UNIVERSIDADE FEDERAL DE PELOTAS

Faculdade de Agronomia Eliseu Maciel

Programa de Pós-Graduação em Agronomia

Tese

Aspectos etnobotânicos e moleculares para delinear estratégias de conservação de palmeiras

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Pelotas, 2022

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Tese apresentada ao Programa de Pós-Graduação em Agronomia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutora em Ciências (área do conhecimento: Fitomelhoramento).

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Pelotas, 2022

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

S587a Silveira, Tatieli

Aspectos etnobotânicos e moleculares para delinear estratégias de conservação de palmeiras / Tatieli Silveira ; Rosa Lia Barbieri, orientadora. — Pelotas, 2022.

86 f.

Tese (Doutorado) — Programa de Pós-Graduação em Agronomia, Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, 2022.

1. *Butia*. 2. *Arecales*. 3. Recursos genéticos. 4. Etnobotânica. 5. Bioinformática. I. Barbieri, Rosa Lia, orient. II. Título.

CDD : 634.4

Elaborada por Gabriela Machado Lopes CRB: 10/1842

Banca examinadora:

Drª. Rosa Lía Barbieri (Presidente)

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Drª. Mercedes Rivas - Universidad de la República.

Aos meus pais

Dedico

Agradecimentos

Seria injusto não abrir essa sessão agradecendo às forças do universo pela possibilidade de estar viva. Foi difícil atravessar a pandemia do Covid 19, por momentos, pensei que não chegaria nesta etapa de conclusão.

Agradeço ao Programa de Pós-graduação em Agronomia da UFPel, pela possibilidade de cursar o doutorado em Fitomelhoramento.

Ao CNPq pela concessão da bolsa de doutorado, para a realização do mesmo. À Embrapa Clima Temperado pela infraestrutura, bem como às relações interpessoais permitidas, as quais contribuíram significativamente para o crescimento e desenvolvimento deste trabalho.

À Rota dos Butiazais e todos os seus integrantes meu muitíssimo obrigada. Em especial à sra. Carmen e a sra. Marta que abriram e permitiram que em suas propriedades fosse executados estudos com os butiás.

À minha dedicada orientadora Dra. Rosa Lia Barbieri, agradeço por todo conhecimento compartilhado, pela compreensão, paciência em todos os momentos, pela confiança e amizade estabelecida.

Aos meus coorientadores PhD. Antônio Costa de Oliveira por todos os ensinamentos, explicações e conselhos, ao Dr. Luciano Carlos de Maia e a Dra. Vivian Ebeling Viana pelos conhecimentos transmitidos, muito obrigada.

Ao pesquisador Dr. Enio Sosinski e à Dra. Mercedes Rivas pelas informações e conhecimentos transmitidos.

À pesquisadora Dra Caroline Marques Castro, pelas portas abertas em meu primeiro contato com a Embrapa, pela excelente orientação durante o mestrado e pela atenção em todas as vezes que precisei de sua ajuda.

À Dra Karine Jenner, pelos ensinamentos, exaustivas explicações, conselhos e amizade, pelos chimas nas manhãs geladas do CGF, e pelo contato virtual durante a pandemia. Ao prof. Dr. Railson, por todos os ensinamentos e conhecimento compartilhado. E ao Dr. Alfonso que mesmo estando no México contribuiu para a realização deste trabalho. Vocês são exemplos de grandes egressos deste PPG e profissionais que sempre buscam a excelência.

Aos professores do Programa de Pós Graduação em Agronomia pelos ensinamentos.

À Jessica, minha grande amiga, conselheira e motivadora. Conseguimos manter uma forte rotina de trabalho, resistimos a pandemia e a eminência de uma terceira guerra mundial. Nada pode nos parar!

À Claudete, pelos conselhos, ensinamentos, pela amizade. Nunca me esquecerei do dia em que lhe contei de uma tese/ trabalho que estava seguindo como exemplo, por ser uma grande referência para os butiás, “de uma tal de Mistura”. Logo depois, descobri que se tratava de sua tese. Obrigada por tudo.

Aos colegas e amigos pelos momentos agradáveis durante o doutorado, Daniela, Marene, Julia, Daiana, Victoria, Marcelo, Jennifer, Natalie, Kamilla e Amanda.

Aos demais colegas e amigos do Fitomelhoramento.

À minha família, diretamente aos meus pais pelo incentivo, dedicação e investimento para que eu chegasse até aqui. À Julia, obrigado por me apoiar e ajudar em tudo, pelo companheirismo, pela paciência e por me ensinar sobre etnobotânica e temas sociais que eu pouco conhecia. Aos teus pais pela acolhida no seu lar e carinho.

Desiderata

Siga tranquilamente entre a inquietude e a pressa, lembrando-se de que há sempre paz no silêncio. Tanto quanto possível sem humilhar-se, mantenha-se em harmonia com todos que o cercam. Fale a sua verdade, clara e mansamente. Escute a verdade dos outros, pois eles também têm a sua própria história.

Evite as pessoas agitadas e agressivas: elas afligem o nosso espírito. Não se compare aos demais, olhando as pessoas como superiores ou inferiores a você: isso o tornaria superficial e amargo. Viva intensamente os seus ideais e o que você já conseguiu realizar.

Mantenha o interesse no seu trabalho, por mais humilde que seja, ele é um verdadeiro tesouro na continua mudança dos tempos. Seja prudente em tudo o que fizer, porque o mundo está cheio de armadilhas. Mas não fique cego para o bem que sempre existe. Em toda parte, a vida está cheia de heroísmo.

Seja você mesmo. Sobretudo, não simule afeição e não transforme o amor numa brincadeira, pois, no meio de tanta aridez, ele é perene como a relva. Aceite, com carinho, o conselho dos mais velhos e seja compreensivo com os impulsos inovadores da juventude. Cultive a força do espírito e você estará preparado para enfrentar as surpresas da sorte adversa. Não se desespere com perigos imaginários: muitos temores têm sua origem no cansaço e na solidão.

Ao lado de uma sadia disciplina conserve, para consigo mesmo, uma imensa bondade. Você é filho do universo, irmão das estrelas e árvores, você merece estar aqui e, mesmo se você não pode perceber, a terra e o universo vão cumprindo o seu destino.

Procure, pois, estar em paz com Deus, seja qual for o nome que você lhe der. No meio do seu trabalho e nas aspirações, na fatigante jornada pela vida, conserve, no mais profundo do seu ser, a harmonia e a paz. Acima de toda mesquinhez, falsidade e desengano, o mundo ainda é bonito. Caminhe com cuidado, faça tudo para ser feliz e partilhe com os outros a sua felicidade".

Max Ehrmann (1872-1945)

Resumo

SILVEIRA, Tatieli. **Aspectos etnobotânicos e moleculares para delinear estratégias de conservação de palmeiras**. 2022. 86f. Tese (Doutorado) – Programa de Pós-graduação em Agronomia. Universidade Federal de Pelotas, Pelotas.

As espécies da família Arecaceae possuem importante valor social, ambiental e econômico, e são amplamente utilizadas para a produção de alimentos e utilizadas na fabricação de utensílios decorativos. O avanço no conhecimento e o uso sustentável dos recursos genéticos é fundamental para garantir a conservação destas espécies. A tese tem como objetivo geral caracterizar o conhecimento compartilhado por participantes da Rota dos Butiazais e a variação genética associados a *Butia* e outras espécies da ordem Arecales, como contribuição à elaboração de estratégias de conservação de palmeiras. No estudo etnobotânico, foram entrevistados participantes chave da Rota do Butiazais que são entusiastas da conservação pelo uso dos butiás, e trabalham diretamente com essas espécies no Brasil, Uruguai e Argentina. Foram citados 16 usos para os butiás, com destaque para o consumo do fruto fresco, licor com os frutos e sucos. As atividades associadas aos ecossistemas de butiazais foram o turismo e a pecuária. As estratégias para conservação dos recursos genéticos que foram indicadas pelos entrevistados reforçam a importância de parcerias entre iniciativas privadas e instituições públicas, juntamente com políticas públicas. Os entrevistados ressaltaram a importância da conservação pelo uso desses recursos genéticos. De forma a integrar o entendimento sobre as espécies de Arecaceae, foi detectado a quantidade, distribuição e organização de Sequências Simples Repetidas (SSRs) plastidiais em 52 espécies da ordem Arecales. Estes genomas foram analisados identificando a posição das sequências SSR, foram gerados em média 76 pares de primers para cada espécie avaliada, visando a transferabilidade de marcadores moleculares. Foram observados polimorfismos nestes SSR e as dissimilaridades foram avaliadas. Foi verificado maior frequência de repetições de mononucleotídeos em regiões intergênicas. A análise de dissimilaridade de cpSSR mostrou que mono e trinucleotídeos foram altamente dissimilares entre si, refletindo a grande abundância desse tipo de SSRs em plastídios. Relações filogenéticas entre espécies do gênero *Butia* foram identificadas ao analisar sequências de fatores de transcrição WRKY com diferentes metodologias em bioinformática. As sequências WRKY usadas neste trabalho estão disponíveis no *National Center for Biotechnology Information* (NCBI). Houve variação nos padrões de agrupamento entre as diferentes regiões WRKY (2, 6, 7, 12, 16, 19, 21), sendo que as espécies nem sempre foram agrupadas devido à sua similaridade morfológica. Os resultados reforçam a importância de estudos desta ordem para maior esclarecimento da origem filogenética deste gênero. Os avanços no conhecimento gerados com este trabalho fornecem subsídios para propor a conservação de espécies da família Arecaceae, especialmente do gênero *Butia*. Estes estudos são importantes para entender a filogenia de *Butia,* buscar esclarecer a evolução deste gênero, contribuindo com informações necessárias para a conservação e uso sustentável dos recursos genéticos.

Palavras-chave: *Butia,* Arecales, Recursos Genéticos, Etnobotânica, Bioinformática

Abstract

SILVEIRA, Tatieli. **Ethnobotanical and molecular aspects to design palm conservation approaches.** 2022. 86f. Tese (Doutorado) – Programa de Pós-graduação em Agronomia. Universidade Federal de Pelotas, Pelotas.

The species of the Arecaceae family have important social, environmental, and economic value, and are widely used for food production and used in the manufacture of decorative utensils. Advances in knowledge and sustainable use of genetic resources are essential to guarantee the conservation of these species. The general objective was to characterize the knowledge shared by participants of the *Butia* Palm Groves Network and the genetic variation associated with *Butia* and other species of the Arecales order, as a contribution to the elaboration of palm conservation strategies. In the ethnobotanical study, key participants of the *Butia* Palm Groves Network who are enthusiasts of conservation through the use of *Butia* palm and work directly with these species in Brazil, Uruguay, and Argentina, were interviewed. Sixteen uses were cited for butiás, with emphasis on the consumption of fresh fruit, liqueur with the fruits and juices. The activities associated with the *Butia* palm groves ecosystems were tourism and livestock. The strategies for the conservation of genetic resources that were indicated by the interviewees reinforce the importance of partnerships between private initiatives and public institutions, together with public policies. Respondents highlighted the importance of conservation through the use of these genetic resources. To integrate the understanding of the species of Arecaceae, the quantity, distribution and organization of plastid Simple Repeat Sequences (SSRs) were detected in 52 species of the order Arecales. These genomes were analyzed to identify the position of the SSR sequences, an average of 76 pairs of primers were generated for each species evaluated, aiming at the transferability of molecular markers. Polymorphisms were observed in these SSRs and dissimilarities were evaluated. A higher frequency of mononucleotide repetitions was observed in intergenic regions. The dissimilarity analysis of cpSSR showed that mono and trinucleotides were highly dissimilar to each other, reflecting the great abundance of this type of SSRs in plastids. Phylogenetic relationships between species of the genus *Butia* were identified by analyzing sequences of WRKY transcription factors with different methodologies in bioinformatics. The WRKY sequences used in this work are available from the National Center for Biotechnology Information (NCBI). There was variation in the clustering patterns between the different WRKY regions (2, 6, 7, 12, 16, 19, 21), and the species were not always clustered due to their morphological similarity. The results reinforce the importance of studies of this order to clarify the phylogenetic origin of this genus. The advances in knowledge generated by this work provide subsidies to propose the conservation of species of the Arecaceae family, especially the genus *Butia*. These studies are important to understand the phylogeny of *Butia*, and seek to clarify the evolution of this genus, contributing with the necessary information for the conservation and sustainable use of genetic resources.

Keywords: *Butia,* Arecales, Genetic Resources, Ethnobotany, Bioinformatics

Lista de Abreviaturas

cpDNA: *Chloroplast genome* – Genoma de cloroplasto

cpSSR: *Chloroplast Simple Sequence Repeats* - Sequências simples repetidas de cloroplasto

DNA: *Deoxyribonucleic Acid* – Ácido desoxirribonucléico

NCBI: *National Center for Biotechnology Information* - Centro Nacional de Informações sobre Biotecnologia

SSRs: *Simple Sequence Repeats* – Sequências simples repetidas

WRKY - Fatores de Transcrição WRKY- Proteínas encontradas em plantas e algas. Estas proteínas constituem uma complexa superfamília gênica com um ancestral muito antigo. São caracterizadas por um domínio conservado na sequência proteica do tipo WRKYGQK, seguido por uma região de *zinc finger* (C2H2 ou C2HC).

SUMÁRIO

1 INTRODUÇÃO GERAL

As palmeiras pertencem à família Arecaceae, que abrange 252 gêneros e aproximadamente 2.600 espécies (THE PLANT LIST, 2019). Esta família tem distribuição Pantropical. No Brasil estão representados 38 gêneros e 270 espécies, distribuídas em todos os ecossistemas e com maior diversidade na Floresta Amazônica e na Mata Atlântica (LORENZI et al., 2010).

Arecaceae possui espécies de estimado valor econômico, estando entre as principais fontes de recursos florestais não madeireiros (BALICK, 1984; RIVAS et al., 2012). O óleo proveniente de palmeiras é utilizado em diversas atividades (INVEST E EXPORT BRASIL, 2016).

Entre os gêneros de Arecaceae com importância econômica, destaca-se *Butia* (Becc.) Becc., composto por palmeiras denominadas butiazeiros e que formam populações conhecidas como butiazais (MARCATO, 2004). As espécies de *Butia* ocorrem naturalmente em áreas das regiões Nordeste (BA), Centro-Oeste (GO, MS), Sudeste (MG, SP) e Sul (PR, SC, RS) do Brasil, no leste do Paraguai, no nordeste da Argentina e no Uruguai (ESLABÃO et al., 2016; HEIDEN et al., 2020). Atualmente considera-se que o gênero compreenda entre 21 espécies (HEIDEN et al., 2018) ou 24 espécies (DEBLE et al., 2017), sendo 20 encontradas no Brasil.

Butia apresenta complexo histórico taxonômico, com casos de equívocos na aplicação de nomes científicos e inúmeras divergências entre os taxonomistas quanto ao número de espécies que o compõem (ELLERT-PEREIRA, 2019; GAIERO et al., 2011).

Os butiás, frutos dos butiazeiros, são amplamente utilizados para o consumo in natura, ou na produção de alimentos como bolos, doces, salgados, sucos, geleias, licores, produção de vinagre e sorvetes, entre outros (ROSSATO, 2007). As fibras das folhas e da polpa dos frutos são utilizadas no artesanato, para produção de utilitários, como cestas, bolsas, redes e chapéus (BÜTTOW et al., 2010). Segundo Fonseca (2012), os frutos de *Butia odorata,* possuem altos níveis de minerais na polpa, compostos bioativos com elevadas concentrações de compostos fenólicos totais, vitamina C e carotenoides. Além disso, os ecossistemas de butiazais são fonte de geração de renda, além de informação cultural, possuindo grande importância na manutenção dos habitats nos quais encontram-se inseridos (BARBIERI et al., 2021; BARBIERI et al., 2022a; RIVAS; BARBIERI, 2018)

Estudos que visam a compreensão de sistemas sócio-ecológicos que envolvem uma espécie ou população vegetal são de grande importância e é um dos principais focos em estudos etnobiológicos (ALBUQUERQUE et al., 2019). A etnobotânica aprofunda o conhecimento sobre as interações humano x plantas. Esta dinâmica exerce um efeito poderoso sobre as relações socioambientais (ALBUQUERQUE et al., 2008), e a contribuição da etnobotânica reflete no resgate e fortalecimento identitário (ALBUQUERQUE et al., 2005, 2019). Pesquisas dessa ordem integram a proteção da diversidade biológica, os costumes de comunidades associadas e a agricultura familiar, através de seus métodos e análises (SILVA et al., 2010). Para o butiá, que possui ações como o projeto da Rota dos Butiazais (BARBIERI et al., 2021; BARBIERI et al. 2022b; MARCHI et al., 2018), essas pesquisas permitem entender e avaliar as dinâmicas estabelecidas e a valorização da espécie (RIVAS, 2004; RIVAS; BARBIERI, 2014; RIVAS; CONDÓN, 2015).

Em um âmbito biotecnológico, poucas são as informações e estudos relacionados a aspectos moleculares do *Butia* (CODINA; MOSQUERA, 2015; MISTURA, 2013; RIVAS; BARILANI, 2004), o que torna difícil o entendimento taxonômico e as relações deste gênero com as demais palmeiras.

Nesse sentido, estudos com os microssatélites ou SSRs (Simple Sequence Repeats), sequências de 1 a 6 nucleotídeos repetidos, presentes em genomas nucleares, plastidiais e mitocondriais de plantas e animais, são importantes. Em análises de genomas é fundamental considerar a distribuição dos SSRs e a possibilidade de transferibilidade. Isso pode contribuir para o entendimento molecular de espécies dentro de famílias de plantas, e permitir uma melhor exploração dos recursos genéticos existentes em diferentes populações vegetais, por consequência viabilizando a elaboração de marcadores moleculares (CODINA; MOSQUERA, 2015; KPATÈNON et al., 2020; LAINDORF et al., 2019; MISTURA et al., 2012).

Pouco se sabe sobre os genomas mitocondriais e plastidiais e suas mutações decorrentes à codificação de proteínas associadas ao metabolismo energético. Os SSRs são mais informativos do que outros marcadores moleculares, uma vez que novos alelos que surgem devido às mutações podem escapar da correção pelo sistema de reparo de incompatibilidade de DNA, logo, diferentes alelos podem existir em um determinado locus SSR.

