



Universidade de Brasília

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

DEPARTAMENTO DE BOTÂNICA

MESTRADO EM BOTÂNICA

**Filogenia molecular do gênero *Hymenoloma* Dusén
(Bryophyta) na Antártica**

Orientador: Prof. Dr. Paulo Eduardo A. S. Câmara PhD

Orientanda: Crislielly de Oliveira Silva Machado

Brasília

Novembro de 2022



Universidade de Brasília

INSTITUTO DE CIÊNCIAS BIOLÓGICAS

DEPARTAMENTO DE BOTÂNICA

MESTRADO EM BOTÂNICA

**Filogenia molecular do gênero *Hymenoloma* Dusén
(Bryophyta) na Antártica**

Dissertação apresentada ao
Programa de Pós-Graduação em
Botânica como parte dos requisitos
para a obtenção do título de
Mestre em Botânica da
Universidade de Brasília (UnB).

Orientador: Prof. Dr. Paulo Eduardo A. S. Câmara

Orientanda: Crislielly de Oliveira Silva Machado

Brasília

2022

Agradecimentos

Primeiramente gostaria de agradecer aos programas de fomento desse trabalho. À CAPES pela bolsa de mestrado concedida, ao Programa Antártico Brasileiro por financiar essa pesquisa e à Universidade de Brasília por custear uma parte da pesquisa, além de ter propiciado em sua instituição a realização desse trabalho.

Agradeço imensamente ao meu orientador Paulo Câmara, que desde nosso primeiro contato foi muito prestativo e amável. Obrigada por ter me dado essa grandiosa possibilidade de trabalhar com você e com as briófitas, por ter sido um mestre, professor e orientador tão gentil e amigo, por ter me passado tanto conhecimento. Te levarei para a vida! Obrigada.

Não poderia deixar de agradecer também às minhas amigas de laboratório, Tamara Poliana e Júlia Mundim. Foram elas que me ensinaram a parte molecular, sem elas não sei se teria conseguido tantos resultados bons. Além disso agradeço pela amizade que deixava todos os perrengues do mestrado mais leves. Amo vocês.

Agradeço imensamente à professora Micheline, esposa do Paulo, que tanto me acolheu e me ajudou em diversas vezes. Obrigada.

Além de todas essas pessoas ilustres, não poderia esquecer dos colegas de laboratório: Pikachu (Diego), Alan, Dafne, Bárbara e Amanda, dos colegas de mestrado: Thaís, Fernanda, Tayara e Rayane, e da minha estagiária Vitória, que fizeram tanta diferença nessa pós graduação, obrigada a todos por entrarem na minha vida.

E por último, gostaria de agradecer à minha família, que tanto me ajudou na pandemia, nos momentos difíceis, vocês são meus pilares. Obrigada.

Lista de Figuras e Tabelas

Introdução geral	7
Tabela 1. Classificações de <i>Hymenoloma</i> Dusén encontradas na literatura.....	9
Molecular phylogeny of <i>Hymenoloma</i> Dusén (Bryophyta) in Antarctica	13
Table 1. Samples of <i>Hymenoloma</i> used for molecular analysis in this study and herbarium where the plants are deposited with their respective codes: CH: Chile; KG: King George Island; LV: Livingston Island; (NY) New York Botanical Garden and (UB) University of Brasília.....	27
Table 2. Primers used in this study.....	28
Figure 1. Figure 1. Phylogram from <i>Hymenoloma</i> species obtained from all used markers (<i>rps4 trnL-F</i> and <i>nad5</i>). Ns above branches are Bayesian posterior probability, bootstrap for maximum parsimony and maximum likelihood, respectively. The (-) indicates no support and MAX indicates maximum support. Names in bold indicate <i>Hymenoloma</i> species. AUT: Austria, CHI: Chile, ESP: Span, KG: King George Island, KAZ: Kazakhstan, LV: Livingston Island and RUS: Russia.....	29
Figure 2. Figure 2. Phylogram from <i>Hymenoloma</i> species obtained from all used markers (<i>rps4 trnL-F</i> and <i>nad5</i>) with the names proposed in this study. Ns above branches are Bayesian posterior probability, bootstrap for maximum parsimony and maximum likelihood, respectively. The (-) indicates no support and MAX indicates maximum support. Names in bold indicate <i>Hymenoloma</i> species. AUT: Austria, CHI: Chile, ESP: Span, KG: King George Island, KAZ: Kazakhstan, LV: Livingston Island and RUS: Russia.....	30
Figure 3. Figure 3. Electro micrography and photomicrography of <i>Hymenoloma</i> specimens from Antarctica and Chile. A, B, C and E: <i>Hymenoloma grimmiaceum</i> , D: <i>Hymenoloma</i> sp. from Livingston, Chile.....	31
Figure 4. Figure 4. Photomicrography of <i>Hymenoloma</i> specie from Chile.....	32

Sumário

Agradecimentos	3
Organização da dissertação.....	7
Introdução geral.....	8
Referências.....	11
Molecular phylogeny of <i>Hymenoloma</i> Dusén (Bryophyta) in Antarctica.....	14
Introduction	15
Material and methods	18
Results	19
Discussion	20
Conclusions	23
Acknowledgements	23
References	23
Table 1.....	27
Table 2.....	28
Figure 1	29
Figure 2	30
Figure 3	31
Figure 4	32
Considerações finais.....	33

Organização da dissertação

A presente dissertação possui uma introdução geral (seguindo as normas do periódico *Acta Botanica Brasilica*), para melhor entendimento dos temas abordados e problemas ao qual o estudo visa resolver, seguido de um capítulo em formato de artigo.

O capítulo contém o trabalho feito sobre a filogenia do grupo de plantas estudado. Esse primeiro artigo pretende-se publicar na revista *Jornal of Bryology* e por isso seguirá a formatação sugerida pela revista.

Por fim, a dissertação é encerrada com as considerações finais (seguindo à *Acta Botanica Brasilica*).

Introdução geral

Descoberto no século XIX por exploradores franceses, russos e britânicos, o continente Antártico é o mais frio, mais seco, mais alto, mais ventoso, mais desconhecido e o mais preservado de todos (Rives 1988; Machado & Brito 2006; Walton 2013). Esse vasto continente começou a se separar de Gondwana no Mesozóico (c.a. 250 ma), e, devido à corrente circumpolar que se formou há 30 milhões de anos (onde a água do oceano quente vinda de latitudes mais baixas ficou isolada) houve declínio gradual das temperaturas locais e o consequente acúmulo de neve e gelo, que hoje se estende em até 4 km desde a superfície terrestre até o platô (Thomas *et al.* 2008; Simões *et al.* 2011; Salleh 2015; Stevenson 2018). Toda a área do planeta acima do paralelo 60° S é considerada Antártica (Capozoli 2001; Câmara & Carvalho-Silva 2020), o qual é dividido geralmente em três zonas biogeográficas terrestres: Subantártica, Antártica Marítima e Antártica Continental (Simões *et al.* 2011). A zona Subantártica fica ao norte do continente congelado, compreende desde o paralelo 40°S até o 60°S (Simões 2011; Leppe *et al.* 2010). Já a zona da Antártica Marítima compreende as ilhas que ficam acima do paralelo 60°S até cerca de 70°S, no continente antártico (Ochyra *et al.* 2008). E a Antártica Continental, como o próprio nome sugere, é o respectivo continente circundado pelo Oceano Austral (ou Oceano Antártico) (Machado & Brito 2006). Estudos revelam que a península Antártica é a área que mais aquece no planeta Terra, promovendo áreas de degelo, cujo ambiente se torna promissor para o estabelecimento de plantas (Turner *et al.* 2005; 2009; Venables & Meredith 2014; Hahn-Woernle 2020).

Quando ainda estava unida à Gondwana, a Antártica compartilhava a flora presente nesse supercontinente (Boger 2011). Após se separar, acreditou-se que a última glaciação, ocorrida há ~10 mil anos, teria dizimado toda a flora Antártica (Biersma *et al.* 2018). Assim, toda a vegetação presente ali teria surgido após o término da glaciação. Entretanto, dados moleculares têm mostrado que algumas plantas se refugiaram em terrenos com atividade vulcânica, conseguindo assim sobreviver às adversidades da glaciação (Biersma *et al.* 2018, Fraser *et al.* 2014; Pisa *et al.* 2014). Sendo assim, a flora local é composta por plantas que sobreviveram às glaciações e plantas que chegaram posteriormente.

