Systematics and Evolution of *Chresta* Vell. ex DC. (Vernonieae, Asteraceae)

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Systematics and Evolution of *Chresta* Vell. ex DC. (Vernonieae, Asteraceae)

Sistemática e Evolução de *Chresta* Vell. ex DC. (Vernonieae, Asteraceae)

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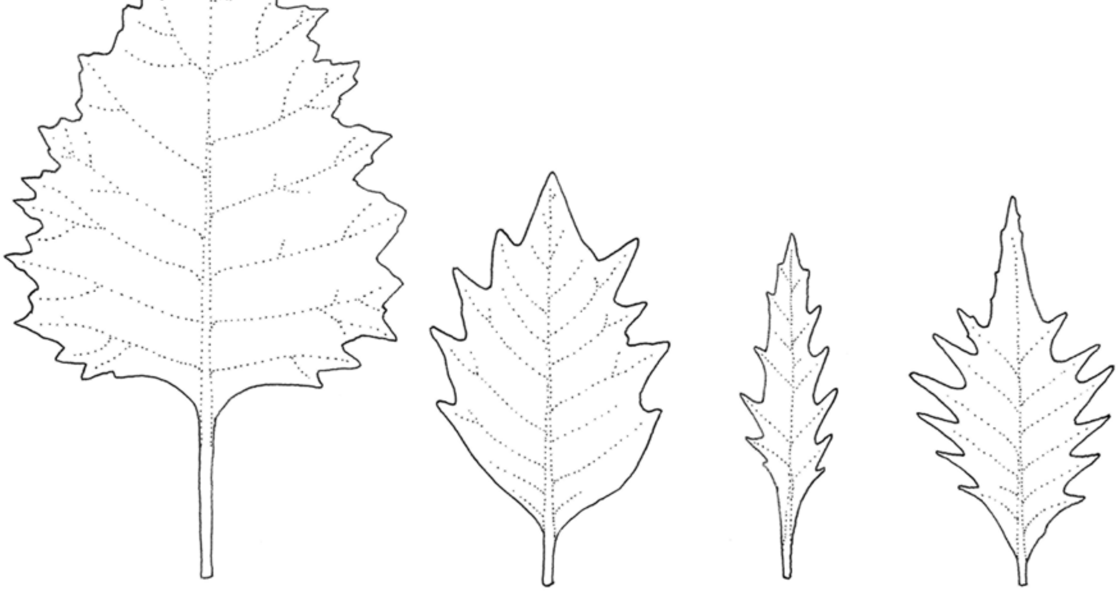
Aos meus pais, Cíntia e Roberto e à minha avó, Judith,
meus pilares.



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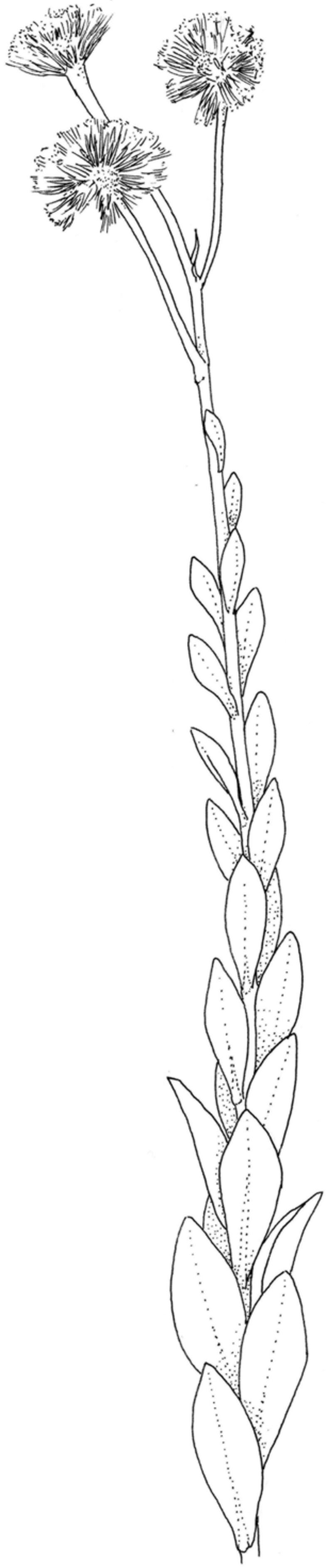
Magno amore in familiam
Synantherearum captus...