Os cpSSRs apresentam grande potencial para uso em filogeografia e análises de impressões digitais de DNA para Arecaceae (LOPES et al., 2018, 2019). Muitos pesquisadores têm buscado utilizar regiões hipervariáveis do cloroplasto ou DNA plastidial (cpDNA) para caracterizar a variação genética de espécies (SHAW et al., 2005). O estudo desses elementos em genomas acessórios de plantas se torna ainda mais significativo, já que esses genomas geralmente são pequenos e apresentam forte pressão de seleção para variações.

A análise dos fatores de transcrição das regiões WRKY, que estão amplamente distribuídas por todo o genoma das plantas (ZANG e WHANG, 2005), além de formarem uma superfamília gênica com um ancestral muito antigo (WU et al., 2005), é uma estratégia para entender a proximidade filogenética de espécies vegetais e das palmeiras, inclusive de *Butia*. Muitos estudos, como Meerow et al. (2009), consideram *Butia* pertencente a um grupo monofilético com os gêneros pertencentes à subtribo Attaleinae, outros consideram *Butia* um grupo irmão de Jubaea (CUENCA et al., 2008). No entanto, não estão totalmente elucidadas suas associações filogenéticas, visto a falta de informações de regiões uteis de DNA para análises com as demais palmeiras.

Os três artigos presentes neste trabalho são o resultado da busca por informações em diferentes áreas do conhecimento que possam servir como base para elaborar estratégias para a conservação das palmeiras, em especial da família Arecaceae e do gênero *Butia*. Através de estudos etnobotânicos, busca-se a compreensão da importância de *Butia* para as comunidades que dele se utilizam. A investigação de cpSSR em palmeiras demonstra a possibilidade de transferibilidade de marcadores microssatélites entre espécies de palmeiras. A análise dos fatores de transcrição WRKY para *Butia* permite o estabelecimento de relações filogenéticas entre espécies deste gênero. As pesquisas desenvolvidas neste trabalho são contribuições para traçar o histórico evolutivo do gênero *Butia*, contribuindo com informações necessárias para a conservação e o uso sustentável dos recursos genéticos.

O presente trabalho foi desenvolvido com o objetivo de caracterizar o conhecimento compartilhado por participantes da Rota dos Butiazais e a variação genética associados a *Butia* e outras espécies da ordem Arecales, como contribuição à elaboração de estratégias de conservação de palmeiras.

Esta tese é composta por três artigos, um deles já publicado, um submetido à publicação e outro a ser submetido a periódico indexado. O primeiro aborda as relações etnobotânicas com o gênero *Butia*, o segundo tem como objetivo detectar a quantidade, distribuição e organização de cpSSRs em 52 espécies da ordem Arecales, além de identificar os SSRs que mais contribuem para as diferenças encontradas entre os genomas plastidiais, visando futura transferabilidade para o *Butia,* e o terceiro trabalho teve o objetivo de inferir as relações genéticas de espécies do gênero *Butia* através da análises de sequências de fatores de transcrição WRKY, disponíveis no banco de dados do *National Center for Biotecnology Information* (NCBI).

2 Use and conservation of *Butia* palm groves: the link that goes beyond borders

Artigo publicado na revista Ethnobotany Research and Applications

Use and conservation of *Butia* palm groves: the link that goes beyond borders

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Ethnobotany Research and Applications 23:21 (2022)

Research

Abstract

Background: Butia is a genus palm species from South America. Currently, the species of this genus are threatened with extinction in Brazil. This work aimed to understand the ethnobotanical relationships with *Butia* palm groves, how this process has been built, and how it contributes to the *in situ* conservation of its native genetic resources.

Methods: The methodology consisted of conducting semi-structured interviews with an ethnobotanical emphasis. Statistical methods and informant indexes were applied, mixing theoretical discussions with data analysis to assist in understanding the results.

Results: 16 uses for **butiá** (the fruit of *Butia* palm) were cited by the interviewed. The most cited was the consumption of fresh fruit. The contact with the *Butia* palm groves varied between daily and seasonal. The activities associated with the *Butia* palm groves ecosystems were tourism and livestock. Proposals for their conservation refer to partnerships between private initiatives and public institutions associated with public policies and legal reserves.

Discussion: The *Butia* palm groves are integral to local communities' identities. Choices related to *Butia* palm groves impact communities, just as the *Butia* palm groves also impact local populations.

Conclusions: The *Butia* palm groves have great sociocultural and biological importance. The bond shared between people and the *Butia* palm groves goes beyond borders, and its conservation can be related to good practices of extraction, sustainable management and also through public policies and specific legislation aimed at protecting this environment.

Keywords: ethnobotany, genetic resources, sociobiodiversity, *Butia* palm groves network, bioeconomy

Manuscript received: 07/08/2021 – Revised manuscript received: 03/04/2022 - Published: 04/04/2022
http://dx.doi.org/10.32859/era.23.21.1-16

Background

Butia (Arecaceae) is a genus of subtropical palms with distribution in southern South America. The genus *Butia* comprises 22 species (Deble *et al.*, 2017; Soares, 2015; Sant'Anna 2021), distributed in Argentina, Paraguay, Uruguay (Geymonat & Rocha, 2009; Noblick, 2010; Soares, 2015; Keller & Paz-Deble 2020) and Brazil, which is the country that has the most significant number of species. In addition, 20 species are distributed in different Brazilian regions: Northeast (Bahia), Midwest (Federal District, Goiás, Mato Grosso do Sul), Southeast (Minas Gerais, São Paulo), and South (Paraná, Santa Catarina, Rio Grande do Sul) (Eslabão *et al.* 2016, Ellert Pereira *et al.* 2017, Sant'Anna 2021).

In a social and economic sense, the *Butia* genus offers raw material for several types of food and drinks, as well as handicrafts (Marchi *et al.*, 2018). However, due to several large-scale anthropic actions in the *Butia* palm groves, which oppose the traditional uses of the ecosystem, the genus currently composes lists of extinction and vulnerability (IUCN 2016). As a strategy to raise society's awareness of the importance of conserving the species and the *Butia* palm groves ecosystems, the project *Butia* Palm Groves Network (Rota dos Butiazais, in Portuguese, and Red Palmar, in Spanish) was proposed. This project is a network for integration between people, places, and countries (Brazil, Uruguay, and Argentina), aiming to promote the conservation and sustainable use of biodiversity associated with *Butia* palm groves, ecosystems where *Butia* palms are predominant (Barbieri *et al.* 2015). Furthermore, the *Butia* Palm Groves Network considers the cultural link with *Butia*, and the products derived from it essential to increase their valuing, leading to the preservation of Butia and the environment (Barbieri *et al.*, 2017). The most common product of the *Butia* palm groves is the fruit, known as **butiá**. The *Butia* Palm Groves Network emerged in 2015 in partnership with the Brazilian Agricultural Research Corporation, Embrapa, and several public and private institutions. The people who idealized the network are the main disseminating agents of the project.

Among the activities conducted by large-scale human activities that threaten the *Butia* palm groves, there is pressure for converting the ecosystem areas into intensive crops and the growth of urban and road areas (Sosinski Júnior *et al.* 2019). Moreover, real estate speculation is also responsible for much of the reduction in the native populations of *Butia* in Brazil (Kumagai & Hanakazi 2013). As a result, besides traditions, historical and cultural knowledge associated with the *Butia* genus are being lost amid the capitalization of the environment.

Understanding socio-ecological systems is one of the main focuses of ethnobiological studies (Albuquerque *et al.* 2019). Therefore, the concern with the conservation and maintenance of biodiversity is of great importance due to the management of the dynamic balance of ecosystems, and the applicability of resources in socio-economic systems. (Watson *et al.*, 2018).

In the case of *Butia*, Rivas and Barilani (2004) and Rivas and Barbieri (2014) suggest the creation of plans for the development and promotion of products derived from *Butia* palm, arguing that the use of *Butia* palm by residents should be part of the conservation strategies of the biodiversity. One of these actions is the *Butia* Palm Groves Network project, which aims to integrate the different areas of knowledge and network people who work or have some connection with the species (Barbieri *et al.*, 2017).

In this sense, ethnobotany studies the interactions between people and plants, contributing to the revitalization of associated knowledge (Albuquerque *et al.* 2005 and 2019). The analysis of data in ethnobotany, using the method of individual interviews, provides an idea of how responses can be combined to create the social and cultural profile of the study population. Such methodology aims to perceive the symbolic value of using a particular plant and its cultural and environmental importance, determining a consensus factor among the informants when recognizing it as a possible identity marker (Silva *et al.* 2010).

Considering the exposed scenario, this work aimed to understand the ethnobotanical relations with the *Butia* palm groves, how this process has been built, and how it has contributed to the *in situ* conservation of genetic resources.

Materials and methods

At the II International Meeting of the Rota dos Butiazais, held in Pelotas (Rio Grande do Sul, Brazil) in August 2018, 14 people of different genders, age groups, occupations, countries, and historical-cultural contexts were interviewed (Table 1). Each respondent answered eight semi-structured questions (Appendix 1).

Due to institutional regulations on work performed with human beings, the study was submitted to the Research Ethics Committee of the Federal University of Pelotas, obtaining a favorable opinion No. 2,565,059. The semi-structured questionnaire was applied to each agent in order to raise knowledge about the sociocultural environment in which they are inserted. The social profiles revealed by the interviews directly involve the practices and uses associated with *Butia* palm groves and *Butia* fruits and leaves. During the interviews, the informants answered the questions and shared other personal narratives in the conversation.

Table 1. List of survey respondents, informing gender, occupation, and institution/location.

Interviewed	Gender	Occupation	Location
A. M.	man	environmentalist	Ubajay, Argentina
E.F.	woman	craftswoman	Ubajay, Argentina
M.P.	woman	businesswoman	Rocha, Uruguai
J.S.M.	woman	confectioner	Tapes, RS, Brazil
M.B.I.	woman	craftswoman	Santa Vitória do Palmar, Brazil
C.H.B.	woman	farmer	Tapes, RS, Brazil
A.A.M.S.	man	farmer	Pescaria Brava, Brasil
M.G.	man	artist	Caxias do Sul, Brazil
M.P.	woman	environmentalist	Ubajay, Argentina
M.E.P.	woman	farmer and businesswoman	Ubajay, Argentina
F.T.	woman	businesswoman	Tapes, RS, Brazil
M.C.V.	woman	environmentalist and tour guide	Guichón, Uruguay
G.M.	woman	student	Imbituba, Brazil
R.J.	man	politician	Giruá, Brazil

The study was centered on an approach that integrated qualitative and quantitative instruments to gather social data from the interviewees about activities associated with the use of *Butia* palm groves, their potentialities, and possible limitations of the plant, among other topics. In this sense, the study aims to understand the ethnobotanical relations with the *Butia* palm groves. The intentional sampling technique was used (Bailey 1982, Bernard 1994) to identify the interviewees.

Data analysis

The qualitative data of the interviews were studied from content analysis (Minayo, 1993; Franco, 2005), based mainly on the interviews conducted and relating them to the theoretical framework researched.

For quantitative data, indices were used to determine the nature of the informant's consensus and the nature of the distribution of knowledge. The calculated indices were: Informant Diversity Value (IDV), Use Diversity Value (UDV), Contact Diversity Value (CDV), Associated Activities Diversity Value (AADV), and Conservation Proposals Diversity Value (CPDV) (Byg & Baslev 2001, Minayo 2010).

The IDV consists primarily of the sum of individual quotes from an interviewee. The total of this sum is divided by the total number of uses, contact, associated activities and conservation proposals, respectively. And to obtain the final IDV values, shown in the tables, the resulting individual indices are added and divided by the total uses in the specification of each category (gender: male or female; Country: Argentina, Brazil and Uruguay; connection: owner or extractive).

The UDV, Contact, AA and CP were calculated considering the total citations of a specific use, or contact, or associated activity or conservation proposal, divided by the total number of citations of uses, contact, associated activities or conservation proposals, respectively in each topic.

The informants were categorized according to gender (woman, man), country where they live (Argentina, Brazil, Uruguay), and their connection with the *Butia* palm groves (extractivist or owner). Then, the results were subjected to statistical analysis using the Kruskal Wallis test (above two categories) and Mann-Whitney (up to two categories) with the aid of the Minitab 19 statistical program.

Results and Discussion

The prospect of using the *Butia* genus

The diversity of plants known and used by humans results from ancient interactions between populations and plants. Primarily, plants supplied food, medicinal and ritualistic purposes and recently, industrial needs (Albuquerque 2002). In this way, ethnobotany is a tool found halfway between nature and culture and can explain how the relationship between plants and humans work. Through the interviews, we observed the importance of *Butia* palm groves for the people who earn their livelihood with them, making clear the concern of all interviewees with the conservation of this environment.

The interactions between nature and culture are fluid and inseparable paths (Ingold 2000, Latour 2014). Such interpretation can be seen in the interviewees' speech, such as E.F., a craftswoman. When asked about her relation to the *Butia* palm groves, she says: "... for me, the palm trees choose the places, there is a whole pathway, a corridor, which takes Corrientes (in Argentina), Brazil, Paraguay..." "...I have lived for many years near the El Palmar National Park, with many palm trees, as the Indigenous called it sour fruits, and worked with products like vinegar, liquor, pulp ...". Keller & Paz-Deble (2020), in an ethnobotanical study with palm trees in Misiones, Argentina, highlight that *Butia* is currently used for ornamentation of gardens. Part of the *Butia* palms leaves was once used to make sandals, which are also widely used by the Guarani people to make mats and mattresses and fresh consumption and used to make a wide variety of drinks.

When asked about the possible age of the *Butia* palm groves in his region, A.A.M.S. exposes the economic importance of *Butia* palms over time, as well as the old ways of using this plant: "... I think they are *restinga* areas, hundreds of years old ... Because you don't see the *Butia* palm groves in other areas, they are particular to some points, and we have known them for many years. In the 40s and 50s (of the 20th century), *Butia* palms were used, the fibers of the leaves became mattress fillers, in São Paulo and Rio (de Janeiro), and some of it (fruits) was consumed as alcoholic beverages, used in the production of cachaça flavored with **butiá**".

The habit of using **butiá** fruits for the flavoring of cachaça was reported by Marcato (2004) in Paraná. Such activity was frequent among many of the residents of the locations visited by the researcher. Thus, choices made by extractivists combined forms and methods in the sense of how such communities dealt with *Butia* palms. On the other hand, Dabezies & Rivas (2020) found several reports when presenting the uses of *Butia odorata* in southeastern Uruguay. The most cited were liqueur production with the fruit, coconut coffee made from seeds, the filling of cushions with leaf fibers, and jelly made from the fruit.

During the interviews, sixteen uses were associated and highlighted by the interviewees to one of the products of the *Butia* palm groves: the fruits. The uses mentioned include production of jellies, juices, cakes, bread, cucas, liquor, salty snacks, vinegar, flavoring of cachaça, cookies, therapeutic, use of coquinho, fresh fruit selling, crafts, art (with leaves and fruits) and consumption of fresh fruit (Table 2). However, among the uses explained by the interviews, the one that stood out was the consumption of fresh fruit, appreciated by all respondents (UDV = 0.23). Statistical differences in the uses given to the *Butia* palm's fruits, leaves, among others, were observed with respect to connection (Owner or extractivist), when observed the IDV ($p=0.02$). This difference is associated with the fact that extractivists' income depends on their contact/marketing of the products obtained from the leaves, fruits, and pulp of the *Butia* palm. However, we had access to only two owners, and such results could be different if there was a larger sample number of respondents of this nature.

When questioned about its relationship with the Tapes *Butia* palm groves, the informant J.M.S. said: "... they (products from *Butia* palm) are present at my house on a daily basis, it has become a routine because of my work At harvest time, and in other times of the year. But when I don't have that daily contact with the environment (*Butia* palm groves), I can use the frozen fruit... Currently, I am working on diversifying. I am working with a variety of **butiá** products. I make juice, liqueur, we have our line of snacks. We do work with **butiá** in the snacks too. There is a line of sweets, chocolates, bread, cakes, cookies. Today, I work with it in practically everything..."

As noted by the statement above, the knowledge of preparation of food related to **butiá** is constant as well as their form of consumption. Such knowledge can be perpetuated in a generational and community way, helping conserve what is necessary for food production (DaMatta, 1987). When analyzing the context of *Butia*, it can be seen that one of its primary uses is focused on cooking (Buttow et al., 2009). Anthropology considers the preparation and consumption of food as identity markers of communities, religions, ethnic groups, and even nations. It is not difficult to associate culinary types with populations (DaMatta 1987, Laplantine 2007, Mintz 2001, Oliveira 1996), such as, for example, Japanese sushi, gaucho barbecue, Mexican taco, Jewish Matzo or Amalá from Afro-Brazilian religions.

Table 2. Number of citations for the different uses of **butiá** (* Production of jellies (PJ), juices (J), cakes (C), bread (B), cucas (C), liquor (L), salty snacks (SL), vinegar (V), cachaça (CA), cookies (CK), therapeutic (TP), use of coquinho (UC), fresh fruit selling (FFS), crafts (CR), art (A) and fresh fruit (FF), with a demonstration of the Informant's Diversity Values (IDV) and Use Diversity Values (UDV) for the different categories, gender of the interviewees, country of origin of the interviewees and working relationship with the *Butia* palm groves or *Butia* palm. Equal letters in the columns indicate no significant difference between the groups, according to the Kruskal Wallis (Country) and Man- Whitney (Gender and Connection) at a 5% error probability.

		Use citations																
Category		PJ*	J*	C*	B*	C*	L*	SL*	V*	CA*	CK*	TP*	UC*	FFS*	CR*	A*	FF*	IDV*
Gender	Man (n=4)	1	2	0	0	0	1	0	0	1	0	1	2	2	1	1	4	0.100A
	Woman (n=10)	4	4	2	2	2	6	1	1	2	1	1	3	3	1	1	10	0.171A
Country	Argentina (n=4)	1	1	0	0	0	2	0	1	0	0	0	0	0	0	0	4	0.112A
	Brazil (n=8)	4	3	2	2	2	3	1	0	3	1	2	4	4	2	2	8	0.175A
	Uruguay (n=2)	0	2	0	0	0	2	0	0	0	0	0	1	0	0	0	2	0.109A
Connection	Owner (n=2)	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0.100B
	extractivist (n=12)	5	6	2	2	2	7	1	0	3	1	1	5	4	2	2	12	0.229A
Total of citations		5	6	2	2	2	7	1	1	3	1	2	5	4	2	2	14	
UDV		0.08	0.10	0.03	0.03	0.03	0.11	0.01	0.01	0.05	0.01	0.03	0.08	0.06	0.03	0.03	0.23	

* Production of jellies (PJ), juices (J), cakes (C), breads (B), cucas (C), liquor (L), salty snacks (SL), vinegar (V), cachaça (CA), cookies (CK), therapeutic (TP), use of coquinho (UC), fresh fruit selling (FFS), crafts (CR), art (A) and fresh fruit (FF)

Use Diversity Value (UDV)= number of citations for each use category divided by the total number of citations for all categories

**Informant Diversity Value (IDV)= Summation of the number of citations of each interviewee divided by the total number of citations in the category (gender=man or woman; country= Argentina, Brazil, Uruguay; connection= owner or extractivist).

