

## MOLECULAR PHYLOGENY OF *BUTIA* (ARECACEAE): AN UPDATE SHOWING THE PARANÁ FOREST AND ATLANTIC FOREST AS A PHYSICAL BARRIER IN THE EVOLUTIONARY HISTORY OF SPECIES<sup>1</sup>

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**Abstract:** *Butia* is endemic to South America, with the highest species count in Brazil. Currently, 22 species of *Butia* are recognized, but little is known about the phylogenetic relationships among them. Thus, this study investigated the phylogenetic relationships of *B. buenopolensis*, *B. capitata*, *B. catarinenses*, *B. eriospatha*, *B. lallemantii*, *B. marmorii*, *B. odorata*, *B. paraguayensis*, *B. yatay*, and specimens related to *B. paraguayensis* and *B. yatay* using *WRKY* gene family markers, applying the Maximum Likelihood analysis. Results confirm the monophyly of *Butia*. A strongly supported dichotomy (100% bootstrap) was observed in the phylogram. The hypothesis that the presence of the Southern Plateau and the forest formations of the Parana Forest and Atlantic Forest in the southern region of Brazil caused the formation of the dichotomy observed in the phylogenetic tree is elucidated. The clades formed reflect more than phylogenetic relationships, also reflect the impact of South America's phytogeography on species' ecological adaptations: while the Chaco-Cerrado Clade includes acaulescent or dwarf species adapted to the Cerrado and Chaco, the Espinal-Pampa Clade has species with elevated stems. This is the first study to demonstrate congruence between molecular and biogeographic data by comparing molecular phylogeny with species' geographic distribution.

**keywords:** arecoideae; attaleinae; cocoseae; evolution; *WRKY*.

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# FILOGENIA MOLECULAR DE *BUTIA* (ARECACEAE): UMA ATUALIZAÇÃO MOSTRANDO A FLORESTA DO PARANÁ E A MATA ATLÂNTICA COMO BARREIRA FÍSICA NA HISTÓRIA EVOLUTIVA DAS ESPÉCIES

**Resumo:** *Butia* é endêmico da América do Sul, com o maior número de espécies no Brasil. Atualmente, 22 espécies de *Butia* são reconhecidas, mas pouco se sabe sobre as relações filogenéticas entre elas. Assim, este estudo investigou as relações filogenéticas de *B. buenopolensis*, *B. capitata*, *B. catarinenses*, *B. eriospatha*, *B. lallemantii*, *B. marmorii*, *B. odorata*, *B. paraguayensis*, *B. yatay* e de espécimes relacionados a *B. paraguayensis* e *B. yatay* utilizando marcadores da família gênica WRKY, aplicando a análise de Máxima Verossimilhança. Os resultados confirmam a monofilia de *Butia*. Uma dicotomia fortemente sustentada (100% bootstrap) foi observada no filograma. A hipótese de que a presença do Planalto Meridional e das formações florestais da Floresta Paranaense e Mata atlântica na região sul do Brasil causaram a formação da dicotomia observada na árvore filogenética é elucidada. Os clados formados refletem mais do que relações filogenéticas, também refletem o impacto da fitogeografia da América do Sul nas adaptações ecológicas das espécies: enquanto o Clado Chaco-Cerrado inclui espécies acaulescentes ou anãs adaptadas ao Cerrado e Chaco, o Clado Espinal-Pampa possui espécies com caules elevados. Este é o primeiro estudo a demonstrar congruência entre dados moleculares e biogeográficos ao comparar a filogenia molecular com a distribuição geográfica das espécies.

**Palavras-chave:** arecoideae; attaleinae; cocoseae; evolução, WRKY.

## 1 INTRODUCTION

Arecaceae is widely distributed around the world, but with a more representative number of species along the tropics (Uhl and Dransfield, 1987). However, in South America, for instance, some native genera, such as *Jubaea* Kunth, thrive in subtropical climates (Reichgelt *et al.*, 2018). *Jubaea* represents the southernmost extreme for the family in the Americas, along with species of *Syagrus* Mart. and *Butia* (Becc.) Becc. genera (Lorenzi *et al.* 2010).

*Jubaea* and *Butia* are sister groups, and this relationship is supported by molecular analysis (Baker *et al.*, 2009; Meerow *et al.*, 2009; 2015) as well as by root anatomy of both genera (Dransfield *et al.* 2008, p. 12). *Butia* is pleonanthic, characterized by interfoliar inflorescences protected by a peduncular bract, a common trait among taxa belonging to the tribe Cocoseae (Dransfield *et al.* 2008). *Butia* comprises small to medium-sized, solitary or clustered pinnate-leaved palms, native to cooler parts of south America; the petioles are usually with toothed margins, the staminate flowers have six stamens, and the endosperm is homogeneous (Dransfield *et al.* 2008).

*Butia* was first treated as a subgenus of *Cocos* L. (Beccari, 1887), and along the time underwent numerous changes in taxonomic limits due to morphological characters among their species. This led some authors to search

for new ways to identify and differentiate its taxa. For example, Glassman (1979) separated the genera *Butia* and *Syagrus*, while Sant'Anna-Santos *et al.* (2015) distinguished *Butia capitata* (Mart.) Becc. from *Butia odorata* (Barb. Rodr.) Becc., both utilizing leaf anatomical characters.

Zanoni *et al.* (2023) analyzing scientific papers published on the genus *Butia* between 1942 and 2021, had found that the most species studied are *B. odorata* and *B. capitata*, focused on physiological and anatomical aspects and biochemical/nutritional features of the fruits, and no work was cited specifically investigating the relationship among species of the genus through molecular data. However, Marcato (2004) was one of the first authors to try explaining the relation between *Butia*'s species throughout molecular data, though his research had focused on the subtribe Butiinae, which is no longer accepted.

Meerow's work using nuclear genes represents the most comprehensive molecular analysis for the subtribe Attaleinae (Meerow *et al.* 2009) and a more recent analysis for the tribe Cocoseae (Meerow *et al.* 2015). However, neither study focused on exploring the relationships within *Butia* at the species level, although they did include molecular data from some species. Based on this, our study aimed to infer the phylogenetic relationships among species of the genus *Butia* using WRKY genes and Maximum Likelihood analyses, including new molecular data for *Butia buenopolensis* Sant'Anna-Santos.