A Antártica apresenta temperaturas que chegam a -93°C no inverno (no interior do continente). Devido a esse ambiente inóspito para a sobrevivência dos organismos, é preciso ter várias adaptações, dessa forma, os únicos registros de plantas que existem até hoje são de briófitas e duas fanerógamas nativas - *Deschampsia antarctica* Desv. (Poaceae) e *Colobanthus quitensis* (Kunth) Bartl. (Caryophyllaceae), além de uma invasora, *Poa annua* L. (Poaceae) (Ochyra *et al.* 2008). O primeiro registro de briófitas na Antártica foi feito pelo cirurgião Willian Smith em 1819, em uma breve anotação em sua caderneta, e o primeiro musgo coletado foi um exemplar do gênero *Polytrichastrum alpinum* (Hedw.) G.L. Sm. (Eights 1833). Desde então, tem aumentado progressivamente a catalogação de briófitas do continente.

Como todas as briófitas são poiquiloídricas e poiquilotérmica, essa característica possibilita sua ocorrência na Antártica, apesar de seu ambiente hostil. Portanto, temos

registro de 116 espécies de musgos, sendo que 11 são endêmicas naquele continente (Ochyra *et al.* 2008; Ellis *et al.* 2013a, b, Sollman 2015, Câmara *et al.* 2019).

Dentre os grupos de briófitas catalogados na Antártica, está o gênero *Hymenoloma* Dúsen, o qual se encontra dentro da família Hymenolomataceae (Grimmiales) (Frey *et al.* 2009). Tal gênero tem oito espécies: *Hymenoloma antarcticum* (Müll. Hal.) Ochyra, *H. grimmiaeum* (Müll. Hal.) Ochyra, *H. crispulum* (Hedw.) Ochyra, *H. brevipes* (Müll. Hal.) Ochyra, *H. brevisetum* (Cardot) Ochyra, *H. compactum* (Schwägr.) Ochyra, *H. conterminum* (Renauld et Cardot) Ochyra e *H. mullahaceni* (Höhn.) Ochyra (Ochyra *et al.* 2008; Werner *et al.* 2013). As características diagnósticas do gênero são: a presença frequente de papilas em seção transversal do filídio, devido a lamelas cuticulares cobrindo ambas as superfícies do filídio; margem inteira, costa única, ocupando cerca de 1/5 da base do filídio; células guia separando os estereóides da parte ventral e dorsal do filídio, filídios crispados (enrolados) quando secos, além do peristômio fortemente crispado com 16 dentes (Ochyra *et al.* 2008; Werner *et al.* 2013).

Na Antártica as espécies presentes são apenas três: *Hymenoloma antarcticum*, *H. grimmiaeum* e *H. crispulum*, que basicamente se diferenciam pelos seus esporófitos e esporos. Quando não estão férteis, a identificação é quase impossível.

Ochyra *et al.* (2008) relaciona geograficamente dois fenótipos de *Hymenoloma antarcticum*: um presente nas ilhas Rei George e Livingston (arquipélago Shetlands do Sul), denominado fenótipo *brevipes*, que possui células laminares basais curtas e faltam aurículas convexas. Esse fenótipo foi nomeado dessa maneira, devido ao tipo (*Blindia brevipes* Müller) estar presente na Geórgia do Sul. E, o fenótipo típico, presente no arquipélago Orcadas do Sul e também na ilha Deception (província das Shetlands do sul), apresenta plantas maiores, folhas largas e células laminares basais bastante longas e lineares. Embora se tenha características tão distintas nos dois fenótipos, nenhum trabalho aborda a possibilidade desses dois morfotipos serem espécies diferentes.

Nenhuma das espécies de *Hymenoloma* é endêmica da Antártica, podendo ser encontradas também na América do Sul, em países como Colômbia e Peru (*H. crispulum*), além da Ásia, na Geórgia do Sul (*H. grimmiaeum*), Tasmânia e Austrália (*H. antarcticum*) e na Oceania em Nova Zelândia (*H. antarcticum*). *Hymenoloma crispulum* é a única espécie bipolar intermediária, ou seja, ocorre tanto na região polar Norte quanto na Sul, além de outras regiões, como na América do Sul e Rússia (Ochyra *et al.* 2008; Ochyra & Bednarek-Ochyra 2015; Fedosov *et al.*, 2016). Mas até o momento não temos estudos moleculares abordando e afirmando a possibilidade dessa espécie ser mesmo bipolar.

O gênero foi descrito por Dúsen (1905), para a colocação de uma espécie patagônica nova - *H. nordenskjoeldii* Dúsen. (espécie tipo). Brotherus (1924) retirou três outras espécies dos gêneros *Blindia* Bruch (Seligeriaceae), *Dicranoweisia* Milde (Rhabdoweisiaceae), e *Verrucidens* Cardot (Rhabdoweisiaceae), e os colocou em *Hymenoloma*. Posteriormente Ochyra *et al.* (2003), em uma revisão taxonômica dos musgos da Polônia, retirou quase todas as espécies do gênero *Dicranoweisia* e os inseriu em *Hymenoloma* (Seligeriaceae), deixando apenas *D. cirrata* (Hedw.) Lindb. ex Milde e

D. africana Dixon em *Dicranoweisia* (Frey *et al.* 2009; Stech *et al.* 2013; Werner *et al.* 2013).

Nesse sentido, há certa confusão na literatura sobre qual família *Hymenoloma* pertence (tabela 1). Há estudos moleculares de algumas espécies para elucidar a real posição de *Hymenoloma* (e. g. Werner *et al.* 2013; Fedosov *et al.* 2016), mas ainda nada muito conclusivo, devido à falta de informações (tanto moleculares, quanto morfológicas) das outras espécies. Werner *et al.* (2013) sugerem uma forte relação molecular do gênero com a família Scouleriaceae, o que foi confirmado por Fedosov *et al.* (2016). Porém, nesse último estudo, eles concluíram que as características morfológicas encontradas em *Hymenoloma* eram únicas e não se encaixavam nessa família, sugerindo assim a criação de uma nova família – Hymenolomataceae, onde incluíram apenas as espécies *Hymenoloma crispulum*, *H. nordenskjoldii* (= *H. antarcticum*) e *H. mulahaceni* (Höhn.) Ochyra.

Resumindo, *Hymenoloma* é um conjunto de espécies que não couberam em outros gêneros, e apesar de ter dois trabalhos (Werner *et al.* 2013; Fedosov *et al.* 2016) mostrando o monofiletismo do grupo, eles não englobam todas as espécies, principalmente as antárticas e o grupo carece de sinapomorfias morfológicas, especialmente no gametófito - uma melhor circunscrição. Além disso, os estudos moleculares podem nos dar respostas acerca da bipolaridade de *Hymenoloma crispulum*, se formam clados monofiléticos ou não. Além da posição familiar do grupo.

Tabela 1. Classificações de *Hymenoloma* Dusén encontradas na literatura.

Ochyra <i>et al.</i> 2003; 2008	Goffinet <i>et al.</i> 2008	Frey <i>et al.</i> 2009	Werner <i>et al.</i> 2013	Fedosov <i>et al.</i> 2016
Seligeriaceae	Rhabdoweisiaceae	Oncophoraceae	Scouleriaceae	Hymenolomataceae

Como mencionado, além de não sabermos a qual família *Hymenoloma* pertence, a identificação das espécies Antárticas apenas com o gametófito é praticamente inviável. São poucas as características que as diferenciam apenas com esse recurso, e mesmo assim é difícil, devido à falta de características diagnosticáveis. As descrições que temos na literatura são muito superficiais e subjetivas, dando brechas para duplas interpretações (como cor do filídio, caulídio, formato do filídio e das células e etc.) (e. g. Ochyra *et al.* 2008).

Dessa forma, o objetivo geral desse trabalho é estudar a taxonomia e com a ajuda de ferramentas moleculares a filogenia do grupo *Hymenoloma* presente na Antártica: *H. grimmiaeum*, *H. antarcticum* e *H. crispulum*.