When asked about the importance of *Butia*, interviewee M.B.I. said: "For us, it is extremely important (the *Butia*), because our city is Santa Vitória do Palmar, so people have a reference to Santa Vitória, to have a large number of palm trees. *Butia* palm groves Network is rescuing the history of *Butia* palms because this is our city's history; there is a cultural rescue. Therefore, the handicrafts produced here (in Santa Vitória do Palmar) represent our city... I work with handicrafts derived from the *Butia* palm; I work with the leaf, the coconut, the fiber, and also with the fruit in the culinary part..." This statement is an example of an identity marker that connects this informant to a sense of belonging when talking about the *Butia* palms that represent her city.

This way, helping the proper management of the species, as can be seen in the speech of G.M.: "The creative economy activity that I am a part of is linked to *Butia* palm leaves. Also, to the **butiá** fruit and the area of the *Butia* palm groves. My activity is related to the articulation and preservation of the species, which is linked to the *Butia* palm groves ecosystem". Sosinski et al. (2019) argue that a strategy to promote the conservation of *Butia* palms is to encourage people to use the products made from *Butia*. The use of the plant generates social significance. It, consequently, moves a productive system associated with financial activities, which requires the maintenance of the resource so that continuous gain occurs following legal requirements, especially when it comes to a native plant threatened with extinction (Brazil Decree Law 25 of 1937 and Brazil, 6,938). However, this approach must respect limits that ensure sustainable exploitation. Sosinski et al. (2019) also highlight that to improve the commercialization chain of native species for conservation, the Rio Grande do Sul Environment Secretariat (SEMA) developed a simplified and tax-free process for environmental regularization for extractivism and cultivation of native plants and products for marketing (including *Butia*). Known as environmental certification of extractivism and agroforestry production (SEMA, 2017), it is a process that meets legal requirements. Also, it aims to improve the understanding of the exploitation chain of these endangered species, such as *Butia*.

This environmental certification allows for the regularization of extractive activities, such as roadside collectors and vendors, farmers who economically exploit these fruits, leaves, and seeds, including seedlings for restoration (Sosinski et al. 2019). In addition, it allows the establishment of a productive structure that contributes to the valorization of the *Butia* species, and at the same time allows the environmental agency to acquire accurate information about the species, populations, different uses, market demands, and conservation opportunities (Sosinski et al. 2019, 2020b).

The contact with *Butia* palm groves

To assess the contact with *Butia* palm groves, results among the five periods obtained from the conversations were observed: daily, weekly, monthly, fruiting and sporadic, the most cited was the daily (n=5), with the highest rate of women (n=3), in Argentina (n=3) and Brazil (n=2), and most frequently by extractivists (n=4). Regarding the distribution of contact with *Butia* palm groves among the interviewees' categories, due to the wide variation in the means of the informants' diversity values (IDV), it was not possible to observe significant differences.This information can be best viewed in Table 3.

Through qualitative research, it was possible to observe an effort to perpetuate the economic and cultural relationship associated with the maintenance of the *Butia* palm groves. There is a continuous effort to maintain the *Butia* palm groves**.** The communities have a special connection that originates in childhood since affective bonds and associated memories with the plant and ecosystem happen at a young age. The emotional bond with Butia palm groves was established for nine interviewees while the interviewees were children. This relationship is an ally in combating environmental threats and promoting the well-being and equality of communities (Vygotsky, 2007). "... I was born here surrounded by the palms. And in my family, a traditional getaway was to get these coconuts..." points out M.C.V.

The results show that sporadic activities conducted in *Butia* palm groves are associated with obtaining income (citation n=2). Furthermore, the frequency of visiting the environment is associated with the seasonality of the fruits. However, the daily actions in the *Butia* palm groves focused on conservation (citation n=6). Therefore, it was found that the most frequent visits are associated with people who work in conservation units of the species, network management, or sustainable associations. As described in the speech by A.M., an employee of the El Palmar National Park: "...yes, I go daily...", "...the palm groves are a component of the ecosystem of this place, and therefore a strategy for biodiversity conservation...".

Table 3. Number of citations of the frequency of contact with *Butia* palm groves, with a statement of the Informant's Diversity Values (IDV) and Contact Frequency Diversity Values (CDV) for the different categories, gender of the interviewees, respondents' country of origin and working relationship with *Butia* palm groves. Equal letters in the columns indicate no significant difference between the groups, according to the Kruskal Wallis (Country) and Man-Whitney (Gender and Connection) test at a 5% error probability.

		Contact with the *Butia* palm groves					
Category		Daily	Weekly	Monthly	Fruiting	Sporadic	IDV*
Gender	Man (n=4)	2	1	0	2	0	0.333 A
	Woman (n=10)	3	2	1	2	2	0.444 A
Country	Argentina (n=4)	3	0	0	0	1	0.444 A
	Brazil (n=8)	2	2	1	3	1	0.360 A
	Uruguay (n=2)	0	1	0	1	0	0.200 A
Connection	Owner (n=2)	1	1	0	0	0	0.200 A
	extractivist (n=12)	4	2	1	4	2	0.520 A
Total of citations		5	3	1	4	2	
	CDV	0.33	0.20	0.07	0.26	0.13	

**Informant Diversity Value (IDV)= Summation of the number of citations of each interviewee divided by the total number of citations in the category (gender=man or woman; country= Argentina, Brazil, Uruguay; connection= owner or extractivist). CDV= Number of citations for each contact category divided by the total number of citations for all categories.

In this context, the extractivists of the **butiá**, those who use the raw material for the elaboration of their activities, do not assume a predatory stance in relation to the environment of their livelihood, since, after the harvesting of a certain amount of material, it is not necessary to return to the collection site. J.M.S., when asked about the frequency of visits to the *Butia* palm groves, says: "... No, I don't go too much, I have (**butiá** fruits) in my work..." "... I have more contact with the fruits at harvest time...". Conscious extractivism focuses on a sustainable lifestyle, respecting nature's time and space, fighting against the conception of domain (Díaz *et al.* 2019, Latour 2014).

Associated Activities with *Butia* palm groves

The interviewees described nine activities associated with the *Butia* palm groves. They are cattle breeding, agriculture, tourism, river stone extraction, eucalyptus plantation, extractivism organization, archaeological excavations, ecological associations with other plant or animal species, and the establishment of a cooperative company.

The activity most mentioned in the conversations was tourism, with a higher rate for women (n=4), extractivists (non-owners) (n=4), residing in Argentina(n=3). Another activity that occurred the most was cattle raising in the *Butia* palm groves. However, due to the large variation in the means, no significant differences were observed for the IDV. The problems involving such activity concerning the environment were mentioned in all reports (Table 4).

The activities associated with the *Butia* palm groves were diverse. In the participants' speeches, it was possible to observe the impact of other economic activities on the *Butia* palm groves, for example in M.C.Vs' dialogue, "... at this moment the problem we have is flowering and monoculture, each day we have less space for *Butia* palm groves. Studies are showing that Uruguay has less and less place for this landscape. What we have to do is look for how to protect the *Butia* palm groves (of *Butia yatay*) in some way. One of the main problems is the forestation with eucalyptus..." This narrative corroborates with Carrasco (2012), where he observes that monoculture has become the predominant system in the world. Between 1988 and 2002, smaller farms with up to 200 ha discontinued their activities, giving space to extensive crops, which implied an excessive use of pesticides. Thus, the ecological threat of monoculture is evident. There is also a great loss of biodiversity, both in terms of landscapes and species. Such disturbances, in some cases, are reversible, depending on their magnitude.

Other problems indicated by the informants' narratives involved activities associated with cattle raising in areas where the *Butia* palm groves occur. F.T., another interlocutor, presented to be positive in relation to livestock that interacts with the Butia palm groves, in the same way, the interviewed states that this must be done strategically "...Without cattle ranching, the *Butia* palm groves cannot be maintained, everything must be done intelligently, and this process should help to conserve the environment...". Still, she demonstrates how this could be done: by "having rotations at different times of the year, which will make the pasture better, have a certain number of animals to leave too, dividing them into paddocks." The interviewee M.P.I. pointed out that the existence of the *Butia* palm groves is also important for the livestock, as they benefit from the space "...When I pass by the *Butia* palm grove, in midsummer, the shade of the *Butia* palms protects the livestock from the sun rays. Also, the livestock feed on the fruits...". The interviewee M.E.P. is also optimistic about the presence of livestock in the *Butia* palm groves "...we have healthy and very fat cattle...". One of the interviewees pointed out that in her place of work, *Butia* palm groves, there is management in relation to livestock in the winter period, to enable the development of new plants, M.P. said: "...there is an area called conservative management, where they take the livestock in winter, precisely so that the *Butia* palms can emerge and not be damaged...".

Table 4. Number of citations of associated activities close to or in the same space where the *Butia* palm groves are (*livestock (LS), agriculture (Agro), tourism and trail(T), river stone extraction (RSE), planting eucalyptus (PE), extractive organization (EO), archaeological excavations (Arch), scientific research (SR), the establishment of a cooperative company (ECOO), Landscaping (Land) with a demonstration of the Informants Diversity Value (IDV) and Associated Activity Diversity Values (AADV) for the different categories, gender of respondents, country of origin of respondents and working relationship with *Butia* palm groves. Equal letters in the columns indicate no significant difference between groups, according to the Kruskal Wallis (Country) and Man-Whitney (Gender and Connection) test at a 5% error probability.

		Associated activity										
Category		**LS***	**Agro***	**T***	**RSE***	**PE***	**EO***	**Arch***	**SR***	**ECOO***	**Land***	**IDV***
Gender	Men (n=4)	0	1	1	1	1	2	0	0	0	1	0.116 A
	Women (n=10)	4	2	4	0	1	0	1	2	1	2	0.212 A
Country	Argentina (n=4)	2	1	3	1	1	0	0	0	0	1	0.133 A
	Brazil (n=8)	1	2	1	0	0	2	0	2	1	2	0.171 A
	Uruguay (n=2)	1	0	1	0	1	0	1	0	0	0	0.100 A
Connection	Owner (n=2)	1	0	1	0	0	0	0	1	0	1	0.111 A
	extractivist (n=12)	3	3	4	1	2	2	1	1	1	2	0.211 A
Total of citations		4	3	5	1	2	2	1	2	1	3	
	AADV	0.16	0.12	0.20	0.04	0.08	0.08	0.04	0.08	0.04	0.12	

*livestock (LS), agriculture (Agro), tourism and trail(T), river stone extraction (RSE), planting eucalyptus (PE), extractive organization (EO), archaeological excavations (Arch), scientific research (SR), the establishment of a cooperative company (ECOO), Landscaping (Land).
**Informant Diversity Value (IDV)= Summation of the number of citations of each interviewee divided by the total number of citations in the category (gender=man or woman; country= Argentina, Brazil, Uruguay; connection= owner or extractivist). AADV= Number of citations for each associated activity category divided by the total number of citations for all categories.

Activities related to livestock affects the management of *Butia* since one of the great difficulties in the establishment of new plants of the species is in the long period that the seed takes to germinate, which can reach more than 24 months, in addition to the long period until the fruiting (Carpenter 1988, Broschat 1998). During the emission of the first pinnate leaves, *Butia* is also more exposed to weather and herbivore attacks, especially in periods of scarcity of more palatable forages (Báez & Jaurena, 2000). In contrast, the presence of cattle is essential in the management of ecosystems in the *Butia* palm groves (Rivas *et al.*, 2017; Sosinski *et al.*, 2020b). Grazing helps to avoid competition for light with other species of plants with faster development and facilitates the regeneration of new *Butia* palms. Research has recommended techniques such as the conservative management of livestock in native fields, where the presence of cattle is restricted during some days of autumn and winter. These are periods of greater scarcity of pasture and greater possibility of damage to *Butia* seedlings (Sosinski Junior *et al.* 2015, 2019, 2020b; Hagemann 2016, Rivas & Barbieri 2014).

In this sense, conservative management is vital for conserving the *Butia* palm groves ecosytem and maintaining the local economy through consortium activities, such as tourism.

Knowing how to conserve also applies to the perspective of tourism. In this context, ecotourism is seen as "a segment of tourist activity that uses, in a sustainable way, natural and cultural heritage, encourages its conservation and seeks the formation of an environmental awareness through the interpretation of the environment, promoting the well-being of the populations" (Brasil 2010, 17). If well planned and to provide tourists with an interpretive educational experience, ecotourism can be an important tool for developing and conserving endangered species. Such activity is cited by the interviewees, who evidenced its use for the conservation of *Butia*. For instance. M.E.P .: "... yes, when they are aware of it, but often the population does not know the importance of these places (*Butia* palm groves), because it can generate jobs for the community. We generate 20 jobs. There is a National Park that needs people to work. Thus, 50% of the families in this community alone have a direct or indirect link with the *Butia* palm groves; there is a need for many people around these palm trees that receive tourists..." "...The environmental benefit of the palm that houses animals, the fauna, there is a nearby forest, wild animals. We have more than 120 birds, other animals, plant species, all of which are protected, which is a benefit. Because the palm is protected, it also protects animals. The *Butia* palm groves are important for nature, for wild animals, to maintain ecosystems and forest ecosystems, also field ecosystems..."

Therefore, the concern with the rational use of space is perceived in the discourse, seeking a balance between the ecological, economic, and sociocultural aspects of the area where the *Butia* palm groves are found. The concern with the generation of minimal negative impact on the natural and sociocultural environment can also be observed, from a long-term perspective (Albuquerque *et al.* 2015), mainly with the involvement of the local community in the process of planning and developing activities such as ecotourism.

Proposals for the conservation of *Butia* palm groves

Several ideas were indicated as tools to increase the value and interest that people attribute to the *Butia* palm groves, as well as actions that contribute to the conservation of *Butia*. With the analysis of the interviews and according to the Conservation Proposals Diversity Value index, the most cited strategy was the development of environmental awareness (n=11). This was the most cited strategy individually, according to the IDV, by the categories of women (0.300), Brazilians (0.285) and extractivists (0.320), and no significant difference was observed. The participants also cited public policies and academic/scientific partnerships as measures to encourage environmental awareness and, consequently, the conservation of the *Butia* palm groves (Table 5).

In general, the respondents showed concern with the conservation of the *Butia* palm groves. However, in their speeches, the lack of knowledge of effectively conserving the *Butia* palm groves was evident. Proposals for the conservation of genetic resources are essential, especially when it comes to native and threatened plants (Oliveira & Bursztyn 2001), such as the case of *Butia*. The participants in this study, without exception, agree that the development of environmental awareness is crucial for the conservation of species. Thus, the respondents demonstrated the will to improve the educational practices and the generation of knowledge associated with *Butia* palm groves.

All the actions mentioned by the participants involved proposals for public or private partnerships, tourist activities, environmental education, ecomuseums, and the creation of buffer zones, as previously mentioned. However, the proposals most mentioned and highlighted were related to academic/scientific partnerships (n=5) and the creation of public policies (n=4), as can be seen in M.P.I.'s narrative, an Argentine businesswoman: "... lately, they have been giving a lot of importance. We, who belong to the place, need support from the government so that we can continue with everything that we may have from activities, tours, tourism, making good crafts, everything we can work with the fruits of *Butia* palm...".

Butia palm groves comprise a valuable diversity of associated native flora and fauna, where trophic chains and energy flows characteristic of communities occur (Barbieri *et al.* 2015, Sosinski Junior *et al.* 2020a). They are the source of various ecosystem services, information/culture, forage and livestock production, habitat maintenance, biodiversity conservation, as well as mitigation of greenhouse gases and regulation of the water cycle (Sosinski Junior *et al.* 2015, 2019 and 2020a).

Table 5: Number of citations of conservation proposals for Butia palm groves (*public policies (PP), environmental awareness (EA), private partnerships (PRIPART), academic/scientific partnerships (ASPART), public partnerships (PUPART), tourist activities (TACT), environmental education (EED), buffer zones (BZ), ecomuseum (Ecomus), Informant diversity value (IDV)), with the statement of Informant Diversity Values (IDV) and Conservation Proposals Diversity Values (CPDV) for the different categories, gender of respondents, country of origin of respondents and employment relationship with *Butia* palm groves. Equal letters in the columns indicate no significant difference between the groups, according to the Kruskal Wallis test and Man-Whitney at a 5% error probability.

		Conservation Proposals									
Category		**PP***	**EA***	**PRIPART***	**ASPART***	**PUPART***	**TACT***	**EED***	**BZ***	**Ecomus***	**IDV***
Gender	Men (n=4)	1	2	2	1	2	0	0	0	0	0.177A
	Women (n=10)	3	9	1	4	0	1	2	1	1	0.300 A
Country	Argentina (n=4)	0	3	1	2	0	0	2	1	0	0.177 A
	Brazil (n=8)	3	7	2	2	2	0	1	0	1	0.285 A
	Uruguay (n=2)	1	1	0	1	0	1	0	0	0	0.111 A
Connection	Owner (n=2)	0	2	0	2	0	0	0	0	0	0.222 A
	extractivist (n=12)	4	9	3	3	2	1	2	1	1	0.320 A
Total of citations		4	11	3	5	2	1	2	1	1	
CPDV		0.13	0.36	0.10	0.16	0.06	0.03	0.06	0.03	0.03	

*public policies (PP), environmental awareness (EA), private partnerships (PRIPART), academic/scientific partnerships (ASPART), public partnerships (PUPART), tourist activities (TACT), environmental education (EED), buffer zones (BZ), ecomuseum (Ecomus).

**Informant Diversity Value (IDV)= Summation of the number of citations of each interviewee divided by the total number of citations in the category (gender=man or woman; country= Argentina, Brazil, Uruguay; connection = owner or extractivist).