The diversification of the genus *Butia* appears intrinsically linked to complex historical processes and landscape features in southern South America. Notably, the extensive forest formations of the Paraná and Atlantic Forests, shaped by ancient geologic events and recognized as distinct phytogeographic provinces, are hypothesized to have acted as significant physical and ecological barriers within the region. These barriers likely restricted populations occurring in open environments, such as the Pampas and Cerrado, potentially leading to the deep phylogenetic split observed in *Butia*. Thus, we propose that the present-day dichotomy between the Espinal-Pampa and Chaco-Cerrado clades reflects a historical scenario in which the forested area isolated populations in adjacent open biomes. This hypothesis will be explored in light of molecular phylogenetic evidence and biogeographical patterns.

## 2 MATERIAL AND METHODS

### 2.1 Database

Sequences of the transcription factors WRKY2, WRKY6, WRKY7, WRKY12, WRKY16, WRKY19, and WRKY21 were retrieved from GenBank, available through the National Center for Biotechnology Information (NCBI). Our dataset included DNA sequences from 68 species across 7 genera of American Attaleinae (see Appendix A). Additionally, novel sequences for the

genes WRKY2, WRKY6, WRKY7, and WRKY21 from *Butia buenopolensis* were generated in this study and have been deposited in GenBank (see Appendix B).

## 2.2 Alignment

For each *WRKY* loci, a matrix was created, and since these are coding genes, it was decided to manually trim the sequences in the region that represented the first start codon (ATG) and the last stop codon (TAA, TAG or TGA) in each of the sequences obtained for each *WRKY* gene in all taxa. Sequences were edited using MEGA version 11 (Tamura *et al.* 2021). The treated sequences were subjected to alignment using the MAFFT-version 7 program (online) (Kato *et al.* 2017). The first alignment was performed with the FFT-NS-2 strategy, and with the result of this alignment, a new alignment using the FFT-NS-i strategy was performed, according to Ghosh *et al.* (2022). The other program parameters were kept at default.

## 2.3 Phylogenetic analyses

Maximum likelihood analysis was used to infer phylogenetic relationships. The analyses were performed using the IQ-Tree software version-2.2.2.6-Windows (Minh *et al.* 2020). The best nucleotide substitution models were sought using this same software for each gene loci (see Appendix C). Using the Mesquite software version 3.81-Windows (Maddison and Maddison, 2023), the seven datasets were concatenated. Using the Partition Finder algorithm through IQ-Tree, the best partition scheme was sought, and subsequently, with the adjusted parameters, the concatenated gene tree was inferred with 10,000 bootstrap replicates, resulting in the phylogram shown in Figure 1.

## 2.4 Mapping species of *Butia*

In order to understand the relationships between the results of the phylogenetic relationships obtained and the geographic distribution of the species of the genus *Butia*, the geographic coordinates of the collections of the 10 specimens used in the analysis carried out by Meerow were sought (Meerow *et al.* 2009): *B. lallemantii*, *B. capitata*, *B. capitata* var. *odorata* FTG: Noblick 5130, *B. capitata* var. *odorata* FTG: Noblick 5465, *B. marmorii*, *B. paraguayensis*, *B. aff. Paraguayensis* FTG Noblick 5459, *B. eriospatha*, *B. yatay*, *Butia* aff. *yatay* FTG: Zardini s.n.

Searches for collection records of each specimen were carried out in the virtual databases of SpeciesLink (Ann *et al.* 2022), Herbarium Virtual Re flora (Souza, 2023), bibliographies and information from the collector himself (see Appendix D). The search was performed using the specimen indicator in Genbank “specime\_voucher”, followed by the collector and collection number. The geographic coordinates of the 10 specimens included in the phylogeny are

presented on the specimen map, which also illustrates the hypothesis of species dispersal in the environment (Figure 2).

In preparing the maps, the phytogeographic classification proposed by Morrone *et al.* (2022) was preferred, as it classifies South and Central America into provinces, refining the earlier phytogeographic framework developed by Cabrera and Willink (1980). The Atlantic Forest was demarcated in Brazilian territory according to the Brazilian Institute of Geography and Statistics (IBGE, 2022) and the Espinal territory was included according to Arana *et al.* (2021), as it is believed that the ecosystem influences the ecology of *Butia*. The biomes Pampa, Chaco, Cerrado, and the subdivisions of the Atlantic Forest were also classified according to Morrone *et al.* (2022).

### 3 RESULTS

#### 3.1 Mapping and Revising Identification of *Butia* Specimens

Due to documented identification errors and subsequent taxonomic changes in the last years (Azambuja & Pereira, 2022), a thorough revision of the identification of *Butia* specimens included in the phylogenetic analysis was necessary (see Appendix D). Elesbão *et al.* (2016) attributed the specimen “*B. aff. paraguayensis*” (Noblick 5459, Rivera/Uruguay) to *B. paraguayensis* and confirmed the identification of *B. lallemantii*, *B. eriospatha*, and *B. yatay*. Meerow *et al.* (2009) incorporated molecular data from two specimens identified as *B. capitata* var. *odorata* (Noblick 5465 and Noblick 5130) into their phylogeny. However, a reassessment of the collection data revealed that Noblick 5130, collected in Torres, Rio Grande do Sul, Brazil, is cited as a paratype of *Butia catarinensis* Noblick & Lorenzi (Noblick, 2010), with its identification as *B. catarinensis* also corroborated by Elesbão *et al.* (2016).

For specimens lacking sufficient identification data, further information provided by Noblick (2023) was consulted. This confirmed the following occurrences: *B. capitata* from Goiás, Brazil; *B. marmorii* from Alto Paraná, Paraguay; *B. yatay* (Noblick 5461) from Paysandu, Uruguay; and *B. capitata* var. *odorata* (Noblick 5465) from Rocha, Uruguay. No additional information was available for the specimen “*B. aff. yatay* Zardini s.n.”; however, it is noteworthy that all *Butia* collections designated as “Zardini s.n.” originate from various localities in Paraguay. This geographical information is consistent with its observed phylogenetic affinities to *B. paraguayensis*.

Lastly, it should be noted that *B. buenopolensis* is geographically restricted to the State of Minas Gerais, Brazil, and is endemic to the Serra do Cabral region. Thus, all specimen identifications were carefully revised to ensure taxonomic accuracy and congruence with current classifications prior to their inclusion in the phylogenetic reconstruction.

