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GEOGRAPHIC DISTRIBUTION, MORPHOLOGICAL DIVERSITY AND CONSERVATION STATUS OF *Chascolytrum parodianum* (POACEAE, POOIDEAE, POEAE): AN ENDEMIC SPECIES OF PAMPA BIOME?

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Trabalho de conclusão de curso apresentado como requisito parcial para obtenção do título de Bacharel em Ciências Biológicas na Universidade do Rio Grande do Sul.

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Geographic distribution, morphological diversity and conservation status of *Chascolytrum parodianum* (Poaceae, Pooideae, Poeae): an endemic species of Pampa Biome?

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Abstract: *Chascolytrum parodianum* has been considered a rare species, endemic to Pampa Biome, only known for Uruguay and Southern Rio Grande do Sul, Brazil. The species was included as Critically Endangered (CR) in the national and regional Red Lists in Brazil. However, records of specimens morphologically similar to *C. parodianum* assigned as *C.* aff *parodianum* in the highland grasslands of Atlantic Forest Biome suggested an expansion on its distribution. In this study we aimed to test the morphological boundaries among known populations from both Biomes, including the variance of selected characters and their correlation with altitude. Through morphometric approaches, we generated box-plots comparing the variation of characters and analyzed the clustering of individuals using Hierarchical Cluster (HCA) and Principal Component (PCA) analyses. We also applied Mantel test for an ecological approach concerning altitude influence among populations. The results confirmed the occurrence of the species in Atlantic Forest Biome, whereas no character assessed showed considerable differentiation to support the circumscription of distinct taxa, confirming the inclusion of the specimens identified as *C.* aff. *parodianum* under the circumscription of *C. parodianum*. Mantel test did not show a significant correlation, but a tendency for altitude influence was found. A map showing the actual known distribution for *C. parodianum* is presented, as well as a reevaluation of its conservation status from CR to Vulnerable (VU). Future studies using niche modeling and phylogeographic techniques are needed to understand the evolutionary history and distribution of the species, since its actual range reveals disjoined populations under different abiotic conditions.

Keywords: Morphometrics, cool-season grasses, conservation, IUCN status reevaluation, geographic range

1. Introduction

Currently, in Brazil, near one new species of angiosperm is described every two days (Sobral & Stehmann, 2009), while worldwide around 2000 new plant species are discovered per year (Christenhusz & Byng, 2016). Many of these new taxa do not necessarily represent new species previously unknown by science, but derive from taxonomic revisions of species already described, where significant morphological and/or genetic differences pointed to a different circumscription of these taxa (Su, 2015; Peichoto *et al.*, 2015; Vigalondo *et al.*, 2019). However, the opposite often happens resulting on synonymities (Essi *et al.*, 2017; Pastori *et al.*, 2018). Species delimitation has been a major problem in Biology, which gained more attention after robust statistical analyses became feasible (Sites Jr & Marshall, 2003; Duminil *et al.*, 2012). An integrative approach using different methods and aspects (morphological, genetic, ecological, etc.) can be useful to understand species boundaries and as distinct evolutionary lineages (Sites Jr & Marshall, 2003; Vogel Ely *et al.*, 2018; Pastori *et al.*, 2018). Therefore, if species are important unities for ecological and evolutionary studies, their correct delimitation is fundamental to comprehend biodiversity, biogeography, ecology and conservation (Duminil *et al.*, 2012; Pessoa *et al*., 2012).

Morphometric analyses have been successfully used to test morphological boundaries between taxa (Newmaster *et al.*, 2008; Peichoto *et al*., 2015), and provide statistical evidence that avoids the split or grouping of taxa based on non-precise interpretations concerning determined phenotypic diversity (Duminil *et al.*, 2012). Morphological data are essential for species delimitation because commonly they are the only data available and represent the outcome of multilocus variation and environmental influence (Ezard *et al.*, 2010). When combined with ecological factors, morphometric analyses provide evidences that help to understand the geographic distribution patterns and the role of abiotic factors acting over phenotypic variations of the species (Vogel Ely *et al.*, 2018).

Poaceae is a cosmopolitan family, dominant in grasslands and open environments, comprising around 768 genera and 11000 species (Osborne *et al*., 2011; Soreng *et al*., 2017). It plays a key role in economics, mainly on human feeding, since the four most cultivated plants in the world are grasses: the wheat (*Triticum aestivum* L.), the rice (*Oryza sativa* L.), the corn (*Zea mays* L.) and the sugarcane (*Saccharum officinarum* L.) (Rúgolo de Agrasar & Puglia, 2004; Boldrini *et al.*, 2008). In Brazil, 224 genera and 1482 species are recorded, of which 493 are endemic (Filgueiras *et al.,* 2018). The classification within the family has changed over the past years, and revisions are still constant, even in well-established groups (Zhang, 2012; Silva *et al.*, 2015; Barberá, 2019; Peterson *et al.*, 2019), as well as recent descriptions of new species (e.g. Newmaster, 2008; Mashau & Coetzee, 2018, Andrade *et al.*, 2019; Oliveira *et al.*, 2019) and new circumscriptions at the genus level (Su *et al*., 2015; Finot *et al.*, 2018; da Silva *et al.*, 2020). Commonly, the morphometric approach is applied to comprehend the delimitation of taxa of grasses (Silva, 2013; Peichoto *et al*., 2015).

The subfamily Pooideae (the cool-season grasses) represents the most diverse lineage of Poaceae, including one third of the grass species, distributed mainly in temperate and subtropical regions worldwide (Saarela *et al*., 2017). In Southern Brazil, the cool-season grasses are important elements of the composition, where the livestock represents part of the local economy (Boldrini *et al.*, 2008; Boldrini, 2009).

Chascolytrum Desv. belongs to Pooideae, tribe Poeae *s.l.*, subtribe Calothecinae (Soreng *et al.*, 2017), and comprises 23 American species. The genus is remarkably diverse in Southern Brazil, where at least 19 species occur (da Silva *et al.*, 2020). The genus, as currently circumscribed, includes species with a complex taxonomic background, somehow recognized in nine different genera in the past (*Briza* L., *Calotheca* Desv., *Chascolytrum*, *Erianthecium* Parodi, *Gymnachne* Parodi, *Lombardochloa* B.Rosengurtt & B.R.Arrill., *Microbriza* Parodi ex Nicora & Rúgolo, *Poidium* Nees, and *Rhombolytrum* Link) (Essi *et al.*, 2008). Consequently, *Chascolytrum* is morphologically diverse and is recognized using a combination of characteristics, such as basal leaf sheaths fibrous, spikelets with 3- to many-flowers, and pedicels straight (Essi *et al.*, 2017; da Silva *et al*., 2020). Based on morphological and molecular data, recently *Chascolytrum* was divided into eight sections (da Silva *et al.*, 2020).