CPDV= Number of citations for each conservation proposals category divided by the total number of citations for all categories.

In situ conservation provides the maintenance and recovery of species populations in their original environments. The strategies carried out in residences, production units, or *Butia* palm groves ecosystems, have contributed to the conservation of diversity at all levels, in addition to enabling the producer / extractivist the accession and use of genetic resources to improve their livelihood.

In Tapes and Barra do Ribeiro, Rio Grande do Sul state, where the largest remnants of Butia palm groves in Brazil are found, the genetic resources are conserved in situ, that is, in the farms (Costa et al., 2017; Mistura, 2013). Furthermore, the *Butia* Palm groves were defined as Legal Reserves areas on each farm. In general, Brazilian environmental legislation provides legal protection for native flora in three ways: protection for those species in official extinction lists at federal and state levels (Brasil, 2014; Rio Grande do Sul, 2014); preventing the plant from being cut, which is allowed only in exceptional cases; and restrictions on the suppression or exploitation of vegetation according to its ecological complexity (Sosinski *et al.* 2019).

In Uruguay, most *Butia* palm groves of *Butia odorata* are also located in private properties where agricultural activities are practiced (primarily rice cultivation and livestock) (Rivas, 2013). The *Butia* palm groves (of *Butia* odorata) are part of the "Bañados del Este" Biosphere Reserve (UNESCO, 1976). By National Law 9872 (1939), (Law nº 9.872, 1939, altered by Law nº 15.939 1987), the damage and the cutting of palm groves was prohibited (Rivas 2005; Dabezies & Rivas 2020). Some strategies for encouraging in situ conservation seek the valorization of **butiá** fruits and the *Butia* palm groves as a part of Uruguay's identity. Furthermore, when properly conducted, cattle management contributes to the conservation of butia palm groves. In Uruguay, there are also initiatives by organizations and institutions that work together to protect *Butia* palm groves (Betancurt and Crosa, 2014).

Since 2009 the *Butia* palm groves have been considered native vegetation by the Argentine legislation through Decree No.91/2009. And in 2017, the Law nº 26.331 for the protection of native vegetation was approved. It is also important to highlight that, since 1966, Argentina has maintained the El Palmar National Park, created to protect *Butia yatay* (Entre Ríos province) (Batista *et al.* 2014; Policelli et al., 2018). There is also regional legislation to protect natural environments (El Palmar, 2015). Also, it is worth noting that Argentina has four other areas with *Butia* palm groves protected in addition to the Parque Nacional El Palmar. In the Refugio de Vida Silvestre La Aurora del Palmar, in the Sitio Ramsar Palmar *Yatay* and in the Parque Nacional Mburucuyá, we find *Butia yatay*, while in National Park Iberá we find *Butia paraguayensis*, *B. yatay* and *B. poñi* (Maranta 2020). Maranta (2020) also emphasizes that Argentina is concerned about conserving its biodiversity through areas of protection and conservation, mainly through the creation of protected areas, which has many advantages but also limitations. Yet, he highlights that the use of native plants is relatively little promoted in Argentina, unlike in Brazil and Uruguay, where conservation is more associated with intended use than with their preservation.

The recognition of legal reserves for the *Butia* palms groves is essential for their conservation. Nevertheless, it can not be the only strategy to promote the conservation of the *Butia* palm groves. Considering the Brazilian example, even if there are legal reserves in the country, the *Butia* palm groves are not adequately recognized in the official vegetation classification systems (Brazilian Institute of Geography and Statistics (IBGE), 2012; Veloso *et al.* 1991, Sosinski *et al.* 2019); however, there is an exception when palm groves are associated with other types of vegetation such as Pampa (Brazilian Institute of Geography and Statistics (IBGE), 2012; Oliveira-Filho, 2009). There is a lack of ecological recognition and legal protection of palm groves as natural and ecologically integral ecosystems in Brazil (Sosinski *et al.*, 2019). Thus, other strategies that aimed at the conservation of Butia palm groves are adopted by those who are concerned about this ecosystem.

The perception of the *Butia* palm groves contexts shown by the interviewees presents some concern about the conservation of the native plants, as can be seen in the speech of M.G. when asked about the importance of the *Butia* palm grove "... The system we use is agroforestry, syntropic agriculture. The palm tree is a source of connection in the ecosystem. It fulfills its function as a fruit rich in vitamin C. For the local fauna, it brings great diversity to the place... It brings many animals there, and they come back... The whole ecosystem then has more importance ... " and also states that " ... currently people are more aware of preserving nature ... ".

A.A.M.S.'s statement points out the environmental interactions with *Butia* palms space and income generation. When asked about the possibility of extractivism and preservation, A.A.M.S. said: "... Every time I drink juice or have some jelly, I drink liquor (from **butiá**), I am helping, I am exercising to improve the generation of income for those on the field, who are extractivists...". A.A.M.S. also adds: "... through the Ecovida network, and participating in Agroecology, and the Slow Food movement, we managed to preserve, conserve, and show the importance of **butiá** for everyone. The issue of fauna, flora, the issue of income generation and the options for sustainably making money...".

The sustainable management of native fruit species is a tool for preserving existing agrobiodiversity in the places where these plants coexist. For the management of ecosystems of *Butia* palm groves in places where there are agricultural activities, the exclusion of grazing during the winter and the continuous grazing of cattle during the rest of the year is a sustainable alternative, which helps in the conservation of the native field. *Butia* seedlings that develop under proper management escape the action of trampling and grazing animals, and already established plants have more significant potential for sprouting (Sosinski *et al., 2015)*. Sustainable extractivism, associated with management, can be planned through a harvesting circuit covering many collection sites and providing for rotation between them. Thus, avoiding excessive harvesting in a single area, some fruits must remain on each palm due to the regeneration of the environment. In addition, it favors the production of seedlings and the feeding of wild animals (Rivas and Barbieri, 2014, Sosinski et al., 2015, 2020b).

The generation of income from the sustainable management of natural resources has contributed to the conservation scenario (Negi *et al.*, 2017). Movements such as Slow Food, which aims to provide food produced consciously, respecting both the environment and the people responsible for the production (Petrini 2009), motivate critical thinking about non-agroforestry systems. Slow Food operates both locally and globally, with international institutions such as FAO - Food and Agriculture Organization of the United Nations. Moreover, it establishes bonds of friendship with governments worldwide (Petrini 2009).

Two statements point to the importance of the existence of the *Butia* Palm Groves Network for this sustainable relationship. First, according to R. J., when asked about the role of the *Butia* Palm Groves Network, "... since the beginning, *Butia* Palm Groves Network has been important. These partnerships that the event has today, with Technical Assistance and Rural Extension Company (Emater) and Embrapa, entities that are also concerned, seeking alternatives with the community. This integration of other regions, for example, strengthens the journey to continue and expand, with knowledge, the exchange of experience, in short... Even though it is an old plant, its use continues, and this shared knowledge guarantees a little plant conservation. People working with **butiá**, having economic goals too, will be more concerned with spreading, expanding, replanting, and conserving the *Butia* palm groves that already exist..." The *Butia* Palm Groves Network has been an ally, providing integrative experiences, which, as an alternative to the traditional economic system, collaborate directly or indirectly to conserve *Butia* palm groves (Rivas *et al.* 2020). This can be observed through C.H.B.'s speech: "...You observe that this consortium with the *Butia* Palm Groves Network has been beneficial, to a certain extent, because as there was no regeneration, we sought out Embrapa. Since then, research has been done, but the property is always managed considering the *Butia* palm groves".

Albuquerque and Andrade corroborate the speeches about the *Butia* Palm Groves Network's interaction networks (2002). The authors reinforce the idea that knowledge accumulated by local populations and integration of different communities in favor of a common goal constitute a powerful tool. Developmentalists and conservationists can use this tool to manage and maintain these areas.

Conclusions

This study sought to compile the view of different participants in the *Butia* Palm Groves Network event that took place in 2018. People from different social spheres, sites, states, and countries share their interest in conserving the *Butia* species and the *Butia* palm groves. However, although the interviews attest to its importance, almost none of the interlocutors knew how to effectively implement a practical way to conserve the *Butia* palm groves beyond conscious extractivism. In order to efficiently conserve such environments, a Statal contribution is necessary through specific legislation, recognition, effective surveillance, and scientific research involving the *Butia* palm groves. Unfortunately, the goodwill of the people who work with the *Butia* palm groves is not enough to defend its ecosystems. Nonetheless, initiatives such as the *Butia* Palm Groves Network, the Solidarity Chain of Native Fruits, the Slow Food, among other organizations, promote the production and sale of products from the sustainable use of the ecosystem, which adds value to the conservation interest of these places. Moreover, the understanding of livestock management methods and other associated activities such as tourism also contributes to *Butia* palm groves' conservation.

The *Butia* palm groves cross countries' borders and harbors a unique sociocultural and biological importance. Given the historical and cultural importance of **butiá** and *Butia* palm groves and their valuation as a heritage, both natural and social, their conservation is necessarily related to good practices of extraction and sustainable management.

Declarations

List of abbreviations: Informant Diversity Value (IDV), Use Diversity Value (UDV), Contact Diversity Value (CDV), Activities Diversity Value (AADV), Proposals for conservation Diversity Value (PCDV) Production of jellies (PJ), Juices (J), Cakes (C), Breads (B), Cucas (C), Liquor (L), Salty snacks (SS), Vinegar (V), Cachaça (CA), Cookies (CK),), therapeutic (TP), use of coquinho (UC), fresh fruit selling (FFS), crafts (CR), Art (A), Fresh Fruit (FF), Livestock (LS), Agriculture (Agro), Tourism (T), River Stone Extraction (RSE), planting eucalyptus (PE), extractive organization (EO), archaeological excavations (Arch), scientific research (SR), the establishment of a cooperative company (ECOO), Landscaping (Land), public policies (PP), environmental awareness (EA), private partnerships (PRIPART), academic/scientific partnerships (ASPART), public partnerships (PUPART), tourist activities (TACT), environmental education (EED), buffer zones (BZ), ecomuseum (Ecomus).

Ethical approval and consent to participate: The study was submitted to the Federal University of Pelotas Research Ethics Committee, obtaining a favorable opinion No. 2,565,059.

Consent to publication: Not applicable in this section

Availability of data and materials: Not applicable in this section

Competing interests: There are no competing interests

Funding: Ministry of Science, Technology and Innovations (MCTI), CNPq (process 441493 / 2017-3) and Transmissão de Energia Eletrica Sul Brasil S.A. (TESB).

Authors' contributions: TS followed the interviews, carried out the statistical analysis of the data, and wrote the work. PSG contacted respondents and conducted the interviews. MKP performed the transcription of the audios of the interviews. JMGD contributed by indicating anthropological references. JGC proofreading. JGW proofreading and statistical support. RLB supervised and guided the development of the work.

Acknowledgments

To the participants in the *Butia* Palm Groves Network (Rota dos Butiazais), for building this great collaborative network. To the Ministry of Science, Technology and Innovations (MCTI), CNPq (process 441493 / 2017-3) and Transmissão de Energia Elétrica Sul Brasil S.A. (TESB), for the financial support for the execution of the Rota dos Butiazais Project.

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Appendix 1

Questions		Answers	Observations
1.	How long has *Butia* palm grove existed? How did it come about?		
2.	How long have you known *Butia* palm groves?		
3.	Do you use *Butia* palm groves? How often?		
4.	Does *Butia* palm groves influence your direct daily activities?		
5.	Do you consider *Butia* palm groves important for the local community and for nature? Because?		
6.	What are the main activities in the *Butia* palm groves area?		
7.	Do *Butia* palm groves favor these activities? Because?		
8.	What would be important for activities with *Butia* palm groves to be maintained? Because?		

3 Variation in palm tree plastidial Simple Sequence Repeats, characterization and potential use

Artigo submetido à revista *Crop Breeding and Applied Biotechnology*

Variation in palm tree plastidial Simple Sequence Repeats, characterization and potential use

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ABSTRACT: *Palm trees compose the third most important botanical family for humans, due to their potential use in oils, drugs, cosmetics and as food/feed. Despite their importance, scarce information on their genetics and molecular variations exist and their better understanding could contribute with breeding programs. This study aimed to detect the amount, distribution, and organization of plastid SSRs (cpSSRs) in 52 species belonging to the Arecales order and their potential use in breeding. Plastid genomes were analyzed identifying cpSSRs in their nature, position and presence in genic or intergenic regions. Primer pairs for the amplification and evaluation of polymorphisms in these SSR as well as their dissimilarity are shown here. Results show a higher frequency of mononucleotide repeats in intergenic regions. Approximately 76 primer pair sequences were generated and are suggested for further studies. The cpSSR dissimilarity analysis shows that mono and trinucleotides are highly abundant in plastid SSRs*.

KEYWORDS: *SSRs, Chloroplast DNA, markers, palm three*

INTRODUCTION

Arecaceae includes most palm trees and represents the third most important botanical family for humans (Johnson 1998). This family is a part of an order of flowering plants called Arecales. This order comprises the Arecaceae and, more recently, the Dasypogonaceae family (The Angiosperm Phylogeny Group, 2016). For Arecales a total of 192 genera and 2,603 species have already been described (Main Tree 2021, NCBI 2021).

Among its species, most have economic importance, given their potential for use in human and animal food, production of inputs, oils, drugs, cosmetics, and decorative utensils, as well as for the ornamentation of gardens and public roads. Some species such as *Cocos nucifera* (coconut), *Phoenix dactylifera* (date palm) and *Elais guineensis* (oil palm) deserve special attention due to their local economic importance and high potential of use. Coconut cultivation reached an area of 12.3 million hectares in 2017, with production of 60.7 million tons of coconut fruits in 92 countries. The Philippines, Indonesia and India accounted for 72.7% of the area. Brazil has a planted area of 215,683 hectares and production of about 2.4 million tons of fruit per year (FAO 2017). The Northeast region is responsible by 74% of national production, and is concentrated in the states of Bahia, Sergipe, Rio Grande do Norte and Pernambuco (IBGE 2017). Despite the progress achieved with coconuts, the little understanding of genetic features of palm species slows down their economical widespread, and their full potential is yet to be exploited. Also, genome sequence and physiological data are still scarce, something that can be even seen in *Butia* spp. (Nazareno et al. 2011).

The Simple Sequence Repeats (SSRs), also called microsatellites, are highly informative due to its size variation, being widely used for breeding and genetics studies in many plant species (Weber 1990, Kashi and King 2006, Palliyarakkal et al. 2012). The development of molecular markers for palm species increased its prominence after the complete sequencing of the nuclear genome of oil palm (Singh et al. 2013) and coconut (Aljohi et al. 2016). Currently, SSR markers are already used in Arecales, for studies regarding ecology, systematics, conservation, phylogeny, and even in breeding programs (Elshibli and Korpelainen 2008, Aljohi et al. 2016, Zhao et al. 2016, Xiao et al. 2017, Bai et al. 2017, Khan et al. 2018, Babu et al. 2019, Bhagya et al. 2020, Kpatènon et al. 2020). Some nuclear SSRs (nSSRs) have been developed for the most important representatives of this order, such as *Phoenix. dactylifera*, *Cocos. nucifera, Calamus simplicifolius, Elaeis oleifera* and *Elaeis guineensis*, however the use of chloroplast or plastidial SSRs (cpSSRs) is still limited.

The cpSSRs show great potential for use in phylogeography and DNA fingerprint analyzes for Arecaceae (Lopes et al. 2018, 2019) and, considering that many researchers have sought to use hypervariable regions of chloroplast or plastidial DNA (cpDNA) to characterize the genetic variation of species (Shaw et al., 2005). Therefore, the aim of this study was to detect the amount, distribution and organization of cpSSRs in 52 species of the order Arecales, as well as to identify the SSRs that most contribute to the differences found among the plastidial genomes.

MATERIAL AND METHODS

In this study, all available plastid genomes from Arecales were downloaded from NCBI (National Center for Biotechnology Information) (**https://www.ncbi.nlm.nih.gov/genomes/GenomesGroup.cgi?opt=plastid&taxid=2759**). We assessed SSRs for coding (genic) and non-coding (intergenic) regions according to the information available in the NCBI database for each species. A total of 52 complete cpDNAs and the coding regions of these (Supplementary material 1) were inserted in SSRlocator (Maia et al. 2008). The minimum repeat size was set to >=8-mono, >=6-di, >=3-tri, tetra, penta, and hexanucleotides (hexamers).

Primer pairs for sequences flanking each SSR were designed also using *SSRLocator* according to the following parameters: GC content 40 to 60%, melting temperature (T_m) ranging from 57 to 60 °C, primer size ranging from 22 to 27 bp and PCR product size ranging from 101 to 300 bp, as suggested by Preethi et al. 2020.

For SSR comparative analysis, a heat map based in their dissimilarity was generated using CIMminer (**https://discover.nci.nih.gov/cimminer/**). Organellar Genome Draw (OGDRAW) was used for representing the SSR annotation (Lohse et al. 2007). A phylogeny based on the RuBisCo sequences was built. Thus, each species of Arecales order had its RuBisCo sequence extracted and aligned with ClustalW. From the identification of the best nucleotide substitution model, a phylogenetic tree was built based on the Bayesian model, using 1 million boostrap replicates. Principal component analysis was used to highlight the reason that most contributed to the differentiation of phylogenetically defined groups. For this analysis we used the statistical software R (R TEAM CORE 2020).

RESULTS AND DISCUSSION

The cpSSRs from the 52 Arecales species reported in NCBI is available in Supplementary material 2. The highest number of cpSSRs is observed in *Wallichia desinflora* (302), while the lowest is found in *Dasypogon bromeliifolius* (200). The largest variation is cpSSR size is found when *Tahina spectabilis* (126,251 pb) (Barrett et al. 2016) and *Caryota obtusa* (159,882 pb) are compared (Gao et al. 2020).

Although we have identified mono-, di-, tri-, -tetra, -penta and hexamers in majority of Arecales species (Supplementary material 2), the highest abundance is found as monomers. Currently, no studies evaluating distribution and organization of cpSSRs across all Arecales are available, but Palliyarakkal et al. (2012), Al-Faifi et al. (2017), Lopes et al. (2018) and Khan et al. (2018) also identified a high number of monomers in in intergenic regions of palm trees (for the species *Acrocomia aculeata, Phoenix dactylifera*, and others species). However, monomers are rarely used as markers in palms, it commonly occurs with dimers, trimers, tetramers, pentamers and hexamers (Preethi et al. 2020). Monomers may deteriorate over short periods of time, as these have a high replacement rate (Ceplitis et al. 2005). Several studies on cpDNAs from palm species (Magnabosco et al. 2020, Zou et al. 2021) make it possible to perform genetic studies using the cpSSRs to analyze populations of these plants.