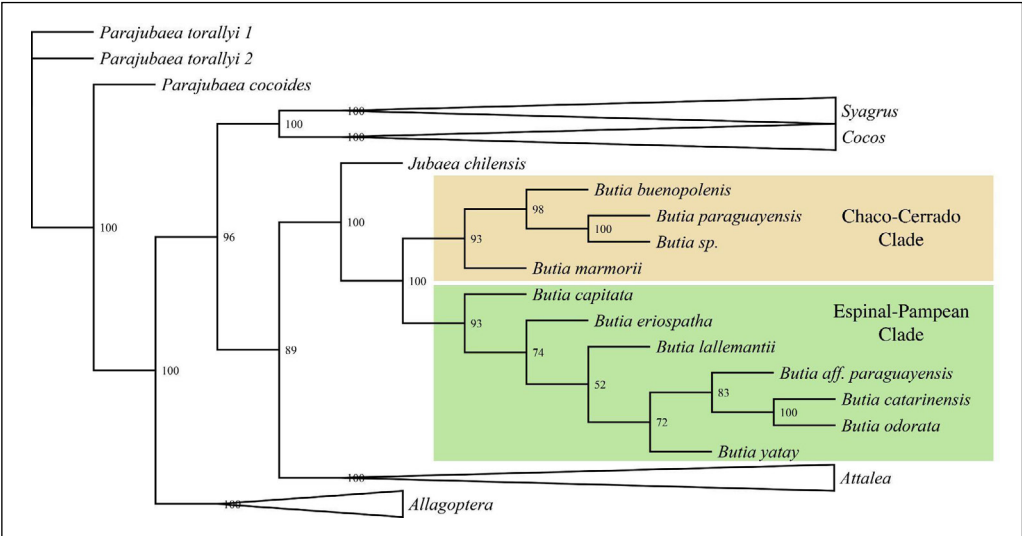
3.2 Phylogenetics Inferences

After analyzing the data obtained and comparing them with results similar to the analyses performed by Meerow *et al.* (2009; 2015) (see Appendix 5) and based on the nucleotide substitution models for each gene loci it was decided to maintain only maximum likelihood as phylogenetic inference due to the satisfactory results obtained.

Results confirm the monophyly of *Butia* and a strongly supported dichotomy (100% bootstrap) was observed in the phylogram. The discussion first focuses on the formation of this cladogenesis where the hypothesis of a physical barrier possibly responsible for forming two groups of species is presented. The clades formed were named *Espinal-Pampa Clade* and *Chaco-Cerrado Clade*, considering phytogeographic patterns of occurrence of the genus. Subsequently, the phylogenetic relationships between the species of each of the clades will be discussed.

The phylogenetic relationships found in th analysis are also supported by biogeography, when considering the geographic distribution among the species of *Butia* analyzed, since geographically close species were also grouped from a phylogenetic point of view (Figure 1).

Fig. 1 A single most likely phylogram resulting from maximum likelihood analysis of the combined molecular datasets. Bootstrap percentage values are indicated at branches



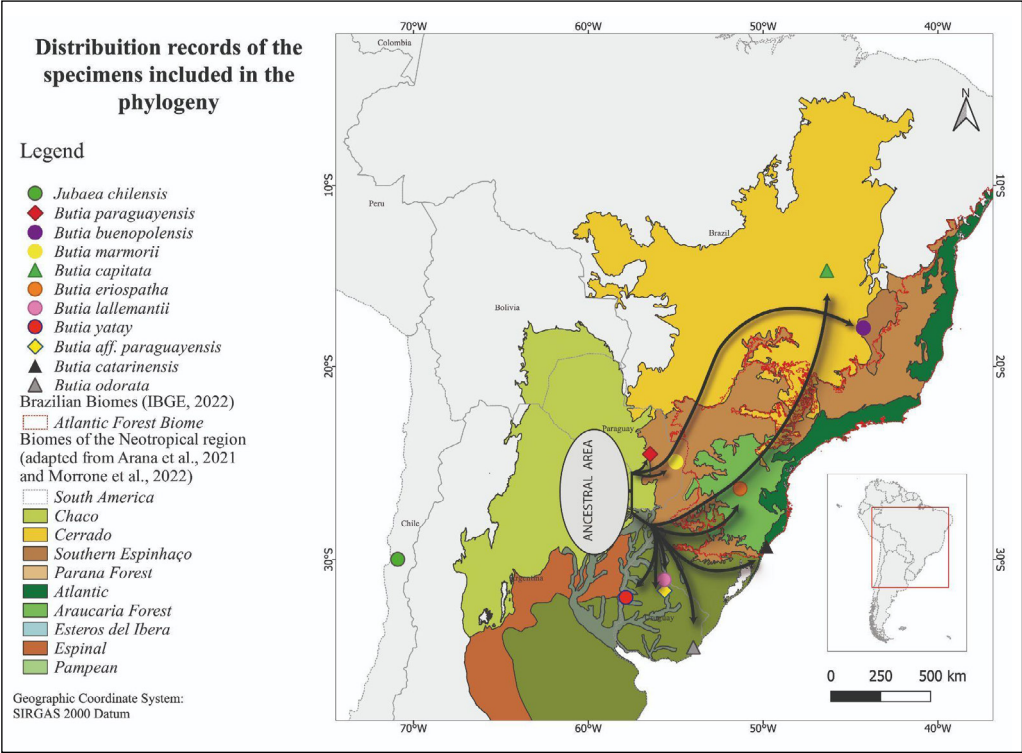
## 4 DISCUSSION

### 4.1 The Paraná and Atlantic Forest as a physical barrier in the evolutionary history of species

Between the Espinal-Pampa and Chaco-Cerrado Clades, the Southern Plateau is located in the south-central part of South America, formed by a set of volcanic spills from the Lower Cretaceous, during the separation of Gondwana. With altitudes ranging from 70 m in the west to over 1000 m in the northeast of Rio Grande do Sul (Robaina *et al.* 2011), the Southern Plateau is currently predominantly forested, with the western portion being composed of the Paraná forest. Cabrera (1976) treated the Paraná forest as a phytogeographic province and recognized that it includes the forests and savannas of the western portion of southeastern and southern Brazil, eastern Paraguay and the province of Misiones in Argentina, as well as the gallery forests of the main rivers of the Plata basin. Furthermore, it includes part of the edaphic savannas that are around the primary forest, generating large ecotones with the Cerrado Grasslands and northern sectors of the grassland areas of the Pampas region and will give way to the Atlantic forest (or Atlantic Phytogeographic Province) near the eastern limit of South America (Cabrera and Willink, 1980, Morrone *et al.* 2022).

Here we found that the presence of forest formations that comprise the Paraná and Atlantic Forests (or phytogeographic provinces), in the central-eastern coastal direction of the continent, along the southern region of Brazil, caused the formation of the dichotomy observed in the phylogenetic tree in two groups of species, which correspond to the current regions where they are found. (Figure 2).

Fig. 2 Occurrence of specimens included in the phylogeny



The morphological adaptations required for establishment in the environment where the species occur provide information about their evolution and dispersion. Noblick (2006) describes several species from the Chaco-Cerrado region as “*Butia grassy*” due to the similarity and camouflage of *Butia* species with the vegetation predominantly composed of grasses and shrub species of the Cerrado biome. Most species from this region are acaulescent or have short stems (Noblick, 2010).

