*) and by having pubescent lemmas (*vs.* glabrous lemmas in *Calotheca*).

We are proposing two new monotypic genera, *Rosengurttia* and *Boldrinia*. The first one encompasses *Chascolytrum monandrum*, a species that was previously included in *C.* sect. *Tricholemma* L.N.Silva, Essi & Souza-Chies, comprising four species (da Silva *et al.*, 2020a), characterized by having margins of the lemma ciliate at the lower third. *Rosengurttia* is sister to the clade consisted of *Chascolytrum poomorphum* (= *Microbriza*). The three other species of *Chascolytrum* sect. *Tricholemma* aligned in a strongly supported clade, recognized here as *Rhombolytrum*, that is sister to the *Rosengurttia* + *Microbriza* clade. This is the first time that the monophyly of sect. *Tricholemma* has been tested with inclusion of all its species sampled together in an analysis. Also, this is the first study to find a close relationship between *C. monandrum* (= *Rosenburttia*) and *C. poomorphum* (= *Microbriza*). *Rosengurttia* differs from *Microbriza* by having 4 to many-flowered spikelets (*vs.* to (2)3-flowered spikelets in *Microbriza*), chartaceous lemmas (*vs.* coriaceous lemmas in *Microbriza*), smooth to scaberulous lemmas (*vs.* echinulate lemmas in *Microbriza*), and membranous and glabrous paleas (*vs.* coriaceous and echinulate paleas in *Microbriza*). *Rhombolytrum* was placed as sister to the *Microbriza* + *Rosengurttia* clade, although with weak support, whereas *Microbriza* and *Rosengurttia* are closely related with strong support.

The new monotypic genus *Boldrinia* was recovered as sister to *Chascolytrum s.s.* These two genera have the same circumscription as proposed by da Silva *et al.* (2020a), under *Chascolytrum* sect. *Obovatae* L.N.Silva, Essi & Souza-Chies and sect. *Chascolytrum*, respectively. Matthei (1975) recognized *C. parodianum* within *Chascolytrum s.s.*, a classification that is phylogenetically supported by our results. However, *Boldrinia* differs significantly from *Chascolytrum s.s.*. The former is characterized by having chartaceous lemmas, without a gibbous back distinct from the margins, and chartaceous and obovate to obovate-truncate paleas, and the latter by having coriaceous lemmas, whit a gibbous back strongly distinct from the margins, and coriaceous and orbicular to elliptic paleas. Given these differences, the acceptance of a single genus would be hindered by the absence of morphological synapomorphies, hence our preference for recognizing *C. parodianum* in the new genus *Boldrinia*.

Relationships within the *Chascolytrum s.s.* clade were mostly resolved, except those among *C. subaristatum*, *C. erectum* and *C. paleapiliferum*. These three taxa are intermixed in a clade. *Chascolytrum scabrum* was recovered as sister to the remaining species of the genus, and *C. lamarckianum* as sister to *C. subaristatum* and its relatives. *Chascolytrum subaristatum* is a polymorphic and widely distributed species, with a complex taxonomic background. For example, Essi *et al.* (2017) included several taxa previously accepted by Longhi-Wagner (1987) in its synonymy, and more recently *C. erectum* was segregated from it (da Silva *et al.*, 2020a). Further studies on *C. subaristatum* morphological variation and the inclusion of *C. altimontanum* in the phylogenetic context may be helpful for understanding the relationships among all these taxa.

5 Taxonomy

Subtribe Calothecinae Soreng—Type: *Calotheca* Desv.

Description— Perennial herbs, cespitose, tufted. Innovations extravaginal, base fibrous, basal internodes of the culms not thickened or thickened, bulb-like. Leaf sheaths glabrous or pilose, smooth or scabrous. Leaf blades linear to linear-lanceolate, involute, convolute or flat. Ligule obtuse, acute or truncate. Panicle open to contracted, erect or pendulous, pedicels glabrous, smooth or scabrous. Spikelets (2-)3- to many-flowered, laterally compressed to cylindrical, without a rachilla prolongation, occasionally with a long, pilose and sharp callus, pale, pale green, greenish to purplish. Glumes herbaceous to chartaceous, subequal, equal or unequal, naviculate or convex, as long as the florets or shorter than the florets, glabrous or pilose, smooth scabrous, 1- to 9-veined. Lemmas membranous, herbaceous, chartaceous or coriaceous, glabrous or pilose, smooth or scabrous, rarely echinulate or papillate, laterally to dorsiventrally compressed, apex acute, obtuse, truncate, bidentate or bifid, 3- to 11-veined, muticous, mucronate or awned, awn basal, middle back or subapical, straight, twisted or geniculate. Paleas membranous, chartaceous or coriaceous, apex acute, obtuse, rounded or truncate. Lodicules linear, linear-lanceolate, or elliptic-lanceolate to flabelliform, rarely bilobate. Stamens 1 to 3. Caryopsis suborbicular, oblong to elliptic. Hilum linear, linear-elliptic, elliptic, or punctiform.

Comments—We propose a new circumscription of Calothecinae, excluding *Laegaardia* and *Paramochloa*, which are placed in the new subtribe Paramochloinae, but including *Trisetum brasiliense* and *T. bulbosum* based on our phylogenetic results. In our plastid and combined plastid + nuclear trees, *Trisetum brasiliense* and *T. bulbosum* were included in a strongly supported clade with the species of *Chascolytrum s.l. Trisetum bulbosum* was placed in the backbone polytomy along with the two major clades of *Chascolytrum s.l.* (Clades A and B), whereas *T. brasiliense* as merged within the Clade A and placed as sister to *Poidium* in the combined nuclear + plastid analyses. The recognition of a single genus encompassing also these two species of *Trisetum* is morphologically unpractical. Thus, we divide *Chascolytrum s.l.* into several smaller genera, of which two are newly described. The two species of *Trisetum* are unplaced to genus, pending further study. Their morphological characteristics, however, are included in the description of the subtribe.

Genera included: *Boldrinia* (1 species), *Calotheca* (1 species), *Chascolytrum* (6 species), *Erianthecium* (1 species), *Lombardochloa* (1 species), *Microbriza* (1 species), *Poidium* (9 species), *Rhombolytrum* (3 species), and *Rosengurttia* (1 species).

Species *incertae sedis*: *Trisetum brasiliense* and *Trisetum bulbosum*.

Boldrinia L.N.Silva, **gen. nov.** ≡ *Chascolytrum* sect. *Obovatae* L.N.Silva, Essi & Souza-Chies, Bot. J. Linn. Soc. 192: 109. 2020–Type: *Boldrinia parodiana* (Roseng., B.R.Arrill. & Izag.) L.N.Silva [≡ *Briza parodiana* Roseng., B.R.Arrill. & Izag. ≡ *Chascolytrum parodianum* (Roseng., B.R. Arrill. & Izag.) Matthei].

Boldrinia parodiana (Roseng., B.R.Arrill. & Izag.) L.N.Silva, **comb. nov.** ≡ *Briza parodiana* Roseng., B.R.Arrill. & Izag., Bol. Fac. Agron. Univ. Montevideo 105: 26. 1968. ≡ *Chascolytrum parodianum* (Roseng., B.R. Arrill. & Izag.) Matthei, Willdenowia 8: 68. 1975. Type: Uruguay. Dpto. Maldonado: Abra de Perdomo, 21 Nov. 1948, *B. Rosengurtt B 5273* (holotype: MVFA0000249 digital image!; isotypes, BAA00000071 digital image!, K000433704!, MVFA0000250 digital image!, MVFA0000251 digital image!, MVFA0000252 digital image!, MVFA0000253 digital image!).

Diagnosis—*Boldrinia* differs from *Chascolytrum s.s.* in having longer and involute leaves, chartaceous lemmas (without a gibbous back distinct from the margins), and chartaceous and obovate to obovate-truncate paleas.

Description—Perennial herbs, cespitose, tufted. Innovations extravaginal, base fibrous, basal internodes of the culms not thickened. Leaf sheaths glabrous, smooth, margins not overlapping. Leaf blades 12.5–25.5 cm long × 0.5–2.5 mm wide, firm, involute, apex acute, glabrous. Ligule 1.5–5.5 mm long, acute or truncate. Panicle open to contracted, flaccid to pendulous, pedicels glabrous and smooth, 7.8–22 cm long. Spikelets 2.5–5.7 mm long × 1.4–3.5 wide, 4-8-flowered, subcylindrical, elliptic-lanceolate. Glumes chartaceous, subequal, naviculate, shorter than the lower lemma, scabrous on the keels, 3-veined; lower glume 2–3.3 mm long × 0.6–

0.9 mm wide; upper glume 2.2–3.5 mm long × 0.8–1 mm wide. Lemmas chartaceous, glabrous, smooth, slightly laterally or dorsiventrally compressed, pale to purplish, without a gibbous back distinct from the margins, margins not ciliate at the lower third, not thickened, without a pair of oil glands or their scars at the base, acute at the apex, muticous, 7-veined; lower lemma 2.7–3.6 mm long × 0.9–1.4 mm wide. Paleas chartaceous, obovate, apex rounded to truncate, glabrous and smooth between the keels, keels ciliate or ciliolate; lower palea 1.3–2 mm long × 0.7–1.1 mm wide. Lodicules elliptic-lanceolate. Stamens 1 or 2. Caryopsis elliptic, concavo-convex. Hilum elliptic.

Etymology—The genus name *Boldrinia* honors Ilsi Iob Boldrini (b. 1947), a Brazilian researcher focused on the southern Brazilian grasslands, with remarkable knowledge of grasses.

Distribution—Brazil and Uruguay.

Comments—In da Silva *et al*. (2020a), *Chascolytrum parodianum* (= *Boldrinia parodiana*) was placed in the monospecific *Chascolytrum* sect. *Obovatae*, characterized by having chartaceous and obovate to obovate-truncate paleas. In the present study, *C. parodianum* was recovered as sister to *Chascolytrum s.s.* in the combined DNA analysis (plastid + nuclear datasets). This topology is consistent with the proposal of Matthei (1975) to include *C. parodianum* within *Chascolytrum s.s.* However, *Chascolytrum parodianum* differs from the species of *Chascolytrum s.s.* by having chartaceous lemmas (*vs.* coriaceous lemmas in *Chascolytrum s.s.*) with a gibbous back not distinct from the margins (*vs.* lemmas with a gibbous back strongly distinct from the margins), and chartaceous and obovate to obovate-truncate paleas (*vs.* coriaceous and elliptic or orbicular paleas). Based on these morphological differences and consistent with the phylogenetic evidence, the monotypic genus *Boldrinia* is here proposed to accommodate this species.

Taxon included: *Boldrinia parodiana*.

Calotheca Desv., Nouv. Bull. Sci. Soc. Philom. Paris 2: 190. 1810 ≡ *Briza* sect. *Calotheca* (Desv.) Benth. & Hook., Gen. Pl. 3(2): 1195. 1883. ≡ *Briza* subg. *Calotheca* (Desv.) Parodi, Revista Fac.

Agron. Veterin. (Buenos Aires) 3: 130. 1920. ≡ *Chascolytrum* sect. *Calotheca* (Desv.) L.N.Silva **syn. nov.**, Bot. J. Linn. Soc. 192: 109. 2020–Type: *Calotheca brizoides* (Lam.) Desv.

