UNIVERSIDADE FEDERAL DO PAMPA

KARINE ELISE JANNER DE FREITAS

INFERÊNCIAS GENÔMICAS E FILOGENÉTICAS DOS GENOMAS ACESSÓRIOS DAS POLYTRICHACEAE ANTÁRTICAS: *Polytrichum strictum* Menzies ex Brid. e *Polytrichum juniperinum* Hedw.

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Dissertação apresentada ao Programa de Pós-Graduação *Stricto Sensu* em Ciências Biológicas da Universidade Federal do Pampa, como requisito parcial para obtenção do Título de Mestre em Ciências Biológicas.

Orientador: Filipe de Carvalho Victoria

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Dedico este trabalho aos meus pais, por me darem a vida e me ensinarem tanto a vivê-la como admira-la. Não há nada no que eu sou, que eu não deva à vocês.

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“Um pássaro pousado em uma árvore nunca tem medo que o galho se rompa, porque sua confiança não está no galho e sim em suas próprias asas”.

Chico Xavier

RESUMO

A família das Polytrichaceae possui diversos representantes no continente Antártico, e entre elas a espécie *Polytrichum strictum* Menzies ex Brid. Considerado um organismo bipolar, pois se desenvolve tanto no Ártico como na Antártica, ainda carece de estudos que esclareçam sua classificação taxonômica, já que por diversas vezes foi conduzido como variante de *Polytrichum juniperinum* Hedw. devido sua morfologia ser similar a espécie e, sua verdadeira origem ainda não estar clara. Em um estudo, sugeriu-se que *P. juniperinum* poderia ser o ancestral materno de *P. strictum*, porém a análise apresentou incongruências. Ainda não se tem estudos moleculares dos espécimes oriundos dos polos e, muito menos abordagens a nível genômico do gênero *Polytrichum*. Com o advento das tecnologias de seqüênciamento de nova geração, os genomas organelares tornan-se uma ferramenta para estudos filogenéticos, já que fornecem dados sobre o conteúdo gênico e arquitetura do genoma, além de inferências filogenéticas complementares sobre a história evolutiva das espécies. Neste trabalho, foi determinada a sequencia parcial dos genomas do cloroplasto (cpDNA) e mitocondrial (mtDNA) de *P. strictum* e *P. juniperinum*, com o objetivo de analisar e caracterizar estruturalmente os genomas acessórios dos exemplares do gênero *Polytrichum* e inferir nas relações filogenéticas entre *P. strictum* e *P. juniperinum*. Os genomas acessórios das espécies foram sequenciados em um sequenciador NGS da *Ion Torrent*. A montagem, anotação, alinhamento, construção da filogenia e análise sintênica foram realizados *in silico* com softwares específicos. O cpDNA de *P. juniperinum* apresenta 55.168 pb compreendendo 51 genes, 31 *tRNAs*, 4 *rRNAs* e 19 proteínas relacionadas ao fotossistema I e II. O mtDNA de *P. juniperinum* compreende um total de 88.021 pb com 67 genes incluindo 19 t*RNAs*, 5 *rRNAs*, e 12 proteínas relacionadas ao metabolismo oxidativo. O cpDNA de *P. strictum* apresenta 20.183 pb compreendendo 45 genes, 14 *tRNAs*, 4 *rRNAs*, e 18 proteínas do fotossistema I e II. O mtDNA de *P. strictum* apresenta 58.896 pb contendo um total de 62 genes, 19 t*RNAs*, 5 *rRNAs*, e 13 proteínas relacionadas ao metabolismo oxidativo. Nas análises filogenéticas com cpDNA e mtDNA as árvores consenso apresentaram algumas diferenças no padrão de ramificação, porém *P. juniperinum* e *P. strictum* foram agrupadas no mesmo clado. Essas informações geradas a partir do cpDNA e mtDNA de *P. juniperinum* e *P. strictum* fornecem um aporte para futuros estudos filogenéticos com os espécimes do Ártico.

Palavras-chave: *Polytrichum*, Antártica, genômica comparativa.

ABSTRACT

The Polytrichaceae family has several representants on the Antarctic continent, including *Polytrichum strictum* Menzies ex Brid. Considered a bipolar organism, because it develops in both the Arctic and Antarctica Continent, it still need studies to clarify its taxonomic classification, since several times it was conducted as a variant of *Polytrichum juniperinum* Hedw due to its morphology be similar to the species and its true origin still not be clear. In study, it was suggested that *P. juniperinum* could be the maternal ancestor of *P. strictum*, but the analysis presented incongruence's. There are still no molecular studies of the specimens from the poles, let alone genomic approaches of the genus *Polytrichum*. With advent of new generation sequencing technologies, organellar genomes become as tool for phylogenetic studies, as they provide data on genome content and genome architecture, as well as complementary phylogenetic inferences about the evolutionary history of species. In this work, the sequence of the chloroplast (cpDNA) and mitochondrial (mtDNA) genomes of *P. strictum* and *P. juniperinum* was determined with the objective of analyze and structurally characterize the accessory genomes of the genera *Polytrichum* and infer in the phylogenetic relationships between *P. strictum* and *P. juniperinum*.The accessory genomes of the species were sequenced on an Ion Torrent NGS sequencer. Assembly, annotation, alignment, phylogeny construction and syntenic analysis were performed *in sílico* with specific software. The *P. juniperinum* cpDNA has 55,168 bp comprising 51 genes, 31 tRNAs, 4 rRNAs and 19 proteins related to photosystem I and II. The *P. juniperinum* mtDNA comprises a total of 88,021 bp with 67 genes including 19 tRNAs, 5 rRNAs, and 12 proteins related to oxidative metabolism. The *P. strictum* cpDNA has 20,183 bp comprising 45 genes, 14 tRNAs, 4 rRNAs, and 18 proteins from photosystem I and II. The *P. strictum* cpDNA has 20,183 bp comprising 45 genes, 14 tRNAs, 4 rRNAs, and 18 proteins from photosystem I and II. The *P. strictum* mtDNA has 58,896 bp containing a total of 62 genes, 19 tRNAs, 5 rRNAs, and 13 proteins related to oxidative metabolism. In the phylogenetic analyzes with cpDNA and mtDNA the consensus trees presented some differences in the branching pattern, but *P. juniperinum* and *P. strictum* were grouped in the same clade. This information generated from cpDNA and mtDNA of *P. juniperinum* and *P. strictum* provide a contribution to future phylogenetic studies with the specimens from Arctic.

Keywords: *Polytrichum*, Antarctic, comparative genomics.

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1. INTRODUÇÃO

1.1. A espécie *Polytrichum strictum* e a problemática da filogenia

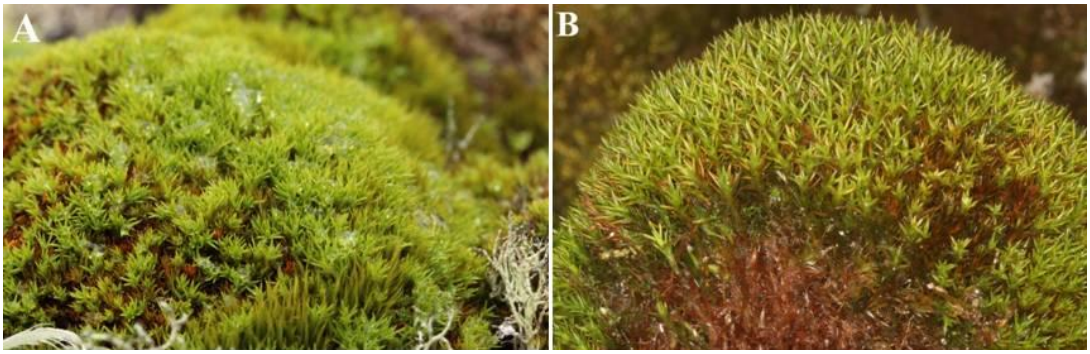
Abordagens baseadas em dados morfológicos, fisiológicos e moleculares em plantas têm identificado as embriófitas como as primeiras plantas terrestres (Bremer *et al.* 1987; Kenrick e Crane, 1997). As embriófitas compreendem seis clados existentes, os quais são denominados de Bryophyta, Marchantiophyta e Anthocerophyta, onde a fase gametofítica é dominante (Qiu *et al*. 2008), além de Lycophyta, Monilophyta e Euphyllophyta. As briófitas *sensu stricto*, assim também denominado o clado da divisão bryophyta que compreende os musgos, são a chave para a compreensão da história evolutiva das plantas terrestres, pois diversas pesquisas, incluindo análises cladísticas de seqüências moleculares mostram que essa divisão emerge há mais de 450 milhões de anos atrás (Kenrick e Crane, 1997). Reconhecidamente um grupo bastante diverso, mais de duzentos anos de estudos briológicos tem levado ao reconhecimento de aproximadamente 13,000 espécies de musgos (Goffinet *et al.*, 2009) distribuídas em todos os continentes (Vanderpoorten e Goffinet, 2009).

Polytrichaceae Schwägr. é uma família da divisão Bryophyta que compreende 23 gêneros, possuindo distribuição natural cosmopolita (Goffinet *et al*., 2009) incluindo também regiões frias como o continente Antártico (Ochyra, 1998) e Ártico (Longton, 1988). Polytrichaceae foi descrita por Schwägrichen (1830) que agrupava as espécies que possuíam gametófitos grandes, caulídios eretos, filídios rígidos com costa lamelada, esporófitos com cápsula angulosa, caliptra pilosa, columela membranacea e epifragma plano. O número de espécies dentro da família Polytrichaceae varia conforme os estudos propostos por diferentes autores (Crosby *et al*. 2000; Churchill e Linares C., 1995). Quanto à classificação, Polytrichaceae é a única família de Polytrichales e, juntamente com Tetraphidales, formam a classe Polytrichopsida. Estudos moleculares recentes (Hyvönen *et al*. 1998, 2004) sustentam Polytrichaceae como um grupo monofilético, com um clado basal constituído pelos gêneros *Alophosia* Cardot, *Atrichopsis* Cardot, *Bartramiopsis* Kindb. e *Lyellia* R. Brown, sendo este o grupo irmão do clado que inclui todos os demais gêneros da família dentre eles *Polytrichum* Hedw.

No Continente Antártico, o gênero *Polytrichum* desempenha um papel importante na vegetação terrestre do bioma, sendo constituinte essencial nas várias comunidades de liquens e musgos (Ochyra, 1998) e compreende três espécies: *Polytrichum juniperinum* Hedw.

(Figura 1B), *Polytrichum piliferum* Hedw. e *Polytrichum strictum* Menzies ex Brid. (Figura 1A) (Greene *et al.* 1970), todas pertencentes a mesma seção.