According to Cassia-Silva *et al.* (2022), the acaulescence of species from the Cerrado region may be a response to environmental selective pressure caused by the incidence of natural fires and the prolonged seasonal dry season recorded for the region. This same pattern can be observed in other palm genera, such as *Allagoptera* (Bacon *et al.* 2017), plants with short to acaulescent stems that occupy seasonally dry regions of the Cerrado and coastal environments. Also related to morphological adaptations, Freitas *et al.* (2021) highlight that the high phylogenetic diversity found in grassland areas of South America indicates a growing adaptation to seasonally dry environments caused by Quaternary climate changes, favoring ecological and evolutionary adaptations for the occupation of new environments.

The Espinal-Pampa Clade is also formed mostly by species naturally occurring in grassland areas of the Pampa Biome, also composed of herbaceous and shrubby plants. Most of the species that make up this clade are found in the Grasslands of Rio Grande do Sul, Argentina, and Uruguay; however, with a surprising diversity of species and a mix of taxa with high or short stems (Soares *et al.* 2015; Deble *et al.* 2017). The high-altitude grasslands constitute the landscapes of the Southern Brazilian Plateau of Rio Grande do Sul and Santa Catarina, giving rise to mosaics with the Araucaria Forest (Behling, 2009). Behling (2004) proposed that the Southern Brazilian Plateau during glacial periods was composed of extensive grassland areas at the beginning of the Holocene.

Therefore, evidence of the predominance of grassland areas in the Southern Region indicates a cold and dry climate, which favored the expansion of the group that naturally occurs on grasslands. However, the presence and advancement of forest ecosystems, which had two flanks of expansion, the first-one from the central region of South America, which corresponds to the advancement of the Paraná forest towards the south and east, and the other flank in the eastern portion, which corresponds to the advancement of the Atlantic forest to the northeastern portion of Rio Grande do Sul, isolated populations of different species in rural remnants. This hypothesis is also corroborated when comparing the quantity and diversity of species of *Butia* occurring in predominantly grassland areas of the Pampa and Cerrado Biomes (19 species in total), with those of the Atlantic Forest Biome (3 species), all of them represented preferably in grassland environments of this Biome (Noblick, 2014; Soares *et al.* 2015). These forest formations likely acted as a historical barrier between the central-western and southern regions of Brazil, as well as northern Argentina and eastern Paraguay, being responsible for the cladogenesis that formed the Espinal-Pampa and Chaco-Cerrado Clades. The occurrence gap observed exactly where the Paraná Forest currently dominates may corroborate this hypothesis. (Figure 2).

Plací and Bitetti (2005, p. 199, figure 1) carried out a historical analysis of the Atlantic Forest of Alto do Paraná, characterized by semi-deciduous vegetation, and highlight that “el Bosque atlántico del Alto Paraná ha sido reducido a sólo el 7,8% de su extensión original. En Brasil sólo queda el 2,7% (771.276 ha) de la superficie original”. Although currently fragmented, the Misionera Subtropical Forest in the province of Misiones in Argentina had natural and lush vegetation that formed a natural barrier between the aforementioned clades, but it was gradually reduced and gave way to crops of economic value and anthropized areas.

Meerow *et al.* (2015) indicated that the uplift of the Andes Mountain range possibly caused the divergence of the “*Butia-Jubaea*” clade through vicariance during the Miocene, contributing to the differentiation of the biota of the Andean region (Antonelli *et al.* 2009). Considering the highly supported

relationship between *Butia* and *Jubaea* as sister groups, where *Jubaea* is restricted to the west coast of Chile, on the western side of the Andes Mountains, and *Butia* is restricted to the eastern side of the Andes Mountains in Argentina, Uruguay, Brazil and Paraguay (Dransfield *et al.* 2008), it is believed that the most recent common ancestor between both genera lived in the region between Chile and Argentina, before the uplift of the Andes Mountains

Soares (2013) suggests that *Butia* originated in the border between Mato Grosso do Sul and Paraguay (Cerrado). However, evidence shows that the ancestor that gave rise to *Butia* initially colonized the grassland ecotone zone between Espinal, Pampa and Chaco (Meerow *et al.* 2009). It then expanded towards the Pampa in south-central Argentina, southern Brazil and Uruguay, and to the Chaco in Paraguay and the Cerrado in western and central Brazil. The environmental variation mentioned above led to the diversification of species in the genus.

Global cooling and dry climate, together with glacial-interglacial cycles that persist to this day, led to the expansion of open and dry areas, predominantly of the savannas type. In South America, these areas are represented by the "Diagonal of open formations", composed of the Caatinga, Cerrado and Chaco (Da Silva *et al.* 2015), which may have favored the distribution of *Butia* species in the biomes with open grassland formations that surround the Southern plateau, which possibly explains the dichotomy of the genus into two clades.

#### 4.2 Phylogenetic relationships of the Espinal-Pampa Clade

The Espinal-Pampa Clade concentrates species from Argentina, Southern Brazil and Uruguay (*B. capitata*, *B. eriospatha*, *B. lallemantii*, *B. yatay*, *B. aff. paraguayensis*, *B. odorata* and *B. catarinensis*) and generally have a short to tall stipe. These species occur in the Pampa and Atlantic Forest biomes, but when occurring in this last biome, they are always associated with grassland areas that comprise the Southeast South American Grassland (SESA grasslands), which includes one diverse grassland ecosystem complex (*sensu* Azpiroz *et al.* 2012). Surprisingly, *B. capitata* emerges as the most external taxon in the clade (Figure 1), although its distribution is the Brazilian Cerrado, in the states of Bahia, Goiás and Minas Gerais (Noblick, 2010; Elesbão *et al.* 2016), which demonstrates that the positioning of *B. capitata* in the phylogram remains uncertain, as it is the only taxon that was not congruent with the biogeography of the species inserted in this phylogeny. The current literature does not cover the distribution area of *B. capitata* to the South of Brazil, however, it was related to the species from the South, since *B. odorata* was long considered a variety of *B. capitata*. However, morphological (Noblick, 2010; 2011) and anatomical (Sant'Anna-Santos *et al.* 2015) differences clearly distinguish and separate these two species. The next species to emerge in the clade is *B. eriospatha*, endemic to southern Brazil and naturalized in Misiones (Argentina) (Soares *et al.* 2015; Krapovickas and Dematteis, 2008). It occurs in grassland areas and on edge

of “capões” (woods) and forests, often associated with *Araucaria angustifolia* (Bertol.) Kuntze. *B. eriospatha* adequately exemplifies the hypothesis of evolution and dispersion of the genus, since it grows in the Atlantic Forest biome, but its range is restricted to grassland formations and on the edge of “capões” (woods) and forests, which impose its area of occupancy.