Description—Perennial herbs, cespitose, tufted. Innovations intravaginal, base fibrous, basal internodes of the culms not thickened. Leaf sheaths glabrous, smooth, margins not overlapping. Leaf blades linear, 4–26 cm long × 0.5–2.4 mm wide, firm, conduplicate, apex obtuse to truncate, glabrous. Ligule 0.7–2.5 mm long, obtuse to truncate. Panicle open to contracted, erect, pedicels smooth, 2–9 cm long. Spikelets 5–10 mm long × 2.2–6 mm wide, 4- to 8-flowered, laterally compressed, oblong. Glumes chartaceous, subequal or unequal, naviculate, shorter than the lower lemma, glabrous, scaberulous or smooth, 3-5-veined; lower glume 2.8–5.8 mm long × 0.6–1.2 mm wide; upper glume 3.4–6.8 mm long × 0.7–1.7 mm wide. Lemmas chartaceous, glabrous, smooth on the back, laterally compressed, pale or stramineous, without a gibbous back distinct from the margins, margins not ciliate at the lower third, margins not thickened, without a pair of oil glands or their scars at the base, bidentate at the apex, awned, awns 1.3–4.4 mm long, 5–veined; lower lemma 5–7 mm long × 2.5–3.6 mm wide. Paleas membranous, lanceolate, apex obtuse to acute, pubescent between the keels, keels ciliate; lower palea 2.4–4.1 mm long × 1–1.4 mm wide. Lodicules bilobate. Stamens 3. Caryopsis oblong to elliptic, concavo-convex. Hilum elliptic.

Distribution—Brazil, Chile, and Uruguay.

Comments—*Chascolytrum brizoides* (=*Calotheca brizoides*) was recently placed in *Chascolytrum* sect. *Calotheca*, along with *Chascolytrum bulbosum* (=*Erianthecium bulbosum*), based on the presence of awned lemmas (da Silva *et al.*, 2020a). However, the two samples of *C. brizoides* included in this study formed a well-supported clade apart from *C. bulbosum*, supporting the reestablishment of the monotypic genus *Calotheca*.

Taxon included: *Calotheca brizoides*.

Chascolytrum Desv., Nouv. Bull. Sci. Soc. Philom. Paris 2: 190. 1810 ≡ *Briza* sect. *Chascolytrum* (Desv.) Benth. & Hook., Gen. Pl. 3(2): 1195. 1883. ≡ *Briza* subg. *Chascolytrum* (Desv.) Parodi, Revista Fac. Agron. Veterin. (Buenos Aires) 3: 120. 1920–Type: *Chascolytrum subaristatum* (Lam.) Desv.

= *Chondrachyrum* Nees, Nat. Syst. Bot. 449. 1836. ≡ *Melica* subsect. *Chondrachyrum* (Nees) W. Hempel, Feddes Repert. 84(7–8): 541. 1973–Type: *Chondrachyrum scabrum* Nees ex Steud. [= *Chascolytrum scabrum* (Nees ex Steud.) Matthei].

Description—Perennial herbs, cespitose, less frequently rhizomatous, tufted. Innovations extravaginal, base fibrous, basal internodes of the culms not thickened. Leaf sheaths glabrous or sparsely pilose, smooth or scabrous, margins not overlapping. Leaf blades linear to linear-lanceolate, 5–50 cm long × 1.6–6.5 mm wide, firm or flaccid, flat, apex acute, obtuse or truncate, glabrous, smooth or scaberulous on the margins. Ligule 0.4–4.5 mm long, truncate or obtuse. Panicle open to contracted, erect or pendulous, pedicels smooth, 3–25 cm long. Spikelets 2.4–9 mm long × 1.9–9 mm wide, (2)3- to 14-flowered, cylindrical, globose or laterally compressed, orbicular, ovate or oblong. Glumes herbaceous, equal to subequal, convex, shorter than the lower lemma, glabrous, smooth or scaberulous, 3- to 11-veined; lower glume 1.5–4.6 mm long × 0.6–1.7 mm wide; upper glume 1.5–4.8 mm long × 0.9–1.9 mm wide. Lemmas coriaceous, glabrous and smooth, or strigose on the back, slightly laterally to dorsiventrally compressed, cream, pale, pale green or purplish, with a gibbous back strongly distinct from the margins, gibbous back pale, yellow, orange to brown, margins not ciliate at the lower third, margins not thickened, without a pair of oil glands or their scars at the base, acute or obtuse, muticous or mucronate (mucron up to 0.5 mm), 5(12)-veined; lower lemma 1.8–6.5 mm long × 0.9–5 mm wide. Paleas coriaceous, orbicular to elliptic, apex obtuse to rounded, glabrous and smooth, strigose or with sparse capitate trichomes between the keels, keels glabrous, occasionally ciliolate; lower palea 1.3–3 mm long × 0.8–1.9 mm wide. Lodicules flabelliform. Stamens 1 to 3. Caryopsis suborbicular, plano-convex. Hilum punctiform or elliptic.

Distribution—Argentina, Bolivia, Brazil, Chile, Colombia, Guatemala, Mexico, Paraguay, Peru and Uruguay.

Comments—The circumscription of *Chascolytrum s.s.* accepted here agrees with the one proposed for *Chascolytrum* sect. *Chascolytrum* by da Silva *et al.* (2020a). It differs from that accepted by Matthei (1975) by the exclusion of *C. parodianum* (= *Boldrinia parodiana*). *Chascolytrum s.s.* is recognized by having coriaceous lemmas with a gibbous back strongly distinct from the margins, and coriaceous and elliptic to orbicular paleas.

Taxa included: *Chascolytrum altimontanum* Essi, Souza-Chies & Longhi-Wagner (not sampled in the phylogenetic analyses), *C. erectum* Desv., *C. lamarckianum* (Nees) Matthei, *C. paleapiliferum* (Parodi) Matthei, *C. scabrum* (Nees ex Steud.) Matthei, and *C. subaristatum*.

Erianthecium Parodi, Notas Mus. La Plata, Bot. 8: 75, fig. 1. 1943–Type: *Erianthecium bulbosum* Parodi.

Description—Perennial herbs, cespitose, tufted. Innovations extravaginal, base fibrous, basal internodes of the culms thickened, bulb-like. Leaf sheaths glabrous or pilose, smooth, margins not overlapping. Leaf blades linear to linear-lanceolate, 4.5–20 cm long × 2.8–5 mm wide, soft, flat, apex acute, pilose on both surfaces. Ligule 1.8–4 mm long, acute to truncate. Panicle subcontracted to contracted, erect, pedicels smooth, 3–12 cm long. Spikelets 7–8 mm long × 2.5–4 mm wide, (3–)4–11-flowered, subcylindrical to laterally compressed, lanceolate. Glumes chartaceous, subequal or unequal, convex, shorter than the lower lemma, glabrous on the back and ciliate at the margins, punctate, 3–5–-7-veined; lower glume 3.8–4.3 mm long × 1–2 mm wide; upper glume 4–5 mm long × 1.3–3 mm wide. Lemmas chartaceous, pubescent on the back, slightly laterally compressed, pale to pale green, without a gibbous back distinct from the margins, margins not ciliate at the lower third, margins not thickened, without a pair of oil glands or their scars at the base, bidentate at the apex, awned, awns 1–3 mm long, 7–9-veined; lower lemma 4–5.7 mm long × 3.5–4 mm wide. Paleas chartaceous, elliptic, apex truncate or lobed, pubescent between the keels, keels ciliate; lower palea 3–3.5 mm long × 1–1.3 mm wide. Lodicules flabelliform. Stamens 3. Caryopsis elliptic, concavo-convex. Hilum punctiform.

Distribution—Brazil and Uruguay.

Comments—*Erianthecium bulbosum* (= *Chascolytrum bulbosum*) was recently placed in *Chascolytrum* sect. *Calotheca* along with *Chascolytrum brizoides* (= *Calotheca brizoides*) based on the presence of awned lemmas (da Silva *et al.*, 2020a). This circumscription was not recovered as monophyletic at the light of the phylogenetic results reported here. Thus, we reestablish the monotypic genus *Erianthecium*, which can be distinguished from *Calotheca* by having leaf blades flat and pilose (*vs.* involute and glabrous in *Calotheca*), lemmas pilose on the back (*vs.* lemmas glabrous on the back) and culms with the basal internodes thickened, bulb-like (*vs.* basal internodes not thickened).

Taxon included: *Erianthecium bulbosum*.

Lombardochloa Roseng. & B.R. Arrill., Anales Fac. Quimica Montevideo 9: 260. 1979. ≡ *Briza* sect. *Lombardochloa* (Roseng. & B.R. Arrill.) Longhi-Wagner, Bol. Inst. Bioci. Univ. Fed. Rio Grande do Sul 41: 22. 1987. ≡ *Chascolytrum* sect. *Lombardochloa* (Roseng. & B.R.Arrill.) L.N.Silva, **syn. nov.**, Bot. J. Linn. Soc. 192: 114. 2020–Type: *Chascolytrum rufum* J.Presl, Reliq. Haenk. 1 (4–5): 282. 1830 [= *Lombardochloa rufa* (J. Presl) Roseng. & B.R. Arrill.].

Description—Perennial herbs, cespitose to stoloniferous, tufted. Innovations extravaginal, base fibrous, basal internodes of the culms not thickened. Leaf sheaths glabrous, smooth, margins not overlapping. Leaf blades linear-lanceolate, 5–47 cm long × 2–12.5 mm wide, soft, flat, apex acute, glabrous. Ligule 1.1–3 mm long, truncate. Panicle contracted, rarely open, erect, pedicels smooth, 3–13 cm long. Spikelets 2–3.4 mm long × 1.4–2.5 mm wide, 3- to 6-flowered, laterally compressed, elliptic to elliptic-lanceolate. Glumes herbaceous, subequal or unequal, convex, shorter than the lower lemma, glabrous or pilose, 3- to 5-veined; lower glume 1.4–2.2 mm long × 0.6–1 mm wide; upper glume 1.4–2.3 mm long × 0.7–1.2 mm wide. Lemmas coriaceous, glabrous or pilose on the back, slightly laterally compressed, greenish, with a rufous gibbous back strongly distinct from the margins, margins not ciliate at the lower third, margins not thickened, with a pair of oil glands or their scars at the base, obtuse at the apex, muticous, 7-veined; lower lemma 1.6–2.4 mm long × 0.9–1.5 mm wide. Paleas membranous, hyaline, lanceolate, apex acute to obtuse, glabrous and smooth between the keels, keels ciliate; lower palea 1.1–1.7 mm long × 0.5–0.9 mm wide. Lodicules linear to elliptic-lanceolate. Stamens 1 or 2. Caryopsis elliptic, concavo-convex. Hilum punctiform or elliptic.

Distribution—Argentina, Brazil, Paraguay, and Uruguay.

Comments—*Chascolytrum rufum* was previously placed in *Poidium* (=*P. rufum*) by Matthei (1975), and few years later the genus *Lombardochloa* was described to accommodate its unusual morphology (Rosengurtt & Arrillaga de Maffei, 1979). More recently, it was recognized in the monotypic *Chascolytrum* sect. *Lombardochloa* (da Silva *et al*., 2020a). Here, we resurrect the genus *Lombardochloa*, which can be distinguished from other genera of Calothecinae by having spikelets 3(–4)-flowered, lemmas coriaceous, with distinct and rufous gibbous back and a pair of oil glands at the base, and membranous paleas.

Taxa included: *Lombardochloa rufa* var. *rufa* and *L. rufa* var. *sparsipilosa* (B.Rosengurtt et al.) B.Rosengurtt & B.R.Arrill.