Referências

- Biersma EM, Jackson JA, Stech M et al. 2018. Molecular Data Suggest Long-Term in Situ Antarctic Persistence Within Antarctica's Most Speciose Plant Genus, *Schistidium*. *Front. Ecol. Evol.*, 05. doi.org/10.3389/fevo.2018.00077
- Boger S. 2011. Antarctica — Before and after Gondwana. *Gondwana Research* 19(2): 335-371. <https://doi.org/10.1016/j.gr.2010.09.003>
- Brotherus VF. 1924. Musci. – In: Engler, A. & K. Prantl (eds.). *Die Natürlichen Pflanzenfamilien*. 2 edn. Berlin, Duncker and Humblot.
- Câmara PEAS, Valente DV, Amorim ET *et al.* 2019. Integrated analysis of intraspecific diversity in the bipolar moss *Roaldia revoluta* (Mitt.) P.E.A.S. Câmara & M. Carvalho-Silva (Bryophyta) in Antarctica. *Polar Biology* 42:485–496. doi.org/10.1007/s00300-018-2437-8
- Câmara PEA, Carvalho-Silva M. 2020. 180 years of botanical investigations in Antarctica and the role of Brazil. *Acta Bot. Bras.* (in press). doi.10.1590/0102-33062020abb0172.
- Capozoli U. 2001. *Antártida a última terra*. São Paulo, Edusp.
- Dusén PKH. 1905. Beiträge zur Bryologie der Magellansländer, von Westpatagonien Westpatagonien und Südchile. *Arkiv för Botanik*, 4: 1–45.
- Eights J. 1833. Description of a new crustaceous animal found on the shores of the South Shetland Islands, with remarks on their natural history. *Transactions of the Albany Institute*, 2: 53-69.
- Ellis LT, Asthana AK, Gupta R *et al.* 2013a. New national and regional bryophyte records, 34. *J Bryol* 35: 62–70.
- Ellis LT, Bednarek-Ochyra H, Ochyra R *et al.* 2013b. New national and regional bryophyte records, 35. *J Bryol* 345:129–139.
- Fedosov VE, Fedorova AV, Troitsky AV, Bobrova VK, Ignatov MS. 2016. On the systematic position of *Hymenoloma* (Bryophyta). *Arctoa*, 25: 119-130.
- Fraser CI, Terauds A, Smellie J, Convey P, Chown SL. 2014. Geothermal activity helps life survive glacial cycles. *PNAS* (in press). doi: 10.1073/pnas.1321437111.
- Frey W, Stech M, Fischer E. 2009. *Bryophytes and seedless vascular plants*. 13th ed. Germany, Borntraeger.
- Goffinet B, Buck WR, Shaw AJ. 2008. Morphology, Anatomy and Classification of Bryophyta. In: Shaw AJ, Goffinet B. (Org.) *Bryophyte biology*. Cambridge, Cambridge University Press. p. 55-138.
- Hahn-Woernle L, Powell B, Lundesgaard O, Wessem M. 2020. Sensitivity of the summer upper ocean heat content in a Western Antarctic Peninsula fjord. *Progress in Oceanography*, 183. doi.org/10.1016/j.pocean.2020.102287
- Leppe M, Moisan P, Abad E, Palma-Heldt S. 2006. Upper Triassic Palaeobotany of Biobío river valley, Chile: Filicopsida Class. *Revista Geológica de Chile* 33 (1) : 81-107

- Machado MC. (Org.), Brito T (Org.). 2006. Antártica: Ensino fundamental e ensino médio. Brasília.
http://portal.mec.gov.br/seb/arquivos/pdf/EnsMed/vol109_meioambientantart.pdf. 8 apr. 2019.
- Ochyra R, Bednarek-Ochyra H. 2015. *H. Dicranoweisia fastigiata*, a new synonym of *Hymenoloma antarcticum* (Seligeriaceae). *Cryptogamie, Bryologie*, 36: 41-45.
- Ochyra R, Żarnowiec J, Bednarek-Ochyra H. 2003. Biodiversity of Poland. Vol. III Census Catalogue of Polish Mosses. Kraków, Cambridge University Press.
- Ochyra R, Smith RIL, Bednarek-Ochyra H. 2008. The illustrated moss flora of Antarctic. Cambridge, Cambridge University Press.
- Pisa S, Biersma EM, Convey P. 2014, The cosmopolitan moss *Bryum argenteum* in Antarctica: recent colonisation or in situ survival? *Polar biology*, 37: 1469–1477.
- Rives ED. 1988. El continente Antartico. *Boletín de informacion*, 210.
- Salleh, A. 2015. Westerlies 'kick-started' Antarctic current. <http://www.abc.net.au/science/articles/2015/07/30/4282773.htm>. 4 May 2019.
- Simões J, Garcia CAE, Evangelista H, Campos LS, Mata MM, Bremer UF. 2011. Antártica e as Mudanças Globais: um desafio para a humanidade. Vol IX. São Paulo, Blucher.
- Sollman P .2015. The genus *Bryoerythrophyllum* (Musci, Pottiaceae) in Antarctica. *Polish Bot J* 50:19–25.
- Stech M, McDaniel SF, Hernández-Maqueda R. *et al.* 2013. Phylogeny of haplolepidous mosses - challenges and perspectives. *Journal of Bryology*, 34: 173-186.
- Stevenson DE. 2018. *Granite Skyscrapers: How Rock Shaped Earth and Other Worlds*. Nottinghamshire, Springer.
- Thomas DN, Fogg GE, Convey P. *et al.* 2008. *The biology of polar regions*. 2nd. Oxford: Oxford University Press.
- Venables HJ, Meredith MP. 2014. Feedbacks between ice cover, ocean stratification, and heatcontent in Ryder Bay, western Antarctic Peninsula. *J. Geophys. Res. Oceans*, 119: 5323–5336.
- Walton, DHA. (Ed.). 2013. *Antarctica: Global Science from a frozen continent*. Cambridge: Cambridge University Press.
- Werner O, Rams S, Kučera J *et al.* 2013. New data on the moss genus *Hymenoloma* (Bryophyta), with special reference to *H. mulahaceni*. *Cryptogamie, Bryologie*, 34: 13-30.

Chapter 1

Molecular phylogeny of
***Hymenoloma* Dusén**

Molecular phylogeny of *Hymenoloma* Dusén (Bryophyta) in Antarctica

C. O. S. Machado (0000-0003-4596-2716)^{a*} and P. E. A. S. Câmara (0000-0002-3944-996X)^b

^a^b*Botany Department, University of Brasilia, Brasilia, Brazil*

cristiellymachado10@gmail.com *corresponding author

Molecular phylogeny of *Hymenoloma* Dusén (Bryophyta) in Antarctica

In Antarctica, there are three species of *Hymenoloma*: *H. antarcticum*, *H. crispulum* and *H. grimmiaceum*. Literature reported that *Hymenoloma crispulum* is an intermediate bipolar species, but no work has tested this hypothesis in depth. Besides, there is also some confusion about the family position of *Hymenoloma*. The objective of this study is to investigate the relationships between Antarctic species and their continental pairs using morphologic and molecular studies. For this study, plants of *Hymenoloma* from South Shetlands islands were used, with the molecular marker's *trnLF*, *rps4* and *nad5*. Phylogenetic analyzes were performed under Maximum Parsimony, Maximum Likelihood and Bayesian Inference. As a result, it was possible to see the monophyly of the genus *Hymenoloma* with good support. The phylogram showed that *Hymenoloma* is a sister group of Drummondiaaceae, your family is Hymenolomataceae and order is Scouleriales. *Hymenoloma crispulum* is not a bipolar specie and a not have *H. crispulum* in Antarctic but *H. brevipes*. *Hymenoloma grimmiaceum* is monophyletic and we have new *Hymenoloma* species for antarctica and Chile.

Keywords: *trnL-F*; *rps4*; *nad5*; monophyly; phylogeny, morphology, Hymenelomataceae.

Introduction

Antarctica is a continent geographically isolated, and despite that, genetic flow occurs with other continents, through the air, migratory birds and even by human influence (Lewis et al. 2014; Viana et al. 2016). Despite of the inhospitable climate some few plants manage to survive and even thrive as it is the case of more than 116 (Walton 2013), bryophyte species that occurs there (Ochyra et al. 2008b; Ellis et al. 2013a; b; Sollman 2015; Câmara et al. 2019). And within the bryoflora there is the genus *Hymenoloma* Dusén (1905) with three species reported to Antarctica: *Hymenoloma antarcticum* (Müll. Hall.) Ochyra, *H. crispulum* (Hedw.) Ochyra and *H. grimmiaceum* (Müll. Hal.) Ochyra (Convey and Smith 2006; Ochyra et al. 2008b; Pereira and Putzke 2013).

The genus *Hymenoloma* was described originally by Dusén (1905) for a Patagonian species – *H. nordenskoeldii* Dusén. Brotherus (1924) removed three species from the genera *Blindia* Bruch (Seligeriaceae), *Dicranoweisia* Milde (Rhabdoweisiaceae), and *Verrucidens* Cardot (Rhabdoweisiaceae), and placed them into *Hymenoloma*. Ochyra et al. (2003), synonymized seven *Dicranoweisia* species in *Hymenoloma*, leaving only *D. cirrata* (Hedw.) Lindb. ex Milde and *D. africana* Dixon in *Dicranoweisia* (Frey et al. 2009; Stech et al. 2012; Werner et al. 2013).