The grouping of the other species also matches what is expected for the biogeographic distribution of the genus. *B. lallemantii*, emerging as the next taxon in the clade, is devoid of aerial stems and has a caespitose habit, and is characterized by emitting sprouts from the central stem (Deble and Marchiori, 2006). This species is exclusive to the “Campos de Areia” (sandy grasslands) region of southwestern Rio Grande do Sul state in Brazil (Soares *et al.* 2013), with records for the municipalities of Quaraí, São Francisco de Assis, Manoel Viana and Alegrete (Cogo *et al.* 2022), and north Uruguay, Rivera department (Alves and Marchiori, 2010).

*Butia yatay* emerges close to *B. lallemantii*, however, there is divergence regarding the taxonomic position and the real geographic distribution of *B. yatay*. The species is considered endemic throughout the Argentine provinces of Corrientes, Entre Ríos, Chaco and Formosa, extending to western Uruguay, in the department of Paysandú (Deble *et al.* 2012a; Deble *et al.* 2012b and Silva *et al.* 2023). On the other hand, the species is cited for the Flora of Brazil, in which the authors give a broad treatment for this taxon, including *Butia poni* (Hauman) Burret, *Butia missionera* Deble & Marchiori and *Butia quaraimana* Deble & Marchiori (Heiden and Sant’Anna-Santos, 2024) in its synonymy.

*Butia* aff. *paraguayensis* is another species that emerges in the Espinal-Pampa Clade; however, it is a taxon that still has uncertainties regarding its taxonomic identification, since this specimen (Noblick 5459) collected in Uruguay was attributed as being related to *B. paraguayensis* (Meerow *et al.* 2009). However, Elesbão *et al.* (2016) analyzed this exsiccate and determined it as *B. paraguayensis*. However, in our phylogenetic analysis, “*B. paraguayensis*” from Uruguay was phylogenetically positioned distant from “*B. paraguayensis*” collected in Paraguay. *B. paraguayensis* is the species with the largest geographic distribution within the genus. It occurs in Paraguay, Argentina, Uruguay and Brazil (Xifreda e Sanso, 1996; Durigan *et al.* 2004; Noblick, 2014; Soares *et al.* 2014; Heiden and Sant’Anna-Santos, 2023).

Gaiero *et al.* (2011) using ISSR markers demonstrated that populations of *B. yatay*, *B. paraguayensis* and *B. lallemantii* occurring in Uruguay have high levels of genetic proximity, indicating occur genetic flow between *B. lallemantii* and *B. paraguayensis*. In addition, the authors consider the occurrence of hybridization between the species in the recent past. Thus, we can infer that the specimen Noblick 5459 from Uruguay is genetically distant of the specimen Noblick 5285 from Paraguay, and are probably distinct species with incorrect identification.

In this work, the nomenclature of the taxa "*B. capitata* var. *odorata*-5465" and "*B. capitata* var. *odorata*-5130" (Morrow *et al.* 2009) was updated to *B. odorata* and *B. catarinensis* respectively. Both species correspond to the last two taxa that make up the Espinal-Pampa clade, emerging as sister groups and genetically close. With morphological differences, as Noblick (2010) reports when describing *B. catarinensis*, such as differences in fruit size and shape, number of persistent leaves and shape of the peduncular bract. Regarding the biogeographic support of sister groups between the species, when we analyze the maps prepared by Soares *et al.* (2014: 136, figure D and F) of the distribution of both species, the continuous geographic distribution from the end of the occurrence domain of *B. odorata* in Rio Grande do Sul and the beginning of the occurrence domain of *B. catarinensis* is evident, extending to the coast of the State of Santa Catarina.

The coastal regions where these two taxa occur underwent several changes due to climate change during the recent geological history of the Pleistocene, between 2.5 Ma and 11,700 thousand years ago (Tomazelli *et al.* 2006). Couto (1961) emphasized that these variations observed in the current geological record of the Atlantic coast in Rio Grande do Sul are influenced by the Pleistocene glaciations in the accumulation of water on the continents, in the form of ice, exposing large areas of land on the continental shelf, which varied as the melting occurred. Thus, the coastal region of Rio Grande do Sul underwent recent geographic changes caused by the accumulation of sediment that formed the four barriers of lagoon systems (Tomazelli *et al.* 2006). This explains the adaptation of the local coastal flora and the hypothesis of recent cladogenesis between *B. catarinensis* and *B. odorata*, given the current establishment of this environment.

The recent colonization of *Butia* in the southern coastal region is evidenced through the analysis of a soil profile, in which Salgado *et al.* (2022) sought to understand the environmental changes in the region where the Tapes butiazal was formed (Rio Grande do Sul, Brazil). The analyses carried out by the authors revealed five distinct moments of environmental changes in the studied region, influenced by variations in temperature and sea level, which influenced the plant colonization of the region. Moment 1 (~ 11,125- 9,500 years ago) stands out, with a humid and cold climate and the presence of the Atlantic Forest in the region, with a low presence of *Butia*; and moment 5 (~ 1,650 years ago) with a dry and hot climate, predominant grassland vegetation, and a significant increase in butiazeiros (Salgado *et al.* 2022). Through the palynological analysis of a soil profile from a rocky outcrop in the southern coastal plain of Rio Grande do Sul, dated to approximately 7,500 years ago, Masetto and Lorscheiter (2019) present data consistent with those later obtained by Salgado *et al.* (2022). Their findings illustrate the fluctuations in grassland and forest taxa throughout the analyzed profile, highlighting recent changes in the coastal region. Of particular note is the period between ~2,000 and 1,760

years ago, marked by a significant reduction in marine remnants, indicating the last marine regression and a notable increase in grassland flora. Thus, we demonstrate that genetic and geographic proximity points to a relationship between *B. catarinenses* and *B. odorata*, but these species present exclusive morphological and anatomical variations (Deble *et al.* 2011, Sant'Anna-Santos *et al.* 2018).

*B. catarinensis* and *B. odorata* emerge as the most recent taxa of the Espinal-Pampa clade, corroborating the hypothesis of dispersal from the center of the continent towards the Atlantic coast. The relationships between the species of the clade are expressed phylogenetically from the region of dominance between the Espinal-Pampa grasslands to the Atlantic Forest biome and towards the southern coast of the continent, characterized as one of the last regions of Rio Grande do Sul to be geologically established, given the recent environmental changes that this region has undergone due to variations in mean sea level.