—Lessing, 1829



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RESUMO

Chresta pertence à tribo Vernonieae e apresenta dezoito espécies distribuídas nos domínios da Caatinga, Cerrado e Mata Atlântica. A extensa variabilidade de caracteres observada no gênero fez com que suas espécies fossem atribuídas a diversos gêneros distintos e até mesmo classificadas em diferentes grupos dentro da tribo. Tentativas anteriores de definir a posição do gênero em relação a outras subtribos de Vernonieae não foram bem-sucedidas. Nesse trabalho, apresentamos uma nova filogenia para *Chresta,* baseada numa amostragem taxonômica completa da qual obtivemos centenas de marcadores moleculares e, pela primeira vez, métodos filogenômicos são aplicados a um grupo neotropical de Asteraceae. Essa análise confirma a monofilia de *Chresta,* resolve as relações infragenéricas com alto suporte, e define com sucesso o grupo-irmão do gênero, embora as relações dessa linhagem com Lychnophorinae e Lepidaploinae ainda sejam duvidosas. Usamos as árvores obtidas para reconstruir estados ancestrais de dez caracteres morfológicos selecionados face a sua provável relevância na história do grupo, e assim propomos um cenário biogeográfico no qual a diversificação do grupo pode ter ocorrido. Também usamos marcadores de microssatélites para estudar a genética de populações de cinco espécies da Caatinga que apresentam populações naturalmente isoladas, devido a sua restrição a afloramentos rochosos de área limitada. Essas espécies apresentam diferentes padrões, variando no grau de diversidade e estruturação genética. Esses dados permitem uma discussão do impacto relativo de fatores como distância espacial, preferência de substrato, tamanho populacional e capacidade de dispersão, sobre o fluxo gênico entre as populações analisadas. Também apresentamos uma sinopse ilustrada do gênero, com uma nova classificação infra-genérica filogenética, condensando o conhecimento acumulado até o momento sobre *Chresta.* A sinopse inclui descrição de quatro espécies novas, chave de identificação das 18 espécies aceitas e três táxons infragenéricos, sinonímia completa, ilustrações e mapas de distribuição das espécies, bem como seu status de conservação atual.

ABSTRACT

Chresta belongs to tribe Vernonieae, subtribe Chrestinae, and presents eighteen species distributed in the Caatinga, Cerrado and Mata Atlântica domains. Its species display a fairly wide range of characters that led the species to be ascribed to several distinct genera, sometimes also classified into different groups inside the tribe. Attempts to define the position of the genus in relation to other Vernonieae subtribes and to understand the relationships within the genus have so far been unsuccessful. In this work, we present a novel phylogeny for *Chresta,* based on a complete set of sampled taxa from which hundreds of molecular markers were assessed, and for the first time, phylogenomics methods are applied to a Neotropical group of Asteraceae. We confirm the monophyly of *Chresta* and resolve infrageneric relationships with high support for all clades. We also successfully define the sister group to the genus, although the relations of this whole lineage with Lychnophorinae and Lepidaploinae are still doubtful, and. We use the obtained trees to reconstruct ancestral states of ten selected characters, which seem to have played an important role during the history of the genus, and propose a biogeographical scenario where the diversification of the group may have taken place. We also use evidence from microsatellite markers to study the population genetics of five species from the Caatinga that naturally present isolated populations restricted to narrow rupiculous habitats. These species present contrasting patterns, varying in their degree of genetic diversity and structuring, and this data enable us to discuss the relative impact of factors such as spatial distance, substrate preference, population size and dispersal ability on the genetic flow among the populations assessed. We also present an illustrated synopsis of the genus, with a new phylogenetic infrageneric classification, summarizing the accumulated knowledge about *Chresta* so far. We provide descriptions of four new species, a key for identification of 18 accepted species and three infrageneric taxa, synonyms, illustrations and distribution maps of the species, as well as their current conservation status.

