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TAXONOMIA INTEGRATIVA EM LIQUENS PARMELIOIDES (*PARMELIACEAE*) DO BRASIL

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RODRIGUES, Andressa Silva, M.S., Universidade Federal de Mato Grosso do Sul, dezembro de 2023. **Taxonomia integrativa em liquens parmelioides (*Parmeliaceae*) do Brasil.** Orientadora: Aline Pedroso Lorenz. Coorientadora: Luciana da Silva Canêz.

Fungos liquenizados compreendem diferentes organismos funcionando como um ecossistema autossustentável, uma complexa forma de vida mutualística benéfica entre fungos, algas verdes e/ou cianobactérias, além de outros microrganismos. Possuem mais de 19 mil espécies conhecidas, sendo *Parmeliaceae* a mais diversa. Ela é dividida em duas subfamílias: *Protoparmelioideae* e *Parmelioideae*. Esta última, ainda é subdividida informalmente em sete clados, sendo que o clado Parmelioide, com representantes predominantemente foliosos, agrupa cerca de 67% da diversidade da família. Por muitos anos os liquens têm sido identificados baseado apenas em características morfológicas, mas com adventos moleculares, novas ferramentas para identificação desses organismos têm ganhado espaço, como o uso do *DNA barcoding*, e mais atualmente a associação desse com a taxonomia integrativa. Esta abordagem tem como objetivo utilizar diferentes fontes de dados para a identificação e/ou delimitação específica, e tem mostrado alta eficiência nos resultados obtidos. Assim, nosso estudo tem como objetivo utilizar a abordagem integrativa e o *DNA barcoding* (baseado no marcador nuITS) nas espécies do clado Parmelioide de *Parmeliaceae* de algumas localidades do Brasil. No primeiro capítulo, realizamos a combinação das espécies *Canoparmelia amazonica* e *Myelochroa lindmanii* no gênero *Parmelinella*. Confirmamos o posicionamento dessas espécies através da filogenia molecular (nuITS), propondo assim, a combinação de ambas sob o gênero *Parmelinella*. Além disso, também fornecemos descrições morfológicas e químicas e imagens, subsidiando a nova classificação. No segundo artigo propomos *Hypotrachyna neohorrescens* como nova espécie (subgênero *Parmelinopsis*). *Hypotrachyna neohorrescens* é proximamente relacionada com *H. mcmulliniana*, porém com baixa, utilizando apenas o marcador nuITS. A distinção filogenética de ambas só foi possível com marcadores concatenados (nuITS+mtSSU). Morfologicamente as espécies também apresentam diferenças sutis. Além disso, não encontramos diferenças morfológicas e químicas entre *Hypotrachyna neohorrescens* e *H. horrescens*, mas geneticamente, possuem grande divergência, sendo, portanto, consideradas espécies crípticas. No terceiro e último capítulo realizamos a identificação integrativa de 90 espécimes de liquens coletados no extremo sul do Brasil. Inicialmente fizemos a identificação morfológica, encontrando 39 morfoespécies. Com a identificação molecular baseada em *DNA barcoding* (marcador nuITS), utilizando o BLASTn para verificar as

espécies mais próximas das sequências, e, posteriormente, reconstruímos as filogenias por gênero e usando como sequências de referências aquelas disponíveis no banco de dados público – GenBank. Baseado nos resultados das três abordagens (*DNA barcoding*, análises do BLASTn e filogenia), entre as 39 morfoespécies, 22 continham sequências homônimas, ou seja, sequências com mesmo nome no banco de dados GenBank. Todavia os resultados das filogenias mostraram que 20 morfoespécies agruparam com sequências de mesma identidade, mas 10 delas apresentaram também diferentes espécies nos clados. Entre as 17 morfoespécies sem sequência homônimas, somente seis mostraram ser geneticamente distintas das demais sequências do GenBank, enquanto as demais apresentaram alguma incongruência entre as abordagens. Encontramos pelo menos 22 casos de possíveis complexos de espécies em nossos dados, os quais não foram solucionados utilizando apenas o marcador nuITS. Nossos resultados corroboram com estudos recentes, os quais destacam a falta de resolução do marcador nuITS em complexos de espécies. Tornando assim a tarefa de usar apenas *DNA barcoding* como ferramenta de identificação específica ainda mais desafiadora.

Palavras-chaves: banco de dados, biodiversidade, *DNA barcoding*, filogenia, nuITS.

RODRIGUES, Andressa Silva, M.S., Universidade Federal de Mato Grosso do Sul, dezembro de 2023. **Integrative taxonomy in parmelioides lichens (*Parmeliaceae*) from Brazil.** Orientadora: Aline Pedroso Lorenz. Coorientadora: Luciana da Silva Canêz.

Lichenized fungi comprise different organisms functioning as a self-sustaining ecosystem, a complex form of beneficial mutualistic life between fungi, green algae, and/or cyanobacteria, in addition to other microorganisms. They have more than 19 thousand known species, with *Parmeliaceae* the most diverse, and divided into two subfamilies: *Protoparmelioideae* and *Parmelioideae*. The latter is still informally subdivided into seven clades, with the Parmelioid clade having predominantly foliose representatives, accounting for around 67% of the family's diversity. For many years, lichens have been identified based only on morphological characteristics, but with molecular advances, new tools for identifying these organisms gained space, such as the use of DNA barcoding, and more currently its association with integrative taxonomy. This approach aims to use different data sources for specific identification or delimitation and has presented the best results among the most current studies. Thus, our study aims to use an integrative approach and DNA barcoding (based on the nuITS marker) in species of the Parmelioid clade of *Parmeliaceae* from some localities in Brazil. In the first chapter, we combined two species *Canoparmelia amazonica* and *Myelochroa lindmanii* into *Parmelinella*. We confirmed the positioning of these species through molecular phylogeny (nuITS), thus proposing the combination of both under the genus *Parmelinella*. In addition, we also provide morphological and chemical descriptions and images, subsidizing the new classification. In the second article, we propose *Hypotrachyna neohorrescens* as a new species (subgenus *Parmelinopsis*). *Hypotrachyna neohorrescens* was closely related to *H. mcmulliniana*, but with low resolution using only the nuITS marker. The phylogenetic distinction of both was possible only with concatenated markers (nuITS+mtSSU). Morphologically, both species also present subtle differences. Moreover, we did not find morphological or chemical differences between *Hypotrachyna neohorrescens* and *H. horrescens*, but genetically, they present high divergences, thus being considered cryptic species. In the third and final chapter, we carried out the integrative identification of 90 specimens of lichens. Initially, we identified based on morphology, finding 39 morphospecies. Then molecular identification was carried out through DNA barcoding (nuITS marker), using BLASTn to verify the species closest to the study sequences and later we reconstructed the

phylogenies by genus and used as homonymous sequences those available in the public database – GenBank. Based on the results of the three approaches (DNA barcoding, BLASTn analysis, and phylogeny), Among the 39 morphospecies, 22 contained homonymous sequences, i.e., sequences with the same name in the GenBank database. However, the phylogeny results show that 20 of these morphospecies grouped with sequences of the same identity, while 10 also presented distinct species in the same clade. Among the 17 morphospecies without homonymous sequences, only six were genetically distinct from the other sequences in GenBank, while the others showed some incongruity between the approaches. We found at least 22 cases of possible species complexes in our data, which were not resolved using only the nuITS marker. Our results corroborate recent studies that have already highlighted the lack of resolution of the nuITS marker in species complexes. Thus, making the task of using only DNA barcoding as a specific identification tool even more challenging.

Keywords: database, diversity, DNA barcoding, nuITS, phylogeny.

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Sumário

Introdução geral

Fungos liquenizados, popularmente chamados de liquens, compreendem diferentes organismos funcionando como um ecossistema autossustentável, uma complexa forma de vida mutualística benéfica entre fungos, algas verdes e/ou cianobactérias, além de outros microrganismos [1]. A diversidade desse grupo é de aproximadamente 115 famílias, 995 gêneros e mais de 19.000 espécies [2]. Por apresentarem respostas rápidas a perturbações ambientais, os liquens são considerados potenciais indicadores de alterações climáticas [3], desta forma são utilizados em diversos trabalhos como agentes bioindicadores e biomonitores da qualidade do ar atmosférico [4-5-6]. Espécies de *Parmeliaceae* normalmente fazem parte de tais estudos [7, 8, 9].

Parmeliaceae, atualmente, é a família mais diversa conhecida entre os fungos liquenizados; conta com mais de 2700 espécies e 77 gêneros [2], possui como principal centro de diversificação o hemisfério sul [10]. Considerando análises filogenéticas temporais, *Parmeliaceae* é dividida em duas subfamílias: *Protoparmelioideae* (incluindo *Protoparmelia* M. Choisy e *Maronina* Hafellner & R.W. Rogers) e *Parmelioideae* (incluindo os demais gêneros) [11]. *Parmelioideae* é ainda subdividida em sete principais clados bem definidos [11], dentre os quais Parmelioide é o maior e mais diverso, representado por cerca de 25 gêneros e mais de 1800 espécies [2, 10, 12]. Predominantemente, os representantes desse clado apresentam forma de crescimento folioso, superfície inferior rizinada, apotécios laminais e ascósporos incolores simples [13]. O processo de identificação dos fungos liquenizados tem mudado e sido aprimorado cada vez mais ao longo do tempo. Até os anos 2000 a identificação era feita a partir de caracteres fenotípicos, principalmente morfológicos e químicos, e atualmente com os avanços da biologia molecular e das ferramentas de bioinformática, novas metodologias têm sido utilizadas na taxonomia dos liquens [14].

Nesse cenário, a abordagem do *DNA barcoding* trouxe o objetivo de sistematizar e agilizar a identificação de táxons a partir de uma região curta, específica e com altas taxas evolutivas do genoma [15]. Para fungos em geral, incluindo os liquenizados, é proposta a região dos espaçadores internos transcritos do DNA ribossomal nuclear (nuITS) como *barcoding* universal [16]. A abordagem de DNA *barcoding* mostrou certa taxa de sucesso em muitos grupos de liquens, principalmente quando os locais de estudo apresentavam bancos de dados locais com identificações acuradas, ou seja, sequências de espécimes devidamente identificados e verificados, quando possível, por especialistas do

grupo [17, 18]. No entanto, a utilização do *DNA barcoding* associado apenas a uma ferramenta de busca rápida de identificação como o BLAST (*Basic Local Alignment Search Tool*) tem mostrado baixa taxas de sucesso, principalmente por fatores como: 1) sequências erroneamente identificadas disponíveis em bancos de dados públicos [19]; 2) falta de bancos de dados locais, principalmente em países em desenvolvimento megadiversos [20, 21]; 3) presença de espécies crípticas e/ou com grande plasticidade fenotípica [22, 23]; e 4) falta de sequências de espécimes-tipos, ou seja, exemplares no qual o autor (a) se baseou para designar a espécie.

Diante disso, a taxonomia integrativa busca vincular evidências de fontes de dados independentes, como morfologia, filogenia molecular, ecologia, biogeografia, entre outros, a fim de identificar e/ou delimitar espécies [24, 25]. Para liquens as principais fontes de dados são obtidas a partir do fenótipo e da filogenia molecular, metodologia que tem demonstrado taxas de identificações mais confiáveis [21, 22, 26, 27]. Na taxonomia tradicional dos liquens, um grande desafio é a falta de padronização dos dados fenotípicos disponíveis, o que dificulta sua integração e comparação com outras abordagens. Assim, buscando organizar e acelerar os processos de descrições e combinações de táxons, sistematizações de práticas taxonômicas foram propostas a fim de uniformizar os resultados, contribuindo assim com a perspectiva integrativa [28]. Outro importante viés da taxonomia integrativa é fornecer diferentes tipos de dados afim de subsidiar recursos para estudos complementares como, por exemplo, as construções de inventários de biodiversidade que podem servir de base para trabalhos ecológicos de biomonitoramento e conservação de regiões ameaçadas. Dessa forma, esse estudo tem como principal objetivo utilizar a taxonomia integrativa em espécies de *Parmeliaceae* do clado Parmelioide, localizados em algumas regiões do centro-oeste, sudeste e sul do Brasil.

Revisão da literatura

Taxonomia e relações filogenéticas em *Parmeliaceae* Eschw.

A família *Parmeliaceae* foi descrita por Eschweiler em 1824 [29], com seis gêneros: *Collema* Weber ex F.H. Wigg., *Cornicularia* (Schreb.) Ach., *Hagenia* Eschw., *Lecanora* Ach., *Parmelia* Ach. e *Sticta* (Schreb.) Ach. Atualmente apenas *Parmelia* e *Cornicularia* se mantém em *Parmeliaceae*, enquanto, *Collema* pertence à *Collemataceae*, *Lecanora* a *Lecanoraceae*, *Sticta* a *Lobariaceae*, e *Hagenia*, hoje sinônimo de *Anaptychia* Körb., a *Physciaceae* [2].

Parmeliaceae é uma das famílias de fungos liquenizados mais bem estudada, taxonômica e filogenéticamente. Grande parte de seus táxons foram propostos a partir do gênero *Parmelia*. Acharius em 1803 [30], descreveu *Parmelia* baseado em características fenotípicas muito amplas, como posição e forma dos apotécios, englobando muitos táxons distintos no gênero, com o tempo, muitos gêneros acabaram sendo retirados desse grupo. Acharius também contribuiu com a descrição de mais três gêneros para a família: *Alectoria* Ach., *Cetraria* Ach. e *Evernia* Ach. Ao longo dos anos, outras famílias foram sinonimizadas em *Parmeliaceae*, tais como: *Alectoriaceae*, *Anziaceae*, *Hypogymniaceae* e *Usneaceae* [10]. A ontologia do ascoma (forma um excípulo cupular), descrita em 1973 por Henssen & Jahns [31], se mantém como a única característica compartilhada pelos membros da família.

Parmeliaceae tem como centro de diversificação o hemisfério sul, abrangendo grande parte da América do Sul, sul da África e Austrália [10]. No Brasil, os estudos liquenológicos iniciaram com a chegada da futura Imperatriz D. Carolina Josefa Leopoldina em 1817, juntamente com Martius e Spix, naturalistas responsáveis pela primeira expedição científica oficial apoiada pelo governo. Posteriormente, demais naturalistas, em sua maioria europeus, vinham, assim como Martius e Spix com a principal intenção de coletar plantas, mas acabavam esporadicamente também coletando alguns espécimes de liquens, os quais foram enviados e estudados por outros liquenólogos da época [32]. O estudo da liquenologia brasileira, teve maior visibilidade com Vainio (a partir de 1885) e Malme (entre 1892 – 1903), inclusive com *Parmeliaceae* [32].

Estudando o gênero *Parmelia*, Vainio [33] criou três seções para agrupar espécies mais semelhantes entre si, como: seção *Amphigymnia* caracterizada pelas margens inferiores dos lobos nuas, presença ou não de cílios e apotécios subpedicelados; seção *Hypotrachyna*, agrupando espécies com abundantes rizinas dispostas até as

margens dos lobos, ou com zona marginal inferior nua muito estreita e papilada; seção *Xanthoparmelia*, espécies com córtex superior amarelado, talo adpresso e apotécios sésseis. Além de descrever vários novos táxons.

Em duas expedições científicas, financiadas pelo Fundo Regnelliano, G.O.A. Malme, durante os anos de 1892-1894 e 1901-1903, coletou cerca de 6 mil amostras de liquens na América do Sul, passando pela Argentina, Brasil, Paraguai e Uruguai [34]. Muitos dos espécimes de *Parmelia*, posteriormente foram estudados por Lynge [35], o qual propôs o gênero *Pseudoparmelia* Lynge e a nova seção *Bicornuta*. Ao longo dos anos, entre os diversos liquenólogos que contribuíram para o conhecimento de *Parmeliaceae*, destacam-se: Fries [36], Tuckerman [37], Krempelhuber [38], Stirton [39], Nylander [40], Hue [41], Zahlbruckner [42], Motyka [43], Berry [44], Asahina [45] e Dodge [46].

Todavia, foi entre os anos 1960-1990, com as inúmeras e fundamentais contribuições de Mason Hale, que *Parmeliaceae* passou a ser melhor compreendida. Ao longo de mais de 20 anos, Hale publicou diversas monografias, novos gêneros e espécies, realizou várias modificações nomenclaturais e designou muitos tipos de espécies [14, 47]. Estudos com *Parmeliaceae* também tiveram contribuições de Culberson & Culberson [48], Ahti [49], Awasthi [50], Esslinger [51], Krog & Swinscow [52], Galloway [53], Elix & Hale [54], Kurokawa [55], Sipman & Aubel [56], Elix [57], Nash III *et al*. [58], Louwhoff & Elix [59] e Hawksworth [60]. Para o Brasil tivemos valiosas contribuições de Osorio *et al.* [61], Fleig [62], Eliasaro & Adler [63], Jungbluth *et al.* [64], Barbosa *et al*. [65], Canêz & Marcelli [66], Marcelli *et al.* [67], Benatti [68], Aptroot *et al*. [69], Buril [70], Cunha *et al*. [71], Hora [72], Honda *et al.* [73], Gerlach *et al.* [74], Zanetti *et al.* [75], Spielmann & Marcelli [76], entre tantos outros.

Estudos baseados em filogenia molecular, iniciaram em *Parmeliaceae* no final dos anos 90 abrindo fronteiras para novas compreensões sobre as relações genéricas e específicas existentes dentro da família [77, 78]. Assim, ao longo desse quase um quarto de século após os primeiros estudos filogenéticos, aprimoramentos da biologia molecular e o desenvolvimento de ferramentas de bioinformática tem contribuído para a compreensão das características utilizadas nas delimitações morfológicas dos táxons, bem como as relações filogenéticas nos gêneros e espécies em *Parmeliaceae*, como em *Hypotrachyna* (Vain.) Hale [79], *Melanelixia* O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw. & Lumbsch e *Melanohalea* O. Blanco, A. Crespo, Divakar, Essl., D. Hawksw.

& Lumbsch [80], *Parmotrema* A. Massal. [81], *Relicinopsis* Elix & Verdon [82], *Remototrachyna* Divakar & A. Crespo [83] e *Xanthoparmelia* (Vain.) Hale [80, 84].

Com mais de 2700 espécies, *Parmeliaceae* é a família mais diversa atualmente conhecida entre os fungos liquenizados [2]. É dividida, ainda, baseado em filogenia temporal, em duas subfamílias: *Protoparmelioideae* (incluindo *Protoparmelia* e *Maronina*) e *Parmelioideae* (incluindo os demais gêneros). A divisão é baseada no tempo de divergência entre os clados, sendo recomendada a classificação em diferentes famílias grupos que divergiram entre 102.13 – 112.88 Ma, e em gêneros entre 29.45 – 32.55 Ma. Dentro da subfamília *Parmelioideae*, sete clados são distinguíveis: Alectorioide (5 gêneros), Anzioide (4), Cetrarioide (5), Hypogymnioide (4), Parmelioide (25) e Usneoide (4) [11, 12]. O clado Parmelioide é o mais diverso e o mais bem estudado. Possui mais de 1800 espécies, e concentra cerca de 67% da diversidade da família [2, 11, 13]. Os representantes desse clado predominantemente apresentam forma de crescimento folioso, superfície inferior rizinada, apotécios laminais e ascósporos incolores simples [13].

DNA barcoding em liquens

Proposto no início dos anos 2000, a técnica do *DNA barcoding* surgiu com o objetivo de facilitar e agilizar o processo de identificação das espécies [15]. A abordagem baseia-se na utilização de uma região curta e específica do genoma para a identificação de táxons, sendo proposto nesse mesmo estudo o gene mitocondrial Citocromo C oxidase I (COI) como *DNA barcode* para animais [15]. Em 2012, os espaçadores internos transcritos do DNA ribossomal nuclear (nuITS), região que compreende dois espaçadores intergênicos (ITS1 e ITS2) e o gene 5.8S foi proposto como *DNA barcode* para fungos e fungos liquenizados [16].

Desde então, a utilização do *DNA barcoding* para discriminação de espécies de liquens tem sido amplamente utilizado. Baseado na técnica *barcoding*, Kelly et al. [17] obtiveram taxas de sucesso de 96% na identificação específica de espécimes de *Usnea* oriundos da Grã-Bretanha e Irlanda. Semelhantemente, Leavitt et al. [103] também demostraram altas taxas de sucesso para a identificação de parmélias marrons da Groelândia, assim como Divakar et al. [18] de *Parmelia* s.str. oriundas de várias regiões do mundo.

Em contrapartida, Orock et al. [20] com amostras de liquens coletadas na República de Camarões, apenas 15% dos espécimes obtiveram porcentagens de identificações ≥ 97 % utilizando o BLASTn NCBI com as sequências disponíveis no banco de dados públicos GenBank. La Torre et al. [106] baseando a identificação molecular também através do BLASTn em espécimes da Antártica, encontraram 11 grupos de espécies com identificações ambíguas. Cenários como esses, tem demostrado que a taxa de sucesso da abordagem *barcoding* é proporcional ao conhecimento da biodiversidade local, ou seja, regiões com bibliotecas de *DNA barcoding* acuradas possuem maior efetividade na identificação específica [105].

Contudo, outros fatores limitantes encontrados na utilização do *DNA barcoding* também estão associados: 1. A baixa resolução do marcador nuITS nos casos de complexos de espécies [24]; 2. Alta taxa de sequências erroneamente identificadas disponíveis em bancos de dados públicos [19, 86]; 3. presença de espécies crípticas e/ou com grande plasticidade fenotípica que ainda não foram taxonomicamente resolvidas [23]; 4. Pouca disponibilidade de sequências das espécies-tipo ou das localidades-tipo para corroborar a proposição de novas espécies que apresentam morfologias muito semelhantes. Assim, uma estratégia que tem ganhado força nos últimos anos para tentar resolver os obstáculos encontrados no processo de identificação ou delimitação das espécies é o uso da taxonomia integrativa.

Taxonomia integrativa

Baseada na utilização de diferentes dados independentes, tais como morfologia, química, filogenia molecular, biogeografia, ecologia entre outros, a taxonomia integrativa busca delimitar táxons [24, 25]. Em estudos com liquens, dados baseados em fenótipo e filogenia molecular são normalmente utilizados, tendo como principal marcador para as reconstruções filogenéticas os espaçadores internos transcritos do DNA ribossomal nuclear (nuITS) [21, 22, 26, 27].

Estudos mais atuais com fungos liquenizados, utilizando a taxonomia integrativa, de maneira geral, seguem os seguintes passos: 1) comparação geral de identidade molecular a partir do BLASTn (*Standard Nucleotide BLAST*) na plataforma https://blast.ncbi.nlm.nih.gov/Blast.cgi, considerando como espécies compatíveis

aquelas que obtiveram porcentagem de identidade ≥ 98 ou 98,5%; 2) BLASTn quando existe um banco de dados local acurado, ou seja, verificado por especialistas; 3) análises filogenéticas baseadas em alinhamentos múltiplos ou a partir de ferramentas de delimitação de espécies (baseadas em distâncias genéticas ou em padrões de diversificação filogenética); e 4) taxonomia integrativa, ou seja, utilização de diferentes conjuntos de dados para a identificação ou delimitação específica, baseado frequentemente em dados fenotípicos e moleculares [21, 22].

Como exemplo dessa abordagem, podemos citar o estudo de Moncada *et al.* [21] com o gênero *Usnea* Dill. ex Adans. (*Parmeliaceae*) na Colômbia. A partir de 15 sequências de nuITS geradas, e contando com um banco local com identificações moleculares acuradas, a compatibilidade de identidade baseada a partir do BLASTn obteve taxa de sucesso entre 7% (com sequências originais do GenBank) a 23% (quando essas sequências foram corrigidas). Quando utilizaram o banco de dados acurado a taxa de identificação subiu para 47%, enquanto a identificação baseada em filogenia molecular obteve sucesso de 80% e a taxonomia integrativa de 100%. Mesmo com apenas três espécies correspondendo aos nomes esperados, enquanto as 12 demais se mostraram espécies candidatas ou hipóteses de espécies, somente com a taxonomia integrativa a identidade das espécies foi confiável [21].

Para a construção de um inventário da diversidade de liquens em uma ilha isolada dos Estados Unidos da América, Leavitt *et al.* [22] usaram a ferramenta de delimitação automática de espécies ASAP, descrita por Puillandre *et al.* [85], para analisar sequências da região nuITS. Foram encontradas 240 espécies candidatas, pertencentes a 24 famílias, sendo 189 espécies identificadas a partir da abordagem integrativa. Destas, baseado no BLASTn, 54% das espécies corresponderam às espécies esperadas, 29 espécies mostraram ser complexos de espécies (correspondendo a porcentagem de identificação valores < 98% - ≥ 97%), enquanto 31% não obtiveram sequências correspondentes no GenBank [22].

Embora cada vez mais estudos sejam desenvolvidos reforçando a necessidade da utilização de abordagens integrativas e melhores práticas taxonômicas para a delimitação e identificação de espécies [28], a ocorrência de sequências de DNA disponíveis no GenBank erroneamente identificadas, principalmente ao nível de espécie, ainda são um fator limitante [19, 86]. Assim, de acordo com Moncada *et al*. [21], estratégias para melhorar a aplicabilidade do *DNA barcoding* nessas situações são: 1)

atualizar as identificações de sequências de referência em bancos de dados públicos ou usar um conjunto de dados de referência verificado por um especialista; 2) realizar o BLAST com um conjunto de dados de referência local com identificações acuradas focando principalmente no grupo de estudo, além de combinar a metodologia com análises filogenéticas; e 3) fechar lacunas geográficas e taxonômicas nos dados de referência existentes.

Por fim, a taxonomia integrativa fornece diferentes evidências para compreender a diversidade de fungos liquenizados, bem como suas relações evolutivas, biogeográficas e ecológicas. Servindo assim como subsídio para diversos estudos, sejam eles taxonômicos, sistemáticos, farmacológicos, de conservação e biomonitoramento ambiental, assim como possíveis fontes de dados para futuros inventários florísticos e reconhecimento de novos táxons.

Objetivo geral

Aplicar a taxonomia integrativa, utilizando a abordagem de *DNA barcoding* combinada com dados fenotípicos em espécies de liquens Parmelioides (*Parmeliaceae*) das regiões centro-oeste, sudeste e sul do Brasil, a fim de ampliar e refinar o conhecimento da diversidade de liquens deste clado nestas regiões.

Objetivos específicos

- Identificar espécies utilizando características fenotípicas (morfologia e química) e moleculares (a partir do *DNA barcoding*);
- Utilizar o *DNA barcoding* na identificação de espécies de *Parmeliaceae* (clado Parmelioide) a partir de bancos de dados públicos, como o GenBank;
- Verificar a posição filogenética das espécies estudadas em nível de gênero;
- Descrever novas espécies utilizando a abordagem integrativa;
- Gerar e disponibilizar uma biblioteca de sequências nuITS acuradas das espécies coletadas;
- Fornecer descrições morfológicas, identificações dos principais componentes químicos e imagens das espécies estudadas;

Artigos

Capítulo I

Canoparmelia amazonica, Myelochroa lindmanii and *Parmelinella salacinifera* belong to *Parmelinella* (Parmeliaceae)

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Canoparmelia amazonica, *Myelochroa lindmanii* and *Parmelinella salacinifera* belong to *Parmelinella* (Parmeliaceae)

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ABSTRACT. The lichen family Parmeliaceae is among the best studied groups of lichens. *Canoparmelia amazonica, Myelochroa lindmanii*, and *Parmelinella salacinifera* are species of Parmeliaceae that have yet to be studied in detail with molecular methods. This study used analyses of ITS sequences to examine the phylogenetic position of these three species. *Canoparmelia amazonica* and *M. lindmanii* were recovered within *Parmelinella* rather than the genera to which they are currently assigned. While for the *P. salacinifera* we confirmed its phylogenetic position within the genus. Chemical and morphological descriptions of species are provided, generic placement is discussed, and new combinations are proposed as needed. These results highlight the need for morphological revision of the delimitation of *Parmelinella*, a small genus of Parmeliaceae that has been considered well-defined morphologically and is phylogenetically closely related to *Bulbothrix* s.l.

KEYWORDS. Lichens, taxonomy, ITS, restinga vegetation, Cerrado vegetation, Brazil.

INTRODUCTION

Parmelinella Elix & Hale, a genus belonging to the Parmeliaceae, was segregated from *Parmelina* s.l. by Elix & Hale (1987) with only three species. The genus was delimited by several characteristics including: a white medulla, emaculate thallus, simple cilia commonly restricted to the lobe axils, salazinic acid, consalazinic acid as main secondary compounds (Benatti 2014; Elix & Hale 1987). *Parmelinella* has a wide geographical distribution, occurring mainly in tropical and subtropical regions (Kirika et al. 2016). Currently, 10 species are known and five of them occur in Brazil: *Parmelinella cinerascens* (Lynge) Benatti & Marcelli, *P. mutata* (Vain.) Benatti, *P. salacinifera* (Hale) Marcelli & Benatti*, P. versiformis* (Kremp.) Marcelli and *P. wallichiana* (Taylor) Elix & Hale (Benatti 2014; Kirika et. al. 2016). *Parmelinella inexplicabilis* Marcelli & C.H.Ribeiro described from Brazil by Marcelli & Ribeiro (2002) is now treated as a synonym of *Remototrachyna costaricensis* (Nyl.) Divakar & A.Crespo (Divakar et al. 2010; Sipman et al. 2009).

The number of species in the genus *Parmelinella* has increased over time, in part, due to morphological reviews of taxa previously placed in other genera, such as *P. cinerascens* and *P. salacinifera*, that were previously placed in *Canoparmelia* Elix & Hale (Benatti 2012, 2014; Elix et al. 1986). *Canoparmelia* species are recognized mainly by the absence of cilia on the margins of the lobes, while *P. cinerascens* and *P. salacinifera* have cilia. Based mainly on this, both were transferred to *Parmelinella* (Benatti 2012, 2014). However, phylogenetic studies have not yet been carried out to confirm the generic placement of these species. *Canoparmelia* is phylogenetically distinct from *Parmelinella*, belonging to the *Parmotrema* clade within the parmelioid lichens (Crespo et al. 2010). *Parmelinella*, while morphologically distinct, is closely related to *Bulbothrix* Hale, and belongs to the *Parmelina* clade, which also includes *Myelochroa* (Asahina) Elix & Hale, *Remototrachyna* Divakar & A.Crespo, and *Parmelina* Hale (Crespo et al. 2010; Divakar et al. 2017).

The identification of lichenized fungal species, primarily based on morphology and chemistry characteristics, has changed over time, often associating with molecular tools (Lendemer 2021; Lücking et al. 2020). Recent studies of Parmeliaceae, the most diverse family of lichenized fungi with ca. 2,700 species (Lucking et al. 2017), have led to reviews of numerous genera and species circumscriptions, resulting in many taxonomic changes (Alors et al. 2016; Blanco et al. 2004, 2005; Boluda et al. 2019; Divakar et al. 2010; Kirika et al. 2017; Leavitt et al. 2018). In *Parmelinella*, phylogenetic studies also have expanded the knowledge about diversity within the genus, indicating that the morphological variation among the species may be much broader than previously believed (Eliasaro et al. 2010; Kirika et al. 2016).

During a preliminary study using the DNA barcode of fungi (ITS region) to identify Brazilian Parmeliaceae species, we found that *Canoparmelia amazonica* (Nylander) Elix & Hale and *Myelochroa lindmanii* (Lynge) Elix & Hale appeared to be more closely related to *Parmelinella* species. The aim of this study was to examine the phylogenetic position of *C. amazonica*, *M. lindmanii* and *Parmelinella salacinifera* within of the *Parmelina* clade.

MATERIALS AND METHODS

Sampling and specimen identification. Samples were collected from trees in two different vegetation domains of Brazil: Cerrado (Brazilian savanna), in the midwest region, and restinga (Atlantic Forest) close to the Brazil-Uruguay border. The Cerrado, distributed between the midwest and northeast regions of Brazil, is considered a global biodiversity hotspot (Mittermeier et al. 2011). It is characterized by heterogeneous vegetation, ranging from grassland formations to dry forests (Bianchi & Haig 2013; Strassburg et al. 2017). On the other hand, the restingas are distributed throughout the entire coastal zone of Brazil, being considered pioneer formations because the vegetation is located on unstable terrain with marine, wind and fluvomarine influences (IBGE 2006; Muylaert et al. 2018). Morphological

examination of specimens was carried out using a Nikon SMZ645 stereomicroscope and an Olympus CX22LED optical microscope. Species identification was based on diagnostic characteristics of the thallus, such as medulla coloration, presence/absence and type of vegetative propagules, lower surface coloration, rhizine shape, presence of cilia on the lobe margins, shape and size of the ascospores and conidia. Species descriptions were produced using the Parmeliaceae description protocol adapted from Canêz & Marcelli (2006).

For the initial chemical identification, we also performed spot tests (K, C, KC, P and UV) on the upper cortex and medulla of the specimens, following Orange et al. (2010). Chemical compounds were further studied with thin layer chromatography (TLC) on all specimens, using solvent G in proportions 139:83:8 of toluene/ethyl acetate/formic acid to identify secalonic and protocetraric acid, while for salazinic and consalazinic acid we used the toluene/ethyl acetate/acetic acid in the ratio of 6:4:1 (Culberson 1972; Culberson & Kristinsson 1970; Orange et al. 2010). All studied specimens were deposited in the Herbarium of the Federal University of Mato Grosso do Sul (CGMS).

DNA extraction, PCR amplification, and sequencing. For the DNA extraction, we removed a fragment of the thallus (ca. 5mm^2). The samples were placed in acetone for 20 minutes to remove the secondary metabolites that can interfere in DNA extraction and amplification. The fragments were air-dried until the acetone evaporated completely. DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega), following the protocol of the manufacturer. The ITS region was amplified using the ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) universal primers. PCR reactions were carried out in 25 µL reactions containing: 1× buffer, 0.2mM dNTPs, 0.2 µM of each primer, 3.0 mM $MgCl_2$, 1U Taq DNA polymerase (Promega) and ca. 20 ng of DNA template. The PCR conditions were: initial denaturation for 2 min at 95°C, followed by 30 cycles of denaturation at 95°C for 30 s, annealing between 54°C to 56°C for 30 s, extension at 72°C for

1.10 min and a final extension at 72°C for 5 min, using a Mastercycler® Gradient thermal cycler. The amplification products were visualized in 1% agarose gel stained with GelRed®. Macrogen Korea performed DNA purification and sequencing.

Dataset selection. Seven new ITS sequences were generated in the study, three of *Canoparmelia amazonica,* two of *Myelochra lindmanii,* and two of *Parmelinella salacinifera.* These new sequences were compared to 35 GenBank sequences (**Supplementary Table S1**) representing species of the genera *Bulbothrix*, *Myelochroa*, *Parmelina*, *Parmelinella* and *Remototrachyna*, members of the *Parmelina* clade (Crespo et al. 2010). Reference sequences, representing all the main taxa belonging to this clade, were selected according to the genetic proximity identified through the Basic Local Alignment Search Tool (BLAST) (Johnson et al. 2008). The chosen outgroup, *Hypotrachyna osseoalba* (Vain.) Y.S.Park & Hale, was selected according to recent Parmeliaceae phylogenetic studies, showing that *Hypotrachyna* (Vain.) Hale is the closest genus of the *Parmelina* clade (Divakar et al. 2017).

Sequence assembly and alignment. For assembly and quality evaluation of the DNA sequences generated, we used Geneious® 9.1.6 (Kearse et al. 2012). For the sequence alignments, we used MAFFT v7 (Katho & Standley 2013), applying the following parameters: G-INS-i algorithm, 200PAM/K=2 scoring matrix, offset value of 0.0 and the remaining parameters default values (Katho & Standley 2013). To remove ambiguous sites and increase the reliability of the final alignments, we used the Gblocks 0.97b webserver (http://molevol.cmima.csic.es/castresana/Gblocks_server.html) selecting all less stringent options (Talavera & Castresana 2007). The final ITS matrix contained 446 aligned nucleotide positions, included 19 species and is available from TreeBASE, reviewer access URL: http://purl.org/phylo/treebase/phylows/study/TB2:S27491?x-access-code=2670b7a1369dacb53e7f387c08e83b45&format=html.

Phylogenetic analyses. We inferred the phylogenetic position of Parmeliaceae specimens previously identified as *Canoparmelia amazonica*, *Myelochroa lindmanii* and *Parmelinella salacinifera* through the construction of phylogenetic trees using maximum likelihood (ML) and Monte Carlo Bayesian Markov chain (B/MCMC) approaches. Both analyses were run on the Cipres Science Gateway webserver (https://www.phylo.org/). The construction of the ML tree was done using the RAxML v8 program (Stamatakis 2014), with the "GTRGAMMA" model and 1000 bootstrap pseudoreplicates to evaluate the branch support. To determine the best-fitting nucleotide substitution model, we used the program JModelTest 2.1.6 (Darriba et al. 2012) using the Akaike information criterion–AIC (Akaike 1974). The model GTR+I+G was selected. For Bayesian inference, we used MrBayes 3.2.7 (Ronquist et al. 2012). We ran two parallel Markov Chain Monte Carlo (MCMC) chains for 10 million generations, saving every 1.000th tree. The first 25% of the sampled trees were discarded as burn-in. We verified the convergence of the Bayesian analysis chains using Tracer 1.7 (Rambaut et al. 2018), considering as good indicative a sample size (ESS) ≥200. Branches with bootstrap values ≥70%, and posterior probabilities (pp) ≥0.95 were considered supported.

RESULTS AND DISCUSSION

Morphological and chemical characters supported the identification of the seven specimens examined as *Canoparmelia amazonica*, *Myelochroa lindmanii* and *Parmelinella salacinifera* (Benatti 2014; Hale 1976a,b). *Canoparmelia amazonica* is characterized by the presence of a white medulla, absence of cilia, laminal isidia, and medullary protocetraric acid**.** *Myelochroa lindmanii* specimens were identified by the presence of yellow medulla (secalonic acid), marginal cilia and laminal isidia. In case of *Parmelinella salacinifera,* the specimen was recognized mainly by the presence of a white medulla, laminal isidia, cilia on

the margins of the lobes, brown lower surface, and presence of salazinic and consalazinic acid in the medulla.

According to the phylogenetic analyses, all genera belonging to the *Parmelina* clade were recovered as monophyletic (support values above 0.95 for posterior probabilities and 70% for bootstrap), except for *Bulbothrix* s.l, which presented two distinct clades (**Fig. 1**). Our results corroborate previous studies that have identified *Bulbothrix* as polyphyletic (Divakar et al. 2017); therefore, we maintained the previous convention in *Bulbothrix*, referring to 'Clade 1' for species closer to *Remototrachyna*, and 'Clade 2' for those closer to *Parmelinella*.

The *Parmelinella* clade was supported in the phylogeny (**Fig. 1**), and included sequences of *Canoparmelia amazonica*, *Myelochroa lindmanii*, *Parmelinella salacinifera P. schimperiana* and *P. wallichiana*. Sequences of *M. lindmanii* group *with Parmelia lindmanii* (also from Brazil, GenBank accession number GQ267190), indicating that they belong to the same species, however in *Parmelinella*.

The analyses recovered *Canoparmelia amazonica* and *Myelochroa lindmanii* as monophyletic species, with high support values (**Fig. 1**). However, *Parmelinella salacinifera* grouped with a sequence of *Parmelinella* aff. *wallichiana* obtained from a Brazilian specimen (Eliasaro et al. 2010). *Parmelinella salacinifera* and *P. wallichiana* share the same medullary chemistry and have isidia, but differ in lobes sizes, lower surface color and ascospore size (Benatti 2014; Kirika et al. 2016a). Since *Parmelinella wallichiana* s.str. (Kirika et al. 2016) is in another clade, the Brazilian specimen identified as *P.* aff. *wallichiana* should be revised. Due to its genetic similarity (95–96% identity in the ITS region), it can be a intraspecific variation of *P. salacinifera* or even another species still unknown.

TAXONOMY

Parmelinella amazonica (Nyl.) A.S.Rodrigues, A.P.Lorenz & Canêz, *comb. nov.* **Fig. 2**

MYCOBANK MB839860

ITS BARCODING SEQUENCE ACCESSIONS: MW364885, MW364886 AND MW364887.

≡ *Parmelia amazonica* Nyl., Flora 68: 611. 1885. ≡ *Pseudoparmelia amazonica* (Nyl.) Hale, Phytologia 29(3): 189. 1974. ≡ *Canoparmelia amazonica* (Nyl.) Elix & Hale, Mycotaxon 27: 278. 1986. TYPE: BRAZIL. PARÁ: Santarém, *R. Spruce 111* (H-NYL 35111 [n.v.] lectotype designated by Hale (1959) as holotype; BM [n.v.], G [n.v.], NY [n.v.], W [n.v.], PC [n.v.], isolectotypes) *fide* Hale (1976a).

Description. Thallus greyish green, lobate, corticolous, 7–8 cm broad. Lobes loosely attached, irregularly branched, contiguous to laterally overlapping, 2.5–6.5 mm wide, continuous, smooth and rarely rugose, apical zone rounded, margin crenate; maculae punctiform on the margins of the lobes; cilia absent. Soralia and pustules absent. Isidia present, laminar, concolor to the upper cortex, cylindrical, simple to branched, caducous, 0.1–0.3 × 0.04–0.06 mm. Medulla white. Lower surface black, lustrous, smooth to veined, marginal zone naked, 1–3 mm wide, light brown to brown, slightly lustrous to opaque, papillate to veined. Rhizines black in the central region and brown in the marginal zone, simple, abundant, 0.2–0.4 × 0.02–0.05 mm, evenly distributed. Apothecia present, disc imperforate, substipitate, laminal, margin crenate and involute, amphithecium isidiate; ascospores ellipsoid, 10–15 × 7–9 µm. Pycnidia present, few, submarginal to laminal, conspicuous and immersed, ostiole black, conidia sublageniform to bifusiform, some may appear to be bacilliform, but have a more inflated extremity, similar to sublageniform, 5–8 µm long.

Chemistry. Atranorin and protocetraric acid. We found traces of two other unidentified substances in the specimens studied. One with lower Rf and one higher than protocetraric acid in solvent G. Spot tests: upper cortex: K+ yellow, UV–; medulla K–, C–, KC–, P+ orange, UV–.

Geographic distribution. Africa: Angola and Madagascar (Aptroot 1991; Hale 1976a); North America: Mexico and United States (Lendemer et al. 2016; Sipman & Wolf 1998); Central America: Cuba, Honduras and Puerto Rico (Hale 1976a); South America: Bolivia, Brazil, Colombia, Guyana, Trinidad and Venezuela (Hale 1976a, Flakus et al. 2016; Sipman & Aptroot 1992); and Asia: China, Sri Lanka, Thailand and Taiwan (Ahti et al. 1999; Breuss & Brunnbauer 1997; Hale 1976a; Mongkolsuk et al. 2011).

Notes. *Parmelinella amazonica* is characterized by the absence of cilia on the margins of the lobes, presence of isidia laminar, and protocetraric acid as a medullary chemical compound. *Parmelinella amazonica* was previously treated in *Canoparmelia* due to the absence of cilia, white medulla, and presence of papillae on the lower margin of the lobes (Elix et al. 1986). The similarity between *C. amazonica* and *P. salacinifera,* when both were treated in *Pseudoparmelia*, had already been observed by Hale (1976a) who differentiated them mainly by the medullary chemistry. Later, both taxa were transferred to *Canoparmelia* (Elix et al. 1986) and *Parmelinella* (Benatti 2012).