Microbriza Parodi ex Nicora & Rúgolo, Darwiniana 23(1): 292. 1981. ≡ *Chascolytrum* sect. *Microbriza* (Parodi ex Nicora & Rúgolo) L.N.Silva, Bot. J. Linn. Soc. 192: 114. 2020–Type: *Microbriza poomorpha* (J.Presl) Parodi ex Nicora & Rúgolo.

Description—Perennial herbs, cespitose to stoloniferous, tufted. Innovations extravaginal, base fibrous, basal internodes of the not culms thickened. Leaf sheaths glabrous, smooth or scaberulous, margins not overlapping. Leaf blades linear, 6–21.5 cm long × 0.8–4.8 mm wide, soft, flat, apex acute, glabrous or pilose on the abaxial surface. Ligule 1.2–5.2 mm long, acute. Panicle open, erect, pedicels smooth, 7–16 cm long. Spikelets 1.2–1.8 mm long × 1–2 mm wide, (2–)3-flowered, laterally compressed, ovate to orbicular. Glumes herbaceous, subequal, convex, shorter than the lower lemma, glabrous, scabrous, 3-veined; lower glume 1–1.6 mm long × 0.3–0.5 mm wide; upper glume 1–1.7 mm long × 0.4–0.6 mm wide. Lemmas coriaceous, glabrous, surface echinate, slightly laterally compressed, pale to purplish, without gibbous back distinct from the margins, margins not ciliate at the lower third, margins not thickened, without a pair of oil glands or their scars at the base, obtuse at the apex, muticous, 5-veined; lower lemma 1–1.5 mm long × 0.5–0.7 mm wide. Paleas coriaceous, elliptic, apex acute, glabrous, echinate between the keels, keels glabrous or ciliolate; lower palea 0.7–1.2 mm long × 0.4–0.6 mm wide. Lodicules linear. Stamens 1. Caryopsis elliptic, concavo-convex. Hilum punctiform or elliptic.

Distribution—Argentina, Brazil, Paraguay, and Uruguay.

Comments—*Chascolytrum poomorphum* was recently recognized in the monospecific *C.* sect. *Microbriza* based on the presence of lemmas and paleas coriaceous and echinulate (da Silva *et al.*, 2020a). In this study, *C. poomorphum* (=*Microbriza poomorpha*) was recovered as sister to *Chascolytrum monandrum*, a species not morphologically related. We resurrect the genus *Microbriza*, excluding *M. brachychaete*, a species phylogenetically related to *Chascolytrum itatiaiae* here placed in *Poidium*.

Taxon included: *Microbriza poomorpha*.

Poidium Nees, Intr. Nat. Syst. Bot. 450. 1836. ≡ *Briza* sect. *Poidium* (Nees) Ekman, Ark. Bot. 13(10): 70. 1913. ≡ *Briza* subg. *Poidium* (Nees) Parodi, Revista Fac. Agron. Veterin. (Buenos Aires) 3: 133. 1920. ≡ *Chascolytrum* sect. *Poidium* (Nees) L.N.Silva, **syn. nov.**, Bot. J. Linn. Soc. 192: 116. 2020–Type: *Poidium brasiliense* Nees ex Steud. [= *Chascolytrum brasilense* (Nees) Essi, Longhi-Wagner & Souza-Chies.]

= *Chascolytrum* sect. *Hildaea* L.N.Silva, Essi & Souza-Chies, **syn. nov.**, Bot. J. Linn. Soc. 192: 112. 202. Type: *Chascolytrum calotheca* (Trin.) Essi, Longhi- Wagner & Souza-Chies.

Poidium juergensii (Hack.) Matthei, Willdenowia 8: 114. 1975. ≡ *Briza juergensii* Hack., Verh. K. K. Zool.-Bot. Ges. Wien 65: 76–77. 1915. ≡ *Chascolytrum juergensii* (Hack.) Essi, Souza-Chies & Longhi-Wagner, Novon 20(2): 153. 2010–Type: Brazil. Rio Grande do Sul: Pinheiral, Mpio. Rio Pardo, Nov. 1909, *C. Jürgens G 951* (holotype, W-19160039367!; isotype, US-865543A digital image!).

Poidium juergensii* var. *angustilemma (Essi, Souza-Chies & Longhi-Wagner) L.N.Silva, **comb. nov.** ≡ *Chascolytrum juergensii* var. *angustilemma* Essi, Souza-Chies & Longhi-Wagner, Novon 20(2): 154, fig. 3. 2010–Type: Brazil. Rio Grande do Sul: Cambará do Sul, perto do Fortaleza, 29°04954.60S, 050°00914.40W, 1 Dec. 2003, *L. Essi, A. Guglieri & S. Hefler 122* (holotype, ICN!; isotypes, K001077945!, MO!).

Poidium latifolium (Essi, Souza-Chies & Longhi-Wagner) L.N.Silva, **comb. nov.** ≡ *Chascolytrum latifolium* Essi, Souza-Chies & Longhi-Wagner, Novon 20(2): 151, fig. 2, 4A. 2010–Type: Brazil. Santa Catarina: Urubici, Faz. Arno Philippi, 7 Dec. 2006, *H.M. Longhi-Wagner, A. Zanin & L. Souza 10228* (holotype, ICN!; isotypes, FLOR!, K001077949!, MO!).

Poidium serranum (L.N.Silva) L.N.Silva, **comb. nov.** ≡ *Chascolytrum serranum* L.N.Silva, Phytotaxa 435(1): 41. 2020–Type: Brazil. Rio Grande do Sul: Cambará do Sul, Parque Nacional da Serra Geral, estrada para Cânion Fortaleza, banhado, 29°04’02.70”S, 50°02’28.70”W, 1022 m, 05 December 2017, *L.N. da Silva, R. Oliveira, R. Marchioretto & A. Christ 1067* (holotype ICN!, isotypes CAN!, RB!, US!).

Description—Perennial herbs, cespitose, tufted. Innovations extravaginal, base fibrous, basal internodes of the not culms thickened. Leaf sheaths glabrous, smooth, scaberulous to scabrous, or pilose, margins not overlapping. Leaf blades linear to linear-lanceolate, 10–50 cm long × 1.3–12 mm wide, soft or firm, flat or convolute, apex acute, glabrous and smooth, or pilose on the abaxial surface. Ligule 0.7–8 mm long, acute, obtuse or truncate. Panicle open, subcontracted to contracted, erect or pendulous, pedicels smooth, scaberulous or scabrous, 4.5–33 cm long. Spikelets 1.9–7.5 mm long × 1.4–5 mm wide, (2)3- to 11-flowered, subcylindrical to laterally compressed, ovate, elliptic, elliptic lanceolate or lanceolate. Glumes herbaceous to chartaceous, subequal, convex to naviculate, shorter than the lower lemma, glabrous, smooth to scabrous, (1)3- to 5-veined; lower glume 1.6–3.6 mm long × 0.4–1.1 mm wide; upper glume 1.7–4 mm long × 0.5–1.6 mm wide. Lemmas herbaceous, chartaceous or coriaceous, glabrous, smooth, tuberculate, papillate or strigose on the back, slightly laterally to laterally compressed, or dorsiventrally compressed, stramineous, pale, pale green to purplish, without a gibbous back distinct from the margins or with a gibbous back slightly distinct from the margins, margins not ciliate at the lower third, margins not thickened, without a pair of oil glands or their scars at the base, acute or bidentate at the apex, muticous or mucronate, 3- to 7-veined; lower lemma 1.6–5 mm long × 0.4–2.5 mm wide. Paleas membranous, elliptic, elliptic-lanceolate, lanceolate to linear-lanceolate, apex acute to obtuse, glabrous or sparsely to densely pilose between the keels, keels ciliate; lower palea 1.2–3.2 mm long × 0.4–1.7 mm wide. Lodicules linear, lanceolate or linear-lanceolate. Stamens 1 to 3. Caryopsis suborbicular or elliptic, concavo-convex. Hilum elliptic or punctiform.

Distribution—Argentina, Brazil, Colombia, Paraguay, and Uruguay.

Comments—*Poidium* as accepted here includes nine species and two varieties, recently recognized in *Chascolytrum* sect. *Hildaea* and *C.* sect. *Poidium* (da Silva *et al.*, 2020a). The phylogenetic results obtained here did not support the recognition of any infrageneric division within *Poidium*. The genus can be recognized by the 4- to many-flowered spikelets, with herbaceous lemmas, not ciliate or thickened at the margins, and membranous paleas.

Taxa included: *Poidium ambiguum, Poidium brachychaetum, Poidium brasilense, Poidium calotheca, Poidium itatiaiae, Poidium juergensii* var. *juergensii, Poidium juergensii* var. *angustilemma*, *Poidium latifolium*, *Poidium serranum,* and *Poidium uniolae*.

Rhombolytrum Link, Hort. Berol. 2: 296. 1833. Type: *Rhombolytrum rhomboideum* Link.

= *Gymnachne* Parodi, **syn. nov.**, Notas Mus. La Plata, Bot. 3: 29. 1938–Type: *Gymnachne jaffuelii* Parodi.

= *Chascolytrum* sect. *Tricholemma* L.N.Silva, Essi & Souza-Chies, **syn. nov.**, Bot. J. Linn. Soc. 192: 117. 2020–Type: *Chascolytrum bidentatum* (Roseng., B.R.Arrill. & Izag.) Essi, Longhi-Wagner & Souza-Chies.

Rhombolytrum bidentatum (Roseng., B.R.Arrill. & Izag) L.N.Silva, **comb. nov.** ≡ *Briza bidentata* Roseng., B.R. Arrill.&Izag., Bol. Fac.Agron.Univ. Montevideo 105: 24. 1968, replacement name for *Eragrostis monandra* Hack., Repert. Spec. Nov. Regni Veg. 7: 320. 1909, *non Briza monandra* (Hack.) Pilg., Notizbl. Bot. Gart. Berlin-Dahlem 10(97): 725. 1929, blocking name. *Rhombolytrum monandrum* (Hack.) Nicora & Rúgolo, Darwiniana 23: 302. 1981. ≡ *Chascolytrum bidentatum* (Roseng., B.R. Arrill. & Izag.) Essi, Longhi-Wagner & Souza-Chies, Novon 21(3): 327. 2011–Type: Brazil. Rio Grande do Sul: Rio Pardo, prope Fazenda Soledade, 70 m, Dec. 1905, *C. Jürgens s.n.* (holotype, W!; isotypes, BAA not seen, US-2941534 digital image!).

Rhombolytrum koelerioides (Trin.) L.N.Silva, **comb. nov.** ≡ *Poa koelerioides* Trin., Mem. Acad. Imp. Sci. Saint-Pétersbourg, Sér. 6, Sci. Math., Seconde Pt. Sci. Nat. 4, 2(1): 62. 1836. ≡ *Gymnachne koelerioides* (Trin.) Parodi, Revista Argent. Agron. 29(1–2): 19. 1962 [1963]. ≡ *Chascolytrum koelerioides* (Trin.) Essi, Longhi-Wagner & Souza-Chies, Novon 21(3): 328. 2011–Type: Chile. Quillota: in pascuis sylvaticis collium editiorum, Oct. 1829, *C. G. Bertero 947* (lectotype, designated by Essi et al., 2011: 328, P!; isolectotypes, LE-TRIN-2641.01 not seen, US-88773 [US00386380**]** [P fragm.] digital image!, BAA00002712 [P fragm.] digital image!, MO 243766).