GENERAL INTRODUCTION

Asteraceae (or Compositae) is one of the largest families of Angiosperms, with around 24,000 species and ca. 1700 genera (~10% of all spermatophytes), distributed in the whole world, except for Antarctica (Funk et al. 2009). Peculiar characters of the family, such as flowers organized in capitula, the fused anthers, the secondary pollen presentation and fruits of the type cypsela with pappus, have contributed to it being acknowledged as natural group since the Middle Age (Small 1919); its monophyly was posteriorly confirmed in several molecular and morphologic phylogenetic analysis (e.g. Jansen & Palmer 1987, Lundberg & Bremer 2003; Keeley & Robinson 2009). Around 2000 species are found in Brazil, 65% of which are endemic to this country (Brazil Flora Group 2015).

The family is currently subdivided in 12 subfamilies and 28 tribes (Panero & Funk 2002), with Vernonieae being one of the largest tribes, with ~129 genera e ~1100 espécies (Keeley & Robinson 2009). Vernonieae was initially delimited by Cassini (1816), based on several characters, including the dorsally curved style arms that are common in the group (Cassini 1819), with the general delimitation being sparsely changed since then. The monophyly of Vernonieae was corroborated in several phylogenetic analyses (e.g. Keeley & Jansen 1991, 1994; Kim & Jansen 1995; Kim et al. 1998; Keeley et al. 2007) and the tribe is currently placed in subfamily Cichorioideae. Recently analysis using large genomic datasets, however, put the monophyly of Cichorioideae in doubt (Mandel et al. 2017), indicating that relationships in that part of the family might not be as stable as it was once thought.

Besides the presence of thin styles, with filiform and pilose arms, other important features of tribe Vernonieae are the variety of habits, from herbs to trees, the phylotaxy that is usually alternate and the discoid, homogamous capitula that usually present purple, lilac or white florets (Keeley & Robinson 2009). One remarkable feature is the widespread presence of secondary heads (i.e. syncephalia) in the tribe, which occur in ca. 14 genera, mostly from the New World (Loeuille et al. 2015a); this character has been historically used to further divide the tribe into subtribes (e.g. Baker 1873). Vernonieae is found in the American continent, in Africa and Asia, and the main diversity centers are Brazil and Africa. Members of the tribe show a peculiar tolerance for soils with unusual composition, such as those with high contents of iron, bauxite and dolomite (Keeley & Robinson 2009). In Brazil, the tribe is especially representative in the Cerrado phytogeographical domain, where many species occupy the *campos rupestres*, an herbaceous and shrubby type of vegetation found in high elevations, in sandy and rocky soils (Loeuille et al 2015a, b).

Among the Brazilian taxa is *Chresta,* composed by 18 species of herbs, subshrubs and shrubs distributed in the Caatinga, Cerrado and Atlantic Forest. Species of the genus are frequently found in rock outcrops and other types of dry and rocky soils. Some of the features that define *Chresta* are the presence of syncephalia, which can have different morphologies, the indument composed of T-shaped trichomes, the non-sclerified cells on the anther appendages, pappus composed of several series of setose elements and a variety of underground systems.

The first phylogenetic studies in Vernonieae were mainly focused on the monophyly and composition of the mega-genus *Vernonia*, which grouped ca. 1000 species, until its dismemberment after several taxonomic (several treatments by Robinson, but specially 1999a, b, c) and phylogenetic works (Kim & Jansen 1995, Keeley& Turner 1990, Keeley et al. 2007), resulting in many monotypic and small genera and a new circumscription for *Vernonia,* containing ca. 20 species (Keeley &

Robinson 2009). More recently, phylogenetic studies were used to investigate the origin of syncephaly in the American taxa of tribe (Loeuille et al. 2015a) and also to understand the relationships within Lychnophorinae, one of the most representative subtribes in Brazil (Loeuille et al. 2015b). These more recent study helped clarify many of the relationships within South American taxa, but still left some unresolved issues, mainly due to poor resolution and support.