Parmelinella amazonica differs from other species of *Canoparmelia* that were later transferred to *Parmelinella*, such as *P. cinerascens* and *P. salacinifera*, in that it does not have cilia on the margins of the lobes and in that it produces protocetraric acid in the medulla. The only morphological characters that suggest this species belongs to *Parmelinella* are the white medulla and the absence or traces of triterpenes. Both characteristics are also found in other genera of Parmeliaceae (e.g., *Canoparmelia*, *Parmotrema* A.Massal., *Hypotrachyna* (Vain.) Hale, among others). Therefore, at the moment, this placement is entirely based on molecular data.

Additional specimens examined. BRAZIL. MATO GROSSO DO SUL: Campo Grande co., Natural Reserve of the Federal University of Mato Grosso do Sul, on bark, 23 May 2019, *A.S. Rodrigues 673, 676, 681* (CGMS).

Parmelinella lindmanii (Lynge) A.S.Rodrigues, Canêz & A.P.Lorenz, *comb. nov.* **Fig. 2**

MYCOBANK MB839859

ITS BARCODING SEQUENCE ACCESSIONS: MW364890 AND MW364891.

≡ *Parmelia lindmanii* Lynge, Ark. Bot. 13(13): 74. 1914. ≡ *Parmelina lindmanii* (Lynge) Hale, Phytologia 28(5): 483. 1974. ≡ *Parmotrema lindmanii* (Lynge) Kurok. *in* Kurokwa & Arakawa, Bull. Bot. Gard. Toyama 2: 42. 1997. ≡ *Myelochroa lindmanii* (Lynge) Elix & Hale, Mycotaxon 29: 241. 1987. TYPE: BRAZIL. RIO GRANDE DO SUL: Porto Alegre, 25 Sept. 1892, *G.O.A. Malme 450* (S [n.v.], holotype) *fide* Lynge (1914).

Description. Thallus greyish green, lobate to sublacinulate, corticolous, 4–6 cm broad. Lobes loosely attached, irregularly branched, contiguous, 2–4.5 mm wide, smooth, continuous to rugose in the older regions, apical zone rounded, margin crenate to slightly crenate; maculae absent; cilia present, black, simple, few and distributed mainly in the axils of the lobes, (0.05) 0.1–0.3 × 0.02–0.05 mm. Soralia and pustules absent. Isidia present, laminar to submarginal, concolor to the upper cortex, cylindrical, simple to slightly branched and very fragile, erect, caducous, 0.1–0.3 × 0.04–0.06 mm. Medulla yellow. Lower surface black, lustrous, smooth to slightly veined, marginal zone naked present, 1–1.5 mm wide, brown, lustrous, papillate to rugose. Rhizines black on the margins with whitish apex becoming totally black in the central region, simple, abundant, 0.2–0.5 × 0.02–0.06 mm, evenly distributed. Apothecia present, disc imperforate, substipitate, laminal, margin smooth, ornamentation absent; ascospores ellipsoid, 10–12 × 6–8 µm. Pycnidia present, submarginal to laminal, conspicuous and immersed, ostiole black, conidia bifusiform 5.5–7 µm long.

Chemistry. Atranorin and secalonic acid. Spot tests: upper cortex: K+ yellow, UV–; medulla K+ weak orange, C–, KC–, P–, UV–.

Geographic distribution. North America: Mexico (Hale 1976b). South America: Argentina, Bolivia, Brazil, Colombia, Paraguay, Uruguay and Venezuela (Flakus et al. 2014; Hale 1976b).

Notes. *Parmelinella lindmanii* is characterized by a yellow medulla, laminal isidia, and secalonic acid as the main medullary chemical compound. Hale (1976b) distinguished two sections in the genus *Parmelina* with distinct morphological and chemical characteristics. Section *Parmelina* included species characterized by and white medulla and absence of triterpenes, while the section *Myelochroa* included species with triterpenes and a pigmented medulla. *Parmelina lindmanii* and *P. immiscens* (Nyl.) Hale, both with yellow medulla and lack of triterpenes, were classified in section *Parmelina*. Elix & Hale (1987) proposed five new genera segregated from *Parmelina* s.l., including *Myelochroa* and *Pamelinella*. The genus *Parmelinella* included species with white medulla, marginal cilia commonly restricted to the lobe axils, salazinic and consalazinic acids, and lack or traces of triterpenes. Species of *Myelochroa* had moderately wide lobes, cilia homogeneously distributed on the margins of the lobes, yellow medulla, presence of medullary secalonic acid, and commonly triterpenes. At that time, *P. immiscens* and *P. lindmanii*, were included in *Myelochroa* (Elix & Hale 1987). Afterwards, Kurokawa & Arakawa (1997) combined *M. lindmanii* into *Parmotrema* because this species had large lobes, with a rounded apex and an evident naked marginal zone in the lower surface. However, *Parmotrema lindmanii* (Lynge) Kurok. was not used by most lichenologists (Eliasaro & Adler 2000; Estrabou et al. 2006; Flakus et al. 2014; Spielmann & Marcelli 2008).

Myelochroa is the sister group to genus *Parmelina*, and together with *Bulbothrix*, *Remototrachyna* and *Parmelinella* belongs to the *Parmelina* clade (Crespo et al. 2010; Divakar et al. 2017). Eliasaro et al. (2010), using ITS sequences, pointed out that *M. lindmanii* probably belonged to *Parmelinella*. They decided not to propose any nomenclatural

changes considering that more molecular analyses were needed, instead treating the species under the basionym *Parmelia lindmanii*. Subsequently, a phylogenetic study with *Parmelinella* (Kirika et al. 2016) used the same sequence but referred to it as *Parmelinella lindmanii*. However, neither Eliasaro et al. (2010) nor Kirika et. al. (2016) proposed a formal new combination. Thus, we formally transfer the species to *Parmelinella*.

Based on the morphological characters, *Parmelinella lindmanii* shares with the other species of the genus the presence of marginal cilia commonly restricted to lobes axils and absence of triterpenes. Differing mainly by the yellow medulla and the presence of secalonic acid.

Additional specimens examined. BRAZIL. RIO GRANDE DO SUL: Pelotas co., Dunas Las Acácias, on bark, 28 Jul. 2017, *A.S. Rodrigues 287* (CGMS). Rio Grande co., Barra Falsa, on bark, 15 Jan. 2018, *A.S. Rodrigues 518* (CGMS).

CONCLUSIONS

Phylogenetic analyses performed in this study recovered *Canaparmelia amazonica*, *Myelochroa lindmanii,* and *Parmelinella salanifera* as members of the genus *Parmelinella*. Thus, the phenotypic characteristics of *Parmelinella* are broader than those expected, including species with white or yellow medulla, presence or absence of marginal cilia that when present are commonly restricted to the lobe axils, and lower surface varying from black to brown. However, to better delimit *Parmelinella*, studies with greater sampling of species and specimens are needed, including from different geographical regions and using integrative approaches, such as morphological and phylogenetic data.

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Supplementary documents online:

Supplementary Table S1. Sequences of the specimens used in the study, containing the collection site and the accession number of the ITS sequences deposited in GenBank. The new sequences generated in the study are in bold.

Figure 1. Phylogenetic relationships of *Parmelina* clade based on a maximum-likelihood (ML) and Bayesian analyses from ITS rDNA sequences. The Bayesian tree is shown here. Branches recovered with support values of Posterior probabilities ≥0.95 from the Bayesian analysis and ML bootstrap values ≥70% are indicated in the figure.

Figure 2. Images of the species studied. **A**–**B**. Thallus, habitat and eciliated lobes of *Parmelinella amazonica*. **C.** Thallus and habitat of *Parmelinella lindmanii*. **D.** Detail of the morphological characteristics of *Parmelinella lindmanii*. Arrows indicate axillary cilia and yellow medulla. **E**–**F**. Upper surface, as well as brown lower surface of *Parmelinella salacinifera*.

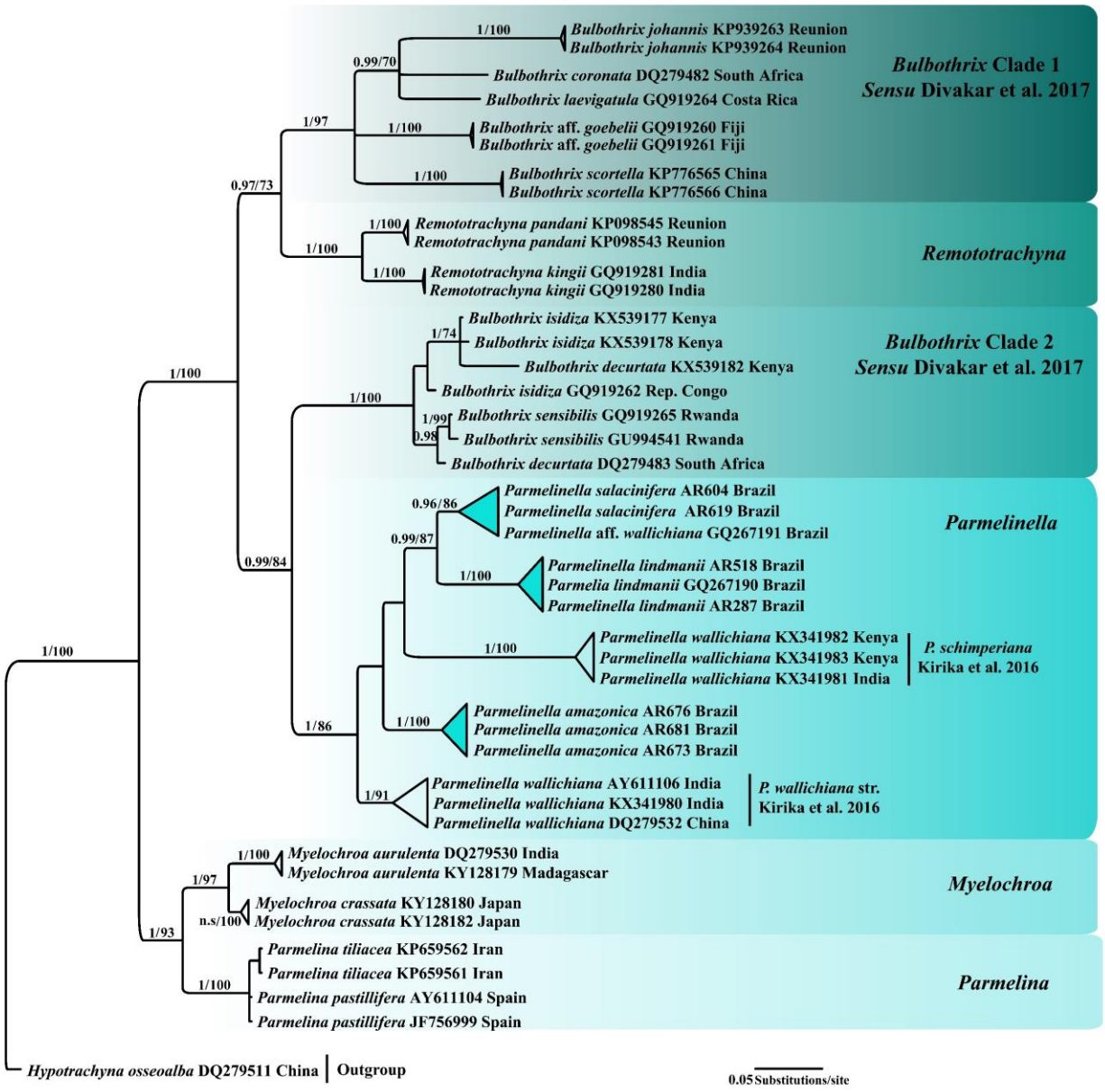
Bulbothrix johannis KP939263 Reunion
Bulbothrix johannis KP939264 Reunion
Bulbothrix coronata DQ279482 South Africa
Bulbothrix laevigatula GQ919264 Costa Rica
Bulbothrix aff. goebelii GQ919260 Fiji
Bulbothrix aff. goebelii GQ919261 Fiji
Bulbothrix scortella KP776565 China
Bulbothrix scortella KP776566 China
Bulbothrix Clade 1 Sensu Divakar et al. 2017
Remototrachyna pandani KP098545 Reunion
Remototrachyna pandani KP098543 Reunion
Remototrachyna kingii GQ919281 India
Remototrachyna kingii GQ919280 India
Remototrachyna
Bulbothrix isidiza KX539177 Kenya
Bulbothrix isidiza KX539178 Kenya
Bulbothrix decurtata KX539182 Kenya
Bulbothrix isidiza GQ919262 Rep. Congo
Bulbothrix sensibilis GQ919265 Rwanda
Bulbothrix sensibilis GU994541 Rwanda
Bulbothrix decurtata DQ279483 South Africa
Bulbothrix Clade 2 Sensu Divakar et al. 2017
Parmelinella salacinifera AR604 Brazil
Parmelinella salacinifera AR619 Brazil
Parmelinella aff. wallichiana GQ267191 Brazil
Parmelinella lindmanii AR518 Brazil
Parmelia lindmanii GQ267190 Brazil
Parmelinella lindmanii AR287 Brazil
Parmelinella wallichiana KX341982 Kenya
Parmelinella wallichiana KX341983 Kenya
Parmelinella wallichiana KX341981 India
P. schimperiana Kirika et al. 2016
Parmelinella amazonica AR676 Brazil
Parmelinella amazonica AR681 Brazil
Parmelinella amazonica AR673 Brazil
Parmelinella wallichiana AY611106 India
Parmelinella wallichiana KX341980 India
Parmelinella wallichiana DQ279532 China
P. wallichiana str. Kirika et al. 2016
Parmelinella
Myelochroa aurulenta DQ279530 India
Myelochroa aurulenta KY128179 Madagascar
Myelochroa crassata KY128180 Japan
Myelochroa crassata KY128182 Japan
Myelochroa
Parmelina tiliacea KP659562 Iran
Parmelina tiliacea KP659561 Iran
Parmelina pastillifera AY611104 Spain
Parmelina pastillifera JF756999 Spain
Parmelina
Hypotrachyna osseoalba DQ279511 China
Outgroup
0.05 Substitutions/site

Figure 1

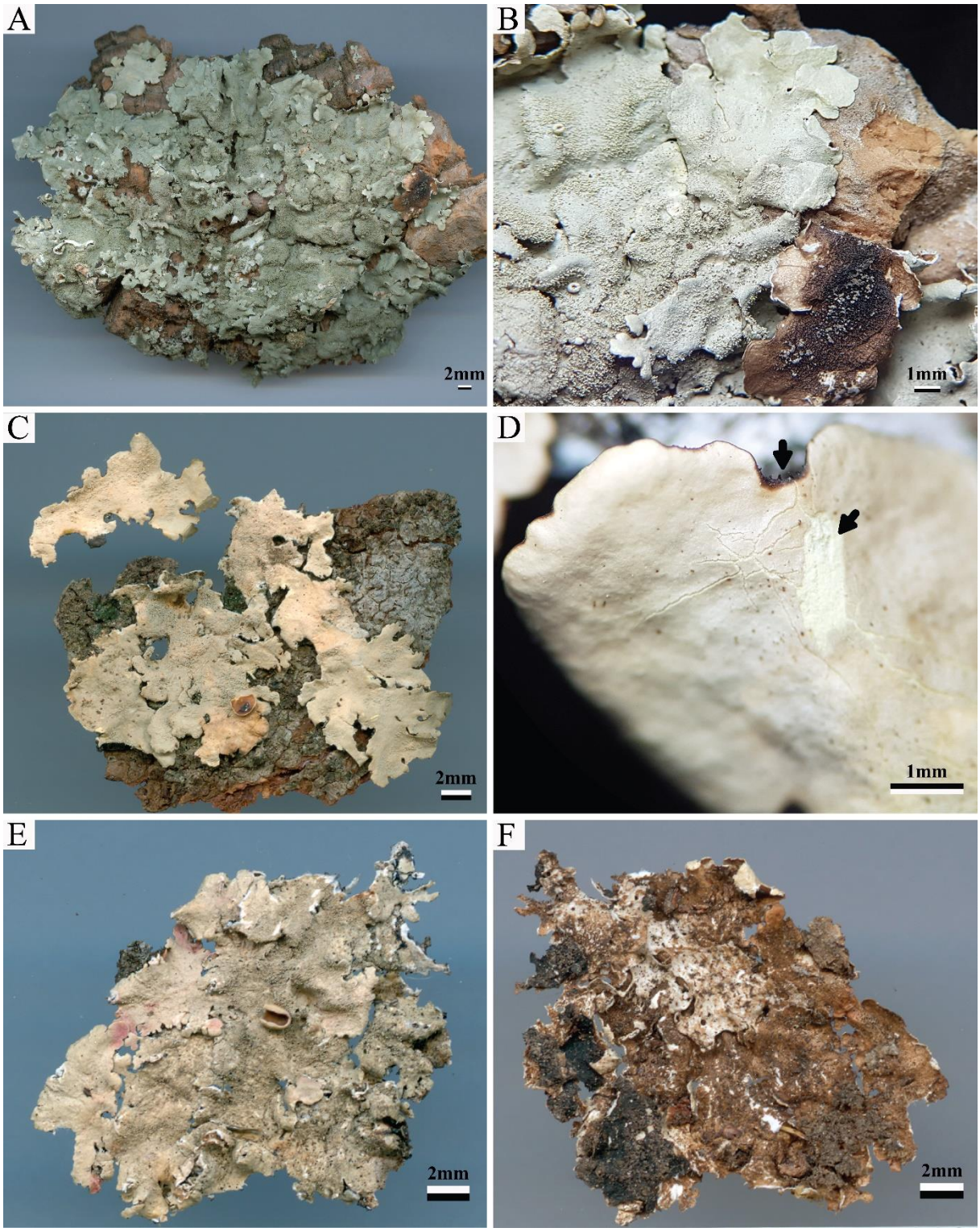

Figure 2.

Supplementary material for: Rodrigues, A. S., L. S. Canêz & A. P. Lorenz. 2021. *Canoparmelia amazonica*, *Myelochroa lindmanii* and *Parmelinella salacinifera* belong to *Parmelinella* (Parmeliaceae). The Bryologist 124(3): XX–XX.

Supplementary Table S1. Sequences of the specimens used in the study, containing the collection site and the accession number of the ITS sequences deposited in GenBank. The new sequences generated in the study are in bold.

Species	**Voucher**	**Locality**	**GenBank no.**
Hypotrachyna ossealba	MAF 10389	China: Yunnan Prov.	DQ279511
Bulbothrix coronata	MAF 13987	South Africa	DQ279482
Bulbothrix decurtata	Kirika 4489	Kenya: Coast Prov.	KX539182
Bulbothrix decurtata	MAF 13988	South Africa	DQ279483
Bulbothrix aff. *goebelii*	F:Lumbsch 19809g	Fiji	GQ919261
Bulbothrix aff. *goebelii*	F:Lumbsch 19817e	Fiji	GQ919260
Bulbothrix isidiza	Kirika, Mugambi & Lumbsch 2829	Kenya: Rift Valley Prov.	KX539177
Bulbothrix isidiza	Kirika 4363B	Kenya: Central Prov.	KX539178
Bulbothrix isidiza	MAF-Lich 15511	Congo	GQ919262
Bulbothrix johannis	LG S3347	Reunion	KP939264
Bulbothrix laevigatula	F:Luecking 15045b	Costa Rica	GQ919264
Bulbothrix scortella	KUN 14-44441	China	KP776565
Bulbothrix scortella	KUN 14-44442	China	KP776566

Bulbothrix sensibilis	BR 11025	Rwanda	GQ919265
Bulbothrix sensibilis	Ertz 11025	Rwanda	GU994541
Bulbothrix johannis	LG S3251	Reunion	KP939263
Myelochroa aurulenta	MAF 13992	India: North Sikkim	DQ279530
Myelochroa aurulenta	BR-Lich 7268-90	Madagascar	KY128179
Myelochroa crassata	MAF-Lich 17779	Japan: Saitama	KY128180
Myelochroa crassata	MAF-Lich 19987	Japan: Ibaraki	KY128182
Parmelina tiliacea	MAF:Lich 19381	Iran	KP659562
Parmelina tiliacea	MAF:Lich 19380	Iran	KP659561
Parmelina pastilifera	MAF 6058	Spain: Cadiz	AY611104
Parmelina pastilifera	MAF-Lich 16472	Spain	JF756999
Parmelinella amazonica	**Rodrigues, A.S. 676 (CGMS)**	**Brazil: Mato Grosso do Sul**	**MW364885**
Parmelinella amazonica	**A.S. Rodrigues 681 (CGMS)**	**Brazil: Mato Grosso do Sul**	**MW364886**
Parmelinella amazonica	**A.S. Rodrigues 673 (CGMS)**	**Brazil: Mato Grosso do Sul**	**MW364887**
Parmelinella lindmanii	**A.S. Rodrigues 518 (CGMS)**	**Brazil: Rio Grande do Sul**	**MW364890**
Parmelinella lindmanii	**A.S. Rodrigues 287 (CGMS)**	**Brazil: Rio Grande do Sul**	**MW364891**
Parmelia lindmanii	3131	Brazil: Paraná	GQ267190
Parmelinella salacinifera	**A.S. Rodrigues 604 (CGMS)**	**Brazil: Rio Grande do Sul**	**MW364888**

Parmelinella salacinifera	**A.S. Rodrigues 619 (CGMS)**	**Brazil: Rio Grande do Sul**	**MW364889**
Parmelinella aff. *wallichiana*	S. Eliasaro 3132	Brazil: Paraná	GQ267191
Parmelinella wallichiana	Kirika & Lumbsch 4678	Kenya: Eastern	KX341982
Parmelinella wallichiana	Kirika & Lumbsch 4715	Kenya: Eastern	KX341983
Parmelinella wallichiana	Lumbsch et al.	India: South India	KX341981
Parmelinella wallichiana	LWG 20-77171	India: Sikkim	AY611106
Parmelinella wallichiana	Divakar s/n	India: Uttaranchal	KX341980
Parmelinella wallichiana	MAF 10411	China: Yunnan Province	DQ279532
Remototrachyna kingii	MAF-Lich 15609	India	GQ919281
Remototrachyna kingii	MAF-Lich 15610	India	GQ919280
Remototrachyna pandani	LG:S3350	Reunion	KP098545
Remototrachyna pandani	LG:S3286	Reunion	KP098543

Artigos

Capítulo II

Hypotrachyna neohorrescens, a new species in the subgenus *Parmelinopsis* (*Parmeliaceae*) from Brazil

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Hypotrachyna neohorrescens, a new species in the subgenus *Parmelinopsis* (*Parmeliaceae*) from Brazil

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Abstract

This study describes a new species of *Hypotrachyna* subgenus *Parmelinopsis* from the south-eastern Cerrado (Brazilian savannah), a biodiversity hotspot. The species is especially common in open vegetation, including urban environments. *Hypotrachyna neohorrescens* sp. nov. is morphologically and chemically similar to *H. horrescens*. Nevertheless, phylogenetic analyses of the nuITS and mtSSU regions revealed that *H. neohorrescens* is a distinct species and closely related to the North American *H. mcmulliniana*, differing by the size of the laciniae and ascospores.

Key words: Cerrado, lichenized fungi, phylogeny, taxonomy

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Introduction

With more than 260 described species, the genus *Hypotrachyna* (Vain.) Hale belongs to *Parmeliaceae*, a hyperdiverse family of lichenized fungi (Lücking *et al*. 2017). Phylogenetic reconstructions indicate a probable generic origin in the neotropical region, where the split of the major *Hypotrachyna* clades occurred between the Eocene and Oligocene, with South America being the main centre of diversification, represented by *c*. 120 species (Sipman *et al*. 2009; Cubas *et al*. 2018). The *Hypotrachyna* clade includes a *sensu stricto* group and several well-supported clades recognized as the subgenera *Cetrariastrum* (Sipman) Divakar *et al*., *Everniastrum* (Hale ex Sipman) Divakar *et al*., *Longilobae* Divakar *et al*., *Parmelinopsis* (Elix & Hale) Divakar *et al.* and *Sinuosae* Divakar *et al*. (Divakar *et al*. 2013).

Currently considered a subgenus of *Hypotrachyna*, *Parmelinopsis* is represented by 28 species, 10 of which occur in Brazil: *Hypotrachyna cryptochlora* (Vain.) D. Hawksw. & A. Crespo, *H. damaziana* (Zahlbr.) Krog & Swinscow, *H. heteroloba* (Zahlbr.) Divakar *et al*., *H. horrescens* (Taylor) Krog & Swinscow, *H. jamesii* (Hale) Divakar *et al*., *H. minarum* (Vain.) Krog & Swinscow, *H. schindleri* (Hale) Divakar *et al*., *H. spathulata* (Kurok.) Krog & Swinscow, *H. spumosa* (Asahina) Krog & Swinscow, and *H. subfatiscens* (Kurok.) Swinscow & Krog (Canêz 2005; Martins *et al*. 2008; Benatti 2012; Lendemer & Allen 2020). *Parmelinopsis* is a genus that was previously segregated from *Parmelina* Hale by Elix & Hale (1987), encompassing species with a white medulla, emaculated thallus, narrow and apically truncated laciniae, simple marginal cilia, simple to dichotomous rhizines, cylindrical to bifusiform conidia (*c*. 3–5 µm) and commonly orcinol tridepsides as the main medullary chemical group (Elix & Hale 1987; Benatti 2012). However, this circumscription of *Parmelinopsis* proved to be polyphyletic in molecular phylogenies, indicating multiple origins of these morphological and chemical characters in the *Hypotrachyna* clade (Divakar *et al*. 2006, 2013). Thus, the subgenus *Parmelinopsis* currently encompasses species with eciliate laciniae

up to 6 mm wide, mostly dichotomous rhizines, and depsides derived from β-orcinol and (or) orcinol as main chemical compounds. These characteristics are not exclusive and can also be found in other subgenera (Divakar *et al*. 2013; Lendemer & Allen 2015, 2020).

In Brazil, species of *Hypotrachyna* subgenus *Parmelinopsis* are common in the Cerrado ecoregion or province, including open areas of savannah and seasonal forests, as well as urban environments (Marcelli 1993). The Cerrado is a biodiversity hotspot characterized by different landscapes, ranging from tropical grasslands to seasonal forests, with numerous species adapted to the frequent fires that occur during the six-month dry season (Batalha 2011; Strassburg *et al*. 2017; Oliveira *et al.* 2021). However, studies of widespread lichenized fungi from this highly seasonal region are still scarce (Jungbluth 2006).

This study introduces *Hypotrachyna neohorrescens* Jungbluth, Marcelli & Lorenz, a new species from the south-eastern Cerrado, including a detailed description and its phylogenetic position.

Material and Methods

Morphological data: study location, sampling and identification

Specimens of *Hypotrachyna neohorrescens* sp. nov. were collected on trees of forest fragments in southern Cerrado (Fig. 1). Furthermore, specimens of *Hypotrachyna minarum*, a species that morphologically resembles *H. horrescens*, were collected in restingas (Atlantic forests adapted to coastal plains) in southern Brazil. Morphological descriptions are based on all specimens examined, using a Nikon SMZ645 stereomicroscope (Nikon Corporation, Japan) and an Olympus CX22LED optical microscope (Olympus Corporation, Japan). Chemical compounds were identified by spot tests (K, C, KC and P) and thin-layer chromatography (TLC), using solvents A and C (Orange *et al.* 2010).

The specimens of *H. neohorrescens* examined were deposited in the herbaria of the Universidade Federal de Santa Maria (PALM) and the Instituto de Botânica (SP), and the specimens of *H. minarum* were deposited in the herbarium of the Universidade Federal de Mato Grosso do Sul (CGMS). In addition, we undertook morphological studies of the lectotype of *Parmelia* [*Hypotrachyna*] *horrescens* (Taylor) Krog & Swinscow (FH-TAYL), kindly loaned to us by the curator of herbarium FH (Farlow Herbarium, Harvard University).

Phylogenetic analyses

Prior to DNA extraction, thallus fragments were immersed in acetone for 20 min to remove the secondary compounds. The fragments were then dried in the open air until the acetone evaporated completely. DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. The nuITS region was amplified using the universal primers ITS1F (Gardes & Bruns 1993) and ITS4 (White *et al.* 1990). To amplify the mtSSU and nuLSU regions, we used the primers mrSSU1 and mrSSU3R (Zoller *et al*. 1999), and LR1R (Döring *et al.* 2000) and LR6 (Vilgalys & Hester 1990), respectively. The 25 µl PCR reactions w contained the following: 1× buffer, 0.2 mM dNTPs, 0.2 µM of each primer, 3.0 mM $MgCl_2$, 1U Taq DNA polymerase (Promega) and *c*. 20 ng of DNA template. The PCR conditions for the amplification of the nuITS region were as follows: initial denaturation for 2 min at 95 °C, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing between 54–56 °C for 30 s, extension at 72 °C for 1 min 10 s and a final extension at 72 °C for 5 min. For the mtSSU and nuLSU regions, we applied the following: initial denaturation for 5 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 1 min, annealing at 54 °C for 1 min, extension at 72 °C for 1 min 30 s and a final extension at 72 °C for 10 min. The PCR reactions were performed in an Eppendorf Mastercycler® Gradient thermal

cycler. The amplification products were visualized in 1% agarose gel stained with GelRed® (Biotium). Macrogen Korea performed DNA purification and sequencing.

For assembly and quality evaluation of the DNA sequences generated, we used Geneious® 9.1.6 (Kearse *et al.* 2012). Sequences from GenBank were selected to encompass the main clades of the *Hypotrachyna* clade (details in Table 1). *Hypotrachyna cirrhata* (Fr.) Divakar *et al*. (subgenus *Everniastrum*) was chosen as outgroup due to its close phylogenetic relationship with *Hypotrchyna* subgenus *Parmelinopsis* (Divakar *et al.* 2013). Multiple sequence alignments for each locus were performed using MAFFT v.7 (Katoh & Standley 2013), with the auto option and subsequent manual inspection. We removed the ambiguous sites from the alignments with Gblocks 0.97b (http://molevol.cmima.csic.es/castresana/Gblocks_server.html), selecting all less stringent options (Talavera & Castresana 2007).

Exploratory analyses using BLAST tools revealed that the nuLSU sequences obtained were up to 85% identical to mtDNA of the green algal genus *Trebouxia* Puymaly, the associated photobiont. Therefore, the nuLSU sequences were excluded from subsequent analyses. Two datasets were used, one with the sequences of the nuITS and mtSSU regions concatenated and one with only the nuITS region, the universal DNA barcode for fungi (Schoch *et al.* 2012). Phylogenetic analyses were based on maximum likelihood (ML) and Bayesian (B/MCMC) approaches, performed on the Cipres Science Gateway webserver (https://www.phylo.org/). The ML analysis was performed using RAxML v.8 (Stamatakis 2014), with the GTRGAMMA model and 1000 bootstrap pseudoreplicates. We used jModelTest 2.1.6 (Darriba *et al.* 2012) to verify the best nucleotide substitution model, using the Akaike information criterion (Akaike 1974). The model GTR + G was selected for the nuITS region, and HKY + I + G for mtSSU. For the tree reconstruction based on Bayesian inference, the program MrBayes 3.2.7 (Ronquist *et al*. 2012) was used with two parallel Markov chain Monte Carlo (MCMC) chains with 10 million generations, saving every 1000th tree. The first 25% of the sampled trees was discarded as burn-in. The convergence of the Bayesian analysis chains was verified using Tracer v.1.7 (Rambaut *et al.* 2018), considering the sample size (ESS) ≥ 200 as indicative. Branches with bootstrap values ≥ 70% for ML, and posterior probabilities ≥ 0.95 for Bayesian inference were considered to be supported. These values were also used to compare maximum likelihood and Bayesian analyses to check for conflicts in the resulting topologies.

Additionally, pairwise genetic distances were calculated for the nuITS region (using the alignment with the ambiguous sites removed) with PAUP v.4.0b10 (Swofford & Sullivan 2003), using ML as a distance measure, while pairwise distances between different sequences are given as the number of nucleotide substitutions per site (s/s). We used the barcode gap values (a threshold close to 0.015–0.017 s/s) proposed for *Parmeliaceae* (Del-Prado *et al.* 2010) to distinguish *H. neohorrescens* from the phylogenetically closest species.

Results

Novel sequences from nuITS and mtSSU regions were obtained for nine specimens of H. neohorrescens (collected from sampling points 1, 2 and 3 in Fig. 1), and two specimens of H. minarum (Table 1). The final data matrix contained 63 concatenated sequences of the markers nuITS and mtSSU with a length of 1271 characters (see Supplementary Material S1, available online). There were no conflicts between the phylogenies based on maximum likelihood and Bayesian inference of the concatenated matrix, so only the Bayesian tree is shown in Fig. 2. Hypotrachyna neohorrescens sp. nov. formed a new clade, closely related to the North American H. mcmulliniana Lendemer & J. L. Allen. The single marker matrix containing 63 nuITS sequences with 472 characters resulted in trees with a similar topology, but they did not recover complete reciprocal monophyly of H. neohorrescens and H. mcmulliniana (Supplementary Material Fig. S1, available online). This lack of resolution

reflects the low divergence among the nuITS sequences. The mean genetic distance between H. neohorrescens and H. mcmulliniana was 0.0185 s/s, ranging from 0.0142 to 0.0205 s/s.

In addition, we also verified the phylogenetic position of H. minarum, a widely distributed species with its type specimen from Brazil. The H. minarum clade included sequences from Australia, Brazil, China, the United States and Spain. (Fig. 2). Interestingly, nuITS distances up to 0.017 s/s indicated that more than one species might be in this clade. The Brazilian specimens analyzed in this study were characterized by laminal isidia without cilia (as described for North American populations in Lendemer & Allen (2020)), marginal lobes with rare cilia, and the presence of atranorin and gyrophoric acid, together with an unidentified compound (migrated above gyrophoric acid in solvent C in the TLC profile).

Thus, based on its morphology, phylogenetic relationships (inferred with concatenated markers) and geographical distribution, we consider H. neohorrescens to be a new species and highlight its main diagnostic characteristics below and in Table 2.

Taxonomy

Hypotrachyna neohorrescens Jungbluth, Marcelli & Lorenz sp. nov.

MycoBank No.: MB 841994

Morphologically similar to *Hypotrachyna horrescens*, but differs by its phylogenetic position (based on concatenated nuITS and mtSSU regions). Additionally, *H. neohorrescens* is phylogenetically close to *H. mcmulliniana,* differing in lacinia size (0.5–1.7 mm vs 1.0–4.0 mm wide), ascospore size (12.5–19.0 × 8.8–11.3 µm vs 9.9–13.8 × 5.1–8.1 µm) and geographical distribution (South America vs North America).

Type: Brazil, São Paulo State, municipality of Itirapina, Estação Experimental de Itirapina, Instituto Florestal, corticolous, tree in a well-preserved area of Cerrado seasonal forest, named Valério, *c.* 818 m alt., 22°13′10.0″S, 47°51′05.7″W, 10 December 2012, *P. Jungbluth, M. J. Kitaura* & *S. A. Adachi* 3317 (PALM—holotype). GenBank Accession nos: MZ919273 (nuITS) and MZ919147 (mtSSU).

(Fig. 3A & B)

Thallus corticolous, greenish grey, laciniate, 2–7 cm diam., adnate. *Proximal upper surface* continuous, densely isidiate. *Distal upper surface* continuous, smooth, becoming densely covered by young isidia, shiny, with a darker line near the tips, evidently thicker at the sinuses of the laciniae. *Laciniae* sublinear, mainly dichotomously branched, contiguous to slightly overlapping laterally, 0.5–1.7(–2.0) mm wide at the base of the branches, 1.5–2.5 mm maximum width; lateral margin smooth to crenate, slightly canaliculated; axils oval; apices subtruncate, flat with an involute tendency, procumbent. *Pruina* absent. *Maculae* absent. *Cilia* black, marginal, shiny, with acute ends, simple, abundant, mainly restricted in the axils and sinuses and in the isidia, up to 0.4 mm long. *Isidia* with pale to dark brown apices, mainly cylindrical, simple becoming very branched and coralloid in old thalli, erect, apices frequently ciliate, including the apices of the lateral branches; laminal, up to 0.5 mm. *Soralia* absent. *Medulla* white. *Distal lower surface* brown, shiny, smooth near the margins becoming papillate (small rhizines). *Proximal lower surface* black, shiny, slightly rugose, and veined. *Rhizines* black, simple, rarely irregularly branched, abundant, evenly distributed, up to 0.75 mm long.

Apothecia rare, concave, adnate, laminal, up to 2.5 mm diam.; margin smooth becoming isidiate; disc brown, shiny, without pruina. *Epithecium* 12.5–17.5 µm high; hymenium 50.0–62.5 µm high; subhymenium 50.0–62.5 µm high. *Ascospores* ellipsoid, (12.5–)16.0(–19.0) ×

(8.8–)10.2(–11.3) µm, episporium 1.0–2.0 µm (apothecia and ascospores found only in *P. Jungbluth* 2294).

Pycnidia rare, laminal to submarginal. *Conidia* bacilliform to bifusiform, (3.0–)4.0–6.0 µm.

Chemistry. Upper cortex K+ yellow, UV−; medulla K−, C−, KC+ pink, P−, UV−. Atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid ('horrescens complex'), 5-*O*-methylhiascic acid and gyrophoric acid.

Etymology. The specific epithet refers to the morphological similarity to *H. horrescens* and its known distribution in the neotropical region.

Distribution and habitat. The species is commonly found in south-eastern Cerrado, mainly in the states of São Paulo and Minas Gerais, Brazil.

Intraspecific morphological variation. There is a conspicuous presence of ciliate isidia in some specimens of this species although there are exceptions. Populations of *H. neohorrescens* from Itirapina (collection point 1 in Fig. 1), for example, show abundantly ramified isidia with conspicuous apical cilia (Fig. 3B). Whereas other specimens do not have ciliate isidia, instead resembling *Hypotrachyna minarum*, a species common in Brazil (Fig. 3C & D). This populational variation was also observed in *H. horrescens* by Hale (1971). However, *H. horrescens* and *H. neohorrescens* can be distinguished from *H. minarum* by the presence of the 3-methoxy-2,4-di-*O*-methylgyrophoric acid.

Additional specimens examined. **Brazil:** *Minas Gerais State*: Catas Altas, Parque Natural do Caraça (Sanctuary of Caraça), tree from the forest between Atlantic and Cerrado seasonal forests, trail to 'Piscina', corticolous, 1365 m, 20°06′08.2″S, 43°30′04.1″W, 2010, *P. Jungbluth* 2294 (PALM). *São Paulo State*: Itirapina, Estação Experimental de Itirapina, Instituto Florestal, corticolous, tree in a well-preserved area of Cerrado seasonal forest, named Valério, *c.* 818 m, 22°13′10.0″S, 47°51′05.7″W, 2012, *P. Jungbluth, M. J. Kitaura & S. A. Adachi* 3160, 3161 (PALM); ; *ibid*., on tree singed by fire in savannah (Cerrado s. str.), 757 m, 22°12′23″S, 47°54′26″W, 2012, *P. Jungbluth, M. J. Kitaura & S. A. Adachi* 3264, 3266 (PALM). *Mogi-Guaçu*: Martinho Prado District, Reserva Biológica de Mogi-Guaçu, Fazenda Campininha, corticolous, on Cerrado seasonal forest, 620 m alt., 22°15′19″S, 47°09′17″W, 2011, *P. Jungbluth, M. M. Marcelli & B. R. da Hora* 2642, 2647 (PALM). *Pratânia*: Fazenda Palmeira da Serra, private reserve area of Cerrado, on seasonal forest inside sugar cane crops, corticolous, 710 m, 22°48′55″S, 48°44′36″W, 2011, *P. Jungbluth & S. B. Bissacot* 2690, 2709 (PALM).

Discussion

The Cerrado is characterized by a patchy landscape with frequently burned areas which have poor or absent lichenized mycobiota because it takes *c*. 20 years for significant coverage and diversity to develop again (Marcelli *et al.* 1998). Species of *Hypotrachyna* subgenus *Parmelinopsis* are especially frequent in these environments (Jungbluth 2006); however, their diversity may be underestimated due to the lack of studies based on morphology and molecular tools (Lendemer & Allen 2020).

Using the nuITS and mtSSU regions, specimens phenotypically similar to *H. horrescens* and *H. mcmulliniana* collected in south-eastern Cerrado were recovered as new species of the *Parmelinopsis* subgenus. *Hypotrachyna neohorrescens* sp. nov. and *H. horrescens* produce frequent ciliated isidia on the upper surface, laciniae 0.5–1.7 mm wide, ellipsoid spores 12.5–19 × 8.8–11.3 µm in *H. neohorrescens* and 16–19 × 10–12 µm in *H. horrescens* according to

Hale (1976) (the studied lectotype did not have apothecia), and the same chemistry (atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid, 5-*O*-methylhiascic acid and gyrophoric acid) (Table 2). Thus, morphological and chemical characters did not distinguish these species, although they belong to different clades and accumulate significant genetic divergence (0.0359–0.0551 s/s in the nuITS; Fig. 2). Phenotypically cryptic or 'near-cryptic' species are commonly found in molecular studies of *Parmeliaceae*, which have revealed higher levels of undescribed diversity among the lichenized fungi in recent decades (Crespo & Lumbsch 2010; Altermann *et al.* 2014; Singh *et al.* 2015; Leavitt *et al*. 2016; Lutsak *et al*. 2020). *Hypotrachyna neohorrescens* and *H. mcmulliniana* also share many morphological features; however, they can be distinguished by the lacinia size (0.5–1.7 mm vs 1.0–4.0 mm wide) and the size of the ascospores ((12.5–)16.0(–19.0) × (8.8–)10.2(–11.3) µm vs 9.9–13.8 × 5.1–8.1 µm). Furthermore, the known geographical distribution of *H. neohorrescens* is the south-eastern Cerrado (South America), while *H. mcmulliniana* is widespread throughout south-eastern North America (Table 2; Lendemer & Allen 2020).

Phylogenetic reconstructions based solely on the nuITS marker did not recover reciprocal monophyly of *H. neohorrescens* and *H. mcmulliniana* (Supplementary Material Fig. S1, available online). Conversely, the two species were separated in distinct clades with the nuITS and mtSSU regions concatenated (Fig. 2). The nuITS genetic distances, widely used as a tool for taxon delimitation in *Parmeliaceae* (Leavitt *et al*. 2014; Divakar *et al*. 2016; Del-Prado *et al*. 2019), do not appear to be universally efficient in discriminating species of the subgenus *Parmelinopsis.* The mean genetic distance between *H. neohorrescens* and *H. mcmulliniana* was 0.018 s/s, ranging from 0.014–0.020 s/s, at the threshold between intra- and interspecific distances of *Parmeliaceae* (0.015–0.017 s/s; Del-Prado *et al.* 2010). Similarly, *H. afrorevoluta* (Krog & Swinscow) Krog & Swinscow and *H. appalachensis* Lendemer & J. L. Allen present nuITS distances between 0.016–0.018 s/s, indicating that some species of the subgenus *Parmelinopsis* may have a recent origin and cannot be differentiated using only the nuITS region.