Most of the SSRs found were monomers in intergenic regions. Tri and tetramers were also observed, which were frequent both in genic and intergenic regions (Supplementary material 2). However, 18 species of the Arecaceae family and one of Dasypogonaceae, present greater abundance of trimer repeats in genic regions, and these present great potential for the development of molecular markers.

Primer pairs for amplification of all SSRs from the 52 analyzed palm species have been designed and are available online (Supplementary material 3). Species of higher economic interest, such as *Cocos nucifera*, *Elaeis guineensis*, *Phoenix canariensis* *Phoenix dactylifera,* and *Syagrus coronata,* present respectively, 74, 83, 75, 76 and 79 cpSSR that could be amplified, while species of the family Dasypogonaceae, such as *Baxteria australis* and *Dasypogon bromeliifolius*, present 66 and 75 cpSSR. The primers were developed based in Preethi et al. (2020), for *Cocos nucifera.* Melting temperature (Tm) ranged from 57 to 60 °C, primer GC content ranged from 40 to 60%, primer size ranged from 22 to 27 bp, and product size ranged from 101 to 300 bp.

A report of 93 polymorphic nuclear SSRs on the palm species *Phoenix dactylifera* has been published (Al-Faifi et al. 2017). Markers based in cpSSRs are known to be more effective indicators of population subdivision and differentiation than nuclear markers in plants even with taxonomic variations, cpSSRs are generally abundant in most plants (Powell et al. 1995a and 1995b, Provan et al. 2001, Petit et al. 2005, Ebert and Peakall, 2009), as observed in this study.

The application of cpSSR markers is especially useful since these can amplify homologous regions in several taxa due to its uniparental inheritance and slow accumulation of mutations throughout the ages, something that makes evolutionary studies easier and more effective. When evaluating the molecular evolution of plastids within the Arecaceae family, using every gene that encodes plastid proteins, high divergence could be observed. This suggests that degeneration process may be taking place within Arecaceae at the level of genus and/or species (Lopes et al. 2018). The distribution of positive signatures across the phylogenomics of Arecaceae suggests convergent evolution of most sites, including genes involved in photosynthesis. Therefore, researchers seek to use non-coding regions, including introns and intergenic spacers to characterize genetic variation (Shaw et al. 2005, 2007). On the other hand, the use of SSRs as markers can help the discovery of genetic and molecular features that are not much explored in Arecaceae.

The cpSSR markers transferability is easier when species belong to the same order and family, helping research in species that still need further studies (Xiao et al. 2016). Transferability studies between *Cocos nucifera* and *Butia odorata* (Mistura et al. 2012) confirms that 66% of these plastid SSR markers present polymorphisms, evidencing its great advantage. Other successful example occurred in *Medemia argun*, a palm species believed to have a total world population of only 1,000 individuals, which are found in Nubia Sudan desert. Transferability also proved to be possible with cpSSR of *Bismarckia nobilis*, that is phylogenetically related with *Medemia argun*, that proved to be extremely important for the study related to conservation of this almost extinct species (Elshibli and Korpelainen 2018). Other authors highlighted the efficiency of the transferability markers in palm, evidencing the importance of identifying SSRs in known species and suggesting an advantage over conventional markers due to their high polymorphism (Ebert and Peakall 2009).

We can observe through the genome map of the *Cocos nucifera* plastid, the most frequent cpSSR in the 52 species studied in this work, for the genes *trnH-psbA*, *matK*, *rcbl* and *rps19* (Supplementary material 4 and 5). The *rbcl* and *matK* genes are used and standardized for DNA barcoding in terrestrial plants (CBOL Plant Working Group 2009), given their high levels of high-quality sequences and acceptable levels of species differentiation and identification (Burgess et al. 2011). *trnH-psbA* is also used for DNA barcoding, in addition to the two genes already mentioned (Hollingsworth et al. 2011). The gene *rps19* was also used since it exists in duplicate in most plastid genomes of arecales species (Magnabosco 2020).

Dissimilarity analysis of cpSSR in palms (Figure 1) show highly dissimilar mono- and trimers, reflecting the great abundance of these SSR types. In addition, we observed that the 52 species evaluated in this study formed two groups based in the SSR frequency. A first group with the species *Dasypogon bromeliifolius* and *Baxteria australis*, both belonging to the dasypogonaceae (Liliopsida), while the second is a large group, subdivided into four subgroups, with all the Magnoliophyta. The second group genetic proximity was already described by Meerow et al. (2009), when analyzing seven WRKY genes between *Cocos nucifera* and *Syagrus coronata.* Still, we observed the proximity between the species *Astrocaryum* sp and *Acrocomia* sp, identified by Meerow et al. (2015) as sibling genera when evaluating six WRKY genes.

Figure 1 shows a number of species previously described as phylogenetically close, dividing the clusters by the families analyzed. Furthermore, the highest frequency of monomers is found in the order Arecales. Therefore, studies that allow the development of cpSSR markers in these species should allow a better understanding of genetic relationships within this order (Zhang et al. 2017). From now on, further tests to amplify these markers and to test their transferability between related species should be performed and may help us analyzing genetic diversity and evolutionary history across these important plants (Doorduin et al. 2011, Ebert and Peakall 2009).

We detected a total of 14,017 SSRs across all the analyzed palm species. A total of 9,087 SSRs were composed by monomers, while 502 were composed by dimers, 3,676 by trimers, 483 by tetramers, 204 by pentamers and 65 by hexamers. The most abundant repetitive motifs found for the 52 species studied were AT and TTC/AAC/TAT for the di- and trimers, respectively. A total of 38,086 SSRs were found for the Arecaceae family, in public domains (Palliyarakkal et al. 2012), while in *Syagrus romanzoffiana* alone1,563 sequences were found (Laindorf et al. 2019). The most frequent motifs in these studies were di- and trimers, respectively. In contrast, our study presented a higher amount of SSRs composed by monomers. The discrepancy of values found in different studies can be attributed to the type of data filtering uses, as well as the increase in the amount of information available in databases or even changes in the pattern of the strings (Laindorf et al. (2019).

The evolution of SSRs has come under different pressures, observed even at the level of closely related species. This statement is evident when we compare the dissimilarity analysis based on the number of SSRs and phylogeny based on the conserved RuBisCo gene (Supplementary material 6). A difference was observed in the positioning of species of specific groups between the two analyses, in which we can highlight that, through the phylogeny, all species of the same genus, in short, were side by side, as is the case with *Arenga* sp, *Astrocaryum* sp and *Areca* sp. It is worth noting that the proximity of *Cocos nucifera* and *Syagrus coronata* is widely discussed in the literature and used in several studies to infer phylogenies with representatives of the Arecaceae family (Meerow et al. 2009 and 2015). Thus, showing that the distribution of SSRs can show differences even between closely related species, as the positioning of the species observed in the dissimilarity does not follow the same organization of the phylogenetic analysis.

The principal component analysis (PCA), seen in Figure 2, shows that the motifs that most affect the grouping of species due to both its presence*absence and intron*exon positioning are TAT3, GAA3, AAG3 and AGA3 which occur in three or four groups formed in the dissimilarity analysis, showing persistence and stability. These data can be useful for diversity analysis due to its capacity for differentiating closely related species and maybe even populations of the same species. Motif ATA3, on the other hand, presented non-linear behavior and was not selected for further tests.

CONCLUSION

In this work, a higher frequency of mononucleotide repeats in intergenic regions was observed, and 76 pairs of primers that can be used in future studies were reported. Through cpSSR dissimilarity analyses, mono and trinucleotides were found to be highly different from each other, and abundant in plastids. When comparing the rubisco SSR of the 52 species studied, in a phylogeny analysis, we observed a distribution pattern of the species very different from what was found in the dissimilarity analysis. Furthermore, we identified that the SSR that most contributed to the grouping of species were TAT3, GAA3, AAG3 and AGA3. Thus, we observe that studies of this order are important because they can give directions to phylogenies, as well as help in the conservation of germplasm and genetic improvement of palm trees.

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FIGURES

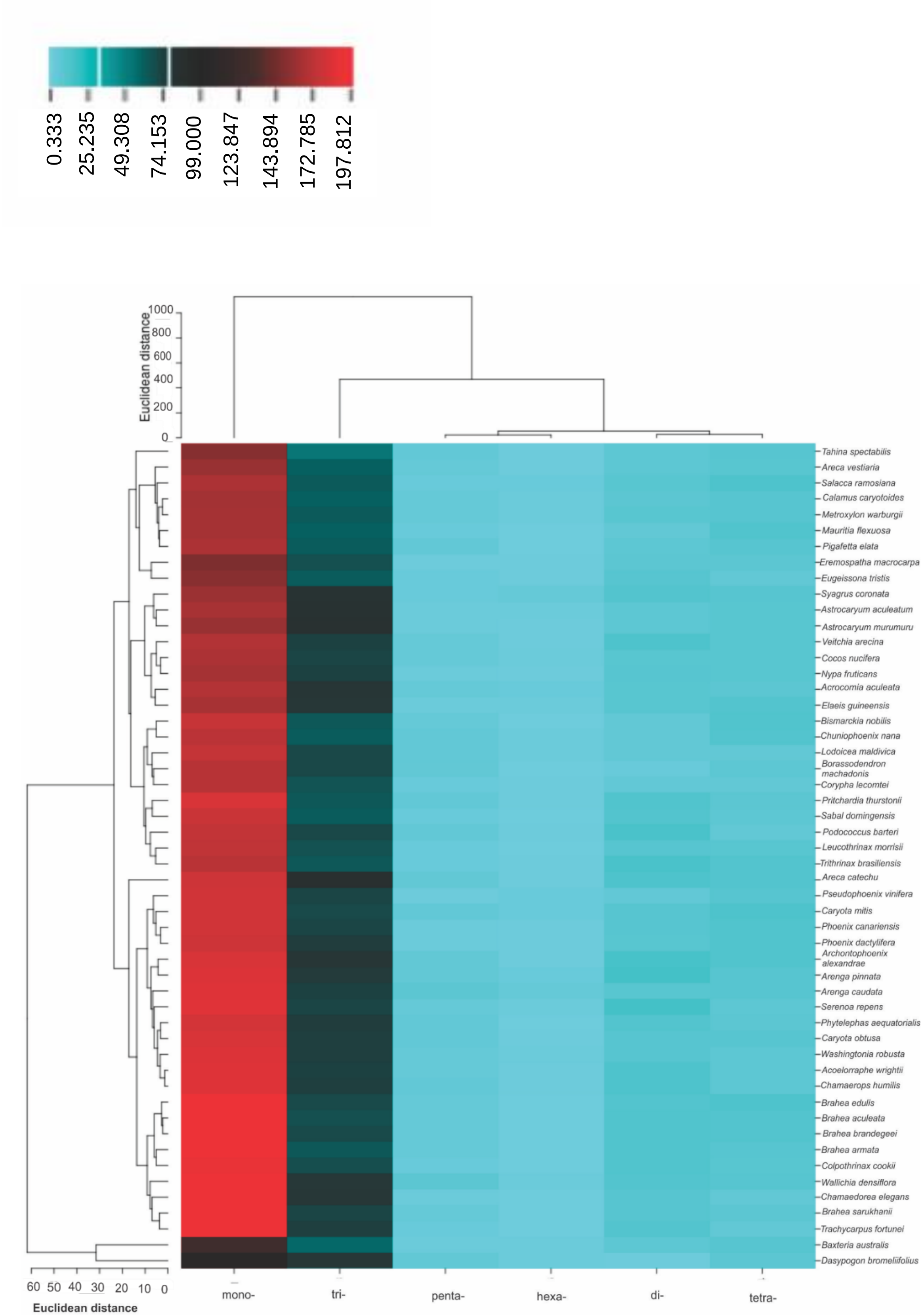

Figure 1. Dissimilarity analysis of 52 species of the order Arecales based on cpSSRs.

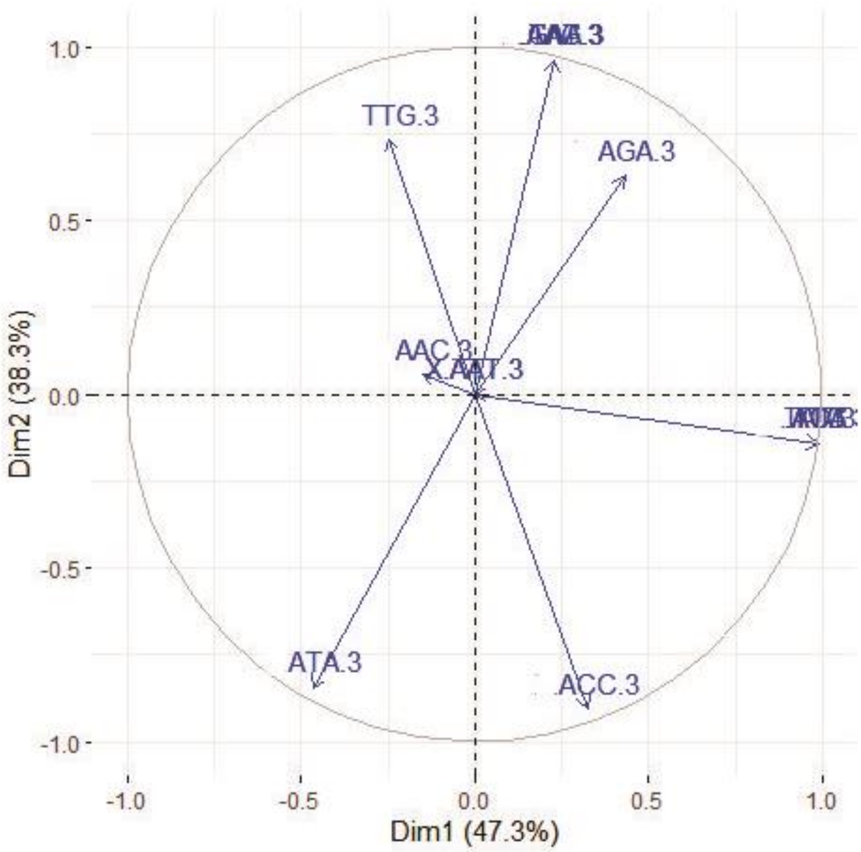

Figure 2. Ordering diagram of the Principal Component Analysis (PCA) performed with the five groups from the dissimilarity analysis of 52 species from the order Arecales based on cpSSRs.

SUPPLEMENTARY MATERIALS

Supplementary material 1. Table 1. Plastome size in each of the 52 Arecales species

Supplementary material 2. The cpSSRs from the 52 Arecales species reported in NCBI.

Supplementary material 3. Primer pairs for amplification of all SSRs from the 52 analyzed palm species have been designed.

Supplementary material 4. SSRs in coding regions of the chloroplast genome 52 species.

Supplementary material 5. The genome map of the *Cocos nucifera* plastid, the most frequent cpSSR in the 52 species studied in this work, for the genes *trnH-psbA*, *matK*, *rcbl* and *rps19.*

Supplementary material 6. Dissimilarity analysis based on the number of SSRs and phylogeny based on the conserved RuBisCo gene.

Arquivo disponibilizado de forma separada em formato .xmlx e .word.

4 Phylogenetic relationships of *Butia* spp. through WRKY transcription factors

A ser submetido à revista *Brazilian Journal of Biology*

Phylogenetic relationships of *Butia* spp. through WRKY transcription factors

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(With 8 figures)

Keywords: Arecaceae, *Butia odorata*, phylogeny

Palavras-chave: Arecaceae, *Butia odorata*, filogenia

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Abstract

The *Butia* is a genus of palm trees of the Arecaceae family, that produces edible fruits and represents potential economic importance in the food and agro-industrial area. There are only few studies regarding its molecular biology and bioinformatics, and little is known about its phylogenetic origin. This work aimed to infer the genetic relationships of species of the genus *Butia* through the analysis of transcription factor sequences (WRKY). All WRKY sequences are available at the NCBI for the *Butia* species and were searched to establish the phylogenetic relationship between these species. Through the results, it was possible to observe a variation in the clustering patterns when the different WRKY regions (2, 6, 7, 12, 16, 19, 21) were evaluated, and the species were not always grouped due to their morphological similarity, widely discussed in the literature. Therefore, the results reinforce the importance of studies of this order for further clarification of the phylogenetic origin of this genus, aiming at its conservation.

Relações filogenéticas de *Butia* spp. **através de fatores de transcrição WRKY**

Resumo

Butia é um gênero de palmeiras da família Arecaceae, que produz frutos comestíveis e representa potencial importância econômica na área alimentícia e agroindustrial. Existem poucos estudos sobre sua biologia molecular e bioinformática, e pouco se sabe sobre sua origem filogenética. Este trabalho teve como objetivo inferir as relações genéticas de espécies do gênero *Butia* através da análise de sequências de fatores de transcrição (WRKY). Todas as sequências de WRKY estão disponíveis no NCBI para as espécies de *Butia* estudadas, elas foram pesquisadas para estabelecer a relação filogenética entre essas espécies. Através da avaliação das diferentes regiões WRKY (2, 6, 7, 12, 16, 19, 21), foi possível observar uma variação nos padrões de agrupamento, e nem sempre as espécies foram agrupadas devido a sua similaridade morfológica, amplamente discutida na literatura. Portanto, os resultados reforçam a importância de estudos desta ordem para maior esclarecimento da origem filogenética deste gênero, visando sua conservação.

1. Introduction

The family Arecaceae is listed as having 2,640 species globally distributed into 252 genera (Dransfield et al. 2008; Lorenzi et al. 2010). These species are predominantly present in the tropics, of which 270 (belonging to 38 genera) can be found in Brazil. (Lorenzi et al. 2010); this diversity has made palm trees symbols of tropical forests by endemism, wealth, or abundance (Galetti et al. 2003; Jones and Dransfield, 1995). The *Butia* (Becc.) Becc., is a South American genus that comprises 24 species (Deble et al. 2017; Soares, 2015; Sant'Anna 2021), distributed in Argentina, Paraguay, Uruguay (Geymonat and Rocha, 2009; Noblick, 2010; Soares, 2015; Keller and Paz-Deble, 2020) and Brazil, which is the country that has the largest number of species.