Adding to this morphological and nomenclatural confusions, there is also some uncertainty in the literature about the familial position of *Hymenoloma*. Ochyra et al. (2003; 2008a; 2008b), based on morphology, classified the genus into Seligeriaceae, Goffinet et al. (2008), based on DNA evidence, moved it into Rhabdoweisiaceae. Still, none of these works presented a well-defined circumscription for the genus. Stech et al. (2012), Werner et al. (2013), Fedosov et al. (2015) and Fedosov et al. (2016) using also molecular tools have shown that *Hymenoloma* does not fit in these families. Fedosov et al. (2016) described a new family – Hymenolomataceae to accommodate the genus, but his studies did not include any of the Antarctic species.

The three species of *Hymenoloma* present in Antarctica are very hard to distinguish when they are not fertile, differing only by the sporophyte (Ochyra *et al.* 2008b). *Hymenoloma grimmiaceum* has capsules with 0.9-1.2 mm length and 0.8-0.9mm wide, brown, peristome teeth are deeply inserted below the mouth and recurved when dry; *Hymenoloma crispulum* has capsules with 1.5-2.0 mm length and 0.6-0.7 wide, light brown, peristome teeth are hyaline or orange-brownish, papillose to obscurely papillose-striate above on both outer and inner surfaces and *Hymenoloma antarcticum* presents capsules with 0.9-1.2 mm length and 0.7-0.9 mm wide, castaneous; peristome teeth are entirely or variously eroded at the apex, orange- brown,

reddish-orange to hyaline-yellow, densely papillose or verrucose distally and finely papillose at the base (Ochyra *et al.* 2003, 2008a, 2008b, Fedosov *et al.* 2016).

Due to the fact of the morphology of the Antarctic species of *Hymenoloma* are based mostly by the sporophyte and specimens are usually found sterile, due to the lack of liquid water during most of the time in Antarctica more molecular investigation is necessary to resolve these taxonomic confusions, like the identification morphology based in the gametophytic, the family of the group and the monophyly of the specimens.

In Antarctica, mosses are in constant morphological plasticity, due to the cold and inhospitable climate. And that is why they need a specialized correct identification and a vast taxonomic knowledge. There are several studies showing this influence of the Antarctic climate on the mosses there (Câmara *et al.* 2019). Therefore, the use of molecular markers has been increasingly significant and relevant for the correct identification of species, thus revealing the underestimated richness of that continent (Câmara *et al.* 2017; Park *et al.* 2018; Câmara *et al.* 2019).

Moreover, Convey and Smith (2006); Ochyra *et al.* (2003; 2008a; b); Pereira and Putzke (2013); Werner *et al.* (2013); Fedosov *et al.* (2016) and Câmara *et al.* (2020) reported the occurrence of *Hymenoloma crispulum* in the northern hemisphere (in Russia and North America) (Ochyra *et al.* 2003; 2008a; Werner *et al.* 2013; Fedosov *et al.* 2016) and the south hemisphere (in Antarctic, Oceania, South America) and considered this species as intermediate bipolar. But this hypothesis has not yet been phylogenetically tested.

The main objectives of this study are: 1. Investigate the monophyly of the genus with the inclusion of more species, including all the ones from Antarctica. 2. Investigate with molecular tools if *Hymenoloma crispulum* is indeed a bipolar species and 3. Investigate the real family position of the Antarctic species of the genus *Hymenoloma*.

Material and methods

Twelve plants of the genus *Hymenoloma* from Livingston and King George Island (South Shetlands Islands) in Antarctica and one plant from Chile, were included in the molecular analyses (table 1). These plants were obtained from the herbaria: NY (New York) and UB (University of Brasilia). Ten Genbank sequences were downloaded to compose the phylogenetic analysis. The chosen outgroup (*Tricodon cylindricus* (Hedw.) Schimp.) was based on Fedosov *et al.* (2016).

We used three molecular markers (table 2), being two from chloroplast: *rps4* (*trnS-rps4* spacer) and *trnL-F* (*trnL-trnF* spacer), using the primers by Nadot *et al.* (1994) and Taberlet *et al.* (1991), respectively. And also, one mitochondrial marker - *nad5* (NADH dehydrogenase subunit 5) using the primers from Câmara and Shaw (2013). DNA was extracted using the CTAB protocol (Doyle and Doyle 1987). The content for the PCR mixes and the temperature cycle in the thermal cycler was the same as used in Câmara *et al.* (2019), modifying only the annealing in 1 min at 54-56° C (*trnL-F*) and 49-51° C (*rps4*). PCR products were purified and sequenced by Macrogen Inc. (Seoul, Korea) and BPI Biotechnology (São Paulo, Brazil).

The edition of sequences and contigs was made with Geneious v. 6.1.6 (Biomatters 2010). Alignment was done initially using Clustal X v. 2.1 (Higgins and Sharp 1988) and manually adjusted using PhyDe 0.9971 (Müller *et al.* 2006). Indels were coded as Single Indel coding, SIC (Simmons & Ochoterena 2000) using Seqstate v. 1.4.1 (Müller 2005). Phylogenetic inferences were made using PAUP v. 4 (Swofford 2002), for maximum parsimony analysis, RaxML v. 6 (Stamatakis 2006), for maximum likelihood analysis and Mr. Bayes v. 3.2.2 (Ronquist *et al.* 2012), for Bayesian analysis. Heuristic searches were done with 100 random addition replicates and tree-bisection - reconnection (TBR) branch swapping, saving a maximum of 10,000 trees; all characters

were unordered and equally weighted, and gaps were treated as missing data. For maximum parsimony and maximum likelihood analysis for clade support was evaluated using the non-parametric bootstrap (Felsenstein 1985). The Bayesian Inference support was evaluated using posterior probabilities. For Bayesian analysis, two parallel Markov Chain Monte Carlo (MCMC) simulations were run for 5 million generations, sampling one tree every 1000 generations. Convergence was established by ensuring that the mean standard deviation of split frequency was < 0.01 . The first 25% of the trees were discarded as “burn-in”. A majority-rule consensus tree was constructed from the resulting trees to estimate the posterior probabilities. We consider unsupported clades when the inference Bayesian $< 0,95$ the maximum parsimony < 60 and maximum likelihood < 60 (Yang and Rannala 2012).

Results

Trees from individual markers as well as analyses treating gaps as missing data or coding them by simple indel coding (SIC) (Simmons and Ochoterena 2000) differed only in the degree of resolution. Consequently, all markers were using in a single matrix. In all our analyses, *Hymenoloma* is resolved as a monophyletic group with maximum support in all analysis (figs 1 and 2). The family of the genus *Hymenoloma* is Hymenolomataceae and the order is Scouleriales.

The support for the *Hymenoloma* group was 1 for Bayesian inference, 100 for maximum parsimony and 100 for maximum likelihood. Within the *Hymenoloma*'s group, five clades were formed figure 1 As follows:

Clade 1: have *Hymenoloma crispulum* from King George and Livingston Island (*H. crispulum*_KG1 and KG2; *H. crispulum*_LV, LV1, LV2 and LV3), *H. antarcticum* from Livingston and *H. brevipes* from Chile (no support, with 0,92 for Bayesian inference, 50 for maximum parsimony and 0 for maximum likelihood).

Clade 2: grouped *Hymenoloma crispulum* from Spain, Austria and Russia with *H. compactum* from Austria (no support, with 0,93 for Bayesian inference, 0 for maximum parsimony and 0 maximum likelihood).

Clade 3: have a group formed for *Hymenoloma grimmiaceum* with maximum support in Bayesian inference, 68 for maximum parsimony and no support for maximum likelihood (56).

Clade 4: The specie *H. mullahaceni* from Russia and Kazakhstan with maximum support in all analysis.

Clade 5: grouped *Hymenoloma antarcticum* and *H. grimmiaceum* from Livingston and *H. crispulum* from Chile as sister group. In this clade we didn't get support in our analysis.

In figure 2, we have a phylogram consensus of Bayesian inference with the names suggested at this study.

Discussion

The monophyly of the genus *Hymenoloma* reported in the literature (Werner et al. 2013, Fedosov et al. 2016) has been confirmed here. In the two trees (figs 1 and 2) the supporting was maximum for all analysis. The sister group for *Hymenoloma* in our study is *Drummondia*, (Drummondiaceae), the order for the genus *Hymenoloma* is Scouleriales and the family is Hymenolomataceae.