Considering the different datasets collected in this study, we propose *Hypotrachyna neohorrescens* as a new species and reinforce the need for more taxonomic and molecular studies to unveil the diversity of *Hypotrachyna* subgenus *Parmelinopsis* in the neotropical region, especially in threatened biodiversity hotspots such as the Cerrado.

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Supplementary Material. To view Supplementary Material for this article, please visit

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Table 1. Sequences of *Hypotrachyna* used in the phylogenetic analyses with voucher information and GenBank Accession numbers. New sequences are shown in bold.

Species	Sample ID	Voucher	Locality	nuITS	mtSSU
Subgenus *Parmelinopsis*					
Hypotrachyna afrorevoluta	01	MAF-Lich-10409	Spain: Canary Islands	DQ279529	DQ287839
	02	NY-2328235	USA: North Carolina	MT482207	MT482225
	03	NY-2328105	USA: North Carolina	MT482206	MT482226
H. appalachensis	01	NY-2606541	USA: North Carolina	MT482160	MT482254
	02	NY-2606549	USA: North Carolina	MT482161	-
H. britannica	01	MAF-Lich-15415	Ireland: Kerry	GQ919273	GQ919221
	02	NY-2795290	USA: North Carolina	MT482155	MT482261
H. cryptochlora	01	MAF-Lich-10398	China: Yunnan	DQ279535	DQ287845
	02	NY-2327559	USA: South Carolina	MT482203	MT482228
	03	NY-1885857	USA: North Carolina	MT482215	-
H. exsecta	01	MAF-Lich-10381	China: Yunnan	DQ279497	DQ287806
	02	MAF-Lich-10380	China: Yunnan	DQ279498	DQ287807
H. horrescens	01	MAF-Lich-9913	Spain: La Coruña	AY581085	AY582321
	02	NY-2357898	USA: North Carolina	MT482181	MT482237
	03	NY-2329051	USA: North Carolina	MT482195	MT482236
	04	NY-2329248	USA: North Carolina	MT482178	MT482240
	05	MAF-10399	Spain: Canary Islands	DQ279536	DQ287846
	06	MAF-10400	Spain: Ponevedra	DQ279537	DQ287847
	07	NY-3722141	USA: Tennessee	MT482187	-
	08	NY-3722347	USA: Tennessee	MT482188	-

Table 1. (*Continued*)

Species		Voucher	Locality	nuITS	mtSSU
H. neohorrescens	**01**	***P. Jungbluth* 3160**	**Brazil: São Paulo**	**MW969682**	**MW980901**
	02	***P. Jungbluth* 2690**	**Brazil: São Paulo**	**MW969683**	**MW980902**
	03	***P. Jungbluth* 2642**	**Brazil: São Paulo**	**MW969684**	**MW980903**
	04	***P. Jungbluth* 2709**	**Brazil: São Paulo**	**MW969685**	**MW980904**
	05	***P. Jungbluth* 2647**	**Brazil: São Paulo**	**MW969686**	**MW980905**
	06	***P. Jungbluth* 3266**	**Brazil: São Paulo**	**MW969687**	**MW980906**
	07	***P. Jungbluth* 3264**	**Brazil: São Paulo**	**MW969688**	**MW980907**
	08	***P. Jungbluth* 3161**	**Brazil: São Paulo**	**MW969689**	**MW980908**
	09	***P. Jungbluth* 3317–TYPE**	**Brazil: São Paulo**	**MZ919273**	**MZ919147**
H. kauffmaniana	01	NY-2356582	USA: North Carolina	MT482200	MT482231
	02	NY-2794602	USA: North Carolina	MT482157	MT482257
H. mcmulliniana	01	NY-2356553	USA: North Carolina	MT482197	MT482234
	02	NY-2356567	USA: North Carolina	MT482174	MT482245
	03	NY-2328946	USA: North Carolina	MT482171	MT482248
	04	NY-2328867	USA: North Carolina	MT482196	MT482235
	05	NY-2329035	USA: North Carolina	MT482180	MT482238
	06	NY-2329184	USA: North Carolina	MT482175	MT482244
	07	NY-2328963	USA: North Carolina	MT482172	MT482247
	08	NY-3721310	USA: Tennessee	MT482186	-
	09	NY-3722066	USA: Tennessee	MT482193	-
H. minarum	01	MAF-Lich-7639	Spain: Cádiz	AY581086	AY582322
	02	NY-2356512	USA: North Carolina	MT482176	MT482243
	03	NY-2329265	USA: North Carolina	MT482179	MT482239
	04	MAF-10401	Spain: Canary Islands	DQ279538	DQ287848
	05	MAF-13968	Australia: Queensland	DQ279539	DQ287849
	06	MAF-10220	China: Yunnan	AY611110	AY611168
	07	NY-3721830	USA: Tennessee	MT482182	-
	09	***A.S. Rodrigues* 594**	**Brazil: Rio Grande do Sul**	**MZ919271**	**MZ919145**
	10	***A.S. Rodrigues* 614**	**Brazil: Rio Grande do Sul**	**MZ919272**	**MZ919146**

Table 1. (*Continued*)

Species		Voucher	Locality	nuITS	mtSSU
H. neodamaziana	01	MAF-Lich-10182	Australia: Queensland	AY611107	AY611166
H. neodissecta	01	MAF-Lich-13986	South Africa: Western Cape	DQ279510	DQ287820
	02	F, MAF-Lich-19622	Kenya: Western Province	JN943836	KR995336
H. pluriformis	01	KRAM-L	Bolivia: Aniceto Arce	KF380912	KF380995
H. revoluta	01	MAF-Lich-6047	Spain: Puerto Urkiola	AY611075	AF351166
	02	NY-2356534	USA: North Carolina	MT482202	MT482229
	03	MAF-13989	South Africa: Western Cape	DQ279523	DQ287833
H. showmanii	01	MAF-Lich-15618	USA: Pennsylvania	GQ919287	GQ919234
	02	NY-1080325	USA: Pennsylvania	KF380916	KF380999
H. spumosa	01	NY-1885632	USA: North Carolina	MT482220	-
	02	NY-1886244	USA: North Carolina	MT482216	-
H. subfatiscens	1	MAF-Lich-6878	Australia: Queensland	AY611108	AF351174
Subgenus *Everniastrum*					
Hypotrachyna cirrhata	01	MAF-13976	Peru	DQ279487	DQ287795
	02	MAF-10374	China: Yunnan	DQ279486	DQ287794

Table 2. Comparison of morphological characters of *Hypotrachyna neohorrescens*, *H. horrescens, H. mcmulliniana* and *H. minarum.* Descriptions for *H. mcmulliniana* are based on Lendemer & Allen (2020) and for *H. horrescens* on the lectotype (FH-TAYL).

	H. neohorrescens	*H. horrescens*	*H. mcmulliniana*	*H. minarum*
Vegetative propagules	laminal isidia with frequent apical cilia	laminal isidia with sparse apical cilia	Laminal isidia with sparse to occasional apical cilia	Laminal isidia lacking apical cilia
Laciniae/lobe (mm)	sublinear, 0.5–1.7 wide at the base of the branches, 1.5–2.5 maximum width	sublinear, (0.7–)1.0–2.5 wide at the base of the branches, 1.5–2.5 maximum width	sublinear, 1.0–4.0 wide (probably maximum width)	sublinear, 1.1–3.5 wide
Marginal cilia	abundant	frequent	infrequent	infrequent
Rhizines	simple	simple to rarely dichotomously branched	simple to dichotomously branched	simple to irregularly branched
Ascospores (µm)	(12.5–)16.0(–19.0) × (8.8–)10.2(–11.3)	16–18 × 10–12 (according to Hale (1976))	9.9–13.8 × 5.1–8.1	not measured (no apothecia found)
Chemistry	Atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid, 5-*O*-methylhiascic acid and gyrophoric acid	Atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid, 5-*O*-methylhiascic acid and gyrophoric acid	Atranorin, 3-methoxy-2,4-di-*O*-methylgyrophoric acid, 5-*O*-methylhiascic acid and gyrophoric acid.	Atranorin, gyrophoric acid and unidentified compound (R_f above gyrophoric acid in solvent C).

Fig. 1. Sampling points of *Hypotrachyna neohorrescens* in the south-eastern Cerrado ecoregion of Brazil. 1, *P. Jungbluth* 3160, *P. Jungbluth* 3317 (type), 3161, 3264 and 3266. 2, *P. Jungbluth* 2690 and 2709. 3, *P. Jungbluth* 2642 and 2647 4, *P. Jungbluth* 2294. The Cerrado distribution is shown in grey.

Fig. 2. Phylogenetic relationships of Hypotrachyna subgenus Parmelinopsis based on maximum likelihood (ML) and Bayesian inference analyses from nuITS and mtSSU sequences. The Bayesian tree is shown here. Thickened branches indicate ML bootstrap values ≥ 70% and posterior probabilities ≥ 0.95. Grey boxes indicate the species for which novel sequences have been generated in this study.

Fig. 3. Morphological features of selected *Hypotrachyna* species in Brazil. A & B, *Hypotrachyna neohorrescens*. A, thallus of the holotype (*P. Jungbluth* 3317). B, distribution and number of ciliated isidia on the upper surface (*P. Jungbluth* 3316). C & D, *Hypotrachyna minarum.* C, thallus (*A. S. Rodrigues* 614). D, laminal isidia lacking cilia (*A. S. Rodrigues* 594). Scales: A = 2 mm; B & C = 1 mm; D = 0.2 mm.

Supplementary Material:

Figure S1. Phylogenetic relationships of Hypotrachyna subgenus Parmelinopsis based on a maximum likelihood (ML) and Bayesian inference analyses from nuITS sequences. The Bayesian tree is shown here. Thickened branches represent bootstrap values ≥ 70 and posterior probabilities ≥ 0.95 are indicated in the figure. Grey boxes indicate the species for which novel sequences have been generated in this study.

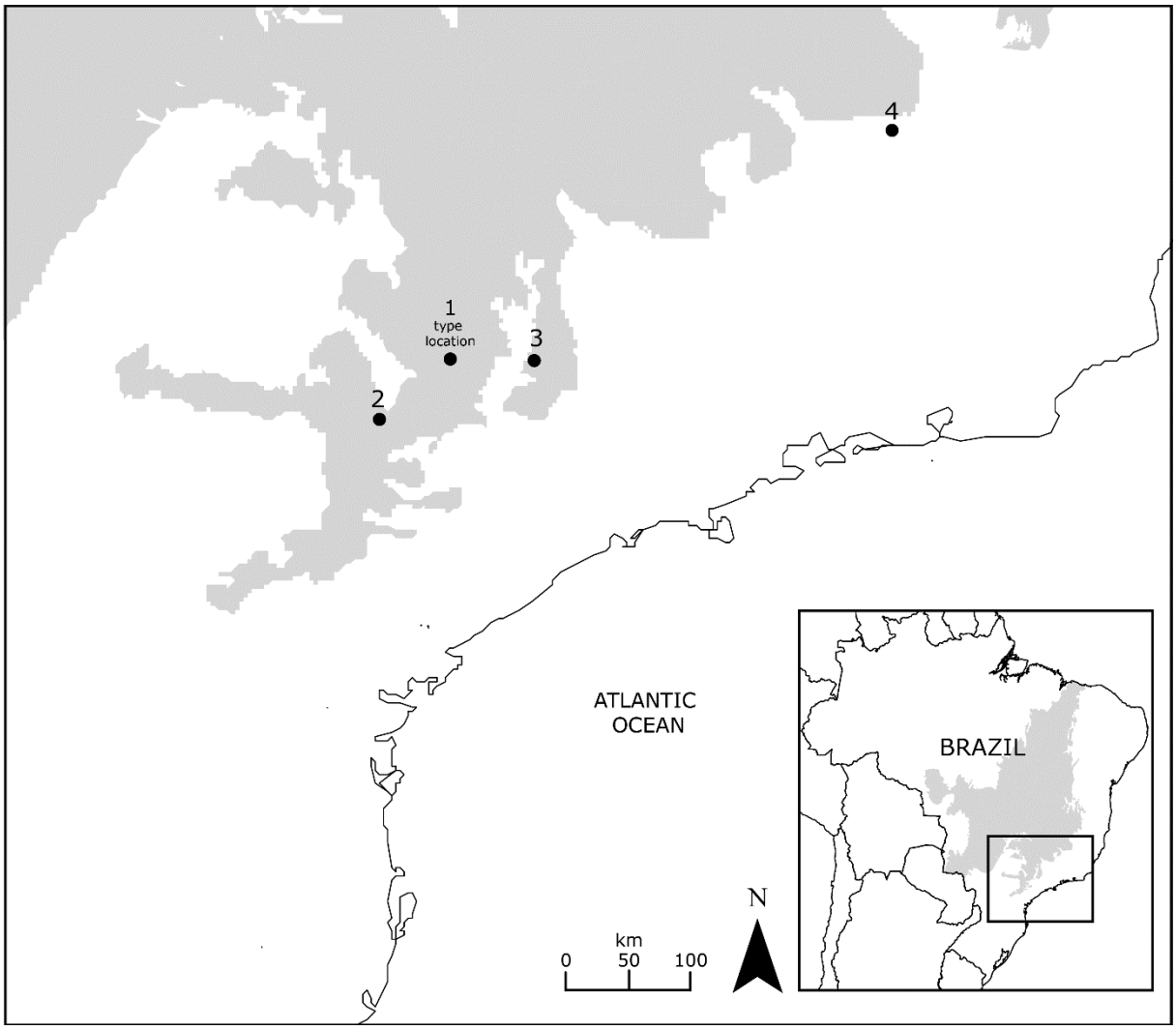
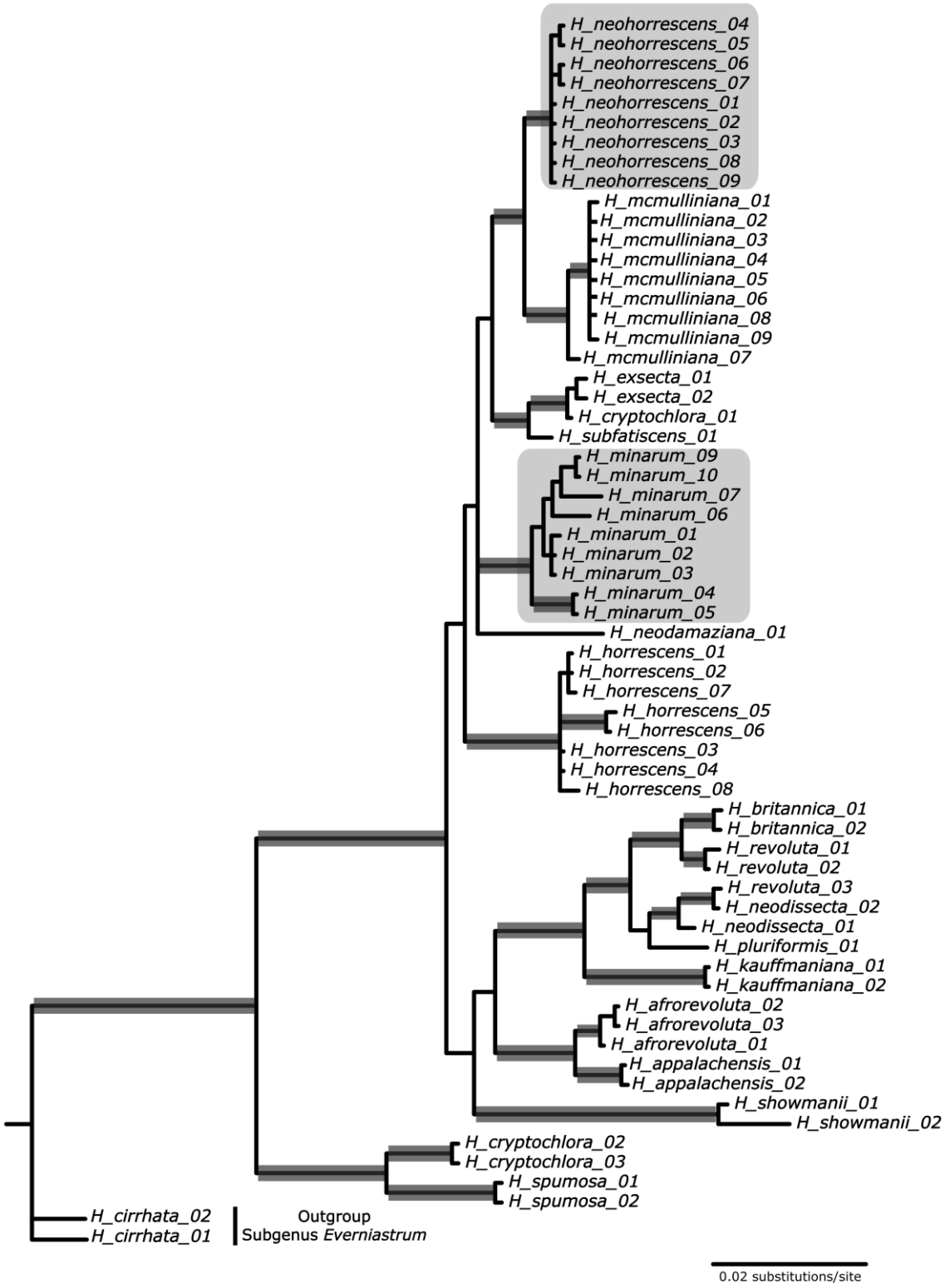


Figure 1.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H_neohorrescens_05
H_neohorrescens_06
H_neohorrescens_07
H_neohorrescens_01
H_neohorrescens_02
H_neohorrescens_03
H_neohorrescens_08
H_neohorrescens_09
H_mcmulliniana_01
H_mcmulliniana_02
H_mcmulliniana_03
H_mcmulliniana_04
H_mcmulliniana_05
H_mcmulliniana_06
H_mcmulliniana_08
H_mcmulliniana_09
H_mcmulliniana_07
H_exsecta_01
H_exsecta_02
H_cryptochlora_01
H_subfatiscens_01
H_minarum_09
H_minarum_10
H_minarum_07
H_minarum_06
H_minarum_01
H_minarum_02
H_minarum_03
H_minarum_04
H_minarum_05
H_neodamaziana_01
H_horrescens_01
H_horrescens_02
H_horrescens_07
H_horrescens_05
H_horrescens_06
H_horrescens_03
H_horrescens_04
H_horrescens_08
H_britannica_01
H_britannica_02
H_revoluta_01
H_revoluta_02
H_revoluta_03
H_neodissecta_02
H_neodissecta_01
H_pluriformis_01
H_kauffmaniana_01
H_kauffmaniana_02
H_afrorevoluta_02
H_afrorevoluta_03
H_afrorevoluta_01
H_appalachensis_01
H_appalachensis_02
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H_showmanii_02
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H_cryptochlora_03
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H_cirrhata_01
Outgroup
Subgenus Everniastrum
0.02 substitutions/site

Figure 2.

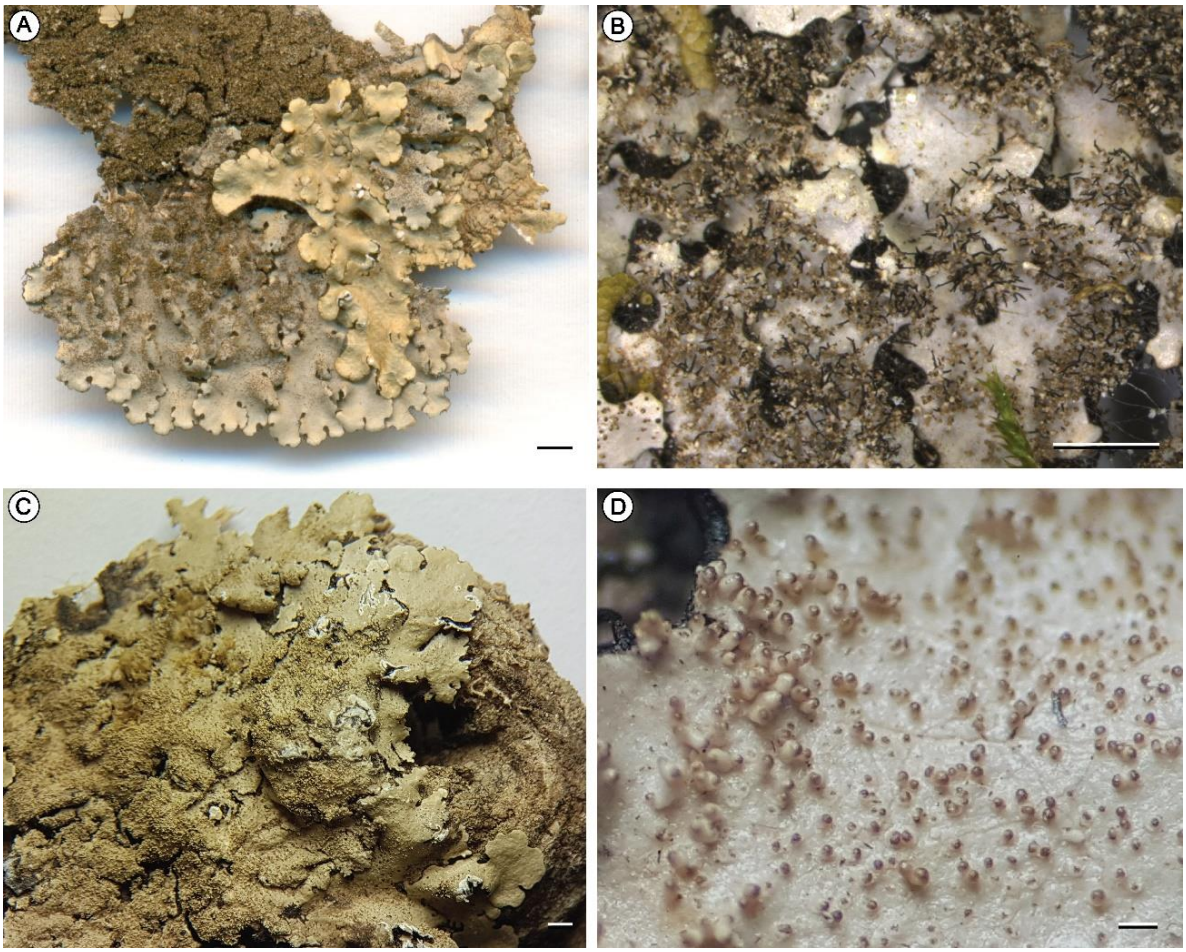
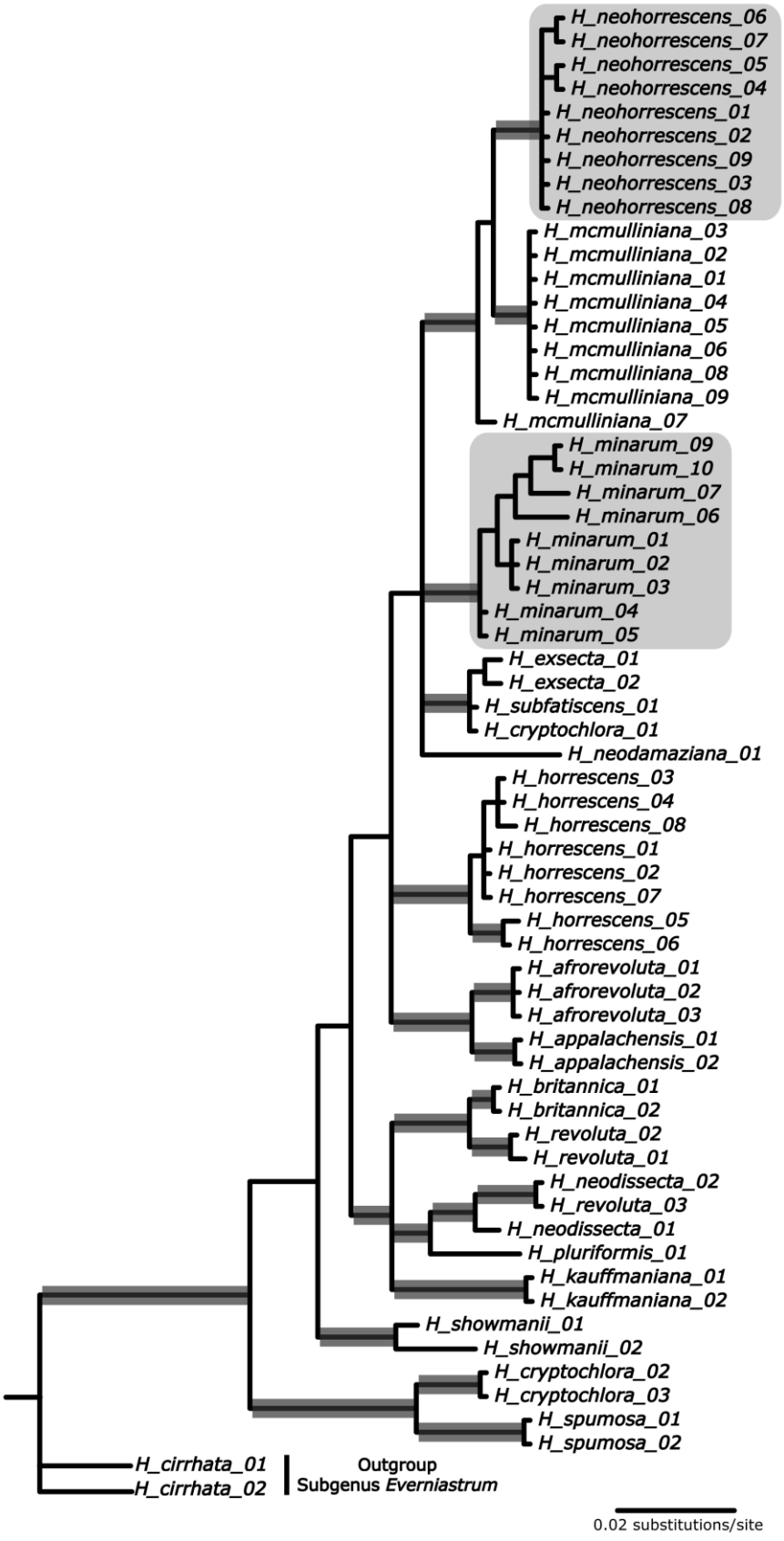


Figure 3.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H_neohorrescens_02
H_neohorrescens_09
H_neohorrescens_03
H_neohorrescens_08
H_mcmulliniana_03
H_mcmulliniana_02
H_mcmulliniana_01
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H_mcmulliniana_07
H_minarum_09
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H_minarum_07
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H_minarum_03
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H_exsecta_01
H_exsecta_02
H_subfatiscens_01
H_cryptochlora_01
H_neodamaziana_01
H_horrescens_03
H_horrescens_04
H_horrescens_08
H_horrescens_01
H_horrescens_02
H_horrescens_07
H_horrescens_05
H_horrescens_06
H_afrorevoluta_01
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H_afrorevoluta_03
H_appalachensis_01
H_appalachensis_02
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H_pluriformis_01
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H_showmanii_01
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H_cryptochlora_02
H_cryptochlora_03
H_spumosa_01
H_spumosa_02
H_cirrhata_01
H_cirrhata_02
Outgroup
Subgenus Everniastrum
0.02 substitutions/site

Figure S1.

Artigos

Capítulo III

Applying integrative taxonomy in parmelioid lichens from Brazil, a challenging task

Revista alvo: *Plant and Fungal Systematics Journal*

Situação: em preparação.

Applying integrative taxonomy in parmelioid lichens from Brazil, a challenging task

Abstract. The identification of lichenized fungi has evolved mainly due to advances in molecular biology, such as DNA barcoding, providing tools to facilitate species recognition. Limitations of this technique include the reliability of the taxonomic identification of sequences deposited in public databases and the low success rate in regions with poor knowledge of their local biodiversity. Thus, this study aimed to make the integrative identification of species of parmelioid lichens (*Parmeliaceae*) from the coastal region of the extreme south of Brazil. Several characteristics of the thallus and its main chemical compounds were analyzed and compared with molecular data based on the nuITS region, using BLASTn NBCI and phylogenetic reconstructions. We analyzed 90 specimens, representing 39 morphologically distinct species. Of these morphospecies, only 22 have already DNA sequences with the same species identification in the GenBank public database. Among these, 20 were recovered in clades with their respective homonymous sequences, yet, in 45%, the clades also included sequences with different identifications. Of the 17 morphospecies without homonymous sequences in GenBank, only six appear to be distinct in molecular approaches from the sequences available in Genbank, being the new records of the species in the database. While the others present some incongruity between the approaches. Based on the phylogenetic and genetic distances results, we found 22 species complexes, mainly encompassing species of *Hypotrachyna*, *Parmotrema*, and *Punctelia*. The low effectiveness of the nuITS marker in resolving recently diverged groups requires increasing the number of genomic regions sequenced. Our data also demonstrates the difficulty of using DNA barcoding in regions with poor biodiversity knowledge. The currently encouraged strategy is developing a regional DNA barcode reference library, an alternative to the limitations encountered in using sequences from public databases. Thus, this study provides a

reference DNA barcode database for parmelioid lichens of the coastal region of the extreme south of Brazil, combined with detailed morphological descriptions of the species.

Key words: Morphology, nuITS marker, *Parmeliaceae*, Phylogeny, Species complex.

Introduction

Lichens are the result of symbiotic relationships between fungi and photobionts, such as green algae and cyanobacteria, as well as other microorganisms (Hawksworth & Grube 2020). They are commonly found in the most diverse environments, mainly in areas of pioneer formations (Favero-Longo et al. 2012; Gheza et al. 2020). Among the great diversity of lichens, *Parmeliaceae* is currently considered the most diverse family, encompassing about 2700 species (Lücking et al. 2017), presenting in the southern hemisphere, its main zone of diversification (Thell et al. 2012). *Parmeliaceae* is phylogenetically divided into two subfamilies - *Protoparmelioideae* (including *Maronina* Hafellner & R.W. Rogers, *Neoprotoparmelia* Garima Singh, Lumbsch & I. Schmitt, and *Protoparmelia* M. Choisy) and *Parmelioideae* (including all other genera), the latter being informally classified in seven main clades (Crespo et al. 2011; Divakar et al. 2017; Pizarro et al. 2018). Among those, the Parmelioid clade is the most diverse, with more than 1800 species (Lücking et al. 2017; Divakar et al. 2013a; 2017). Parmelioid species are predominantly characterized by having foliose thallus, rhizinate lower surface, laminal apothecia, and simple colorless ascospores (Divakar et al. 2013a).

Lichens are complex associations and their nomenclature is based on the primary mycobiont, therefore, the process of specific phenotype-based identification in this group is not always an easy and quick task (Lücking et al. 2021; Singh et al. 2019). The evolution of techniques to facilitate this process has gained strength in recent decades, as is the case of DNA barcoding, a tool proposed for the rapid identification of species (Lücking et al. 2020a; Hebert et al.

2003). For fungi, the nuITS region is used as the universal marker (Schoch et al. 2012). Although it has a high representation of sequences in public databases and is widely used in the most diverse groups of lichenized fungi, the nuITS marker has demonstrated low effectiveness in the identification or delimitation of species complexes (Lücking et al. 2020a; 2020b).

Therefore, the identification based on phenotype in *Parmeliaceae* has still been an important approach for the recognition of taxa; however, it has been shown that some morphological characters used for the delimitation of genera and species do not always corroborate the results obtained by molecular phylogenies (Blanco et al. 2005; Divakar et al. 2013b; Kirika et al. 2016; Leavitt et al. 2018; Rodrigues et al. 2021). This scenario can result from the presence of cryptic species (Leavitt et al. 2016; Haugan & Timdal 2019; Garrido-Huéscar et al. 2022), species with high phenotypic plasticity (Pérez-Ortega et al. 2012; Del-Prado et al. 2019), or even species complexes (Del-Prado et al. 2016; Dos Santos et al. 2019; Boluda et al. 2019; Lucking et al. 2020b). As mentioned above, using only DNA barcoding for specific inference also has significant limitations, mainly related to the lack of confidence in the sequences deposited in public databases due to the high rates of errors found in them (Nilsson et al. 2018; Pentinsaari et al. 2020; Cheng et al. 2023). Another great challenge of using DNA barcoding has been its application for species from tropical and subtropical megadiverse regions where the diversity of lichenized fungi is poorly known, sequenced, and represented in public databases (Orock et al. 2012; La Torre et al. 2023).

Strategies and protocols for best practices for the identification of lichenized fungi have been recommended (Lucking et al. 2021; Lendemer 2021). In this way, integrative taxonomy emerges as a proposal to use different datasets to delimit taxa, thus providing diverse and concrete evidence about their identity (Lucking et al. 2020b). In addition, creating regional DNA barcode reference libraries has shown to be a potential alternative in the molecular

identification of lichenized fungi, considerably minimizing error rates compared to sequence-based identification based only on public databases (Kerr & Leavitt 2023).

Thus, this study aims to perform integrative taxonomy based on morphological and molecular data of lichen species from the Parmelioid clade found in the coastal region of extreme southern Brazil. We provided accurate sequences, phylogenies based on the nuITS region, images, and morphological descriptions of the studied species, and discussed the application of integrative taxonomy as a tool to improve lichen species identification.

Materials and Methods

Collection sites

The collections occurred in the municipalities of Chuí, Pelotas, and Rio Grande in the State of Rio Grande do Sul, Brazil. The specimens were collected from native trees of the coastal vegetation of the Pampa biome, in the extreme south of Brazil, as illustrated in Figure 1 and summarized in Table 1. The geographic region is characterized by a humid subtropical climate and is dominated by grasslands and shrub communities adapted to the local edaphic conditions (IBGE 2019). The collection methodology followed Filgueiras (1994). The specimens were removed from the phorophytes using a knife and stored in paper sacks with information on location, type of substrate, lighting, and collection point corresponding to geographic coordinates. After the samples remained for 15 days in open paper sacks in a ventilated environment to dry naturally.

Morphological studies

For morphological identification, specimens were examined using a Nikon SMZ645 stereomicroscope (Nikon Corporation), Olympus CX22LED optical microscope (Olympus Corporation), and specialized bibliographies. Chemical compounds were identified by spot

tests (K - Potassium hydroxide solution; C - Sodium hypochlorite solution; KC - addition of the K test followed by the C; and P - Paraphenylenediamine) and thin layer chromatography (TLC), using solvents A, B, C, and EA, according to the chemical components present in the samples (Orange et al. 2010). A morphological matrix and a species description protocol were adapted from Canêz & Marcelii (2006), analyzing in this matrix the size, shape, and color of the lobes/lacinia, upper/lower surfaces, cilia, rhizines, medulla, vegetative propagules, apothecia, ascospores, conidia, and the main chemical compounds of the specimens. The samples were deposited in the CGMS Herbarium at the Universidade Federal de Mato Grosso do Sul.

Molecular Analyses

Before the DNA extraction, thallus fragments were immersed in acetone for 20 minutes to remove the secondary compounds. Afterward, they were dried in the open air until the acetone evaporated completely. DNA extraction was performed using the Wizard® Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. The nuITS region was amplified using the ITS1F (Gardes & Bruns 1993) and ITS4 (White et al. 1990) universal primers. The PCR conditions followed the described in Rodrigues et al. (2021). The amplification products were visualized in 1% agarose gel stained with GelRed®. Macrogen Korea performed DNA purification and sequencing. For assembly and quality evaluation of the DNA sequences generated, we used Geneious® 9.1.6 (Kearse et al. 2012). Subsequently, the generated sequences were tested using the Basic Local Alignment Search Tool (BLAST) (https://blast.ncbi.nlm.nih.gov/Blast.cgi), selecting Nucleotide BLAST (BLASTn) to confirm the identification at the family or genus level to confirm identification at the family or genus level with sequences available in the GenBank database.

Phylogenetic analyses were performed at the genus level, using all sequences nuITS available on GenBank (accessed June 2023). Here we designate homonymous sequences as those with same-species named sequences available in the GenBank database. The outgroups were selected based on the Parmeliaceae phylogenetic relationships described by Divakar et al. (2017). Sequences erroneously identified at the generic level, clearly revealed during exploratory alignments and phylogenetic analyses, were excluded from the subsequent steps. Multiple sequence alignments were performed using MAFFT v7 (https://mafft.cbrc.jp/alignment/server/), applying the following parameters: G-INS-i algorithm, 1PAM/K=2 scoring matrix, offset value of 0.1 and the remaining parameters with default values (Leavitt et al. 2021), followed by manual inspection. The phylogenetic reconstructions were based on the maximum likelihood (ML) approach, using the RAxML v8 program (Stamatakis 2014), with the "GTRGAMMA" model and 1000 bootstrap pseudoreplicates. Analyses were performed using the Cipres Science Gateway (https://www.phylo.org/). The trees were edited through the FigTree (http://tree.bio.ed.ac.uk/software/figtree/).

Integrative taxonomy

The species-level identifications of the samples were based on phenetic and molecular taxonomies. Thus, to verify the results of the species hypotheses generated according to the two approaches, we followed three steps:

I. Morphological identification: We identified the species using identification keys, including descriptions and digital images of the species' holotypes (when available on herbarium websites or in studies that examined the types). We identify the main chemical components of the thallus, firstly by spot tests and later by thin-layer chromatography. Species identified as “sp.” have morphological differences from the

closest species found by the keys and bibliographies used, thus needing further studies for confirmation. All studied characters were described in a morphological matrix (Supplementary Material S1)

II. Molecular analyses: We used the default parameters to search the generated sequences in BLASTn, selecting the three results with the Maximum Score (MS) and the three with the highest percentage of identity (PI). Following Lücking et al. (2020a), Moncada et al. (2020), and Leavitt et al. (2021), the sequences were classified as "potential candidate", when the PI values were ≥ 99%; as "species complex" when PI values ranged from 97 to 98 %; and "mismatch" when PI values were < 97%. The phylogenetic relationships were recovered with maximum likelihood trees constructed for each genus, considering as highly supported groups only branches with bootstrap values ≥ 70%.

III. Multiple data integration: We organized the analysis of the datasets into four different types of cases found in the results. Case I – morphospecies (identification based on morphology) + homonymous sequences phylogenetically recovered in the same clade; Case II – morphospecies + homonymous sequences recovered in the same clade, but also containing sequences from different species; Case III – morphospecies + sequences with corroborated molecular identity (sequences without homonyms in GenBank and distinct from those existing in the database); Case IV– morphospecies + sequences without homonyms in GenBank and with phylogenetic reconstructions grouping these sequences with other species different from the database, or with sequences of different identity generated by the study. In all cases, the percentage of identity obtained through NCBI BLASTn in each situation was demonstrated.

Results

Morphology

Among the 90 specimens examined, we identified eight genera and 39 morphologically distinct species distributed in eight genera (number of species per genus is in parentheses): *Bulbothrix* Hale (3), *Canoparmelia* Elix & Hale (3), *Flavoparmelia* Hale (2), *Hypotrachyna* (Vain.) Hale (3), *Parmelinella* Elix & Hale (2), *Parmotrema* A. Massal. (20), *Punctelia* Krog (5), and *Relicina* (Hale & Kurok.) Hale (1). We developed a morphological matrix analyzing about 68 states of morphological characters of the main structures of the thallus (Supplementary Material S1). Descriptions of the species in text generated from the morphological matrix are in Supplementary Material S2. Based on phenotypic distinctions, four potential candidates for new species were found, being identified as "sp. nov.".

Molecular data

We generated 90 nuITS sequences containing lengths between 500-700 bp (Table 1). According to the BLASTn results, 34 (37.7%) were classified as "potential candidates" (PI ≥ 99%); 33 (36.6%) as "species complexes" (PI between 98-97%); and 23 (25.5%) as "mismatches" (PI < 97%). Details are described in the Supplementary Table S3.

For the phylogenetic reconstructions, more than 1400 nuITS sequences were retrieved from GenBank (Supplementary Table S4). The phylogenetic trees, constructed for each genus, are presented in Supplementary Figures 1–7. Among the morphospecies identified, 22 contained homonymous sequences in GenBank, of which 20 were recovered in clades with their respective homonymous sequences; however, in 45% of these, the clades also included sequences with different identifications. Examples are *Hypotrachyna livida* (Taylor) Hale, *Parmelinella salacinifera* (Hale) Marcelli & Benatti, *Parmotrema cetratum* (Ach.) Hale, *P. clavuliferum* (Räsänen) Streimann, *P. haitiense* (Hale) Hale, *P. reticulatum* (Taylor) M.

Choisy, *P. perlatum* (Huds.) M. Choisy, *Crespoa carneopruinata* (Zahlbr.) Lendemer & B.P. Hodk., and *C. crozalsiana* (B. de Lesd. ex Harm.) Lendemer & B.P. Hodk. (Supplementary Figures 1, 4, and 5). The morphospecies *Parmotrema pilosum* and *Relicina subabstrusa* did not group with their homonymous sequences.

Among the 17 morphospecies without homonymous sequences, six were not grouped with the sequences available in Genbank, corroborating the phylogenetic positioning, and BLASTn results with identity percentage values lower than 97% - *Parmotrema eliasaroanum* Benatti, Marcelli & Elix, *P. madilynae* A. Fletcher, *P. subrugatum* (Kremp.) Hale, *Parmotrema* sp. nov. 1, *Punctelia borrerina* (Nyl.) Krog, and *P. riograndensis* (Lynge) Krog (Supplementary Figures 5 and 6 and Supplementary Table S3). The remaining species showed some type of inconsistency between morphological and molecular identification, grouping with sequences from other species available in GenBank or with sequences generated in this study, presenting identity values between 97-99%.

Integrative Taxonomy

We combined the morphological and molecular main results in Table 2. Among the 22 species with homonymous sequences available in GenBank, only 10 obtained the same identification considering morphology, BLASTn, and phylogeny: *Canoparmelia albaniensis* (C.W. Dodge) Divakar & Kirika, *C. caroliniana* (Nyl.) Elix & Hale, *C. texana* (Tuck.) Elix & Hale, *Flavoparmelia soredians* (Nyl.) Hale, *Hypotrachyna minarum* (Vain.) Krog & Swinscow, *H. spumosa* (Asahina) Krog & Swinscow, *Parmelinella lindmanii* (Lynge) A.S. Rodrigues, Canêz & A.P. Lorenz, *Parmotrema austrosinense* (Zahlbr.) Hale, *P. praesorediosum* (Nyl.) Hale, and *P. tinctorum* (Despr. ex Nyl.) Hale. These represent only 25.6 % of the 39 morphospecies identified and 45.4 % of those with homonymous sequences available in GenBank.

On the other hand, among the 17 morphospecies without homonymous sequences available, 10 presented inconsistencies between the morphological and molecular identifications (phylogenetic relationships and BLASTn), such as *Bulbothrix bulbilosa* Benatti, Spielmann & Bungartz, *B. cassa* Jungbluth, Marcelli & Elix, and *B. ventricosa* (Hale & Kurok.) Hale, *Flavoparmelia exornata* (Zahlbr.) Hale, *P. commensuratum* (Hale) Hale, *Parmotrema eciliatum* (Nyl.) Hale, *P. homotomum* (Nyl.) Hale, *Punctelia* sp. nov. 1, *Punctelia* sp. nov. 2, and *Punctelia* sp. nov. 3.