Chascolytrum parodianum (Roseng., Arrill. & Izag.) Matthei has its occurrence cited for Uruguay and Rio Grande do Sul, inhabiting grasslands with granite outcrops (Longhi-Wagner, 1987; Essi *et al.*, 2017) (Fig. 1-3). Traditionally, *C. parodianum* was included in *Chascolytrum s.s.* (Mathei, 1975) or *Briza* section *Chascolytrum* (Longhi-Wagner, 1987). However, based on molecular and morphological data, the species was transferred to the monospecific *Chascolytrum* section *Obovatae* and it is recognized by the presence of chartaceous lemma, obovate to obovate-truncate palea (da Silva *et al.*, 2020). Until recently, *C. parodianum* has been considered endemic to Pampa Biome (Boldrini, 2009; Essi *et al.*, 2017). Nevertheless, populations with the same spikelet morphology (treated as *C.* aff. *parodianum* by da Silva *et al.*, 2020), but with apparent smaller height, smaller lemmas and wider paleas were recorded in higher altitude regions within the Atlantic Forest Biome. These records have suggested the extension of the geographic range and raised questions about the endemism of *C. parodianum* to the Pampa Biome (da Silva *et al*., 2020). According to the Red

List of Brazilian Flora (Filgueiras *et al.*, 2013) and the Red List of the Flora of Rio Grande do Sul (Red List RS, 2014) *C. parodianum* is considered Critically Endangered (CR), being the habitat loss for silviculture and overgrazing its major threats. The species was also included in the list of priority species for conservation in Uruguay, considered as threatened based on its endemism and restricted distribution in Uruguay (Marchesi *et al*, 2013).

In this study we aim to: (1) perform a morphometric analysis, including populations of *Chascolytrum parodianum* and *C.* aff. *parodianum*, to access their morphological diversity; (2) recognize the delimitation of *C. parodianum*, testing if *C.* aff. *parodianum* would be included under its circumscription; (3) investigate the influence of altitude and habitat on the morphological characters among the populations from Pampa and Atlantic Forest; and (4) reevaluate the geographic distribution and the conservation status of *C. parodianum*.

Figure 1. Individuals of *Chascolytrum parodianum* inhabiting granitic hills in Torrinhas (Rio Grande do Sul, Brazil). (Photo: Leonardo da Silveira de Souza)

Figure 2. Detail of the panicle of *Chascolytrum parodianum*. Torrinhas (Rio Grande do Sul, Brazil). (Photo: Leonardo Nogueira da Silva).

Figure 3. Habitat of *Chascolytrum parodianum* in Torrinhas (Rio Grande do Sul, Brazil). (Photo: Leonardo Nogueira da Silva).

2. Materials and methods

2.1 Taxon sampling

Specimens from herbaria and collected in fieldwork expeditions were included in the analyses. The following herbaria were revised: ICN, BLA and HAS (acronyms following Thiers, 2019 onwards). Specimens from MVFA were consulted through digital images and only included for geographical distribution. Field trips occurred from 2014 to 2019, covering Uruguay (Rocha and Lavalleja Departments) and Rio Grande do Sul State (Serra do Sudeste and South Brazilian highland regions). The total sampling included 19 specimens, 14 identified as *C. parodianum* and five identified as *C.* aff. *parodianum* (Table 1).

Table 1. Voucher information, location, altitude and Biome from each specimen included in this study. Specimens from Atlantic Forest Biome are treated as *Chascolytrum* aff. *parodianum*. * indicates specimens not included in the morphometric and ecological analyses, but considered for geographic distribution. BR = Brazil; RS = Rio Grande do Sul State UY = Uruguay.

ID (Voucher)	Location	Altitude (m)	Biome
da Silva, 674 (ICN180285)	BR – RS - Piratini	430	Pampa
da Silva, 711 (ICN194831)	UY – Rocha Dp. (Cerro Aguirre)	190	Pampa
da Silva, 673 (ICN180284)	BR – RS - Piratini	387	Pampa
da Silva, 834 (ICN199530)	BR – RS - Cambará do Sul (Parque Nacional dos Aparados da Serra)	920	Atlantic Forest
da Silva, 667 (ICN180280)	BR – RS - Torrinhas	419	Pampa
da Silva, 672 (ICN180283)	BR – RS - Piratini	275	Pampa
da Silva, 706 (ICN194833)	UY – Lavalleja Dp. (Parque Salus)	195	Pampa
Essi, 290 (ICN134887)	BR – RS - Piratini	272	Pampa
Essi, 291 (ICN134888)	BR – RS - Piratini	272	Pampa

da Silva, 1080 (ICN199468)	BR – RS - Cambará do Sul (Parque Nacional dos Aparados da Serra)	919	Atlantic Forest
Essi, 292 (ICN134889)	BR – RS - Piratini	272	Pampa
Valls, 2254 (ICN023082)	BR – RS - Between Piratini and Pinheiro Machado	272	Pampa
Rosengurtt, 6357 (ICN033215)	UY – Lavalleja Dp. (Parque Ute)	225	Pampa
Windisch, 9334 (ICN134635a)	BR – RS - Morro Reuter (Morro da Embratel)	630	Atlantic Forest
Leo 1090	BR – RS - Morro Reuter (Morro da Embratel)	633	Atlantic Forest
Rosengurtt, 5674 (BLA11444)	UY – Lavalleja Dp. (Parque Salus)	195	Pampa
Montoro, 2802 (BLA11443)	UY – Maldonado Dp.	366	Pampa
HB	BR – RS - Estrela Velha	225	Atlantic Forest
Leo S 37	BR – RS - Pedras Altas	377	Pampa
*Ziliane *et al*., 20403 (MVFA)	UY - Lavalleja Dp. (Cerro Arequita)	-	Pampa
*Montoro, 2821 (MVFA)	UY – Maldonado Dp. (Sierra de las Ánimas)	-	Pampa
*Izaguirre, 42 (MVFA)	UY – Maldonado Dp. (Cerro Tupamba)	-	Pampa
*Rosengurtt, B-6357 (MVFA)	UY – Lavalleja Dp. (Parque Ute)	-	Pampa
*Montoro, s/n. (MVFA)	UY – Maldonado Dp.	-	Pampa

2.2 Morphological characters

Quantitative and qualitative characters were selected based on taxonomic revisions for *Chascolytrum* (Longhi-Wagner, 1987; Essi *et al*., 2017) and field observations pointed previously by da Silva *et al.* (2020). Character states were defined according to The Kew Plant Glossary (Beentje, 2010) and Plant Identification Terminology (Harris & Harris, 1995). Seventeen characters were primarily selected, fifteen quantitative and two qualitative (Table

2). Only material with complete spikelets were measured. For each specimen, three leaves were measured; the measures of reproductive characters were taken in five distinct unities. Average and ratios of morphological quantitative variables were calculated and used for morphometric analyses and to test correlation with altitude (Table 3). The qualitative characters of paleas were excluded from analyses since they show variation within the same individual and panicle.

Table 2. Quantitative and qualitative characters of *C. parodianum* selected for measurement and posterior assessment, according to taxonomic revisions (Longhi-Wagner, 1987; Essi *et al.*, 2017) and field observations (da Silva *et al.*, 2020).

Quantitative characters
Plant hight (cm)
Panicle lenght (cm)
Panicle widht (mm)
Leaf lenght (cm)
Spikelet lenght (mm)
Spikelet widht (mm)
Lower glume lenght (mm)
Lower glume widht (mm)
Upper glume lenght (mm)
Upper glume widht (mm)
Lemma lenght (mm)
Lemma widht in lateral view (mm)
Lemma widht in dorsal view (mm)
Palea lenght (mm)
Palea widht (mm)
Qualitative chatacters
Palea apex (truncate or obovate)
Palea margin (ciliate or glabrous)

Table 3. Averages and ratios of characters of *Chascolytrum parodianum* used for morphometric and ecological analyses

Characters
Plant hight (cm)
Panicle length (cm)
Panicle width (mm)
Leaf length (cm)
Spikelet length (mm)

Spikelet width (mm)
Length x width ratio of the spikelet (mm)
Lower glume length (mm)
Lower glume width (mm)
Length x width ratio of the lower glume (mm)
Upper glume length (mm)
Upper glume width (mm)
Length x width ratio of the upper glume (mm)
Lemma length (mm)
Lemma width in lateral view (mm)
Length x width ratio of the lemma in lateral view (mm)
Lemma width in dorsal view (mm)
Length x width ratio of the lemma in dorsal view (mm)
Palea length (mm)
Palea width (mm)
Length x width ratio of the palea (mm)

2.3 Statistical analyses

All analyses were performed with RStudio version 1.2.1335 (RStudio Team, 2015), using packages cluster, ggplot2 and vegan. (1) We analyzed the variation of morphological characters among *Chascolytrum parodianum* and *C.* aff. *parodianum* specimens through Student's t-test; (2) we tested if both groups can be well-delimited by multivariate analyses; (3) we seek to understand what variables were related to each other; and (4) we search for a correlation between selected characters with altitude, which is a significant abiotic factor that drives differences among Pampa and Atlantic Forest grasslands using Mantel test.