Hymenoloma crispulum not is a bipolar specie (figure 1 and 2). The *Hymenoloma crispulum* from northern hemisphere is not nested with Antarctic and Southern hemisphere species. We suggest the use of *H. brevipes* for the southern hemisphere specimens because the type of is from South Georgia in Antarctic.

In the two papers published (Fedosov et al. 2016, Werner et al. 2013) for *Hymenoloma*, *H. brevipes* is separated from of the *H. crispulum*. This occur because *H.*

crispulum only happens so far in Europe, in South America not happens. In literature have a proximate on the morphological of the *H. crispulum* and *H. brevipes*. According to Werner et al. (2013), the only difference between the two species is the larger spore in *H. brevipes*, which may occur due to environmental conditions acting on the morphology of the species. May be the cause of misidentification.

European *Hymenoloma crispulum* grouped with *H. compactum* (figs 1 and 2, clade 2), the two species needs of the revision and may be synonymous. *H. crispulum* of the Chile (fig 1, clade 5) is a specie unknown, your characteristics genetics and morphological are not of the no species described.

Hymenoloma grimmiaceum was resolved monophyletic with the three markers (figs 1 and 2, clade 3). *H. grimmiaceum* that grouped with *H. crispulum* of the Chile, could be a new specie (figs 1 and 2 clade 5). The characteristic distinguishable of the *H. grimmiaceum* is the peristomal teeth facing out when dry (fig 3a, 3b and 3c), what does not happen with this species that joined with *H. crispulum* of the Chile (fig 3d). It is genetically and morphologically different.

Hymenoloma crispulum from Chile (figs 1 and 2, clade 5) is the more different morphologically of the all *Hymenoloma* specimens. She present allar cells very evident (fig 4i), leaf very flaccid and the base is wedged when pulled from the stem (fig 4h) (remember the *Bryum* Hedw.), characteristics never seen in *Hymenoloma*.

We do not have enough data to say for sure the position of *Hymenoloma antarcticum*. Despite the low support in Figures 1 and 2 in the clade 1 and 5, *H. antarcticum* separated.

Hymenoloma mulahaceni is monophyletic. In the all analysis grouped with high support, in the two figure the support was 1 for Bayesian inference, 100 for maximum parsimony and 100 for maximum likelihood (figs 1 and 2, clade 4).

The proximity of the genus *Hymenoloma* with the Drummondiaceae family has been related in the literature (Stech et al. 2012, Werner et al. 2013; Inoue and Tsubota 2014; Fedosov et al. 2015; Fedosov et al. 2016), and confirmed here. Ochyra et al. (2003, 2008a, 2008b) classified the genus based on morphology in the order Grimmeriales (Seligeriaceae). The characteristics described fit into *Hymenoloma*, but the molecular phylogeny contradicts it, since the taxa representing the order Grimmeriales (*Grimmia* and *Ceratodon*) are not close to the genus. The genus is not grouped with Seligeriaceae species. Goffinet et al. (2008), based on DNA classifies *Hymenoloma* within Dicranales. Still, in their description, this order's plants do not have a basal membrane in the peristome, and some taxa in *Hymenoloma* have this characteristic (e.g. *H. crispulum*). Moreover, the phylogeny showed here and confirmed in several other works (e.g. Stech et al. 2012, Werner et al. 2013, Fedosov et al. 2015; Fedosov et al. 2016) illuminated us about the right order of *Hymenoloma* – Scouleriales. Plants of this order are blackish, acro or cladocarpous, saxicolous in riparian habitats; calyptrae mitrate, smooth; annulus not differentiated; capsules urceolate to globose, these characters corroborate with *Hymenoloma*.

A better circumscription of *Hymenoloma* is still in need. The family's various classification found is not plausible, and the insertion of *Hymenoloma* in Drummondiaceae (based in phylogeny here) is not plausible. Drummondiaceae presents cladocarpous plants and reduced peristomy. *Hymenoloma* is acrocarpous, and its peristomy has 16 teeth (sometimes more). In some cases, as in *Hymenoloma grimmiceum* and *H. antarcticum* (phenotype *brevipes*), the peristome is so long that it curves out of the mouth of the capsule. The only justification for creating a new family in Fedosov et al (2016) – Hymenolomataceae, was the morphologic and molecular distinction of the *Hymenoloma* of Scouleriaceae and Drummondiaceae. The family is

accepted but does not include all species and still needs a better description of these ridges present on the cell wall unique to *Hymenoloma*.

Conclusions

The genus is monophyletic, the family of the *Hymenoloma* is Hymenolomataceae and the order of *Hymenoloma* is Scouleriales. We showed proximity with the Drummondiaceae family, but the insertion of *Hymenoloma* in Drummondiaceae is not supported based on the molecular and morphology evidence. The Hymenolomataceae family needs a better circumscription. *Hymenoloma crispulum* is not a bipolar species, and a not have *H. crispulum* in Antarctic but *H. brevipes*. *Hymenoloma grimmiaceum* is monophyletic and we have unknown *Hymenoloma* species for antarctica and Chile. In Antarctic have *H. antarcticum*, *H. brevipes* and *H. grimmiaceum*.

Acknowledgements

The authors are grateful for the support granted by ProAntar and UnB, and to the herbaria of NY and SP for the loan of material. The first author is grateful to the Higher Education Personnel Improvement Coordination (CAPES) for a master's scholarship at the Graduate Program in Botany at the University of Brasília.

References

- Beckert S, Steinhauser S, Muhle H, Knoop V. 1999. A molecular phylogeny of bryophytes based on nucleotide sequences of the mitochondrial *nad5* gene. *Plant Syst. and Evol.* 218: 179–192.
- Buck WR, Cox CJ, Shaw AJ, Goffinet B. 2005. Ordinal relationships of pleurocarpous mosses, with special emphasis on the Hookeriales. *Syst. and Biodivers.* 2: 121–145.
- Câmara PEAS, Shaw J. 2013. A Molecular Phylogeny of the Moss Genus *Taxithelium* (Pylaisiadelphaceae) Based on Plastid, Mitochondrial and Nuclear Markers. *Syst. Bot.* 38(4): 861–868.
- Câmara PEAS, Costa Silva BGC, Carvalho-Silva M, Knop DH. The moss flora of Ostrov Geologov (Geologists Island), Maxwell Bay, King George Island, Antarctica. *Bol. Soc. Argent. Bot.* 52(2): 1851-2372.

- Câmara PEAS, Valente DV, Amorim ET, Henriques DK, Carvalho-Silva M, Convey P, Stech M. 2019. Integrated analysis of intraspecific diversity in the bipolar moss *Roaldia revoluta* (Mitt.) P.E.A.S. Câmara & M. Carvalho-Silva (Bryophyta) in Antarctica. *Polar Biol.* 42:485–496. <https://doi.org/10.1007/s00300-018-2437-8>.
- Câmara PEAS, Valente DV, Sancho LG. 2020. Changes in the moss (Bryophyta) flora in the vicinity of the Spanish Juan Carlos I Station (Livingston island, Antarctica) over three decades. *Polar Biol.* 43(11):1-8. <https://doi.org/10.1007/s00300-020-02740-0>.
- Convey P, Smith LRI. 2006. Geothermal bryophyte habitats in the South Sandwich Islands, Maritime Antarctic. *J. of Vegetation Sci.* 17: 529-538.
- Doyle JJ, Doyle JL. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. *Phytochem Bull* 19:11–15.
- Dusén, PKH. 1905. Beiträge zur Bryologie der Magellansländer, von Westpatagonien und Südchile [Contributions to the bryology of the Magellanic countries, western Patagonia and southern Chile]. *Arkiv för Bot.* 4: 1–45. German.
- Ellis LT, Asthana AK, Gupta R, Nath V, Sahu V, Bednarek-Ochyra H, Ochyra R, Cykowska B, Aranda SC, Fischer E et al. 2013a. New national and regional bryophyte records, 34. *J. Bryol.* 35:62–70. 10.1179/1743282012Y.0000000042.
- Ellis LT, Bednarek-Ochyra H, Ochyra R, Benjumea MJ, Saïs LV, Caparrós R, Lara F, Mazimpaka V, Dulin MV, Garilleti R et al. 2013b. New national and regional bryophyte records, 35. *J. Bryol.* 345:129–139. 10.1179/1743282013Y.0000000049.
- Fedosov VE, Fedorova AV, Ignatova EA, Bobrova VK, Troitsky AV. 2015. RPS4 and NAD5 Sequences Indicate the Polyphyly of Ditrichaceae and Parallelisms in the Evolution of Haplolepidous Mosses. *Molecular Biol.* 49: 890–894.
- Fedosov VE, Fedorova AV, Troitsky AV, Troitsky AV, Bobrova VK, Ignatov MS. 2016. On the systematic position of *Hymenoloma* (Bryophyta). *Arctoa* 25:119-130. <http://doi: 10.15298/arctoa.25.10>.
- Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evol.* 39: 779–783.
- Frey W, Stech M, Fischer E. 2009. Bryophytes and seedless vascular plants. Stuttgart (DE): Borntraeger Verlagsbuchhandlung.
- Frey W, Stech M, Meissner K. 1999. Chloroplast DNA-relationship in palaeoaustrian *Lopidium concinnum* (Hypopterygiaceae, Musci). An example of steno-evolution