### 4.3 Phylogenetic relationships of the Chaco-Cerrado Clade

The Chaco-Cerrado clade concentrates species from Eastern Paraguay, Western and Central Brazil (*B. marmorii*, *B. buenopolensis*, *B. paraguayensis* and "*B. aff. yatay*"), a region highlighted by Elesbão *et al.* (2016) as the center of diversity of the genus. This clade is notable for the strong bootstrap support in the relationships between species, grouping together acaulescent species or those with short stems, a characteristic also presents in species of *Allagoptera* Nees. and *Syagrus* Mart. that occur in the same region. (Noblick, 2010).

Noblick (2006) described three species of *Butia* from the Argentina-Brazil-Paraguay border, giving the species occurring in this region the term "*grassy Butia*" due to their reduced habit and similarity to the grasses of the region (Cerrado). Among them is *B. marmorii*, the first taxon to emerge in the Chaco-Cerrado Clade, followed by *B. buenopolensis*, whose genetic data included in this study are unpublished.

When describing the species, Sant'Anna-Santos (2021) reports that although similar in size, *B. buenopolensis* was not related to the "*grass-like Butia*". However, this work shows that, genetically, the species are close, although geographically separated, since *B. buenopolensis* is endemic to the Espinhaço range, a mountainous area of the State of Minas Gerais, Brazil. *B. buenopolensis* presented morphoanatomical novelties for the genus, possibly being apomorphies of the species that do not reflect the evolutionary and genetic history of the group, based on the *WRKY* genes. However, it should be taken into account that this phylogenetic relationship was based on 4 of the 7 *WRKY* genes, since it was not possible to amplify the *WRKY2*, *WRKY12* and *WRKY19* regions for the species (Azambuja *et al.* 2023). Based on this, it is believed that the low population density associated with the restricted geographic distribution of the species may be related to a recent speciation of

the taxon, or inverse, with this population being relictual and currently isolated in the Serra do Cabral where it occurs. New field work in the area may better elucidate these hypotheses.

The last two taxa of the Chaco-Cerrado clade are difficult to discuss in terms of their proximity. One of them is *B. paraguayensis* (see Appendix D), with DNA extracted from a specimen collected in Paraguay. When Barbosa-Rodrigues (1899, 1903) described *B. paraguayensis* (under the name *Cocos paraguayensis*), he cited: "Acaulis, rara caulescens; Caudex nullus v. 1m-2m alt." This differs greatly from those cited for Argentina, Rio Grande do Sul and Uruguay, because, according to Noblick (2010), it has an erect and generally elevated stem. Thus, this species presents the greatest morphological variation in *Butia* or comprises a complex of species that has not yet been adequately understood.

Regarding "*Butia* aff. *yatay* voucher Zardini s.n", no collection record was found. *Butia yatay* was considered endemic to Argentina and western Uruguay, with its northern limit of occurrence being the department of General Paz, approximately 40 km east of Capital, province of Corrientes, Argentina (Deble *et al.* 2012b, Silva *et al.* 2023). In Paraguay, there are few species with a medium to high stipe that could be linked to *B. yatay* due to this characteristic. Given the taxonomic uncertainty of the specimen, the taxon was named "*Butia* sp." in the phylogram.

#### 4.4 Environment and dispersion

Although we are conditioned to think of geographic limits through political-administrative boundaries, beings develop, evolve and adapt to the environment according to the factors that delimit certain areas (Nodari, 2016). Based on this, it is evident that although the species of the genus occupy distinct territories and biomes, they have given rise to similar habits and physical characteristics of environments, which facilitated the colonization of these distinct habitats. Brazeiro *et al.* (2019) showed with *B. yatay* the diversity of mammals that use *Butia* ecosystems as food resources and can sometimes ingest fruits and disperse their seeds throughout the environment, providing an ecosystem service of colonizing new areas for species. Barbieri *et al.* (2015) demonstrated in their study the diversity of invertebrates that exert important ecological value in the pollination of *Butia* species, as well as vertebrates, and the importance of the species of the genus in the ecosystems where they are inserted. Thus, it is evident that physical-chemical characteristics of the environment acted in the diversification of species of the genus *Butia*, but the fauna plays an important role in the dispersion and colonization of new environments for the species.

However, we must also take into account the dispersal of *Butia* seeds through human action by ancient indigenous populations, since Bonomo and

Capeletti (2014) report traces of carbonized endocarps of *B. yatay* and *Syagrus romanzoffiana* were found in archaeological sites dating back to the early Holocene. It is important to highlight the role of human action in the selection and artificial occurrence that species of the genus undergo, since the presence of *Butia* in the sociocultural aspect (Rivas and Barbieri, 2017) influences individuals that produce larger and sweeter fruits, to be selected by local human populations for planting the generated seeds (Soares *et al.* 2014), due to the consumption of its fruits in natura and processed and the current wide use of the species in urban landscaping (Rivas and Barbieri, 2017). Therefore, all these characteristics may have influenced the colonization of the environments that the current species occupy. In view of the above, this work sought to address the phylogenetic relationships between the species of the genus *Butia* through evolutionary evidence, such as molecular biology, biogeography, anatomy and direct observations.

We sought to understand the differences and similarities between the biomes and the influence on the evolution and dispersion of the species that compose them, providing valuable information on how current environmental changes will impact the biodiversity of palm trees of the genus *Butia* in the near future. Finally, it is known that the sample of species included in our analysis does not represent all of the 22 species currently attributed to *Butia* (Azambuja and Pereira, 2022); however, it provides us with initial information to discuss the evolutionary history of the genus and encourages us to include more species in our next work in order to fill the gaps in knowledge about the genus through the genetic data of the remaining species.

## 5 CONCLUSION

This was the first study to show congruence between molecular and biogeographic data when comparing the relationship between molecular phylogeny and geographic distribution of some species of the genus *Butia*. The results indicate that in the evolutionary history of the species included in the phylogenetic analysis, there was a dichotomy, dividing the species into 2 groups. This dichotomy was possibly caused by the preference for grassland environments and pressure from the presence of forest formations in the areas dominated by the Paraná Forest, which formed a physical barrier that limited the dispersion of the species of the genus in the forested region of the biome, so that, considering that the most recent common ancestor that originated the current species lived in the grassland region of the central portion of Argentina, it dispersed to the regions surrounding where the Atlantic Forest is today, due to the selection of the grassland habitat most favorable to the ecology of the species.