Finally, considering all morphospecies, at least 22 (56.4 %) appear to belong to species complexes, mainly members of *Hypotrachyna*, *Parmotrema*, and *Punctelia*. The obtained PI values were often in the 97–98% threshold (Supplementary Table S3), and/or in the phylogenetic trees the sequences were grouped with homonymous and non-homonymous sequences (Supplementary Figures 4, 5, and 6). Some cases, despite having values of 99% with a different species, also presented this value with other species, therefore were considered species complexes. We found cases of species complexes in our dataset in the following situations:

A – Distinct species with different morphologies grouped in the same clade, as in *Crespoa carneopruinata, C. crozalsiana*, *Hypotrachyna livida*, *Parmotrema cetratum*, *P. clavuliferum*, *P. commensuratum*, *P. haitiense*, *P. homotomum,* and *P. reticulatum*.

B – Morphologically similar species, normally varying by the presence/absence of vegetative propagules or variations in these reproductive structures, being recovered in the same clade, such as *Flavoparmelia exornata*, *Parmotrema eciliatum*, *P. eitenii*, *P. perlatum*, *P. pilosum*, *Punctelia* sp. nov. 1, *Punctelia* sp. nov. 2, and *Punctelia* sp. nov. 3.

C – Species with morphologies consistent with the description, but with genetic variation with the homonymous sequences available in GenBank, as *Flavoparmelia*

soredians, *Hypotrachyna minarum*, *H. spumosa*, *Parmelinella salacinifera*, and *P. praesorediosum*.

Below, we present the results for each species separated according to the cases described previously:

Case I

Number of morphospecies found: 10. The specimen of *Canoparmelia albaniensis* was confirmed according to the results obtained by BLASTn (98 – 100% identity), and its phylogenetic position (Supplementary Figure 2). The sequences of *Canoparmelia caroliniana* show variable identity values between 98-100% with the closest sequences (Supplementary Table S3). In the phylogenetic reconstruction, *C. caroliniana* sequences were recovered in two clades, grouping the study sequences into the largest (Supplementary Figure 2). Specimens of *Canoparmelia texana* also grouped with sequences of the same identity available in GenBank (Supplementary Figure 2), and presented PI values (percentage of identity) between 98-99% with the most similar sequences.

Flavoparmelia soredians sequences were phylogenetically recovered in two distinct clades (clades I and II in Supplementary Figure 3) and strongly supported, indicating the existence of two lineages under the same name. The sequence from this study clustered with sequences identified as *F.* aff. *soredians* (KF017378 and KF017379) in clade II (Supplementary Figure 3), showing similarity above 99% according to BLASTn. Sequences from *Hypotrachyna minarum* and *H. spumosa* were recovered with their respective homonymous sequences; however, both showed high intraspecific variation (Supplementary Figure 4 and Supplementary Table S3). Specimens of *Hypotrachyna minarum* presented identity values with the closest homonymous sequences between 97-98%, while the two study sequences

obtained 100% similarity. Specimens of *H. spumosa* obtained values between 96-97% with their respective homonymous sequences.

Parmelinella lindmanii obtained precise identifications with morphological and molecular approaches, grouping with the homonymous sequences and obtaining PI values above 99% (Supplementary Figure 1 and Supplementary Table S3). Specimens of *Parmotrema austrosinense* clustered with their homonymous sequences and showed PI values between 98-99% (Supplementary Figure 5 and Supplementary Table S3). The sequences of *Parmotrema praesorediosum* also grouped with the homonymous sequences, but showed high intraspecific variation, obtaining identity values between 96-97%. *Parmotrema tinctorum* was recovered with the homonymous sequences and showed an IP value above 99% with the closest sequences.

Case II

Number of morphospecies found: 10. *Hypotrachyna livida* grouped with sequences of the same identity and with *H. dactylifera* (Vain.) Hale (AY251422) (Supplementary Figure 4). The specimen AR162 showed a similarity value above 99% with *H. livida* (KF380906 and KF380905), while the AR163 sample has 98-98.6% similarity with the same sequences. Specimens of *Parmelinella salacinifera* grouped with the sequence of *P.* aff. *wallichiana* (Taylor) Elix & Hale (GQ267191) (Supplementary Figure 1), obtaining PI value between 94-95%.

The specimen *Parmotrema cetratum* (AR640) was closely related to the sequences of *P. reticulatum* and *P. cetratum* in the clade named cetratum/reticulatum clade I, showing PI values of 96-98% (Supplementary Figure 5). While the other specimen (AR536) was recovered as a sister group to cetratum/reticulatum clade II and obtained similarity percentages between 96-97% with the closest sequences. The sequences identified as *Parmotrema clavuliferum*

clustered with sequences of *P. clavuliferum* and *P. reticulatum* in the clade of the same name (Supplementary Figure 5). Both sequences obtained identity values between 97-98% with the closest sequences.

Parmotrema haitiense formed a strongly supported group with sequences of *P. haitiense*, *P. recipiendum* (Nyl.) Hale, *P. subtinctorium* (Zahlbr.) Hale, and *P. subsumptum* (Nyl.) Hale (Supplementary Figure 5). The PI values varied from 97-98% between the study sequences and the others. The sequences corresponding to *P. perlatum* were recovered in distinct clades. The specimen AR498 was recovered as sister group to the *P. internexum* clade (Supplementary Figure 5), obtaining PI values of 97% with sequences closest to *P. perlatum* and *P. internexum*. While specimen AR641 clustered with sequences from *P. perlatum* (ON312517) and *P.* aff. *perlatum* (HM017027), obtaining a similarity value of 99% with the *P. perlatum* sequence (ON312517) and 96% with *P. internexum* sequences.

In the case of *P. pilosum* (Stizenb.) Krog & Swinscow formed an unsupported clade with *P. consors* (Nyl.) Krog & Swinscow (Supplementary Figure 5), and showed about 98% similarity. *Parmotrema reticulatum* sequences grouped with *P. reticulatum* and *P. cetratum* in the clade named cetratum/reticulatum clade II (Supplementary Figure 5), obtaining percentages of identifications between 97-99% with the closest sequence.

In the subgenus *Crespoa*, specimens identified as *C. carneopruinata* and *C. crozalsiana* grouped with sequences from the same species and also with *C. inhaminensis* (C.W. Dodge) Lendemer & B.P. Hodk. and *C. schelpei* (Hale) Lendemer & B. P. Hodk. (Supplementary Figure 5). BLASTn results indicated PI values ≥ 99% between the species *C. carneopruinata*, *C. crozalsiana,* and *C. inhaminensis*.

Case III

Number of morphospecies found: 6. *Parmotrema eliasaroanum* Benatti, Marcelli & Elix, was recovered as closer to *P. perlatum* and *P. internexum* sequences (Supplementary Figure 5), obtaining PI values of 94% with the closest sequences. *Parmotrema madilynae* A. Fletcher grouped with sequences from *P. robustum*, *P. mellissii*, *P. paulense*, and *P. flavotinctorum*, and obtained PI values ranging from 92-95%. While the four sequences from *P. subrugatum* (Kremp.) Hale formed a single clade and obtained between 91-96% with the closest sequences from GenBank.

Parmotrema sp.1 was recovered as phylogenetically close to *P. hababianum* (Gyeln.) Hale (MK722216) and *Parmotrema* sp. (MK722235), with no PI values being found between both species. *Parmotrema* sp. nov. 1 proved to be a potential new species, but more studies are needed, mainly to better understand and confirm the distinction of *Parmotrema* sp1. with the species of *P. hababianum* (Gyeln.) Hale (phylogenetically close as illustrated in Supplementary Figure 5), and *P. indicum* Hale, morphologically similar to *Parmotrema* sp.1, but neither sequence of the species in GenBank was found.

Punctelia borrerina was recovered as a sister group to the clade containing sequences from *P. borreri* and *P. stictica* (Supplementary Figure 6), obtaining PI values between 94-96 %. While the sequence of *P. riograndensis* was recovered as sister a group to *P. stictica* (Supplementary Figure 6), showing PI values of 95%.

Case IV

Number of morphospecies found: 11. *Bulbothrix bulbillosa* clustered with sequences of *B. apophysata* (Hale & Kurok.) Hale and *B. laevigatula* (Nyl.) Hale, showing a PI of 99.8 and 99.2%, respectively. *Bulbothrix cassa* and *B. ventricosa* sequences clustered together and did not show similar sequences in the database (Supplementary Figure 1). *Flavoparmelia*

exornata sequences were recovered as closely related to a sequence from *F. papillosa* (Lynge ex Gyeln.) Hale (Supplementary Figure 3), although BLASTn results did not provide identity values between the two species.

The specimens morphologically identified as *Parmotrema commensuratum* and *P. homotomum* grouped with sequences from *P. reticulatum* and *P. cetratum* in the cetratum/reticulatum clade II (Supplementary Figure 5), obtaining percentages of identifications between 97-99% with the closest sequence. The specimens of *Parmotrema eciliatum* formed a clade with sequences from *P. crinitum* (Ach.) M. Choisy (AY251442) and *P.* aff. *gardneri* (CW Dodge) Serus. (HM017034), was also sister group to the largest clade containing sequences from *P. perlatum* and *P. crinitum* (Supplementary Figure 5). The BLASTn results showed a PI value above 99% with the *P. crinitum* sequence, while for *P.* aff. *gardneri* we found no comparisons.

The specimen *P. eitenii* (AR149) was not phylogenetically distinct from *P. tinctorum* (Supplementary Figure 5), presenting similarity values above 99% with the closest *P. tinctorum* sequences. In contrast, the other specimens of *P. eitenii* (AR154 and AR569) were close to a sequence of *P. tinctorum* and *Parmotrema* sp., forming a clade that was distinct from the other sequences of the species (Supplementary Figure 5). The BLASTn results of *P. eitenii* with the clade sequences were 97-98%.

Punctelia sp.1 sequences, except for specimen AR263, clustered together and showed proximity to *P. subpraesignis* (Nyl.) Krog (AY267010) (Supplementary Figure 6). BLASTn results showed PI values varying from 97 to 98 % between *Punctelia* sp.1 and some sequences from *P. subpraesignis*, *P. borreri* (Sm.) Krog, *Punctelia* sp., *P. perreticulata* (Räsänen) G Wilh. & Ladd, and *P. subrudecta* (Nyl.) Krog.

Specimens of *Punctelia* sp. nov. 2 formed an unsupported clade with *P. borreri* (KR024450), and sister group to the larger clade containing sequences from *P. borreri*, *P. perreticulata*, *P.*

subrudecta, and *Punctelia* sp. (Supplementary Figure 6). Two specimens of *Punctelia* sp.2 (AR471 and AR492) showed 99.0% similarity with the sequences of *P. borreri* (AY773115) and *Punctelia* sp. (JQ004658), while specimen AR550 obtained values of 98.4% with the same sequences.

Besides, *Punctelia* sp. nov. 3 formed an unsupported clade with sequences from *P. missouriensis* G. Wilh. & Ladd (GU384892), *P. rudecta* (Ach.) Krog (KR024458), and *P.* aff. *rudecta* (KR024424) (Supplementary Figure 6). BLASTn results showed PI values of 98.6% between *Punctelia* sp. nov. 3 (specimen AR156) and *P. missouriensis*, while specimen AR574 has 99.1% with *P.* aff. *rudecta* (KR024424).

Two morphospecies did not fit the above cases - *Parmotrema permutatum* (Stirt.) Hale was confirmed only by morphology and phylogeny (Supplementary Table S3 and Supplementary Figure 5). While *Relicina subabstrusa* did not obtain specific confirmation by molecular approaches (Supplementary Figure 7 and Supplementary Table S3).

Discussion

Our study demonstrated the difficulty of applying DNA barcoding to specimens collected in specific regions of Brazil. Represented in 10 cases of phylogenetically recovered species with distinct species, 22 possible species complexes, in addition to 11 species without homonymous sequences, but which were shown to be genetically related to distinct species. This scenario, in part, reflects one of the difficulties encountered in this study - the lack of reliability in sequences from public databases, due to high error rates in species-level identifications found in sequences deposited on these servers (Nilsson et al. 2018; Pentinsaari et al. 2020; Cheng et al. 2023). Not unlike our case, this situation has been a significant obstacle in work using DNA barcoding as a tool for the specific identification of lichenized fungi (Lücking et al. 2020a; Moncada et al. 2020; Leavitt et al. 2021).

Based on the results from species that obtained the same identifications in the three approaches, *Canoparmelia caroliniana* was phylogenetically recovered in two well-supported clades, and the two sequences of the species generated in this study grouped into the largest clade as illustrated in Supplementary Figure 2. Previous studies have pointed to the presence of two lineages and high morphological variation in *C. caroliniana*, but without sufficient evidence to distinguish the two species (Lendemer & Ruiz 2015), more recently being proposed as cryptic lineages (Garrido-Huéscar et al. 2022). *Canoparmelia texana* was retrieved with several unsupported clades (Supplementary Figure 2), showing values ranging from 99-98% identity between the query sequences and the database (Supplementary Table S3). Whereas, the specimen *C.albaniensis* presented subtle morphological differences in comparison to specimens of *C. texana*, such as the presence of laminal pustules on the upper surface of the thallus, and confirmed its position in both molecular approaches within *Canoparmelia albaniensis*, a recently resurrected species of *C. texana* (Kirika et al. 2022) (Supplementary Figure 2).

In the cases of *Flavoparmelia exornata* and *Parmotrema pilosum*, the phylogeny grouped the study sequences with their respective species pairs, that is, *F. exornata* (apotheciate pair) with *F. papillosa* (sorediate pair) and *Parmotrema pilosum* (sorediate pair) with *P. consors* (apotheciate pair) (Supplementary Figures 3 and 5, respectively). The quest to understand if species pairs are distinct or not has been discussed for a long time (Mattsson & Lumbsch 1989; Buschbom & Mueller 2005; Crespo & Pérez-Ortega 2009), and recent studies have pointed out that species pairs are generally species complexes (Lücking et al. 2020b). In our results, *Flavoparmelia exornata* formed a well-supported clade with the unique sequence of *F. papillosa* (Supplementary Figure 3), while the grouping of the two *Parmotrema* species was not recovered with support (Supplementary Figure 5).

Furthermore, our study found several incongruities in species identities (summarized in Table 2), mainly within the genus *Parmotrema*. The specimens identified as *P. perlatum* were genetically distinct from each other. While the sequence AR498 was recovered as a sister group of *P. internexum*, the sample AR641 clustered with sequences of *P.* aff. *perlatum* (HM017027) and *P. perlatum* (ON312517) in an unsupported clade (Supplementary Figure 5). *Parmotrema perlatum* (sorediate), is phylogenetics very close to *P. crinitum* (isidiate), both being recovered in two lineages – a larger group containing sequences of *P. crinitum* and *P perlatum*, and another with two sequences of *P. perlatum* (Stelate et al. 2022). Our results demonstrated that the specimen *P. perlatum* (AR641) clustered with the divergent lineage and was distinct from the clade *P. perlatum* and *P. crinitum* obtained by Stelate et al. (2022) (Supplementary Figure 5). On the other hand, the sequences of *P. eciliatum*, a species morphologically characterized by presenting only apothecia, and as the others cited above, having compounds of the stictic complex as medullary chemistry, were recovered in a clade supported with sequences of *P. crinitum* (AY251442), *P. perlatum* (LC700474), and *P.* aff. *gardneri* (HM017034), and a sister group of the lineage of *P. perlatum* and *P. crinitum* (Stelate et al. 2022), as illustrated in Supplementary Figure 5.

Another intriguing case was from a group of species commonly known and delimited by morphological and chemical characters, that showed conflicts in their phylogenetic structures, such as *Parmotrema cetratum* (apotheciate), *P. clavuliferum* (soralia on the lacinulae apices), and *P. reticulatum* (soralia marginal to submarginal). All these species produce medullary salazinic and consalazinic acid and showed to be closely related to specimens of *P. commensuratum* (soralia submarginal and/or on the lacinulae apices), *P. homotomum* (apotheciate), both containing norlobaridone and loxodin as medullary components (Table 2 and Supplementary Figure 5). For a long time, studies of morphological and phylogenetic reviews have tried to delimit *P. clavuliferum* and *P. reticulatum* (Ahn & Moon 2016;

Spielmann & Marcelli 2020), also seeking to understand the different lineages within the *P. reticulatum* complex (Divakar et al. 2005; Del-Prado et al. 2016). Phenotypic variations were found between the species studied, but the phylogeny based only on the nuTS region was not enough to genetically delimit the groups, thus possibly being another case of species complex, a common situation in lichenized fungi (Leavitt et al. 2013; Del-Prado et al. 2016; Dos Santos et al. 2019; Boluda et al. 2019; Lucking et al. 2020b).

Our study found at least 22 potential species complexes, which were not possible to resolve using only the nuITS marker, supporting previous studies that demonstrated low marker resolution in these situations (Lücking et al. 2020a). Not only in *Parmeliaceae* but species complexes have been widely found in lichens, according to results found by Leavitt et al. (2021) in a large study using DNA barcoding for specific identification. Cases of species complexes make the process of species identification and delimitation difficult because each case must be analyzed very carefully, since we do not know whether the species is still in the process of speciation (Boluda et al. 2021) or whether the species has high phenotypic plasticity, possibly derived from environmental influences (Dal Grande et al. 2017; Ellis 2019). Another curious factor for this morphological variation and little genetic difference between the primary mycobiont may be related to the photobionts present in the association, since the understanding of the role played by these organisms in the symbiosis, as well as in the structure of the thallus, has shown interesting perspectives in understanding phenotypic characteristics of lichens (Spribille et al. 2022). Species of photobiont can show great specific variation (Muggia et al. 2020), indicating another promising approach in the delimitation of lichen species complexes (Steinová et al. 2022).

As demonstrated in our data, studies with similar approaches to the identification of lichenized fungi through DNA barcoding have also found low percentages of accurate identifications using BLASTn and molecular phylogeny with sequences from global

databases (Lücking et al. 2020a; Moncada et al. 2020; Leavitt et al. 2021; Munger et al. 2022; La Torre et al. 2023). Furthermore, the identification of lichenized fungi using DNA barcoding usually demonstrates high success rates in sites with known and sequenced species (Kelly et al. 2011; Leavitt et al. 2014; Divakar et al. 2016; Lendemer & Allen 2020; Moncada et al. 2020) in contrast to studies in megadiverse countries and with low knowledge about their biodiversity, with even smaller representation of these in global databases (Orock et al. 2012; La Torre et al. 2023), as is the case in this study. An alternative in this scenario has been the creation of a regional DNA Barcode reference library, representing a considerable tool for more accurate identification of lichen species (Marthinsen et al. 2019; Moncada et al. 2020; Kerr & Leavitt 2023). Barcode sequences from Brazilian lichenized fungi in public databases are very rare, but our results indicate that when there are sequences available from the studied country or nearby countries, the study sequences tend to cluster with those, thus indicating that the creation of local databases may facilitate the identification and delimitation of lichen species, mainly in megadiverse places such as Brazil.

Conclusion

Our study demonstrated that the use of DNA barcoding in countries with poorly known diversity of lichenized fungi, together with the absence of a local database, makes the use of this tool challenging for specific identification. Even with the use of integrative approaches, we were unable to infer accurate identifications for all specimens, mainly due to incongruities found between phenotypic results and nuITS-based phylogenetic reconstruction of some parmelioid lichen species. Thus, two critical points are inferred based on our data – an integrative review, using more approaches and molecular markers is necessary to confirm and delimit the species that obtained inconsistencies between the approaches, especially cases of species complexes. Besides, the need to develop a regional reference library of DNA barcodes

for species of lichenized fungi in Brazil is also evident to improve the understanding, identification, and delimitation of this biodiversity. Thus, these points are essential not only for future taxonomic and evolutionary studies based on DNA barcoding but also to enable the development of studies on environmental preservation, control, and ecological monitoring using lichenized fungi in such a diverse country.

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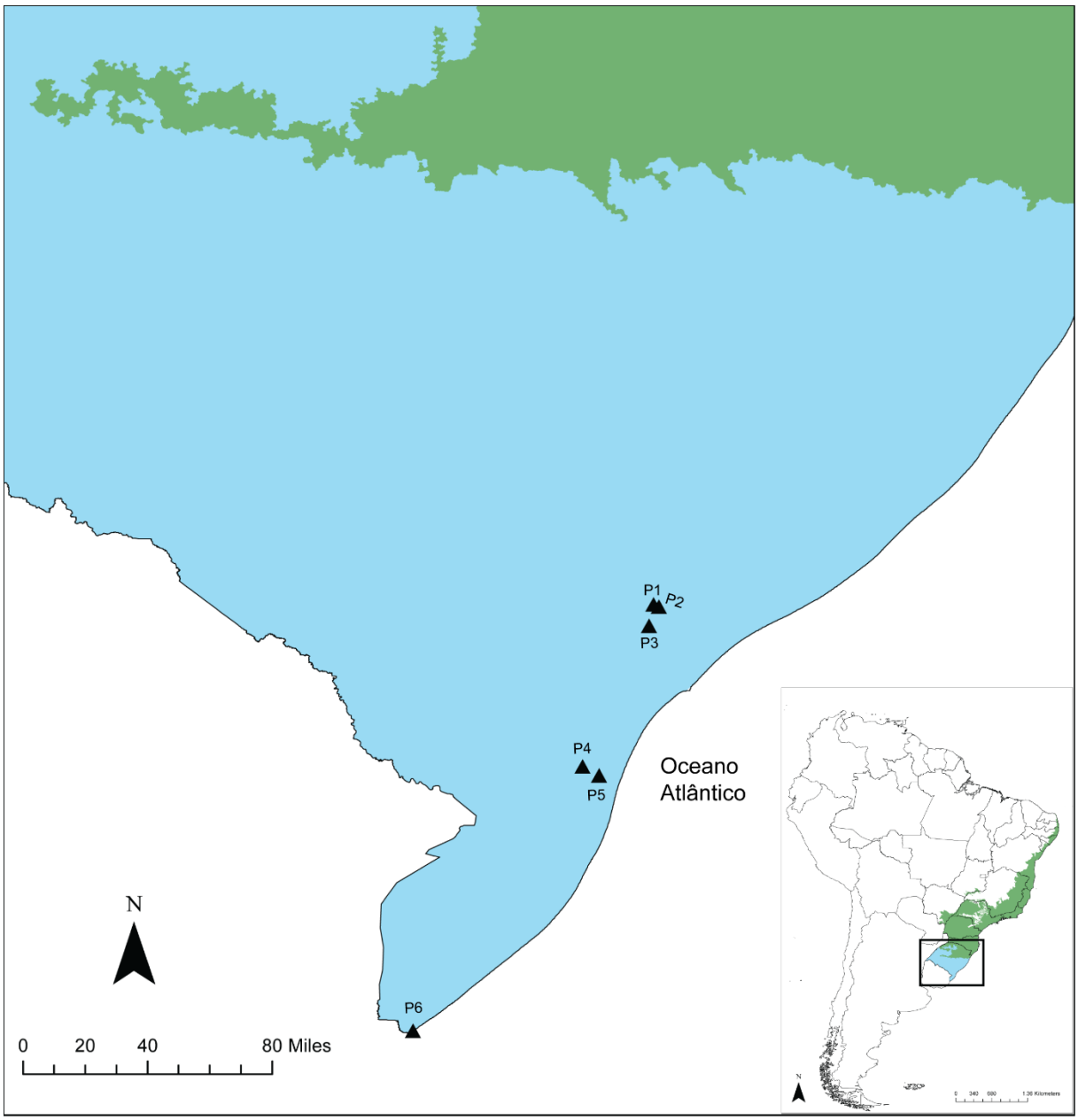


Figure 1. Collection points for parmelioid lichen specimens in the coastal region of the extreme south of Brazil. P1= Dunas Las Acácias; P2= Praia do Laranjal; P3= Capão Seco; P4= Praia da Capilha; P5= Estação Ecológica do Taim; P6= Praia do Chuí. The Pampa biome is represented in blue and the Atlantic Forest biome in green.

Table 1. Sequences of the parmelioid lichens generated in this study.

Species	Locality	Collector	GenBank no. ITS
Bulbothrix			
B. bulbillosa	Rio Grande: Praia da Capilha	A.S. Rodrigues (AR) 257	
B. bulbillosa	Rio Grande: Praia da Capilha	A.S. Rodrigues 233	
B. cassa	Rio Grande: Praia da Capilha	A.S. Rodrigues 474	
B. cassa	Rio Grande: Praia da Capilha	A.S. Rodrigues 475	
B. cassa	Rio Grande: Praia da Capilha	A.S. Rodrigues 493	
B. ventricosa	Rio Grande: Praia da Capilha	A.S. Rodrigues 487	
B. ventricosa	Rio Grande: Capão Seco	A.S. Rodrigues 521	
Canoparmelia			
C. albaniensis	Rio Grande: Capão Seco	A.S. Rodrigues 519	
C. caroliniana	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 581	
C. caroliniana	Chuí: Praia Barra do Chuí	A.S. Rodrigues 625	
C. texana	Rio Grande: Capão Seco	A.S. Rodrigues 559	
C. texana	Rio Grande: Capão Seco	A.S. Rodrigues 561	
C. texana	Chuí: Praia Barra do Chuí	A.S. Rodrigues 627	
Flavoparmelia			
F. exornata	Pelotas: Dunas Las Acácias	A.S. Rodrigues 164	
F. exornata	Rio Grande: Capão Seco	A.S. Rodrigues 551	
F. soredians	Chuí: Praia Barra do Chuí	A.S. Rodrigues 634	
Hypotrachyna			
H. livida	Pelotas: Dunas Las Acácias	A.S. Rodrigues 162	
H. livida	Pelotas: Dunas Las Acácias	A.S. Rodrigues 163	
H. minarum	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 594	
H. minarum	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 614	
H. spumosa	Rio Grande: Praia da Capilha	A.S. Rodrigues 240	
H. spumosa	Rio Grande: Estação Ecológica do Taim	A.S Rodrigues 606	
Parmelinella			
P. lindmanii	Pelotas: Dunas Las Acácias	A.S. Rodrigues 287	
P. lindmanii	Rio Grande: Capão Seco	A.S. Rodrigues 518	
P. salacinifera	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 604	
P. salacinifera	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 619	
Parmotrema			
P. austrosinense	Rio Grande: Praia da Capilha	A.S. Rodrigues 245	
P. austrosinense	Rio Grande: Praia da Capilha	A.S. Rodrigues 252	
P. cetratum	Rio Grande: Capão Seco	A.S. Rodrigues 640	
P. aff. *cetratum*	Rio Grande: Capão Seco	A.S. Rodrigues 536	
P. clavuliferum	Pelotas: Dunas Las Acácias	A.S. Rodrigues 303	
P. clavuliferum	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 588	
P. commensuratum	Rio Grande: Praia da Capilha	A.S. Rodrigues 234	
P. commensuratum	Rio Grande: Capão Seco	A.S. Rodrigues 552	
P. eciliatum	Pelotas: Praia do Laranjal	A.S. Rodrigues 132	

P. eciliatum	Chuí: Praia Barra do Chuí	A.S. Rodrigues 651
P. eciliatum	Chuí: Praia Barra do Chuí	A.S. Rodrigues 661
P. eitenii	Pelotas: Dunas Las Acácias	A.S. Rodrigues 149
P. eitenii	Pelotas: Dunas Las Acácias	A.S. Rodrigues 154
P. eitenii	Rio Grande: Capão Seco	A.S. Rodrigues 569
P. eliasaroanum	Rio Grande: Capão Seco	A.S. Rodrigues 513
P. haitiense	Pelotas: Dunas Las Acácias	A.S. Rodrigues 175
P. haitiense	Rio Grande: Praia da Capilha	A.S. Rodrigues 280
P. homotomun	Pelotas: Praia do Laranjal	A.S. Rodrigues 128
P. madilynae	Rio Grande: Capão Seco	A.S. Rodrigues 549
P. madilynae	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 589
P. perlatum	Rio Grande: Capão Seco	A.S. Rodrigues 498
P. perlatum	Chuí: Praia Barra do Chuí	A.S. Rodrigues 641
P. permutatum	Pelotas: Dunas Las Acácias	A.S. Rodrigues 141
P. permutatum	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 598
P. pilosum	Rio Grande: Praia da Capilha	A.S. Rodrigues 251
P. pilosum	Rio Grande: Praia da Capilha	A.S. Rodrigues 284
P. praesorediosum	Pelotas: Dunas Las Acácias	A.S. Rodrigues 172
P. praesorediosum	Rio Grande: Praia da Capilha	A.S. Rodrigues 485
P. praesorediosum	Rio Grande: Capão Seco	A.S. Rodrigues 520
P. praesorediosum	Rio Grande: Capão Seco	A.S. Rodrigues 656
P. reticulatum	Rio Grande: Capão Seco	A.S. Rodrigues 506
P. reticulatum	Chuí: Praia Barra do Chuí	A.S. Rodrigues 636
Parmotrema sp.1	Rio Grande: Capão Seco	A.S. Rodrigues 557
Parmotrema sp.1	Rio Grande: Capão Seco	A.S. Rodrigues 571
P. subrugatum	Pelotas: Dunas Las Acácias	A.S. Rodrigues 184
P. subrugatum	Rio Grande: Praia da Capilha	A.S. Rodrigues 277
P. subrugatum	Pelotas: Dunas Las Acácias	A.S. Rodrigues 297
P. subrugatum	Rio Grande: Praia da Capilha	A.S. Rodrigues 484
P. tinctorum	Pelotas: Praia do Laranjal	A.S. Rodrigues 133
Parmotrema* subgenus *Crespoa		
C. carneopruinata	Pelotas: Dunas Las Acácias	A.S. Rodrigues 144
C. carneopruinata	Chuí: Praia Barra do Chuí	A.S. Rodrigues 630
C. crozalsiana	Pelotas: Dunas Las Acácias	A.S. Rodrigues 136
C. crozalsiana	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 608
Punctelia		
Punctelia borrerina	Rio Grande: Praia da Capilha	A.S. Rodrigues 276
Punctelia borrerina	Rio Grande: Capão Seco	A.S. Rodrigues 516
P. riograndensis	Pelotas: Dunas Las Acácias	A.S. Rodrigues 298
Punctelia sp. nov. 1	Rio Grande: Capão Seco	A.S. Rodrigues 499
Punctelia sp. nov. 1	Rio Grande: Praia da Capilha	A.S. Rodrigues 491
Punctelia sp. nov. 1	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 609
Punctelia sp. nov. 1	Rio Grande: Praia da Capilha	A.S. Rodrigues 271
Punctelia sp. nov. 1	Rio Grande: Capão Seco	A.S. Rodrigues 554
Punctelia sp. nov. 1	Pelotas: Dunas Las Acácias	A.S. Rodrigues 134
Punctelia sp. nov. 1	Rio Grande: Capão Seco	A.S. Rodrigues 565
Punctelia sp. nov. 1	Rio Grande: Capão Seco	A.S. Rodrigues 572
Punctelia sp. nov. 1	Rio Grande: Capão Seco	A.S. Rodrigues 502
Punctelia sp. nov. 1	Chuí: Praia Barra do Chuí	A.S. Rodrigues 629
Punctelia sp. nov. 1	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 495

Punctelia sp. nov. 1	Rio Grande: Praia da Capilha	A.S. Rodrigues 263
Punctelia sp. nov. 2	Rio Grande: Praia da Capilha	A.S. Rodrigues 492
Punctelia sp. nov. 2	Rio Grande: Praia da Capilha	A.S. Rodrigues 471
Punctelia sp. nov. 2	Rio Grande: Capão Seco	A.S. Rodrigues 550
Punctelia sp. nov. 3	Pelotas: Dunas Las Acácias	A.S. Rodrigues 156
Punctelia sp. nov. 3	Rio Grande: Estação Ecológica do Taim	A.S. Rodrigues 574
Relicina		
R. subastrusa	Pelotas: Dunas Las Acácias	A.S. Rodrigues 155

Table 2. Result of the identifications for each of the three approaches used.

Specimen	Morphological identification	BLASTn identification* (% identity)	Phylogenetic position**	Inferred ID	Comments	Source‡
Bulbothrix						
AR233	*bulbillosa*	*B. apophysata* (99.8)	*B. apophysata/laevigatula* clade	*bulbillosa*	*B. apophysata* has medullary lobaric acid, while *B. bulbillosa* specimens have gyrophoric acid. *B. laevigatula* does not have ciliary bulbs on the upper surface or the isidia as in *B. bulbillosa*. It produces medullary lecanoric acid.	Benatti 2010; Bungartz et al. 2013
AR257	*bulbillosa*	*apophysata* (99.8)	*apophysata/laevigatula* clade	*bulbillosa*		
AR474	*cassa*	*tabacina* (91.5)	*ventricosa/cassa* clade	*cassa*	*B. ventricosa* has medullary norstictic acid, while *B. cassa* has no medullary chemical compound.	Jungbluth et al. 2008
AR475	*cassa*	tabacina (91.5)	*ventricosa/cassa* clade	*cassa*		
AR493	*cassa*	*Remototrachyna awasthii* (92.3)	*ventricosa/cassa* clade	*cassa*		
AR487	*ventricosa*	*tabacina* (91.8)	*ventricosa/cassa* clade	*ventricosa*		
AR521	*ventricosa*	*tabacina* (91.8)	*ventricosa/cassa* clade	*ventricosa*		
Canoparmelia						
AR519	*albaniensis*	*albaniensis* (100)	*albaniensis*	*albaniensis*		
AR581	*caroliniana*	*caroliniana* (100)	*caroliniana*	*caroliniana*		
AR685	*caroliniana*	*caroliniana* (99.8)	*caroliniana*	*caroliniana*		
AR559	*texana*	*texana* (99.8)	*texana* clade	*texana*		
AR561	*texana*	*texana* (99.6)	*texana* clade	*texana*		
AR627	*texana*	*texana* (99.4)	*texana* clade	*texana*		
Flavoparmelia						
AR164	*exornata*	*caperata* (92.0)	*exornata/papillosa* clade	*exornata*	*F. papillosa* has isidia, and *F. exornata* only apothecia.	Hale 1976
AR551	*exornata*	*caperata* (92.0)	*exornata/papillosa* clade	*exornata*		
AR634	*soredians*	aff. *soredians* (99.3)	aff. *soredians*	aff. *soredians*		

Table S2. Cont.

Specimen	Morphological identification	BLASTn identification* (% identity)	Phylogenetic position**	Inferred ID	Comments	Source‡
Hypotrachyna						
AR162	*livida*	*livida* (99.2)	*livida/dactylifera* clade	*livida*	*H. dactylifera* has sidioid-dactyls, and lividic – 4-O-methylphysodic acid chemosyndrome in the medulla. *H. livida* does not have vegetative propagules and contains medullary lividic and colensoic acids.	Sipman et al. 2005
AR163	*livida*	*livida* (98.6)	*livida/dactylifera* clade	*livida*		
AR594	*minarum*	*minarum* (100)	*minarum* clade	*minarum*		
AR614	*minarum*	*minarum* (100)	*minarum* clade	*minarum*		
AR240	*spumosa*	*spumosa* (96.8)	*spumosa* clade	*spumosa*		
AR606	*spumosa*	*spumosa* (97.5)	*spumosa* clade	*spumosa*		
Parmelinella						
AR287	*lindmanii*	*lindmanii* (99.7)	*lindmanii*	*lindmanii*		
AR518	*lindmanii*	*lindmanii* (99.7)	*lindmanii*	*lindmanii*		
AR604	*salacinifera*	*salacinifera* (98.9)	*wallichiana*/*salacinifera* clade	*salacinifera*		
AR619	*salacinifera*	*salacinifera* (98.9)	*wallichiana*/*salacinifera* clade	*salacinifera*		
Parmotrema						
AR245	*austrosinense*	*austrosinense* (99.6)	*austrosinense*	*austrosinense*		
AR252	*austrosinense*	*austrosinense* (99.8)	*austrosinense*	*austrosinense*		
AR640	*cetratum*	*cetratum* (98.5)	*cetratum*/*reticulatum* clade I	*cetratum*	*P. cetratum* has only apothecia, *P. reticulatum* soredia.	Spielmann & Marcelli 2020
AR536	*cetratum*	*cetratum* (97.1)	grupo irmão *cetratum/reticulatum* clade II	*cetratum*		
AR303	*clavuliferum*	*reticulatum* (98.5)	*clavuliferum*/*reticulatum* clade	*clavuliferum*	*P. clavuliferum* presents soredia on the lacinulae apices, while *P. reticulatum* does not form lacinules and the soralia are normally distributed on the margins of the lobes.	

Table S2. Cont.

Specimen	Morphological identification	BLASTn identification* (% identity)	Phylogenetic position**	Inferred ID	Comments	Source‡
Parmotrema						
AR588	*clavuliferum*	*reticulatum* (98.7)	*reticulatum/clavuliferum* clade	*clavuliferum*		
AR234	*commensuratum*	*cetratum* (99.4)	*cetratum/reticulatum* clade II	*commensuratum*	*P. commensuratum* has medullary norlobaridone and loxodin, while *P. reticulatum* and *P. cetratum* have salazinic and consalazinic acids. *P. cetratum* has only apothecia, whereas *P. reticulatum* and *P. commensuratum* have soredia.	Spielmann & Marcelli 2009, 2020
AR552	*commensuratum*	*cetratum* (99.8)	*cetratum/reticulatum* clade II	*commensuratum*		
AR132	*eciliatum*	*crinitum* (99.1)	*crinitum/perlatum* clade	*eciliatum*	*P. crinitum* has isidia, *P. perlatum* soredia, and *P. eciliatum* does not have vegetative propagules. We found in specimens of *P. eciliatum* stictic, constictic, and cryptostictic acids as medullary chemical components. *P. crinitum* and *P. perlatum* normally contain stictic, menegazic, and hypostictic acids.	Benatti & Marcelli 2010; Stelate et al. 2022
AR651	*eciliatum*	*crinitum* (99.1)	*crinitum/perlatum* clade	*eciliatum*		
AR661	*eciliatum*	*crinitum* (99.)	*crinitum/perlatum* clade	*eciliatum*		
AR149	*eitenii*	*tinctorum* (99.6)	*tinctorum*	*tinctorum*	It differs from *P. tinctorum* in the shape of the isidia. In specimens, the isidia are agglutinated forming globular structures that break into soredia. This feature is similar to the description of *P. eitenii*.	Benatti & Marcelli 2009
AR154	*eitenii*	sp. (98.6)	sp./*tinctorum*	*eitenii*		
AR569	*eitenii*	sp. (98.6)	sp./*tinctorum*	*eitenii*		
AR513	*eliasaroanum*	*internexum* (94.7)	sister group of *internexum*/*perlatum* clade	*eliasaroanum*		
AR175	*haitiense*	*subtinctorium* (98.3)	*subtinctorium/haitiense* clade	*haitiense*	*P. haitiense* has norlobaridone and loxodin medullary, while *P. subtinctorium* has salazinic, consalazinic,	Hale 1959 and 1965;

					and norlobaridone acids. Both species have isidia.	
AR280	*haitiense*	*subtinctorium* (98.4)	*subtinctorium/haitiense* clade	*haitiense*		

Specimen	Morphological identification	BLASTn identification* (% identity)	Phylogenetic position**	Inferred ID	Comments	Source‡
Parmotrema						
AR128	*homotomum*	*cetratum* (99.4)	*cetratum/reticulatum* clade II	*homotomum*	Similar to *P. commensuratum*, differing by the absence of vegetative propagules in *P. homotomum*. This presents medullary norlobaridone and loxodin, whereas *P. cetratum* (apothecia) and *P. reticulatum* (soredia) have salazinic and consalazinic acids.	Spielmann & Marcelli 2009, 2020
AR549	*madilynae*	*dilatatum* (95.7)	sister group of *P. robustum*	*madilynae*		
AR589	*madilynae*	*dilatatum* (95.7)	sister group of *P. robustum*	*madilynae*		
AR498	*perlatum*	*perlatum* (97.9)	sister group of *P. internexum*	*perlatum*	*P. internexum* has non-ciliated isidia and scarce marginal cilia. *P. perlatum* has soredia and abundant marginal cilia. Both species contain medullary stictic complex compounds, but *P. internexum* also contains norlobaridone.	Benatti & Marcelii 2010; Stelate et al. 2022
AR641	*perlatum*	*perlatum* (99.0)	*perlatum* clade	*perlatum*		
AR141	*permutatum*	*tinctorum* (95.8)	*permutatum*	*permutatum*		
AR598	*permutatum*	*tinctorum* (95.8)	*permutatum*	*permutatum*		
AR251	*pilosum*	*consors* (98.3)	*pilosum/consors* clade	*pilosum*	*P. pilosum* has soredia, while *P. consors* only apothecia.	Spielmann & Marcelli 2009
AR284	*pilosum*	*consors* (98.3)	*pilosum/consors* clade	*pilosum*		
AR172	*praesorediosum*	*praesorediosum* (97.7)	*praesorediosum* clade	*praesorediosum*		
AR485	*praesorediosum*	*praesorediosum* (97.7)	*praesorediosum* clade	*praesorediosum*		
AR520	*praesorediosum*	*praesorediosum* (97.7)	*praesorediosum* clade	*praesorediosum*		

AR656	*praesorediosum*	*praesorediosum* (97.7)	*praesorediosum* clade	*praesorediosum*		

Specimen	Morphological identification	BLASTn identification* (% identity)	Phylogenetic position**	Inferred ID	Comments	Source‡
Parmotrema						
AR506	*reticulatum*	*cetratum* (99.3)	*cetratum/reticulatum* clade II	*reticulatum*	*P. reticulatum* has soredia, *P. cetratum* only apothecia.	Spielmann & Marcelli 2020
AR636	*reticulatum*	*cetratum* (99.0)	*cetratum/reticulatum* clade II	*reticulatum*		
AR557	sp. nov.1	*tinctorum* (96.0)	grupo irmão de *hababianum/sp* clade	sp. nov. 1	*P. tinctorum* presents isidia and medullary lecanoric acid. *P. hababianum* has soredia, medullary norlobaridone, and protolichesterinic acid. *Parmotrema* sp.1 has soredia and medullary gyrophoric acid.	Hale 1965; Spielmann & Marcelli 2009
AR571	sp. nov. 1	*tinctorum* (96.0)	sister group of *hababianum*/sp clade	sp. nov. 1		
AR184	*subrugatum*	*reticulatum* (95.8)	*subrugatum*	*subrugatum*		
AR277	*subrugatum*	*reticulatum* (96.7)	*subrugatum*	*subrugatum*		
AR297	*subrugatum*	*cetratum* (96.0)	*subrugatum*	*subrugatum*		
AR484	*subrugatum*	*fistulatum* (96.1)	*subrugatum*	*subrugatum*		
AR133	*tinctorum*	*tinctorum* (99.8)	*tinctorum*	*tinctorum*		
Crespoa						
AR144	*carneopruinata*	*crozalsiana* (99.6)	*carneopruinata/crozalsiana* clade	*carneopruinata*	The difference between both species is mainly in the size of the lobes/laciniae and the disposition of the soralia, being more linear in *C. crozalsiana* and globular in *C. carneopruinata*.	Hale 1976; Canêz 2005

Table S2. Cont.