This genus of palm trees includes species of great economic and social interest, and has traditionally been used in gastronomy and handicrafts. In nature, the *Butia* palm groves shelter enormous wealth of animal and plant species that interact in it’s ecosystem (Barbieri et al. 2015; Barbieri et al. 2022).

The hybridisation is common among different species of palms (Uhl and Dransfield, 1987). The taxonomic classification of *Butia* is complex and controversial, with no consensus regarding the number of recognized species (Ellert-Pereira, 2019). This taxonomic confusion can also be explained by the formation of intergeneric hybrids of *Butia x Syagrus*: *Butyagrus nabonnandii* (Prosch.) V orster [*B. odorata* × *S. romanzoffiana* (Cham.) Glassmann)] and × *Butyagrus alegretensis* K. Soares (*B. lallemantii* × *S. romanzoffiana*) (Brusa and Grela, 2007; Soares et al. 2014; Soares, 2015). Therefore, hybridisation among some species could have taken place at some point in their recent past. It could still be happening, though this is difficult to state, probably because their identification presents several difficulties. For example, as reported by

Gaiero et al. (2011) current or recent hybridisation between *B.* p*araguayensis* and *B. lallemantii* could explain the high number of individuals with admixed genotypes observed in both species. In Uruguay, for example, the population of *B. paraguayensis* is more geographically isolated and suffers from the impacts of cattle and sheep grazing, due to the absence of adequate management, so hybridization may be a way to avoid its drift in the near future (Gaiero et al. 2010). Thus, no danger of negative effects of introgression or depression by exogamy is observed, as it is not substantially differentiated from populations of *B. lallemantii* (Allendorf et al. 2010).

Molecular and bioinformatics tools have helped to solve taxonomic problems, understanding genetic diversity between species. In this context, Multiloculos sequence typing is used for phylogenetic studies and involves the amplification of constitutive genes or housekeeping by PCR, followed by DNA sequencing (Kämpfer and Glaser, 2012). These genes are conserved and essential for the maintenance of basic cellular functions in the organism and are expressed at relatively constant levels. In multilocus sequence typing (MLTS), DNA sequences are compared and phylogenetic analyzes are performed with bases in similarity matrices or directly from the sequence. Thus, the differences in the nucleotides in a variable number of genes make it possible to distinguish between genotypes or sub-genotypes, depending on the degree of discrimination considered (Turchetto-Zolet et al. 2017)

For the genus *Butia*, studies are still at very early stages, at first, a molecular characterization with ISSR markers of some species of the genus (Rossato et al., 2007; Gaiero et al., 2011) who developed the. Furthermore, molecular characterization was recently performed by AFLP (Büttow et al. 2010) and microsatellites (Mistura, 2013) where the latter work detected significant genetic variability within populations phylogenetically associated with *Cocos nucifera*.

One of the molecular strategies is the analysis of WRKY transcription factors, which are widely distributed throughout the plant genome (Zang and Whang, 2005), these proteins seem to constitute a complex gene superfamily with a very ancient ancestor (Wu et al. 2005). To answer diversity and phylogenetic questions, the monophyly of the genera belonging to the Attaleinae subtribe, including the *Butia* was suggested *(*Meerow et al., 2009). Another study points to *Butia* as a sister group of *Jubaea* (Cuenca et al. 2008), close to two clades composed by the genera *Syagrus*, *Allagoptera*, *Attalea* and *Cocos*. However, the phylogenetic association and relationships between their species have not yet been entirely elucidated. This is justified by the lack of information on DNA regions useful for phylogenetic research in palm trees. More complete studies involving the genome or parts of the genome of *Butia* have not been carried out to date and are essential to assist in the conservation of the species of the *Butia* genus.

This work aimed to infer the genetic relationships of species of the genus *Butia* through the analysis of transcription factor sequences (WRKY2, WRKY6, WRKY7, WRKY12, WRKY16, WRKY19 and WRKY 21) available in the NCBI database.

2. Material and methods

Database:

The sequences of transcription factors WRKY2, WRKY6, WRKY7, WRKY12, WRKY16, WRKY19 and WRKY 21 of the analyzed species, including the sister group and outgroup (Table 1), were obtained from the database of the National Center for Biotechnology and Information of United States (NCBI) through GenBank. The NCBI is charged with creating automated systems to store and analyze knowledge about molecular biology, biochemistry and genetics; facilitate the use of such databases and software by the scientific community; coordinate efforts to gather information on biotechnology and conduct research into advanced methods of computerized information processing to analyze the structure and function of biologically important molecules (NCBI, 2022). These sequences were chosen because they are available in databases and are also used by Meerow *et al*. (2009).

Alignment:

Seven databases were built from each WRKY regions. The sequences were fetched and loaded into MEGA X using GenBank accession numbers. Subsequently, each transcription factor was ordered and aligned on the MAFFT v. 7 (Katoh et al. 2019). Finally, the derived files were exported to MEGA X and later the sequences were cut until reaching a consensus number of nucleotides.

Phylogenetic Analysis:

To determine the phylogenetic relationships, likelihood and Bayesian analyzes were performed. In the case of likelihood, the model selected was the range of taxa heterogeneity. The analysis was performed using the CIPRES Science Gateway platform with the RAxML 132 HPC BlackBox v. 8.2.11. For the Bayesian analysis, the best replacement model was selected using the Mrmodel tool within the PAUP program; version 4.0b10; Sinauer Associates, Sunderland, MA) (Swofford, 2002). The model was applied in Mrbayes v. 3.2.1 (Ronquist et al. 2012). in the CIPRES Science Gateway platform and a six-chain Markov Chain Monte Carlo (MCMC) algorithm was used to generate phylogenetic trees with random probabilities for 10,000,000 generations, later the trees were sampled every 1000 generations where 25% were discarded (burn-in fraction). The remaining trees were used to calculate the posterior probability (PP). A heating parameter (temperature) of 0.15 was used for all taxa analyzed. Trees were revised and tweaked in Geneious 2021.1.1 and customized in Corel Draw.

3. Results and discussion

WRKY transcription factors play an important role in plant developmental and physiological processes. For example, the transcription factor WRKY11 in rice controlled flowering time and plant height. Also, some WRKY proteins seem to be involved in mechanisms of induction of systemic acquired resistance (SAR).

The conserved domain WRKY binds to a conserved sequence in DNA called the w box (TTGACC/T) (Ulkner and Somssich, 2004) and this sequence can be observed in some gene promoters. WRKY-like proteins have been associated with several physiological situations, including the development and defense response of plants to biotic and abiotic agents (Ulkner and Somssich, 2004). Therefore, these proteins were observed as positive or negative regulators of gene expression in different plant defense responses (Eulgem, 2006).

WRKY 2 **phylogeny**

When observing the results regarding the grouping of the species of *Butia*, with *C. nucifera* and the out-group *S. coronata*, we noticed the grouping of the species of *C.nucifera* and *S. coronata* were isolated and the formation of a clade with the *Butia* species. We visualized for *WRKY 2* (Figure 1), *B. odorata* and *B. lallemantii* were close in this grouping, despite being plants with very different structures, their sizes for example are very variable, *B. odorata* can reach 10 meters (Lorenzi, 2010) while *B. lallemantii* does not exceed 1 m. However, they are similar plants in the appearance of woody and glabrous peduncular bracts, yellow flowers or in some situations purple. In addition to different shapes, for *B. odorata* they have fruits with different sizes (wider than long) and for *B. lallemantii* elongated, ovoid or cone-shaped fruits, the fruits are similar in size (2.0 to 3.5 cm), containing 1-3 seeds and homogeneous endosperm. In a study with pollen from *Butia* species, significant differences were observed between *B. odorata* (61-29 um) and *B. lallemantii* (45-24 um) species, the first species has the largest pollen grains and the second the smaller pollen grains (Mourelle et al. 2015). The species of *B. eriosphata* and *B. yatay* are close, being similar species in aspects such as having a solitary stipe, fruits containing 1-3 seeds and homogeneous endosperm, in other aspects they are distinct plants (Soares, 2015). In a previous study by ISSR analysis, *B. eriospatha* and *B. yatay* were classified as belonging to the same group (Rossato et al. 2007). In a study with *Arabidopsis*, the WRKY 2 transcription factor interacts with the VQ20 protein to modulate pollen development and function, therefore, mutations in WRKY2, WRKY34 and VQ20 simultaneously can result in male sterility, in addition to affecting pollen tube growth (Lei et al. 2017). Studies conducted to determine the functions controlled by WRKY proteins in coconut using homology modeling, identified WRKY 2 as β-strands with random coils, and it shares homology with sequences that have chains β (Sreesmitha et al. 2010). Therefore, it was shown that its nature is highly conserved and stress-induced structural transition suggested that the coconut protein WRKY2 shares similar hypothetical functions. Function assignment of this protein revealed that the DNA binding property was due to the highly conserved motif at the N-terminus.

WRKY 6

The transcription factor *WRKY* gene family is involved in several pathways and includes components from specific plants and old regulatory networks. WRKY genes contain one or two highly conserved DNA binding domains interrupted by an intron (Borrone et al. 2007).

When differences in the transcription factor WRKY 6 were used to build the clades, it was possible to distinguish the genus *Cocos* with *Butia,* however, the formation of a monophyletic group in the species of the genus *Butia* was evidenced. This means that this transcription factor is probably not indicated for the differentiation of cryptic species or with high genetic similarity. WRKY 6 is involved in controlling processes related to pathogen senescence and defense in plants (Robaztek and Somssich 2002), as it can modulate phosphate homeostasis and Pi translocation, regulating PHO1 expression (Su et al. 2015).

WRKY 7

When differences in the transcription factor *WRKY 7* (Figure 3) were used to build the clades, they showed topological relationships similar to those observed with *WRKY6*. However, among the *Butia* species, the relationship between *B. paraguayensis* and *B. marmorii* stands out, possibly due to the occurrence of the solitary stipe and the fact that in both species, this one remains with some of its centimeters underground. Diameters close to 10-35 cm, with slightly sweet-acidified mesocarp. When the sequences of these two species are aligned and evaluated, there is no loss of exons, with the sequences in their total size of 373 bp. In a study with palm oil (varieties XJS30 and SJ64) and WRKY transcription factors, especially WRKY 7, specimens of this species were subjected to several cold treatments and analysed by PCR and quantitative fluorescence in real time (qPCR), in order to identify the role of WRKY in the palm oil process in response to low temperature. WRKY7 transcription factor expression levels at the end of low temperature acclimation were higher than those at the end of cold treatment, playing a regulatory role in the cold resistance of palm oil varieties XJS30 and SJ64 during low temperature acclimation. Therefore, they observed that the transcription factor *WRKY 7*, together with other *WRKYs* studied, such as *WRKY1*, *WRKY22*, *WRKY40* and *WRKY55* in palm oil, belong to the cold stress response genes (Zhou et al. 2018).

WRKY 12

The associations established for *WRKY 12* (Figure 4) indicated that the genus *Butia* remained homogeneous for the vast majority of species, without major distinctions, with the exception of *B. lallemantii* and *B. yatay*, which are similar in terms of pseudo - petiole with toothed margins, glaucous leaflets, flowers that can be yellow or purple, staminate flowers of similar size (9-10mm), fruits with similar coloring: yellow, orange or red. And yet, with 1-3 seeds and homogeneous endosperm (Soares, 2015).

When evaluating the gene structure, all the species analyzed for *WRKY 12* presented two exons of similar size and quantity. Therefore the gene was well conserved.

Little is known about *WRKY 12* for palms or representatives of the *Arecaceae* family. However, in general and in studies with the reference plant *Arabidopsis thaliana*, this transcription factor interacts specifically with the W box (5 '-(T)TGAC [CT] -3'), a cis-action element responsive to frequently occurring elicitor (by similarity) (Arabidopsis Project, 2021).

WRKY 16

Regarding the groups obtained with the differences in the *WRKY 16* transcription factors (Figure 5) little is known from the literature about this factor, however, the rule of binding non-W-box sequences in tobacco (*Nicotiana tabacum*), for example, NtWRKY12 seems to bind to a SURE-like element, but not to the W box (Sun et al. 2003). NtWRKY12 has an amino acid sequence GKK following WRKY rather than the more common GQK and specifically binds to the WK box (TTTTCCAC). Mutation of the GKK motif into GQK or GEK abolished the interaction with the WK box, suggesting that these amino acids may be important for binding site recognition (Van et al. 2008; Rushton et al. 2010).

In this work, we observed the separation of *C. nucifera* from the *Butia* species, it is worth highlighting the strongly related clade (0.9916) between *B. marmorii* and *B. paraguayensis*, both species of solitary stipe, with parts of the stipe subterranean, height not exceeding 2 m. They are common in Paraguay (Soares, 2015). Also, we highlight the clade derived from the species *B. yatay*; *B. capitata* with *B. odorata*, strongly related (0.9982). In the study by Rossato et al. (2007), the analyzed Brazilian population of *B. yatay,* geographically closer to the Uruguayan populations, also belonged to the cluster composed by *B. odorata* and *B. capitata*.

WRKY 19

We evaluated the distribution of the species studied regarding the differences found in the transcription factor *WRKY 19* (Figure 6), since this sequence was found in the NCBI for all species in the study, widely discussed and used in phylogenetic and conservation studies for palm species (Meerow et al. 2009, 2014; Ellert-Pereira, 2019, Mauro-Herrera et al. 2006). We identified that in this phylogenetic proposal based on *WRKY 19*, we highlighted the formation of the phylogenetic tree was interesting, we observed the outgroup *S. coronata* and in conspecific relation the *B. lallemantii* and *B. marmorii*. Therefore, we visualize the formation of a clade (0.626), which is subdivided from the species *B. odorata*, with the species *B. capitata* and a clade containing *B. yatay* (0.7778) and another clade strongly related (0.9683) and divided again into two clades, containing the species *B. yatay* and *B. paraguayensis* (0.9343), corroborating information from the literature, since some botanists considered *B. paraguayensis* a dwarf variety of *B. yatay*, due to similarities in the size and shape of fruits, flowers and endocarps. This information was also observed by Ellert-Pereira (2019), when attempt to establish a phylogeny for palm species using WRKY 19 transcription factors, since in their work there was a formation of a clade composed of. *B. paraguayensis*, *B. catarinensis* and *B.*

yatay, and in B. *lallemantii*. The autor also identified *B. lallemantii* as a sister species of the clade composed by *B. exospadix* and *B. marmorii*, which was also observed in this work the proximity of *B. lallemantii* with *B. marmorii*.

The phylogenetic proximity between some species of graminiform habit previously observed in the analysis of the WRKY19 region is again found. Likewise, observing an important character for the identification of *Butia* species, such as the morphology of the fruits and the coloration, it is noted that *B. lallemantii*, which has yellow-orange fruits, is identified as a sister species and in a conspecific relationship. with the species *B. marmorii*, with purple fruits when ripe. It is worth noting that *B. marmorii* is an endemic species of Paraguay, in a region close to the border with the state of Paraná, in Brazil, it is a species of shrubby habit, which according to Lorenzi (2010) does not exceed 40 cm in height, corroborating with the morphological characteristics of *B. lallemantii*, which does not exceed 1.3 m in height and is found in Rio Grande do Sul (Brazil), in the Pampa Biome. In a study by Gaiero et al. (2011), with ISSR markers, the genetic distance between *Butia* species was considerably low (0.12 on average) compared to the data obtained by Rossato et al. (2007) for the species from Rio Grande do Sul state (from 0.019 to 0.74). The non-formation of well-defined groups among *Butia* species is reported in the scientific literature (Gaiero et al. 2011; Rossato et al. 2007), due to the fact that the species of this genus have high genetic similarity.

The proximity between *B. paraguayensis* and *B. yatay* was expected, considering that the delimitation of species between them has been doubtful, with *B. paraguayensis* considered a subspecies within *B. yatay* in a morphological sense (Xifreda and Sanso, 1996). Furthermore, both have chemically similar fatty acid composition in the endocarp (Rossato et al. 2007).

In turn, the transcription factor WRKY 19 specifically interacts with the W box (5'- (T)TGAC [CT]-3'), a frequently occurring elicitor-responsive cis-acting element. It may also act as a disease-resistant protein with serine/threonine-protein kinase activity (by similarity), reported for the species *Arabidopsis thaliana* (Arabidopsis Project, 2021).

WRKY 21

In the phylogenetic tree associated with the transcription factor *WRKY 21 gene*, we highlight the proximity between species of the same genus, through the formation of two large clades, one with all the species of *C. nucifera* analyzed in this work, and another clade with the species of *Butia*, which are grouped in clades because they are of the same species, since the other proximities were similar to *WRKY 19*.

Associated WRKY phylogeny

When observing the approach on the Bayesian method and Bootstrap (Figure 8) with all the WRKY regions studied in this work, we highlight that *B. paraguayensis* was again grouped with *B. yatay* and in a sister clade with B. *capitata*, which in turn is distinct of *B. paraguayensis*, in aspects related to height and diameters, with *B. capitata* being slightly larger (Gaiero et al. 2011; Soares, 2015). As expected, the combination of WRKY genes produced a phylogeny that agrees with taxonomists in all/most cases, except for the grouping of *B. lallemantii* and *B. odorata* that tend to be less closer. In the proposed groupings across WRKY regions by Meerow et al. (2009) the proximity of these two species was 1, however, in this study we found the value of 0.52, presenting greater discrepancies. In relation to the grouping of *Butia* with species of *C. nucifera*, we observed that they were properly separated and in different groups, as in the study by Meerow et al. (2009).

This analysis proposal, as well as the methods applied, are widely utilized in the literature and can be confirmed as an excellent strategy in the use and phylogenetic analysis. However, to propose or answer the phylogeny of the genus in question, a greater number of *Butia* species would need to be studied, with their sequences of WRKY regions already previously discovered or worked on.

Therefore, the utility of WRKY transcription factors to determine infraspecific relationships has been demonstrated by genetic mapping in *Theobroma cacao* L. (Borrone et al. 2004a) and by differentiating individuals from one another within *T. cacao* germplasm collections (Borrone et al. 2004b) and *C. nucifera* (Mauro-Herrera et al. 2006; Mauro-Herrera et al. 2007). The utility of WRKY genes for phylogenetic inference in Malvaceae was reported (Borrone et al. 2007). Overall, the presence of WRKY domains from different subfamilies suggests that this mechanistic shortcut evolved on several occasions.