The hypothesis was corroborated by genetic data, which grouped species from the same geographic regions (except *B. capitata*), and morphological and ecological characters, since the groupings also united species predominantly

acaulescent or with short stems in the Chaco-Cerrado clade, and species with short to predominantly elevated stems in the Espinal-Pampa clade, which correspond to adaptations to the environment. These results advance the discussion on phylogenetic relationships within *Butia*, extending the work of Meerow *et al.* (2009) contributing to the relevance of using WRKY genes in phylogenetic reconstructions in Arecaceae.

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Appendix Legends

Appendix A: List of species and DNA sequences of the seven WRKY genes obtained from GenBank for 68 species from native South American genera.

Species included in the phylogeny	WRKY2	WRKY6	WRKY7	WRKY12	WRKY16	WRKY19	WRKY21
t0 Parajubaea torallyi1	ok	ok	ok	ok	ok	ok	ok
t1 Parajubaea torallyi2	ok	not	not	ok	ok	ok	not
		GENBANK	GENBANK				GENBANK
t2 Parajubaea cocoides voucher Pinatud 282	ok	ok	ok	ok	ok	ok	ok
t3 Syagrus petraea voucher FTG:Noblick 5126	ok	ok	ok	ok	ok	ok	ok
t4 Syagrus macrocarpa voucher FTG:Noblick 4841	ok	ok	ok	ok	ok	ok	ok
t5 Syagrus flexuosa voucher FTG:Noblick 5108	ok	ok	ok	ok	ok	ok	ok
t6 Syagrus campylospatha voucher FTG:Noblick 5128	ok	ok	ok	ok	ok	ok	ok
t7 Jubaea chilensis voucher Pintaud 287	ok	ok	ok	ok	ok	ok	ok
t8 Butia lallemantii voucher FTG:Noblick 5457	ok	ok	ok	ok	ok	ok	ok
t9 Butia yatay voucher FTG:Noblick 5461	ok	ok	ok	ok	ok	ok	ok
t10 Butia capitata var. odorata voucher FTG:Noblick 5130	ok	ok	ok	ok	ok	ok	ok
t11 Butia capitata var. odorata voucher FTG:Noblick 5465	ok	ok	ok	ok	ok	ok	ok
t12 Butia marmorii voucher FTG:Noblick 5336	ok	ok	ok	ok	ok	ok	not
							GENBANK
t13 Butia paraguayensis voucher FTG:Noblick 5285	ok	ok	ok	ok	ok	ok	ok
t14 Butia eriospatha voucher FTG:Noblick 4878	ok	ok	ok	ok	ok	ok	ok
t15 Butia capitata voucher FTG:Noblick 5090	ok	ok	ok	ok	ok	ok	ok
t16 Butia aff. yatay_Zardini s.n. voucher FTG:Zardini s.n.	ok	ok	ok	ok	ok	ok	ok
t17 Butia buenopolensis	ok	ok	ok	x	x	x	ok
t18 Attalea speciosa voucher FTG:Noblick 4950	ok	Ok	ok	ok	ok	ok	ok
t19 Attalea cichleri voucher George Eiton 26	ok	ok	ok	ok	ok	ok	ok
t20 Attalea brejinhoensis voucher FTG:Noblick 4614	ok	ok	ok	ok	ok	ok	ok
t21 Attalea phalerata voucher FTG:Noblick 5018	ok	ok	ok	ok	not	ok	ok
					GENBANK		
t22 Attalea anisitsiana voucher FTG:Noblick 5291	ok	ok	ok	ok	ok	ok	ok
t23 Attalea guacuyule voucher FTG:Noblick 4928	ok	ok	ok	ok	ok	ok	ok
t24 Attalea cohune voucher FTG:Noblick 5519	ok	ok	ok	ok	ok	ok	ok
t25 Attalea butyracea voucher FTG:Noblick 5513	ok	ok	ok	ok	ok	ok	ok
t26 Attalea speciosa voucher FTG:Noblick 4963	ok	ok	ok	ok	ok	ok	ok
t27 Attalea crassispata voucher FTG:Noblick 5516	ok	ok	ok	ok	ok	ok	ok
t28 Attalea seabrensis voucher BF:Noblick 4600	ok	ok	ok	ok	ok	ok	ok
t29 Attalea oleifera voucher IPA:Noblick 5133	ok	ok	ok	ok	ok	ok	ok
t30 Attalea burretiana voucher:Noblick 4574	ok	ok	ok	ok	ok	ok	ok
t31 Attalea brasiliensis voucher IPA:Noblick 5162	ok	ok	ok	ok	ok	ok	ok
t32 Attalea humilis voucher BAH BRZ :Noblick 4575	ok	ok	ok	ok	ok	ok	ok
t33 Attalea pindobassu voucher IPA:Noblick 5141	ok	ok	ok	ok	ok	ok	ok
t34 Attalea funifera voucher IPA:Noblick 5136	ok	ok	ok	ok	ok	ok	ok
t35 Attalea phalerata voucher FTG:Noblick 5518	ok	ok	ok	ok	ok	ok	ok
t36 Attalea sp. Noblick 5517	ok	Ok	Ok	ok	ok	ok	ok
t37 Allagoptera leucocalyx voucher FTG:Zardini 54930	ok	ok	ok	ok	ok	ok	ok
t38 Allagoptera arenaria voucher NA:Meerow 3200	ok	ok	ok	ok	ok	ok	not
							GENBANK
t39 Allagoptera arenaria voucher IPA:Noblick 5168	ok	ok	ok	ok	ok	ok	ok
t40 Cocos nucifera voucher NA:Meerow 3206	ok	ok	ok	not	ok	ok	ok
				GENBANK			
t41 Cocos nucifera voucher NA:Meerow 3205	ok	ok	ok	not	not	ok	ok
				GENBANK	GENBANK		
t42 Cocos nucifera voucher NA:Meerow 3203	ok	ok	ok	not	ok	ok	ok
				GENBANK			
t43 Cocos nucifera voucher NA:Meerow 3202	ok	ok	ok	ok	ok	ok	ok
t44 Cocos nucifera voucher NA:Meerow 3204	ok	ok	ok	not	not	ok	ok
				GENBANK	GENBANK		
t45 Cocos nucifera voucher NA:Meerow 3201	ok	ok	ok	ok	ok	ok	ok
t46 Syagrus romanzoffiana voucher PY:Noblick 5167	ok	ok	ok	ok	ok	ok	ok
t47 Polyandrococos caudescens voucher IPA:Noblick 5003	ok	ok	ok	ok	ok	ok	ok
t48 Polyandrococos caudescens voucher FTG:Noblick 5135	ok	ok	ok	ok	ok	ok	ok
t49 Syagrus picrophylla voucher IPA:Noblick 5156	ok	ok	ok	ok	ok	ok	ok
t50 Syagrus cearensis voucher IPA:Noblick 5132	ok	ok	ok	ok	ok	ok	ok
t51 Syagrus oleracea voucher FTG:Noblick 5084	ok	ok	ok	ok	ok	ok	ok
t52 Syagrus coronata2 voucher NA:Meerow 3209	ok	ok	ok	ok	ok	ok	ok
t53 Syagrus coronata1 voucher FTG:Noblick 4833	ok	ok	ok	ok	ok	ok	ok
t54 Syagrus ruschiana voucher IPA:Noblick 5169	ok	ok	ok	ok	not	ok	ok
					GENBANK		
t55 Syagrus schizophylla2 voucher NA:Meerow 3210	ok	ok	ok	ok	ok	ok	ok
t56 Syagrus schizophylla1 voucher IPA:Noblick 5134	ok	ok	ok	ok	ok	ok	ok
t57 Syagrus vermicularis voucher FTG:Noblick 4974	ok	ok	ok	ok	ok	ok	ok
t58 Syagrus stenopetala2 voucher FTG:Noblick 5515	ok	ok	ok	ok	ok	ok	ok
t59 Syagrus stenopetala1 voucher FTG:Noblick 4936	ok	ok	ok	ok	ok	ok	ok
t60 Syagrus amara voucher NY:Hahn 7649	ok	ok	ok	ok	ok	ok	ok
t61 Syagrus orinocensis voucher FTG:Noblick 4946	ok	ok	ok	ok	ok	ok	ok
t62 Syagrus botryophora voucher FTG:Noblick 5002	ok	ok	ok	ok	ok	ok	ok
t63 Syagrus cocoides voucher FTG:Noblick 4954	ok	ok	ok	ok	ok	ok	ok
t64 Syagrus sancona voucher FTG:Noblick 5514	ok	ok	ok	ok	ok	ok	ok
t65 Lytocaryum sp. Lorenzi 6496	ok	ok	ok	not	ok	ok	ok
				GENBANK			