Chresta was sampled in two previous phylogenies (Keeley et al. 2007, Loeuille et al. 2015a), which showed the genus to be monophyletic, but placed doubt about its relationships with other Vernonieae subtribe, mainly Lychnophorinae and Vernoniinae. The relationships within the genus showed the presence of two clades, one with Caatinga species and other with Cerrado species, but the internal resolution was not enough to draw conclusions about morphological evolution within the genus.

Molecular phylogenetic studies in Asteraceae have been challenging, mainly due to the abundant polyploidization in the family, both ancient and current, what makes the identification of orthologous nuclear markers difficult (Badouin et al. 2017, Mandel et al. 2017). Besides, the chloroplast genome (plastome) in the family has been shown to be highly conserved at the sequence level, reducing its usefulness in studies of closely related taxa (Shaw et al. 2005, Timmee et al. 2007).

The advances on sequencing techniques in the last two decades (Mardis 2008), with the development of parallel, high-throughput sequencing (Delseny et al. 2010), has been seen as promising to tackle these types of issues (Bräutigam & Gowik 2010), using different approaches, like shotgun sequencing, RADseq (Davey & Blaxter 2010), and Hyb-Seq (Weitemeier et al. 2014) for different types of data. Methods that combine targeting of specific genomic regions with large-scale sequencing are the most promising methods for phylogenetic studies, as they allow the sequencing of hundreds of markers at once and use DNA as source, instead of RNA, as in transcriptome sequencing. On the other hand, they require previous information about the regions of interest, such as a reference genome or EST database. A set of probes based on sunflower ESTs has been developed to be used across the Asteraceae (Mandel et al. 2014), with promising results (Mandel et al. 2015, 2017).

Whereas phylogenies allow the study of the genus as a whole, having species as terminals of the analysis, when this morphological variation is observed among members of a same species, studies focusing on population genetics and phylogeography are more informative. Field observations and study of herbarium collections have evidenced interesting cases of infraspecific variation in the group of *Chresta* species from the Brazilian Caatinga.

Chresta artemisiifolia, C. harleyi H.Rob., *C. hatschbachii* H.Rob. and *C. subverticillata* all occur in a short stretch of Espinhaço Septentrional and Chapada Diamantina (Alkmim 2012), where they form distinct populations delimited by the rock outcrops where they occur. These four species are morphologically similar, what raises interesting questions about the patterns of occupation and speciation in fragmentary rocky environments in the Caatinga.

Chresta martii (DC.) H.Rob. has a wider distribution, forming three geographic clusters around the Northern portion of Rio São Francisco. The geographic clusters are very isolated from each other, and there are some morphological differences between populations in each one of them, with plants on the western portion of the distribution being robust, profusely ramified, with wide leaves, while the plants on the eastern end are smaller, with less ramifications and more delicate, narrower leaves. This geographical separation associated to morphological differentiation is an interesting setting to study effects of gene flow and reproductive isolation in natural populations.

This has implications for conservation, as small, isolated populations often show strong population structuring, with little genetic variation among members of a given population, but with some variation among members of different populations (Gitzendanner & Soltis 2000). Regarding *Chresta* species from the Caatinga, the majority of their populations are outside of conservation units, sometimes presenting scarce populations, each with a restricted number of individuals, practically isolated from other populations of the same species, posing a challenge to their conservation.

Phylogenomics datasets have been used in the past few years as a source for microsatellite mining, as they usually present large portions of coding and non-coding portions of the genome, facilitating the process of developing and validating these markers (Jennings et al. 2011). Computational resources can be used to identify putative repetitive regions, even allowing the comparison with homologous sequences from other species, thus contributing to the development of microsatellite markers transferable among different species, which is an advance to studies comparing multiple species. Even though the advances in sequencing have brought over new methods to study populations, like RAD-Seq and Pool-Seq (Schlötterer et al. 2014), microsatellites are still widely used, proving to be valuable resources in several types of population studies (Hodel et al. 2016).