We carried out a *F*-test to confirm if Pampa and Atlantic Forest groups exhibit homogeneity of variances, and a Shapiro-Wilk test to check the normality of the variables. All variables were parametric. The variation of the 21 characters (Table 3) was portrayed in the box-plots and their significant differences tested through Student's t-test by average comparison at $P < 0.05$. The Mantel test, based on Pearson's product-moment correlation, was performed to test if altitude can explain the variation of the 21 characters.

For the subsequent tests, hierarchical cluster analysis (HCA) and principal component analysis (PCA), we selected six characters based on preliminary Student's t-test analyses and Pearson's product-moment correlation, in which those with high correlation ($r > 0.7$) were excluded from the analyses. The selected characters were: "lower glume length", "spikelet width", "length x width ratio of the upper glume", "length x width ratio of the lemma in lateral view", "palea length", and "palea width". In order to evaluate the morphological differentiation between *C. parodianum* and *C.* aff. *parodianum*, we performed a HCA, using average distance method for grouping and also carried out a PCA. We used the Gower distance as a dissimilarity measure (Gower, 1971) and we selected the result with the highest cophenetic value for the HCA.

2.4 Distribution and conservation status

The map of geographic distribution for *Chascolytrum parodianum* was constructed using the ArcGIS 10.5 software (https://www.arcgis.com/features/index.html). The area of occupancy (AOO), extent of occurrence (EOO), number of subpopulations and number of locations were calculated using the package ConR (Dauby *et al*., 2017) with grid resolution of 2 km for AOO, 10 km for locations and radius of 5 km for delimiting sub-populations. We also took into account the monitoring of two populations, one located in Torrinhas (Pampa Biome), with observations made in 2014 and 2019; and another from Morro Reuter (Atlantic Forest Biome), with observations made in 2018 and 2019. We visually checked if any visible decline in number of individuals and habitat quality occurred during the visits. The conservation status was evaluated according to guidelines of the International Union for Conservation of Nature (IUCN, 2019).

3. Results

3.1 Morphometric and ecological analyses

The Student's t-test showed statistical differences at $P < 0.05$ on "leaf length", "spikelet width", "lower glume length", "length x width ratio of the lower glume", "upper glume length", "length x width ratio of the upper glume", "lemma length", "length x width ratio of the lemma in lateral view", "palea length", and "palea width", between the two groups, as represented in the box-plots (Fig. 4-13). Only the character "length x width ratio of the upper glume" did not presented overlapping boxplots.

In the hierarchical cluster analysis, the specimens of Atlantic Forest and Pampa did not group according the biome (Fig. 14). The group I is composed by the sample ICN134488, a material from Pampa Biome. In the cluster III, two major groups can be visualized: V, with a collection within the Atlantic Forest Biome (HB) clustering with samples from Pampa Biome;

and VI, with only specimens from Pampa Biome. The samples ICN199530 and ICN199468, both from same locality in Parque Nacional dos Aparados da Serra, did not cluster together, as we can see in the groups inside the cluster IV. In VIII, samples from Atlantic Forest nested with material from Pampa Biomes.

The first two PCA axis explained 77.7% of the morphological variation, showing a better tendency to separate groups than the HCA analysis, but still with mixed specimens (Fig. 15). A sample from Pampa Biome (ICN194831) placed close to Atlantic Forest representants, while a sample from Atlantic Forest Biome (HB) is shown to be morphologically related to other Pampa specimens.

The Mantel test did not support correlation between altitude and the morphological characters (r_{Mantel} = 0.4284; P = 0,386). However, its "r_{Mantel}" value was sensible to the data, showing a tendency to variation, even though not significantly enough to result in considerable differences among the taxa.

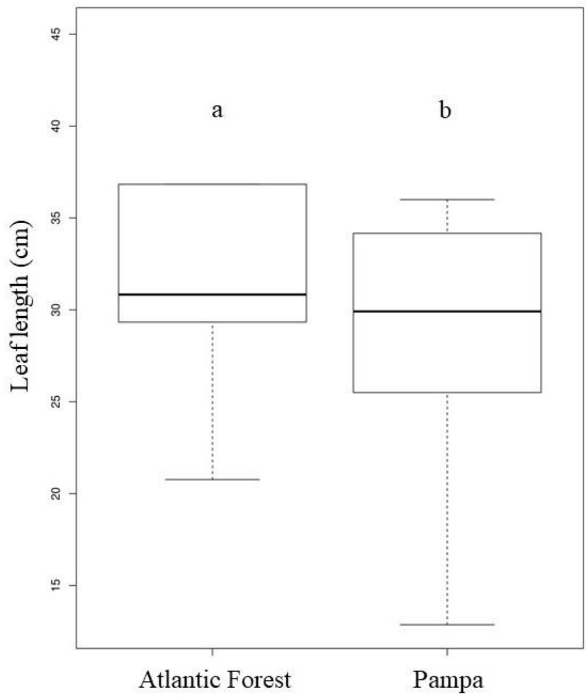

Figure 4. Box-plots representing the variation of leaf length at P < 0.05. The box represents 75% of the variation and the horizontal bars the other 25% of variation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

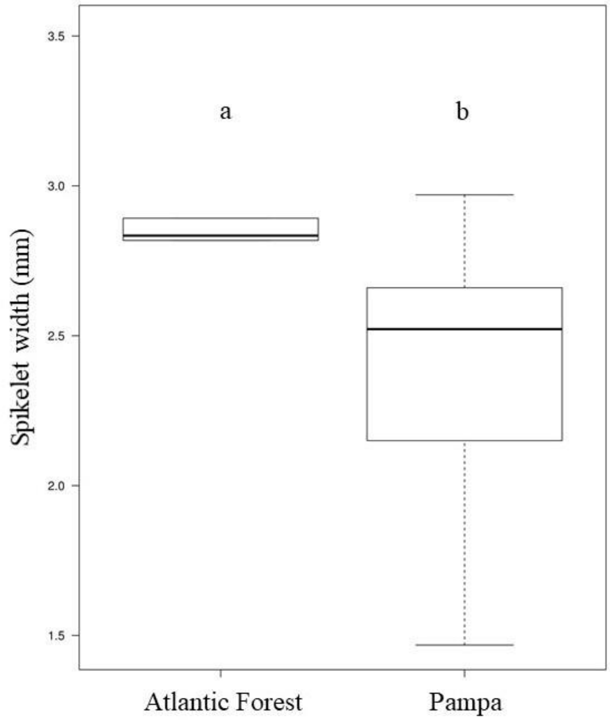

Figure 5. Box-plots representing the variation of "spikelet width" at P < 0.05. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