WRKY regions have a high level of conservation (Borrone et al. 2007), so they can be used in phylogenies. The results of the present study brings important basis for the understanding of the phylogeny of the genus *Butia* and can help in the conservation of the species.

Acknowledgment - To the Ministry of Science, Technology and Innovations (MCTI), CNPq (process 441493 / 2017-3) and Transmissão de Energia Elétrica Sul Brasil S.A. (TESB), for the financial support for the execution of the Rota dos Butiazais Project.

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Tables and Figures

Table 1. Details of *Butia* species subjected to analysis in this study.

Figure 1. Consensus phylogram (50 % majority rule) of *Butia* species, from a Bayesian and Bootstrap analysis of the combined *WRKY 2*. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend).

Figure 2. Consensus phylogram (50 % majority rule) of *Butia* species, from a Bayesian and Bootstrap analysis of the combined *WRKY 6*. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend).

Figure 3: Consensus phylogram (50 % majority rule) of *Butia* species, from a Bayesian and Bootstrap analysis of the combined *WRKY 7*. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend).

Figure 4: Consensus phylogram (50 % majority rule) of *Butia* species, from a Bayesian and Bootstrap analysis of the combined *WRKY 12*. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend).

Figure 5: Consensus phylogram (50 % majority rule) of *Butia* species, from a Bayesian and Bootstrap analysis of the combined *WRKY 16* genes. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend).

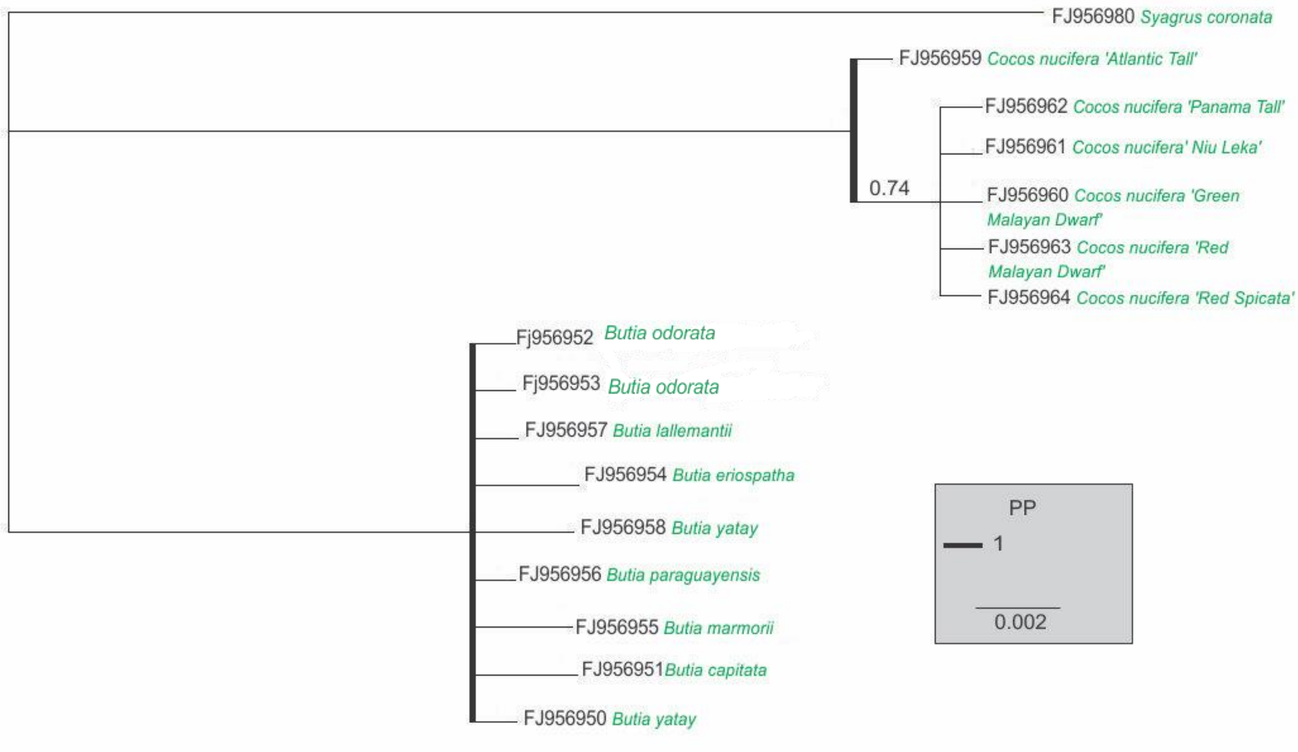
Figure 6: Consensus phylogram (50 % majority rule) of *Butia* species, from a Bayesian and Bootstrap analysis of the combined *WRKY 19*. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend).

Figure 7: Consensus phylogram (50 % majority rule) of *Butia* species, from a Bayesian and Bootstrap analysis of the combined *WRKY 21*. Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend).

Figure 8: Consensus phylogram (50 % majority rule) of *Butia* species, from a Bayesian and Bootstrap analysis of the combined 7-gene sequence alignment (*WRKY 2*, *WRKY 6*, *WRKY 7*, *WRKY 12*, *WRKY 16*, *WRKY 19*, *WRKY 21*). Bayesian posterior probabilities are indicated with color-coded branches and numbers (see legend).

Species	Num. herbário	Access number						
		WRKY 2	WRKY 6	WRKY 7	WRKY 12	WRKY 16	WRKY19	WRKY 21
Butia odorata	Noblick 5465	FJ956953	FJ957098	FJ957172	FJ957244	FJ957313	FJ957383	FJ957025
Butia odorata	Noblick 5130	FJ956952	FJ957097	FJ957171	FJ957243	FJ957312	FJ957382	FJ957024
Butia capitata	Noblick 5090	FJ956951	FJ957096	FJ957170	FJ957242	FJ957311	FJ957381	FJ957023
Butia lallemantii	Noblick 5457	FJ956957	FJ957100	FJ957174	FJ957246	FJ957315	FJ957385	FJ9570027
Butia eriospatha	Noblick 4878	FJ956954	FJ957099	FJ957173	FJ957245	FJ957314	FJ957384	FJ957026
Butia marmorii	Noblick 5336	FJ956955	FJ957101	FJ957175	FJ957247	FJ957316	FJ957386	-
Butia yatay	Noblick 5461	FJ956958	FJ957103	FJ957177	FJ957249	FJ957318	FJ957388	FJ957029
Butia yatay	Elsa Zardini s.n	FJ956950	FJ957095	FJ957169	FJ957241	FJ957310	FJ957380	FJ957022
Butia paraguayensis	Noblick 5285	FJ956956	FJ957102	FJ957176	FJ957248	FJ957317	FJ957387	FJ957028
Butia paraguayensis	Noblick 5459	-	FJ957094	FJ957168	FJ957240	FJ957309	FJ957379	FJ957021
Cocos nucifera 'Atlantic Tall'	Meerow 3201 (NA)	FJ956959	FJ957104	FJ957178	FJ957250	FJ957319	FJ957389	FJ957030
Cocos nucifera' Niu Leka'	Meerow 3203 (NA)	FJ956961	FJ957106	FJ957180	--	FJ957320	FJ957391	FJ957032
Cocos nucifera 'Panama Tall'	Meerow 3204 (NA)	FJ956962	FJ957107	FJ957181	--	--	FJ957392	FJ957033
Cocos nucifera 'Green Malayan Dwarf'	Meerow 3202 (NA)	FJ956960	FJ957105	FJ957179	FJ957251	--	FJ957390	FJ957031
Cocos nucifera 'Red Malayan Dwarf'	Meerow 3205 (NA)	FJ956963	FJ957108	FJ957182	--	--	FJ957393	FJ957034
Cocos nucifera 'Red Spicata'	Meerow 3206 (NA)	FJ956964	FJ957109	FJ957183	--	FJ957321	FJ957394	FJ957035
Syagrus coronata	Noblick 4833	FJ956980	FJ957125	FJ957198	FJ957267	FJ957337	FJ957411	FJ957051