In face of the accumulated knowledge about *Chresta*, and with the possibility of applying new methods and new approaches to study the relationships of the genus with other Vernonieae subtribes, as well as the intraspecific relations within the genus, this work proposed to study the systematics and evolution of *Chresta* and summarize the current knowledge about its species. The results of each study are organized in the following chapters:

Chapter 1 presents a new phylogenetic hypothesis for *Chresta,* reevaluating the relationships within Vernonieae, focusing on other representative Brazilian taxa. The target capture method developed by Mandel et al. (2014) was used to obtain a molecular matrix composed by ca. 700 nuclear markers, which provided complete resolution and good support for almost all nodes. The resulting tree was used to study morphological evolution in the genus, through mapping of different character states. The phylogeny was also used to propose a biogeographical scenario explaining the occupation of the Caatinga and Cerrado by the genus. This part of the study was conducted in partnership with the Mandel Lab at the University of Memphis, TN, USA, and will be co-authored by Dr. Benoit Loeuille, Dr. Jennifer Mandel and Dr. José R. Pirani.

Chapter 2 is divided in two parts; Part A contains the process of developing and validating microsatellite markers transferable among species of *Chresta* from the Caatinga, which will be co-authored by Dr. José R. Pirani and Dr. Jennifer Mandel. Part B presents a population genetics study of five Caatinga species, focusing on genetic diversity and structuring among populations of these species, aiming to understand what processes are at play on the speciation in this group. This part will be co-authored by Dr. Benoit Loeuille, Dr. José R. Pirani and Dr. Jennifer Mandel. This chapter is also a product of the partnership with the University of Memphis.

Chapter 3 summarizes all the taxonomic and systematics knowledge of the genus in a synopsis, also proposing a new infra-generic classification. The synopsis is also an attempt to standardize the nomenclature within the genus, as no comprehensive treatments have been published since the 1980s, when it was split into five different taxa, and because new species and synonyms were published in the last two decades. This chapter also presents illustrations of species that were not previously illustrated, as well as maps and photographs, and also evaluation of conservation status for all species. It will be co-authored by Marcelo T. Kubo, Dr. Benoit Loeuille and Dr. José R. Pirani.

Chapter 4 is divided in three parts and presents four new species of *Chresta.* Part A contains a paper already published in the period *Phytotaxa* (Siniscalchi et al. 2016), describing *C. filicifolia,* the only species endemic to the Atlantic Forest in the genus. Part B contains a work that is already submitted at *Systematic Botany*, containing two new species of the Caatinga. Part C contains another paper that will be submitted to *Phytotaxa*, describing a new species from Bahia, fruit of a partnership with researchers from the herbarium HVASF, in Petrolina, Pernambuco.

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Chapter 1

Phylogenomics of Vernonieae sheds light on morphological evolution of *Chresta* (Asteraceae: Vernonieae) and relationships of South American subtribes

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Phylogenomics of Vernonieae sheds light on morphological evolution of *Chresta* (Asteraceae: Vernonieae) and relationships of South American subtribes

Abstract

Chresta has 18 species distributed in the Brazilian Cerrado and Caatinga, presenting striking morphological and ecological traits, which seem to constitute a turning point within some lineages in Vernonieae, as *Chresta* presents a combination of characters like secondary heads, common in Lychnophorinae, with pollen type C, usually found in some Vernoniinae. Previous phylogenetic studies of the genus sampled few species and did not provide enough resolution and support to define its sister group neither for a proper assessment of this morphological diversity in an evolutionary framework. In this study, we used recently developed HybSeq methods in order to resolve the relationships within *Chresta* species and to define its sister group and its position within Vernonieae. We used ~700 nuclear markers obtained from 60 terminals of *Chresta* and related taxa in Vernonieae aiming to get fully resolved and well-supported trees. We also analyzed a smaller subset of markers to understand the effect of missing data on the analysis. *Chresta* is strongly supported as a monophyletic genus, and its sister group is consistently recovered as a clade formed by North, Central and South American genera of Vernoniinae, while the relative position of *Chresta* in relation to Lychnophorinae and Lepidaploinae remains doubtful, probably due to insufficient sampling within these subtribes. Most clades obtained within *Chresta* are also strongly supported, and this allowed us to discuss the evolution of their morphological features in relation to ecological and environmental requirements, and to propose a biogeographical scenario in which this lineage may have diversified.