= *Gymnachne jaffuelii* Parodi, Notas Mus. La Plata, Bot. 3:(17): 30. 1938–Type: Chile. Concepción: *F. Jaffuel 1884* (holotype, BAA not seen; isotypes, BAA00000181 digital image!, BAA00003593 digital image!).

= *Rhombolytrum berteroanum* E. Desv., Fl. Chil. 6: 387. 1854–Type: Chile: Los Llanos, provincia de Valdivia, *[C.] Gay [Mouret] 298* (lectotype, designated in Essi *et al.*, 2011: 329, P00740288 digital image!; isolectotypes, K, US-90608 digital image!).

Distribution—Brazil, Chile, and Uruguay.

Description—Perennial herbs, cespitose, tufted. Innovations extravaginal, base fibrous, basal internodes of the culms not thickened. Leaf sheaths glabrous or pilose, smooth or scabrous, margins not overlapping or overlapping. Leaf blades linear to linear-lanceolate, 5.2–35 cm long × 0.6–5.2 mm wide, firm or flaccid, flat or convolute, apex acute, glabrous or pilose on the abaxial surface, or pilose on both surfaces. Ligule 1.5–4 mm long, truncate. Panicle open to contracted, erect or pendulous, pedicels smooth or scabrous, 4.5–20 cm long. Spikelets 3.2–8 mm long × 1.1–4 mm wide, 4- to 10-flowered, laterally compressed, rhomboid to oblong. Glumes chartaceous, equal to subequal, convex to naviculate, almost the size of the lower lemma, sometimes covering the lower lemma, glabrous, scabrous, 3-5-veined; lower glume 1.6–5 mm long × 0.4–1.3 mm wide; upper glume 1.5–6 mm long × 0.4–1.3 mm wide. Lemmas herbaceous to chartaceous, glabrous or pilose, smooth or scaberulous, slightly laterally to dorsiventrally compressed, pale to pale green, without a gibbous back distinct from the margins, margins ciliate at the lower third, margins thickened, without a pair of oil glands or their scars at the base, obtuse, acute or bilobate at the apex, muticous or mucronate (mucron up to 0.5 mm), 3-5-9-veined; lower lemma 2–4.5 mm long × 0.6–1.1 mm wide. Paleas membranous, lanceolate, elliptic lanceolate or linear-lanceolate, apex obtuse to acute, glabrous or pilose between the keels, smooth or scaberulous, keels ciliate; lower palea 1.6–3.2 mm long × 0.4–1 mm wide. Lodicules linear or lanceolate. Stamens 1 or 2. Caryopsis oblong to elliptic, concavo-convex. Hilum punctiform.

Comments—*Rhombolytrum* includes three species recently recognized in *Chascolytrum* sect. *Tricholemma* [along with *Chascolytrum monandrum* (= *Rosengurttia monandra* (Hack.) L.N.Silva) by da Silva *et al.* (2020a)], morphologically supported by having lemmas with margins ciliate at the lower third. According to the phylogenetic results obtained in this study *C.* sect.

Tricholemma is not monophyletic due to *C. monandrum* being recovered as sister to *Microbriza poomorpha*, whereas the other *C. bidentatum*, *C. koeleirioides*, and *C. rhomboideum* formed a well-supported clade in the combined DNA analyses. Of these three, two of them were already accepted in *Rhombolytrum* by Nicora & Rúgolo de Agrasar (1981) and Bayón (1998) [*R. rhomboideum* and *Rhombolytrum bidentatum* (= *R. monandrum*)], whereas *C. koelerioides* have been included in *Gymnachne* (Essi *et al.* 2017). Our results revealed that *C. rhomboideum* and *C. koeleriodes* are phylogenetically and morphologically related, along with *C. bidentatum*. Thus, we resurrect the name *Rhombolytrum* due to its priority over the name *Gymnachne*. *Rhombolytrum*, as circumscribed here, is recognized by the rhomboid to oblong spikelets, chartaceous glumes, almost the size of the lower lemmas, and lemmas with thickened margins.

Taxa included: *Rhombolytrum bidentatum*, *R. koelerioides*, and *R. rhomboideum*.

Rosengurttia L.N.Silva, **gen. nov.**–Type: *Chascolytrum monandrum* [= *Rosengurttia monandra* (Hack.) L.N.Silva].

Rosengurttia monandra (Hack.) L.N.Silva, **comb. nov**. ≡ *Poa monandra* Hack., Oesterr. Bot. Z. 52(10): 376–377. 1902. ≡ *Briza monandra* (Hack.) Pilg., Notizbl. Bot. Gart. Berlin-Dahlem 10(97): 725. 1929. ≡ *Poidium monandrum* (Hack.) Matthei, Willdenowia 8: 103. 1975. ≡ *Chascolytrum monandrum* (Hack.) Essi, Longhi-Wagner & Souza-Chies, Novon 21(3): 329. 2011–Type: [Peru.] Prope Cutervo, May 1879, *C. von Jelski 402* (holotype, W-41656!; isotypes, US-88757 [US00386398] [W fragm.] digital image!, W-14194!, BAA00002721 [W fragm.] digital image!).

Diagnosis—*Rosengurttia* is morphologically similar to *Rhombolytrum* due to the presence of lemmas with margins ciliate at the lower third, but differs from that by having spikelets ovate to elliptic, glumes herbaceous, shorter than the lower lemmas, and lemmas with margins not thickened.

Description—Perennial herbs, cespitose, tufted. Innovations extravaginal, base fibrous, basal internodes of the culms not thickened. Leaf sheaths glabrous, smooth, margins not overlapping. Leaf blades 7–22 cm long × 1–3 mm wide, flaccid, flat or involute, apex acute, glabrous, pilose on the adaxial surface, or pilose on both surfaces. Ligule 0.6–2.2 mm long, truncate. Panicle open, erect, pedicels scaberulous, 3.5–18 cm long. Spikelets 3–6 mm long × 1–3.5 mm wide, 4- to 6-flowered, laterally compressed, ovate to elliptic. Glumes herbaceous, subequal, convex to naviculate, shorter than the lower lemma, glabrous, scaberulous or smooth, subequal, 3-veined; lower glume 1.5–3 mm long × 0.5–0.8 mm wide; upper glume 1.5–3 mm long × 0.4–0.9 mm wide. Lemmas herbaceous, glabrous, smooth to scaberulous, laterally compressed, pale green to purplish, without a gibbous back distinct from the margins, margins ciliate at the lower third, not thickened, without a pair of oil glands or their scars at the base, obtuse at the apex, muticous, 5-veined; lower lemma 1.8–2.3 mm long × 0.7–1 mm wide. Paleas membranous, elliptic to elliptic-lanceolate, apex obtuse to acute, glabrous between the keels, smooth or sparsely punctuate, keels ciliate; lower palea 1.3–2 mm long × 0.4–0.7 mm wide. Lodicules lanceolate. Stamens 1(3). Caryopsis elliptic, concavo-convex. Hilum elliptic or punctiform.

Etymology—The name *Rosengurttia* honors Bernardo Rosengurtt (1916–1985), a Uruguayan botanist who notably contributed to the knowledge of the cool-season grasses in southern South America.

Distribution—Argentina, Bolivia, Brazil, Colombia, Ecuador, and Peru.

Comments—In da Silva *et al.* (2020a) *Chascolytrum monandrum* was included within the section *Tricholemma* along with *C. bidentatum, C. koelerioides* and *C. rhomboideum,* based on the lemmas with margins ciliate at the lower third. In the present study *C. monandrum* was recovered as sister to *Microbriza poomorpha* (= *Chascolytrum poomorphum*), although both species are not morphologically similar. Thus, we place *C. monandrum* into the new monotypic genus *Rosengurttia*, which differs from *Microbriza* by having spikelets 4- to many-flowered (*vs.*

3-flowered in *Microbriza*), lemmas herbaceous (*vs.* lemmas coriaceous), smooth or tuberculate (*vs.* echinulate), and paleas membranous (*vs.* paleas coriaceous).

Taxon included: *Rosengurttia monandra*.

Subtribe Paramochloinae L.N.Silva & Saarela, **subtribe nov.—**Type: *Paramochloa* P.M. Peterson, Soreng, Romasch. & Barberá, Phytoneuron 2019–39: 13, f. 4. 2019.

Description— Perennial herbs, cespitose, rhizomatous, tufted. Innovations intravaginal, base fibrous, basal internodes of the culms not thickened. Leaf sheaths glabrous, smooth. Leaf blades linear, involute. Ligule obtuse, acute or truncate. Panicle open to contracted, erect, pedicels glabrous, smooth or scabrous. Spikelets 1(2)-flowered, slightly laterally compressed, with a hairy rachilla prolongation, purplish to dark-purple. Glumes herbaceous, subequal, lanceolate, as long or longer than the florets, glabrous, smooth to scabrous, 1- to 3-veined. Lemmas membranous, glabrous to scabrous, slightly laterally compressed, apex obtuse or emarginate to denticulate, 5-veined, muticous or awned, awn dorsal. Paleas membranous to hyaline, apex bidentate. Lodicules bilobate. Stamens 3. Caryopsis ovate to elliptic-ovate. Hilum linear.

Comments—*Laegaardia* and *Paramochloa* were included in the subtribe Calothecinae by Peterson *et al.* (2019), based on unpublished phylogenetic results. Our combined analyses recovered these two genera into a strongly supported clade placed as sister to the *Chascolytrum s.l.* + *T. bulbosum* + *T. brasiliense* clade, consistent with the topology first published by Saarela *et al.* (2017) with sampling of *Chascolytrum s.l.* and *Paramochloa effusa*. Species of *Laegaardia* and *Paramochloa* differ from *Chascolytrum s.l.* by having 1-flowered spikelets [*vs.* (2-)3 to many-flowered spikelets in *Chascolytrum s.l.*], with a hairy rachilla prolongation (absent in *Chascolytrum s.l.*). Thus, we propose here the new subtribe Paramochloinae to accommodate *Laegaardia* and *Paramochloa*.

Genera included: *Laegaardia* (1 species) and *Paramochloa* (2 species).

Taxonomic key to the genera of subtribes Calothecinae and Paramochloinae (excluding *Trisetum bulbosum* and *T. brasiliense*)

1 Spikelets 1(2)-flowered. Florets with a hairy rachilla prolongation (Paramochloinae) ……….. 2

1 Spikelets (2)3 to many-flowered. Florets without a hairy rachilla prolongation (Calothecinae) ……… 3

2 Lemmas awned …… *Paramochloa*

2 Lemmas muticous …… *Laegaardia ecuadoriense*

3 Lemmas awned, awns more than 1 mm long ………… 4

3 Lemmas muticous, occasionally mucronate, mucron up to 0.5 mm ……. 5

4 Culms with the basal internodes thickened, bulb-like. Leaf blades flat, pilose on both surfaces. Lemmas pilose ….. *Erianthecium bulbosum*

4 Culms with the basal internodes not thickened. Leaf blades involute, linear, glabrous on both surfaces. Lemmas glabrous ….. *Calotheca brizoides*

5 Paleas chartaceous to coriaceous …… 6

5 Paleas membranous to hyaline …. 8

6 Paleas chartaceous, obovate to obovate-truncate ….. *Boldrinia parodiana*

6 Paleas coriaceus, orbiculate to elliptic ….. 7

7 Lemmas with a gibbous back distinct from the margins, surface glabrous or strigose. Paleas glabrous, smooth, strigose or with sparse capitate trichomes … *Chascolytrum*

7 Lemmas without a gibbous back distinct from the margins, surface echinulate. Paleas glabrous, echinulate ……. *Microbriza poomorpha*

8 Lemmas with gibbous back strongly distinct from the margins, back rufous, with a pair of oil glands or their scars at the base …… *Lombardochloa rufa*

8 Lemmas without gibbous back or with a gibbous back slightly distinct from the margins, back pale, greenish or purplish, without a pair of oil glands or their scars at the base …. 9

9 Margins of the lemma glabrous …… *Poidium*

9 Margins of the lemma ciliate or ciliolate at the lower third ….. 10

10 Spikelets rhomboid to oblong. Glumes chartaceous, almost the size of the lower lemma. Margins of the lemma thickened ……. *Rhombolytrum*

10 Spikelets ovate to elliptic. Glumes herbaceous, shorter than the lower lemma. Margins of the lemma not thickened …… *Rosengurttia monandra*

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Table 1. Voucher information and GenBank accessions numbers of the samples used in the phylogenetic analyses.