- in mosses Studies in austral temperate rain forest bryophytes 2. *Plant Syst. Evol.* 218: 67-75.
- Goffinet B, Buck WR, Shaw AJ. 2008. *Morphology, Anatomy and Classification of Bryophyta*. Cambridge (EN): Cambridge University Press.
- Higgins DG, Sharp PM. 1988. Clustal: a package for performing multiple sequence alignment on a microcomputer. *Gene* 73:237–244.
- Inoue Y, Tsubota H. 2014. On the systematic position of the genus *Timmiella* (Dicranidae, Bryopsida) and its allied genera, with the description of a new family Timmiellaceae. *Phytotaxa* 181:151–162.
- Lewis LR, Behling E, Gousse H, Qian E, Elphick CS, Lamarre JF, Bêty J, Liebezeit J, Rozzi R, Goffinet B. 2014. First evidence of bryophyte diaspores in the plumage of transequatorial migrant birds. *PeerJ* 2:e424. <https://doi.org/10.7717/peerj.424>.
- Müller K. 2005. SeqState - primer design and sequence statistics for phylogenetic DNA data sets. *Appl. Bioinform.* 4:65-69.
- Müller K, Quandt D, Müller J, Neinhuis C. 2006. PhyDE: phylogenetic data editor. [Accessed 20 Oct. 2019]. www.phyde.de.
- Nadot S, Bajon R, Lejeune B. 1994. The chloroplast gene *rps4* as a tool for the study of Poaceae phylogeny. *Plant Syst. and Evol.* 191: 27–38.
- Ochyra R, Żarnowiec J, Bednarek-Ochyra H. 2003. *Census Catalogue of Polish Mosses. Biodivers. of Poland 3*. Cracow (PL): Polish Academy of Sciences.
- Ochyra R, Stebel A, Bednarek-Ochyra H. 2008a. *Bryophytes of the Polish Carpathians. Poznari (PL): Sorus*.
- Ochyra R, Smith RIL, Bednarek-Ochyra H. 2008b. *The illustrated moss fora of Antarctica*. Cambridge (EN): Cambridge University Press.
- Park CH, Hong SG, Elvebakk A. 2018. *Psoroma antarcticum*, a new lichen species from Antarctica and neighbouring areas. *Polar Biol.* 41: 1083–1090.
- Pereira AB, Putzke J. 2013. The Brazilian research contribution to knowledge of the plant communities from Antarctic ice-free areas. *An. Acad. Bras. Ci.* 85: 923-935.
- Ronquist F, Teslenko M, Van DMP, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP. 2012. Mrbayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across A Large Model Space. *Syst. Biol.* 61(3):539–542.

- Sollman P. 2015. The genus *Bryoerythrophyllum* (Musci, Pottiaceae) in Antarctica. *Polish Bot. J.* 50:19–25.
- Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinform.* 22:2688–2690.
- Stech, M, Mcdaniel SF, Hernandez-Maqueda R, Ros RM, Werner O, Muñoz J, Quandt D. 2012. Phylogeny of haplolepidous mosses – challenges and perspectives. *J. of Bryol.* 34: 173–186.
- Swofford DL. 2002. *Phylogenetic Analysis Using Parsimony (and Other Methods)*. Version 4. Massachusetts (USA): Sinauer Associates.
- Taberlet P, Gielly L, Pautou G, Bouvet J. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Mol. Biol.* 17: 1105-1109
- Viana DS, Santamaría L, Figuerola J. 2016. Migratory birds as global dispersal vectors. *Trends Ecol. Evol.* 31:763–775.
- Walton DHA. 2013. *Antarctica: Global Science from a frozen continent*. Cambridge (EN): University Press Cambridge.
- Werner O, Rams S, Kučera J, Larraín J, Afonina OM, Pisa S, Ros RM. 2013. New data on the moss genus *Hymenoloma* (Bryophyta), with special reference to *H. mulahaceni*. *Cryptogam. Bryol.* 34:13-30.
<http://dx.doi.org/10.7872/cryb.v34.iss1.2013.13>
- Yang Z, Rannala B. Molecular phylogenetics: principles and practice. *Nat. Rev.* 13: 303-314.

Table 1. Samples of *Hymenoloma* used for molecular analysis in this study and herbarium where the plants are deposited with their respective codes: CH: Chile; KG: King George Island; LV: Livingston Island; (NY) New York Botanical Garden and (UB) University of Brasília.

Specie	Voucher	Local	Latitude	Longitude	Herbarium
<i>H. antarcticum_LV</i>	Valente, D. V. 2292	Livingston Island, Juan Carlos I base	62°39'47" S	60°23'39" W	UB
<i>H. crispulum_CH</i>	J. Larraín 38536 NY Barcode: 02689337	Chile Última Esperanza. Comuna de Torres del Paine	-	-	NY
<i>H. crispulum_KG</i>	Câmara, P. E. A. S., Henriques, D. K. & Guedes, B. 4088	Fildes Peninsula, King George	62°06'51" S	58°33'24" W	UB
<i>H. crispulum_KG1</i>	Henriques, D. K. 2152	King George Island, Punta Ulma	62°07'86" S	58°35'2" W	UB
<i>H. crispulum_KG2</i>	Henriques, D. K. 2095	King George Island, Punta plaza	62°08'88" S	58°40'24" W	UB
<i>H. crispulum_LV</i>	Valente, D. V. 2571	Livingston island, Hill going down to Argentina Calleta	62°40'06" S	60°23'43" W	UB
<i>H. crispulum_LV1</i>	Henriques, D. K. H. 2154	Livingston island, Punta Ulma	62°07'86" S	58°35'2" W	UB
<i>H. crispulum_LV2</i>	Valente, D. V. 2444	Livingston island, Near the Spanish base	62°40'06" S	60°23'12" W	UB
<i>H. crispulum_LV3</i>	Valente, D. V. 2483	Livingston island, Hill above the Juan Carlos I base	62°39'51" S	60°23'09" W	UB
<i>H. grimmiaceum_KG</i>	Câmara, P. E. A. S.; Henriques, D. K. & Guedes, B. 4085	Fildes Peninsula, King George	62°08'97" S	58°40'83" W	UB
<i>H. grimmiaceum_LV</i>	Valente, D. V. 2449	Livingston island, Near the Spanish base	62°40'06" S	60°23'12" W	UB
<i>H. grimmiaceum_LV1</i>	Valente, D. V. 2560	Livingston island	62°40'06" S	60°23'43" W	UB

Table 2. Primers used in this study.

Primer	Sequence	Direction	Source
<i>rps5</i>	ATG TCC CGT TAT CGA GGA CCT	Forward	Nadot et al. 1994
<i>trnas</i>	TACCGAGGGTTCGAATC	Reverse	Souza-Chies et al. 1997
<i>trnF-FM</i>	ATT TGA ACT GGT GAC ACG AG	Forward	Taberlet et al. 1991
<i>trnL-CM</i>	CGA AAT TGG TAG ACG CTG CG	Reverse	Taberlet et al. 1991
<i>Nad5-ki</i>	ACT YGG TTA CCY GAT GCA ATG GAG GGT	Forward	Beckert et al. 1999
<i>Nad5-3R</i>	AAA ACG CCT GCT GTT ACC AT	Reverse	Buck et al. 2005
<i>Nad5-Li</i>	GCT GCA TGA ATC RAA GCR GAT ACT GG	Reverse	Beckert et al. 1999
<i>Nad5-L</i>	AAC TTT GGC CAA GGA TCC TAC AAA	Reverse	Beckert et al. 1999
<i>Nad5-4F</i>	GAAGGAGTAGGTCTCGCTTCA	Forward	Buck et al. 2005

Figure 1. Phylogram from *Hymenoloma* species obtained from all used markers (*rps4 trnL-F* and *nad5*). Ns above branches are Bayesian posterior probability, bootstrap for maximum parsimony and maximum likelihood, respectively. The (-) indicates no support and MAX indicates maximum support. Names in bold indicate *Hymenoloma* species. AUT: Austria, CHI: Chile, ESP: Spain, KG: King George Island, KAZ: Kazakhstan, LV: Livingston Island and RUS: Russia.