Figura 1. Vista geral dos exemplares de Polytrichaceae na Ilha Ardley - Antártica. (**A**) À esquerda, a espécie *Polytrichum strictum.* (**B**) À direita, a espécie *Polytrichum juniperinum.*



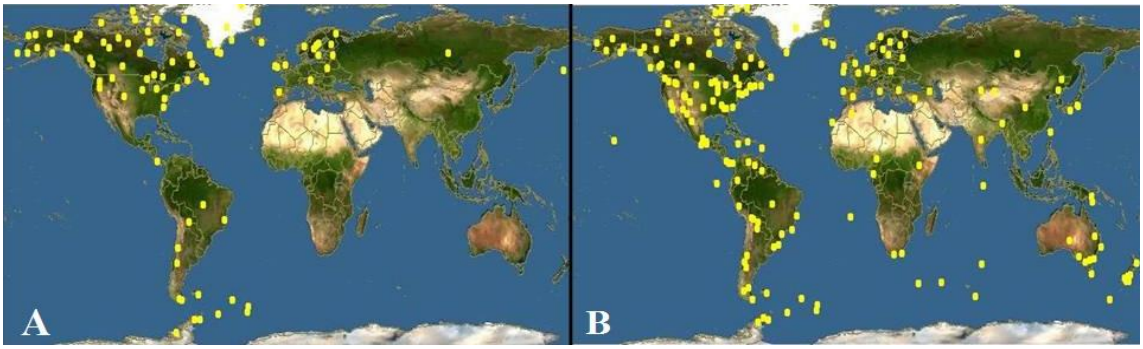
Fonte: Foto de Adriano Spielmann

A região Antártica, é compreendida entre a porção continental e o Oceano Austral, corresponde à extensão de aproximadamente 14 milhões de km^2, representando toda região ao sul do paralelo 60º S (Brasil, 2014). Ambiente dos superlativos, a Antártica apresenta temperaturas severamente baixas; baixa umidade, além de ciclos de descongelamento e congelamento, durante a primavera e outono, e altos níveis de radiação ultravioleta (UV) durante o verão (Lud *et al*., 2001; Kosugi *et al*., 2010), características essas bem suportadas por seres extremófilos, capazes de se desenvolver em ambientes com tais especificidades (Longton e Holdgate 1979; Brasil, 2014). Apesar da inospitalidade, que desfavorece a presença de muitos organismos, principalmente plantas, o continente Antártico possui uma flora criptogâmica significativa. As espécies vegetais lá ocorrentes dividem-se, geograficamente, em endêmicos da Antártica, Subantárticos, sul temperado, bipolar, cosmopolita e tropicais (Øvstedal e Lewis Smith 2001, Ochyra *et al.*, 2008). Predominantemente criptogâmica, a flora é composta por mais de 110 espécies de musgos, sendo 11 espécies endêmicas e 50 musgos bipolares (Ochyra *et al*., 2008). A flora liquênica na Antártica é composta, aproximadamente, por 500 espécies de liquens com cerca de 130 espécies endêmicas (Ochyra *et al*., 2008, Øvstedal e Lewis Smith, 2001; Spielmann e Pereira 2012) e 148 liquens bipolares (Ochyra *et al*., 2008). Segundo Bergh (1947), Stepanjants (1996; 1997) bipolaridade é uma distribuição de espécies idênticas ou estritamente

relacionadas (ou nível de taxa superior) de flora ou fauna nas zonas polares, temperadas ou subtropicais em ambos os hemisférios, sendo caracterizado pela ausência dos indivíduos nos trópicos. Quanto a origem das espécies bipolares, apesar do isolamento geográfico gerado pela deriva continental, as espécies bipolares teriam sua distribuição justificada pela origem holoártica, na qual esses indivíduos teriam chegado às regiões austrais através de dispersão a longa distância (DLD), passando pelas cadeias de montanhas tropicais, sem influência da deriva continental (Ochyra *et al.* 2008). Nesse sentido, o estudo dessas espécies (filogeografia molecular) é fundamental para o entendimento dos fatores que modelam as distribuições naturais bem como suas interações e evolução dentro das áreas onde espécies de ampla distribuição ocorrem.

Polytrichum strictum Menzies ex Brid. (Figura 1A) é amplamente distribuída pelos pólos e próximo dos trópicos (Figura 2A), ocorrendo geralmente em habitats úmidos. Segundo Groeneveld (2007) é pioneiro em locais onde houve perturbações. Na Antártica é encontrado na Ilha Ardley e Península Barton (Ochyra, 1998). Devido sua semelhança com *Polytrichum juniperinum*, desde 1966, *P. strictum* tem motivado estudos taxonômicos para a identificação dos possíveis ancestrais e sua relação com *P. juniperinum* e até outras espécies ao qual foi atribuído como variedade. Morfologicamente, as espécies são bastante semelhantes, muitas vezes não sendo possível a distinção por meio do uso dos caracteres morfológicas utilizados nas chaves de identificação. A espécie difere de *P. juniperinum* na medida em que ocorre em habitats de zonas úmidas de latitude norte (América do Norte) e possui, dentre outras características morfológicas, uma cobertura notável de rizóides brancos (Derda e Wyatt, 2003). Mas tais características não ocorrem como regra, o que torna a diferenciação das duas espécies bastante complicada. Por vezes o táxon foi disposto como uma espécie pelos autores Lawton (1971), Steere (1978), Koponen *et al.* (1977), e Anderson *et al.* (1990), e como uma variedade por Crum e Anderson (1981: "var. Affine") e Osada (1966: "var. Gracilius"). *Polytrichum juniperinum* Hedw. (Figura 1B) é uma espécie de distribuição cosmopolita (Figura 2B) e em alguns locais esta ameaçado de extinção (Hyvönen *et al.*, 2004), adaptado a ambientes abertos, secos e solos arenosos, crescendo sobre uma variedade de turfeiras, especialmente em habitats drenados (Nagelherken e Van der Velde, 2003). Na Antártica é encontrado na maioria das Ilhas da Antártica marítima (Ochyra, 1998). Segundo Derda e Wyatt (2003) a espécie é um grande musgo acrocárpico com ampla distribuição no Hemisfério Norte e Sul, sendo bastante comum em latitudes e elevações mais altas e também pioneiro em certos locais que sofreram perturbações.

Figura 2. Distribuição global da espécie *Polytrichum strictum* (**A**) e distribuição global da espécie *Polytrichum juniperinum* (**B**).



Fonte: http://eol.org

Existem poucos estudos sobre a diversidade genética que envolva uma espécie bipolar e seu respectivo correspondente polar. Em um estudo conduzido por Neil Bell e Jaakko Hyvönen (2010), utilizando regiões conservadas do genoma, os autores sugerem que a espécie bipolar *Polytrichum strictum* poderia ter surgido a partir da hibridização entre a linhagem de *Polytrichum juniperinum* e um táxon basal de *P. juniperinum* com outra Polytrichaceae. Devido a grande semelhança entre as duas espécies e através das análises filogenéticas obtidas a partir de regiões do cloroplasto, mitocôndria e núcleo, os autores propõem que *P. juniperinum* seja o ancestral materno de *P. strictum.* No estudo, foram utilizadas duas amostras de *P. strictum* oriundos do Chile e Finlândia na qual foram avaliados genes e regiões conservadas do genoma plastidial (*rbcL*, espaço intergênico *rps4-trnS, trnL-F*, mitocondrial (gene *nad5*) e nuclear (gene 18S). Devido a existência de eventos de mutação ou introgressão e/ou polimorfismo, eventos normais na evolução das plantas, os resultados da análise bayesiana acabaram tornando-se difíceis de interpretar, apresentando incongruência entre o genoma plastidial, mitocondrial e nuclear. Genes como *rbcl*, possuem uma taxa de mutação muito lenta, assim como a região nuclear *18S*. Essas regiões são utilizadas em pesquisas entre gêneros relacionados por que mostram eventos mais antigos na evolução, porém não é suficientemente variável para mostrar o fluxo gênico entre espécies e distinção filogenética (Judd *et al*, 2009).

Levando em conta a importância das briófitas, tanto para a história evolutiva das plantas e possível potencial biotecnológico dessas plantas (Saidi *et al*. 2005) aliado a modernas tecnologias de sequenciamento de DNA (Hamilton e Buell, 2012) e as diferentes metodologias de montagem e anotação de seqüência, o estudo do genoma de exemplares das briófitas torna-se possível e de fundamental importância. Com as informações anteriores, e

com base no estudo de Bell e Hyvönen (2010), observou-se a necessidade de se obter dados plastidiais e mitocondriais de *Polytrichum strictum* e *Polytrichum juniperinum*, já que filogenias organelares podem proporcionar uma visão complementar, ao invés de redundante, sobre a história evolutiva reticulada de muitos grupos de plantas (Govindarajulu *et al*. 2015). A filogenia organelar dará suporte ao estudo com os espécimes oriundos do Ártico.

1.2. Análise organelar: ferramenta evolutiva e filogenética

1.2.1. Genoma plastidial

Os cloroplastos são organelas pertencentes a uma classe de estruturas intercelulares denominadas plastídios, presente nas plantas e que contém o aparato completo para o processo fotossintético (Bogorad, 2012). A descoberta, que o cloroplasto possui seu próprio DNA, abriu discussão para estudos sobre sua origem, que provavelmente está relacionada a um evento de endossimbiose, pelo qual um organismo protozoário unicelular, através do processo de fagocitose, englobou e manteve uma cianobactéria fotossintetizante (Reyes-Prieto *et al*., 2007), permitindo assim a transição de heterotrofia para autotrofia, adquirindo a capacidade de utilizar fotoenergia (Wicke *et al*., 2011). A integração funcional e genética da antiga cianobactéria na nova célula eucariótica fotossintética foi acompanhada por uma mistura e reestruturação de genomas (Maier e Schmitz-Linneweber, 2004), ou seja, a nível genômico, esta integração envolveu a perda de genes e a transferência de muitos destes genes para o genoma nuclear do hospedeiro (Martin *et al*., 1998, Rujan e Martin, 2001, Martin *et al*., 2002). A maioria dos genes remanescentes no genoma do plastídio tem função reguladora e codificam os componentes do sistema fotossintético, aparato de transcrição e tradução (Maier e Schmitz-Linneweber, 2004).

Considerando que os genomas de plastídios de embriofítas (plantas terrestres) são altamente conservados, o genoma plastidial dos musgos particularmente, consiste de cadeias de dupla fita de DNA de 120 a 160 pb, dispostos em círculos monoméricos e multiméricos (por exemplo, Kolodner e Tewari, 1972; Kowallik e Herrmann, 1972; Lilly *et al*., 2001), bem como em moléculas lineares (Oldenburg e Bendich, 2004). Geralmente, uma unidade básica é subdividida em quatro seções com duas cópias idênticas de uma região de repetição invertida (IR_A e IR_B) de 20 a 30 pb separando uma região grande (LSC) e pequena (SSC). Todos são idênticos em relação a composição gênica, mas existem em duas conformações equimolares que diferem apenas na orientação das regiões de cópia única. No geral, comparando os

genomas nuclear e mitocondrial, o genoma do plastidio é bastante conservado entre as embriofítas, com a observação de algumas exceções na arquitetura geral e tamanho, sendo essa última atribuida principalmente a expansões e contrações evolutivas das regiões IR (Maier e Schmitz-Linneweber, 2004). Quanto a ordem dos genes, apenas uma pequena divergência é observada no cromossomo plastidial das briofitas, sendo geralmente explicado por eventos de inversões. Os genes são geralmente organizados em operons e os poucos rearranjos encontrados na maioria dos casos têm seus limites entre operons (Palmer, 1991). Assim como as mitocôndrias, os plastídeos são, em geral, de origem materna (Harrison e Kidner, 2011).

O primeiro genoma de cloroplasto de musgo a ser totalmente sequenciado foi o da espécie *Physcomitrella patens* (Sugiura *et al*., 2003) que compreendia um tamanho de 122,890 pb (Tabela 1), mantendo a disposição circular do cromossomo e a estrutura quadripartida bem conservada quando comparado com os representantes de hepática e Antoceros, que possuem seus genomas plastidiais sequenciados (Ohyama *et al*., 1986; Kugita *et al*., 2003). Porém quanto à estrutura geral do cloroplasto, houve uma diferença substancial, além disso, observou-se a ausência de genes como *rpoA, cysA, cysT, ccsA.* Quanto ao tamanho do genoma de cloroplasto nos musgos, o maior genoma até hoje já sequenciado é o de *Takakia lepidozioides* (149.016 pb), em contraste o menor foi apresentado pela espécie *Syntrichia ruralis* (122.630 pb) (Tabela 1). Já o conteúdo gênico (número de genes) apresentado pelos cloroplastos das espécies de musgo parece permanecer estável, entre 125 genes, sendo o menor número observado em *Orthotrichum* e o maior em *Takakia* com 138 genes, apesar da disponibilidade de dados sobre genomas de organelas em musgos ser ainda limitada. Segundo informações retiradas do *Organelle Genome Resources* do NCBI (*https://www.ncbi.nlm.nih.gov/genome/browse/?report=5*), atualmente 14 genomas plastidiais de bryophyta *sensu lato* estão disponíveis (Tabela 1).

Tabela 1. Exemplares do grande grupo briófita com o genoma plastidial completo seqüenciado: 8 bryophytas *sensu stricto*, 4 Marchantiophytas, e 2 Anthocerophytas.