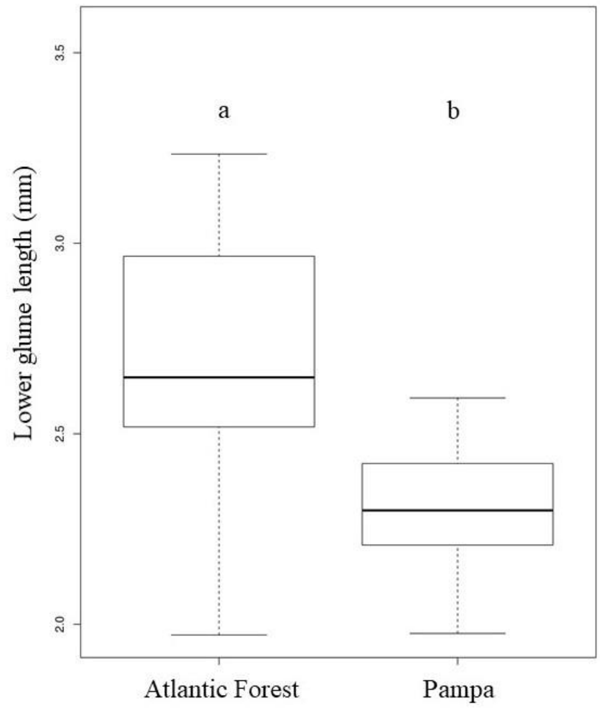

Figure 6. Box-plots representing the variation of "lower glume length" at P < 0.05. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

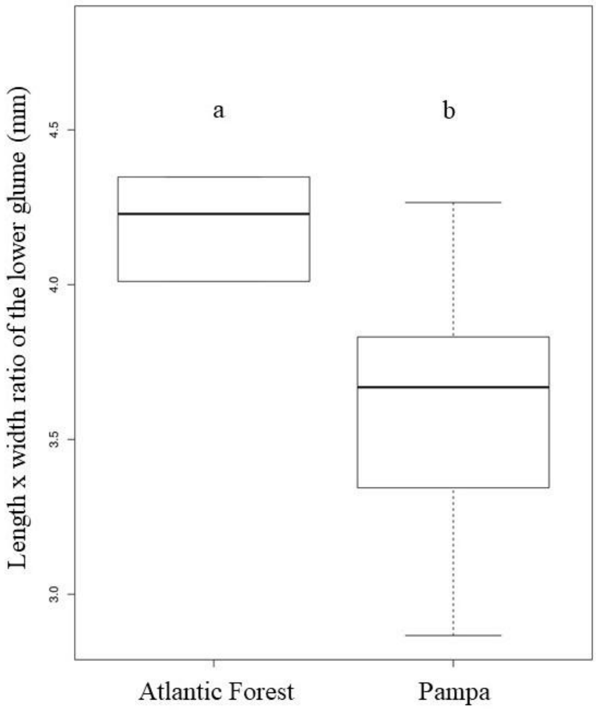

Figure 7. Box-plots representing the variation of "length x width ratio of the lower glume" at P < 0.05. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

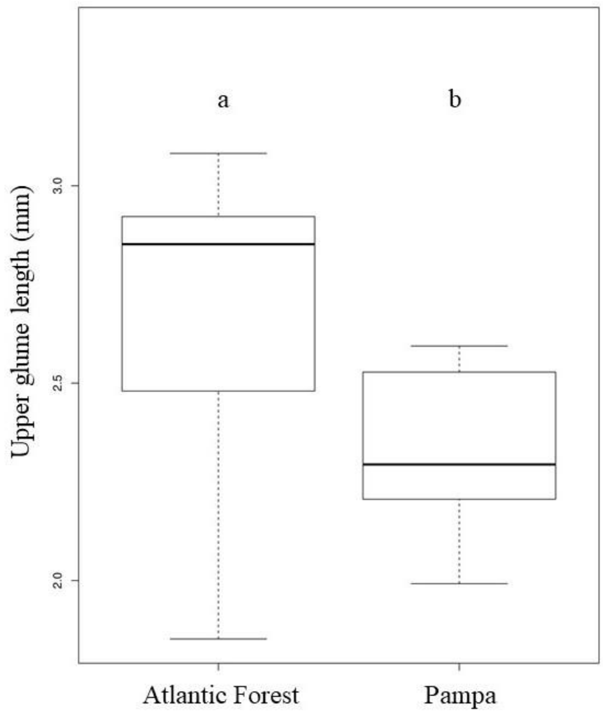

Figure 8. Box-plots representing the variation of "upper glume length" at $P < 0.05$. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

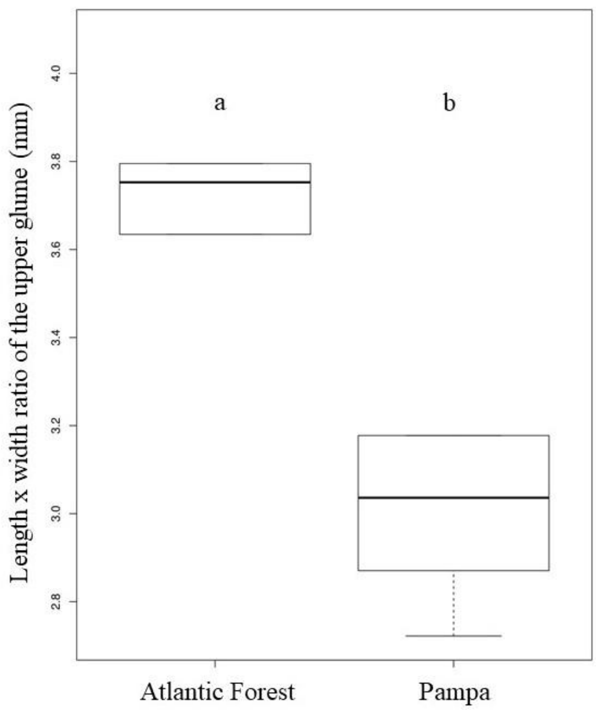

Figure 9. Box-plots representing the variation of "length x width ratio of the upper glume" at

P < 0.05. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

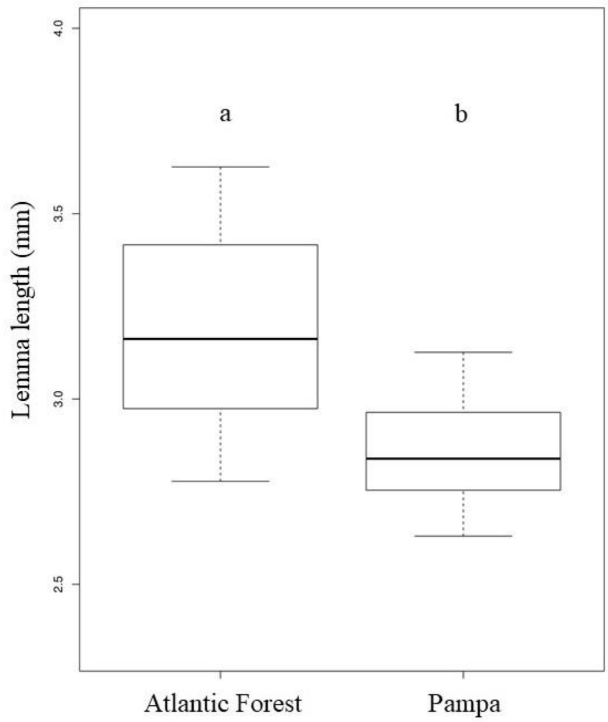

Figure 10. Box-plots representing the variation of “lemma length” at P < 0.05. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