		Plastid				**Nuclear**	
Species	**Colector (Herbarium)**	***atpF-atpH***	***matK***	***trnL-trnF***	***rps16***	**ITS**	**ETS**
Agrostis capillaris	da Silva *et al.* 1070 (ICN)	new	new	new	new	new	new
Agrostis lenis	da Silva *et al.* 1089 (ICN)	new	new	new	new	new	new
Agrostis lenis	da Silva *et al.* 1126 (ICN)	new	new	new	new	new	-
Agrostis longiberbis	Zanin 1057 (ICN)	new	new	new	new	new	new
Agrostis montevidensis	da Silva *et al.* 1087	new	new	new	new	new	new
Agrostis scabra	Peterson *et al.* 18491 (CAN)	KX871930	KX873207	KX872604	-	KX872909	KX872281
Agrostis stolonifera	Peterson *et al.* 18382 (CAN)	KX871934	-	KX872606	-	FJ377622	KX872284
Ammophila arenaria	Peterson *et al.* 19705 (CAN)	KX871941	KX873216	KX872614	-	KX872916	KX872289
Amphibromus fluitans	Beauglehole 82449 (MELU)	KX871944	KX873219	KX872617	-	KX872918	KX872292
Amphibromus macrorhinus	Thomas 586 (MELU)	-	KX873221	KX872624	-	KX872925	KX872298
Amphibromus neesii	Stajsic 4243 (MELU)	-	KX873222	KX872620	-	KX872921	KX872295
Amphibromus pithogastrus	Clarke 2535 (MELU)	-	KX873223	KX872621	-	KX872922	KX872296
	da Silva *et al.* 780 (ICN)	new	new	new	new	new	new
Amphibromus quadridentulus	da Silva *et al.* 823 (ICN)	new	new	new	new	new	new
Amphibromus recurvatus	Walker s.n. (MELU)	-	KX873224	KX872622	-	KX872923	KX872297
Amphibromus scabrivalvis	Soreng & Soreng 7013 (US)	KX871946	KX873225	KX872623	-	KX872924	-
Amphibromus sinuatus	Paget 2257	KX871943	KX872616	KX873218	-	KX872917	KX872291
Briza maxima	Essi 45 (ICN)	new	new	MK821314	MK821363	new	-
Briza media	Essi 256 (ICN)	new	new	new	new	new	new
Briza minor	Essi 01 (ICN)	new	new	MK821313	MK821364	new	new
Bromidium tandilense	da Silva & Essi 693 (ICN)	new	new	MK821312	MK821366	new	new
Calamagrostis epigeios	Soreng *et al.* 7637 (US)	KX872005	KX873286	KX872672	-	KX872968	KX872347
Calamagrostis holciformis	Soreng *et al.* 7697 (US)	KX872016	KX873297	KX872676	-	KX872972	KX872355
Calamagrostis varia	Pazko (KRAM)	KX872095	KX873377	KX872733	-	KX873019	KX872415
Chascolytrum ambiguum	da Silva *et al.* 1033 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 1029 (ICN)	new	new	MK821319	MK821369	new	new

	da Silva *et al.* 869 (ICN)	new	new	MK821320	MK821370	new	new
	da Silva *et al.* 1042 (ICN)	new	new	new	new	new	new
Chascolytrum bidentatum	da Silva 577 (ICN)	new	new	new	-	new	new
	da Silva & Essi 671 (ICN)	new	new	new	new	new	new
Chascolytrum brachychaetum	Essi 151 (ICN)	new	new	MK821324	MK821374	new	new
	da Silva *et al.* 884 (ICN)	new	new	new	new	new	new
Chascolytrum brasilense	da Silva *et al.* 1055 (ICN)	new	new	MK821325	MK821375	new	new
	Viana *et al.* 3190 (ICN)	new	new	new	new	new	new
	Ribeiro *et al.* 357 (ICN)	new	new	new	new	new	new
Chascolytrum brizoides	Soreng & Soreng 7014 (US)	KX87210	KX873386	KX872740	-	KX873026	-
	da Silva *et al.* 710 (ICN)	new	new	new	new	new	new
Chascolytrum bulbosum	Essi 60 (ICN)	new	new	MK821327	MK821377	new	new
	da Silva *et al.* 656 (ICN)	new	new	new	new	new	new
	da Silva & Forgiarini 690 (ICN)	new	new	new	new	new	new
	da Silva & González 714 (ICN)	new	new	new	new	new	new
Chascolytrum calotheca	Longhi-Wagner 9662 (ICN)	new	new	MK821328	MK821378	new	new
	Longhi-Wagner 8062	new	new	new	new	new	new
	da Silva & Ferreira 752	new	new	new	new	new	new
Chascolytrum erectum	da Silva & González 715 (ICN)	new	new	MK821331	MK821381	new	new
	da Silva & Oliveira 782 (ICN)	new	new	new	new	new	new
	da Silva & Oliveira 783 (ICN)	new	new	MK821332	MK821382	new	-
Chascolytrum itatiaiae	da Silva *et al.* 776 (ICN)	new	new	MK821336	MK821386	new	new
	da Silva 1032 (ICN)	new	new	MK821334	MK821384	new	new
	da Silva 1039 (ICN)	new	new	new	new	new	new
	da Silva 1049 (ICN)	new	new	MK821335	MK821385	new	new
Chascolytrum juergensii var. *angustilemma*	da Silva *et al.* 850 (ICN)	new	new	MK821338	MK821388	new	new
	da Silva *et al.* 862 (ICN)	new	new	MK821339	MK821389	new	new

	da Silva *et al.* 1054 (ICN)	new	new	MK821337	MK821387	new	new
Chascolytrum juergensii var. *juergensii*	Essi 186 (ICN)	new	new	MK821343	MK821393	new	new
	da Silva & Valduga 800 (ICN)	new	new	MK821341	MK821391	new	new
	da Silva *et al.* 824 (ICN)	new	new	new	new	new	new
	da Silva 864 (ICN)	new	new	new	new	new	new
	da Silva 927 (ICN)	new	new	MK821342	MK821392	new	new
	da Silva *et al.* 858 (ICN)	new	new	new	new	new	new
Chascolytrum koelerioides	Soreng 7035 (US)	new	new	new	new	new	new
Chascolytrum lamarckianum	da Silva & Valduga 802 (ICN)	new	new	MK821317	MK821367	new	new
	Longhi-Wagner 8060	new	new	MK821344	MK821394	new	new
	da Silva 879 (ICN)	new	new	new	new	new	new
	Renvoize 2940 (US)	-	new	new	new	new	new
	da Silva *et al.* 929 (ICN)	new	new	new	new	new	new
	da Silva & Oliveira 942 (ICN)	new	new	new	new	new	new
Chascolytrum latifolium	da Silva 859 (ICN)	new	new	MK821345	MK821395	new	new
	da Silva *et al.* 890 (ICN)	new	new	MK821346	MK821396	new	new
	da Silva *et al.* 870 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 1139 (ICN)	new	new	new	new	new	new
Chascolytrum monandrum	Peterson & Soreng 21861 (US)	KX872106	KX873388	KX872742	-	KX873028	KX872424
	Beck 30397 (ICN)	new	new	MK821347	MK821397	new	new
	Valls *et al.* 11499 (ICN)	new	new	new	new	new	new
	Essi *et al.* 240 (ICN)	new	new	new	new	new	new
	Campestrini *et al.* 218 (FLOR)	new	new	new	new	new	new
Chascolytrum paleapiliferum	Zuloaga 8119 (BAA)	new	new	MK821349	MK821399	new	new
	Zuloaga 8173 (BAA)	new	new	MK821350	MK821400	new	new
Chascolytrum parodianum	da Silva *et al.* 834 (ICN)	new	new	MK821318	MK821368	new	new
	Essi *et al.* 290 (ICN)	new	new	MK821352	MK821402	new	new

	da Silva *et al.* 1090 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 1080 (ICN)	new	new	new	new	new	new
	da Silva & González 706 (ICN)	new	new	new	new	new	new
	da Silva & González 711 (ICN)	new	new	new	new	new	new
	da Silva & Tacuatiá 667 (ICN)	new	new	MK821351	MK821401	new	new
Chascolytrum poomorphum	da Silva & González 703 (ICN)	new	-	MK821353	MK821403	new	new
	da Silva & Oliveira 938 (ICN)	new	new	new	new	new	new
	da Silva 818 (ICN)	new	new	new	new	new	new
	da Silva 789 & Valduga (ICN)	new	new	MK821354	MK821404	new	new
	da Silva & Essi 643 (ICN)	new	new	new	new	new	new
Chascolytrum rhomboideum	Claude-Joseph 1429 (US)	new	new	new	new	new	new
Chascolytrum rufum var. *rufum*	da Silva 877 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 805 (ICN)	new	new	new	new	new	new
	Trevisan 125 (ICN)	new	new	MK821355	MK821405	new	new
Chascolytrum rufum var. *sparsipilosum*	da Silva *et al.* 872 (ICN)	new	new	new	new	new	new
	da Silva & Oliveira 944 (ICN)	new	new	new	new	new	new
Chascolytrum scabrum	Essi *et al.* 101 (ICN)	new	new	MK821358	MK821408	new	new
	da Silva & Tacuatiá 686 (ICN)	new	new	MK821357	MK821407	new	new
	da Silva *et al.* 695 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 1094 (ICN)	new	new	new	new	new	new
Chascolytrum serranum	da Silva *et al.* 1067 (ICN)	new	new	new	MK821409	new	new
	da Silva *et al.* 1132 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 1138 (ICN)	new	new	new	new	new	new
Chascolytrum subaristatum	da Silva & Valduga 811 (ICN)	new	new	MK821359	MK821411	new	new
	da Silva *et al.* 932 (ICN)	new	new	MK821361	MK821412	new	new
	da Silva 750 (ICN)	new	new	new	new	new	new
	da Silva & González 702 (ICN)	new	new	new	new	new	new
	da Silva & Oliveira 785 (ICN)	new	new	new	new	new	new
	Peterson *et al.* 21078 (US)	new	new	new	new	new	new
	Peterson *et al.* 21072 (US)	new	new	new	new	new	new