Espécie	Acesso NCBI	Tamanho do genoma (pb)
Aneura mirabilis	NC_010359.1	108.007
Anthoceros angustus	NC_004543.1	161.162
Apopellia endiviifolia	NC_019628.1	120.546
Marchantia polymorpha	NC_001319.1	121.024
Nothoceros aenigmaticus	NC_020259.1	153.208

Nyholmiella obtusifolia	NC_026979.1	122.895
Orthotrichum rogeri	NC_026212.1	123.363
Physcomitrella patens	NC_005087.1	122.89
Ptilidium pulcherrimum	NC_015402.1	119.007
Sanionia uncinata	NC_025668.1	124.374
Sphagnum palustre,	NC_030198.1	140.04
Syntrichia ruralis	NC_012052.1	122.063
Takakia lepidozioides	NC_028738.1	149.016
Tetraphis pellucida	NC_024291.1	127.489

Fonte: *https://www.ncbi.nlm.nih.gov/genome/browse/?report=5 modificado pelo autor*.

A ampla quantidade de informações contida no genoma dos plastídeos tem demonstrado que o material genético desta organela pode ser empregado como uma ferramenta adequada e de valor inestimável para a filogenia molecular (Gao *et al*., 2010). Além de oferecer um grande conjunto de genes para análises filogenômicas, também revelam as características estruturais do genoma para complementação das filogenias (Turmel *et al*., 2008). Atualmente, com relação ao uso da filogenômica (utilização de todos os genes do plastídio), a filogenia com base nas sequencias de DNA oriundas do cloroplasto de musgos tem sido proposta por Qiu *et al.* (2006), em contraste, outros estudos como Qiu *et al.* (2007) propõem diferentes topologias, as quais são inferidas com base em algumas regiões do cloroplasto ou, até poucos genes conservados (Bell e Hyvönen, 2010; Chang e Graham, 2012 e outros).

1.2.2.Genoma mitocondrial

As mitocôndrias são organelas ubíquas encontradas em quase todas as células eucarióticas. A principal função delas é providenciar a energia química necessária para as atividades biossintéticas e motoras da célula (Fawcett, 1966). Nas plantas, além de prover a energia celular e a respiração, estas organelas estão envolvidas em outras vias metabólicas incluindo a assimilação do nitrogênio, fotorrespiração, metabolismo do dióxido de carbono, metabolismo ácido das crassuláceas, armazenamento de carbono e nitrogênio durante a germinação das sementes (Douce, 1985), além de outras funções.

Supõe-se que as mitocôndrias originaram-se a partir de um procarioto de vida livre consumidor de oxigênio que por endossimbiose foi capturado por outra célula hospedeira procariótica. Durante a simbiose na célula hospedeira, o genoma mitocondrial de plantas sofreu uma perda massiva de conteúdo gênico, assim como ocorrido no cloroplasto, onde

parte dos genes foram transferidos para o núcleo da célula hospedeira, codificando apenas um conjunto parcial dos componentes das membranas de transdução de energia e componentes da maquinaria de expressão gênica (Hammani e Giege, 2014).

Para a linhagem dos musgos, *Physcomitrella patens* foi a primeira espécie de musgo a ter seu genoma mitocondrial seqüenciado. A sequência completa de nucleotídeos da mitocôndria de *P. patens* consiste em 105.340 pb e contém 3 *rRNAs*, 24 *tRNAs* e 42 proteínas que codificam genes. Esse genoma perde 5 *tRNA*, que supostamente são codificados pelo genoma nuclear. E um grande número de íntrons dentro dos genes foi identificado, e desses íntrons, 9 foram compartilhados com angiospermas e hepáticas. As inversões e translocações observadas no genoma plastidial de *P. patens* podem ser facilmente identificadas entre *Marchantia polymorpha* e *Chara vulgaris*. Observa-se que o genoma mitocondrial de *Phy*scomitrella *patens* perde sintenia quando é comparado com o genoma das angiospermas e clorofitas (Terasawa *et al*., 2007).

O tamanho (Tabela 2) e o conteúdo gênico dos genomas mitocondriais não varia muito na linhagem dos musgos. A espécie *Sphagnum palustre* (Tabela 2) aparece com o maior genoma entre a linhagem dos musgos, apresentando 141.276 pb, porém se comparado exemplares do grande grupo das briófitas, as hepáticas e os Antóceros, *Marchantia polymorpha* apresenta o maior genoma entre as hepáticas, com 186.609 pb, e *Phaeoceros laevis*, o maior entre os Antóceros, com 209.486 pb. O menor genoma entre as espécies de musgo é registrado em *Buxbaumia aphylla*, com 100.725 pb. Segundo Liu *et al.*, (2014), o tamanho da maioria dos musgos é de aproximadamente 100 kb de tamanho e, portanto, o menor entre as plantas terrestres, assim, estima-se que a estrutura do genoma da mitocôndria de musgos permaneceu praticamente congelado durante 350 milhões de anos. Quanto ao conteúdo gênico, o genoma mitocondrial de alguns exemplares da família Grimmiaceae (Gêneros *Bucklandiella, Codriophorus, Racomitrium*) possuem o menor conteúdo gênico, 66 genes. O maior conteúdo gênico entre os musgos foi apresentado por *Anomodon rugelii* com 76 genes (Liu *et al*., 2014). Nos musgos e outras plantas terrestres, o tamanho das regiões de exons permanece bastante constante. Mudanças no tamanho do genoma nos musgos é devido a variações nos introns, e além disso, maiores íntrons são registrados em espécies mais basais de musgo (Liu *et al*. 2014). Segundo informações retiradas do *Organelle Genome Resources* do NCBI (*https://www.ncbi.nlm.nih.gov/genome/browse/?report=5*), atualmente 33 genomas mitocondriais de bryophyta *sensu lato* estão disponíveis (Tabela 2).

Tabela 2. Exemplares do grande grupo bryophyta com o genoma mitocondrial totalmente sequenciado: 28 bryophytas *sensu stricto*, 4 Marchantiophytas e 2 Anthocerophytas.

Espécie	Acesso NCBI	Tamanho do genoma (pb)
Aneura pinguis	NC_026901. 1	165.603
Anomodon attenuatus	NC_021931. 1	104.252
Anomodon rugelii	NC_016121. 1	104.239
Atrichum angustatum	NC_024520. 1	115.146
Bartramia pomiformis	NC_024519. 1	106.198
Bucklandiella orthotrichacea	NC_026974. 1	107.215
Buxbaumia aphylla	NC_024518. 1	100.725
Climacium americanum	NC_024515. 1	105.048
Codriophorus aciculare	NC_026784. 1	106.818
Codriophorus laevigatus	NC_025931. 1	106.809
Codriophorus varius	NC_026891. 1	106.358
Funaria hygrometrica	NC_024523. 1	109.586
Hypnum imponens	NC_024516. 1	103.83
Orthotrichum diaphanum	NC_029356. 1	104.744
Orthotrichum macrocephalum	NC_029355. 1	104.624
Orthotrichum speciosum	NC_026121. 1	104.747
Orthotrichum stellatum	NC_024522. 1	104.131
Oxystegus tenuirostris	NC_028040. 1	105.001
Phaeoceros laevis	NC_013765. 1	209.482
Physcomitrella patens	NC_007945. 1	105.34
Pleurozia purpúrea	NC_013444. 1	168.526
Ptychomnion cygnisetum	NC_024514. 1	104.48
Racomitrium elongatum	NC_026890. 1	106.746
Racomitrium emersum	NC_026975. 1	107.186
Racomitrium ericoides	NC_026540. 1	106.727
Racomitrium lanuginosum	NC_029452. 1	106.795
Sanionia uncinata	NC_027974. 1	104.497
Sphagnum palustre	NC_024521. 1	141.276
Syntrichia filaris	NC_027515. 1	106.343
Tetraphis pellucida	NC_024290. 1	107.73
Tetraplodon fuegianus	NC_028191. 1	104.741
Treubia lacunosa	NC_016122. 1	151.983
Ulota hutchinsiae	NC_024517. 1	104.608

Fonte: *https://www.ncbi.nlm.nih.gov/genome/browse/?report=5* modificado pelo autor

O genoma mitocondrial pode ser utilizado em filogenia de plantas devido a baixa taxa de substituição nucleotídica, menor que no cloroplasto (< 3×) e genes nucleares (<10×), o que resulta em menor saturação e, logo menos homoplasias (Nickrent *et al*. 2000; Qiu *et al*.

2010); geralmente compreendem mais que 40 genes e; as preocupações com o impacto da edição de RNA na reconstrução filogenética (Palmer e Herbon, 1988; Zhang *et al*.,2011; Wolfe *et al.*,1987; Graur e Li, 2000; Drouin *et al*., 2008; Petersen *et al*., 2006 ; Sloan *et al*., 2009; Mower *et al*., 2012) e a suposição de que a história evolutiva dos cloroplastos reflete a da mitocôndria devido à herança materna compartilhada de organelas de plantas (Rieseberg e Soltis, 1991; Olson e McCauley, 2000). Nos musgos foi observado que o genoma mitocondrial possui maior número de genes ortólogos identificados quando comparado com a linhagem das hepáticas. Este achado está em forte concordância com vários estudos anteriores que mostraram hepáticas e musgos como clados irmãos (Qiu *et al.*,2006; 2007; 2008; Chang e Graham, 2012) e a linhagem dos Antóceros foi identificado como uma transição entre as briófitas e pteridófitos (Shanker e Sharma, 2012). Atualmente, com relação ao genoma mitocondrial, as briófitas são classificadas conforme Liu *et al.* (2014).

2. OBJETIVOS

2.1. Objetivo Geral

Este trabalho tem como objetivo determinar a sequência do genoma do cloroplasto e o genoma mitocondrial das Polytrichaceae Antárticas, *Polytrichum juniperinum* e *Polytrichum strictum* visando inferir nas relações filogenéticas entre essas duas espécies na Antártica, além de analisar a estrutura dos genomas acessórios dessas duas espécies.

2.2. Objetivos Específicos

- Sequenciar, montar, anotar o genoma do cloroplasto e mitocôndria de *Polytrichum juniperinum*;
- Sequenciar, montar, anotar o genoma do cloroplasto e mitocôndria de *Polytrichum strictum*;
- Analisar a estrutura dos genomas acessórios de dois representantes de *Polytrichum* na Antártica e comparar com as espécies de briófita que possuem o genoma sequenciado.
- Realizar uma análise filogenética entre *Polytrichum juniperinum e Polytrichum strictum*, os inserindo no grande grupo das briófitas.

3. ARTIGO:

Caracterização e filogenia do cloroplasto e mitocôndria das Polytrichaceae Antárticas, *Polytrichum juniperinum* Hedw. e *Polytrichum strictum* Menzies ex Brid. (Polytrichales, Bryophyta).

(Artigo submetido para a revista Genome Research, conforme normas da revista)

Characterization and phylogenetic analysis of chloroplast and mitochondria from the Antarctic Polytrichaceae, *Polytrichum juniperinum* Hedw. and *Polytrichum strictum* Menzies ex Brid. (Polytrichales, Bryophyta).

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The draft of chloroplast and mitochondria genome sequences of the Antarctic mosses *Polytrichum juniperinum* Hedw. and *Polytrichum strictum* Menzies ex Brid. (Polytrichaceae, Bryophyta) are presented and compared with known mosses plastomes. The size of cpDNA in *P. juniperinum* and *P. strictum* are estimated in 55168 and 20183 base pairs. The size of mtDNA in the species respectively is 88021 and 58896 base pairs. The genomes are very similar to each other with the possible loss of the *petN* gene in cpDNA, which also presented some gene inversions when compared to *Tetraphis pellucida* Hedw. and *Physcomitrella patens* Brid. In mtDNA *rps10* gene was lost. In contrast, Antarctic Polytrichaceae remains with the genes *nad7* and *orf187,* without the occurrence of rearrangement events. The assembly of *P. juniperinum* and *P. strictum* genomes showed high similarity between the genomic features. Phylogenetic analyses with plastid and mitochondria revealed that majoritary rule tree present some differences in the branching pattern, however *P. juniperinum* and *P. strictum* species, were grouped in the same clade. This study presented details that contributed to understand the evolution of plastomes and chondromes in Polytrichaceae family although not propose news phylogenetic hypotheses related to the origin of the Antarctic moss, *P. strictum.*

Polytrichum is a cosmopolitan genus with a continental distribution (from Arctic to the Antarctic Continents) (Ochyra, 1998). In Antarctic, three species have been reported, all confined to the maritime Antarctic: *Polytrichum juniperinum* Hedw., *Polytrichum piliferum* Hedw. and *Polytrichum strictum* Menzies ex Brid. (Greene *et al.*, 1970). They play an important role in the terrestrial vegetation in this biome as essential constituents in the various communities of moss turf subformation as well as in the fruticose lichen (Ochyra, 1998). Phylogenetic relationships of Polytrichales are particularly relevant to consideration of the evolutionary history of mosses since the group is probably among the first of the lineages that diverged from the common ancestor of all mosses (Mishler and Churchill, 1984). Recent development in plastome sequencing is the use of total genomic DNA as template for next generation sequencing (Nock *et al*., 2010, Atherton *et al*., 2010). The outcome of these new

developments was the huge improvements in our understanding of phylogenetic relationship among plants and still most specific, the mosses. Previous work suggested that *P. strictum* arose from a reticulation event and *P. juniperinum* species is probably its maternal ancestor (Bell and Hyvönen, 2010). However, the phylogenetic position of *P. strictum* is still unclear. Thus, know these relationship it is necessary to expand the quantity and quality of the phylogenetic characters available to inform relationships among *P. juniperinum* and *P. strictum* in Antarctic continent.