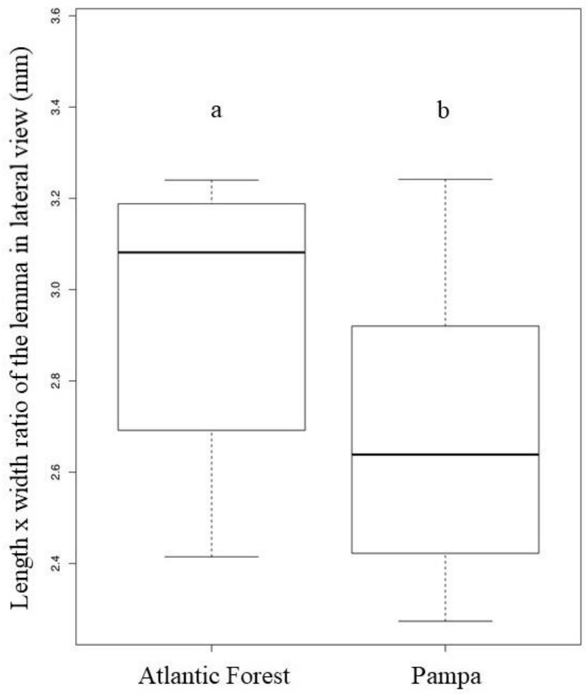

Figure 11. Box-plots representing the variation of "length x width ratio of the lemma in lateral view" at $P < 0.05$. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

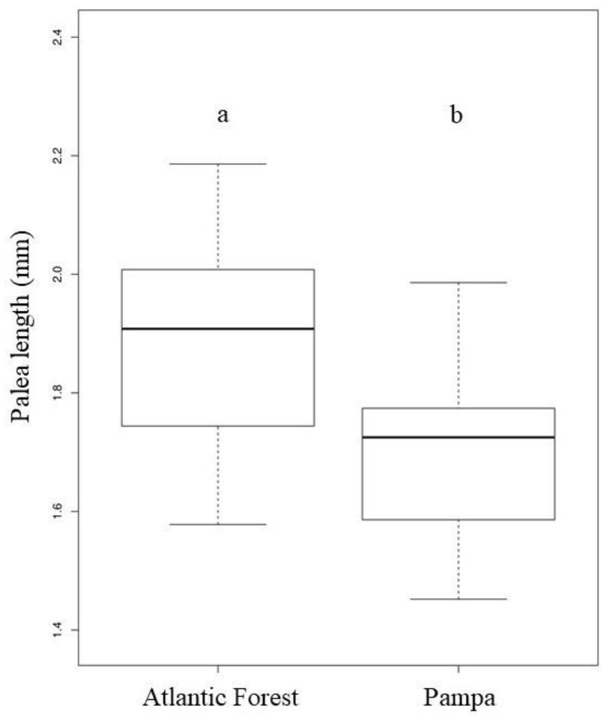

Figure 12. Box-plots representing the variation of "palea length" at P < 0.05. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

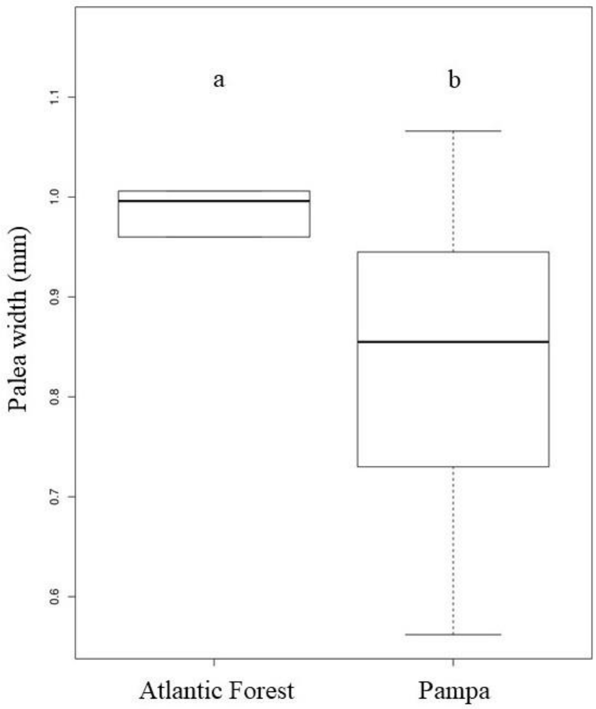

Figure 13. Box-plots representing the variation of "palea width" at P < 0.05. See Fig. 4 for box-plot structure explanation. The bar in the middle of the box represent the median. Different letters represent significant difference between the groups.

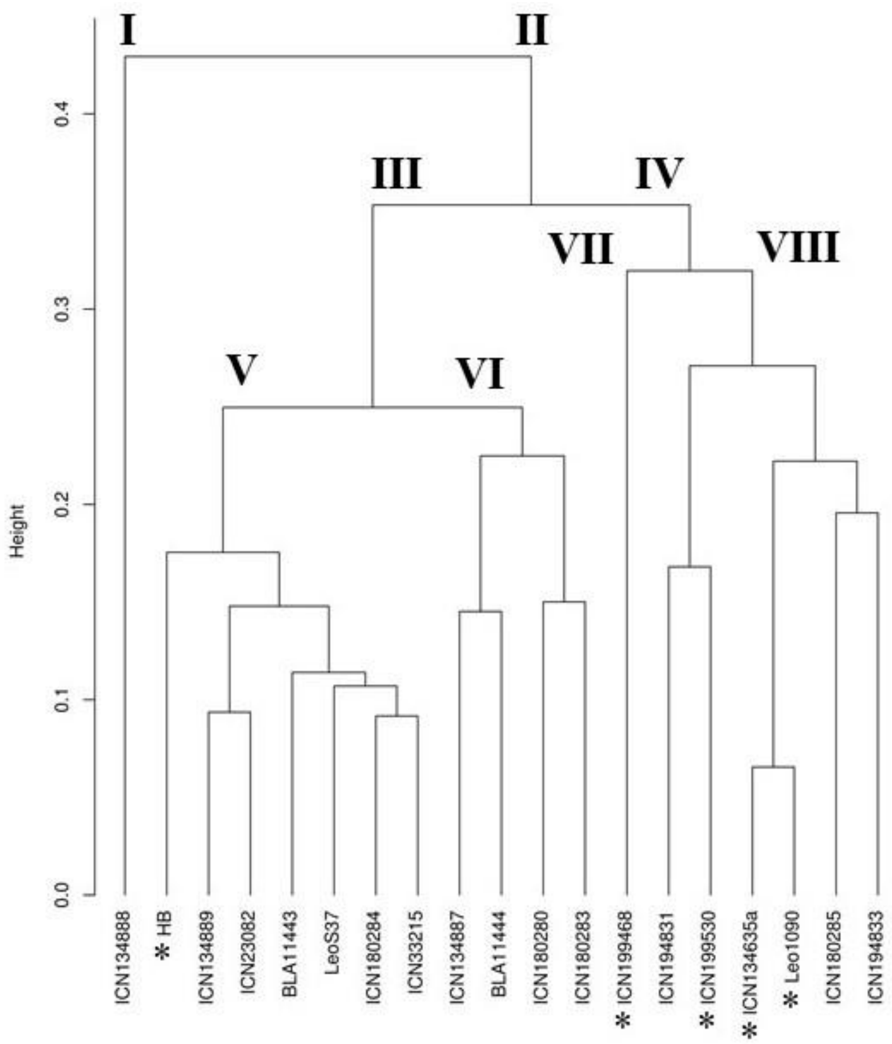

Figure 14. Cluster dendrogram of *Chascolytrum parodianum* based on six selected morphological characters: “lower glume length”, “spikelet width”, “length x width ratio of the upper glume”, “length x width ratio of the lemma in lateral view”, “palea length”, and “palea width". * indicates samples from Atlantic Forest Biome.