	da Silva 856 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 679 (ICN)	new	new	new	new	new	new
	da Silva & González 713 (ICN)	new	new	MK821360	MK821410	new	new
	da Silva *et al.* 960 (ICN)	new	new	new	new	new	new
Chascolytrum uniolae	da Silva & Tacuatiá 696 (ICN)	new	new	MK821362	MK821413	new	new
	da Silva *et al.* 729 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 991 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 1062 (ICN)	new	new	new	new	new	new
Cinnagrostis alba	da Silva & Essi 664 (ICN)	new	new	new	new	new	new
Cinnagrostis rupestris	da Silva 796 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 1079 (ICN)	new	new	new	new	new	new
Cinnagrostis viridiflavescens	da Silva & Coelho 1053 (ICN)	new	new	new	new	new	new
	da Silva 724 (ICN)	new	new	new	new	new	new
	da Silva *et al.* 1063 (ICN)	new	new	new	new	new	-
Deschampsia cespitosa	Peterson *et al.* 18367	KX872112	-	KX872744	-	KX873031	KX872430
Deschampsia cespitosa	da Silva 822 (ICN)	new	new	new	new	new	new
Dichelachne crinita	Peterson *et al.* 14489 (US)	new	new	new	new	new	new
Echinopogon caespitosus	Soreng *et al.* 5900 (US)	KX872184	KX873476	KX872821	-	KX873109	KX872512
Greeneochloa coarctata	Peterson & Saarela 20818 (CAN)	new	new	new	new	new	new
	Duncan 10470 (US)	new	new	new	new	new	new
	Duncan 13152 (US)	new	new	new	new	new	new
Koeleria capensis	Smook 10302 (US)	KX872190	KX873484	KX872828	-	KX873117	KX872517
Laegaardia ecuadoriense	Laegaard 19339 (US)	new	new	new	new	new	new
Paramochloa effusa	Stergios 19875 (US)	-	MF346087	KX872796	-	KX873053	KX872483
	Gomez 21 (US)	KX872135	KX873416	KX872765	-	KX873052	KX872452
Peyritschia deyeuxioides	Peterson *et al.* 21403 (CAN)	KX872199	KU883568	KX872838	FJ590484	KX873126	-
Peyritschia viridis	Peterson & Valdes-Reyna 18783 (US)	KX872256	KX873549	KX872892	-	FJ377676	KX872584
Phalaris arundinacea	Peterson *et al.* 18383 (CAN)	KX872202	KX873497	KX872841	-	FJ377669	KX872527
Podagrostis aequivalvis	Saarela & Percy 1307 (CAN)	KX871907	KX873503	KX872847	-	KX873136	KX872262
Polypogon australis	Peterson *et al.* 1551 (US)	KX872212	FJ394582	FJ394582	-	FJ377671	KX872536
Polypogon chilensis	da Silva *et al.* 1122 (ICN)	new	new	new	new	new	-
Polypogon elongatus	da Silva & Valduga 952 (ICN)	new	new	new	new	new	-
	da Silva *et al.* 1122 (ICN)	new	new	new	new	new	new
Relchela panicoides	Peterson *et al.* 17364 (US)	KX872219	KX873514	KX872856	-	KX873147	KX872544

	Peterson *et al.* 17334 (US)	KX872220	KX873515	KX872855	-	KX873148	KX872545
Rostraria pumila	Peterson & Rosenberg 14444 (US)	KX872221	KX873516	KX872857	-	KX873149	KX872548
Torreyochloa pallida	Saarela & Percy 1187 (CAN)	KX872228	KX873524	KX872865	-	KX873157	KX872556
Trisetum brasiliense	Chase 8304 (US)	-	new	new	-	new	new
Trisetum bulbosum	Claude-Joseph 5720 (US)	new	new	new	new	new	new
Trisetum flavescens	Kalheber 379 (CAN)	KX872238	KX873534	KX872873	-	KX873165	KX872564
Trisetum spicatum	Peterson *et al.* 19768 (CAN)	KX872253	KX873546	KX872889	-	FJ377674	KX872581

Table 2. Summary information for DNA sequences, alignment length, number of indels and model of evolution for each DNA region and combined DNA datasets.

	atpF-atpH	*matK*	*rps16*	*trnL-trnF*	**Plastid combined**	ITS	ETS	**Nuclear**	**Total**
Missing sequence	7	3	31	0	**41**	0	9	**9**	**50**
New sequences	118	119	78	81	**396**	120	114	**234**	**630**
Published sequences	26	29	42	70	**167**	31	28	**59**	**226**
Sequences included in the analyses	**144**	**148**	**120**	**151**	**563**	**151**	**142**	**293**	**856**
Alignment length	705	892	990	804	**3391**	646	629	**1275**	**4666**
Indels	4	-	5	6	**15**	-	-	-	**15**
Substitution model	F81+I (*atpF*) GTR (spacer and *atpH*)	GTR+G	GTR+G	GTR	**-**	SYM+G (ITS1) SYM+I (5.8S) GTR+G (ITS2)	GTR+G	**-**	-

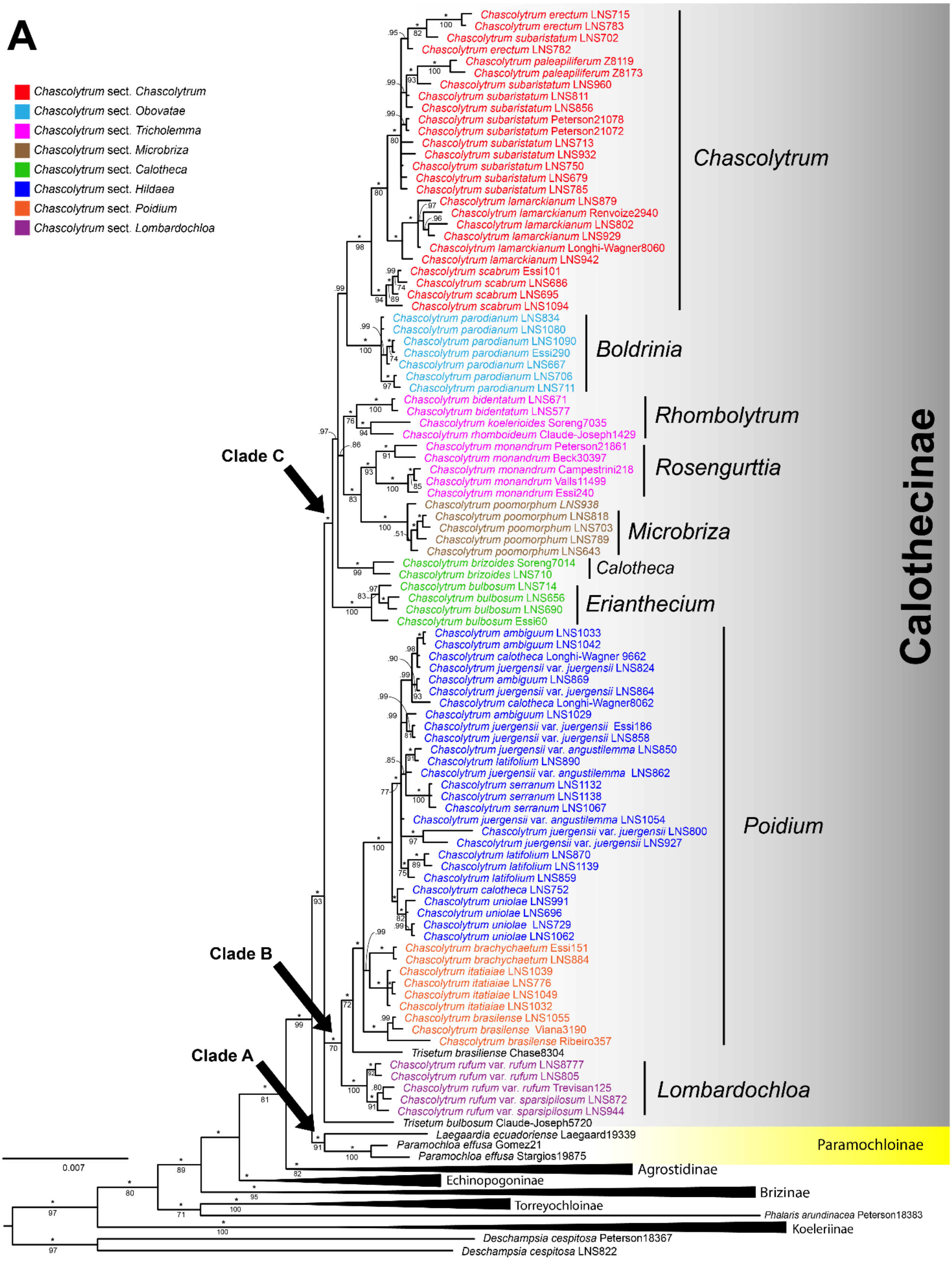


Figure 1. A. Bayesian (BI) consensus phylogram from the analysis of the combined DNA dataset (*atpF-atpH*, *matK*, *rps16* intron, *trnL-trnF*, ITS, and ETS). Numbers above the branches indicates the Bayesian (BI) posterior probabilities (PP) and bellow the branches indicate Maximum Likelihood (ML) bootstrap supports (BS). Asterisk indicates full support (PP = 1) for the BI analyses. Species names in color indicate previous sectional classification for *Chascolytrum* s.l. (da Silva *et al.*, 2020A). Shaded colors (gray and yellow) were used to differentiate subtribes.