Specimen	Morphological identification	BLASTn identification* (% identity)	Phylogenetic position**	Inferred ID	Comments	Source‡
Crespoa						
AR630	*carneopruinata*	*crozalsiana* (100)	*carneopruinata/crozalsiana* clade	*carneopruinata*		
AR136	*crozalsiana*	*crozalsiana* (100)	*carneopruinata/crozalsiana* clade	*crozalsiana*		
AR608	*crozalsiana*	*crozalsiana* (100)	*carneopruinata/crozalsiana* clade	*crozalsiana*		
Punctelia						
AR276	*borrerina*	*stictica* (96.3)	sister group of *borreri/stictica* clade	*borrerina*		
AR516	*borrerina*	*stictica* (95.9)	sister group of *borreri/stictica* clade	*borrerina*		
AR298	*riograndensis*	*stictica* (95.2)	sister group of *stictica* clade	*riograndensis*		
AR134	sp. nov. 1	*subpraesignis* (98.2)	sister group of *subpraesignis*	sp. nov. 1	*P. subpraesignis* does not have vegetative propagules. *Punctelia* sp.1 has phyllidia.	Canêz 2009
AR263	sp. nov. 1	*borreri* (97.2)	sister group of *subpraesignis*/sp.1	sp. nov. 1	The specimen has the same characteristics as the others. *P. borreri* has soredia.	Canêz 2009
AR271	sp. nov. 1	*subpraesignis* (97.6)	sister group of *subpraesignis*	sp. nov. 1		
AR491	sp. nov. 1	*subpraesignis* (97.6)	sister group of *subpraesignis*	sp. nov. 1		
AR495	sp. nov. 1	*subpraesignis* (97.6)	sister group of *subpraesignis*	sp. nov. 1		
AR499	sp. nov. 1	*subpraesignis* (98.3)	sister group of *subpraesignis*	sp. nov. 1		
AR502	sp. nov. 1	*subpraesignis* (98.2)	sister group of *subpraesignis*	sp. nov. 1		
AR554	sp. nov. 1	*subpraesignis* (98.2)	sister group of *subpraesignis*	sp. nov. 1		
AR565	sp. nov. 1	*subpraesignis* (98.2)	sister group of *subpraesignis*	sp. nov. 1		

Table S2. Cont.

Specimen	Morphological identification	BLASTn identification* (% identity)	Phylogenetic position**	Inferred ID	Comments	Source‡
Punctelia						
AR575	sp. nov. 1	*subpraesignis* (97.4)	sister group of *subpraesignis*	sp. nov. 1		
AR609	sp. nov. 1	*subpraesignis* (98.0)	sister group of *subpraesignis*	sp. nov. 1		
AR629	sp. nov. 1	*subpraesignis* (98.0)	sister group of *subpraesignis*	sp. nov. 1		
AR471	sp. nov. 2	*borreri*/sp. (99.0)	*borreri* clade	sp. nov. 2	*Punctelia* sp. nov. 2 has phyllidia isidioide and/or soredioide, while *P. borreri* has soredia.	
AR492	sp. nov. 2	*borreri*/sp. (99.0)	*borreri* clade	sp. nov. 2		
AR550	sp. nov. 2	*borreri*/sp. (98.4)	*borreri* clade	sp. nov. 2		
AR156	sp. nov. 3	*missouriensis* (98.69)	*missouriensis*/*rudecta* clade	sp. nov. 3	*P. missouriensis* has corticate granules and isidia that develop into lobules or phyllidia. *P. rudecta* has isidia. *Punctelia* sp. nov. 3 has lacinules on the upper surface and the margins of the lobes. The species produce lecanoric acid.	Canêz 2009, Alors et al. 2016
AR574	sp. nov. 3	aff. *rudecta* (99.1)	*missouriensis*/*rudecta* clade	sp. nov. 3		
Relicina						
AR155	*subabstrusa*	*sydneyensis* (88.3)	sister group of *sydneyensis* clade	*subabstrusa*		

****Blast identification:*** name of the sequence with the highest percentage of identity with the query sequence - % identity represented in parentheses; *****Phylogenetic position:*** clade in which the specimen clustered or showed close phylogenetic proximity in the ITS phylogeny.; ‡***Source***: bibliographies used to support the comments.

Supplementary Material S1 and S4. Available for download at the link: https://drive.google.com/drive/folders/14dksUBMWRQ9HSXwuP8SK4lWhKWnKjGaa?usp=sharing

Supplementary Material S2. Morphological descriptions of species with sequences generated in this study.

Bulbothrix bulbillosa Benatti, Spielmann & Bungartz (Sup. Fig. 8A and B)

MycoBank MB 805787

Type: Ecuador, Galapagos, Isabela, Volcan Darwin, 613 m, 2007, Bungartz 7393 (CDS 37880 – holotype [n.v.]).

Description: Thallus greyish green, lacinulate, corticolous, 1–5 cm broad. Laciniae irregularly branched, 0.6–1.5 mm wide, continuous and smooth with presence of laminal black ciliary bulbs, apical zone round to truncated. Maculae absent. Cilia present, bulbate, abundant, k- reaction, 0.06–0.18 × 0.02 mm, evenly distributed on the margins of the lobes. Lacinulae, phyllidia, pustules, and soralia absent. Isidia present, laminar, concolor, some with brown apex, cylindrical, simple, ornate with black ciliary bulbs, 0.06–0.12 × 0.02–0.04 mm. Medulla white, pigment absent. Lower surface black, smooth; marginal zone naked absent, light brown to dark brown. Rhizines completely black or black central and marginals with beige or white tips, simple to dichotomous, 0.1–0.2 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia not found.

Chemistry: atranorin and gyrophoric acid. Spot tests: upper cortex: K+ yellow, UV–; medulla K–, C+ pink, KC+ pink, P–, UV–.

Remarks. *Bulbothrix bulbillosa* is characterized by the presence of isidia and ciliary bulbs on the surface of the upper cortex, also distributed over the isidia and the presence of lecanoric or gyrophoric acids in the medulla. The variation in chemical compounds is frequently mentioned in the species. In the type specimen and several samples from the Galápagos, Bungartz et al. 2013 found lecanoric acid as a medullary chemical component, even though a

few specimens had gyrophoric acid. Among the specimens studied we found only gyrophoric acid, a case also described by Benatti et al. 2015 for other samples also examined and collected in Rio Grande do Sul.

Localities

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′40.6″S, 52º34′57.3″W, 6 m, 28 Jul. 2017, A. Rodrigues 233 (CGMS Herbarium), same locality, 32º30′40.8″S 52º34′58.1″W, 6 m, 28 Jul. 2017, A. Rodrigues 257 (CGMS Herbarium).

Bulbothrix cassa Jungbluth, Marcelli & Elix (Sup. Fig. 8D)

MYCOBANK MB 511166

Basionym: *Bulbothrix cassa* Jungbluth, Marcelli & Elix, Mycotaxon 104: 52. 2008.

Type: Brazil, São Paulo, Itirapina Municipality, Estação Experimental de Itirapina, 770 m, 2004, P. Jungbluth, A. A. Spielmann, L. S. Canêz & J. C. Sfair 840 (SP, B – isotype [n.v.]).

Description: Thallus greyish green, lobate and sublinear, corticolous, 2.5–4.5 cm broad. Lobes irregularly branched, 1.0–3.4 mm wide, continuous and smooth with presence of laminal black ciliary bulbs, apical zone round to truncated. Maculae absent. Cilia present, bulbate, abundant, k- reaction, 0.15–0.37 × 0.02–0.05 mm, communly distributed in the axillar crenae. Lacinulae, phyllidia, pustules, and soralia absent. Isidia present, laminar to marginal, concolor with brown apex, cylindrical, simple to branched, 0.06–0.2 × 0.04–0.09 mm. Medulla white, pigment absent. Lower surface light brown to dark, smooth to veined; marginal zone naked absent, brown to light brown. Rhizines completely black or black central and marginals with beige or white tips, simple, 0.15–0.4 × 0.02–0.05 mm, abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia bacilliform, 5–7 µm.

Chemistry: atranorin. Spot tests: upper cortex: K+ yellow, UV–; medulla K–, C-, KC-, P–, UV–.

Remarks. *Bulbothrix cassa* is characterized by considerably wide sublacinia (up to 3.0 mm), presence of isidia, and absence of medullary chemical compounds. According to the species' protologue, the specimens studied have the expected morphological and chemical characteristics (Jungbluth et al. 2008). However, we found laminal ciliary bulbs on the upper surface, and the color of the lower surface varies from dark to light brown in the study specimens, while in the type they vary from black, brown to ivory depending on the portion of the thallus. *Bulbothrix ventricosa* is morphologically very similar to *B. cassa*, it also has isidia, but it differs mainly by the presence of medullary norstictic acid and also by the presence of laminal ciliary bulbs (Jungbluth et al. 2008; Benatti 2010).

Localities

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′39.4″S, 52º34′58.4″W, 6 m, 14 Jan. 2018, A.S. Rodrigues 474, 475, and 493 (CGMS Herbarium).

Bulbothrix ventricosa (Hale & Kurok.) Hale (Sup. Fig. 8C)

MYCOBANK MB 341620

Basionym: *Parmelia isidiza* var. *domingensis* Vainio. Annales Academiae Scientarum Fennicae 6A (7): 17. 1915.

Type: Dominican Republic, Santo Domingo, La Cumbra, ad corticem arboris, 1906, leg. C. Raunkiaer 492, (TUR-V- lectotype [n.v.]).

≡ *Parmelia ventricosa* Hale & Kurokawa. Contributions from the United States National Herbarium 36: 140. 1964

Description: Thallus greyish green, lobate and sublinear, corticolous, 2–4 cm broad. Lobes irregularly branched, 1.0–5.0 mm wide, continuous and smooth with presence of laminal black ciliary bulbs, apical zone round to truncated. Maculae absent. Cilia present, bulbate, abundant, k- reaction, 0.17–0.37 × 0.02–0.04 mm, communly distributed in the axillar crenae. Lacinulae, phyllidia, pustules, and soralia absent. Isidia present, laminar, concolor with brown apex, cylindrical, simple to branched, 0.08–0.26 × 0.04–0.06 mm, ornamentation absent. Medulla white, pigment absent. Lower surface dark brown in the center, smooth to veined; marginal zone naked absent, light brown. Rhizines completely black or brown central and marginals with beige or white tips, simple, 0.16–0.36 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia absent. Pycnidia and conidia not found.

Chemistry: atranorin and norstictic acid. Spot tests: upper cortex: K+ yellow, UV–; medulla K+ yellow turning to orange, C-, KC-, P+ yellow, UV-.

Remarks. See *Bulbothrix cassa*.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′39.4″S, 52º34′58.4″W, 6 m, 14 Jan. 2018, A.S. Rodrigues 487 (CGMS Herbarium); Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A.S. Rodrigues 521 (CGMS Herbarium).

Canoparmelia albaniensis (C.W. Dodge) Divakar & Kirika (Sup. Fig. 8E and F)

MYCOBANK MB 841885

Basionym: *Parmelia albaniensis* C. W. Dodge, Ann. Miss. Bot. Gard. 46, 121. 1959.

Type: South Africa, Cape of Good Hope, forests of Albany, Zeyher 3 (FH, Taylor Herbarium – holotype [n.v.]).

Description: Thallus greyish green, lobate and sublinear, corticolous, 3 cm broad. Lobes irregularly branched, 2.0–4.3 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae present, evident, punctiform, restricted to marginal zone. Cilia absent. Isidia, lacinulae, phyllidia, and soralia absent. Pustules present, entire and not soredioid, laminal. Medulla white, pigment absent. Lower surface black, smooth, marginal zone naked present, light brown. Rhizines black central and marginal with beige or white tips, simple, 0.14–0.5 × 0.04 mm, abundant and evenly distributed. Apothecia absent. Pycnidia and conidia not found.

Chemistry: atranorin, divaricatic and nordivaricatic acids. Spot tests: upper cortex: K+ yellow, UV–; medulla K-, C-, KC+ rose, P-, UV+ bluish white.

Remarks. According to Kirika et al. 2022 *Canoparmelia albaniensis* is very similar to *C. texana*, differing mainly by the size of the ascospore (larger in *C. albaniensis*) and the inconspicuous maculae on the upper surface. In the study, we found only one small specimen without apothecia so that the ascospores could be examined. However, comparing the specimen with those of *C. texana*, the main difference found was the presence of pustules in *C. albaniensis* developing soralia. In the specimens of *C. texana* examined, we did not find this ontogeny of soralia formation. Another species containing pustules found in Brazil is *C. pustulifera* Benatti, S.M. Martins, Vos & Holt, however, this one has medullary perlatolic acid (Benatti et al. 2016), while *C. albaniensis* has divaricatic and nordivariatic acids, just like *C. texana*.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A.S. Rodrigues 519 (CGMS Herbarium).

Canoparmelia caroliniana (Nylander) Elix & Hale (Sup. Fig. 9A)

MYCOBANK MB 128704

Basionym: *Parmelia caroliniana* Nylander, Flora 68: 614. 1885.

Type: U.S.A., South Carolina, Ravenel 404 (H – lectotype [n.v.]; FH Tuck. –isolectotype [n.v.]).

Description: Thallus greyish green, lobate and sublinear, corticolous, 7–11.5 cm broad. Lobes irregularly branched, 1.6–5.0 mm wide, continuous, smooth, central zone rugose, marginal zone little foveolate, apical zone round. Maculae present, evident, reticular, restricted to marginal zone. Cilia absent. Lacinulae, phyllidia, pustules, and soralia absent. Isidia present, laminar, concolor with brown apex, cylindrical, simple to branched, 0.08–0.36 ×0.04–0.06 mm, ornamentation absent. Medulla white, pigment absent. Lower surface dark brown to black, smooth to veined; marginal zone naked present, light brown. Rhizines black central and marginals with beige or white tips, simple to irregularly branched, 0.17–1.5 × 0.02–0.15 mm, abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia sublageniform, 5–6 µm.

Chemistry: atranorin, perlatolic and glomelliferic acids. Spot tests: upper cortex: K+ yellow, UV–; medulla K-, C-, KC-, P-, UV+ bluish white.

Remarks. *Canoparmelia caroliniana* is recognized by the presence of isidia, reticular maculae, and presence of medullary perlatolic acid. The description of the species varies in some aspects depending on the bibliographies. The color of the lower cortex varies from brown and beige margins according to Spielmann & Marcelli (2008), Michlig (2014) found the lower surface to be black and rarely brown, while Canêz (2005) describes it as black. The specimens studied ranged from dark brown to black. The KC spot test also varies between literature, finding the evanescent pink KC+ reaction in most descriptions, however this was not found in the specimens studied. Furthermore, in the descriptions by Canêz (2005) and

Spielmann & Marcelli (2008), the UV reactions are negative, while in the others they are UV+ bluish-white, as also found in the specimens studied here.

There are few references about the conidia of the species, Michlig (2014) describes them as bifusiform to bacilliform 4-6 µm, while Lynge (1914) found conidia described as having an acute or thickened apex, rarely narrow-bifusiform or subcylindrical, 5-7 µm, similar to that found in this study.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Estação Ecológica do Taim, 32º33′11.5″S 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 581 (CGMS Herbarium); Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S, 53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 625 (CGMS Herbarium).

Canoparmelia texana (Tuck.) Elix & Hale (Sup. Fig. 9B)

MYCOBANK MB 128723

Basionym: *Parmelia texana* Tuck., American Journal of Science and Arts, series 2, 25: 424. 1858.

Type: U.S.A, Texas, thickets of the Blanco, leg. Wright (FH – lectotype [n.v.]; M, US – isolectotype [n.v.]).

Description: Thallus greyish green, lobate and sublinear, corticolous, 2.5–4.5 cm broad. Lobes irregularly branched, 1.7–4.5 mm wide continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia absent. Isidia, lacinulae, phyllidia, and pustules absent. Soralia present, capitate, soredia farinose, laminal to marginal. Medulla white, pigment absent. Lower surface black, smooth to veined, marginal zone naked present, dark brown. Rhizines black central and marginal with beige or white tips, simple, 0.10–0.6 ×

0.04–0.06 mm, abundant and evenly distributed. Apothecia absent. Pycnidia and conidia not found.

Chemistry: atranorin, divaricatic and nordivaricatic acids. Spot tests: upper cortex: K+ yellow, UV–; medulla K-, C-, KC+ rose, P-, UV+ bluish white (AR627 reacted C+ pink in medulla).

Remarks. See *Canoparmelia albaniensis*.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.4″S, 52º16.3′15.9″W, 18m, 15 Jan. 2018, A.S. Rodrigues 559 (CGMS Herbarium). Same locality, 31º51′21.5″S, 52º16.3′12.3″W, 18m, 15 Jan. 2018, A.S. Rodrigues 561 (CGMS Herbarium). Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S, 53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 627 (CGMS Herbarium).

Flavoparmelia exornata (Zahlbr.) Hale (Sup. Fig. 9C)

MYCOBANK MB 103354

Basionym: *Parmelia caperata* var. *exornata* Zahlbruckner, Annalles Mycologici 10:379. 1912.

Type: Uruguai, Cerillos Canelones, Felippone 431 (W – lectotype [n.v.]; G – isotype [n.v.]).

Description: Thallus yellowish green, lobate, corticolous, 6–12 cm broad. Lobes irregularly branched, 2.0–4.0 mm wide, continuous, smooth in the marginal zone, very rugose on the center, apical zone round to truncated. Maculae absent. Cilia absent. Isidia, lacinulae, phyllidia, pustules, and soralia absent. Medulla white, pigment absent. Lower surface black, rugose to veined, marginal zone naked present, light brown. Rhizines black central and marginal with beige or white tips, simple to irregularly branched, 0.10–0.3 × 0.04–0.06 mm, abundant and evenly distributed. Apothecia present, laminal, disk naked, unperforated,

ornamentation absent, ascospores ellipsoid, 16–20 × 9–11 µm, episporium 1 µm. Pycnidia present, ostiole black, conidia bifusiform, 6–8 µm.

Chemistry: usnic and protocetraric acids, as well as another substance that migrates less than protocetraric acid. Spot tests: upper cortex: K-, UV–; medulla: K+ yellow-orange, C-, KC-, P+ orange, UV-.

Remarks. *Flavoparmelia exornata* is recognized by the presence of apothecia, cortical usnic acid, and medullary protocetraric acid. Morphological differences with *F. rutidota* (Hooker & Taylor) Hale vary according to the literature. Hale (1976) differentiates both mainly by the presence of chemical compounds from the "conformata" complex in *F. exornata*. Nash et al. (2002) found, in addition to protocetraric acid in *F. rutidota*, caperatic acid and secalonic acid. However, among the bibliographies, there are variations in components also found for *F. exornata*. Canêz (2005) found fumarprotocetraric acid associated with protocetraric acid, while Eliasaro & Adler (2002) found malonprotocetraric acid. The specimens analyzed in this study showed another compound associated with protocetraric acid, but it was not possible to identify it.

In the specimens studied, we found bifusiform conidia in *F. exornata*, while Nash et al. (2002) describe bacilliform conidia for *F. rutidota*. *Flavoparmelia exornata* also morphologically and chemically resembles *F. papillosa*, but differs in the presence of isidia.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 Abr. 2017, A.S. Rodrigues 164 (CGMS Herbarium). Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.4″S, 52º16.3′15.9″W, 18m, 15 Jan. 2018, A.S. Rodrigues 551 (CGMS Herbarium).

Flavoparmelia soredians (Nyl.) Hale (Sup. Fig. 9D)

MYCOBANK MB 103364

Basionym: *Parmelia* soredians Nyl., Flora (Regensburg) 55: 426 1872.

Type: Spain, Força-Réale, Nylander (H Nylander herbarium number 34690 – lectotype [n.v.]).

≡ *Parmelia conspersa* var. *soredians* (Nyl.) Boistel, Nouv. Fl. Lichens 2: 64. 1903.

≡ *Parmelia caperata* var. *soredians* (Nyl.) Hillmann, Rabenh. Krypt.-Fl., Edn 2 (Leipzig) 9(5.3): 240. 1936.

≡ *Pseudoparmelia soredians* (Nyl.) Hale, Phytologia 29 (3): 191, 1974.

Description: Thallus yellowish green, lobate, corticolous, 4.5 cm broad. Lobes irregularly branched, 1.3–4.0 mm wide, continuous, smooth to rugose in the central region, apical zone round to truncated. Maculae absent. Cilia absent. Isidia, lacinulae, phyllidia, and pustules absent. Soralia present, laminal, capitate, soredia farinose. Medulla white, pigment absent. Lower surface black, rugose to veined; marginal zone naked present, light brown. Rhizines black central and marginal with beige or white tips, simple to irregularly branched, 0.14–0.3 × 0.03–0.06 mm, abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia not found.

Chemistry: usnic and salazinic acids. Spot tests: upper cortex: K-, UV–; medulla: K+ yellow turning to red, C-, KC-, P+ orange, UV-.

Remarks. *Flavoparmelia soredians* is characterized by the presence of soredia, cortical usnic acid, and medullary salazinic and consalazinic acids. Another sorediated species that occurs in Brazil is *F. subamplexa*, but it differs due to the presence of protocetraric acid. *Flavoparmelia subcapitata* is similar, but this species also presents protocetraric and caperatic acid as medullary metabolites (Nash et al. 2002).

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S, 53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 634 (CGMS Herbarium).

Hypotrachyna livida (Taylor) Hale (Sup. Fig. 9E)

MYCOBANK MB 342288

Basionym: *Parmelia livida* Taylor, London Journal of Botany 6: 171. 1847.

Type: U.S.A, Louisiana, New Orleans, Hooker Herbarium (FH-Tayl – lectotype [n.v.]; BM, H – isolectotypes [n.v.]).

Description: Thallus greyish green, lobate and sublinear, corticolous, 6–12 cm broad. Lobes irregularly branched, 1.0–4.0 mm wide, continuous, smooth in the marginal zone, very rugose on the center, apical zone truncated. Maculae absent. Cilia absent. Isidia, lacinulae, phyllidia, pustules, and soralia absent. Medulla white, pigment absent. Lower surface black, rugose to veined; marginal zone naked absent, dark brown. Rhizines white, dichotomous to irregulary branched, 0.15–0.67 × 0.03–0.05 mm, abundant and evenly distributed. Apothecia present, laminal, disk naked, unperforated, ornamentation absent, ascospores ellipsoid, 7–10 × 3–4 µm, episporium 1 µm. Pycnidia present, ostiole black, conidia bifusiform, 5–7 µm.

Chemistry: atranorin, lividic and colensoic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ light pink, C-, KC+ light pink, P-, UV+ bluish white.

Remarks. *Hypotrachyna livida* is recognized by the absence of vegetative propagules, absence of cilia, and the presence of lividic and colensoic acids. It presents similarities with *H. degelii*, differing by the absence of lividic acid (Spimann et al. 2005), for the same reason as it differs from *H. palmarum* (Lynge) Hale.

According to Canêz (2005), the species presents considerable morphological variations, such as the colors obtained by KC during the spot test. Between the two specimens analyzed, we

can observe differences mainly in the rugosity of the upper surface and the size of the lobes. Such differences are individually described in Supplementary Table S1.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 Abr. 2017, A.S. Rodrigues 162 (CGMS Herbarium), A.S. Rodrigues 163 (CGMS Herbarium).

Hypotrachyna minarum (Vain.) Krog & Swinscow

MYCOBANK MB 130747

Basionym: *Parmelia minarum* Vain., Acta Soc. Fauna Flora Fenn. 7: 48. 1890.

Type: Brasil, Minas Gerais, Antônio Carlos (Sítio), Vainio s.n. in Lichenes Brasiliensis Exsiccati n° 1040 (TUR, Vainio herbarium 2689 [n.v.] – lectotype designated by Hale 1971: 13, as "holotype"; BM, FH, UPS – isolectotypes).

≡ *Parmelina minarum* (Vain.) Skorepa, Phytologia 53 (4): 445. 1983.

≡ *Parmelinopsis minarum* (Vain.) Elix & Hale, Mycotaxon 29: 243. 1987.

Description: Thallus greyish green, lobate and sublinear, corticolous, 4 cm broad. Lobes irregularly branched, 1.1–3.5 mm wide, continuous and smooth, apical zone round to truncated. Maculae absent. Cilia present, simple, k- reaction, 0.10–0.36 × 0.02–0.03 mm, rare, marginal. Lacinulae, phyllidia, pustules, and soralia absent. Isidia present, cylindrical, concolor with brown apex, 0.10–0.30 × 0.03–0.05 mm, simple to branched. Medulla white, pigment absent. Lower surface black, smooth; marginal zone naked absent, light brown. Rhizines black, simple to irregularly branched, 0.10–0.58 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia absent. Pycnidia and conidia not found.

Chemistry: atranorin and gyrophoric acid. We found another compound that migrated above the of gyrophoric acid in the solvent C. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C+ pink, KC+ pink, P-, UV-.

Remarks. *Hypotrachyna minarum* is recognized by the presence of eciliated laminal isidia, eciliated lobe margins, and the presence of medullary gyrophoric acid. It differs from other isidiated species with gyrophoric acid such as *H. mcmulliniana*, *H. horrescens,* and *H. neohorrescens* mainly due to the absence of apical cilia on the isidia and the medullary compound 3-methoxy-2,4-di-O-methylgyrophoric acid (Rodrigues et al. 2022).

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Estação Ecológica do Taim, 32º33′11.5″S, 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 594 (CGMS Herbarium), A.S. Rodrigues 614 (CGMS Herbarium).

Hypotrachyna spumosa (Taylor) Hale (Sup. Fig. 9F)

MYCOBANK MB 130749

Basionym: *Parmelia spumosa* Asahina, Journal of Japanese Botany 26: 259. 1951.

Type: Japan, Musashi, Higashi-Murayama, Kita-Tama-gun, Y. Asahina s.n. (TNS – lectotype [n.v.] designated by Hale 1976).

≡ *Parmelina spumosa* (Asahina) Hale, Phytologia 28 (5): 483. 1974.

≡ *Parmelinopsis spumosa* (Asahina) Elix & Hale, Mycotaxon 29: 243. 1987.

Description: Thallus greyish green, lobate and sublinear, corticolous, 3–3.5 cm broad. Lobes irregularly branched, 1.2–2.7 mm wide, continuous and smooth (in one specimen the upper surface shows peeling in some areas of the surface), apical zone truncated. Maculae absent. Cilia present, simple, k- reaction, 0.08–0.20 × 0.02–0.04 mm, rare, marginal. Isidia, lacinulae, phyllidia, and soralia absent. Pustule present, entire to breaking into soredia, laminal to

marginal. Medulla white, pigment absent. Lower surface completely black or dark brown to black, smooth; marginal zone naked absent, light brown. Rhizines black, simple to irregularly branched, 0.10–0.36 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia absent. Pycnidia and conidia not found.

Chemistry: atranorin and gyrophoric acid. We found another compound that migrated above the of gyrophoric acid in the solvent C. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellowish, C+ pink, KC+ pink, P+ light orange, UV-.

Remarks. *Hypotrachyna spumosa* is characterized by the presence of laminal pustules, marginal cilia, and medullary gyrophoric acid. *H. subfatiscens* (Kurokawa) Elix & Hale is very similar, differing by the rupture of the pustules into soredioid structures, and the presence of compounds belonging to the "horrescens" complex (Jungbluth 2006). Among the bibliographies used, there is variation related to the rupture of pustules in soredia, but in the specimens studied, the majority of pustules are entire, and others rupture into coarse soredia. *Hypotrachyna cryptochlora* also presents a similar morphology to *H. spumosa* but differs due to the presence of soredia.

Hypotrachyna spumosa is also commonly recognized by the presence of a chemical compound with a UV + bluish-white reaction on TLC. However, according to Lendemer & Allen 2020, not all specimens present this characteristic. The analyzed specimens showed this reaction on TLC. Another compound described by Lendemer & Allen 2020 is the presence of methyl hiascate, however, we were unable to confirm the presence of this compound. In the TLC results, we identified gyrophoric acid and another compound that migrated above it in solvent C, which based on the chromatography plate demonstrated by Lendemer & Allen 2020 (containing the same solvent) could be methyl hiascate.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′40.6″S, 52º34′57.3″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 240 (CGMS Herbarium); Estação Ecológica do Taim, 32º33′11.5″S 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 606 (CGMS Herbarium).

Parmotrema austrosinense (Zahlbr.) Hale (Sup. Fig. 10A)

MYCOBANK MB 343014

Basionym: *Parmelia austrosinensis* Zahlbr., Symbolae Sinica 3: 180, 192 .1930.

Type: China, Kweitschou: Gwanyinschen near Guiyang, Setschwan, Handel-Mazzetti 10580 (BPI [n.v.] – lectotype designated by Hale 1959, isosyntypes BPI [n.v.]).

Description: Thallus greyish green, lobate, corticolous, 5.5–7.5 cm broad. Lobes irregularly branched, 2.5–15 mm wide, continuous and smooth, apical zone round. Maculae present, weak, effigurate, laminal. Cilia absent. Lacinulae, isidia, and pustules absent. Soralia present, linear continuous, marginal, soredia farinose. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light brown. Rhizines dark brown to black, simple to irregularly branched, 0.2–0.8 × 0.04–0.1 mm, few and grouped. Apothecia and pycnidia absent.

Chemistry: atranorin and lecanoric acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C+ pink, KC+ pink, P-, UV-.

Remarks. *Parmotrema austrosinense* is characterized by the presence of linear soralia on the margins of the lobes, eciliated lobes, and the presence of medullary lecanoric acid. *Parmotrema praesorediosum* has similar morphology but differs in the presence of praesoredioic and protopraesoredioic acids. Another species with linear soralia similar to *P. austrosinense* and commonly found in Brazil is *Parmotrema sancti-angeli* (Lynge) Hale, however, it has marginal cilia and medullary gyrophoric acid.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′42″S, 52º34′57.7″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 245 (CGMS Herbarium); same locality, 32º30′40.8″S, 52º34′58.1″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 252 (CGMS Herbarium).

Parmotrema cetratum (Ach.) Hale (Sup. Fig. 10B and C)

MYCOBANK MB 343018

Basionym: *Parmelia cetrata* Ach., Synopsis Methodica Lichenum: 198. 1814.

Type: USA, Pennsylvania, Muhlenberg s.n. (H-ACH 1329 – lectotype [n.v.]; UPS – isolectotype [n.v.]).

≡ *Parmelia perforata* var. *cetrata* (Ach.) Fr., Lichenographia europaea reformata: 58. 1831.

≡ *Rimelia cetrata* (Ach.) Hale & Fletcher, The Bryologist 93(1): 26. 1990.

= *Parmelia perforata* Ach. var. *corniculata* Kremp. Vi- denskabelige meddelelser fra den Naturhistoriske foreningi Kjöbenhavn 5: 11. 1873.

Type: Brazil, Rio de Janeiro, Warming 323 (M – lectotype [n.v.]).

≡ *Parmelia cetrata* var. *corniculata* (Kremp.) Müll. Arg., Hedwigia 30: 228. 1891.

Description: Thallus greyish green, lobate, corticolous, 9 cm broad. Lobes irregularly branched, 2.0–6.0 mm wide, continuous to cracked reticulate, smooth to rugose in the central region, apical zone lacinulate. Maculae present, evident, reticular, and laminal. Cilia present, simple, K- reaction, 0.14–0.7 × 0.02 mm, abundant and marginal. Isidia, phyllidia, pustules, and soralia absent. Lacinulae present, plane, simple, 1.0–4.0 × 0.4–1.0 mm. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked absent, light brown. Rhizines black, simple to irregularly branched, 0.14–0.60 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia present, submarginal, disk naked, perforated, ornamentation

absent, ascospores ellipsoid, 8–15× 5–8 µm, episporium 1 µm. Pycnidia present, ostiole black, conidia filiform, 10–14 µm.

Chemistry: atranorin, salazinic and consalazinic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellow turning red, C-, KC-, P+ orange, UV-.

Remarks. *Parmotrema cetratum* is characterized by the absence of vegetative propagules, evident reticular maculae, usually with lacinules on the margins of the lobes, and the presence of medullary salazinic and consalazinic acids. See Spielmann & Marcelli 2020 for a more in-depth discussion of species characteristics and comparisons based on type.

The two specimens studied show a difference in the number of lacinules. Specimen AR640 presents the characteristics expected for the species and described by Spielmann & Marcelli 2020. However, the specimen AR536 has abundant lacinules and may be a distinct species.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 536 (CGMS Herbarium); Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S, 53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 640 (CGMS Herbarium).

Parmotrema clavuliferum (Räsänen) Streimann (Sup. Fig. 10D)

MYCOBANK MB 129346

Basionym: *Parmelia clavulifera* Räsänen, Annales Botanici Societatis Zoologicae Botanicae Fennicae "Vanamo" 20 (3): 4 .1944.

Type: Oceania, Tahiti: ad corticem arboris, 1868, Dr. med. Bouffon vel E. Vieillard (H – lectotype [n.v.]; H – paratype [n.v.]).

≡ *Rimelia clavulifera* (Räsänen) Kurok., Journal of Japanese Botany 66: 158 .1991.

= *Parmelia urceolata* var. *subcetrata* Müll. Arg., Flora (Regensburg) 66: 46 .1883.

Type: Australia. Queensland: prope Toowoomba, 1881, Hartmann s.n. (G – holotype [n.v.]).

Description: Thallus greyish green, lobate, corticolous, 6.5–15 cm broad. Lobes irregularly branched, 2.0–8.5 mm wide, continuous to cracked reticulate, smooth to rugose in the central region, apical zone lacinulate. Maculae present, evident, reticular, laminal. Cilia present, simple, k- reaction, 0.3–1.37 × 0.02 mm, few, marginal. Isidia, phyllidia, and pustule absent. Lacinulae present, plane to few canaliculate, simple, 0.7–2.5 × 0.3–0.6 mm. Soralia present, capitate, on the lacinules apices, soredia granular. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked absent, light brown, and black on the lacinules. Rhizines black, simple to irregularly branched, 0.2–1.7 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia present, laminal, sorediate, disk naked, unperforated, ascospores not found. Pycnidia present, ostiole black, conidia filiform, 8–14 μm.

Chemistry: atranorin, salazinic and consalazinic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellow turning red, C-, KC-, P+ orange, UV-.

Remarks. *Parmotrema clavuliferum* is characterized by the presence of soralia on the lacinules apices, presence of cilia, evident reticular maculae, and salazinic and consalazinic acids in the medulla. See Spielmann & Marcelli 2020 for a more in-depth discussion of species characteristics and comparisons based on type.

Parmotrema reticulatum presents morphology and chemistry very similar to *P. clavuliferum*, however, we distinguish both mainly by the arrangement of the soralia, being marginal to submarginal in *P. reticulatum*, and frequently arranged on the lacinules apices in *P. clavuliferum*.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 28 Jul. 2017, A.S. Rodrigues 303 (CGMS

Herbarium); Rio Grande Municipality, Estação Ecológica do Taim, 32º33′11.5″S 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 588 (CGMS Herbarium).

Parmotrema commensuratum (Sup. Fig. 10E)

MYCOBANK MB 343020

Basionym: *Parmelia commensurata* Hale, Phytologia 22 (1): 31 .1971.

Type: Mexico, Veracruz: 9 Km E Jalapa, M.E. Hale 19405 (US – holotype [n.v], TNS, UPS – isotypes [n.v]).

≡ *Rimelia commensurata* (Hale) Hale & A. Fletcher, The Bryologist 93 (1): 27 .1990.

Description: Thallus greyish green, lobate, corticolous, 6–14 cm broad. Lobes irregularly branched, 3.0–12 mm wide, continuous to cracked reticulate, smooth to rugose in the central region, apical zone lacinulate. Maculae present, evident, reticular, laminal. Cilia present, simple, k- reaction, 0.1–0.4 × 0.02 mm, few, marginal. Isidia, phyllidia, and pustules absent. Lacinule present, plane to few canaliculate, simple, 0.3 –1.5 × 0.3 – 1.0 mm. Soralia present, capitate to linear, submarginal or in the lacinules apices, soredia granular. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked absent, brown, and black on the lacinules. Rhizines black, simple to irregularly branched, 0.15–1.5 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia present, submarginal, sorediate, disk naked, unperforated, ascospores not found. Pycnidia present, ostiole black, conidia filiform, 8–10 µm.

Chemistry: atranorin, norlobaridone and loxodin. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC+pink, P-, UV-.

Remarks. *Parmotrema commensuratum* is recognized by the presence of soredia, which are normally submarginal and on the lacinules apices, the presence of marginal cilia, evident reticular maculae, and medullary norlobaridone and loxodin. *Parmotrema homotomum* has

similar morphology and chemistry but does not have vegetative propagules. *Parmotrema clavuliferum* and *P. reticulatum* are also species with similar morphology to *P. commensuratum*, differing by the presence of salazinic and consalazinic acids in the medulla.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′42.6″S, 52º34′56.8″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 234 (CGMS Herbarium); Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.4″S, 52º16.3′15.9″W, 18m, 15 Jan. 2018, A.S. Rodrigues 552 (CGMS Herbarium).

Parmotrema eciliatum (Nyl.) Hale (Sup. Fig. 10F)

MYCOBANK MB 343045

Basionym: *Parmelia crinita* var. *eciliata* Nyl., Flora (Regensburg) 52: 291 .1869.

Type: Mexico, Orizaba: Bourgeau s.n. (H-NYL35295 – holotype [n.v.], P – isotype [n.v.]).

≡ *Parmelia eciliata* (Nyl.) Nyl., Mexic. Pl.: 3 .1872.

≡ *Parmelia latissima* var. *eciliata* (Nyl.) Nyl., Mexic. Pl. 1: 3 .1872.

Description: Thallus greyish green, lobate, corticolous, 6.5–12 cm broad. Lobes irregularly branched, 2.0–12 mm wide, continuous, smooth to rugose in the central region, apical zone cropped. Maculae present, weak, effigurate, restricted to amphithecium. Cilia present, simple, K- reaction, 0.3–1.8 × 0.04–0.06 mm, abundant and marginal. Isidia, lacinulae, phyllidia, pustules, and soralia absent. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light brown. Rhizines black, simple to irregularly branched, 0.14–1.3 × 0.02–0.06 mm, abundant and evenly distributed. Apothecia present, laminal, disk naked, unperforated, ornamentation absent, ascospores ellipsoid, 20–29 × 11–15 µm, episporium 2–3 µm. Pycnidia present, ostiole black, conidia bacilliform, 5–9 µm.

Chemistry: atranorin, stictic, constictic, and cryptostictic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellow, C-, KC-, P+ orange, UV-.

Remarks. *Parmotrema eciliatum* is recognized by the absence of vegetative propagules, marginal cilia, and the presence of compounds belonging to the "stictic complex" in the medulla. *Parmotrema eliasaroanum* has similar morphology and chemistry but has lacinules. Other similar species such as *P. crinitum* and *P. perlatum* have isidia and soredia, respectively.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Laranjal beach, 31º46′5.7″S, 52º13′38.8″W, 7 m, 26 Mar. 2017, A.S. Rodrigues 132 (CGMS Herbarium); Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S, 53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 651 and 661 (CGMS Herbarium).

Parmotrema eitenii Marcelli & Benatti (Sup. Fig. 11A)

MYCOBANK MB 532094

Type: BRAZIL, São Paulo, Praia Grande, M.P. Marcelli & O.Yano 14345 (SP – holotype [n.v.]).

Description: Thallus greyish green, lobate, corticolous, 8–10 cm broad. Lobes irregularly branched, 3.0–20 mm wide, continuous and smooth, apical zone round. Maculae absent. Cilia absent. Lacinulae, phyllidia, and pustules absent. Isidia present, cylindrical, simple becoming branched and inflated or postulated; pustule breaking into in soredia, often concolor with brown apex, laminal to marginal, 0.1–0.5 × 0.06–0.2 mm, ornamentation absent. Soralia present, formed by agglomerations of isidia, inflated to capitate, laminal to marginal; soredia isidioid. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal

zone naked present, light brown. Rhizines black, simple to irregularly branched, 0.2–1.0 × 0.04–0.12 mm, few and grouped. Apothecia and pycnidia absent.

Chemistry: atranorin and lecanoric acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C+ pink, KC+ pink, P-, UV-.

Remarks. *Parmotrema eitenii* is characterized by presenting cylindrical isidia that can develop into soredia due to the agglomeration of isidia that become pustular and rupture into soredia, absence of cilia and medullary lecanoric acid. *Parmotrema tinctorum* is extremely similar, differing by the presence of only isidia.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 03 April 2017, A. S. Rodrigues 149 (CGMS Herbarium), and 05 April 2017, A. S. Rodrigues 154 (CGMS Herbarium); Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′21.5″S, 52º16.3′12.3″W, 18m, 15 Jan. 2018, A. S. Rodrigues 569 (CGMS Herbarium).

Parmotrema eliasaroanum Benatti, Marcelli & Elix (Sup. Fig. 11B)

MYCOBANK MB 511426

Type: Brasil, São Paulo, Cananéia, Ilha do Cardoso, 1981, M. P. Marcelli 1757 (SP – holotype [n.v.]).

Description: Thallus greyish green, lobate, corticolous, 7 cm broad. Lobes irregularly branched, 2.5–6 mm wide, continuous, smooth to rugose in the central region, apical zone lacinulate. Maculae present, weak, effigurate, laminal. Cilia present, simple, K- reaction, 0.1–0.8 × 0.02–0.06 mm, few and marginal. Lacinulae present, plane, irregularly branched, 0.9–3.5 × 0.3–1.0 mm. Isidia, phyllidia, pustules, and soralia absent. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light brown, and

brown on the lacinules. Rhizines black, simple to irregularly branched, 0.18–1.2 × 0.02–0.04 mm, few and grouped. Apothecia absent. Pycnidia present, ostiole black, conidia bacilliform, 6–9 µm.

Chemistry: atranorin, stictic, constictic, and cryptostictic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellow, C-, KC-, P+ orange, UV-.

Remarks. See *Parmotrema eciliatum*.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 513 (CGMS Herbarium).

Parmotrema haitiense (Hale) Hale (Sup. Fig. 11C)

MYCOBANK MB 343061

Basionym: *Parmelia haitiensis* Hale, The Bryologist 62: 20. 1959.

Type: Jamaica, Blue Mountains, 1927, C. R. Orcutt 2987 (U. S. National Herbarium – holotype [n.v.]).

≡ *Rimeliella haitiensis* (Hale) Elix, Mycotaxon 47: 127. 1993.

≡ *Canomaculina haitiensis* (Hale) Elix, Mycotaxon 65: 477. 1997.

Description: Thallus greyish green, lobate, corticolous, 6.5–7 cm broad. Lobes irregularly branched, 2.5–13 mm wide, continuous, smooth to rugose in the central region, apical zone round to truncated. Maculae present, weak, effigurate, laminal. Cilia present, simple, K- reaction, 0.2–1.3 × 0.02–0.06, abundant and marginal. Lacinulae, phyllidia, pustules, and soralia absent. Isidia present, cylindrical, simple to branched arbuscular, concolor, some with brown apex, ornamentation absent, 0.1–0.3 × 0.06–0.1 mm. Medulla white, pigment absent. Lower surface dark brown to black, smooth to veined; marginal zone naked absent, light

brown. Rhizines black, simple to irregularly branched, 0.12–2.0 × 0.02–0.12 mm, abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia not found.

Chemistry: atranorin, norlobaridone and loxodin. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC+ light pink, P-, UV-.