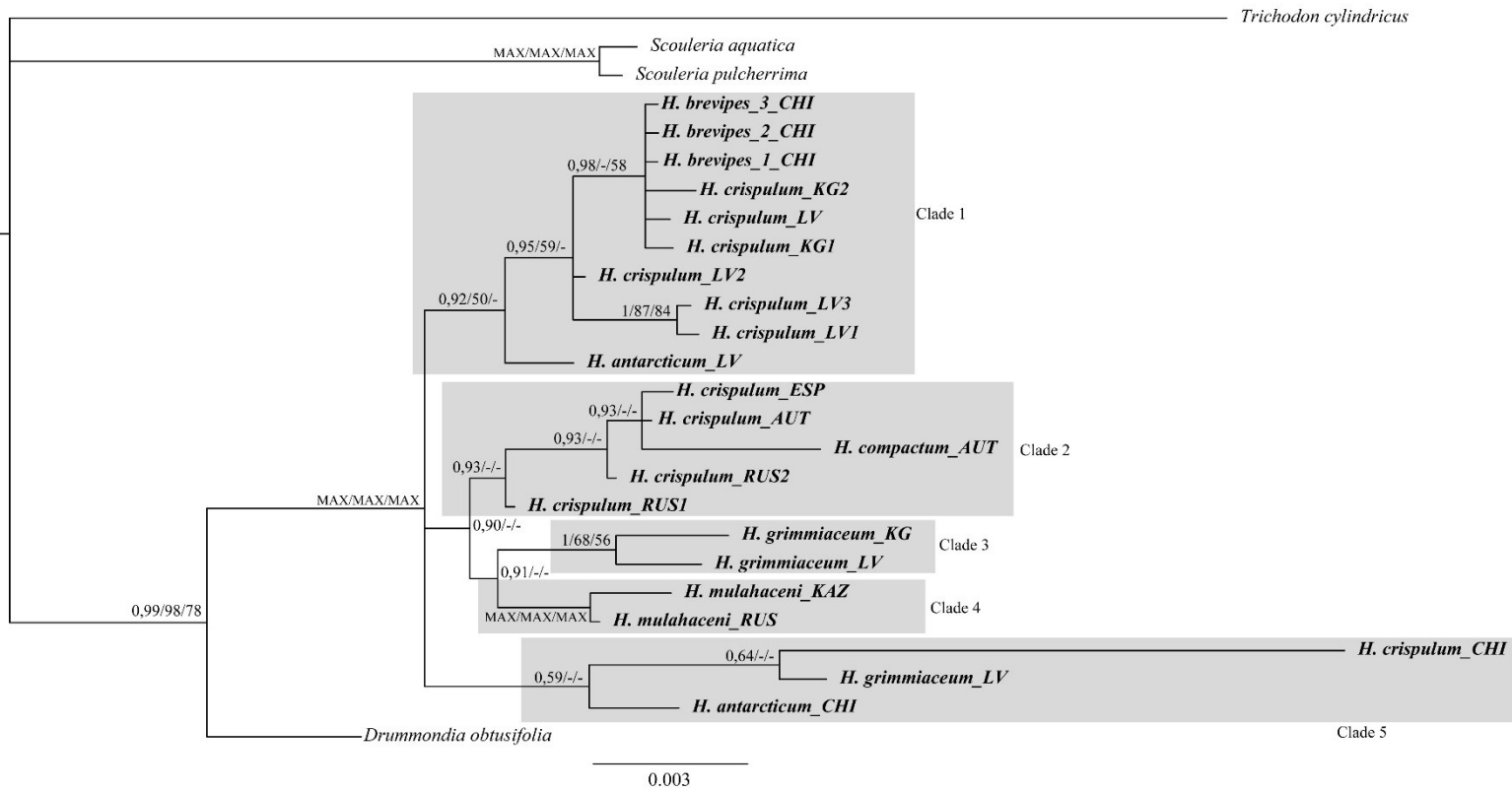


Figure 2. Phylogram from *Hymenoloma* species obtained from all used markers (*rps4*, *trnL-F* and *nad5*) with the names proposed in this study. Ns above branches are Bayesian posterior probability, bootstrap for maximum parsimony and maximum likelihood, respectively. The (-) indicates no support and MAX indicates maximum support. Names in bold indicate *Hymenoloma* species. AUT: Austria, CHI: Chile, ESP: Spain, KG: King George Island, KAZ: Kazakhstan, LV: Livingston Island and RUS: Russia.

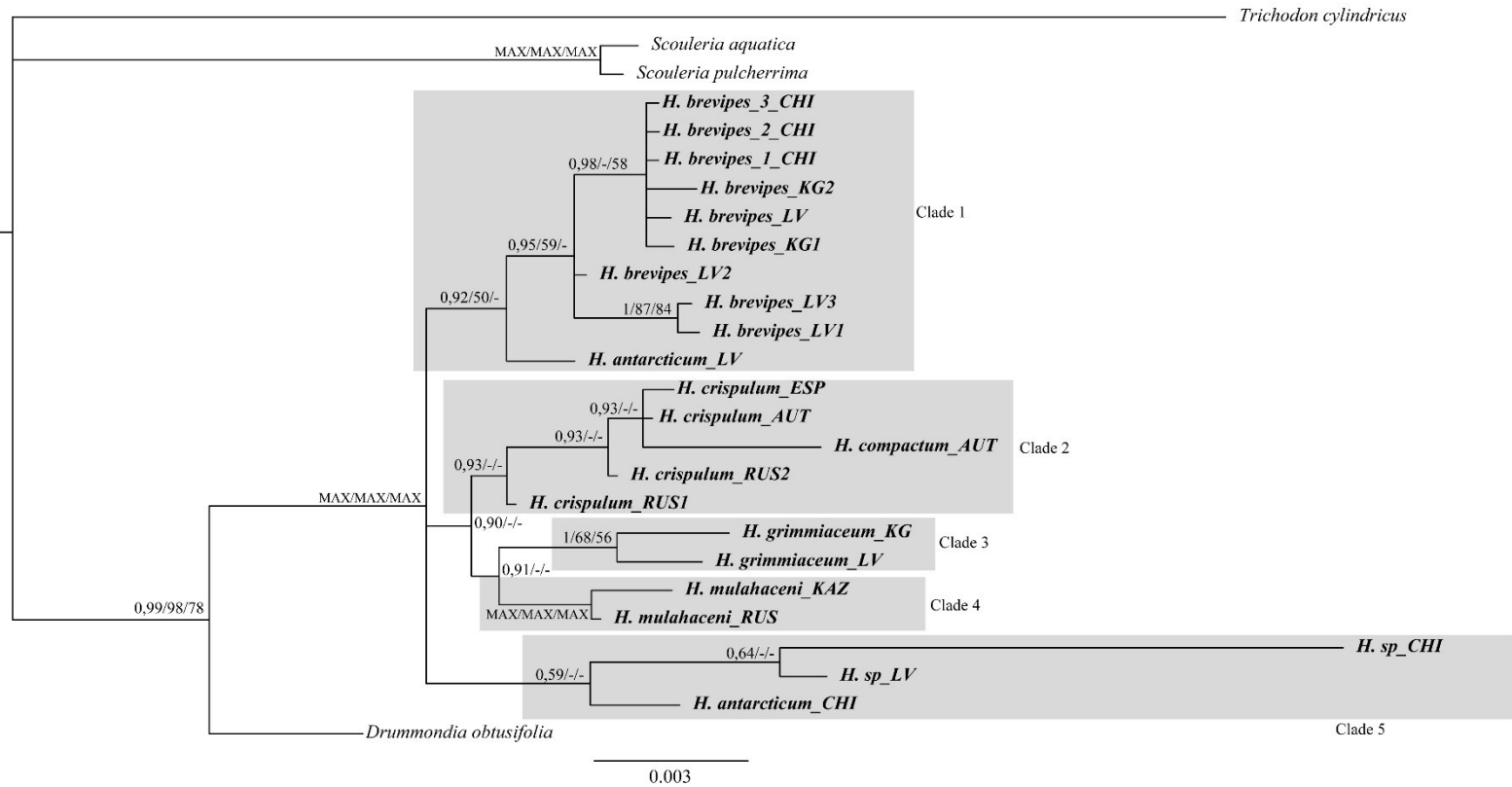


Figure 3. Electro micrography and photomicrography of *Hymenoloma* specimens from Antarctica and Chile. A, B, C and E: *Hymenoloma grimmiaeum*, D: *Hymenoloma* sp. from Livingston, F: *Hymenoloma* sp. from Chile.