Results

Genomic organization and gene content

After assembly the *Polytrichum juniperinum* plastid genome (cpDNA) obtained was 55168 bp in lengh and presented a G+C content of 44.9% (Additional table 3), including 51 putative coding genes, 31 *tRNAs*, and 4 *rRNAs*. Furthermore, the cpDNA revealed 19 putative protein-coding genes related to photosynthesis, such as photosystem I and II putative proteins. The *Polytrichum strictum* cpDNA assembly generated a draft genome of 20183 bp with a G+C content of 46.8% (Additional table 3), similar to *P. juniperinum*. The cpDNA of *P. strictum* also presented 44 putative coding genes, 14 *tRNAs*, and 4 *rRNAs*, and 18 putative protein-coding genes related to photosynthesis, such as photosystem I and II putative proteins.

Our data confirmed the absence of *rpoA* gene in cpDNA of *P. juniperinum* and *P. strictum* (Table 1). Another gene that was absent in *Tortulla ruralis* (Oliver *et al.*, 2010) and *Tetraphis pellucida* Hedw. (Bell *et al.*, 2014), is the *petN* (Table 1) that is likely translocated to the nucleus in *Polytrichum juniperinum* and *Polytrichum strictum*. The BLAST analyses showed that *ycf66* gene had 90% identity between Antarctic Polytrichaceae. In *P. juniperinum* this gene presented 100% identity with the homologous *Sanionia uncinata* (Hedw.) Loeske and in *P. strictum* presented 94.3% identity with the homologous *Tortulla ruralis*. Some gene regions were found with an identity smaller than 90% compared to the reference, for example

psaB, *trnV* (Fig. 1) and *ndhD* gene (Fig. 1 and 2). We also observed one inversion event in the gene *psaI* between *Tetraphis pellucida* and *Polytrichum juniperinum* (Fig. 2), and two inversions between *Physcomitrella patens* and the two species of the *Polytrichum* genera with the genes *ndhA* and *ycf2* (Fig. 1).

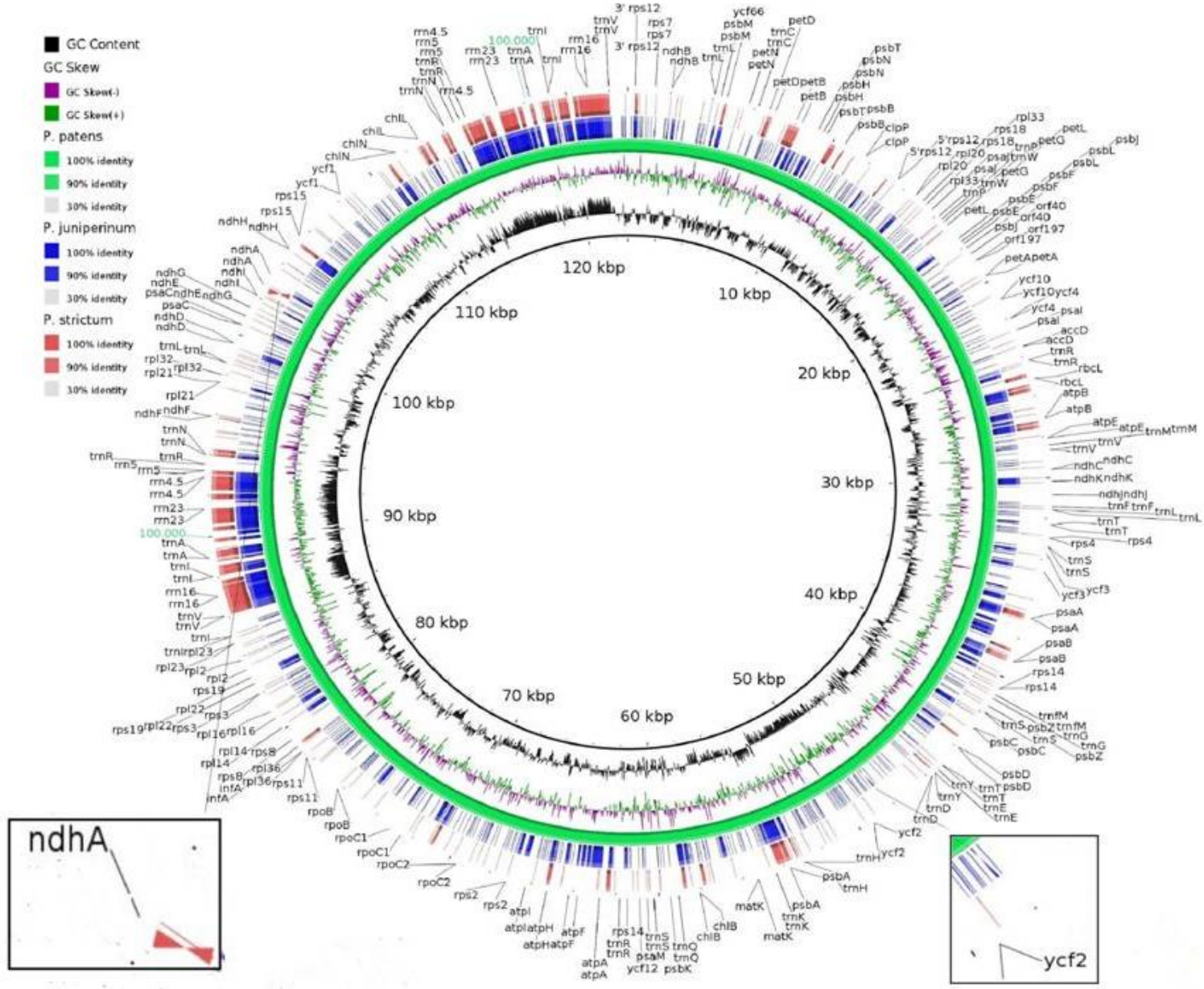

Figure 1: BRIG output image of draft chloroplast genome comparing *Physcomitrella patens* and Antarctic *Polytrichum* species**.** The internal ring represents the *Physcomitrella patens* chloroplast genome (green). The BLAST comparisons with *Polytrichum juniperinum* and *Polytrichum strictum* are depicted by blue and red respectively. The innermost rings show the GC skew (purple/green) and GC content (black). The highlighted blocks show the inversions observed between *P. strictum* and *P. juniperinum* with the reference genome.

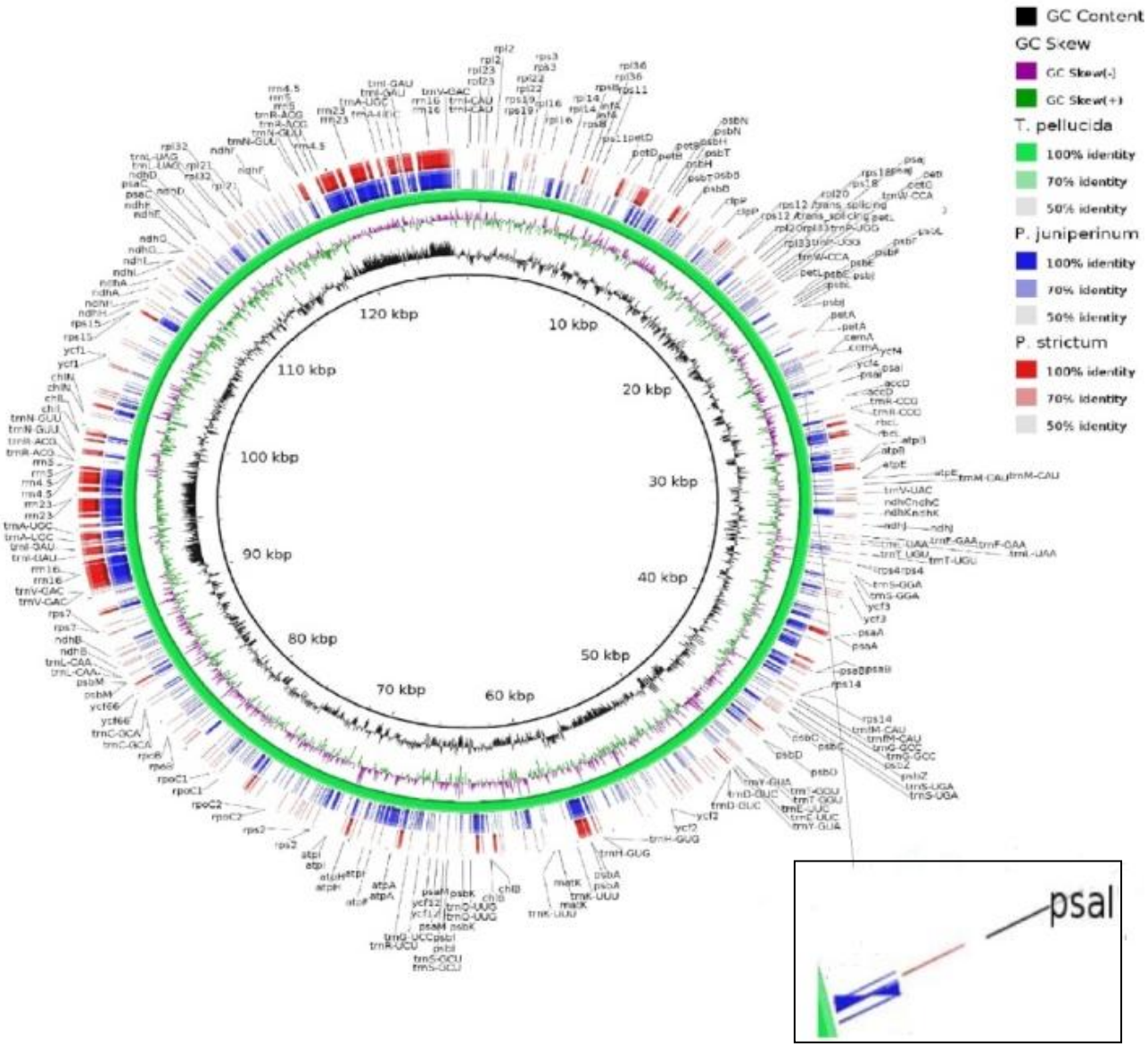

Figure 2. BRIG output image of draft chloroplast genome comparing *Tetraphis pellucida* and Antarctic *Polytrichum* species**.** The internal rings represent the *Tetraphis pellucida* genome (green), the BLAST comparisons with *Polytrichum juniperinum* (blue) and *Polytrichum strictum* (red). Around of the map it is indicated the referred chloroplast genes. The innermost rings show GC skew (purple/green) and GC content (black). The highlighted block shows the inversions observed between *P. juniperinum* and *Tetraphis pellucida*.