*Origen: FTBG = Fairchild Tropical Botanic Garden; MBC = Montgomery Botanical Center.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FJ956961 Cocos nucifera' Niu Leka'
0.74
FJ956960 Cocos nucifera 'Green Malayan Dwarf'
FJ956963 Cocos nucifera 'Red Malayan Dwarf'
FJ956964 Cocos nucifera 'Red Spicata'
Fj956952 Butia odorata
Fj956953 Butia odorata
FJ956957 Butia lallemantii
FJ956954 Butia eriospatha
FJ956958 Butia yatay
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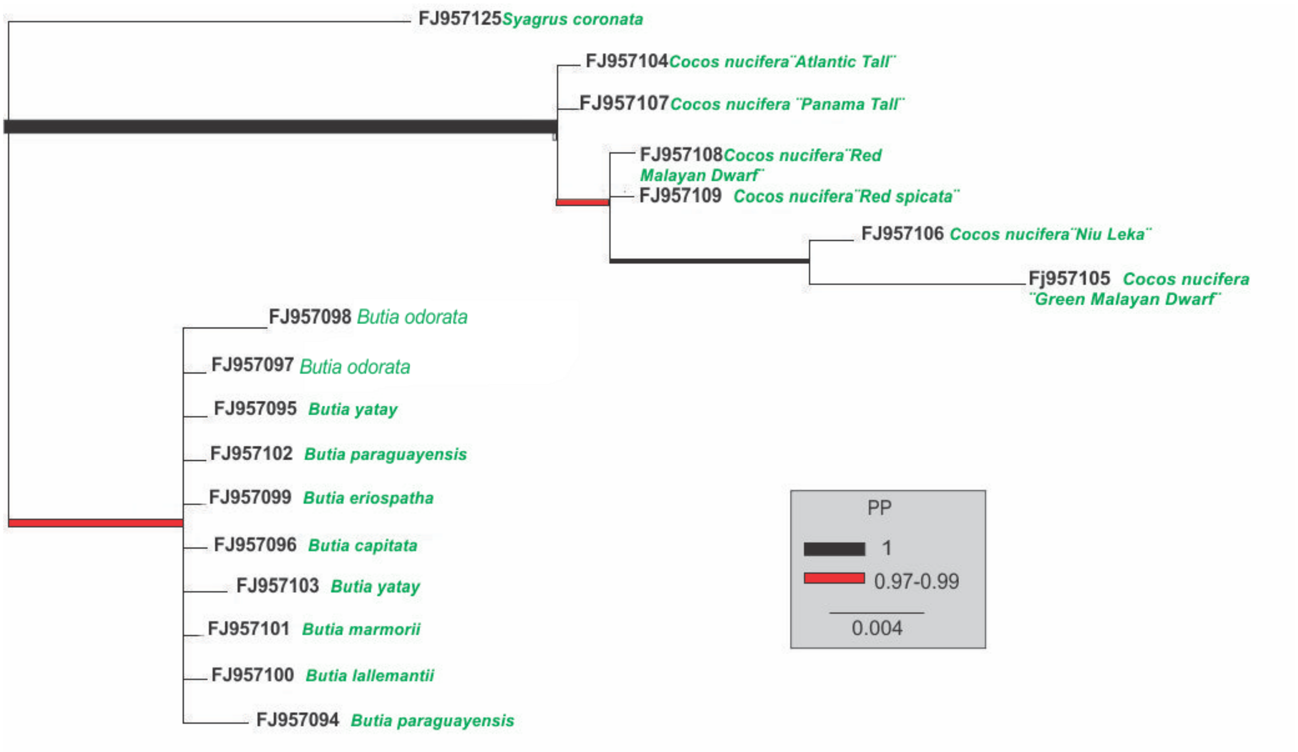
0.002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FJ957109 Cocos nucifera "Red spicata"
FJ957106 Cocos nucifera "Niu Leka"
Fj957105 Cocos nucifera "Green Malayan Dwarf"
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FJ957097 Butia odorata
FJ957095 Butia yatay
FJ957102 Butia paraguayensis
FJ957099 Butia eriospatha
FJ957096 Butia capitata
FJ957103 Butia yatay
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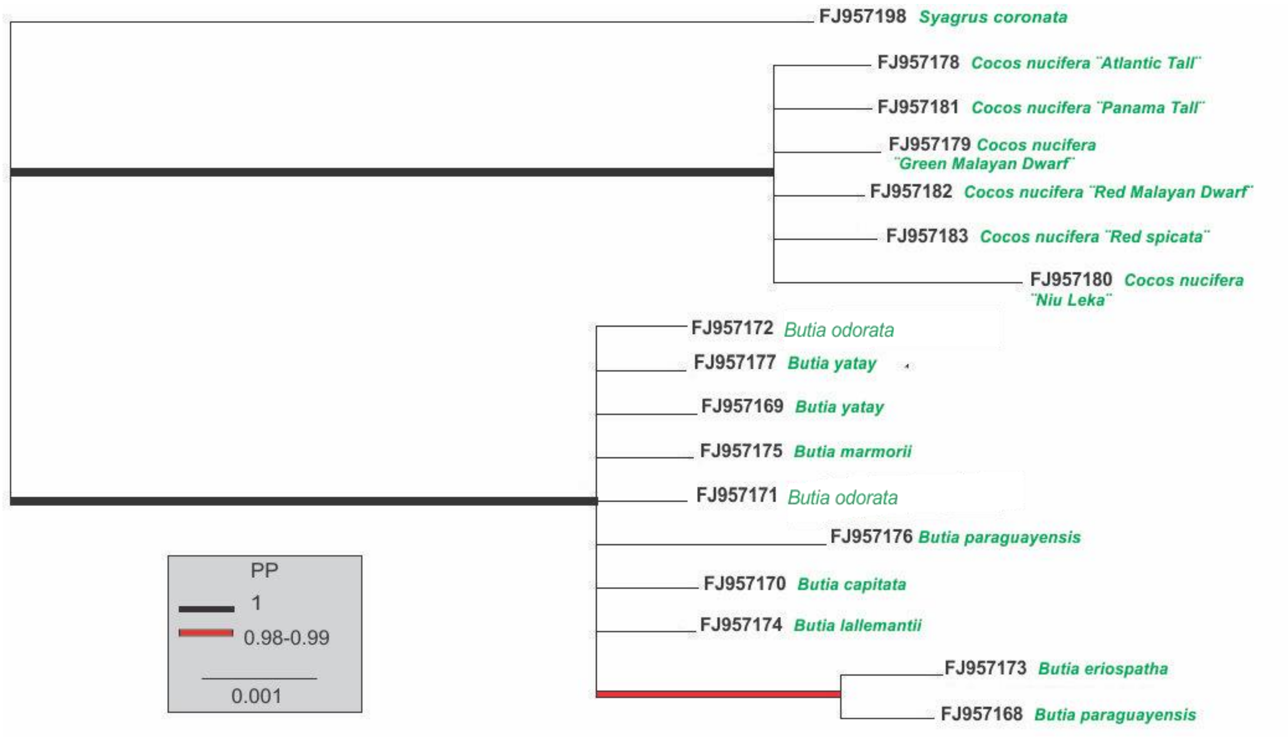
0.004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FJ957183 Cocos nucifera "Red spicata"
FJ957180 Cocos nucifera "Niu Leka"
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FJ957177 Butia yatay
FJ957169 Butia yatay
FJ957175 Butia marmorii
FJ957171 Butia odorata
FJ957176 Butia paraguayensis
FJ957170 Butia capitata
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PP
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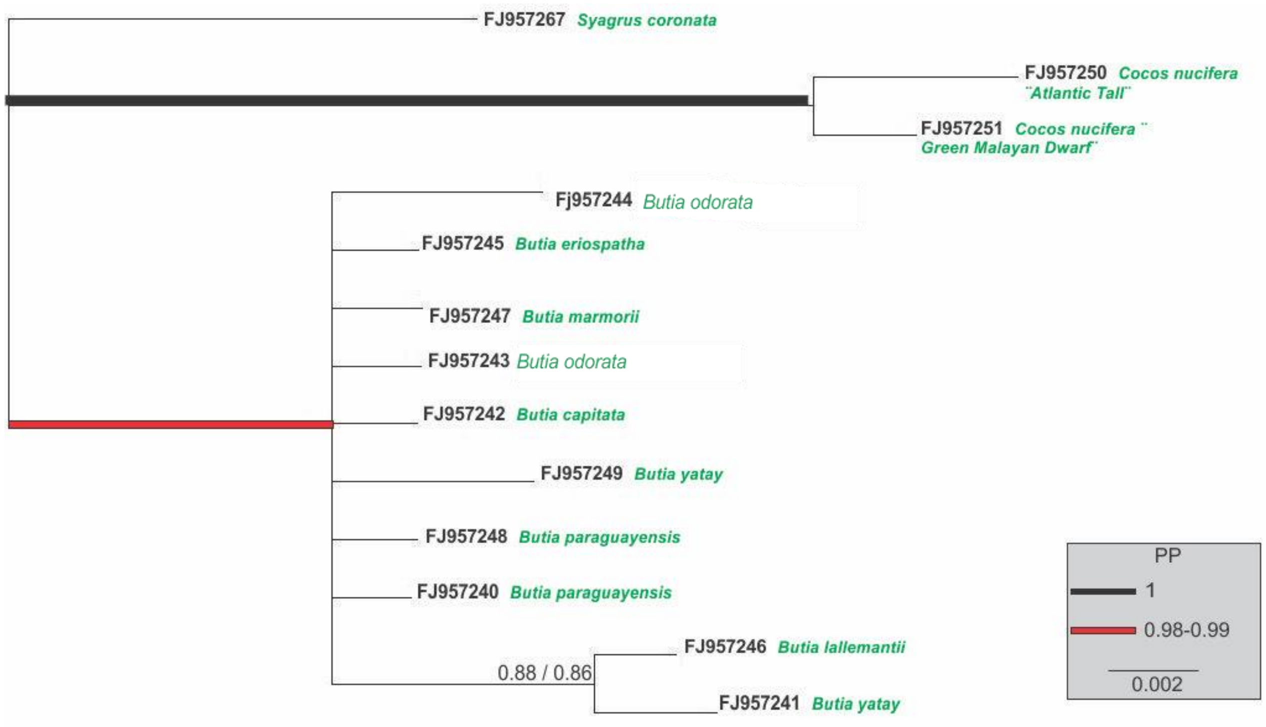
0.98-0.99
0.001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FJ957247 Butia marmorii
FJ957243 Butia odorata
FJ957242 Butia capitata
FJ957249 Butia yatay
FJ957248 Butia paraguayensis
FJ957240 Butia paraguayensis
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0.88 / 0.86
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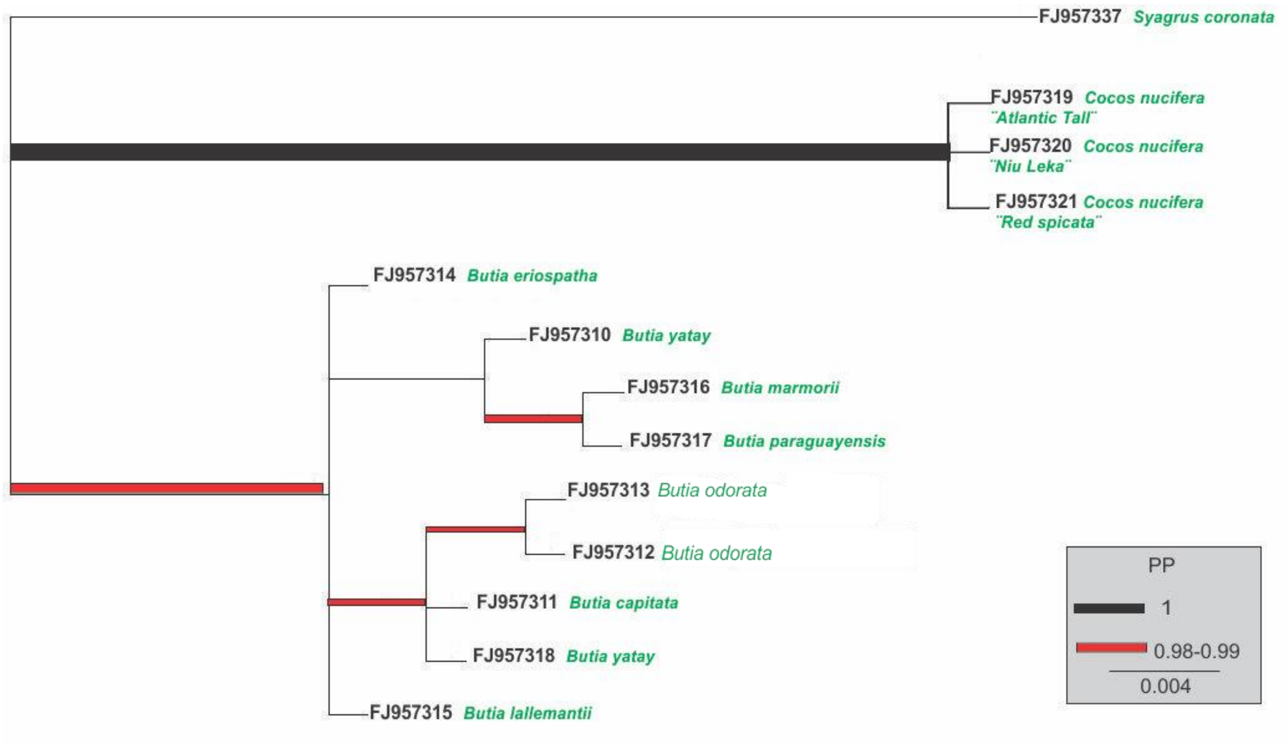
0.98-0.99
0.002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FJ957310 Butia yatay
FJ957316 Butia marmorii
FJ957317 Butia paraguayensis
FJ957313 Butia odorata
FJ957312 Butia odorata
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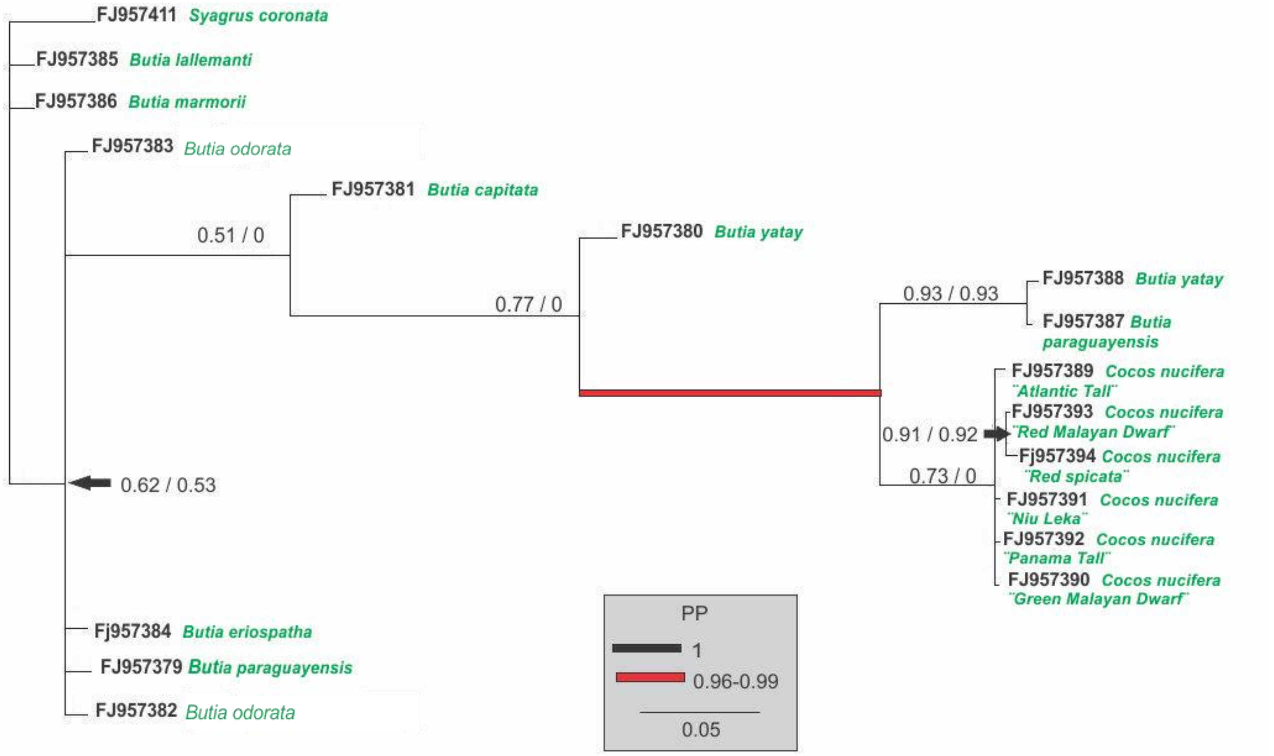
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0.77 / 0
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0.93 / 0.93
FJ957387 Butia paraguayensis
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FJ957393 Cocos nucifera "Red Malayan Dwarf"
0.91 / 0.92
Fj957394 Cocos nucifera "Red spicata"
0.73 / 0
FJ957391 Cocos nucifera "Niu Leka"
FJ957392 Cocos nucifera "Panama Tall"
FJ957390 Cocos nucifera "Green Malayan Dwarf"
0.62 / 0.53
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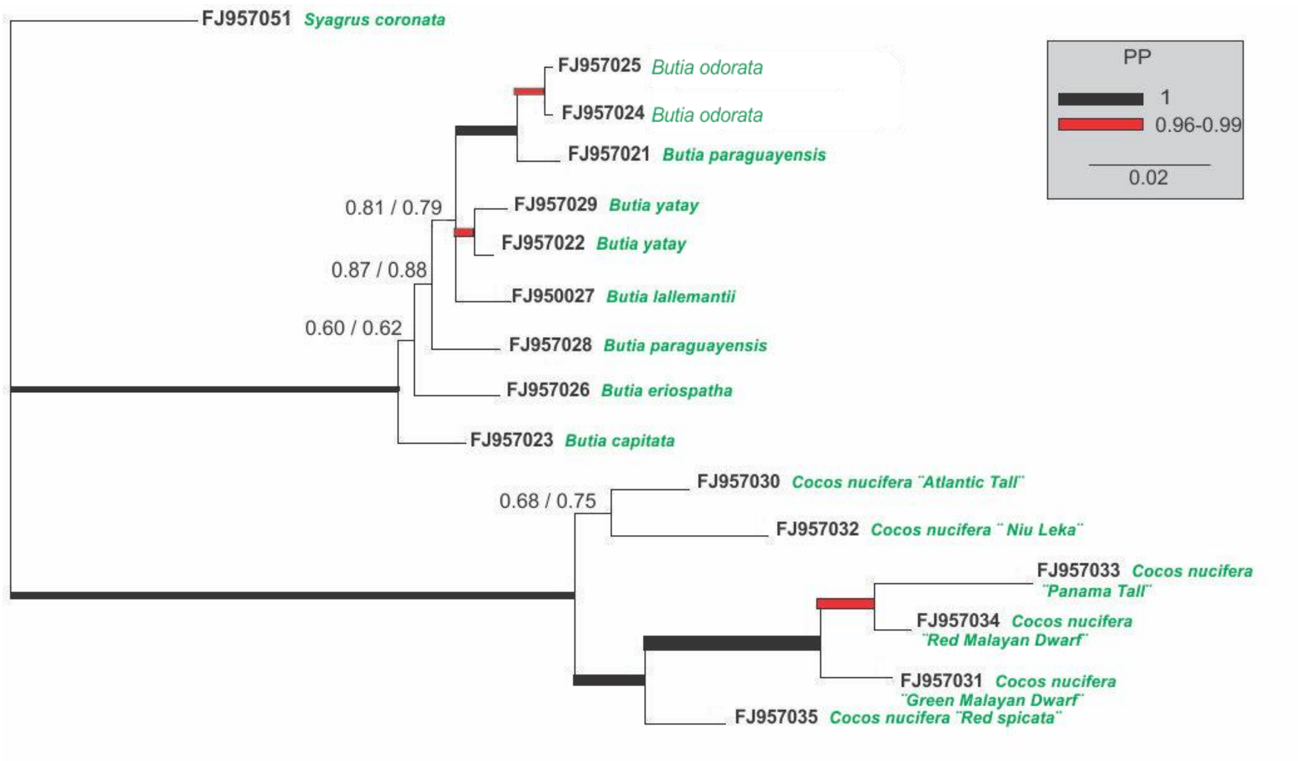
0.96-0.99
0.05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FJ957022 Butia yatay
0.87 / 0.88
FJ950027 Butia lallemantii
0.60 / 0.62
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FJ957023 Butia capitata
0.68 / 0.75
FJ957030 Cocos nucifera "Atlantic Tall"
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FJ957034 Cocos nucifera "Red Malayan Dwarf"
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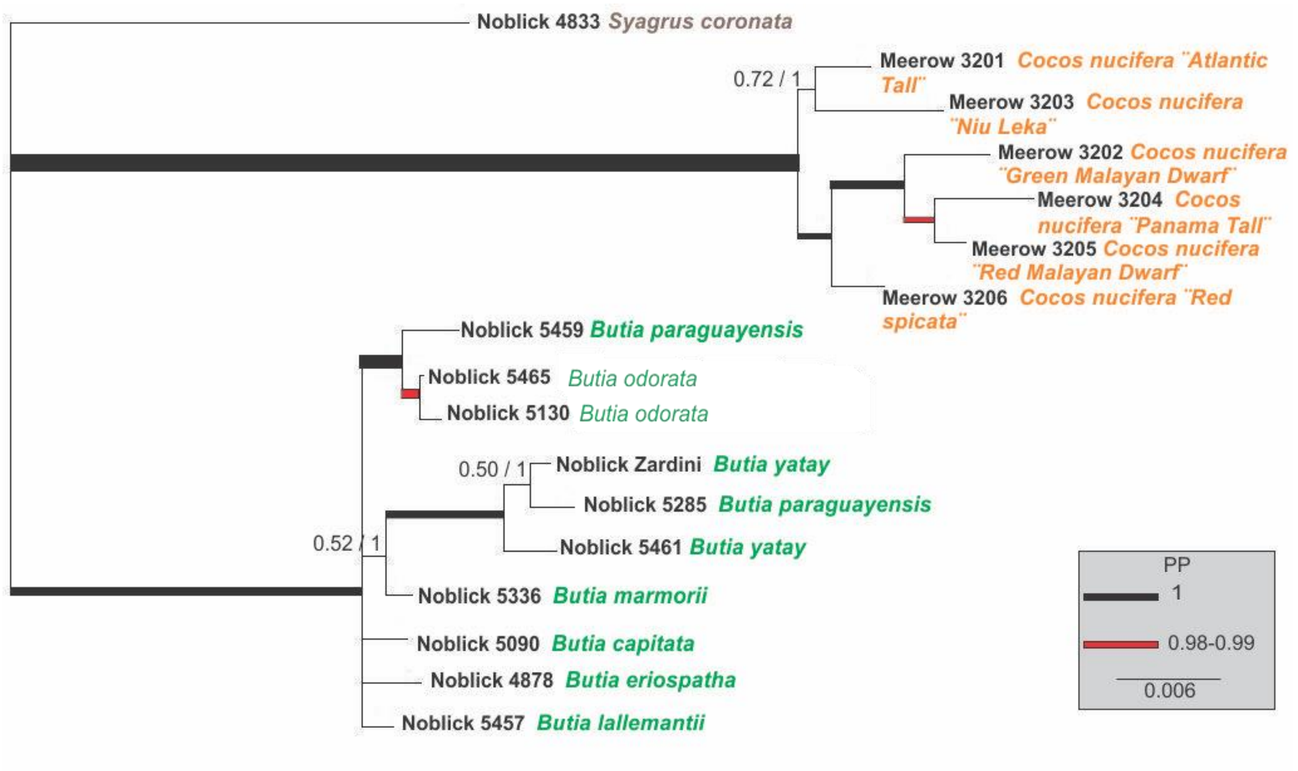
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0.72 / 1
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Meerow 3204 Cocos nucifera "Panama Tall"
Meerow 3205 Cocos nucifera "Red Malayan Dwarf"
Meerow 3206 Cocos nucifera "Red spicata"
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Noblick 5461 Butia yatay
0.52 / 1
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0.006

5 CONSIDERAÇÕES FINAIS

O presente trabalho contribui com a geração de informações acerca de aspectos sociais, biológicos e moleculares relacionados com várias espécies de palmeiras, especialmente do gênero *Butia*. Esses avanços no conhecimento são importantes considerando que existe uma carência de informações relacionadas a etnobotânica e biologia molecular de espécies de palmeiras, como é o caso das espécies de *Butia.*

O trabalho sistematizou a visão de vários participantes da Rota dos Butiazais que residem no Brasil, na Argentina e no Uruguai, com históricos de vida diferentes, mas que compartilham seu grande interesse em conservar e promover o uso sustentável das espécies de *Butia* e dos ecossistemas de butiazais. Os butiazais atravessam as fronteiras dos países e possuem uma importância sociocultural e biológica ímpar. Dada a sua importância histórica e cultural, além da sua valorização como patrimônio, tanto natural quanto social, sua conservação está necessariamente relacionada a boas práticas de extrativismo, manejo sustentável, pesquisa, políticas públicas, e legislação adequada.

O conhecimento dos butiazais e das dinâmicas estabelecidas em seu interior, permite o uso racional e sustentável dos seus recursos, o que resulta em sua conservação. Os princípios da Convenção sobre Diversidade Biológica (CDB), de 1992, defendem que a conservação da biodiversidade deve ser uma preocupação comum da humanidade e que os Estados também são responsáveis pela conservação e utilização sustentável de seus recursos biológicos. Só há efetiva conservação se houver preocupação com os ecossistemas e habitats naturais. Nesse sentido, a valorização dos produtos com butiá, na gastronomia, artesanato e artes plásticas, realização de festas que divulgam a cultura da conservação, o turismo, educação ambiental, extensão e pesquisa acadêmica, entre outras, são ações importantes para a efetiva conservação dos butiazais.

Por sua vez, o conhecimento da estrutura genética das populações de palmeiras é importante para poder traçar estratégias de conservação. Para isto, as ferramentas de bioinformática podem auxiliar na medida em que permitem correlacionar informações disponíveis em bancos de dados. O banco de dados utilizado na tese foi o *National Center for Biotechnology Information* (NCBI), que disponibiliza para uso público um grande conjunto de informações que auxiliam na compreensão de processos moleculares e genéticos. O NCBI cria sistemas automatizados para armazenar e analisar conhecimentos sobre biologia molecular, bioquímica e genética.

Um resultado de destaque deste trabalho foi a verificação da existência de uma maior frequência de repetições de mononucleotídeos em regiões intergênicas nos genomas plastidiais das espécies de palmeiras avaliadas, indicando que sofreram uma menor pressão seletiva ao longo do processo de evolução. A partir dessa análise foi possível sugerir uma média 76 pares de primers por espécie que podem ser utilizados em estudos futuros para caracterização molecular por SSR das 52 espécies de palmeiras analisadas. Os SSRs são uma ótima ferramenta para a análises genéticas, apresentam alto conteúdo de informação de polimorfismo e extensa cobertura genômica. Os primers sugeridos neste trabalho para uma determinada espécies de palmeira poderão ser usados em espécies geneticamente próximas e até mesmo entre gêneros, mas seu grau de transferibilidade para análises de SSR poderá ser bastante variável, o que deverá ser testado em laboratório.

Por fim, como as regiões de fatores de transcrição WRKY em espécies da família Arecaceae possuem um alto nível de conservação evolutiva das sequências, podem ser utilizadas em estudos filogenéticos. Os resultados obtidos trazem avanços na compreensão da filogenia do gênero *Butia,* associando *B. capitata, B. eriospatha, B. lallemantii, B. marmorii, B. odorata, B. paraguayensis* e *B yatay*, apontando suas similaridades e distâncias genéticas, corroborando com informações morfológicas discutidas na literatura científica. No entanto, ressalta-se que árvores filogenéticas são hipóteses acerca do parentesco entre organismos e por isso carregam incertezas. Através do entendimento das relações evolutivas e de parentesco estabelecidas entre as espécies de *Butia*, bem como a identificação de suas características e particularidades, é possível estabelecer estratégias para o seu manejo e conservação. Existe a necessidade de compreender a diversidade filogenética com o objetivo de propor ações de conservação, uma vez que, essas espécies estão sofrendo com a perda de áreas naturais, pela falta de manejo adequado, pelo avanço da fronteira urbana e ou agrícola, pressão de pastoreio, fogo sobre recrutamento (desenvolvimento de novas plantas até a fase reprodutiva).

A caracterização de aspectos etnobotânicos e moleculares associados a *Butia* e demais palmeiras contribuíram para um maior entendimento a respeito destas espécies, o que pode servir de base para delinear estratégias para a sua conservação.

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