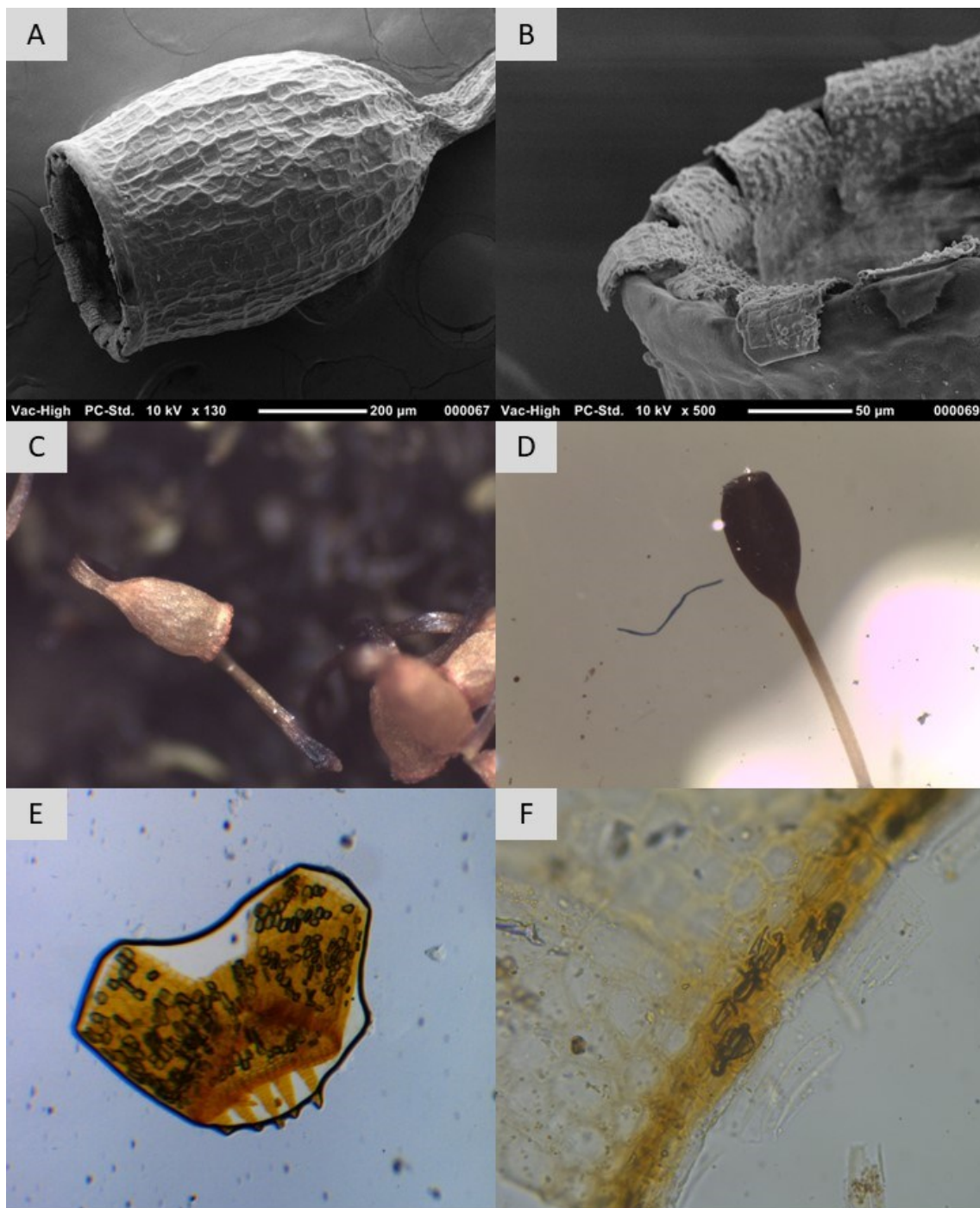
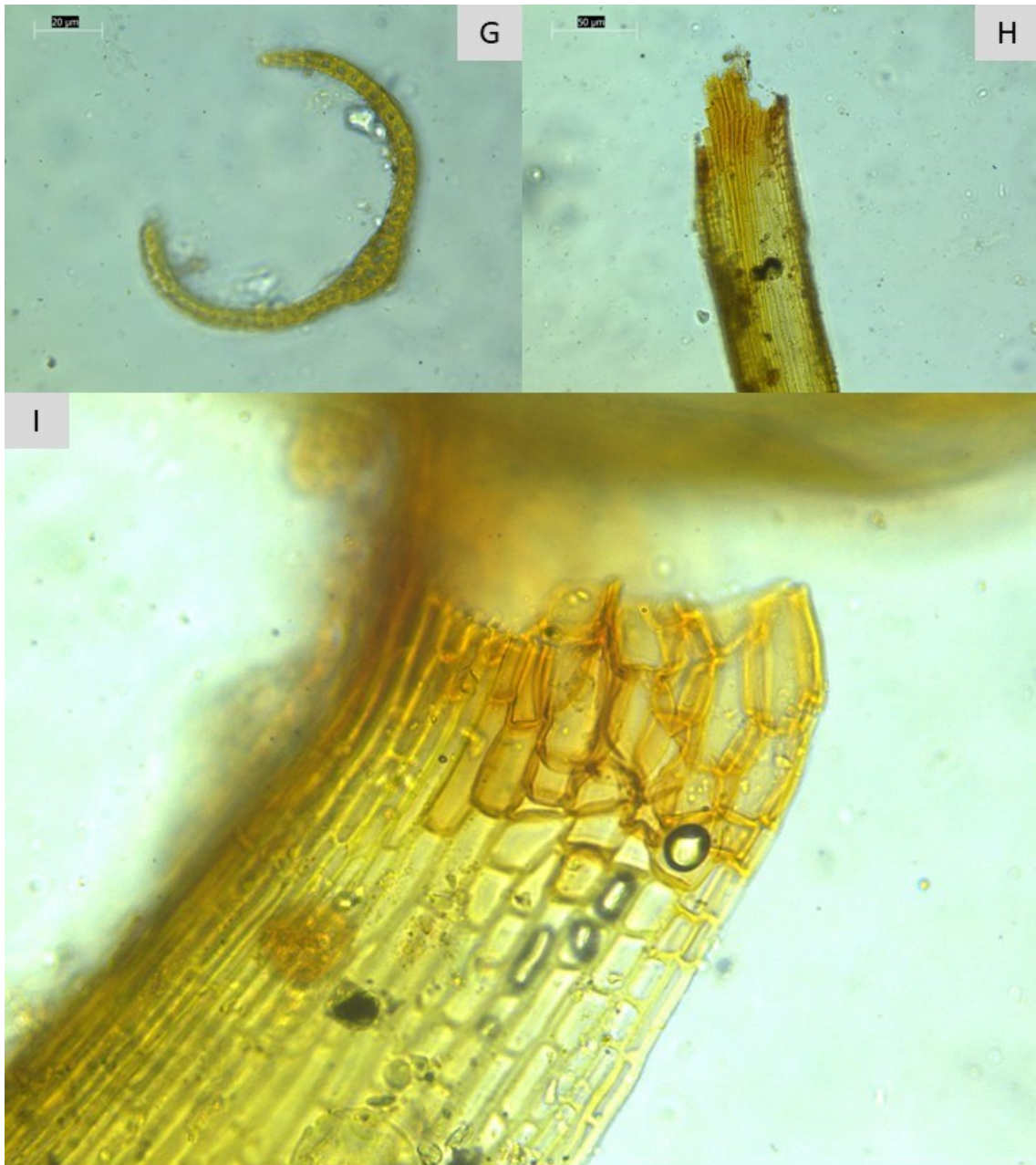


Figure 4. Photomicrography of *Hymenoloma* specie from Chile.



Considerações finais

Com a realização deste trabalho, obtivemos 39 novas sequências de DNA para o gênero *Hymenoloma*, sendo 15 para *rps4*, 17 para o marcador *trnL-F* e 7 para *nad5*. Além disso, pudemos contribuir com a confirmação do monofiletismo do grupo, elucidar a posição familiar (Hymenolomataceae) bem como a ordem (Scouleriales). Revelamos também que uma das espécies encontradas na Antártica (*H. crispulum*) foi identificada erroneamente, sua bipolaridade foi esclarecida, e seu nome foi atualizado: *Hymenoloma brevipes*. Nossa grande contribuição pôde revelar espécies desconhecidas de *Hymenoloma* presente na Antártica, aumentando a riqueza de espécie neste continente tão inexplorado.