The genes *psaJ, psaM, atpE, rpl36, rps14* are absent in the two draft genomes, but has been reported in *Physcomitrella patens* (Sugiura *et al*., 2003). Other genes were analyzed separately, as they were not found with the tools used to annotation in this study. For example, the *psaM* and *ccsA* cpDNA genes in *Polytrichum juniperinum* and *psaI, rpl23, rpl32, rps7,ycf4, ccsA, matK* well as certain *tRNA* in cpDNA for *Polytrichum strictum*, were found only with BLAST (Altschul *et al*, 1997) using total genome Polytrichaceae, and a lower *e-value* (e^{-5}). It is possible that these regions were not sequenced and therefore are not included in the percentage of genome coverage or, these genes probably have high degree of rearrangement (deletions, tandem duplications, and inversions) and substitutions. According to Silva (2013), Scaffold builder is not effective in the assembly when the sequences have high degree of rearrangements, and the sequences of these genes and the sequences of the genome has at least 80% identity. These genes were not accounted for these analyses, and further studies of presence/absence of these genes in moss Polytrichaceae are needed.

The *Polytrichum juniperinum* mitochondrial genome (mtDNA) has a total 88021 bp and GC content of 41.4%. In total, this genome contains 67 genes including two ribosomal RNA genes (one *rnl* and one *rns*), 19 transfer *RNAs*, 3 *rRNAs*, 3 *Open reading frames* (*ORF533, ORF622* and *ORF187*) and 12 protein-coding genes related to mitochondrial oxidative metabolism. Among these, 4 ribosomal proteins (4 *rpl* and 8 *rps*) with absence of *rps10* gene. The *Polytrichum strictum* mtDNA have 58896 bp in length, GC content 41.1%. The genome contains an total of 62 genes, the same content of ribosomal RNA genes than in *P. juniperinum* (one *rnl* and one *rns*), 19 transfer *RNAs*, 3 *rRNAs*, 3 *Open reading frames* (ORF533, *ORF622* and *ORF187*), 13 protein-coding genes related to mitochondrial oxidative metabolism and 13 ribosomal proteins (4 *rpl* and 9 *rps*). The *rps10* gene that encoded a protein from 40S subunit of ribossome apparently was lost in Antarctic Polytrichaceae. This absence is evidenced in Tetraphidaceae (Bell *et al.*, 2014), and Funaraceae (Terasawa *et al.*,

2007). The *nad7* pseudogene in *Marchantia polymorpha* and *ORF187* is frequent in mtDNA of *M. polymorpha* (Oda *et al*., 1992) and contradictorily does not happen in *Tetraphis pellucida* but seems present in *Polytrichum* studded (table 1). In analysis with BLAST, the *nad7* gene has 55.3% identity between species studied here, and 43.8% for the *ORF187*. The *nad7* gene from *P. juniperinum* has 98% of identity with your homologous in *Sanionia uncinata* and *P. strictum* has 97.4% identity with homologous *Atrichum angustatum*. The *ORF187* of *P. juniperinum* presented 96.8% of identity with the *ORF187* in *Marchantia paleaceae* and *P. strictum* presented 98.6% identity with your homologous *A. angustatum.* This demonstrates that in relation to the analysis of these genes, *ycf66, nad7* and *ORF187*, between the two species of *Polytrichum*, appears to be conserved independent, presenting significant rates of mismatch in the sequence, possessing homologous genes in different species. In the same way as in cpDNA, the genes *rps19, nad3, sdh3, atp8* and *atp9* were not found in mtDNA of *P. strictum*.

By another hand, in mtDNA is evident that *Polytrichum juniperinum* and *Polytrichum strictum* share more blocks with *Physcomitrella patens* (fig. 3) than with *Tetraphis pellucida* (Supplementary figure 1). In mitochondria genome were not observed rearrangement.

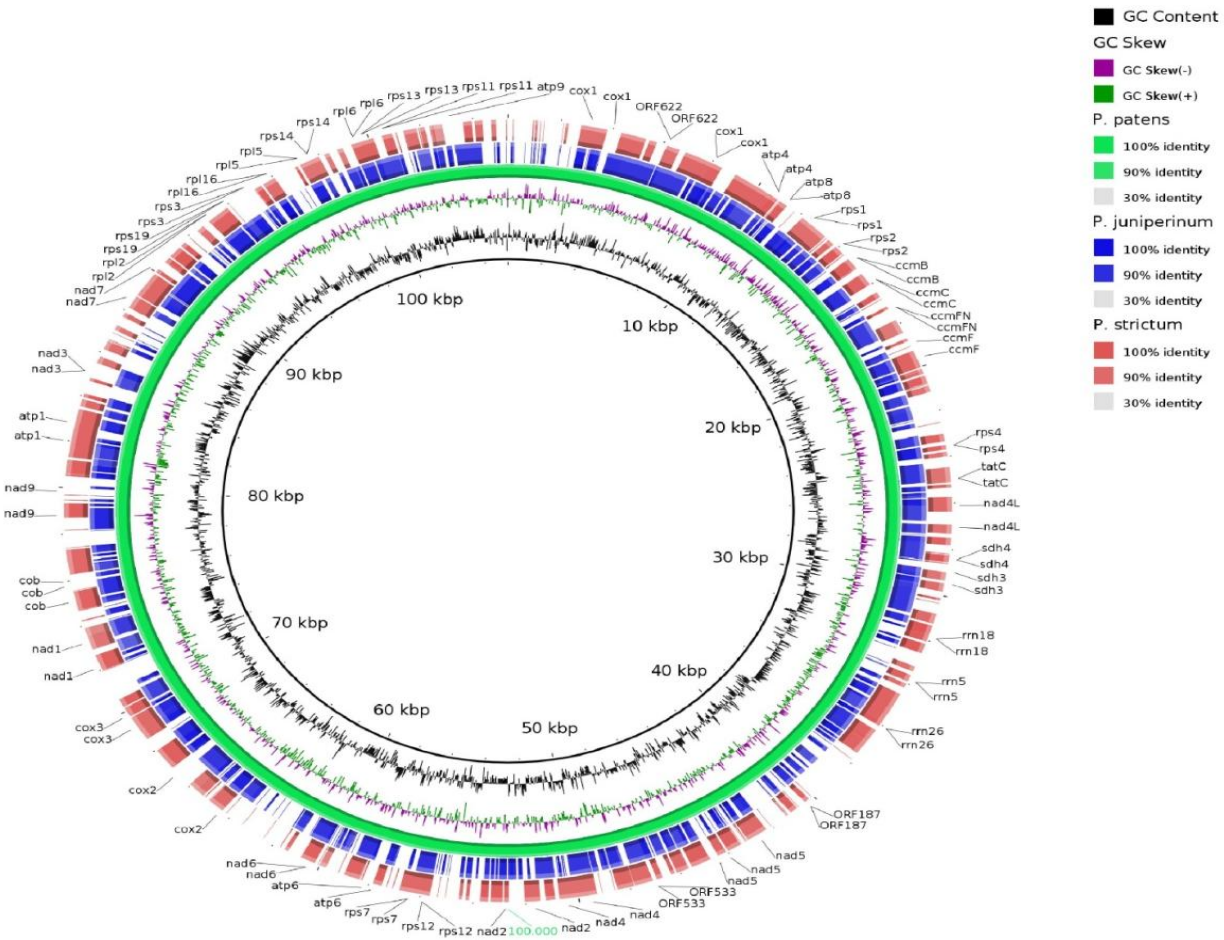

Figure 3: BRIG output image of draft mitochondrial genome**.** The internal rings represent the *Physcomitrella patens* genome (green), the BLAST comparisons with *Polytrichum juniperinum* (blue) and *Polytrichum strictum* (red). Around of the map it is indicated the referred mitochondrial genes. The innermost rings show GC skew (purple/green) and GC content (black).

The differences in gene content in cpDNAs and mtDNAs of the three classes of bryophytes, including the representatives of Polytrichaceae family and seed plants are summarized in Table 1. In chloroplast and mitochondria genome, the two algae lineage diverge in the content of preserved genes and those that have been lost, this shows how the algal lineage varies in both size and gene content due to the various rearrangements that occurred during its evolution (Graham, 1996). *Marchantia polymorpha* with its large size cpDNA (Ohyama *et al*., 1986) and mtDNA (Ohyama *et al.,* 1996) genome remains with some genes compared with other species include many *ORFs* predicted as possible genes, as *ORF187* that is shared with *Polytrichum* but not *Tetraphis pellucida. Marchantia polymorpha* share with some mosses, but not *Physcomitrella patens*, the lack of *petN* gene from cpDNA. This specie has as pseudogene, the mitochondrial *nad7* and *Anthoceros formosae* (Kugita *et al*., 2003) has as pseudogenes, *maturase K* and *rps15,* characterizing these two pseudogenes in cpDNA from Anthocerophyta (Kugita *et al*., 2003). Also, pseudogene is considered the *nad7* from *Tetraphis pellucida* (Bell *et al*., 2014). The mosses share practically the same gene lack between their representants, only *T. pellucida* that lack *rps10* gene in mtDNA, moreover of absence of the *ORF187*. Seed plants has usually an gene content which apparently not differ substantially, and, in recently study, gene loss of plastid is associated to increase the parasitism (Funk *et al.,* 2007).

Table 1: Gene content of cpDNA and mtDNA from alga, bryophytes and land plants.

Plants	chloroplast					mitochondrial		
	rpoA	***ycf66***	***petN***	***matK***	***rps15***	***rps10***	***nad7***	***ORF187***
Chlorella sp.	+	-	-	-	-	+	+	-
Chaetosphaeridium globosum	+	+	+	+	+	+	+	-
Marchantia polymorpha	+	+	-	+	+	+	Ψ	+
Anthoceros formosae	+	-	+	Ψ	Ψ	0	0	0
Physcomitrella patens	-	+	+	+	+	+	+	+
Polytrichum juniperinum	-	+	-	-	+	-	+	+
Polytrichum strictum	-	+	-	-	+	-	+	+
Tetraphis pellucida	-	+	-	+	+	-	Ψ	-
Arabidopsis thaliana	+	-	-	+	+	-	+	+
Oryza sativa	+	-	+	+	+	+	+	+
Triticum aestivum	+	-	-	+	+	-	+	-

The presence (+) or absence (-) of each molecular character, absence of molecular data (0) and pseudogene (Ψ) are shown. The data comes from NCBI database (*https://www.ncbi.nlm.nih.gov/*).

Phylogenetic analysis: chloroplast and mitochondria

The phylogenetic analysis of chloroplast included sequences of 22 genes (*psaA, psaC, psbB, psbI, psbF, psbJ, psbM, psbN, psbT, psbZ, petD, petG, atpH, atpI, chlL, rbcL, rpl16, rpl20, rpl21, rps15, rpoB, rrn5*) of 8 species of the moss division, 4 species of the Marchantiophyta division, 2 species of Anthocerophyta as outgroup (already chosen due to be outside the clade of interest), and additionally the *Polytrichum* species. The majoritary rule constructed with plastid genes show a branching into three clades that agrees the taxonomic classification of division Bryophyta *sensu lato* and *Marchantia polymorpha* L. as the most basal lineage (Fig.5). Peristomate mosses (Edwards *et al*., 1984) were resolved as a clade supported by 0, 99 Bayesian posterior probability (pP) with the representatives of *Sphagnum* as basal lineage. A sister relationship between *Polytrichum juniperinum* and *Polytrichum strictum* received high support (pP = 1). Furthermore, these species were grouped with *Tetraphis pellucida*, indicating *Polytrichum* as an apparent basal lineage and so these grouping characterizes the nematodontous mosses (Bell *et al*., 2014; Magombo, 2003), supported by a pP of 1. These positioning is corroborate by other studies (Volkmar and Knoop, 2010; Ligrone and Ducket,

2011; Cox *et al*., 2004; Hyvönen *et al*., 2004; Newton *et al*., 2000). However, contradictorily other authors reported Tetraphidopsida as basal group of Polytrichopsida (Qiu *et al*., 2006). The remaining species of Marchantiophyta division formed a clade with little support (Pp = 0, 60) but is according to the phylogenomic study of Qiu *et al*., (2006). Finally the species of Anthocerophyta, *Nothoceros aenigmaticus* and A*nthoceros angustus* form a supported group (pP = 0, 93) and is according Qiu *et al*. (2006). These tree topology showed no conflicting clade being according with Newton *et al*. (2000) and Goffinet and Buck (2004), with exception the clade that place *Takakia lepidozioides* with *Syntrichia ruralis* and according Qiu *et al*. (2006) differs only in positioning the nematodontous mosses and the moss, *T. lepidozioides*.