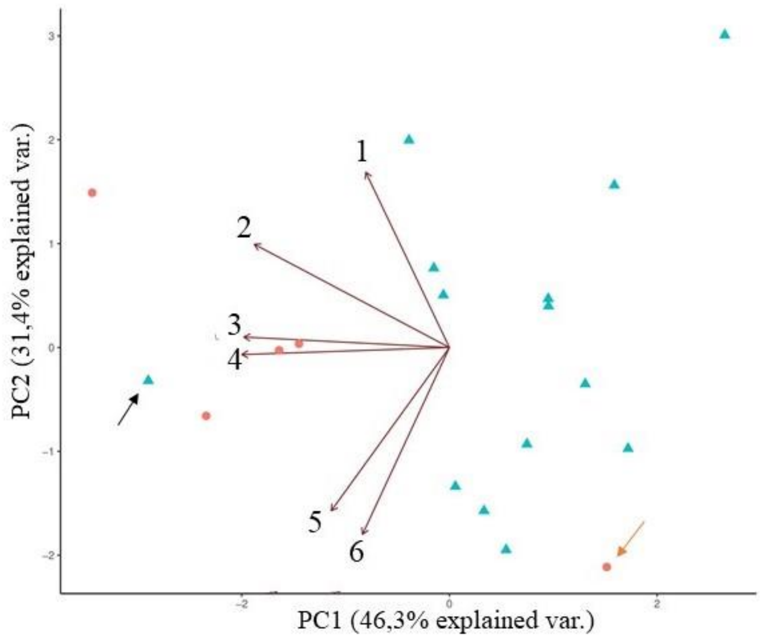

Figure 15. Principal component analysis (PCA) using six selected morphological characters and the projection of these variables on the first and second axis of PCA ordination. ▲ = *Chascolytrum parodianum*; ● = *C.* aff. *parodianum*; 1 = length x width ration of the lemma in lateral view; 2 = length x width ratio of the upper glume; 3 = lower glume length; 4 = spikelet width; 5 = palea length; 6 = palea width; → is indicating the sample "ICN194831" and → is indicating the sample "HB" (see Table 1).

3.1 Geographic distribution

In total, 24 collection points were available for analyses. Duplicates from same locality were not considered, totalizing 22 occurrences. During the field trips, five new populations were found: one from Uruguay (*da Silva 711*), and three from Brazil (*da Silva 834* e *da Silva 1080*; *HB; Leo S 37*). One record was a misidentification (*Windisch 6357*), and its identity was confirmed after fieldtrips to Morro Reuter, in Southern Brazil.

The updated geographic distribution of *C. parodianum* (Fig. 16) covers 120,066 km² (EOO), in both Pampa and Atlantic Forest Biomes, with elevations from 190 m to 920 m. The area of occupancy (AOO) comprises 72 km², with 11 sub-populations and 14 locations (Fig. 17), according the package ConR (Dauby *et al*., 2017).

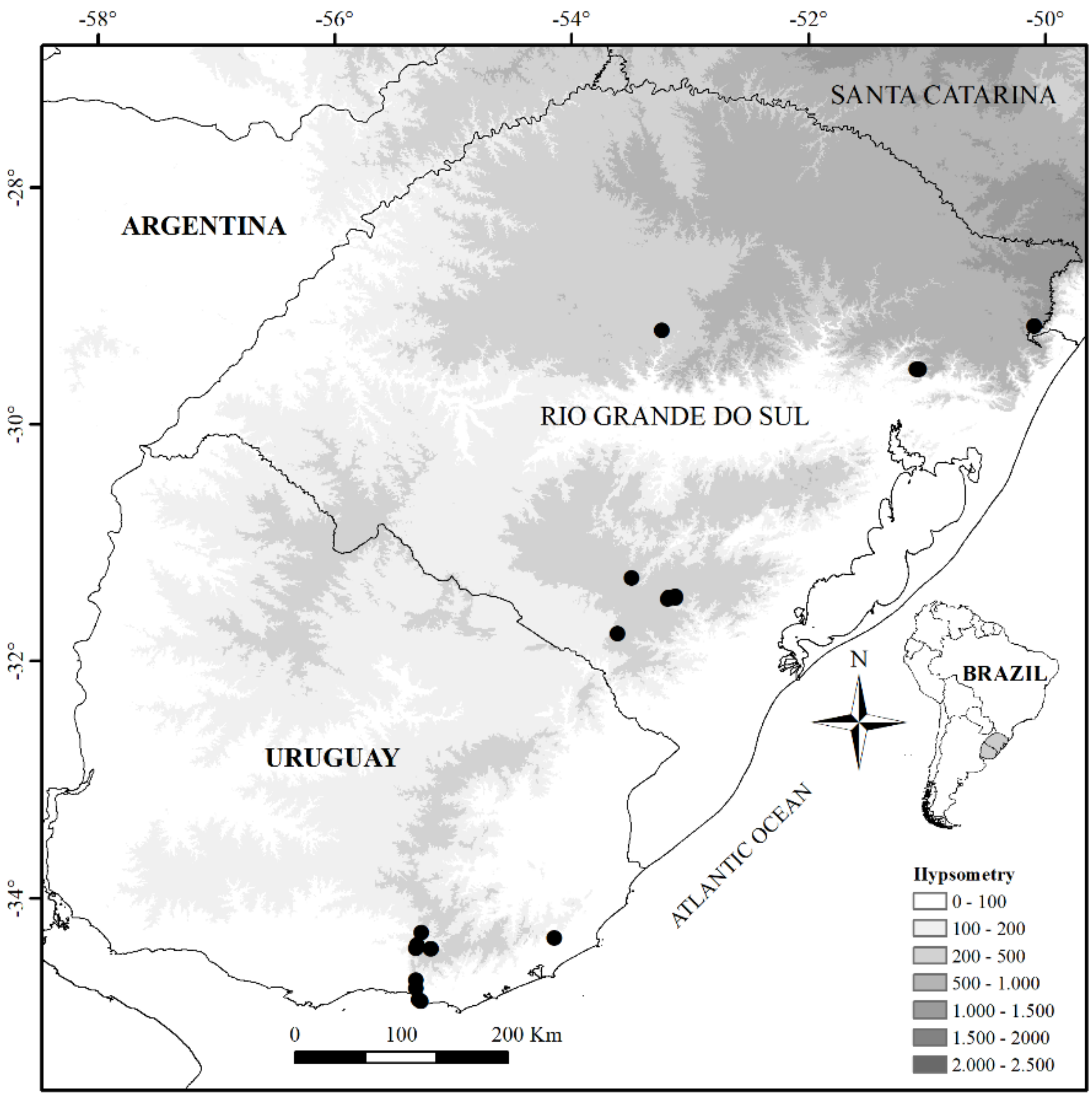

Figure 16. Updated map of the geographic distribution of *Chascolytrum parodianum*.