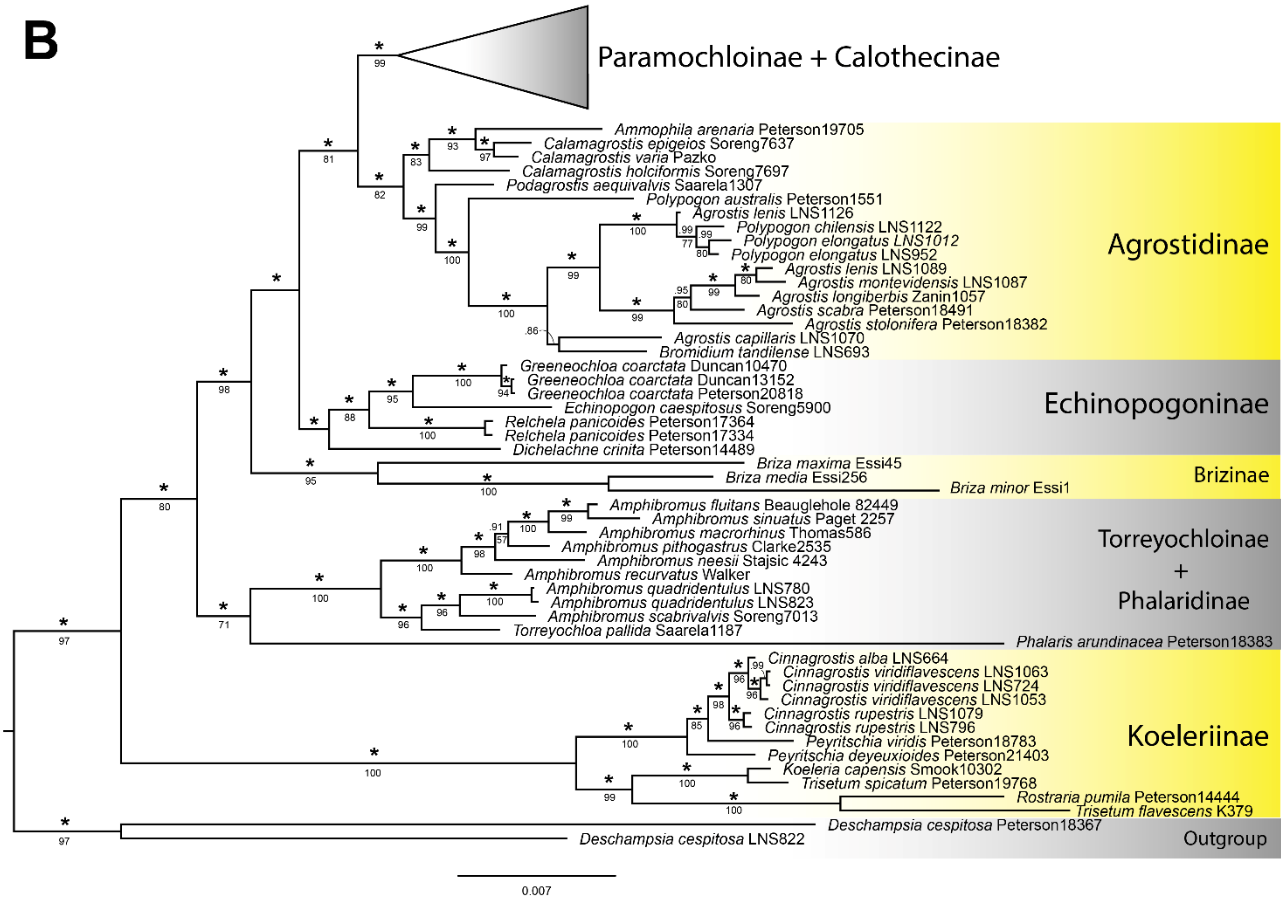
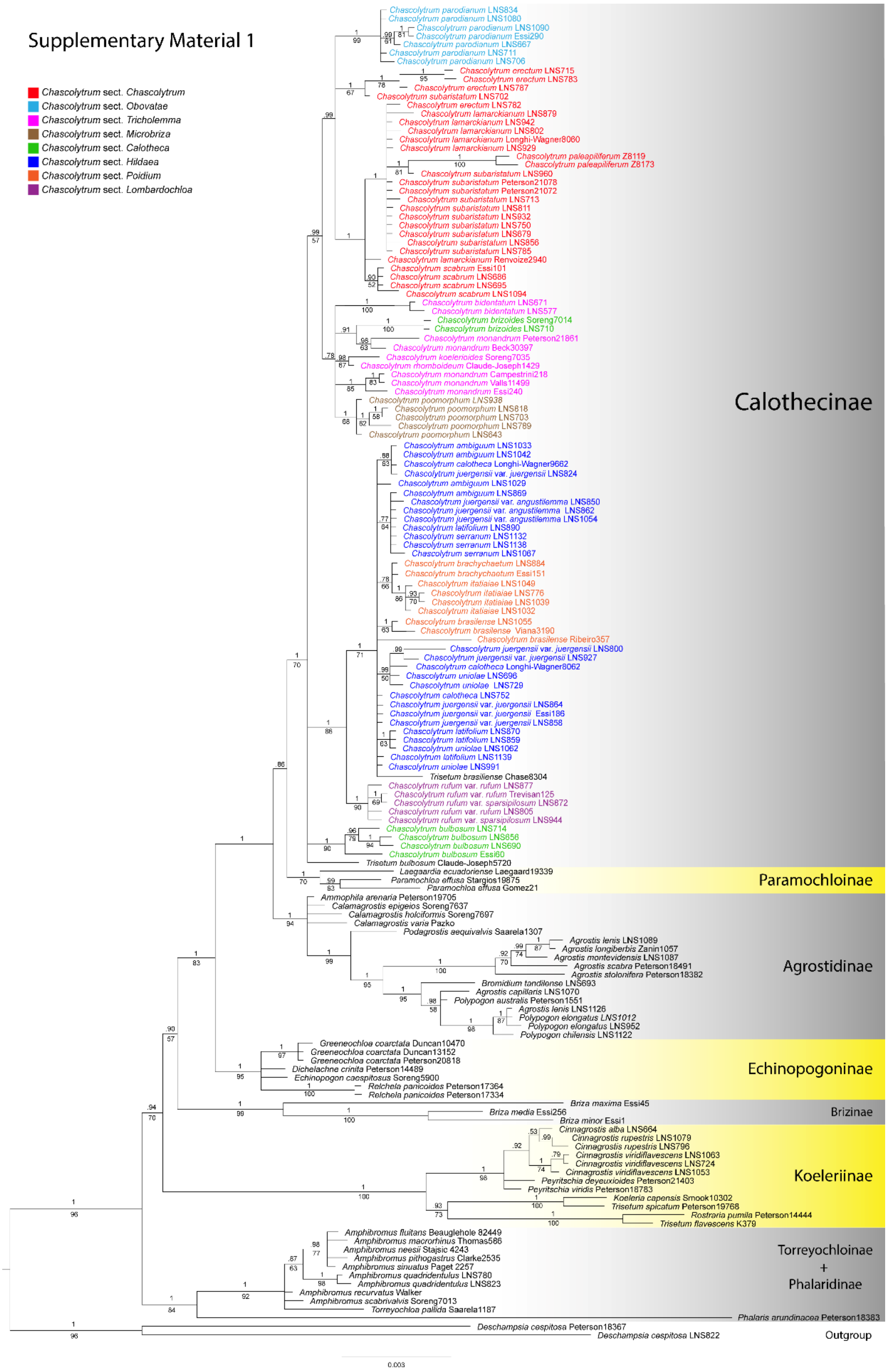
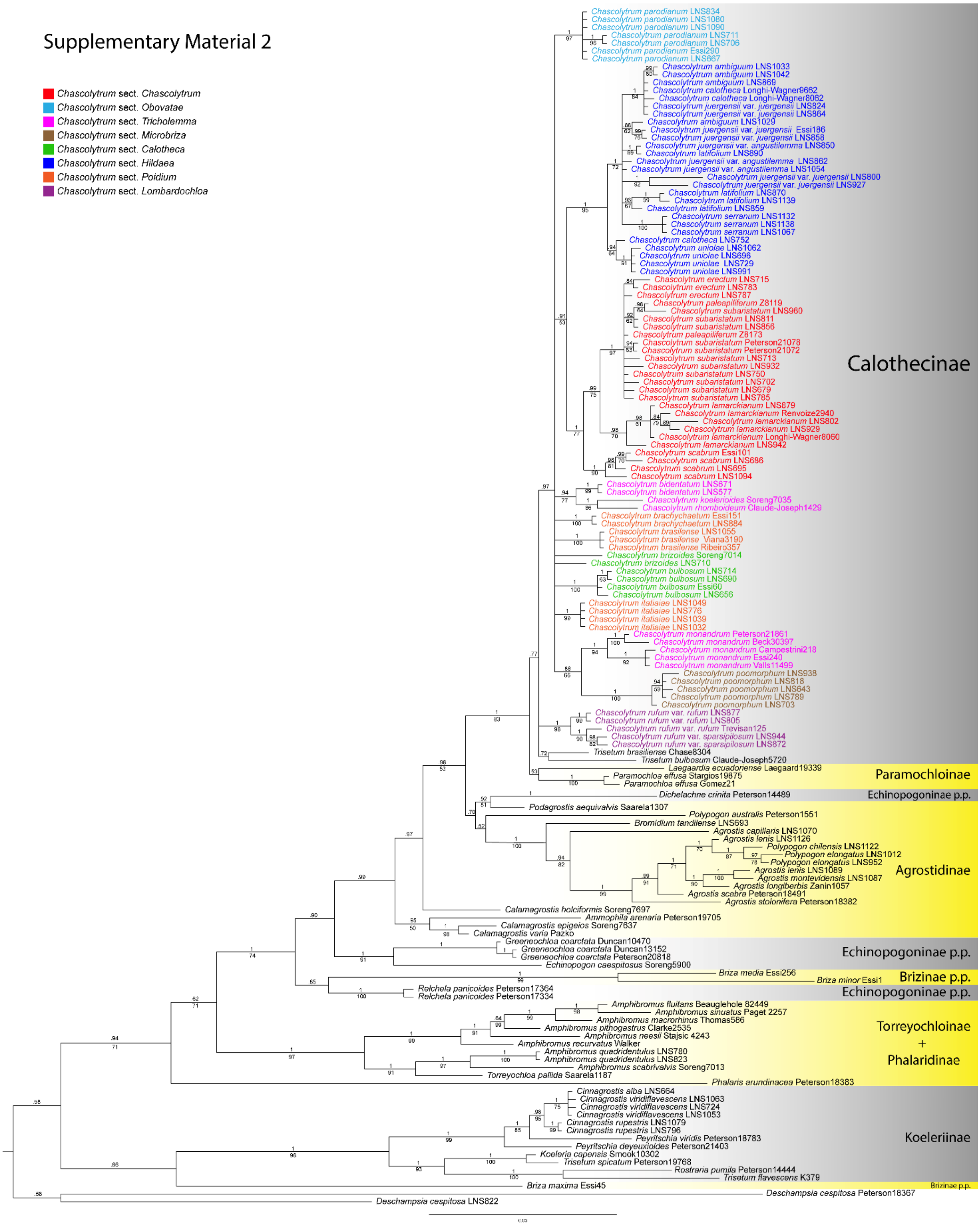


Figure 1. B. Bayesian (BI) consensus phylogram from the analysis of the combined DNA dataset (*atpF-atpH*, *matK*, *rps16* intron, *trnL-trnF*, ITS, and ETS). Numbers above the branches indicates the Bayesian (BI) posterior probabilities (PP) and bellow the branches indicate Maximum Likelihood (ML) bootstrap supports (BS). Asterisk indicates full support (PP = 1) for the BI analyses. Shaded colors (gray and yellow) were used to differentiate subtribes.



Supplementary Material 1. Bayesian (BI) consensus phylogram from the analysis of the combined plastid DNA dataset (*atpF*-*atpH*, *matK*, *rps16* intron, *trnL*-*trnF*). Numbers above the branches indicates the Bayesian (BI) posterior probabilities (PP) and bellow the branches indicate Maximum Likelihood (ML) bootstrap supports (BS). Asterisk indicates full support (PP = 1) for the BI analyses. Species names in color indicate previous sectional classification for *Chascolytrum s.l.* (da Silva *et al.*, 2020A). Shaded colors (gray and yellow) were used to differentiate subtribes.

Supplementary Material 2



Supplementary Material 1. Bayesian (BI) consensus phylogram from the analysis of the combined nuclear DNA dataset (ITS and ETS). Numbers above the branches indicates the Bayesian (BI) posterior probabilities (PP) and bellow the branches indicate Maximum Likelihood (ML) bootstrap supports (BS). Asterisk indicates full support (PP = 1) for the BI analyses. Species names in color indicate previous sectional classification for *Chascolytrum* s.l. (da Silva *et al.*, 2020A). Shaded colors (gray and yellow) were used to differentiate subtribes.