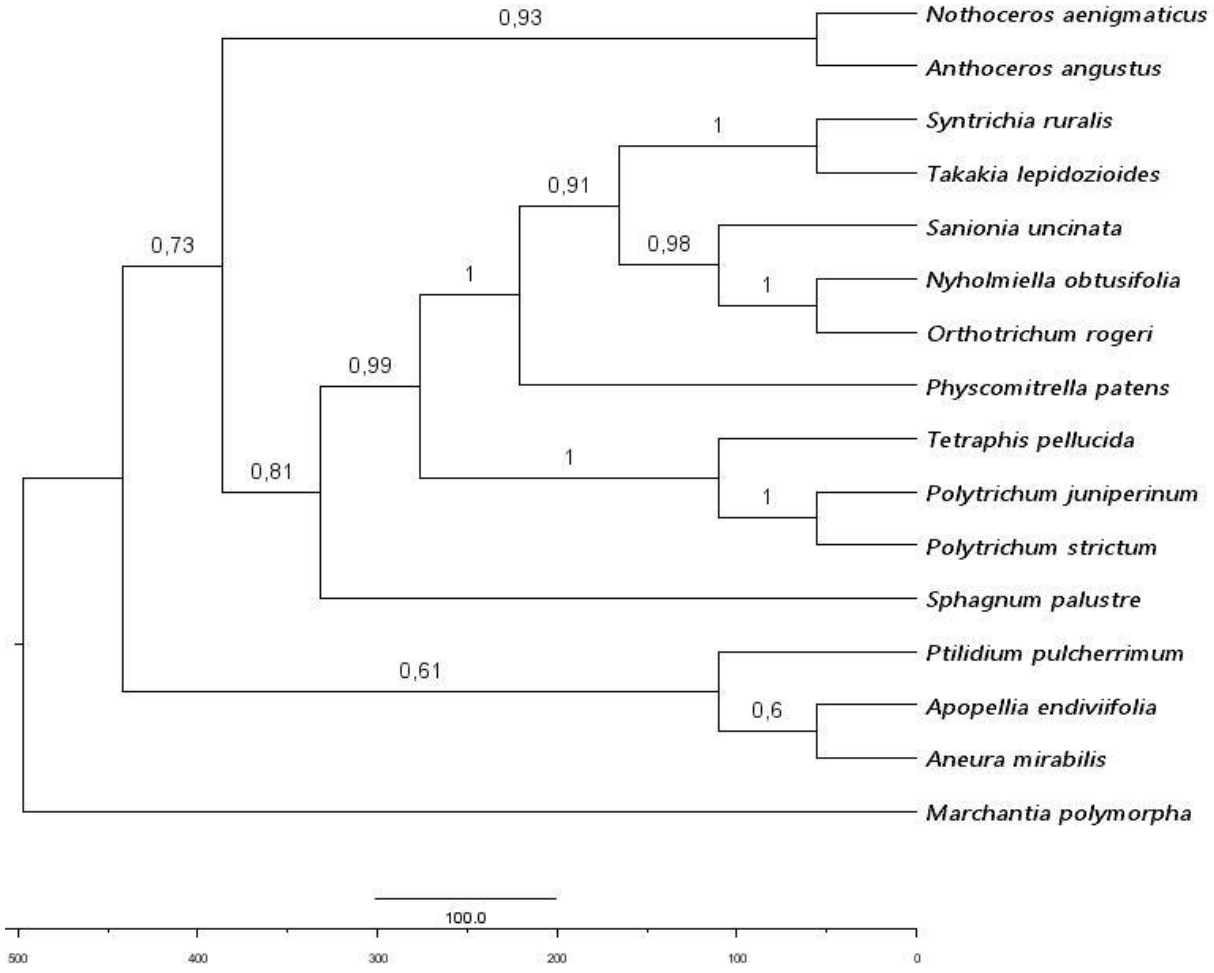

Figure 5. Maximum clade credibility tree resulting from Bayesian analysis of chloroplast genes dataset. The robustness of each node is represented by posterior probability value (Pp) that varies between 0 and 1, and was obtained after 10.000.000 Monte Carlo Markovian chains (MCMC). The tree was reroot utility *M. polymorpha* due to be outside the clade of interest. Time scale root age estimated in 497 ma (http://www.timetree.org/).

Phylogenetic relationship resulting from majoritary rule inferred from 9 sequences of mitochondrial genes (*atp1, ccmB, cob, nad3, nad4, rpl5, rpl6, rps1, rps11*) of 31 mosses species, included *Polytrichum* species studied, 2 species of hornworts and 4 species from liverworts are shown in figure 6. The species of Marchatiophyta and Anthocerophyta are considered the outgroup (already chosen due to be outside the clade of interest). The analyzed species in this study are placed in three separate clades, one corresponding to Bryophytes, one to Marchantiophyta species and one to Anthocerophyta, in addition include *Treubia lacunosa* as basal lineage. The two representative species of Antarctic *Polytrichum* and Funariaceae family closely related species of mosses, forming a little supported clade (pP = 0, 83). The Polytrichaceae *Atrichum angustatum* are grouped to clade with *Tetraphis pellucida*. The phylogenetic relationships of *Polytrichum juniperinum* and *Polytrichum strictum* are little supported by 0, 91 pP, despite of this grouped cannot contribute to origin of *P. strictum* and seems be more basal position that *P. juniperinum*. The topology of tree is according with the proposed by Liu *et al*., (2014) that utility total mitochondrial data for positioning Bryophytes. The nematodontous mosses have been grouped according to Ligrone and Duckett (2011).

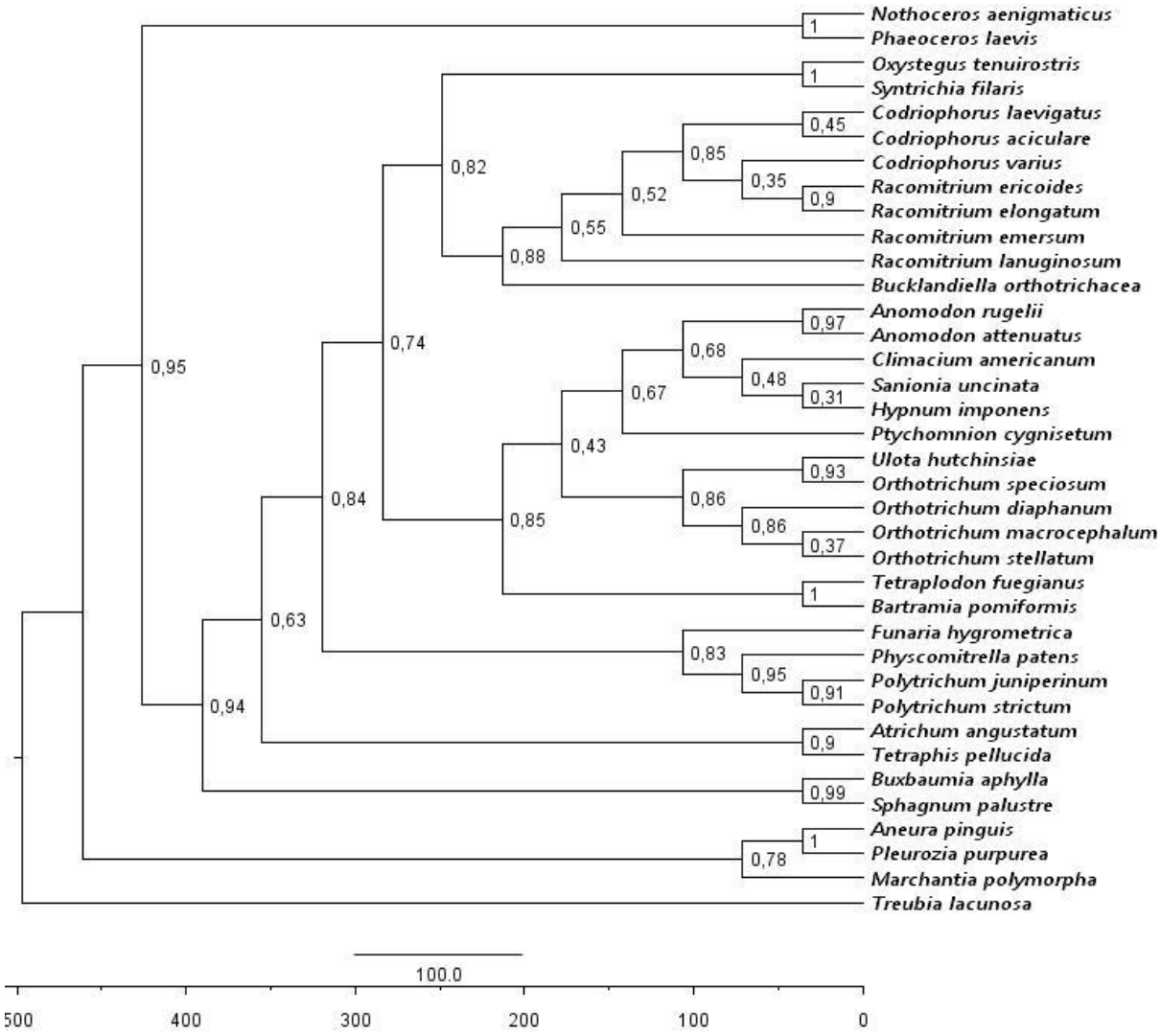

Figure 6. Maximum clade credibility tree resulting from Bayesian analysis of mitochondria genes dataset. The robustness of each node is represented by posterior probability value obtained after 10.000.000 Monte Carlo Markovian chains (MCMC). The tree was reroot utility *T. lacunosa* due to be outside the clade of interest. Time scale root age estimated in 497 ma (http://www.timetree.org/).

Discussion

The chloroplast draft genome of *Polytrichum juniperinum* and *Polytrichum strictum* presented a smaller cpDNA if it is compared to the moss, liverworts and hornworts species

(Shanker, 2012). But these little size is related to the percentage of coverage from cpDNA genomes of these two species, but this not interfere in G + C content, what is enough higher, although plastid genomes of the closest *Polytrichum* species present GC percentage genomes between 28 to 33% (Sugiura *et al.,* 2003; Bell *et al.,* 2014) and in seed plants ranges between 34% and 40% (Jansen and Ruhlman, 2012). For Cai *et al.,* (2008), high G + C contents are observed in the chloroplast coding regions, and certain regions have even higher percentages than others, such as the IR region with its four genes having high levels of guanine and cytosine. In this sense, there is an unequal distribution of GC content in the chloroplast, and perhaps the higher content presented here, by the Polytrichaceas, refers to low coverage present and to the significantly number of coding regions that were scaffolded. The mitochondria of the Polytrichaceae, present small size compared to other mosses, similar to the mtDNA from *Buxbaumia aphylla,* although the mitochondrial genome of mosses has a size between 100 000 and 141 000 bp, and the Anthocerophyta have the largest, present an size chondriome of 209 482 bp. Despite the small size of the mitochondrial genome presented, the % GC is in agreement with the mitochondrial GC content of other species of mosses (Bell *et al.,* 2014; Teresawa *et al.,* 2007; Liu *et al.,* 2011).

The gene content of cpDNA in the two Polytrichaceae genome is similar to *Tetraphis pellucida,* however, Polytrichaceae did not present the *maturase K* in their genome, but more studies are needed to evidence this loss. The *rpoA* gene seems to have been lost in Polytrichaceae. These gene absence had been reported in *T. pellucida* as well as in all arthrodontous groups (Goffinet *et al.,* 2000, Cox *et al*., 1999). Goffinet *et al.,* (2005) shows that *rpoA* seems not to have been lost in *Polytrichum pallidisetum* Funck. but in our data we did not identify this gene, and it's possible that in Antarctic Polytrichaceae this gene has been lost or translocated. According Sheveleva *et al.,* (2002), presence of *rpoA* gene is quite variable from species to species. The membrane tilakoide gene *ycf66* is absent in *Anthoceros*

formosae Steph (Kugita *et al.,* 2003), *Arabidopsis thaliana* (Sato *et al.,* 1999) and *Chlorella vulgaris* (Wakasugi, 1997) but remains more stable in Polytrichaceae than in ferns (Gao *et al.,* 2011). For the *petN* gene, only two species of bryophyte are currently known to lack this gene (Bell *et al*., 2014; Oliver *et al*., 2010), part of the photosynthetic cytochrome b6lf complex in the chloroplast, and it is possible, according Oliver *et al.*,(2010) that another nuclear-encoded gene product performs the same function as a subunit of the complex.