Species included in the phylogeny	WRKY2	WRKY6	WRKY7	WRKY12	WRKY16	WRKY19	WRKY21
t66 <i>Lytocaryum_weddellianum_voucher_NA:Meerow_3207</i>	ok	ok	ok	ok	ok	ok	ok
t67 <i>Syagrus_glaucescens_voucher_FTG:Noblick_5511</i>	not GENBANK	ok	ok	ok	ok	ok	ok
t68 <i>Butia_aff._paraguayensis_Noblick_5459_voucher_FTG:Noblick_5459</i>	not GENBANK	ok	ok	ok	ok	ok	ok

**Appendix B:** Sequencies from *B. buenopolensis* used in the study with voucher specimens and GenBank accession numbers for the WRKY sequences.

Sequence_ID	Specimen_voucher	WRKY	GENBANK Accession Number
Seq1	<i>B. buenopolensis</i> _DIAM 8256	WRKY2	PQ537155
Seq2	<i>B. buenopolensis</i> _MBM 323217	WRKY2	PQ537156
Seq3	<i>B. buenopolensis</i> _IBGE 83300	WRKY2	PQ537157
Seq4	<i>B. buenopolensis</i> _UFG 70074	WRKY6	PQ537163
Seq5	<i>B. buenopolensis</i> _IBGE 83300	WRKY6	PQ537164
Seq6	<i>B. buenopolensis</i> _UFG 70074	WRKY7	PQ537161
Seq7	<i>B. buenopolensis</i> _IBGE 83300	WRKY7	PQ537162
Seq8	<i>B. buenopolensis</i> _DIAM 8256	WRKY21	PQ537158
Seq9	<i>B. buenopolensis</i> _UFG 70074	WRKY21	PQ537159
Seq10	<i>B. buenopolensis</i> _IBGE 83300	WRKY21	PQ537160

### Appendix C

Table 2. Results of nucleotide substitution models by maximum likelihood analyses with seven WRKY loci.

Locus	WRKY2	WRKY6	WRKY7	WRKY12	WRKY16	WRKY19	WRKY21
Nt substitution model	KP2+G4	HKY+F	TN+F+G4	TPM3U+F+G4	HKY+F+G4	HKY+F	HKY+F+G4

**Appendix D:** Information and occurrence points of *Butia* specimens included in the phylogeny

Specimen/ Genbank	Collector	number	Collection location	Herbarium-Access number	species	Taxonomic confirmation
<i>B. lallemantii</i>	Noblick	5457	Rivera/Uruguay	NYBG-02388331	<i>B. lallemantii</i>	SpeciesLink; Elesbão <i>et al.</i> , (2016)
<i>B. eriospatha</i>	Noblick	4878	Paraná/Brasil	MBM-150255	<i>B. eriospatha</i>	SpeciesLink; Elesbão <i>et al.</i> , (2016)
<i>B. aff. paraguayensis</i>	Noblick	5459	Rivera/Uruguay	FTG-20060222	<i>B. aff. paraguayensis</i>	Elesbão <i>et al.</i> , (2016)

Specimen/ Genbank	Collector	number	Collection location	Herbarium- Access number	species	Taxonomic confirmation
<i>B. paraguayensis</i>	Noblick	5285	Distrito de San Pedro/ Paraguay	NYBG-02565374	<i>B. paraguayensis</i>	SpeciesLink
<i>B. capitata</i> var. <i>dorata</i>	Noblick	5465	Castillos: Rocha/ Uruguay	FTG-20060233	<i>B. odorata</i>	Dr. Larry Noblick
<i>B. capitata</i> var. <i>odorata</i>	Noblick	5130	Torres-RS/Brasil	NYBG-567914	<i>B. catarinensis</i>	Noblick, 2010; Elesbão <i>et al.</i> , (2016)
<i>B. capitata</i>	Noblick	5090	Goiás/Brasil	FTG-96109	<i>B. capitata</i>	Dr. Larry Noblick
<i>B. marmorii</i>	Noblick	5336	Alto Paraná/ Paraguay	FTG-20040185	<i>B. marmorii</i>	Dr. Larry Noblick
<i>B. yatay</i>	Noblick	5461	Paysandú/ Uruguay	FTG-20060224	<i>B. yatay</i>	Elesbão <i>et al.</i> (2016); Dr. Larry Noblick;
<i>B. aff. yatay</i>	Zardini	s.n.	-	-	<i>Butia</i> sp.	-
<i>B. buenopolensis</i>	Sant'Anna-Santos	214	Minas Gerais/ Brasil	MBM-323217	<i>B. buenipolensis</i>	Sant'Anna-Santos (2021)

**Appendix 5:** Maximum likelihood bootstrap consensus tree of seven concatenated WRKY gene.