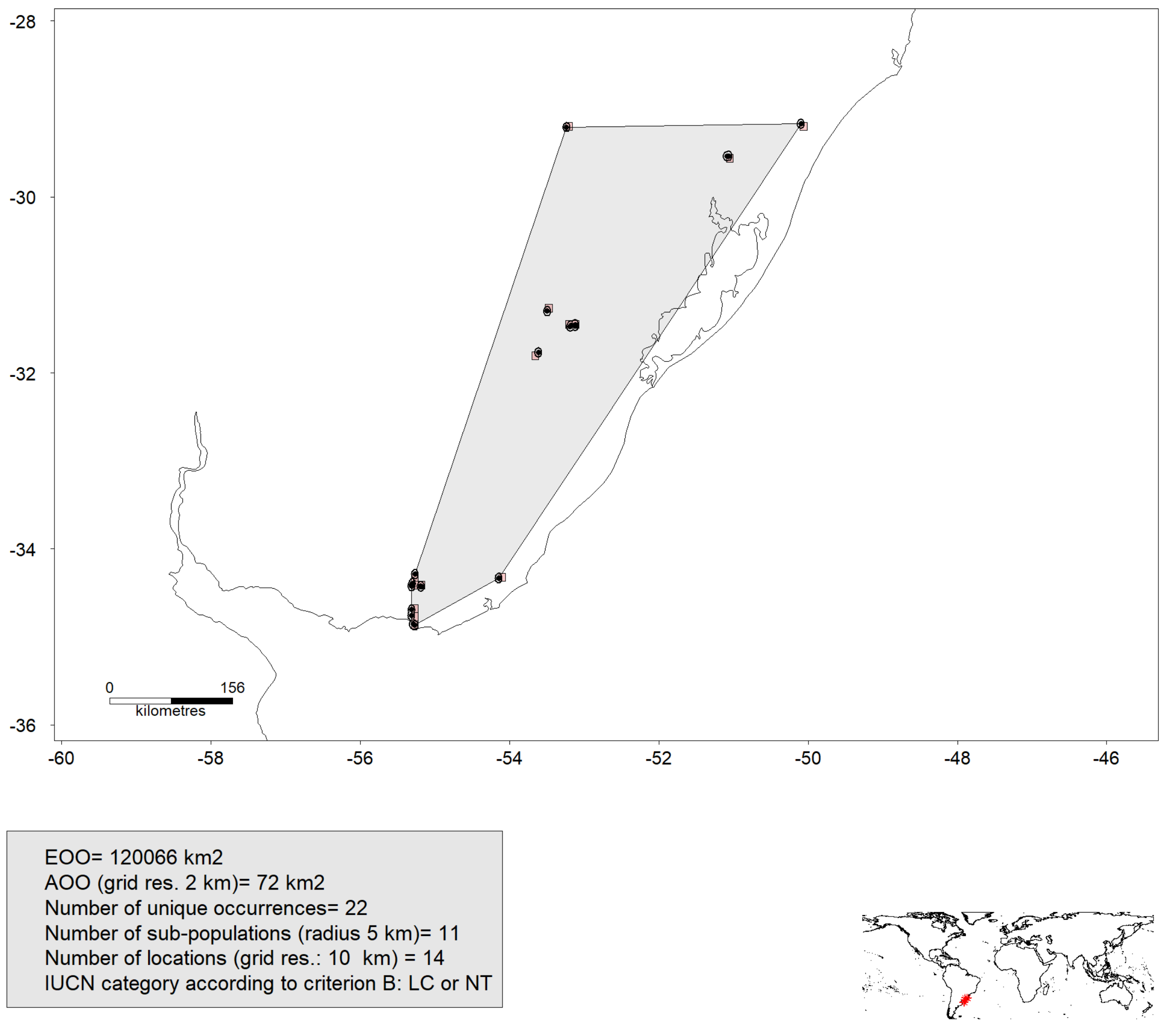

Fig. 17. Map for evaluation of *C. parodianum* conservation status according to criteria B of IUCN. EOO = Extension of occurrence (gray area on the map); AOO = Area of Occupancy.

4. Discussion

4.1 Morphological diversity of Chascolytrum parodianum

Recent revisions of *Chascolytrum* have shown considerable rearrangements, with species complexes being solved (Essi *et al.*, 2017) and ongoing works in this field (da Silva *et al.*, 2020). *Briza macrostachya* (Presl) Steud. and *Briza subaristata* Lam., treated as different taxonomic entities by Longhi-Wagner (1987), are now recognized as a single species, *Chascolytrum subaristatum* (Lam.) Desv. (Essi *et al.*, 2017), for example. When well-based, decisions like this avoid taxonomic inflation and its effects on conservation decisions (Isaac *et al.*, 2004). Our analyses had shown no statistically supported differentiation among the Atlantic

Forest and Pampa populations analyzed in this study, which represent only artificial groups. Thus, *Chascolytrum* aff. *parodianum* must be included into the morphological diversity of *C. parodianum*.

We here understand species as a lineage, according to a concept proposed by De Queiroz (2007), in which morphological differentiation appears as one of the criteria for determining boundaries between species. Morphometric approaches contribute for this criterion as a set of rigorous statistical tests (Ezrad *et al.*, 2010), assessing the relationship among the samples according to specimen placement in multivariate analyses and the variation of characters (Henderson, 2006).

However, our results show that a certain morphological variation among the groups can be found. Populations from Atlantic Forest showed a higher range of variability concerning some characters, such as “lower glume length” (Fig. 6) and “upper glume length” (Fig. 8) when compared to populations from Pampa. The only character that showed differentiation between these two groups, without overlapping in the box-plot, was the “length x width ratio of the upper glume” (Fig. 9). However, even with the Pampa specimens exhibiting higher variation in the “length x width ratio of the lower glume” (Fig. 7), it is interesting to notice that in general Atlantic Forest samples tend to have bigger glumes.

The character “Leaf length” also showed significant difference (Fig. 4), even though both groups have a considerable internal variation. “Leaf length” is considered a plastic character easily influenced by environmental conditions and in monocots environmental fluctuations play an important role in its development (Walter *et al.*, 2009). For *C. parodianum*, leaf variation probably reflects its now bigger range of distribution. Atlantic Forest samples also show a small variation of spikelet width (lesser than 0.25mm) that overlaps with the huge variation among the Pampa group (Fig. 5), among other examples. Even if we consider these two groups as different taxa, none character could virtually distinguish them considering all range and known populations.

Both HCA and PCA results did not support the recognition of two taxa (Fig. 14-15). All samples were mixed into clusters without any geographic structure. This variation can be considered an intrinsic variation of *C. parodianum* considering the expansion of its geographic distribution (Fig. 16), in which each population is susceptible to different conditions. However, the PCA ordination showed a better tendency on grouping (Fig. 15). Maybe the four samples from Atlantic Forest populations found in Cambará do Sul and Morro Reuter are representants of morphological extremes in a continuous distribution, since they came from the highest altitudes among the specimens (Table 1) and placed close in the PCA ordination (along with a specimen from Pampa Biome). Furthermore, the Mantel test, even with a non-significant P-value, showed a tendency for positive correlation between the characters and elevation above sea level. Altitude also differentiates them from the HB sample, also from Atlantic Forest Biome and found in an altitude level at least 400 m lower (Fig. 18). For non-contiguous populations and/or populations inhabiting very different types of habitat, it is expected to find a distinct ecotype pattern of variation (Walter & Briggs, 1997). HB is one of the recent discoveries, being the only record of the species inhabiting a rocky wall (Fig. 19). This sample presented the highest values of the "palea width" in our sampling, and its placing in PCA ordination reveals it has some particularities (Fig. 15). Further attention must be given to this population because, apparently, the rocky wall environment led to its isolation and then morphological differentiation.

Figure 18. Tussok of *Chascolytrum parodianum* inhabiting a rocky wall in Estrela Velha (Rio Grande do Sul, Brazil). (Photo: Henrique Mallmann Büneker).