The overall gene content of the mitochondria genome from the two Polytrichaceae is very similar to *Physcomitrella patens,* as observed in figure 3. The *rps10* gene was absent from Polytrichaceae mitochondrial genome but remains in *P. patens,* Adams *et al*., (2000) reports that *rps10* gene had a frequent loss (26 times) and transfer to the nucleus among 277 diverse angiosperms, and suggests in their study that gene loss is a frequent event. The mitochondrial genes that seems remained in Polytrichaceae species are *nad7* and the open reading frame 187, these identified initially in *M. polymorpha* (Ohyama, 1996) and after in *P. patens* mtDNA (Terasawa *et al.,* 2007). The *nad7* mitochondrial gene absence in *Nicotiana sylvestris* CMSII mutant caused abnormal phenotype, poor growth and male sterility and this has caused by total deletion of *nad7* gene (Lelandais *et al.,* 1998). In Antarctic Polytrichaceae, perhaps *nad7* gene has a key role in sustaining phenotype.

According Jansen and Ruhlman (2012), some genes seem to have been lost multiple times in chloroplast and mitochondrial genomes during the evolution and, other genes appear to be distinctly present or absent in particular clades. For example, a diversity of genes is lack in mosses and liverworts as *rps16,* but the gene is present in hornworts and some vascular plants. The gene *psaM* is lacking from the three polypod ferns (*Adiantum capillus-veneris, Cheilanthes lindheimeri* and *Pteridium aquilinum*), as well as from the two *Selaginella* plastomes and the majority of seed plant plastomes. Seed plant plastomes lack *rpl21,* as do the two *Selaginella* plastomes. In angiosperms far most of the gene transfer to the nuclear genome

affects genes for subunits of the ribosomal proteins as *rps* and *rpl* (Adams *et al.*, 2000). In contrast, some genes remained present, as the plastid gene *ycf66* that looks like an independent loss in multiple clades in land plants including hornworts, ferns and seed plants (Lei *et al.,* 2009). The gene transfer is a continuous event in plant evolution and, this is promoted, possibly by high frequency translocation of gene rich organelle DNA into the nucleus and the relatively rare, or entirely absent, transfer of DNA encoding complete genes from the nucleus to the organelles (Doolittle, 1998). To infer in the overall structure and specific presence or absence gene of the plastidial and mitochondrial genome from the two *Polytrichum* will be necessary more analyzes.

The chloroplast and mitochondrial genome between *Polytrichum juniperinum* and *Polytrichum strictum* are identical in overall gene content and structure as shown on the maps, although *P. strictum* genomes present few coverage. Were observed variations in chloroplast genomes, as example, inversions. Inversions represent one type of rearrangement, on here were observed two gene inversions between the cpDNA from *P. juniperinum* which is not shared with *Physcomitrella patens* and *Tetraphis pellucida*, and one inversion between the cpDNA of *Polytrichum strictum* and *Physcomitrella patens.* The gene content and gene arrangement of the chloroplast is highly conserved in land plants (Palmer, 1991). Large inversions and other chloroplast genome rearrangements are relatively uncommon among land plants (Downie and Palmer, 1992), but small inversions are commons and widespread in the plant plastid genomes, and have been reported from a variety of plants include bryophytes (Quandt *et al.,* 2003, Huttunen and Ignatov, 2004; Hernandez-Maqueda *et al.,* 2008). Small inversions such as these, on a general plane, provide a rather interesting phylogenetic marker between the species, and at the same time, provide a vision of relationships from groups. These inversions that occurred seems restricted to the species, and do not characterize the genera. The mitochondrial genome shows conserved between Antarctic *Polytrichum* and the

closest species. Studies show that the structural evolution of mitochondrial genome is highly conservative not only within each individual lineage, but also across mosses, however this is most evident when compared to more distant orders within the large group Bryophyta, that present some rearrangements but even so are very conserved (Jansen and Ruhlman, 2012). It was observed the occurrence of some rearrangements when compared to *Marchantia polymorpha* mtDNA and *P. patens*, being that these species diverged more than 375 million years ago (Allen *et al.,* 2007).

Over the last decades single gene phylogenetic analyses have served as powerful tools for reconstructing the evolutionary history of every major lineage of life on earth (Donoghue and Cracraft, 2004). Indeed, with next-generation sequencing technologies, complete plastome sequences are now being generated at an ever increasing rate (Cronn *et al.,* 2008; Wolf *et al.,* 2011; Henson *et al*., 2012). We sought to analyze the phylogenetic position of *Polytrichum juniperinum* and *Polytrichum strictum* using the plastid and mitochondrial gene data, of representatives mosses families, hornworts and liverworts deposited in *GenBank*. The question that we would like to answer or provide hipotesis is the origin of the specie *P. strictum*, since exist uncertainty of her origin and definition as specie or variant of *P. juniperinum. Polytrichum strictum* has morphological characteristics similar to *P. juniperinum* (Lawton, 1971, Steere, 1978, Koponen *et al.,* 1977, Anderson *et al.,* 1990) and differs from *P. juniperinum* in that it occurs in habitats of north latitude wetlands (North America) and has, among other morphological characteristics, a remarkable coverage of white rhizomes (Derda e Wyatt, 2003). Bell and Hyvönen (2010), conducting a study on the phylogeny of mosses of the class Polytrichopsida, proposed that the origin of *P. strictum* (samples used were from Chile and Finland) could be from a cross linking event. *P. strictum* could be the product of the hybridization between the *P. juniperinum* linhage (sample used from Finland) and a basal linhage of another Polytrichaceae representant not estabilished. According to the topology

presented by the study, the samples of the specie *P. strictum* were grouped in a same branch, and *Polytrichum juniperinum* appears in a branch brother to the *Polytrichum strictum* as maternal ancestor of *P. strictum*. And still, as reported by the authors, given the lack of well supported resolution for the position of *Polytrichum hyperboreum* and *Polytrichum piliferum,* one of these species, or a related extinct taxon, could easily be the paternal progenitor. In another study with Polytrichales, molecular and morphological data suggests grouping of *P. juniperinum* and *Polytrichum piliferum* (Hyvönen *et al.,* 2004).

The phylogenetic analysis from chloroplast partial data presented relatively support branch (Fig. 5). The Bayesian analysis was chosen in this study due to would be more effective to the large number of data used in phylogenetic analysis. The nematodontous mosses are grouped in the same clade suggesting that *Polytrichum strictum* is a sister of *Polytrichum juniperinum* and *Tetraphis pellucida* appears as a merge of this species. This topology is according several authors that study nematodontous mosses (Bell *et al.,* 2014) and not agrees with Cox *et al.,* (2014). The Bryopsida clade comprising the species *Nyholmiella obtusifolia, Orthotrichum rogeri, Syntrichia ruralis, Sanionia uncinata* and the Takakiopsida *Takakia lepidozioides* was supported, but not agrees with some authors that have proposed different topologies for the class Bryopsida (Cox *et al.,* 2010; La Farge *et al*., 2000; De Luna *et al*., 1999; De Luna *et al*., 2000). The clade that group *Takakia lepidozioides* and *Syntrichia ruralis* be conflict with some authors. This can occur in this study due to the large number of genes have been used in the attempt for a broader analysis (but not all genes of chloroplast). Many of these genes provide insufficient information to the high-resolution necessary to differentiate some clade (Daniell *et al.* 2016, Liu *et al*., 2014) but some genes or regions have undergone a higher selection pressure than others and may lead to different tree topologies than those already proposed in other studies in relation to certain taxa. An example is the study proposed by Newton *et al*. (2000) showing that the spacer region *trnL-trnF* presented a

different topology for the clade containing *Takakia lepidozioides* in relation to the topology presented from *rbcL* and *rps4*. Though a smaller number of chloroplast genomes are available for mosses species compared with mitochondria genomes, with larger number of date it could be reconstruct the phylogenetic positioning of some branches.

In analysis with mitochondrial genes we observed a high branch support for most nodes and little support in some branch as the node of *Racomitrium* and *Codriophorus* but even so agrees with Sawicki *et al*., (2015) that grouping species from Grimniaceae family. The same occurred with node *Climacium americanum, Sanionia uncinata, Hypnum imponens* with Orthotrichaceae. Polytrichaceae representants that appears forming a clade with *Physcomitrella patens* and *Funaria hygrometrica,* but *Atrichum angustatum* appears outside to this clade. Despite *A. angustatum* be more basal specie in Polytrichaceae phylogeny (Bell and Hyvönen, 2010), here the specie seems to be misplaced in mitochondria tree. This possible error can occur due to *Polytrichum* assembly genome was based in one reference genome and this can induce the genomes be more similar to *P. patens* and *A. angustatum*, consequently will remain outside to this clade.

Our mitochondrial phylogenomic tree (Fig. 6) not matches with reconstructed plastid tree (Fig. 5) presenting another topology. Differential inheritance of organelles in the same cytoplasm can break the typically expected linkage equilibrium between chloroplast and mitochondrion (Thyssen *et al.,* 2012, Tsujimura *et al.,* 2013) and if this happened, phylogenetic reconstructions of these two organellar genomes can conflict. The uniparental inheritance in *Rhizomnium* moss for chloroplast as mitochondria genomes was already reported (Jankowiak *et al.,* 2005). In contrast to higher plants, have been few studies of organellar inheritance in bryophytes (Guillon and Raquin, 2000), although the maternal inheritance of the chloroplast of mosses has already been reported. Another study reported that incongruence can be caused by a very small number of characters that are in conflict with

other sources of data and leaving out part of the data would be warranted only if we knew a priori which part of our data is unreliable (Hyvönen *et al*., 2004). Potential incongruence between chloroplast DNA and mitochondrial DNA markers has been previously reported (Mahoney *et al.*, 2010, Njuguna *et al.*, 2013).

Molecular phylogenies derived from plastidial, mitochondrial and nuclear plant genomes can provide insight into the evolutionary history of plant groups influenced by reticulation events (Govindarajulu *et al.*, 2015). The phylogeny here presented suggests that *Polytrichum juniperinum* is a sister species of *Polytrichum strictum*, but unlike the study reported by Bell and Hyvönen (2010), it was not possible to confirm the suggested hypothesis. The authors' utility neighboring species to construct the phylogeny from Polytrichaceae, and hear, the phylogeny was limited to the taxon deposited in GenBank. Currently, the simple step of generating a robust phylogeny for a group of poorly studied organisms can require substantial research investment. One attempt to elucidate the question above is the possible use of different Polytrichaceae samples collected from different locations distribution of species. This study present details that contribute to understand the evolution of plastomes and chondromes in Polytrichaceae family although not propose news phylogenetic hypotheses in relation to the origin of the Antarctic moss *Polytrichum strictum.*

Nucleotide sequence accession numbers. This draft genome BioProject has been deposited at GenBank under the accession number SUB2397616. The genome accession numbers are KY795004, KY795005, KY795006 and KY795007 from *Polytrichum juniperinum* cpDNA and mtDNA, and *Polytrichum strictum* cpDNA and mtDNA, respectively.