Remarks. *Parmotrema haitiense* is recognized by the presence of isidia normally with cilia at their apex, norlobaridone and loxodine as medullary chemical components. *Parmotrema subtinctorium* is very similar morphologically to *P. haitiense*, differing by the presence of salazinic acid together with norlobaridone and loxodine. Kurokawa (1991) proposed the synonymization of both species, considering that specimens may present this chemical variation within populations. Elix 1993 separated the two species, differentiating both mainly by medullar chemistry. Salazinic acid was not found in the specimens studied.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 April 2017, A. S. Rodrigues 175 (CGMS Herbarium); Rio Grande Municipality, Praia da Capilha, 32º30′42.6″S, 52º34′56.8″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 280 (CGMS Herbarium).

Parmotrema homotomum (Nyl.) Hale (Sup. Fig. 11D)

MYCOBANK MB 343064

Basionym: *Parmelia homotoma* Nyl., Flora (Regensburg) 68: 613. 1885.

Type: Brasil, Rio de Janeiro, Weddell s.n. (H-NYL – lectotype [n.v.]).

≡ *Rimelia homotoma* (Nyl.) Hale & A. Fletcher, The Bryologist 93 (1): 28. 1990.

Description: Thallus greyish green, lobate, corticolous, 8 cm broad. Lobes irregularly branched, 3.5–12 mm wide, continuous to cracked reticulate, smooth to rugose in the central region, apical zone round. Maculae present, evident, reticular, and laminal. Cilia present,

simple, K- reaction, 0.2–0.8 × 0.02–0.04 mm, few and marginal. Isidia, lacinulae, phyllidia, pustules, and soralia absent. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked absent, dark brown. Rhizines black, simple to irregularly branched, 0.16–1.2 × 0.02–0.06 mm, abundant and evenly distributed. Apothecia and pycnidia absent.

Chemistry: atranorin, norlobaridone and loxodin. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC+ pink, P-, UV-.

Remarks. See *Parmotrema commensuratum*.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Laranjal beach, 31º46′5.7″S, 52º13′38.8″W, 7 m, 26 Mar. 2017, A.S. Rodrigues 128 (CGMS Herbarium).

Parmotrema madilynae A. Fletcher (Sup. Fig. 11E)

MYCOBANK MB 104596

Type: Brasil, São Paulo, Ilha Comprida, 4 km SE of Cananéia, G. & W. D. Clayton 61 32-b (US – holotype [n.v.]).

Description: Thallus greyish green, lobate, corticolous, 8–10 cm broad. Lobes irregularly branched, 2.5–6.5 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia present, simple, K- reaction, 0.3–1.5 × 0.04–0.08 mm, few and marginal. Isidia, lacinulae, phyllidia, and soralia absent. Pustules present, entire to breaking into soredia, commonly marginal. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light brown. Rhizines black, simple to irregularly branched, 0.2–1.0 × 0.02–0.1 mm, few and grouped. Apothecia absent. Pycnidia present, ostiole black, conidia not found.

Chemistry: atranorin and protocetraric acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC-, P+ orange, UV-.

Remarks. *Parmotrema madilynae* is recognized by the presence of pustules, marginal cilia, and medullary protocetraric acid. It was a species found in abundance in the coastal region, a characteristic also found by Benatti & Marcelli 2011. *Parmotrema schindleri* Hale, another common species in Brazil, is morphologically similar but differs in the absence of pustules and the presence of gyrophoric acid together with protocetraric acid (Benatti & Marcelli 2011).

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.4″S, 52º16′15.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 549 (CGMS Herbarium); Estação Ecológica do Taim, 32º33′11.5″S, 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 589 (CGMS Herbarium).

Parmotrema perlatum (Huds.) M. Choisy (Sup. Fig. 11F)

MYCOBANK MB 368896

Basionym: *Lichen perlatus* Huds., A natural arrangement of British plants 1: 448. 1762.

Type: Dillenius Herbarium (OXF), illustrated in the plate 20, figure 39B in Dillenius, Historia Muscorum. p. 197, 1742.

≡ *Lobaria perlata* (Huds.) Hoffm., Deutschlands Flora oder botanisches Taschenbuch. Zweyter Theil für das Jahr 1795. Cryptogamie: 148. 1796.

≡ *Parmelia perlata* (Huds.) Ach., Methodus qua Omnes Detectos Lichenes Secundum Organa Carpomorpha ad Genera, Species et Varietates Redigere atque Observationibus Illustrare Tentavit Erik Acharius: 216. 1803.

≡ *Parmelia perlata* subsp. *perlata* (Huds.) Ach., Methodus qua Omnes Detectos Lichenes Secundum Organa Carpomorpha ad Genera, Species et Varietates Redigere atque Observationibus Illustrare Tentavit Erik Acharius: 216. 1803.

≡ *Platysma perlatum* (Huds.) Frege, Deutsches Botanisches Taschenbuch für Liebhaber der deutschen Pflanzenkunde 2: 167. 1812.

≡ *Imbricaria perlata* (Huds.) Körb., Lichenographiae Germanicae specimen: *Parmeliacearum* familiam continens: 8. 1846.

Description: Thallus greyish green, lobate, corticolous, 5.5–8.5 cm broad. Lobes irregularly branched, 2.0–3.5 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia present, simple, K- reaction, 0.2–0.8 × 0.03–0.04 mm, few to abundant and marginal. Lacinulae present, simple, plane, 0.3–1.4 × 0.2–0.6 mm. Isidia, phyllidia, and pustules absent. Soralia present, capitate to interrupted, marginal to submarginal or in the lacinules apice, soredia granular. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light brown to variegated on the lobes, and black to variegated on the lacinules. Rhizines black, simple to irregularly branched, 0.20–1.2 × 0.02–0.06 mm, abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia not found.

Chemistry: atranorin, stictic acid, constictic acid, and traces of cryptostictic acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellow, C-, KC-, P+ orange, UV-.

Remarks. See *Parmotrema eciliatum.*

Among the two specimens studied, only sample AR498 showed lacinules, however, the sample consists of a small specimen, so it was not possible to verify other morphological characteristics that could differentiate both. Specimen AR641 presented small projections on the margins of the lobes, which we do not consider lacinules.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 498 (CGMS Herbarium); Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S, 53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 641 (CGMS Herbarium).

Parmotrema permutatum (Stirt.) Hale (Sup. Fig. 12A)

MYCOBANK MB 343104

Basionym: *Parmelia permutata* Stirt., Scottish Naturalist 4: 252. 1878.

Type: Austrália, near Brisbane, Bailey s.n. (BM – holotype [n.v.]; GLAM – isotype [n.v.]).

Description: Thallus greyish green, lobate, corticolous, 6.5–7 cm broad. Lobes irregularly branched, 3.0–20.0 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia present, simple, K- reaction, 0.3–3.5 × 0.04–0.1 mm, few to abundant and marginal. Isidia, lacinulae, phyllidia, and pustules absent. Soralia present, linear interrupted, some continuous, marginal, soredia granular. Medulla white and yellow, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light to dark brown. Rhizines black, simple to irregularly branched, 0.2–1.8 × 0.04–0.06 mm, few and grouped. Apothecia and pycnidia absent.

Chemistry: atranorin and gyrophoric acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellowish, C+ pink, KC+ pink, P-, UV-.

Remarks. *Parmotrema permutatu*m is recognized for presenting soredia, bicolor medulla (white on the upper portion and yellow on the lower portion), marginal cilia, and medullary gyrophoric acid. There are variations in the medullary chemical components found for the species according to the literature, such as Benatti & Marcelli (2009) found gyrophoric acid and eumitrin-type pigments in the medulla, Canêz (2005) report secalonic C and gyrophoric acids, Fleig (1997) gyrophoric and secalonic A acids, while Swinscow & Krog (1988)

gyrophoric acid. In the specimens studied, we did not find other compounds associated with gyrophoric acid. *Parmotrema subochraceum* is similar to *P. permutatum* but differs due to the presence of orange portions in the medulla with K+ red reaction, in addition to producing protocetraric acid (Benatti & Marcelli 2009).

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 April 2017, A. S. Rodrigues 141 (CGMS Herbarium). Rio Grande Municipality, Estação Ecológica do Taim, 32º33′11.5″S, 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 598 (CGMS Herbarium).

Parmotrema pilosum (Stizenb.) Krog & Swinscow (Sup. Fig. 12B)

MYCOBANK MB 109155

Basionym: *Parmelia pilosa* Stizenb., Bericht über die Tätigkeit der St. Gallischen Naturwissenschaft lichen Gesellschaft 1888-1889: 165. 1890.

Type: South Africa, Orange Free, Taaibosch Kranz Montains, Rio Rhenoster, Rehmann s.n. (ZT – lectotype [n.v.])

≡ *Parmelina pilosa* (Stizenb.) Hale, Phytologia 28 (5): 483. 1974.

≡ *Canomaculina pilosa* (Stizenb.) Elix & Hale, Mycotaxon 29: 240. 1987.

Description: Thallus greyish green, lobate, corticolous, 4–9.5 cm broad. Lobes irregularly branched, 2.0–7.5 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae present, evident, effigurate, and laminal. Cilia present, mixed - simple, furcated and branched, K- reaction, 0.2–1.3 × 0.06–0.12 mm, abundant and marginal. Isidia, lacinulae, phyllidia, and pustules absent. Soralia present, capitate, laminal to marginal, soredia granular. Medulla white, pigment absent. Lower surface black, smooth; marginal zone naked absent, light brown. Rhizines black, simple to irregularly branched, 0.2–1.4 × 0.02–0.1 mm,

abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia filiform, 8–12 µm.

Chemistry: atranorin. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC-, P-, UV-.

Remarks. *Parmotrema pilosum* is recognized for presenting soredia, marginal cilia, and the absence of chemical compounds in the medulla. *Parmotrema consors* is similar but does not have vegetative propagules.

Localities.

BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′42.6″S, 52º34′56.8″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 251 and 284 (CGMS Herbarium).

Parmotrema praesorediosum (Nyl.) Hale (Sup. Fig. 12C and D)

MYCOBANK MB 343106

Basionym: *Parmelia praesorediosa* Nyl., Sertum Lichenaeae Tropicae e Labuan et Singapore: 18. 1891.

Type: SINGAPORE, 1879, E. Almquist (H-NYL35547 – holotype [n.v.]; S – isotype [n.v.]).

Description: Thallus greyish green, lobate, corticolous, 3–9 cm broad. Lobes irregularly branched, 2.0–15.0 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia absent. Isidia, phyllidia, and pustules absent. Lacinulae present, canaliculate, simple, 0.2-1.0x0.2-0.8 mm. Soralia present, linear continuous to capitate, marginal to submarginal, can be stipitated and/or present on the lacinules apices, soredia granular. Medulla white, pigment absent. Lower surface black, veined; marginal zone naked present, light brown. Rhizines completely black or black central and marginal with beige or white tips, simple, 0.10–0.40 × 0.02–0.04 mm, few and grouped. Apothecia absent. Pycnidia present, ostiole black, conidia sublageniform, 5–7 µm.

Chemistry: atranorin, praesorediodic and protopraesorediodic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC-, P-, UV-.

Remarks. *Parmotrema praesorediosum* is recognized by the presence of soredia, absence of marginal cilia, and presence of medullary praesorediodic and protopraesorediodic acids. Among the identified specimens we found variation according to the presence of lacinules and arrangement of the soralia. Specimen AR520 presented developed lacinules containing soralia on their apices, whereas specimen AR636 has structures similar to lacinules, but we consider stipitated soralia. The other two specimens have linear soralia on the margins of the lobes, a characteristic expected for the species. *Parmotrema mordenii* (Hale) Hale is similar, but differs due to the presence of atranorin (yellow K+ reaction) in the medulla, and generally presenting a saxicolous habit.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 April 2017, A. S. Rodrigues 172 (CGMS Herbarium); Rio Grande Municipality, Praia da Capilha, 32º30′39.4″S, 52º34′58.4″W, 6 m, 14 Jan. 2018, A.S. Rodrigues 485 (CGMS Herbarium); Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 520, 656 (CGMS Herbarium).

Parmotrema reticulatum (Taylor) M. Choisy (Sup. Fig. 12E)

MYCOBANK MB 357464

Basionym: *Parmelia reticulata* Taylor, Flora Hibernica 2: 148. 1836.

Type: Ireland, Kerry, near Dunkerron, on rocks, common, T. Taylor s.n. (FH –lectotype [n.v.], BM – isolectotype [n.v.]).

≡ *Rimelia reticulata* (Taylor) Hale & Fletcher, The Bryologist 93(1): 28. 1990.

= *Parmelia praeperlata* Nyl., Comptes Rendues des Séances Hebdomadaires de l'Académie des Sciences Paris 81: 725. 1875.

Type: French Southern and Antarctic Lands, St. Paul Island, Fenzl s.n. (H-NYL 35548 – lectotype [n.v.] designated by Nylander 1886: 319).

Description: Thallus greyish green, lobate, corticolous, 6–9 cm broad. Lobes irregularly branched, 2.0–6.5 mm wide, continuous to cracked reticulate, smooth to rugose in the central region, apical zone round. Maculae present, evident, reticular, and laminal. Cilia present, simple, K- reaction, 0.2–0.8 × 0.02–0.04 mm, few to abundant and marginal. Isidia, phyllidia, and pustules absent. Lacinulae present, plane, simple, 0.5–1.7 × 0.5–1.0 mm. Soralia present, capitate to linear, submarginal or in the lacinules apices, soredia granular. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked absent, light to dark brown, black on the lacinules. Rhizines black, simple to irregularly branched, 0.18–1.4 × 0.02–0.06 mm, abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia filiform, 7–12 µm.

Chemistry: atranorin, salazinic and consalazinic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellow turning red, C-, KC-, P+ orange, UV-.

Remarks. See *Parmotrema clavuliferum.*

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 506 (CGMS Herbarium); Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S, 53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 636 (CGMS Herbarium).

***Parmotrema* sp. nov. 1** (Sup. Fig. 12F)

Description: Thallus greyish green, lobate, corticolous, 9–15 cm broad. Lobes irregularly branched, 2.5–13.0 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia present, simple, K- reaction, 0.3–1.6 × 0.04–0.06 mm, few and marginal. Isidia, lacinulae, phyllidia, and pustules absent. Soralia present, linear continuous to interrupted, marginal, soredia granular. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light brown. Rhizines black, simple to irregularly branched, 0.20–1.6 × 0.04–0.06 mm, few and grouped. Apothecia absent. Pycnidia present, ostiole black, conidia filiform, 9–12 µm.

Chemistry: atranorin and gyrophoric acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C+ rose, KC+ rose, P-, UV-.

Remarks. *Parmotrema* sp. nov. 1 is recognized by the presence of soredia, marginal cilia, and medullary gyrophoric acid. *Parmotrema indicum* is similar, but in addition to gyrophoric acid, it also contains norlobaridone according to Hale (1977). In the specimens studied, we did not find associated norlobaridone. Another species commonly found in Brazil and similar is *P. sancti-angelii*, which has sublageniform conidia of 5.0−9.0 µm (Spielmann & Marcelii 2009), while *Parmotrema* sp. nov. 1 has filiform conidia 9–12 µm.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′21.5″S, 52º16.3′12.3″W, 18m, 15 Jan. 2018, A. S. Rodrigues 557 and 571 (CGMS Herbarium).

Parmotrema subrugatum (Kremp.) Hale (Sup. Fig. 13A)

MYCOBANK MB 343135

Basionym: *Parmelia subrugata* Kremp., Verhandlungen der Kaiserlich-Königlichen Zoologisch-Botanischen Gesellschaft in Wien 18: 320. 1868.

Type: BRAZIL, Rio de Janeiro, Serra dos Órgãos, Helmreichen s.n. (M – holotype [n.v.]; US – isotype [n.v.]).

Description: Thallus greyish green, lobate to lacinulate, corticolous, 3–9.5 cm broad. Lobes irregularly branched, 1.0–12.0 mm wide, continuous and smooth, can be rugose in the central region, apical zone varied between specimens: round, cropped or lacinulate. Maculae present, weak, punctiform, and restricted to amphithecium. Cilia present, simple, K- reaction, 0.3–2.0 × 0.03–0.06 mm, few to abundant and marginal. Isidia, phyllidia, pustules, and soralia absent. When lacinulae are present, plane, simple, 0.6–1.7 × 0.4–1.0 mm. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light brown to variegated. Rhizines black, simple to irregularly branched, 0.2–1.6 × 0.02–0.06 mm, few and grouped. Apothecia present, submarginal, disk naked, unperforated, often crenate or lacinulate, ascospores ellipsoid, 24–36 × 14–18 µm, episporium 3–4 µm. Pycnidia present, ostiole black, conidia unciform, 5–7 µm.

Chemistry: atranorin, alectorinic acid and α-collatolic. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC+ rose, P-, UV+ greenish.

Remarks. *Parmotrema subrugatum* is recognized by the absence of vegetative propagules, presence of marginal cilia, unciform conidia 5–7 µm, medullary alectorinic acid, and α-collatolic acid (UV+ greenish reaction). *Parmotrema restingense* Marcelli, Benatti & Elix is another Brazilian species containing alectorinic acid, but based on the description of the prototype (Marcelli & Benatti 2011) both species present subtle differences, in *P. restingense* the lacinules are considered adventitious, the lower surface has cream or white only in lobes

that have apothecia, while in *P. subrugatum* the white or cream coloration is continuous on the margins. *Parmotrema restingense* has smaller unciform conidia, between (3) 4-5 (6) µm, while the specimens have unciform conidia between 5-7 µm. Furthermore, in the description of the species of *P. restingense*, it was described that the margins of the apothecia are toothed or short-lacinulated. In the specimens studied, some apothecia have very evident projections. The presence of maculae in the amphithecia of *P. restingense* is not described, while *P. subrugatum* has amphithecium maculate, as well as the specimens examined. Another similar species is *Parmotrema pycnidiocarpum* Benatti, Marcelli & Elix, but is differentiated by the presence of smaller unciform conidia (3.0–5.0 µm) and orange-pigmented medulla around the hymenium (Marcelli & Benatti 2011)

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 April 2017, A. S. Rodrigues 184 (CGMS Herbarium), and 28 Jul. 2017, A.S. Rodrigues 297 (CGMS Herbarium); Rio Grande Municipality, Praia da Capilha, 32º30′43.8″S, 52º34′56.5″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 277 (CGMS Herbarium), 32º30′39.4″S, 52º34′58.4″W, 6 m, 14 Jan. 2018, A.S. Rodrigues 484 (CGMS Herbarium).

Parmotrema tinctorum (Despr. ex Nyl.) Hale (Sup. Fig. 13B)

MYCOBANK MB 343140

Basionym: *Parmelia tinctorum* Despr. ex Nyl., Bulletin de la Société Linnéenne de Normandie 6 (2): 269. 1872.

Type: Canary Islands, Dèspréaux s.n. (H-NYL35365 – holotype [n.v.])

≡ *Parmelia tinctoria* Despr. ex Nyl. Flora, Regensburg 55: 547. 1872.

Description: Thallus greyish green, lobate, corticolous, 12 cm broad. Lobes irregularly branched, 3.5–15 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia absent. Lacinulae, phyllidia, pustules, and soralia absent. Isidia present, cylindrical, simple to branched, concolor with brown apex, laminal to marginal, 0.08–0.2 × 0.06–0.1 mm, ornamentation absent. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, light brown. Rhizines black central and marginals with beige or white tips, simple to irregularly branched, 0.2–1.0 × 0.04–0.1 mm, few and grouped. Apothecia absent. Pycnidia present, ostiole black, conidia filiform, 11–15 µm.

Chemistry: atranorin and lecanoric acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C+ pink, KC+ pink, P-, UV-.

Remarks. See *Parmotrema eitenii*.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Laranjal beach, 31º46′5.7″S, 52º13′38.8″W, 7 m, 26 Mar. 2017, A.S. Rodrigues 133 (CGMS Herbarium).

Parmotrema* subgenus *Crespoa: (Sup. Fig. 13C)

Crespoa carneopruinata (Zahlbr.) Lendemer & B.P. Hodk.

MYCOBANK MB 564130

Basionym: *Parmelia carneopruinata* Zahlbr., Sitzungsberichte der Kaiserlichen Akademie der Wissenschaften Math.-naturw. Klasse Abt. I 111 (1): 419. 1902.

Type: Brasil, Rio de Janeiro, Höhnel 164 (W– lectotype [n.v.]).

≡ *Pseudoparmelia carneopruinata* (Zahlbr.) Hale, Phytologia 29 (3): 189. 1974.

≡ *Canoparmelia carneopruinata* (Zahlbr.) Elix & Hale, Mycotaxon 27: 278. 1986.

≡ *Parmotrema carneopruinatum* (Zahlbr.) D. Hawksw., The Lichenologist 43 (6): 648 .2011.

Description: Thallus greyish green, sublacinulate, corticolous, 5-6 cm broad. Lobes irregularly branched, 0.5–1.7 mm wide, continuous, foveolate and rugose in the central region, apical zone round. Maculae present and evident, reticular, over the foveolos. Cilia absent. Isidia, lacinulae, phyllidia, and pustules absent. Soralia present, capitate to linear, laminal to marginal, soredia granular. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, brown. Rhizines black, simple to irregularly branched, 0.2–0.8 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia absent. Pycnidia present, ostiole black, conidia not found.

Chemistry: atranorin, stictic, constictic, and cryptostictic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellow, C-, KC-, P+ orange, UV-.

Remarks. *Crespoa carneopruinata* is recognized by the presence of soredia, narrow and sublacinated lobes, absence of cilia, and presence of the "stictic complex" in the medulla. The distinction between *C. carneopruinata* and *C. crozalsiana* is very subtle, generally based on the size of the lobes, with *C. crozalsiana* having wider lobes (Jungbluth 2006; Canêz 2005), and also on the arrangement of the soralia - *C. crozalsiana* normally has linear, while *C. carneopruinata* generally has globular (Hale 1976).

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 03 April 2017, A. S. Rodrigues 144 (CGMS Herbarium); Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S, 53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 630 (CGMS Herbarium).

Crespoa crozalsiana (B. de Lesd. ex Harm.) Lendemer & B.P. Hodk (Sup. Fig. 13D)

MYCOBANK MB 564134

Basionym: *Parmelia crozalsiana* de Lesd.: 555. 1910.

Type: França, Hérault, Agde, col. De Crozals, May 1909 (US – lectotype [n.v.])

≡ *Pseudoparmelia crozalsiana* (de Lesd. ex Harm.) Hale, Phytologia 29 (3): 189. 1974.

≡ *Canoparmelia crozalsiana* (de Lesd.) Elix & Hale, Mycotaxon 27: 278. 1986.

≡ *Parmotrema crozalsianum* (B. de Lesdain ex Harmand) D. Hawksworth, The Lichenologist 43 (6): 648. 2011.

Description: Thallus greyish green, lobate, corticolous, 4.5-5.0 cm broad. Lobes irregularly branched, 1.2–4.3 mm wide, continuous, foveolate and rugose in the central region, apical zone round. Maculae present and evident, reticular, over the foveolos. Cilia absent. Isidia, lacinulae, phyllidia, and pustules absent. Soralia present, capitate to linear, laminal to marginal, soredia granular. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, brown. Rhizines black, simple to irregularly branched, 0.25–1.25 × 0.03–0.07 mm, abundant and evenly distributed. Apothecia and pycnidia absent.

Chemistry: atranorin, stictic, constictic, and cryptostictic acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K+ yellow, C-, KC-, P+ orange, UV-.

Remarks. See *Crespoa carneopruinata*.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 April 2017, A. S. Rodrigues 136 (CGMS Herbarium); Rio Grande Municipality, Estação Ecológica do Taim, 32º33′11.5″S, 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 608 (CGMS Herbarium).

Punctelia borrerina (Nyl.) Krog (Sup. Fig. 13E)

MYCOBANK MB 110967

Basionym: *Parmelia borrerina* Nyl., Les Lichens des Environs de Paris 36. 1896.

Type: Brazil, Rio Grande do Sul, Pelotas, J. Newton, 1892/1893 (H-NYL 35036 – lectotype [n.v.] designated by Canêz 2009).

= *Parmelia borreri* var. *allophyla* Kremp. Flora 61: 438. 1878.

Type: Argentina, Lorentz & Hieronymus (M – lectotype [n.v.] designated by Canêz 2009).

Description: Thallus greyish green, lobate, corticolous, 6–10 cm broad. Lobes irregularly branched, 1.5–7.5 mm wide, continuous, foveolate and rugose in the central region, apical zone round. Maculae absent. Cilia absent. Isidia, lacinulae, phyllidia, pustules, and soralia absent. Pseudocyphellae present, plane, punctiform, laminal. Medulla white, pigment absent. Lower surface completely black or black to variegated, smooth to veined; marginal zone naked present, brown to dark brown. Rhizines black to black with beige or white tips, simple, 0.14–1.0 × 0.02–0.06 mm, abundant and evenly distributed. Apothecia present, laminal, immature. Pycnidia present, ostiole black, conidia filiform, 15–19 µm.

Chemistry: atranorin and unidentified fatty acids. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC-, P-, UV-.

Remarks. *Punctelia borrerina* is recognized by the absence of vegetative propagules, filiform conidia, and the presence of fatty acids in the medulla. *Punctelia riograndensis* is similar but differs in that it has unciform conidia, in addition to having traces of gyrophoric acid.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′43.8″S, 52º34′56.5″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 276 (CGMS Herbarium); Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 516 (CGMS Herbarium).

Punctelia riograndensis (Lynge) Krog (Sup. Fig. 13F)

MYCOBANK MB 110979

Basionym: *Parmelia riograndensis* Lynge, Ark. Bot. 13 (13): 26. 1914.

Type: Brazil, Rio Grande do Sul, Porto Alegre, 1892, Malme 461 (S – lectotype [n.v.] designated by Hale 1960)

≡ *Parmelia microsticta* Müll. Arg. var. *riograndensis* (Lynge) Lynge, Nytt Magazin for Naturvidenskaberne 62: 90. 1924.

Description: Thallus greyish green, lobate, corticolous, 4 cm broad. Lobes irregularly branched, 2.0–6.0 mm wide, continuous, foveolate and rugose in the central region, apical zone round. Maculae absent. Cilia absent. Isidia, lacinulae, phyllidia, pustules, and soralia absent. Pseudocyphellae present, plane, punctiform, laminal. Medulla white, pigment absent. Lower surface black, smooth to veined; marginal zone naked present, dark brown. Rhizines black to black with beige or white tips, simple, 0.2–0.8 × 0.02–0.04 mm, abundant and evenly distributed. Apothecia present, laminal, disk naked, unperforated, ornamentation absent, ascospores ellipsoid, 21–25 × 12–16 µm, episporium 1–2 µm. Pycnidia present, ostiole black, conidia unciform, 5–8 µm.

Chemistry: atranorin, unidentified fatty acids and traces of gyrophoric acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C-, KC-, P-, UV-.

Remarks. See *Punctelia borrerina* above, and Canêz (2009) for a more in-depth discussion of species characteristics based on type.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º46′13″S, 52º15′21.8″W, 7 m, 28 Jul. 2017, A. S. Rodrigues 298 (CGMS Herbarium).

***Punctelia* sp. nov. 1** (Sup. Fig. 13A)

Description: Thallus greyish green, lobate, corticolous, 5–12 cm broad. Lobes irregularly branched, 1.0–6.5 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia absent. Isidia, lacinulae, pustules, and soralia absent. Phyllidia

present, simple to branched becoming arbuscular (beginning as isidioid in some specimens), ascendant to erect, laminal to marginal. Pseudocyphellae present, plane, punctiform, laminal. Medulla white, pigment absent. Lower surface black, often smooth, veined in some specimens; marginal zone naked present, varied colors among specimens - light brown, light brown to variegated, beige or brown to variegated. Rhizines black to black with beige or white tips, simple to irregularly branched, 0.16–1.2 × 0.02–0.08 mm, abundant and evenly distributed. Apothecia present, laminal a submarginal, disk naked, unperforated, ornamentation absent, ascospores ellipsoid, 11–17 × 7–9 µm, episporium 1 µm. Pycnidia present, ostiole black, conidia unciform, 5–7 µm.

Chemistry: atranorin and gyrophoric acid. We also found an unidentified compound with a higher Rf than gyrophoric acid in the E/A solvent. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C+ pink, KC+ pink, P-, UV-.

Remarks. *Punctelia* sp. nov. 1 is recognized by the presence of phyllidia, unciform conidia, small ascospores (up to 20 µm long), black lower surface, and medullary gyrophoric acid. *Punctelia subpraesignis* is similar but differs in the absence of vegetative propagules. *Punctelia* sp. nov. 2 has similar morphology, but the phyllidia have isidioid and soredioid shapes and may have coarse soredia.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 03 April 2017, A. S. Rodrigues 134 (CGMS Herbarium); Rio Grande Municipality, Praia da Capilha, 32º30′42.6″S, 52º34′56.8″W, 6 m, 28 Jul. 2017, A.S. Rodrigues 263, 491 (CGMS Herbarium), 32º30′40.8″S, 52º34′58.1W, 6 m, 28 Jul. 2017, A.S. Rodrigues 271, 495 (CGMS Herbarium); Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.8″S, 52º16.3′3.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 499, 502, 554, 565 (CGMS Herbarium); Chuí Municipality, Barra do Chuí Beach, 33º44′19.2″S,

53º22′9.8″W, 20 m, 10 Jan. 2018, A.S. Rodrigues 629 (CGMS Herbarium); Rio Grande Municipality, Estação Ecológica do Taim, 32º33′11.5″S, 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 609 (CGMS Herbarium).

***Punctelia* sp. nov. 2**

Description: Thallus greyish green, lobate, corticolous, 5–10 cm broad. Lobes irregularly branched, 1.5–5.0 mm wide, continuous, smooth to rugose in the central region, apical zone round. Maculae absent. Cilia absent. Isidia, lacinulae, pustules, and soralia absent. Phyllidia present, isidioid to soredioid, some simple, ascendant to erect, laminal to marginal. Pseudocyphellae present, plane, punctiform, laminal. Medulla white, pigment absent. Lower surface black, smooth; marginal zone naked present, brown to variegated. Rhizines black to black with beige or white tips, simple, 0.2–1.0 × 0.02–0.08 mm, abundant and evenly distributed. Apothecia present, laminal, disk naked, unperforated, ornate with phyllidia isioid, ascospores ellipsoid, 12–15 × 8–10 µm, episporium 1 µm. Pycnidia absent.

Chemistry: atranorin and gyrophoric acid. We also found an unidentified compound with a higher Rf than gyrophoric acid in the E/A solvent. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C+ pink, KC+ pink, P-, UV-.

Remarks. See *Punctelia* sp. nov. 1.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Rio Grande Municipality, Praia da Capilha, 32º30′39.4″S, 52º34′58.4″W, 6 m, 14 Jan. 2018, A.S. Rodrigues 471, 492 (CGMS Herbarium); Rio Grande Municipality, Capão Seco (Barra Falsa), 31º51′25.4″S, 52º16′15.9″W, 18m, 15 Jan. 2018, A. S. Rodrigues 550 (CGMS Herbarium).

***Punctelia* sp. nov. 3** (Sup. Fig. 14B)

Description: Thallus greyish green, lobate, corticolous, 8–12 cm broad. Lobes irregularly branched, 2.0–3.5 mm wide, continuous, smooth to rugose in the central region, apical zone lacinulate. Maculae absent. Cilia absent. Isidia, phyllidia, pustules, and soralia absent. Lacinulae present, plane, simple to irregular, 0.3–1.4 × 0.16–0.32 mm. Pseudocyphellae present, plane, punctiform, laminal. Medulla white, pigment absent. Lower surface beige, smooth; marginal zone naked present, beige. Rhizines beige, simple, 0.3–1.8 × 0.02–0.06 mm, abundant and evenly distributed. Apothecia present, laminal to submarginal, disk naked, unperforated, ascospores ellipsoid, 12–14 × 5–7 µm, episporium 1 µm. Pycnidia present, ostiole black, conidia unciform, 5–7 µm.

Chemistry: atranorin and lecanoric acid. Spot tests: upper cortex: K+ yellow, UV-; medulla: K-, C+ pink, KC+ pink, P-, UV-.

Remarks. *Punctelia* sp. nov. 3 is recognized by the presence of lacinules, white lower surface, unciform conidia, and medullary lecanoric acid. *Punctelia missouriensis* is differentiated by the presence of corticated granules and isidia that develop into lobules or phyllidia (Canêz 2009).

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 April 2017, A. S. Rodrigues 156 (CGMS Herbarium); Rio Grande Municipality, Estação Ecológica do Taim, 32º33′11.5″S, 52º30′21.4″W, 16 m, 16 Jan. 2018, A.S. Rodrigues 574 (CGMS Herbarium).

Relicina subabstrusa (Gyeln.) Hale (Sup. Fig. 14C)

MYCOBANK MB 343556

Basionym: *Parmelia subabstrusa* Gyeln., Feddes Repertorium Specierum Novarum Regni Vegetabilis 29: 288. 1931.

Type: Boca da Serra, Serra da Chapada, Mato Grosso, Brasil, Malme s.n. (S – lectotype [n.v.]) based on *P. abstrusa* f. *laevigata* Lynge by Hale 1975.

≡ *Parmelia abstrusa* f. *laevigata* Lynge, Ark. Bot. 13 (13): 147. 1914.

≡ *Parmelia kilaueae* f. *laevigata* (Lynge) Gyeln. 1935.

Description: Thallus greyish green, sublacinulate, corticolous, 7 cm broad. Lobes irregularly branched, 0.7–2.0 mm wide, continuous, smooth to rugose in the central region, apical zone round to truncated. Maculae absent. Cilia present, bulbate, K- reaction, 0.2–0.9 × 0.04–0.06 mm, abundant and marginal. Isidia, lacinulae, phyllidia, pustules, and soralia absent. Medulla white, pigment absent. Lower surface black, smooth; marginal zone naked absent, dark brown. Rhizines black, simple to irregularly branched, 0.3–1.8 × 0.04–0.06 mm, abundant and evenly distributed. Apothecia present, laminal to submarginal, ciliate, disk naked, unperforated, ascospores ellipsoid, 7–9 × 4–5 µm, episporium 1 µm. Pycnidia present, ostiole black, conidia bifusiform, 7–9 µm.

Chemistry: usnic acid and norstictic acid. Spot tests: upper cortex: K-, UV-; medulla: K+ yellow, C-, KC-, P+ orange, UV-.

Remarks. *Relicina subabstrusa* is recognized by the absence of vegetative propagules, presence of marginal bulbate cilia, cortical usnic acid, and medullary norstictic acid. Hale (1975) reports that specimens may also present salazinic acid, however, in the study specimen we found only norstictic acid in the medulla. *Relicina abstrusa* is differentiated by the presence of isidia.

Localities.

Specimens examined. BRAZIL. Rio Grande do Sul State, Pelotas Municipality, Dunas Las Acácias, 31º45′33″S, 52º15′4.1″W, 7 m, 05 April 2017, A. S. Rodrigues 155 (CGMS Herbarium).

Supplementary Table S3. Specific identification results obtained through the NCBI BLASTn of the specimens generated in this study. The criteria for the selection of results are described in material and methods. **Query:** study specimen; **MI:** morphological identification; **HS:** presence of homonymous sequence in Genbank; **Accession:** GenBank accession number of the corresponding sequence; **MS:** maximum score; **QC:** query cover; **PI:** percentage identity; **Class.:** classification based on percent identity in ≥ 99% "potential" candidate to be the same species, between 98-97% possible "species complex", and < 97% "mismatch"; **BI:** identity according to BLASTn. When the MS results were the same as those obtained with %PI, we did not repeat the information. The sequences in bold were the identifications based on the highest percentages of identity considered and presented in Table 2.

Bulbothrix								
Query	**MI**	**HS**	**Accession**	**MS**	**QC (%)**	**PI (%)**	**Class.**	**BI**
AR233	*bulbilosa*	no	**DQ279481**	**900**	**90**	**99.80**	**potential**	***apophysata***
			GQ919264	771	78	99.29	potential	*laevigatula*
			AY251412	628	77	93.63	mismatch	sp.
AR257	*bulbilosa*	no	**DQ279481**	**904**	**90**	**99.80**	**potential**	***apophysata***
			GQ919264	771	78	99.29	potential	*laevigatula*
			AY251412	628	77	93.63	mismatch	sp.
AR474	*cassa*	no	LC573992	643	98	89.49	mismatch	*subscortea*
			LC573993	638	98	89.49	mismatch	*subscortea*
			KT729546	619	94	89.40	mismatch	*mammillaria*
			KX539187	**420**	**59**	**91.59**	**mismatch**	***tabacina***
			KX539188	416	59	91.05	mismatch	*tabacina*
			OQ971988	503	74	90.26	mismatch	*isidiza*
AR475	*cassa*	no	LC573992	641	100	89.61	mismatch	*subscortea*
			LC573993	636	100	89.41	mismatch	*subscortea*
			KT729546	603	100	89.21	mismatch	*mammillaria*
			KX539187	**420**	**60**	**91.59**	**mismatch**	***tabacina***
			KX539188	416	61	91.05	mismatch	*tabacina*
			OQ971988	503	76	90.26	mismatch	*isidiza*
AR493	*cassa*	no	LC573992	614	100	88.74	mismatch	*subscortea*
			LC573993	608	100	88.54	mismatch	*subscortea*

			KT729546	580	94	88.48	mismatch	*mammillaria*
			GQ919272	**392**	**54**	**92.39**	**mismatch**	***Remototrachyna awasthii***
			GQ919271	392	54	92.39	mismatch	*Remototrachyna awasthii*
			KX539178	429	60	92.18	mismatch	*isidiza*
AR487	*ventricosa*	no	LC573992	654	98	89.75	mismatch	*subscortea*
			LC573993	649	98	89.56	mismatch	*subscortea*
			KT729546	619	94	89.40	mismatch	*mammillaria*
			KX539187	**420**	**58**	**91.80**	**mismatch**	***tabacina***
			KX539188	416	59	91.26	mismatch	*tabacina*
			OQ971988	514	75	90.56	mismatch	*isidiza*
AR521	*ventricosa*	no	LC573992	667	98	90.14	mismatch	*subscortea*
			LC573993	662	98	89.94	mismatch	*subscortea*
			KT729546	640	100	88.60	mismatch	*mammillaria*
			KX539187	**420**	**57**	**91.80**	**mismatch**	***tabacina***
			KX539188	416	58	91.80	mismatch	*tabacina*
			OQ971988	514	74	90.56	mismatch	*isidiza*
Canoparmelia								
Query	**MI**	**HS**	**Accession**	**MS**	**QC (%)**	**PI (%)**	**Class.**	**ID**
AR519	*albaniensis*	yes	KP659642	972	100	99.81	potential	*albaniensis**
			KP659643	929	95	99.80	potential	*albaniensis**
			KY929412	**907**	**92**	**100**	**potential**	***albaniensis****
			GU994547	891	95	98.42	complex	*albaniensis**
AR581	*caroliniana*	yes	**KP659632**	**966**	**97**	**100**	**potential**	***caroliniana***
			KP659629	965	97	100	potential	*caroliniana*
			KP659628	965	97	100	potential	*caroliniana*
			KP659623	965	97	100	potential	*caroliniana*

			KP659633	907	95	98.45	complex	*caroliniana*
			KP659627	907	95	98.45	complex	*caroliniana*
AR625	*caroliniana*	yes	**KP659632**	**959**	**96**	**99.81**	**potential**	***caroliniana***
			KP659631	952	96	99.62	potential	*caroliniana*
			KP659629	952	96	99.81	potential	*caroliniana*
			KP659628	952	96	99.81	potential	*caroliniana*
			KP659633	909	95	98.45	complex	*caroliniana*
			KP659627	909	95	98.45	complex	*caroliniana*
AR559	*texana*	yes	**KP659644**	**992**	**97**	**99.82**	**potential**	***texana***
			KP659648	989	97	99.81	potential	*texana*
			KP659647	989	97	99.81	potential	*texana*
			AY251413	856	86	98.96	complex	*texana*
			KC978849	824	83	98.71	complex	*texana*
AR561	*texana*	yes	**KP659644**	**989**	**97**	**99.63**	**potential**	***texana***
			KP659648	985	97	99.63	potential	*texana*
			KP659647	985	97	99.63	potential	*texana*
			AY251413	850	86	98.75	complex	*texana*
			KC978849	819	83	98.49	complex	*texana*
AR627	*texana*	yes	KP659644	970	96	99.44	potential	*texana*
			KP659648	966	96	99.44	potential	*texana*
			KP659647	966	96	99.44	potential	*texana*
			KP659649	952	96	98.88	complex	*texana*
			KP659641	952	96	98.88	complex	*texana*

Flavoparmelia

Query	MI	HS	Accession	MS	QC (%)	PI (%)	Class.	ID
AR164	*exornata*	no	LC669627	728	97	91.68	mismatch	*caperata*
			LC669626	728	97	91.68	mismatch	*caperata*

			LC657570	728	97	91.68	mismatch	*caperata*
			OK577919	**702**	**92**	**92.05**	**mismatch**	***caperata***
AR551	*exornata*	no	LC669627	728	96	91.68	mismatch	*caperata*
			LC669626	728	96	91.68	mismatch	*caperata*
			LC657570	728	96	91.68	mismatch	*caperata*
			OK577919	**702**	**91**	**92.05**	**mismatch**	***caperata***
AR634	*soredians*	yes	**KF017378**	**839**	**100**	**99.35**	**potential**	**aff. *soredians***
			KF017379	833	100	99.14	potential	aff. *soredians*
			MK812200	579	100	94.89	mismatch	*soredians*
			HM014221	477	81	96.25	mismatch	*soredians*
Hypotrachyna								
Query	**MI**	**HS**	**Accession**	**MS**	**QC (%)**	**PI (%)**	**Class.**	**ID**
AR162	*livida*	yes	**KF380906**	**907**	**100**	**99.20**	**potential**	***livida***
			KF380905	898	99	99.00	potential	*livida*
			GQ919282	865	97	98.57	complex	*livida*
AR163	*livida*	yes	**KF380906**	**918**	**99**	**98.65**	**complex**	***livida***
			KF380905	913	99	98.46	complex	*livida*
			GQ919282	874	96	98.02	complex	*livida*
AR594	*minarum*	yes	**MZ919272**	**1107**	**100**	**100**	**potential**	***minarum***
			MT482183	1022	97	98.29	complex	*minarum*
			MT482191	1005	97	97.78	complex	*minarum*
			AY611110	695	65	98.72	complex	*minarum*
			KM250211	684	64	98.70	complex	*minarum*
AR614	*minarum*	yes	**MZ919271**	**1107**	**100**	**100**	**potential**	***minarum***
			MT482183	1022	97	98.29	complex	*minarum*
			MT482191	1005	97	97.78	complex	*minarum*
			AY611110	695	65	98.72	complex	*minarum*