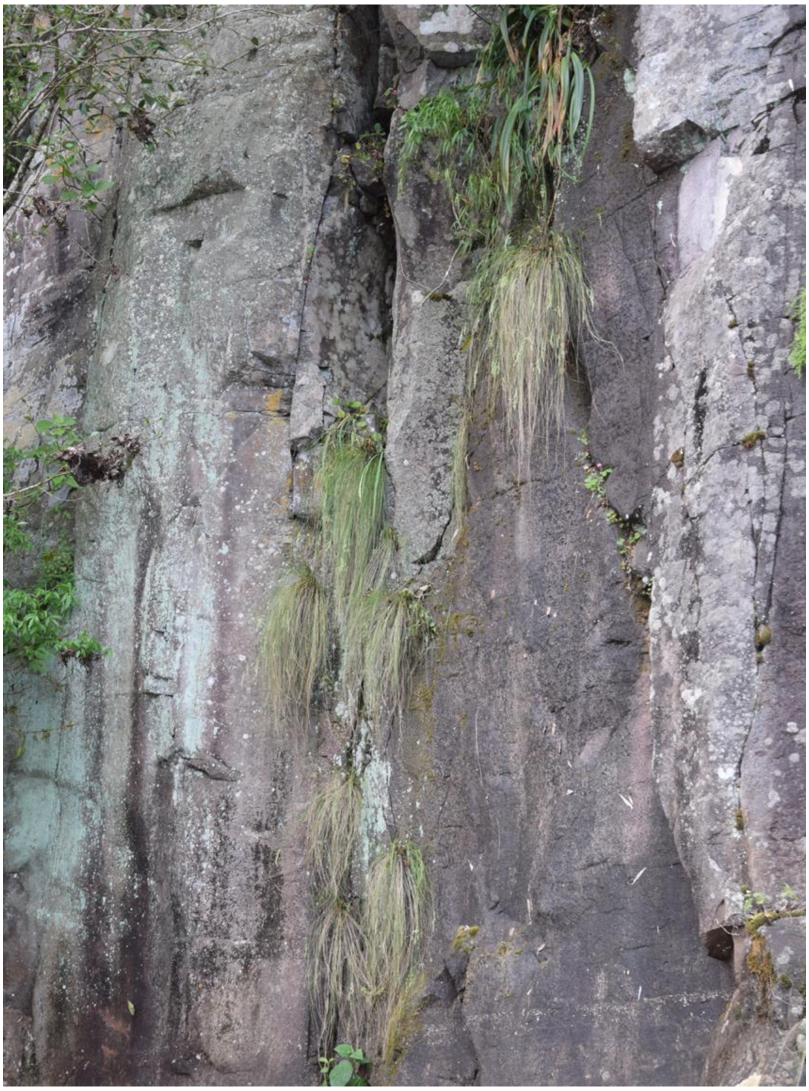

Figure 19. Tussoks of *Chascolytrum parodianum* inhabiting a rocky wall in Estrela Velha (Rio Grande do Sul, Brazil). (Photo: Henrique Mallmann Büneker).

4.2 Geographic distribution and habitat

Until recently, *Chascolytrum parodianum* was a poorly known species, due to scarce records. During a long time, only few populations were known, with a gap of occurrence between Southern Uruguay and Southern Rio Grande do Sul. Our new findings expanded significantly its geographical range, with several new populations being recorded in the past years. *C. parodianum* now has its occurrence confirmed more northerly than previously known, by Longhi-Wagner (1987) and Essi (2007), within the Atlantic Forest Biome. The most outstanding collection was at the border of the Itaimbezinho Canyon (Cambará do Sul), a region with highest altitude and geologically different from the granitic formations of Serra do

Sudeste, in the Pampa Biome, where the species was thought to be restrict. An abiotic factor that continues to influence its occurrence is rocky fields and gaps between boulders, since granite outcrops do not seem to be vital for the species establishment. The population from Estrela Velha found vegetating on steep rocky wall also extends its habitat range (Fig. 18-19). Based on the number of records, Northern Rio Grande do Sul seem to be the place with the most unknown populations, since three of the five new populations found in this study are from the Atlantic Forest Biome (Morro Reuter, Cambará do Sul and Estrela Velha).

4.3 Conservation status

Chascolytrum parodianum had its conservation status evaluated mainly based on its distribution, endemism and local threats (Filgueiras *et al.*, 2013; Marchesi *et al*, 2013; Red List RS, 2014). The species has not been collected in Uruguay since 1950, which was interpreted as local extinction of subpopulations (Essi *et al.*, 2017). However, recent populations were found in Cerro Aguirre (Rocha Department) and Parque Salus (Lavalleja Department) in 2016, which suggest that new populations still can be found. In Brazil, based on the IUCN Guidelines on Red Lists of Threatened Species (IUCN, 2001; IUCN, 2019), *C. parodianum* was considered Critically Endangered (CR) in both National and Rio Grande do Sul Red Lists (criteria B2ab(iii)). At that point, the conservation status was based on few collections from Pampa Biome (Essi, 2007), a region dominated by grasslands under serious threats due to the advance of forestry of *Acacia* P. Miller *s.l.*, *Eucalyptus* L'Hér. and *Pinus* L., as well as the lack of protected areas and conservation actions (Caporal & Boldrini, 2007; Overbeck *et al.*, 2007). We here make a reevaluation of *C. parodianum* conservation status based on its range, comprising Brazil and Uruguay. Similar to other plant species, data concerning population features and reproductive traits necessary for a more accurately estimate of the threat level are absent (Forgiarini *et al*., 2017). Besides distribution, it is known that: (1) four populations of Serra do Sudeste present a remarkable genetic diversity and are very likely to have an intricate dynamic involving migration and dispersal events (da Silva, 2016); (2) two populations (from Torrinhas and Morro Reuter) do not show apparent decrease, based on monitoring made on 2014 and 2018, respectively, and then again in 2019, with a new population found in Pedras Altas municipality; and (3) only one population is found inside a protected area, the Parque Nacional dos Aparados da Serra, in Cambará do Sul, Brazil, with few samples.

The area of occupancy (AOO) of 72 km² (grid resolution of 2 km) places *C. parodianum* into the Endangered (EN) category, while its extent occurrence (EOO) of 120,066 km² (Fig.

17), around six times the threshold for Vulnerable (VU), does not put the species into any category, being a nonpractical measure in this case, since populations of *C. parodianum* are highly disjoined with few individuals each, showing a fragmented distribution in 14 locations (grid resolution of 10 km²). As mentioned above, populations of *C. parodianum* in Serra do Sudeste seem to be stable, but a decrease in the quality of the habitat surrounding those populations caused by forestry and mining is visible and documented (Pilar *et al.,* 2009, Oliveira *et al.*, 2017) and due to its habitat specificity, *ex situ* conservation seems to be a difficult strategy (da Silva, 2016).

The range expansion of *C. parodianum* demonstrates that CR is a magnified placement. However, LC (least concern) or NT (not threatened), as results from package ConR (Dauby *et al*., 2017), also seem unrealistic regarding particularities of the species and the locations in which most of known populations occur. Hence, based on values of range, number of locations and habitat threats, we recommend that *C. parodianum* should be considered as Vulnerable (VU) according the criteria B2ab(iii).

5. Conclusion and future perspectives

We were able to confirm that *Chascolytrum parodianum* has a wider geographical range, and cannot be considered as restricted or endemic to Pampa Biome. The new collections presented here also expand significantly the number of potential occurrences, mainly in the gaps of its distribution. The dispersion events of the species need to be assessed through niche modelling techniques and phylogeographic approaches. This way, we can interpret if so-called isolated populations of *C. parodianum* are incipient, resultant of long distant dispersion; or represent relicts derived by vicariance processes, having a better understanding of the evolutionary dynamics of the species and Brazilian southern grasslands.

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