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

A presente tese apresentou contribuições significativas para um melhor entendimento sobre a sistemática da tribo Poeae *s.l.*, elucidando diversas relações filogenéticas entre as subtribos reconhecidas no grupo "Aveneae *type*", especialmente as relações internas da subtribo Calothecinae e do gênero *Chascolytrum s.l.* Com base em evidências moleculares e morfológicas, uma nova taxonomia para este grupo foi proposta. No total, foram descritas três novas seções para *Chascolytrum s.l.*, um novo táxon para a ciência, dois novos gêneros para Calothecinae e uma nova subtribo. Além disso, foram resgatados seis gêneros anteriormente incluídos na sinonímia de *Chascolytrum s.l.* e uma espécie, incluída anteriormente na sinonímia de *C. subaristatum,*

Em um primeiro momento, foram investigadas as relações de parentesco entre as espécies do gênero *Chascolytrum s.l.*, através do uso de marcadores moleculares combinados com dados morfológicos (evidência total). Neste primeiro capítulo, já publicado (da Silva *et al.*, 2020a), foi possível corroborar que *Chascolytrum s.l.* é um grupo morfologicamente variável, com um histórico taxonômico complexo. Embora algumas relações de parentesco ainda tenham permanecido pouco resolvidas, principalmente em virtude de uma politomia posicionada na base do clado *Chascolytrum s.l.*, o uso combinado de marcadores moleculares e dados morfológicos permitiu reconstruir uma árvore filogenética com maior resolução e suporte comparado aos estudos de Essi *et al.* (2008) e Persson & Rydin (2016). Desta forma, foi possível propor uma classificação infragenérica para *Chascolytrum s.l.*, com respaldo nos princípios do monofiletismo. Foram reconhecidas oito seções morfologicamente diagnosticáveis, sendo três novas e três monoespecíficas: *Calotheca*, *Chascolytrum*, *Hildaea*, *Lombardochloa*, *Microbriza*, *Obovatae, Poidium* e *Tricholemma*. Este estudo foi o primeiro a reconhecer uma classificação interna para o gênero. Do ponto de vista prático, uma classificação infragenérica permite que grupos morfológicos sejam identificados com mais facilidade por outros pesquisadores que trabalham com vegetação campestre, principalmente no Sul do Brasil, onde o gênero é mais diversificado. Além de descrição morfológica para cada seção, foram apresentadas imagens das espécies tanto em lupa, evidenciando características de importância taxonômica, quanto em campo, além de uma chave para a identificação das seções propostas.

As análises filogenéticas conduzidas no primeiro capítulo também permitiram resolver a posição taxonômica de *Chascolytrum erectum*, anteriormente incluída na sinonímia de *C. subaristatum* (Essi *et al.*, 2017), como uma espécie distinta. Os acessos de *C. erectum* incluídos na análise [*sensu* Longhi-Wagner (1987)] formaram um clado fortemente suportado em todas as análises, sem mistura com nenhum acesso de *C. subaristatum.* Esta última espécie, no entanto, não seria monofilética. Análises filogenéticas mais recentes, realizadas no terceiro capítulo desta tese, evidenciaram que ainda existe mistura entre os acessos de *C. erectum* e *C. subaristatum* e estudos futuros para compreender melhor as relações filogenéticas, evolutivas e morfológicas entre estes dois táxons ainda são necessárias.

Durante expedições de coleta a campo, populações de *Chascolytrum* com morfologias distintas foram registradas e consideradas potencialmente como novos táxons para a ciência. Após análises morfológicas mais detalhadas, uma destas populações (*LNS834*), coletada no Parque Nacional dos Aparados da Serra – Itaimbezinho, em Cambará do Sul (RS), foi temporariamente denominada “*Chascolytrum* aff. *parodianum*” (da Silva *et al.*, 2020a), devido a suas semelhanças com esta espécie, até então considerada restrita ao Bioma Pampa. Nas análises filogenéticas do primeiro capítulo, o acesso (*LNS834*) pertencente à população do Itaimbezinho incluído na análise se agrupou com os acessos típicos de *C. parodianum* coletados no Pampa. Expedições de coletas subsequentes permitiram o registro de três novas populações de *C. parodianum* para a região dos Aparados da Serra, aumentando significativamente a distribuição geográfica da espécie. Sete acessos de *C. parodianum*, representando toda sua amplitude geográfica, foram incluídas nas análises filogenéticas e formaram um clado com suporte total. Entretanto, diferenças no tamanho e altura das plantas, inflorescências e espiguetas, chamam atenção nas populações encontradas em altitudes mais elevadas. Por isso, um estudo baseado em dados morfométricos vem sendo conduzido em parceria com o estudante Leonardo da Silveira de Souza, buscando compreender a influência da altitude na diversidade morfológica de *C. parodianum*. Futuramente, análises filogenéticas e filogeográficas serão importantes para entender melhor a história evolutiva deste táxon.

Uma segunda população com morfologia distinta (*LNS1067*), coletada nos arredores e dentro do Parque Nacional da Serra Geral, em Cambará do Sul (RS), foi primeiramente identificada como *Chascolytrum ambiguum*. Contudo, uma análise morfológica mais detalhada em lupa permitiu a visualização de tricomas distribuídos na superfície da pálea, que é glabra em *C. ambiguum*. O táxon morfologicamente mais próximo, com registro para a mesma região, e que apresenta páleas pilosas é *Chasolytrum juergensii* var. *angustilemma*. No entanto, este último táxon apresenta lemas estrigosos, característica ausente nos exemplares coletados da população *LNS1067*. Um acesso de *LNS1067* foi incluído nas análises filogenéticas realizadas no primeiro capítulo [(sob *Chascolytrum* sp. em da Silva *et al.*, (2020a)], o qual não agrupou com nenhum acesso dos dois táxons mencionados acima. Durante a realização Período de Doutorado Sanduíche no Exterior (PDSE) do autor no Canadian Museum of Nature - Canadá, foram realizadas análises de microscopia eletrônica de varredura (MEV) comparando a ornamentação dos lemas e páleas de *Chascolytrum* sp. *C. ambiguum* e *C. juergensii* var. *angustilemma*, revelando diferenças morfológicas significativas entre os três táxons. Estes dados compõem o segundo capítulo da tese, e foram úteis para o reconhecimento e publicação de um novo táxon para a ciência, *Chascolytrum serranum* (da Silva *et al.*, 2020b). Nas análises filogenéticas mais recentes, realizadas no terceiro capítulo da tese, os três acessos de *C. serranum* formaram um clado com forte suporte, aparte das demais espécies morfologicamente semelhantes mencionadas, corroborando seu reconhecimento como uma espécie distinta.

Devido à pouca resolução obtida nas filogenias apresentadas no primeiro capítulo da tese (da Silva *et al.*, 2020a), a necessidade de incluir as amostras de *Chascolytrum s.l.* em um contexto filogenético mais amplo incluindo representantes de outras subtribos próximas, veio à tona. Desta forma, durante o período de Doutorado Sanduíche do autor, buscou-se reconstruir as relações filogenéticas entre as subtribos Calothecinae, Agrostidinae e Echinopogoninae, evidenciadas como filogeneticamente próximas, porém sem resolução, por Saarela *et al.* (2017). Os resultados obtidos foram incorporados no terceiro e último capítulo desta tese.

Este último capítulo trouxe novas evidências acerca da sistemática e taxonomia de Calothecinae e *Chascolytum s.l.* Com a inclusão de uma amostragem mais compreensiva (mais de 150 acessos) e com o aumento no número de marcadores moleculares, foi possível resolver as principais politomias evidenciadas no primeiro capítulo da tese (da Silva *et al.*, 2020a). Este estudo representa a mais completa filogenia reconstruída para Calothecinae até o momento, por incluir também representantes dos gêneros *Laegaardia* e *Paramochloa*, posicionados na subtribo por Peterson *et al.* (2019). Além disso, 23 das 24 espécies de *Chascolytrum s.l.* foram amostradas, incluindo *C. koelerioides* e *C. rhomboideum*, não incluídas anteriormente em da Silva *et al.* (2020a). Este estudo revelou que tanto Calothecinae quanto *Chascolytrum s.l.* são polifiléticos, devido ao posicionamento de *Trisetum brasiliense* e *T. bulbosum* dentro do clado *Chascolytrum s.l.*, embora suas relações taxonômicas não tenham sido totalmente esclarecidas. Como resultado, estas duas espécies foram formalmente incluídas em Calothecinae como *incertae sedis*, e novos estudos incluindo estas e outras espécies Sul-Americanas de *Trisetum s.l.* são de extrema importância para determinar seus posicionamentos genéricos. Com estas evidências e devido à escassez de informações sobre estas espécies, um projeto de Pós-Doutorado Júnior (PDJ) foi submetido ao CNPq, visando a continuidade dos estudos sobre a história evolutiva deste grupo. Para isto, pretendemos usar o sequenciamento completo do plastoma, através de *next-generation sequencing.* Este projeto será executado em parceira com o Dr. Jeffery M. Saarela, do Canadian Museum of Nature, supervisor do período Sanduíche do autor, e da Dra. Verônica Aydos Thode, atualmente Pós-Doutoranda do PPG Botânica da UFRGS.

Ao final desta tese, o reconhecimento de *Chascolytrum s.l.*, como até então circunscrito, tornou-se impraticável. Desta forma, as 24 espécies anteriormente reconhecidas em *Chascolytrum s.l.* foram divididas em nove gêneros distintos, respeitando grupos monofiléticos e morfologicamente diagnosticáveis: *Boldrinia* (uma espécie), *Calotheca* (uma espécie), *Chascolytrum s.s.* (seis espécies), *Erianthecium* (uma espécie), *Lombardochloa* (uma espécie), *Microbriza* (uma espécie), *Poidium* (nove espécies), *Rhombolytrum* (três espécies) e *Rosengurttia* (uma espécie). Os gêneros *Boldrinia* e *Rosengurttia* são novos para a ciência. Além disso, devido à morfologia contrastante de *Laegaardia* e *Paramochloa* em relação aos demais gêneros de Calothecinae, a subtribo Paramochloinae foi proposta para melhor abrigar estes dois gêneros. Desta forma, o número de gêneros formalmente reconhecidos em Calothecinae aumentou de um (Soreng *et al.*, 2017) para nove, além de duas espécies provisoriamente definidas como *incertae sedis*. Além de descrições morfológicas e novas combinações, o terceiro capítulo apresenta uma chave de identificação para os gêneros de Calothecinae e Paramochloinae.

Em um contexto mais amplo, os resultados do terceiro capítulo desta tese suportaram a subtribo Agrostidinae como irmã do clado formado por Calothecinae e Paramochloinae, enquanto Echinopogoninae é o grupo-irmão destas três subtribos. Contudo, algumas incongruências foram observadas entre as árvores baseadas em marcadores nucleares e aquelas baseadas em marcadores plastidiais, principalmente em relação ao posicionamento de *Briza maxima* (Brizinae) e *Dichelachne* (Echinopogoninae). Estas incongruências também foram evidenciadas recentemente no estudo de Tkach *et al.* (2020), e supostamente causadas devido a eventos de hibridação entre diferentes linhagens. Além disso, os gêneros *Agrostis*, *Polypogon* e *Bromidium* não são monofiléticos, e suas afinidades serão futuramente averiguadas em um estudo filogenético baseado em dados moleculares gerados durante o Período de Doutorado Sanduíche do autor. Estes resultados, contudo, não fazem parte do contexto da tese.

Por fim, o reconhecimento de gêneros distintos para o clado *Chascolytrum s.l.* permitirá que estudos mais detalhados sejam realizados em grupos cujas relações internas ainda permanecem pouco resolvidas, como os gêneros *Chascolytrum* e *Poidium*. Neste sentido, um estudo usando dados morfométricos, investigando as afinidades e limites morfológicos entre as espécies *Poidium ambiguum*, *P. calotheca*, *P. juergensii* var. *angustilemma* e *P. juergensii* var. *juergensii*, bem como a diversificação do gênero *Poidium* nos campos de altitude do Sul do Brasil estão em andamento.

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ANEXO

PRODUÇÃO BIBLIOGRÁFICA DURANTE O DOUTORADO

I. Artigos publicados em periódicos

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