			KM250211	684	64	98.70	complex	*minarum*
AR240	*spumosa*	yes	**MT482220**	**750**	**98**	**96.88**	**mismatch**	***spumosa***
			MT482219	750	98	96.88	mismatch	*spumosa*
			MT482218	750	98	96.88	mismatch	*spumosa*
AR606	*spumosa*	yes	**MT482220**	**828**	**98**	**97.52**	**complex**	***spumosa***
			MT482219	828	98	97.52	complex	*spumosa*
			MT482218	828	98	97.52	complex	*spumosa*
Parmelinella								
Query	**MI**	**HS**	**Accession**	**MS**	**QC (%)**	**PI (%)**	**Class.**	**ID**
AR287	*lindmanii*	yes	GQ267190	904	96	99.01	potential	*lindmanii*
			MW364890	**854**	**88**	**99.78**	**potential**	***lindmanii***
			MW364889	632	88	91.45	mismatch	*salacinifera*
AR518	*lindmanii*	yes	GQ267190	909	96	99.02	potential	*lindmanii*
			MW364891	**854**	**88**	**99.78**	**potential**	***lindmanii***
			GQ267191	640	94	90.00	mismatch	aff. *wallichiana*
AR604	*salacinifera*	yes	**MW364889**	**824**	**88**	**98.92**	**complex**	***salacinifera***
			GQ267191	743	91	94.77	mismatch	aff. *wallichiana*
			GQ267190	656	92	91.22	mismatch	*lindmanii*
AR619	*salacinifera*	yes	GQ267191	832	90	95.92	mismatch	aff. *wallichiana*
			MW364888	**830**	**81**	**98.92**	**complex**	***salacinifera***
			GQ267190	699	91	91.08	mismatch	*lindmanii*
Parmotrema								
Query	**MI**	**HS**	**Accession**	**MS**	**QC (%)**	**PI (%)**	**Class.**	**ID**
AR245	*austrosinense*	yes	MG241383	959	100	99.43	potential	*austrosinense*
			LC669650	935	97	99.42	potential	*austrosinense*
			MW311306	**905**	**94**	**99.60**	**potential**	***austrosinense***
			KY929418	850	91	98.55	complex	*austrosinense*

			DQ394372	881	95	98.41	complex	*austrosinense*
AR252	*austrosinense*	yes	MG241383	1055	100	99.65	potential	*austrosinense*
			LC669650	1040	98	99.65	potential	*austrosinense*
			MW311306	**1011**	**95**	**99.82**	**potential**	***austrosinense***
			KY929418	881	85	98.79	complex	*austrosinense*
			EU266116	861	84	98.76	complex	*reticulatum*
AR640	*cetratum*	yes	MG271854	1086	99	97.34	complex	*cetratum*
			KM983347	1064	99	96.86	mismatch	*reticulatum*
			KM983362	1062	99	96.86	mismatch	*reticulatum*
			AY251449	**861**	**76**	**98.57**	**complex**	***cetratum***
			AY642843	863	78	97.80	complex	*reticulatum*
			OQ622389	843	76	97.76	complex	sp.
AR536	*cetratum*	yes	MG271854	1057	99	96.84	mismatch	*cetratum*
			KM983347	1050	98	96.68	mismatch	*reticulatum*
			KU354446	1044	98	96.52	mismatch	*reticulatum*
			KY929419	**841**	**77**	**97.18**	**complex**	***cetratum***
			JN166394	819	76	97.11	complex	*reticulatum*
			AY586579	841	78	97.00	complex	*reticulatum*
AR303	*clavuliferum*	yes	KU354445	1184	100	97.55	complex	*clavuliferum*
			KU354438	1179	100	97.40	complex	*clavuliferum*
			KU354437	1179	100	97.40	complex	*clavuliferum*
			AY642843	**872**	**71**	**98.58**	**complex**	***reticulatum***
			JN166396	861	71	98.17	complex	*reticulatum*
			JN166395	861	71	98.17	complex	*reticulatum*
AR588	*clavuliferum*	yes	KU354445	1201	100	97.98	complex	*clavuliferum*
			KU354438	1195	100	97.83	complex	*clavuliferum*
			KU354437	1195	100	97.83	complex	*clavuliferum*

			AY642843	**878**	**71**	**98.78**	**complex**	***reticulatum***
			JN166393	828	67	98.71	complex	*reticulatum*
			JN166396	867	71	98.38	complex	*reticulatum*
AR234	*commensuratum*	no	KM983347	1175	99	97.67	complex	*reticulatum*
			KU354446	1170	99	97.52	complex	*reticulatum*
			HQ671310	1170	99	97.52	complex	*reticulatum*
			KY929419	**902**	**72**	**99.40**	**potential**	***cetratum***
			JN166374	843	68	99.15	potential	*reticulatum*
			JN166372	843	68	98.94	complex	*reticulatum*
AR552	*commensuratum*	no	KM983347	1166	99	98.20	complex	*reticulatum*
			KU354446	1160	99	98.05	complex	*reticulatum*
			KM983351	1160	99	98.05	complex	*reticulatum*
			KY929419	**913**	**74**	**99.80**	**potential**	***cetratum***
			JN166374	854	70	99.57	potential	*reticulatum*
			JN166372	854	70	99.36	potential	*reticulatum*
AR132	*eciliatum*	no	KX132923	920	90	96.75	mismatch	*crinitum*
			KX132915	920	90	96.75	mismatch	*perlatum*
			MZ391141	909	90	96.38	mismatch	*perlatum*
			AY251442	**883**	**80**	**99.18**	**potential**	***crinitum***
			ON312521	870	84	97.10	complex	*perlatum*
			ON312504	889	87	96.81	mismatch	*perlatum*
AR651	*eciliatum*	no	KX132915	920	91	97.07	complex	*perlatum*
			KX132923	918	91	97.07	complex	*crinitum*
			MZ391141	907	91	96.70	mismatch	*perlatum*
			AY251442	**883**	**81**	**99.18**	**potential**	***crinitum***
			LC700474	791	77	97.42	complex	*perlatum*
			ON312504	889	87	97.15	complex	*perlatum*

AR661	*eciliatum*	no	KX132915	931	91	97.44	complex	*perlatum*
			KX132923	929	91	97.44	complex	*crinitum*
			MZ391141	918	91	97.07	complex	*perlatum*
			AY251442	**883**	**81**	**99.18**	**potential**	***crinitum***
			ON312504	900	87	97.53	complex	*perlatum*
			ON312510	898	87	97.52	complex	*perlatum*
AR149	*eitenii*	no	**KF129455**	**1140**	**99**	**99.68**	**potential**	***tinctorum***
			KF129468	1136	99	99.52	potential	*tinctorum*
			KF129464	1134	99	99.52	potential	*tinctorum*
			KU354447	1133	99	99.68	potential	*tinctorum*
			KM817767	1129	98	99.68	potential	*tinctorum*
AR154	*eitenii*	no	KF129464	931	95	96.95	mismatch	*tinctorum*
			MN814004	926	95	96.77	mismatch	*tinctorum*
			MN814002	926	95	96.77	mismatch	*tinctorum*
			JQ673443	**924**	**90**	**98.66**	**complex**	**sp.**
			KF129420	776	77	97.37	complex	*tinctorum*
			KF129414	815	82	97.30	complex	*tinctorum*
AR569	*eitenii*	no	**JQ673443**	**907**	**88**	**98.63**	**complex**	**sp.**
			KF129464	905	94	96.95	mismatch	*tinctorum*
			MN814004	900	94	96.36	mismatch	*tinctorum*
			KF129420	767	79	96.94	mismatch	*tinctorum*
			KF129414	806	83	96.90	mismatch	*tinctorum*
AR513	*eliasaroanum*	no	**MG257789**	**824**	**87**	**94.73**	**mismatch**	***internexum***
			KP943758	824	87	94.73	mismatch	*internexum*
			KP943756	824	87	94.73	mismatch	*internexum*
AR175	*haitiense*	yes	ON312514	929	100	97.78	complex	*subtinctorum*
			GU593037	928	100	97.77	complex	*subtinctorum*

			AY586558	922	98	97.75	complex	*subtinctorum*
			KC978853	**824**	**87**	**98.30**	**complex**	***subtinctorum***
			GU994578	893	94	98.24	complex	*subtinctorum*
			KY929428	863	91	98.18	complex	*recipiendum*
AR280	*haitiense*	yes	ON312514	907	100	98.08	complex	*subtinctorum*
			GU593037	907	100	98.08	complex	*subtinctorum*
			AY586558	904	99	98.07	complex	*subtinctorum*
			MN038165	**902**	**98**	**98.44**	**complex**	***subsumptum***
			KC978853	824	90	98.30	complex	*subtinctorum*
			GU994578	893	97	98.24	complex	*subtinctorum*
AR128	*homotomun*	no	KM983347	1175	99	97.80	complex	*reticulatum*
			KU354446	1170	99	97.65	complex	*reticulatum*
			HQ671310	1170	99	97.65	complex	sp.
			KY929419	**902**	**72**	**99.40**	**potential**	***cetratum***
			JN166374	843	68	99.15	potential	*reticulatum*
			JN166372	843	68	98.94	complex	*reticulatum*
AR549	*madilynae*	no	ON312515	821	100	92.82	mismatch	*mellissii*
			KX132915	811	99	92.62	mismatch	*perlatum*
			ON312516	811	95	93.91	mismatch	*flavotinctum*
			ON312501	**787**	**85**	**95.71**	**mismatch**	***dilatatum***
			KY929427	773	86	95.12	mismatch	*praesorediosum*
			ON312500	795	89	94.74	mismatch	*dilatatum*
AR589	*madilynae*	no	ON312515	845	100	92.61	mismatch	*mellissii*
			KF129464	821	95	92.96	mismatch	*tinctorum*
			KF129453	821	100	92.09	mismatch	*andinum*
			ON312501	**778**	**82**	**95.33**	**mismatch**	***dilatatum***
			KY929427	763	83	94.74	mismatch	*praesorediosum*

			ON312500	785	86	94.37	mismatch	*dilatatum*
AR498	*perlatum*	yes	MG257789	946	96	97.47	complex	*internexum*
			ON312517	**935**	**93**	**97.96**	**complex**	***perlatum***
			KP943756	911	93	97.38	complex	*internexum*
AR641	*perlatum*	yes	**ON312517**	**918**	**87**	**99.03**	**potential**	***perlatum***
			MG257789	880	89	96.78	mismatch	*internexum*
			KP943758	880	89	96.78	mismatch	*internexum*
AR141	*permutatum*	yes	**MN814004**	**850**	**91**	**95.84**	**mismatch**	***tinctorum***
			MN814003	850	91	95.84	mismatch	*tinctorum*
			MN814002	850	91	95.84	mismatch	*tinctorum*
AR598	*permutatum*	yes	**MN814004**	**850**	**94**	**95.84**	**mismatch**	***tinctorum***
			MN814003	850	94	95.84	mismatch	*tinctorum*
			MN814002	850	94	95.84	mismatch	*tinctorum*
AR251	*pilosum*	yes	GQ344481	955	94	98.18	complex	*consors*
			GQ344482	**933**	**91**	**98.31**	**complex**	***consors***
			ON312515	869	99	93.81	mismatch	*mellissii*
AR284	*pilosum*	yes	GQ344481	955	96	98.18	complex	*consors*
			GQ344482	**933**	**94**	**98.31**	**complex**	***consors***
			ON312515	845	100	93.67	mismatch	*mellissii*
AR172	*praesorediosum*	yes	**KY929427**	**850**	**87**	**97.77**	**complex**	***praesorediosum***
			JQ673446	848	90	96.50	mismatch	sp.
			KM250224	826	90	95.73	mismatch	*Canoparmelia aptata*
AR485	*praesorediosum*	yes	**KY929427**	**850**	**86**	**97.77**	**complex**	***praesorediosum***
			JQ673446	848	90	96.34	mismatch	sp.
			KM250224	832	90	95.75	mismatch	*Canoparmelia aptata*
AR520	*praesorediosum*	yes	KM250224	887	89	97.50	complex	*Canoparmelia aptata*

			KM250223	861	89	96.74	mismatch	*praesorediosum*
			JQ673446	854	89	96.35	mismatch	sp.
			KY929427	**85**	**85**	**97.77**	**complex**	***praesorediosum***
AR656	*praesorediosum*	yes	KM250224	887	89	97.50	complex	*Canoparmelia aptata*
			KM250223	854	89	96.88	mismatch	*praesorediosum*
			KY929427	**850**	**86**	**97.77**	**complex**	***praesorediosum***
AR506	*reticulatum*	yes	KU354446	1210	100	98.40	complex	*reticulatum*
			KM983351	1210	100	98.40	complex	*reticulatum*
			KM983347	1210	100	98.40	complex	*reticulatum*
			KY929419	**889**	**71**	**99.39**	**potential**	***cetratum***
			AY586579	874	70	99.18	potential	*reticulatum*
			JN166376	872	70	99.17	potential	*reticulatum*
AR636	*reticulatum*	yes	KU354446	1216	100	98.14	complex	*reticulatum*
			KM983351	1216	100	98.14	complex	*reticulatum*
			KM983347	1216	100	98.14	complex	*reticulatum*
			KY929419	**893**	**71**	**99.00**	**potential**	***cetratum***
			AY586579	880	70	98.79	complex	*reticulatum*
			JN166376	878	70	98.79	complex	*reticulatum*
AR557	sp. nov. 1	no	MN814004	869	91	94.66	mismatch	*tinctorum*
			MN814003	869	91	94.66	mismatch	*tinctorum*
			MN814002	869	91	94.66	mismatch	*tinctorum*
			KF129414	**785**	**78**	**96.07**	**mismatch**	***tinctorum***
			KF129438	763	77	95.79	mismatch	*pseudotinctorum*
			KF129424	848	86	95.65	mismatch	*pseudotinctorum*
AR571	sp. nov. 1	no	MN814004	870	91	94.67	mismatch	*tinctorum*
			MN814003	870	91	94.67	mismatch	*tinctorum*
			MN814002	870	91	94.67	mismatch	*tinctorum*

			KF129414	**785**	**78**	**96.07**	**mismatch**	***tinctorum***
			KF129438	763	77	95.79	mismatch	*pseudotinctorum*
			KY929431	795	80	95.57	mismatch	*tinctorum*
AR184	*subrugatum*	no	ON312516	963	98	92.39	mismatch	*flavotinctum*
			KU208013	924	95	92.05	mismatch	*xanthinum*
			KU208012	917	91	93.07	mismatch	*xanthinum*
			KM983347	**852**	**76**	**95.85**	**mismatch**	***reticulatum***
			KM983351	846	76	95.66	mismatch	*reticulatum*
			KM983362	845	78	94.86	mismatch	*reticulatum*
AR277	*subrugatum*	no	ON312516	948	100	91.90	mismatch	*flavotinctum*
			KU354446	893	84	94.54	mismatch	*reticulatum*
			HQ671310	893	84	94.54	mismatch	sp.
			AY586579	**817**	**71**	**96.75**	**mismatch**	***reticulatum***
			AY642820	815	70	96.75	mismatch	*reticulatum*
			KM983347	874	76	96.60	mismatch	*reticulatum*
AR297	*subrugatum*	no	ON312516	874	99	93.71	mismatch	*flavotinctum*
			KU208013	845	99	92.74	mismatch	*xanthinum*
			KU208012	845	99	92.74	mismatch	*xanthinum*
			AY586576	**743**	**76**	**96.07**	**mismatch**	***cetratum***
			OQ600640	741	76	96.07	mismatch	*cetratum*
			AY642847	741	76	96.07	mismatch	*cetratum*
AR484	*subrugatum*	no	ON312516	942	98	93.69	mismatch	*flavotinctum*
			KU208013	911	97	92.90	mismatch	*xanthinum*
			KU208012	911	97	92.90	mismatch	*xanthinum*
			AY251415	**798**	**75**	**96.14**	**mismatch**	***fistulatum***
			KY929430	802	76	95.98	mismatch	*reticulatum*
			AY586579	795	75	95.94	mismatch	*reticulatum*

AR133	*tinctorum*	yes	KF129455	1057	100	99.15	potential	*tinctorum*
			KF129468	1053	100	98.98	complex	*tinctorum*
			KF129464	1051	100	98.98	complex	*tinctorum*
			KY929431	**911**	**84**	**99.80**	**potential**	***tinctorum***
			HQ650684	1007	94	99.46	potential	*tinctorum*
			KF129414	869	82	99.17	potential	*tinctorum*
Subgenus *Crespoa*								
Query	**MI**	**HS**	**Accession**	**MS**	**QC (%)**	**PI (%)**	**Class.**	**ID**
AR144	*carneopruinata*	no	EF042904	920	90	99.41	potential	*carneopruinata*
			AY586571	915	90	99.21	potential	*crozalsiana*
			KY929414	**902**	**88**	**99.60**	**potential**	***crozalsiana***
			GU994544	896	88	99.39	potencial	*inhaminensis*
AR630	*carneopruinata*	no	EF042904	933	90	99.80	potential	*carneopruinata*
			AY586571	928	90	99.61	potential	*ccrozalsiana*
			KY929414	**915**	**88**	**100**	**potential**	***crozalsiana***
			GU994544	909	88	99.18	potencial	*inhaminensis*
AR136	*crozalsiana*	no	EF042904	933	89	99.80	potential	*carneopruinata*
			AY586571	928	89	99.61	potential	*crozalsiana*
			KY929414	**915**	**87**	**100**	**potential**	***crozalsiana***
			GU994544	909	87	99.80	potencial	*inhaminensis*
AR608	*crozalsiana*	no	**KY929414**	**915**	**90**	**100**	**potential**	***crozalsiana***
			EF042904	915	90	99.80	potential	*carneopruinata*
			GU994544	909	90	99.80	potencial	*inhaminensis*
			AY586571	909	90	99.60	potential	*crozalsiana*
Punctelia								
Query	**MI**	**HS**	**Accession**	**MS**	**QC (%)**	**PI (%)**	**Class.**	**ID**
AR276	*borrerina*	no	MZ836018	953	100	95.17	mismatch	*subrudecta*

			GU593038	942	97	95.90	mismatch	*borreri*
			LC669679	924	98	95.08	mismatch	*borreri*
			MK812448	**905**	**91**	**96.38**	**mismatch**	***stictica***
AR516	*borrerina*	no	MZ836018	876	100	94.82	mismatch	*subrudecta*
			GU593038	869	97	95.44	mismatch	*borreri*
			LC669679	856	98	94.76	mismatch	*borreri*
			MK812448	**92**	**92**	**95.95**	**mismatch**	***stictica***
AR298	*riograndensis*	no	GU593038	828	85	94.60	mismatch	*borreri*
			MK812448	**817**	**82**	**95.20**	**mismatch**	***stictica***
			OQ717581	815	86	93.91	mismatch	*borreri*
AR134	sp. nov. 1	no	MZ836018	941	99	97.11	complex	*subrudecta*
			GU593038	933	97	97.79	complex	*borreri*
			LC669679	920	98	97.07	complex	*borreri*
			AY267010	**872**	**90**	**98.20**	**complex**	***subpraesignis***
			JQ004658	874	91	97.83	complex	sp.
			AY773115	861	90	97.80	complex	*borreri*
AR263	sp. nov. 1	no	MZ836018	1018	100	97.00	complex	*subrudecta*
			LC669679	996	98	97.12	complex	*borreri*
			GU593038	**992**	**97**	**97.26**	**complex**	***borreri***
			AY773124	846	83	97.20	complex	*perreticulata*
			KM250195	902	88	97.19	complex	*subrudecta*
			MN562210	893	88	97.16	complex	*borreri*
AR271	sp. nov. 1	no	MZ836018	931	100	96.59	mismatch	*subrudecta*
			GU593038	922	97	97.25	complex	*borreri*
			LC669679	909	98	96.55	mismatch	*borreri*
			AY267010	**89**	**89**	**97.60**	**complex**	***subpraesignis***
			AY773115	850	89	97.40	complex	*borreri*

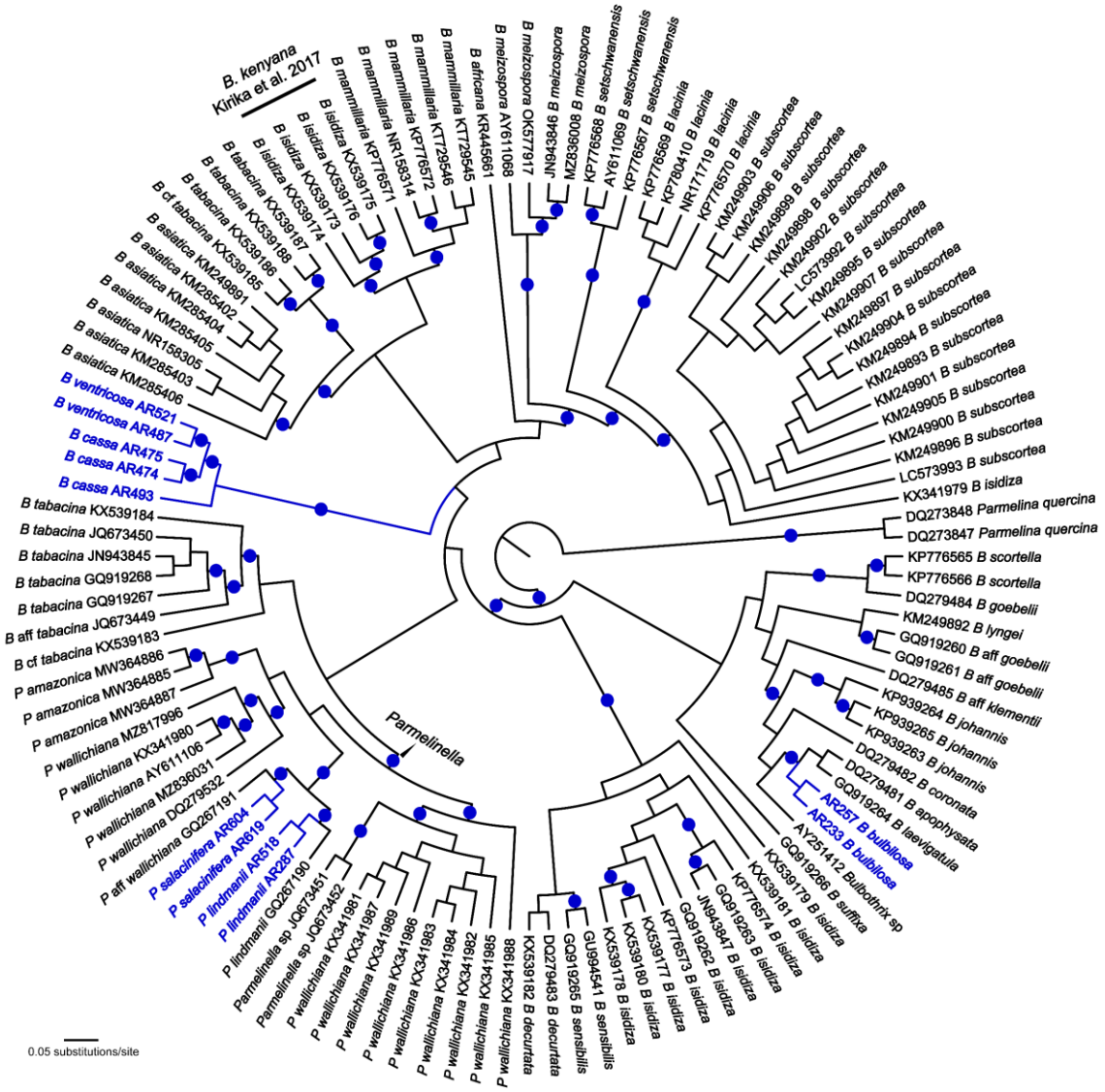
			JQ004658	863	91	97.26	complex	sp.
AR491	sp. nov. 1	no	MZ836018	926	100	96.73	mismatch	*subrudecta*
			GU593038	920	98	97.41	complex	*borreri*
			LC669679	907	99	96.70	mismatch	*borreri*
			AY267010	**856**	**90**	**97.60**	**complex**	***subpraesignis***
			JQ004658	861	91	97.43	complex	sp.
AR495	sp. nov. 1	no	MZ836018	924	99	96.89	mismatch	*subrudecta*
			GU593038	924	98	97.59	complex	*borreri*
			LC669679	911	99	96.88	mismatch	*borreri*
			AY267010	865	90	97.99	complex	*subpraesignis*
			JQ004658	865	91	97.62	complex	sp.
			AY773115	854	90	97.59	complex	*borreri*
AR499	sp. nov. 1	no	MZ836018	933	99	97.09	complex	*subrudecta*
			GU593038	928	97	97.94	complex	*borreri*
			LC669679	915	98	97.22	complex	*borreri*
			AY267010	**869**	**89**	**98.38**	**complex**	***subpraesignis***
			JQ004658	869	90	98.00	complex	sp.
			AY773115	857	89	97.98	complex	*borreri*
AR502	sp. nov. 1	no	MZ836018	948	100	97.12	complex	*subrudecta*
			GU593038	933	97	97.78	complex	*borreri*
			LC669679	920	98	97.07	complex	*borreri*
			AY267010	**874**	**89**	**98.20**	**complex**	***subpraesignis***
			JQ004658	874	91	97.83	complex	sp.
			AY773115	863	89	97.80	complex	*borreri*
AR554	sp. nov. 1	no	MZ836018	941	100	97.10	complex	*subrudecta*
			GU593038	933	97	97.78	complex	*borreri*
			LC669679	920	98	97.07	complex	*borreri*

			AY267010	**874**	**90**	**98.20**	**complex**	***subpraesignis***
			JQ004658	874	91	97.83	complex	sp.
			AY773115	863	90	97.80	complex	*borreri*
AR565	sp. nov. 1	no	MZ836018	946	100	96.96	mismatch	*subrudecta*
			GU593038	939	98	97.63	complex	*borreri*
			LC669679	926	98	96.93	mismatch	*borreri*
			AY267010	**874**	**89**	**98.20**	**complex**	***subpraesignis***
			AY773115	863	89	97.80	complex	*borreri*
			JQ004658	880	91	97.66	complex	sp.
AR572	sp. nov. 1	no	MZ836018	915	100	96.22	mismatch	*subrudecta*
			GU593038	904	97	96.86	mismatch	*borreri*
			LC669679	896	98	96.34	mismatch	*borreri*
			AY267010	**848**	**90**	**97.40**	**complex**	***subpraesignis***
			JQ004658	845	91	96.84	mismatch	sp.
AR609	sp. nov. 1	no	MZ836018	939	100	96.77	mismatch	*subrudecta*
			GU593038	931	98	97.44	complex	*borreri*
			LC669679	918	98	96.74	mismatch	*borreri*
			AY267010	**869**	**89**	**98.00**	**complex**	***subpraesignis***
			AY773115	857	89	97.60	complex	*borreri*
			JQ004658	872	91	97.46	complex	sp.
AR629	sp. nov. 1	no	MZ836018	929	100	96.77	mismatch	*subrudecta*
			GU593038	922	97	97.42	complex	*borreri*
			LC669679	909	98	96.71	mismatch	*borreri*
			AY267010	**865**	**89**	**98.00**	**complex**	***subpraesignis***
			AY773115	854	90	97.60	complex	*borreri*
			JQ004658	863	91	97.44	complex	sp.
AR471	sp. nov. 2	no	MZ836018	972	100	98.19	complex	*subrudecta*

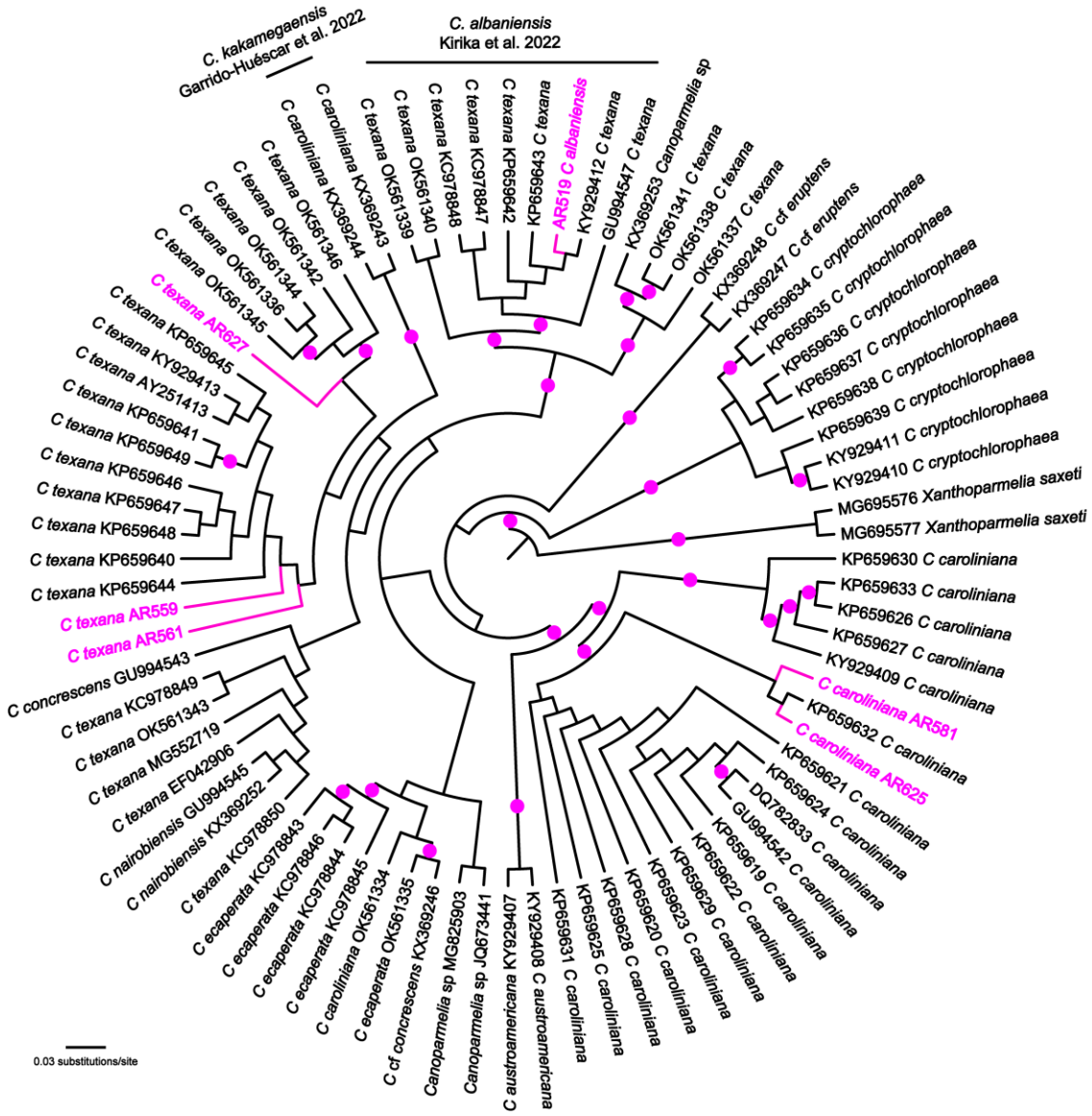
			GU593038	965	98	98.89	complex	*borreri*
			LC669679	952	98	98.17	complex	*borreri*
			JQ004658	**905**	**91**	**99.01**	**potential**	**sp.**
			AY773115	**894**	**90**	**99.00**	**potential**	***borreri***
AR492	sp. nov. 2	no	MZ836018	977	100	98.19	complex	*subrudecta*
			GU593038	968	97	98.89	complex	*borreri*
			LC669679	955	98	98.17	complex	*borreri*
			JQ004658	**909**	**91**	**99.01**	**potential**	**sp.**
			AY773115	**896**	**90**	**99.00**	**potential**	***borreri***
AR550	sp. nov. 2	no	MZ836018	959	100	97.65	complex	*subrudecta*
			GU593038	952	98	98.34	complex	*borreri*
			LC669679	939	98	97.63	complex	*borreri*
			JQ004658	**893**	**91**	**98.43**	**complex**	**sp.**
			AY773115	**880**	**90**	**98.40**	**complex**	***borreri***
AR156	sp. nov. 3	no	**GU384892**	**948**	**95**	**98.69**	**complex**	***missouriensis***
			OK577923	918	95	97.76	complex	*rudecta*
			KR024422	894	94	97.35	complex	*rudecta*
			AY613402	863	89	97.99	complex	aff. *rudecta*
			KM250192	859	88	97.98	complex	*rudecta*
AR574	sp. nov. 3	no	GU384892	948	96	98.69	complex	*missouriensis*
			OK577923	915	95	97.75	complex	*rudecta*
			KR024458	907	92	98.45	complex	*rudecta*
			KR024424	**86**	**86**	**99.16**	**potential**	**aff. *rudecta***
				Relicina				
Query	**MI**	**HS**	**Accession**	**MS**	**QC (%)**	**PI (%)**	**Class.**	**ID**
AR155	*subabstrusa*	yes	LC742649	448	98	82.84	mismatch	*Menegazzia caviisidia*
			GU994581	**448**	**70**	**88.36**	**mismatch**	***sydneyensis***

JX466240	440	98	88.22	mismatch	*Parmelina tiliacea*

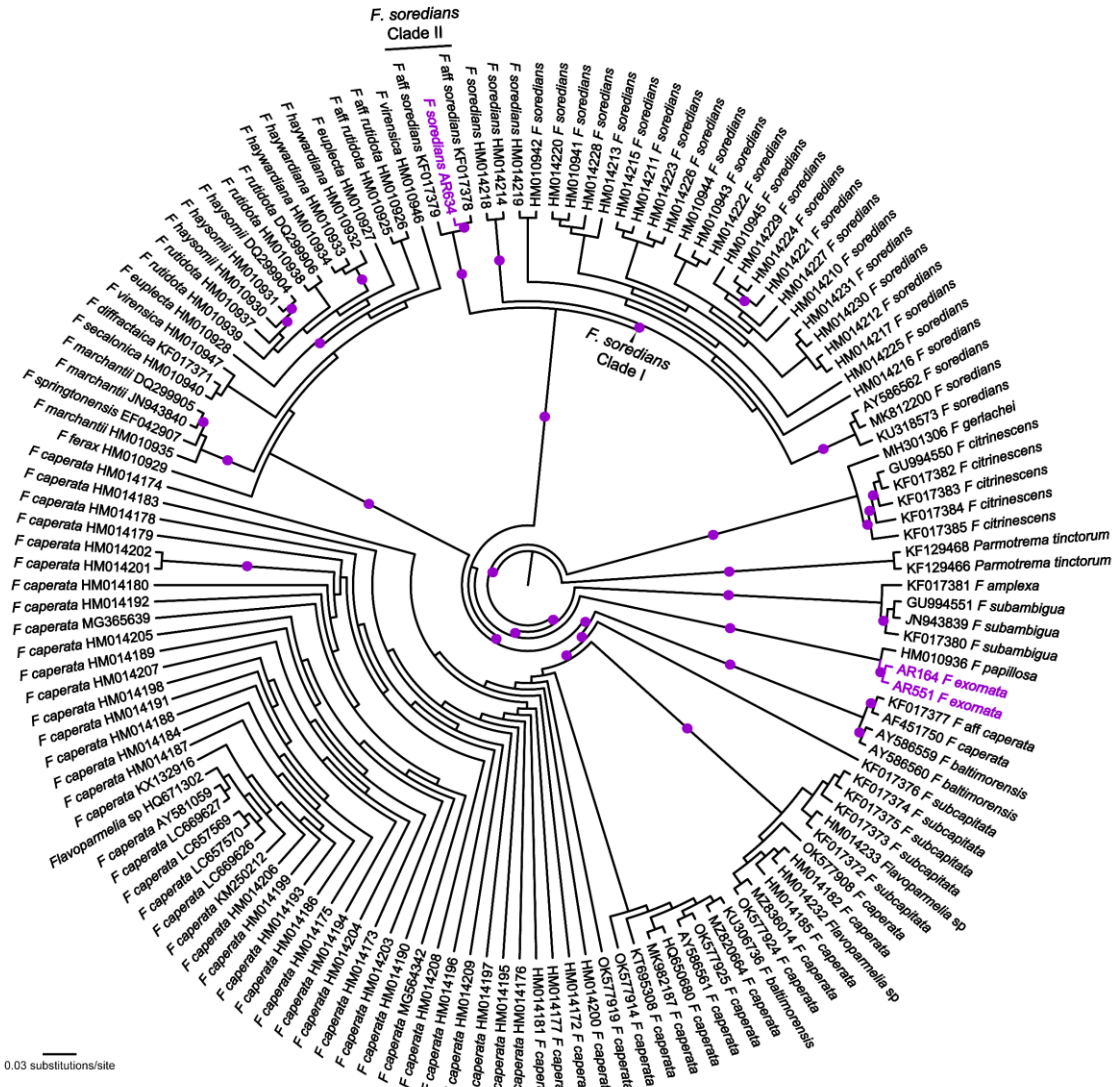
*According to Kirika et al. (2022) the correct identification of the sequences is *Canoparmelia albaniensis*, however in GenBank they are named as *C. texana*.



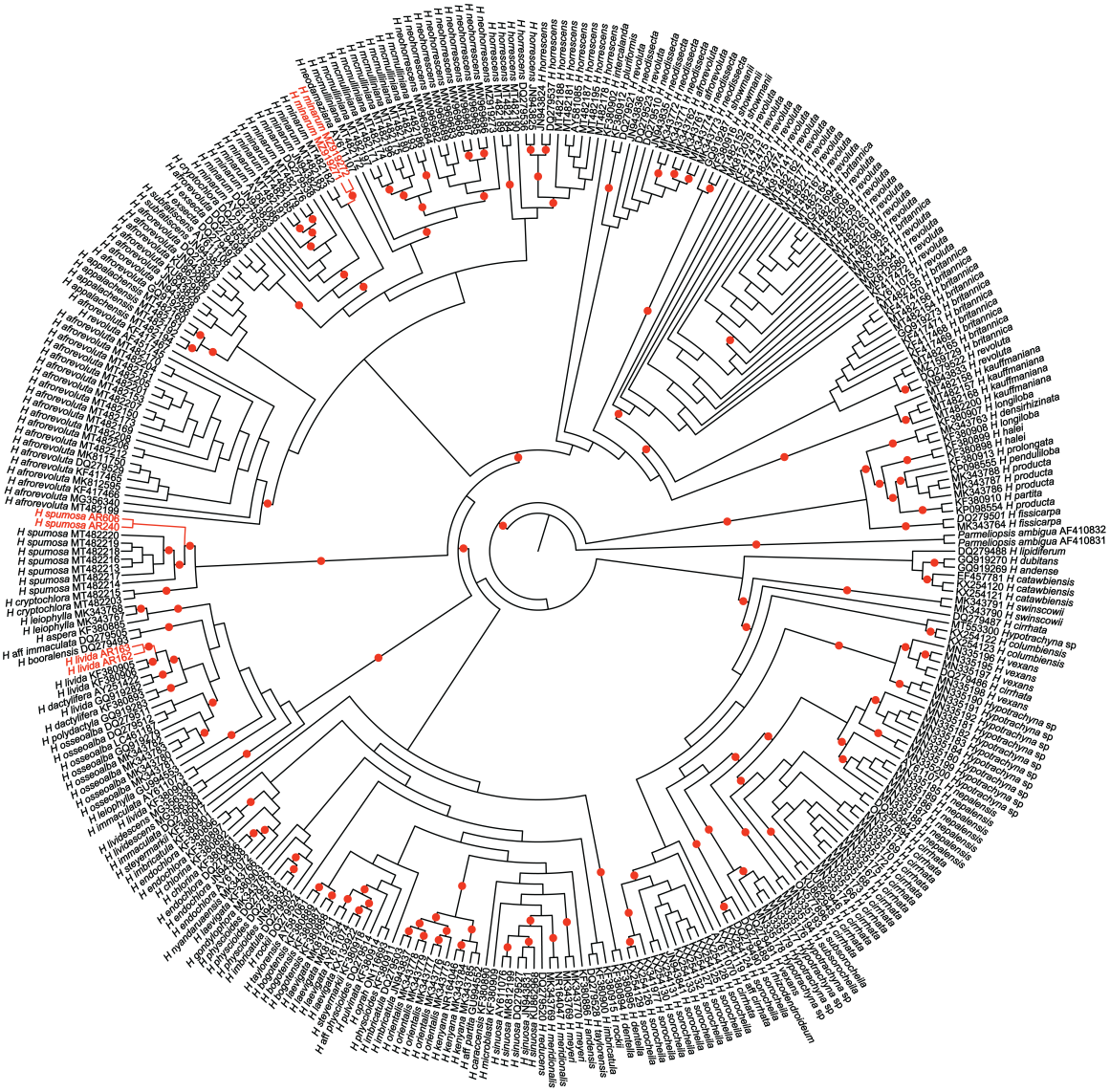
Suplementar Figure 1. Phylogenetic relationships of *Bulbothrix* and *Parmelinella* based on maximum likelihood (ML) from nuITS sequences. Circles on the branches indicate bootstrap values ≥ 70%. Sequence names in blue correspond to those generated in this study.



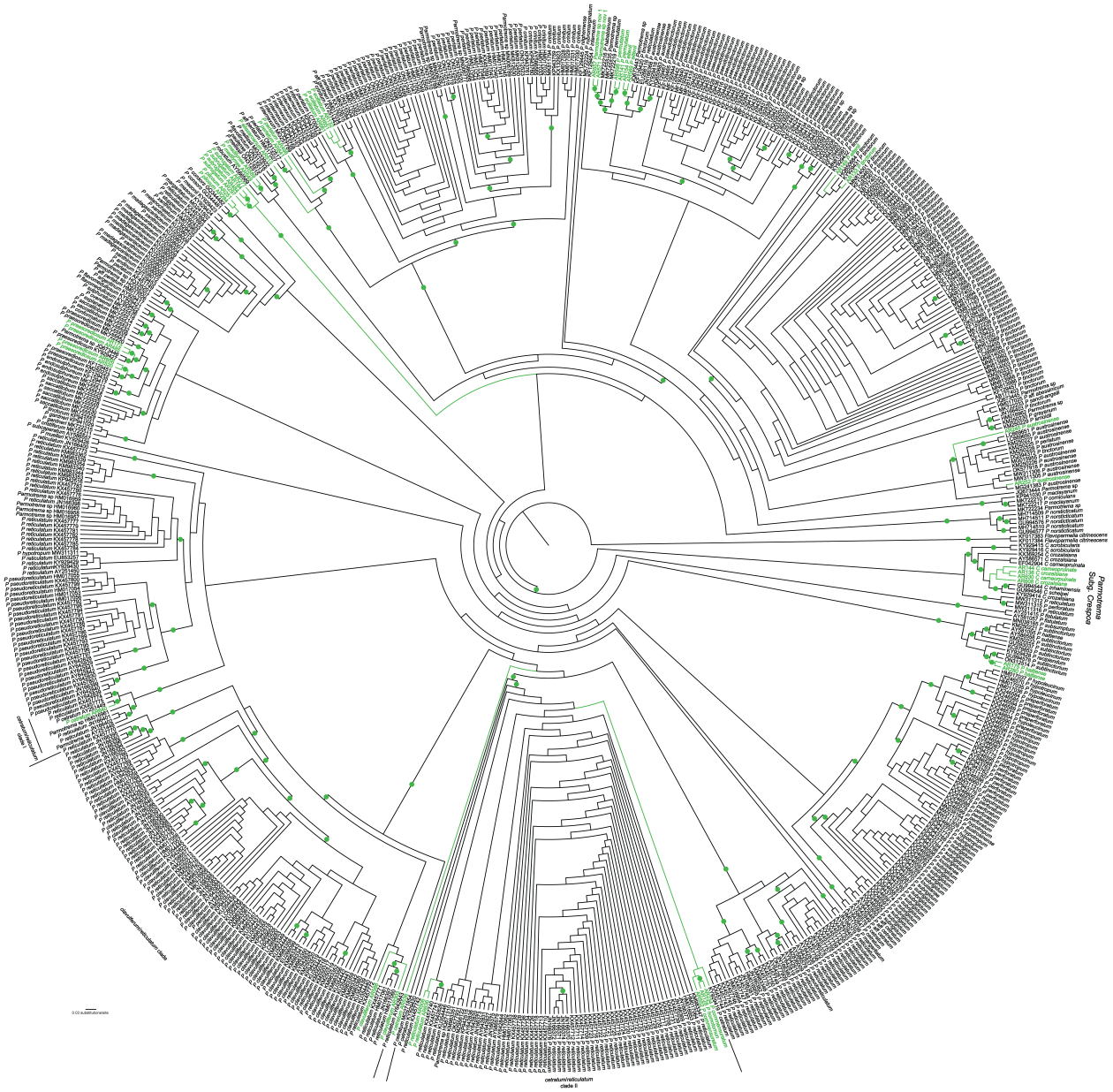
Supplementary Figure 2. Phylogenetic relationships of *Canoparmelia* based on maximum likelihood (ML) from nuITS sequences. Circles on the branches indicate bootstrap values ≥ 70%. Sequence names in pink correspond to those generated in this study.