Methods

Gametophytes samples of *P. juniperinum* (62°12'41,93" S and 58°55'44,61" O) and *P. strictum* (62°12'37,36" S and 58°57'49,87" O) were collected in Ardley Island during the austral summer of 2014/2015, during the Brazilian Antarctic Expedition XXXIII (2014-2015). Total genomic DNA was extracted using a modified CTAB extraction procedure as described by Shaw (2000). After the DNA extraction, samples were quantified with NanoVue^{TM} Plus Spectrophotometer (GE healthcare) and Qubit® 2.0 Fluorometer (Invitrogen) to ensure the quality of samples. Genome sequencing from Polytrichaceae DNA samples was performed using the Ion Torrent PGM platform (Life Technologies). Three genomic DNA libraries were prepared using the Ion one touch template Kit. The amplified library was sequenced using Ion PGM™ Hi-Q™ Sequencing Kit within the 318 Chip. A total of 16 333 496 sequences from *P. juniperinum* and 16 679 733 sequences from *P. strictum*, from maximum 389 bp in length, were sequenced. Following, the filtering of the reads for quality with Galaxy plataform (https://mississippi.snv.jussieu.fr), and the best value of cutoff estimated by *FastQC quality* control tool (Leggett *et al.,* 2013). The assembly of contigs was conducted by Velvet assembler for short reads (Zerbino *et al.,* 2008) using the reference genome of *Physcomitrella patens* Brid. (Funaraceae, Bryophyta) and the best *Kmer* estimated by Kmergenie (Chikhi and Medvedev, 2014). Scaffold assembler from cpDNA and mtDNA was performed in Scaffold Builder assembler version 2.2, utility the *P. patens* as reference genome (Silva *et al.,* 2013). The annotation of chloroplast was performed by web-based DOGMA (Wyman *et al.,* 2004) and same parameters adjusted (percent identity cutoff for protein coding genes estimated in 25; percent identity cutoff for RNAs estimated in 25; *e-value* estimated in $1e^{-5}$) and cpGAVAS with *e-value* estimated in $1e^{-5}$ (Liu *et al.,* 2012). Mitochondrial annotation was conducted by Mitofy version 1.3.1 of tRNAscan-SE and version 2.2.28 of the NCBI blast (Alverson *et al.,* 2010). The annotation of cpDNA and mtDNA genes was manually corrected

by comparison with complete chloroplast and mitochondrial genomes of other bryophytes with BLASTn (Altschul *et al.*, 1997). The species were compared with reference genomes of *Physcomitrella patens* and *Tetraphis pellucida* for generated chloroplast and mitochondrial circular maps to provided coverage visualization, gene content, presence/absence of genes with BRIG 0.95 (Alikhan *et al.*, 2011). For the phylogenetic analyzes individual alignments were performed for each gene in MEGA 5.05 (Tamura *et al.*, 2011) and all alignments were concatenated with sequence matrix 1.8 to create a super alignment. The best model of nucleotide substitution, TN93 model, was established by MEGA 5.05 in each gene alignment. The tree was built based on Bayesian statistic analysis with 10,000,000 million Monte Carlo Markov chains to avoid errors in the posterior probability support in BEAST package (Drummond *et al.*, 2012). The base frequence are estimated and data set was partitioned (e.g., códon positions) in two partitions (1+2), 3 with Beauti (Beast package). The majoritary rule was constructed with TreeAnnotator (Beast package). The support of nodes was calculated through posterior probability that varies of 0 and 1. The frequency convergence of trees and 25 % *burn-in* was confirmed with Tracer (Rambaut *et al*., 2004) and this program was used also for estimate when the sampling of trees was stabilized.

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4. CONSIDERAÇÕES FINAIS

Considerando os dados obtidos no presente estudo pode-se concluir que os genomas de organelas fornecem dados filogeneticamente informativos. A caracterização de tais genomas mostra como as espécies de diferentes famílias divergem em conteúdo gênico e outras características que aliadas a análises filogenéticas permitem a reconstrução de relações taxonômicas entre espécies próximas. O genoma plastidial dos dois musgos Antárticos apresenta-se relativamente variável quando é comparado ao bem conservado genoma mitocondrial observado, apresentando algumas variações como pequenas inversões em determinados genes. Essas inversões possivelmente caracterizam *P. juniperinum* e *P. strictum* na Antártica e podem ser úteis como marcadores filogenéticos entre as duas espécies. O conteúdo gênico apresentado pelo draft do genoma do cloroplasto e mitocôndria de *Polytrichum juniperinum* e *Polytrichum strictum* foi bastante semelhante, compartilhando a presença e ausência de genes com espécies próximas como *Tetraphis pellucida* e *Physcomitrella patens.* Porém a ausência do gene *rpoA,* parece ser específica para as espécies de *Polytrichum* da Antártica. A ausência deste gene pode indicar que o possível isolamento dessas espécies pode ter influencia na ausência deste gene, mas tal inferência deve ser corroborada com a comparação com grupos taxonomicamente mais próximos em ambientes extremos.

Contudo, o posicionamento de *P. juniperinum* no mesmo clado com *P. strictum* nas inferências filogenéticas com o genoma plastidial e mitocondrial não sugere novas hipóteses e por estar utilizando apenas dois táxons próximos não corrobora a hipótese da ancestralidade materna de *P. juniperinum* em *P. strictum*. Porém abre caminho para o aprimoramento deste estudo incluindo outra espécie do gênero *Polytrichum* na Antártica. Os dados gerados a partir do sequenciamento dos genomas acessórios dos espécimes oriundos da Antártica fornecerão um aporte para estudos futuros mais aprofundados com os espécimes do Ártico a fim de entender os fatores que modelam as distribuições naturais bem como suas interações e evolução dentro das áreas onde *P. strictum* e *P. juniperinum* ocorrem.

5. PERSPECTIVAS FUTURAS

- Gerar mais sequências dos genomas das organelas na tentativa de completar a cobertura, a fim de realizar a análise filogenômica do cpDNA e mtDNA.
- Após o fechamento do genoma, realizar nova anotação destes genomas visando o entendimento da evolução dos genomas acessórios para confirmar a atual anotação e buscar possíveis novos genes.
- Incluir a espécie *Polytrichum piliferum* nas análises filogenéticas, pois se faz necessário observar a relação desta espécie com *Polytrichum strictum* e *Polytrichum juniperinum*, já que na Antártica marítima apenas três exemplares do gênero *Polytrichum* são observados.
- Realizar uma análise funcional das seqüências do cloroplasto e mitocôndria de *Polytrichum juniperinum* e *Polytrichum strictum* oriundos da Antártica.
- Realizar uma busca de regiões repetidas no genoma de *Polytrichum* a fim de gerar dados para estudos de diversidade genética e filogenética.
- Sequenciar o genoma nuclear de *Polytrichum.*

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7. ANEXOS

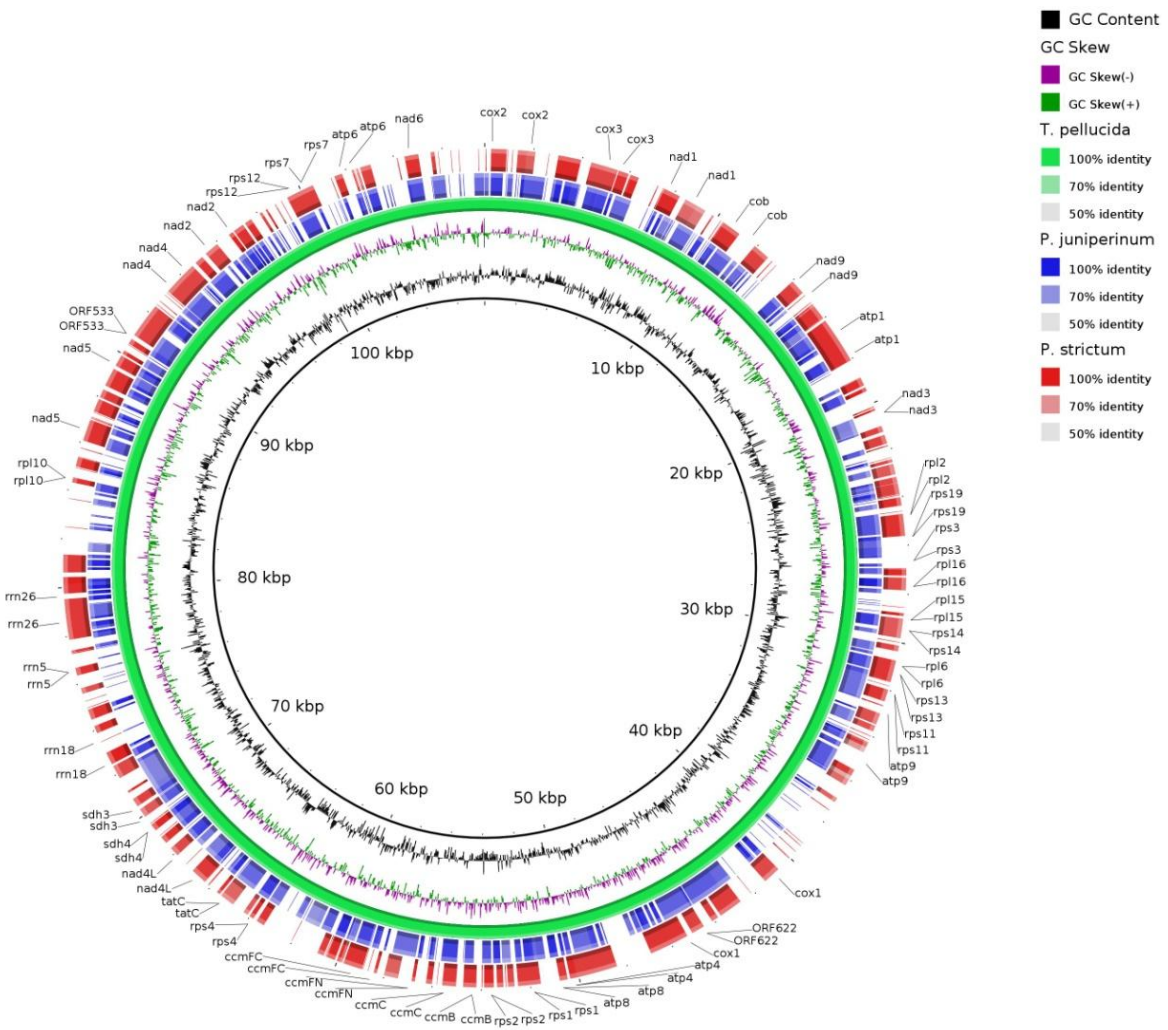

Figura 3: Imagem de saída do genoma mitocondrial por BRIG. Os anéis internos representam o genoma de *Tetraphis pellucida* (verde), as comparações do BLAST com *Polytrichum juniperinum* (azul) e *Polytrichum strictum* (vermelho). Ao redor do mapa são indicados os genes mitôcondriais. Os anéis do interior mostram GC skew (violeta/verde) e conteúdo GC (preto).

Table 2: Identity analysis with blast EMBL plataform.				
Gene/species	*lenght*	*score*	*Identity %*	*e-value*
ycf66 / P. juniperinum and P. strictum	105 pb	435.0	90.5%	-
ycf66 / P. juniperinum and Sanionia uncinata	124.374 pb	208.6	100%	2.7e-50
ycf66 / P. strictum and Tortulla ruralis	139 pb	69.3	94.3%	1.7e-13
nad7 / P. juniperinum and P. strictum	3115 pb	7381.0	55.3%	-
nad7 / P. juniperinum and Sanionia uncinata	104.497 pb	2010.6	98.0%	0.0
nad7 / P. strictum and Atrichum angustatum	115.146 pb	2012.6	97.4	0.0
ORF187 / P. juniperinum and P. strictum	11.225 pb	717.5	43.8%	-
ORF187 / P. juniperinum and Marchantia paleacea	186.609 pb	537.7	96.8%	2.5e-148
ORF187 / P. strictum and Atrichum angustatum	115.146 pb	1731.1	98.6%	0.0
The lenght, score, identity and e-value for this analysis is present. Date of EMBL database *(http://www.ebi.ac.uk/Tools/sss/ncbiblast/)*				

Tabela 3. Análise de identidade utilizando a plataforma blast EMBL apresentando o gene plastidial *ycf66,* o gene mitocondrial *nad7*, e *ORF187.* A espécie que mostra maior identidade com os genes em *Polytrichum juniperinum* e *Polytrichum strictum* é representado na coluna à esquerda. O tamanho, escore, porcentagem de identidade e *e-value* são mostrados na coluna a direita.

	Chloroplast		Mitochondria	
	P. juniperinum	*P. strictum*	*P. juniperinum*	*P. strictum*
Total lenght	55.168 bp	20.183 bp	88.021 bp	58.896
Adenine, A (bp [%])	15.707	5536	25756	17320
Cytosine, C (bp [%])	11872	4693	17643	11588
Guanine, G (bp [%])	12901	4753	18828	12624
Thymine, T (bp [%])	14673	5201	25694	17360
% GC	44,90%	46,80%	41,40%	41,10%

Tabela 4. Características gerais do draft do genoma de *Polytrichum juniperinum* e *Polytrichum strictum* mostrando o tamanho do genoma, conteúdo de bases e conteúdo GC.