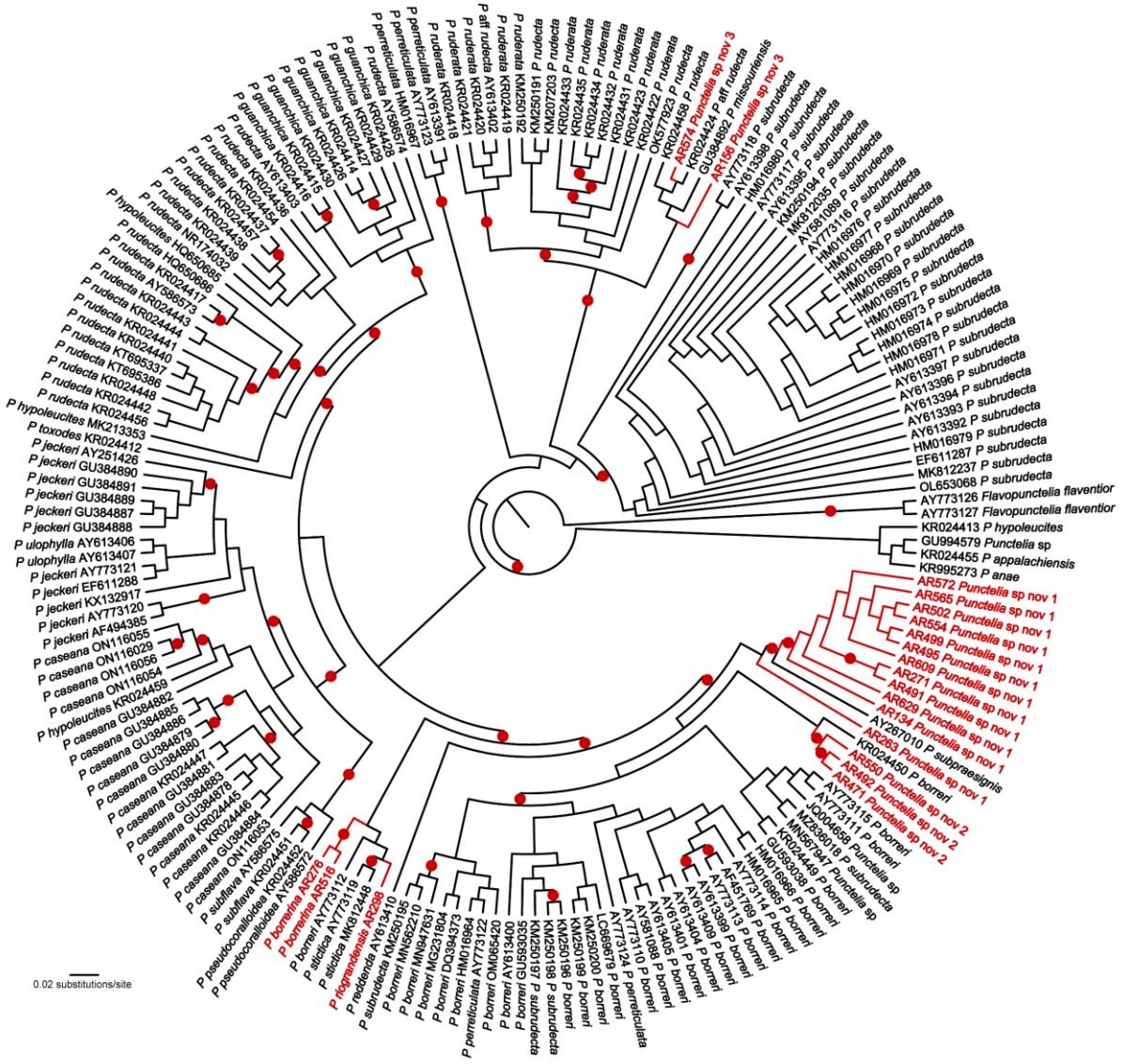
Supplementary Figure 3. Phylogenetic relationships of *Flavoparmelia* based on maximum likelihood (ML) from nuITS sequences. Circles on the branches indicate bootstrap values ≥ 70%. Sequence names in purple correspond to those generated in this study.



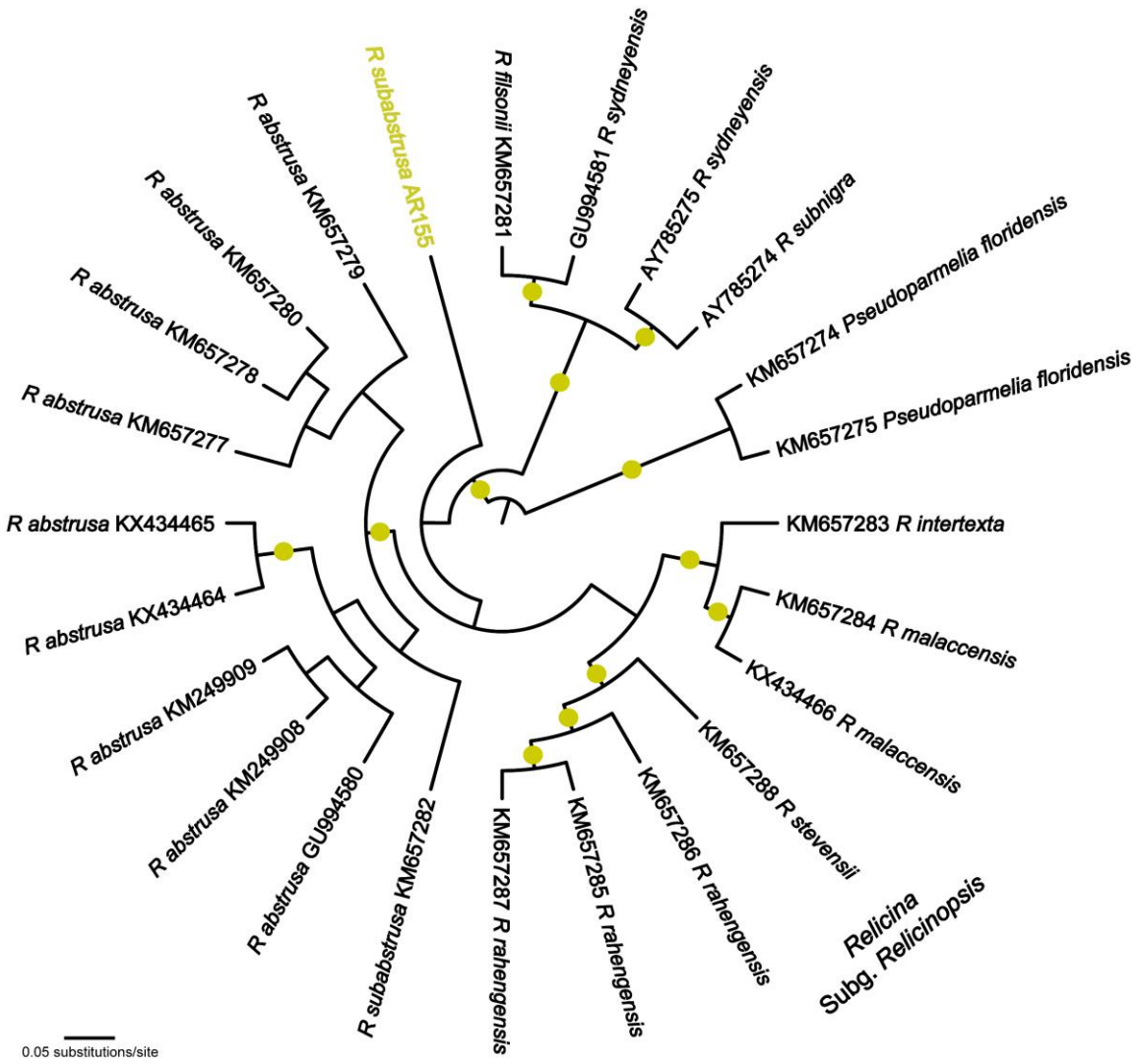
Supplementary Figure 4. Phylogenetic relationships of *Hypotrachyna* based on maximum likelihood (ML) from nuITS sequences. Circles on the branches indicate bootstrap values ≥ 70%. Sequence names in orange correspond to those generated in this study.



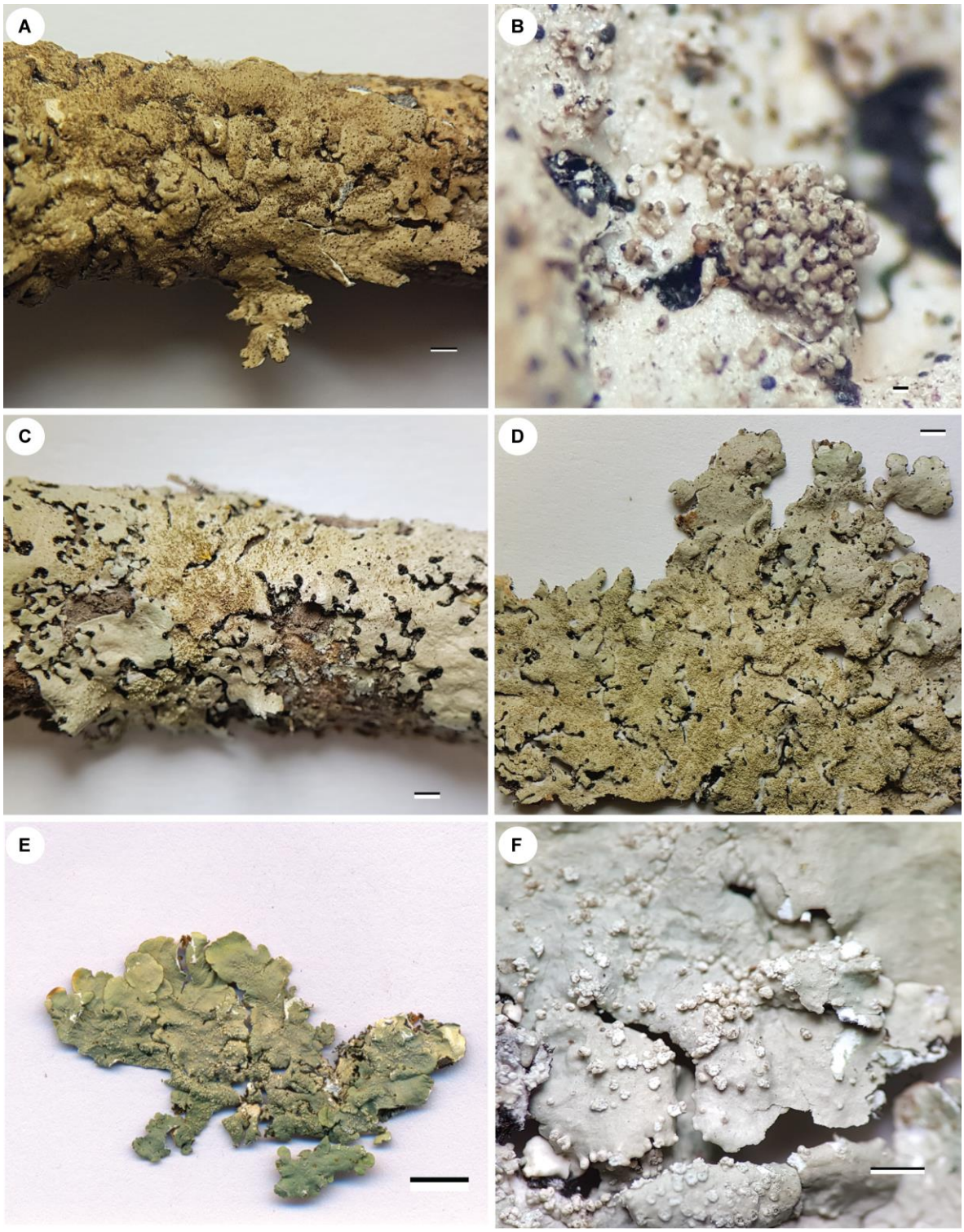
Supplementary Figure 5. Phylogenetic relationships of *Parmotrema* based on maximum likelihood (ML) from nuITS sequences. Circles on the branches indicate bootstrap values ≥ 70%. Sequence names in green correspond to those generated in this study.



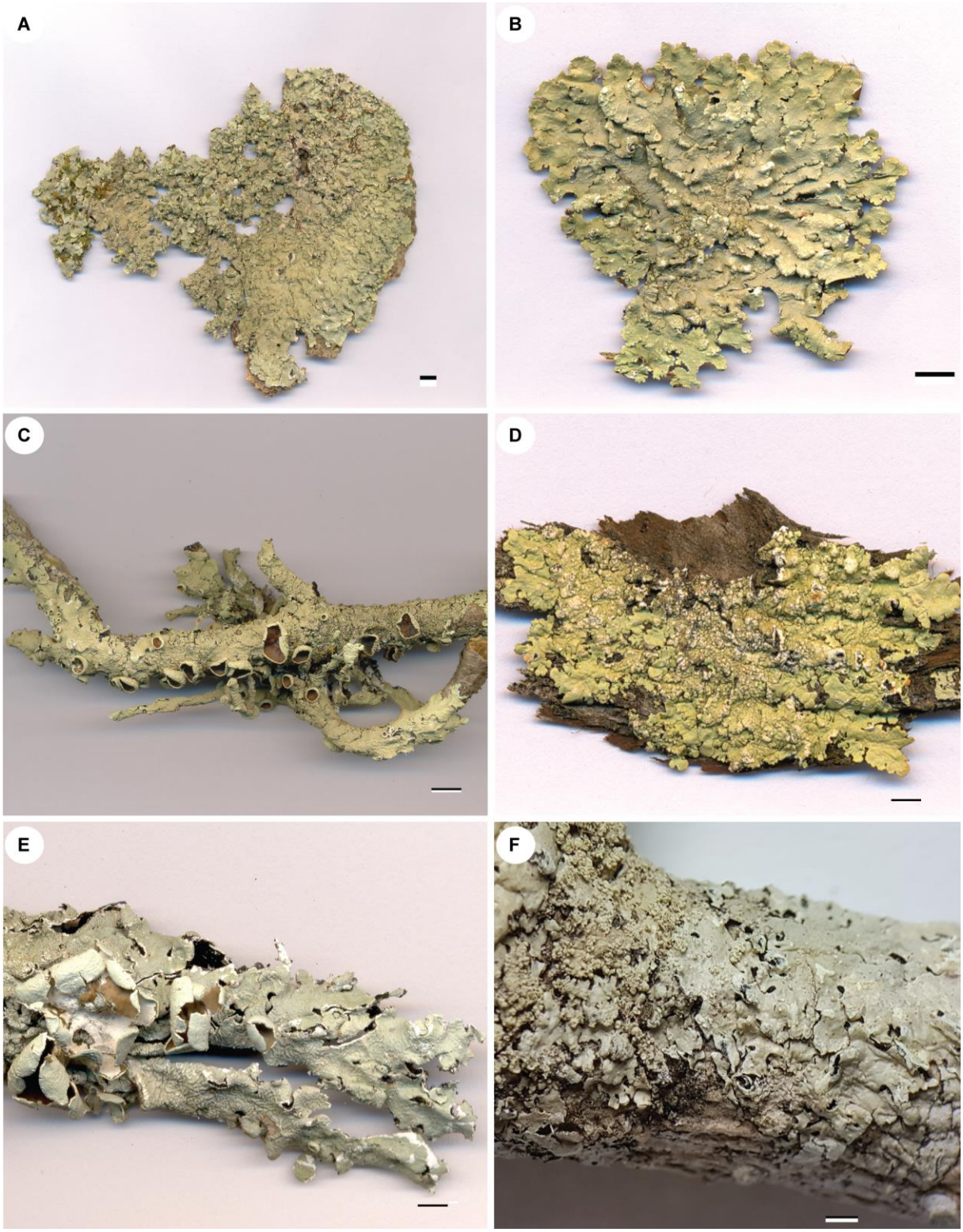
Supplementary Figure 6. Phylogenetic relationships of *Punctelia* based on maximum likelihood (ML) from nuITS sequences. Circles on the branches indicate bootstrap values ≥ 70%. Sequence names in red correspond to those generated in this study.



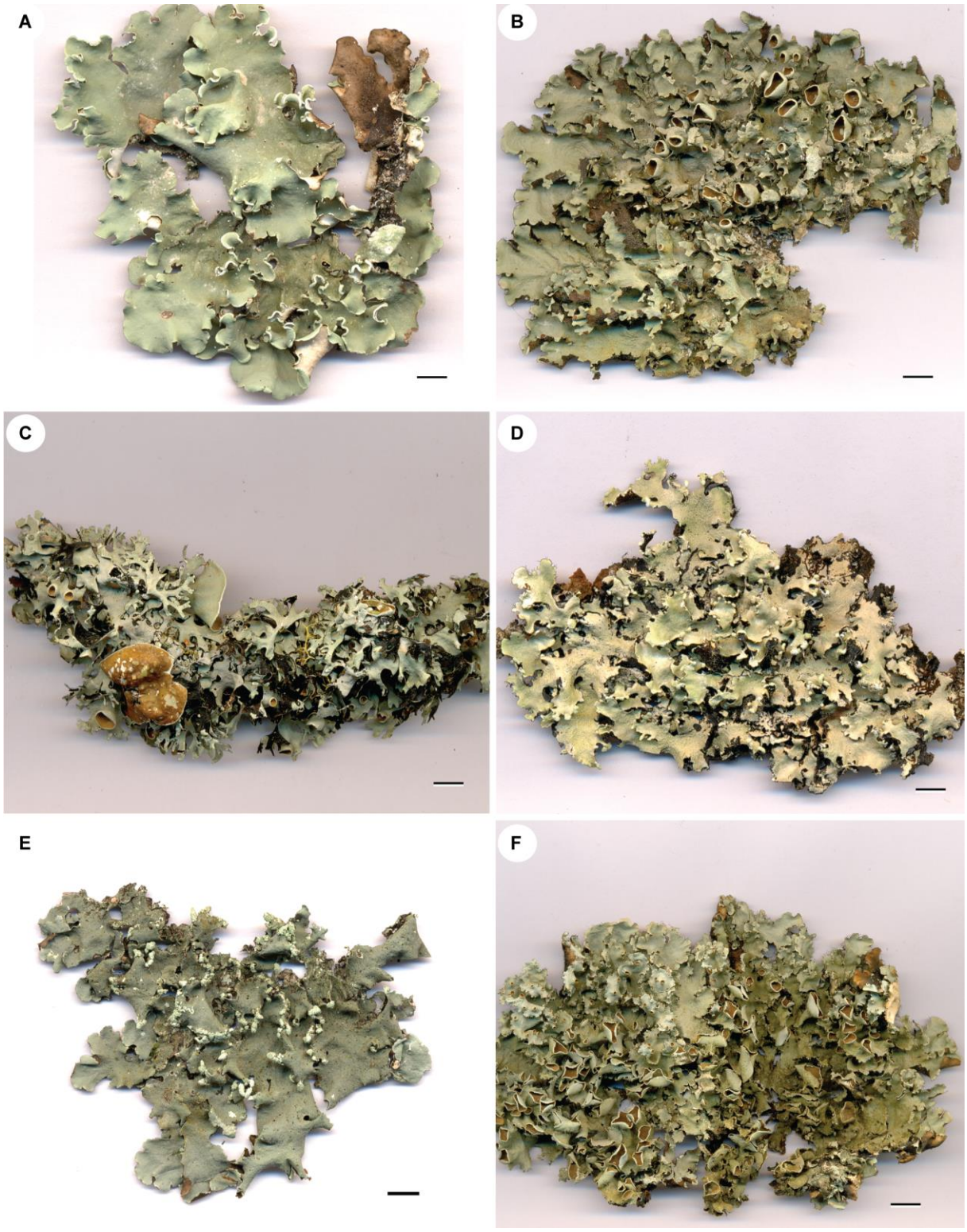
Supplementary Figure 7. Phylogenetic relationships of *Relicina* based on maximum likelihood (ML) from nuITS sequences. Circles on the branches indicate bootstrap values ≥ 70%. Sequence names in yellow correspond to those generated in this study.



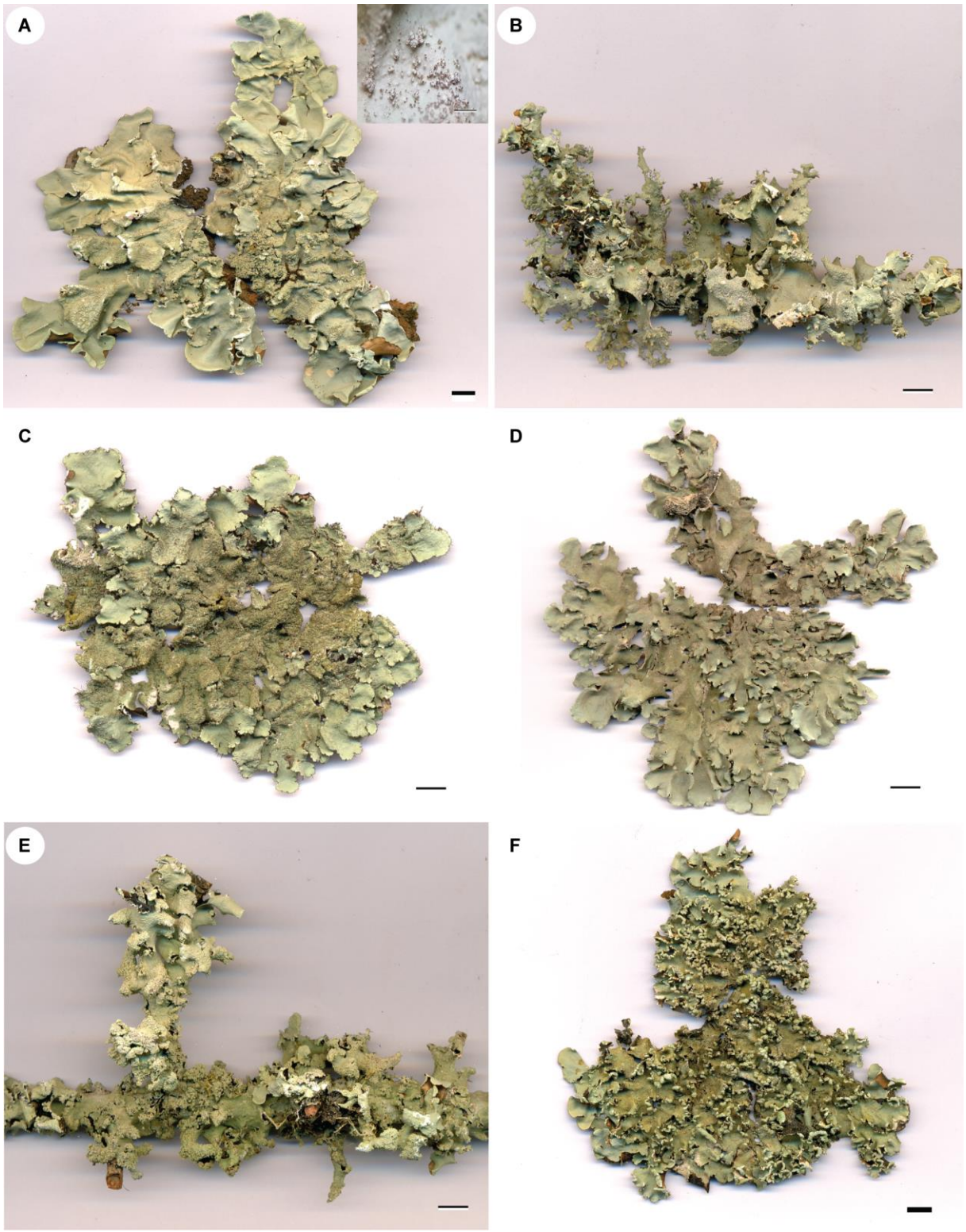
Supplementary Figure 8. *Bulbothrix* and *Canoparmelia* Specimens. A. *Bulbothrix bulbillosa* (AR 233). B. Details of the isidia ornamented with ciliary bulbs of *B. bulbillosa* (AR 233). C. *B. ventricosa* (AR 487). D. *B. cassa* (AR 493). E. *Canoparmelia albaniensis* (AR 519). F = Details of the pustules of the *C. albaniensis* (AR 519). Scales: A = 1 mm; B = 0.02 mm; C & D = 1 mm; E= 5 mm; F = 1mm.



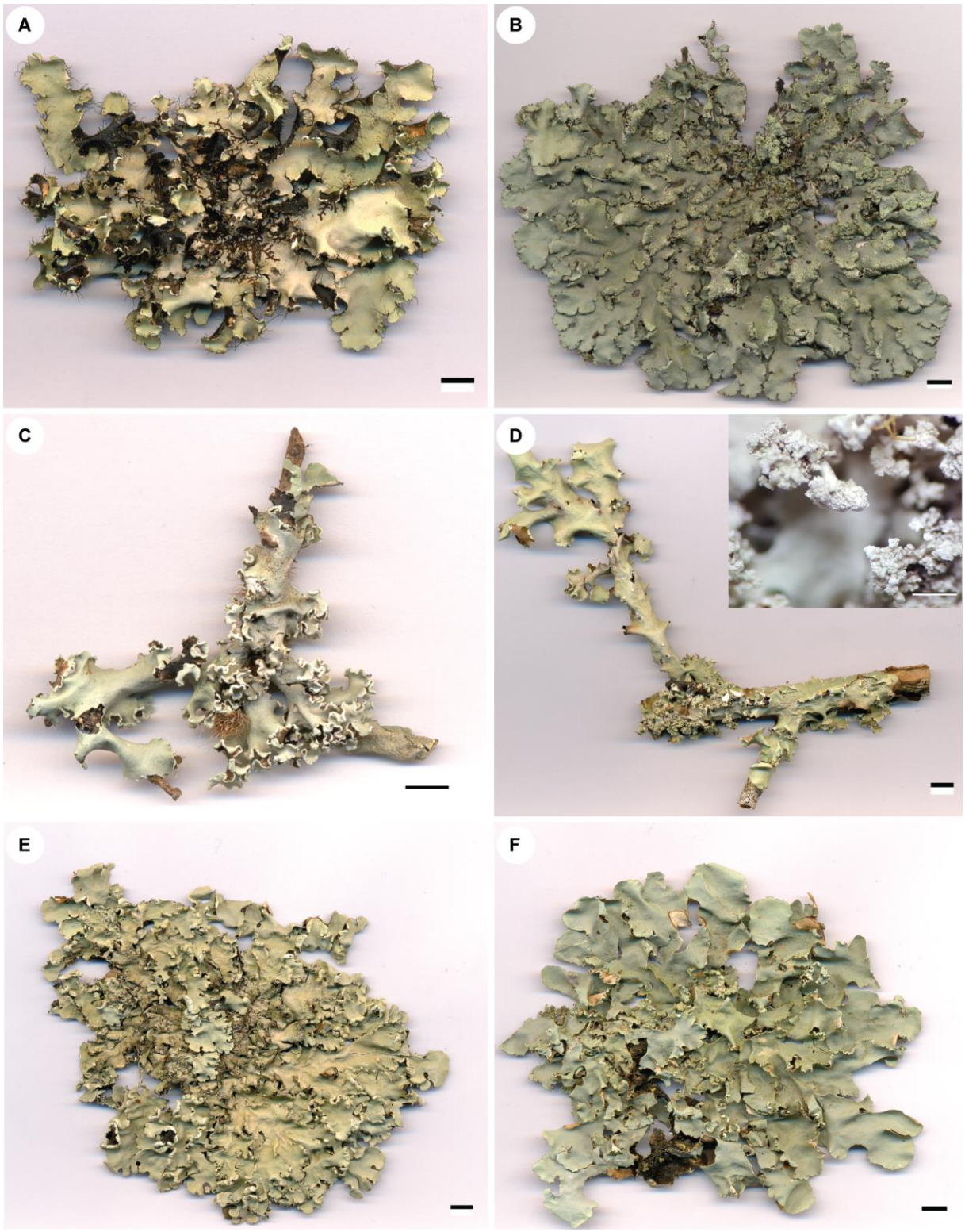
Supplementary Figure 9. Specimens of *Canoparmelia, Flavoparmelia*, and *Hypotrachyna*. A. *Canoparmelia caroliniana* (AR 581). B. *Canoparmelia texana* (AR 559). C. *Flavoparmelia exornata* (AR 551). D. *F. soredians* (AR 634). E. *Hypotrachyna livida* (AR 163). F = *H. spumosa* (AR 240). Scales: A - E= 5 mm; F = 1mm.



Supplementary Figure 10. *Parmotrema* Specimens. A. *Parmotrema austrosinense* (AR 245). B. *P. cetratum* (AR 640). C. *P. cetratum* (AR 536). D. *P. clavuliferum* (AR 588). E. *P. commensuratum* (AR 234). F = *P. eciliatum* (AR 651). Scales: 5 mm.



Supplementary Figure 11. *Parmotrema* Specimens. A. *Parmotrema eitenii* – above the image in detail of the soredioid isidia (AR 569). B. *P. eliasaroanum* (AR 513). C. *P. haitiense* (AR 280). D. *P. homotomun* (AR 128). E. *P. madilynae* (AR 549). F = *P. perlatum* (AR 641). Scales: 5 mm; A = detail photo 1 mm.



Supplementary Figure 12. *Parmotrema* Specimens. A. *Parmotrema permutatum* (AR 598). B. *P. pilosum* (AR 251). C. *P. praesorediosum* (AR 172). D. Specimen of *P. praesorediosum* with stipitate soralia shown in detail in the accompanying figure (AR 656) E. *P. reticulatum* (AR 636). F = *Parmotrema* sp. nov. 1 (AR 571). Scales: 5 mm; A = detail photo 1 mm.

Supplementary Figure 13. *Parmotrema* and *Punctelia* Specimens. A. *Parmotrema subrugatum* (AR 297). B. *P. tinctorum* (AR 133). C. *Crespoa carneopruinata* (AR 144). D. *C. crozalsiana* (AR 136) E. *Punctelia borrerina* (AR 276). F = *P. riograndensis* (AR 298). Scales: 5 mm; C = each line is 1 mm.

Supplementary Figure 14. *Punctelia* and *Relicina* Specimens. A. *Punctelia* sp. nov. 1 - phillidia shown in detail in the accompanying image (AR 263). B. *Punctelia* sp. nov. 3 (AR 156). C. *Relicina subastrusa* (AR 155). Scales: 5 mm; A = detail photo 1 mm.

Conclusões Finais

Rearranjos taxonômicos em *Parmeliaceae* baseado em marcadores moleculares

Com os avanços na identificação molecular nos fungos liquenizados, normalmente baseado em reconstruções filogenéticas, diversos grupos em *Parmeliaceae* foram reconhecidos e/ou reposicionados taxonomicamente. Essa crescente, impulsionada, principalmente a partir do início dos anos 2000, abriu novas fronteiras para novas e distintas abordagens na identificação taxonômica. Os marcadores nuITS, nuLSU e mtSSU foram os principais utilizados nos primeiros trabalhos, e continuam sendo até o presente momento amplamente empregados em estudos de taxonomia molecular, evolução, filogeografia, entre outros. A revolução da abordagem molecular em *Parmeliaceae* mostrou que alguns gêneros com circunscrições fenotípicas delimitadas pertenciam ao mesmo nível genérico, como nos casos de *Bulborrhizina* em *Bulbothrix* [87]; *Hypotrachyna* – contendo atualmente em nível de subgêneros *Cetrariastrum*, *Everniastrum*, *Longilobae*, *Parmelinopsis* e *Sinuosae* [79]; *Parmotrema* – englobando *Concamerella*, *Canomaculina*, *Crespoa* e *Rimelia* [81]; *Relicinopsis* dentro de *Relicina* [82]; *Xhantoparmelia* – atualmente contendo *Almbornia, Chrondropsis, Karoowia, Namakwa, Neofuscelia, Omphalodiella, Paraparmelia e Xanthomaculina* [80, 84], entre outros. Além disso, novos gêneros foram segregados a partir de reconstruções filogenéticas, como *Austroparmelina* [88], *Melanelixia* e *Melanohalea* [89], *Remotrachyna* [83], entre outros.

A taxonomia molecular permitiu que muitas espécies também tivessem novos rearranjos taxonômicos, como foi o caso de *Canopamelia scrobicularis* em *Crespoa* (atualmente *Parmotrema*) [90], *Canoparmelia albaniensis* segregada de *C. texana* [91]; *Cetraria annae* Oxner atualmente reconhecida em *Nephromopsis* [92]; *Maronina saxicola* e *M. rogersii* em *Neoprotoparmelia* [93]; bem como no atual estudo - *Canoparmelia amazonica* e *Myelochroa lindmanii* em *Parmelinella* Elix & Hale. Utilizamos a filogenia molecular (baseado no *DNA barcoding* - nuITS), juntamente com os dados morfológicos e químicos, confirmamos o posicionamento das espécies e propomos a combinação dessas no seu respectivo gênero. Além de confirmar que *P. salacinifera* (Hale) Marcelli & Benatti se tratava de uma espécie distinta de *P. wallichiana* (Taylor) Elix & Hale. Dentro desse grupo o marcador nuITS obteve boa resolução no posicionamento a nível genérico dessas duas espécies, mesmo que ainda hajam desafios a serem resolvidos em algumas espécies do gênero, bem como entre as relações filogenéticas ainda incertas de *Parmelinella* e *Bulbotrix* s.l., como já evidenciado em estudos

anteriores [11]. Baseado em nossos resultados, assim como nos mencionados acima, podemos inferir que *Parmelinella* engloba variações fenotípicas mais amplas do que se imaginava, mostrando a necessidade de um estudo integrativo mais aprofundado para que sejam encontradas quais características realmente delimitam o gênero.

DNA barcoding de *Parmeliaceae* e a descoberta de novas espécies

Propostas de novas espécies em *Parmeliaceae* baseadas em características fenotípicas são comumente encontradas, e por muito tempo foi a principal ferramenta para identificação e delimitação de espécies [76, 94, 95, 96]. Visto que ainda hoje a taxonomia morfológica é amplamente utilizada e necessária, a identificação molecular tem sido uma fonte de dados imprescindível para a descoberta de novas espécies e tem contribuído enormemente para a compreensão da diversidade da família [97, 98, 99]. Estudos moleculares desempenham um papel fundamental também na descoberta de espécies que acabam passando despercebidas quando é utilizado apenas a taxonomia morfológica, como é o caso das espécies crípticas e dos complexos de espécies, por exemplo.

Espécies crípticas, ou seja, espécies que apresentam características morfológicas muito semelhantes e indiferenciáveis, mas alta divergência genética, não são situações incomuns na família [100, 101, 102]. Não distante, nosso estudo encontrou que *Hypotrachyna neohorrescens*, espécie nova aqui descrita é críptica com *H. horrescens*, já que não foi possível encontrar diferenças morfológicas e químicas quando comparada ao lectótipo de *H. horrescens*, embora ambas espécies apresentem grande variação genética, baseado nas sequências disponíveis do GenBank. Em contrapartida, *Hypotrachyna neohorrescens*, utilizando apenas o marcador nuITS, não foi possível uma resolução nítida na delimitação interespecífica com *H. mcmulliniana*. O resultado do BLASTn entre o holótipo de *H. neohorrescens* com sequências de *H. mcmulliniana* mostrou porcentagem de identidade de cerca de 97,5%, ou seja, valor dentro de um possível complexo de espécies. Todavia, utilizando os marcadores nuITS e mtSSU concatenados, ambas as espécies foram recuperadas com monofilia recíproca.

Outro caso facilmente encontrado com a taxonomia molecular são os complexos de espécies. De forma geral, complexos de espécies são grupos intimamente relacionados, que geralmente apresentam pouca diferença fenotípica e/ou genética, assim sendo dificilmente delimitados. Em nosso estudo utilizando o *DNA barcoding* para a identificação específica, encontramos pelo menos 22 casos de possíveis complexos de espécies, sendo recuperadas com espécies morfologicamente distintas e com valores de similaridade genética da região nuITS

variando entre 97-98%, limiar nesse estudo adaptado de trabalhos anteriores, mas normalmente utilizado para designar complexo de espécies [21, 22]. Cenários semelhantes com liquens tem sido evidenciado em estudos recentes contendo abordagens similares, como Leavitt et al. [22], encontrando cerca 1/3 das candidatas a espécies pertencendo a complexos de espécies; Moncada et al. [21] que também enfrentaram o desafio de testar a identificação através do *DNA barcoding* espécies de *Usnea* da Colômbia, deparando-se também com vários casos de complexo de espécies.

Os complexos de espécies em nosso estudo, retrataram três grupos não solucionados utilizando apenas o *DNA barcoding*, corroborando estudos anteriores que já apontaram a falta de resolução do marcador nessas situações [24]. Diante disso, uma alternativa encontrada para a delimitação específica de *H. neorroescens* e *H. mcmulliniana* em nosso estudo foi usar a taxonomia integrativa baseada em três critérios: filogenia molecular utilizando dois marcadores (nuITS + mtSSU), variações morfológicas e distribuição geográfica. Assim, nos casos mencionados acima faz-se necessário estudar caso a caso e aumentar o número de dados analisados para verificar se essas espécies pertencem a uma única espécie com alta plasticidade fenotípica, ou realmente são espécies distintas, mas necessitam de mais marcadores para verificar a sua posição filogenética.

DNA barcoding de fungos liquenizados e levantamentos de diversidade

O *DNA barcoding* foi proposto como uma ferramenta para a identificação rápida e universal dos táxons, mostrando grande importância principalmente em estudos de levantamentos florísticos [15]. Sua utilização nesse contexto tem demostrado altas taxas de sucesso quando aplicado em locais com conhecimento estabelecido da sua biodiversidade, e contendo bancos de dados acurados, como na Grã-Bretanha e Irlanda que com a utilização do *DNA barcoding* em *Usnea* obteve-se taxas de identificação específica correta de 96% [17]. Divakar et al. [18] também obtiveram alta eficiência na identificação das espécies de *Parmelia* s.str. a partir da geração de um banco de dados com sequências acuradas de várias regiões do mundo, assim como Leavitt et al. [103] com espécies de parmélias marrons da Groenlândia.

Em contrapartida, quando o *DNA barcoding* tem sido aplicado em locais megadiveros e com baixo conhecimento da biodiversidade local, a realidade muda de contexto, e geralmente a abordagem apresenta baixa precisão na identificação em nível de espécie. Orock et al. [20] mostrou que as taxas de sucesso na identificação específica utilizando o BLASTn espécimes de

liquens coletas na República de Camarões mostraram que apenas 15% das amostras apresentaram valores de identidade ≥ 97%. Em espécies de *Usnea* da Colômbia, Moncada et al. [21] conseguiram identificar apenas 7% dos espécimes utilizando o BLASTn com sequências disponíveis do GenBank. Já para os liquens alpinos de uma região do Colorado (EUA) baseado em um amplo conjunto de dados, Leavitt et al. [22] encontraram que 68% das sequências geradas não tiveram confiança estatística para confirmação em nível de espécie. Nosso estudo, também realizado em um país megadiverso, e com poucas sequências representadas nos bancos de dados públicos, encontrou resultados semelhantes aos demais. Entre as 39 morfoespécies de fungos liquenizados identificadas, apenas 10 (25,6%) obtiveram correspondência específica entre as abordagens utilizadas (morfológica e molecular). Apenas seis, das 17 morfoespécies sem sequências homônimas foram confirmadas como distintas das sequências disponíveis do GenBank ou do próprio conjunto de dados gerado nesse estudo, sendo então os primeiros registros das espécies no banco de dados. Enquanto 22 morfoespécies mostram estar dentro de complexos de espécies, como apresentado no tópico anterior.

Algumas alternativas frente a esses cenários têm se mostrado promissoras, como é o caso da taxonomia integrativa, que visa utilizar diferentes fontes de dados para a identificação/delimitação acurada dos táxons [104]. Além de evidenciar incongruências intra e interespecíficas para que mais estudos possam ser desenvolvidos no intuito de buscar compreender e solucionar esses casos, como ocorreu com *Hypotrachyna neohorrescens*, *H. mcmulliniana* e *H. horrescens*. Essas foram classificadas como espécies distintas baseado na taxonomia integrativa, caso contrário, provavelmente, *H. neohorrescens* seria tratada como um complexo de espécies. Outra estratégia que tem se mostrado efetiva na melhora da taxa de sucesso do *DNA barcoding* no contexto de levamento de biodiversidade baseia-se na criação e utilização de bibliotecas barcode de sequências referências regionais [105]. Utilizando um banco de dados de *DNA barcoding* regional, Kerr & Leavitt [105] obtiveram taxas de identificações específicas acima de 70%, contrastando com aquelas obtidas a partir de banco de dados públicos que ficaram em torno de 30%. Essa alternativa também visa diminuir as taxas de erro nas identificações de sequências quando utilizamos como base de dados apenas aquelas depositadas em banco de dados públicos, uma vez que a falta de confiabilidade nessas sequências por muitas vezes dificulta a identificação molecular das espécies.

Por fim, de acordo com os resultados obtidos nos três capítulos, podemos inferir de modo geral que:

- O marcador nuITS demostra boa resolução em táxons com alta divergência genética, como foram os casos de *Parmelinella amazonica* e *P. lindmanii*, anteriormente inseridas em outros gêneros (*Canoparmelia* e *Myelochroa*, respectivamente). Mas em gêneros ou espécies com relações muito próximas o marcador sozinho não é efetivo para a identificação ou delimitação precisa dos táxons, como nos casos de *Hypotrachyna neohorrescens* e os demais complexos de espécies encontrados no estudo.
- A utilização da abordagem integrativa é fundamental para a identificação/delimitação das espécies, assim como no caso de complexos de espécies e espécies crípticas. Além de fornecer maior confiança nas identificações inferidas e fornecimento de sequências acuradas.
- A criação de bibliotecas de sequências barcodes regionais tem se mostrado uma boa estratégia para as identificações moleculares. Desta forma, sendo o Brasil um país megadiverso, mas pobremente representado em bancos de dados moleculares públicos, esse método pode ser aliado dos processos taxonômicos e levantamentos florísticos desenvolvidos no país.

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