Keywords: Compositae, Chrestinae, phylogeny, Caatinga, Cerrado.

Introduction

Vernonieae is one of the most representative tribes of Asteraceae in Brazil, especially in the drier physiognomies of Central Brazil. Among the ca. 52 genera of the Vernonieae that are found in Brazil is *Chresta* Vell. ex DC., with 18 species (BFG 2015). Due to the presence of secondary heads (or syncephalium, i.e. capitula aggregated into second order capitula) in all species of the genus, *Chresta* has long been associated to *Eremanthus* Less. and some other genera usually placed on subtribe Lychnophorinae (Robinson 1980, 1999a).

The taxonomic history of the genus is rather complex. The generic name *Chresta* was proposed by Velloso (1831) in *Flora fluminensis*, including only plates of two eastern Brazilian species (*C. cordata* Vell. and *C. lanceolata* Vell.). Subsequently, a formal description of the genus was presented by Candolle (1836), who also added two new species. The latter author also transferred some *Vernonia* species described by Lessing (1831) to the genus *Pycnocephalum* (Less.) DC., whose main differences from *Chresta* are the synflorescence (scapose vs. branched), the shape of the involucre and the presence of secondary bracts on the syncephalia. Candolle (1836) also described two other genera containing herbaceous plants with secondary heads: *Pithecoseris* Mart. ex DC. and *Stachyanthus* DC. [= *Argyrovernonia* MacLeish], both restricted to the Brazilian Caatinga and presenting deeply lobed leaves.

Gardner (1842, 1846) merged *Chresta* under *Pycnocephalum*, pointing out that these differences did not reflect two natural groups, and described more species. In the Compositae monograph for *Flora Brasiliensis,* Baker (1873) grouped several genera with secondary heads in *Eremanthus,* including all species previously described as *Chresta*, *Pycnocephalum* and *Stachyanthus.* Some new species placed in *Eremanthus* were described after that, and in 1947, the genus *Glaziovianthus,* containing one species with red flowers and secondary heads, clearly related to this group, was described by Barroso (1947).

No taxonomic treatments for these genera appeared for more than a hundred years, until several works were published in the 1980s. In a series of articles, Robinson (1980, 1992) resurrected *Chresta* and placed on it herbaceous species that were previously included on *Eremanthus.* In the same period, MacLeish (1984a) concluded a taxonomic reevaluation of *Eremanthus*, publishing revisions for *Chresta, Glaziovianthus* and *Pycnocephalum* as separate entities (MacLeish 1985a, b), and also proposing *Argyrovernonia* MacLeish as a new name for *Stachyanthus,* because this is a later homonym of a genus in Icacinaceae (MacLeish 1984b).

In the meantime, *Chresta* continued placed in Lychnophorinae. In 1992, Robinson removed *Chresta* and *Soaresia* from Lychnophorinae, due to secondary chemistry (presence of glaucolides sesquiterpene lactones instead of heliangolides typical of Lychnophorinae), pollen (weakly lophate with continuous perforate tectum, type A, in Lychnophorinae vs. lophate, type C, in *Chresta*) and anther appendage morphology (sclerified in Lychnophorinae vs. non-sclerified in *Chresta*). In 1999, Robinson creates subtribe Chrestinae and transfers *Chresta, Pithecoseris* and *Soaresia* to it, based on the characters mentioned above (Robinson 1999a). Robinson also pointed out that Chrestinae could represent a divergence point between the Lychnophorinae and Vernoniinae, because despite presenting secondary heads typically related to Lychnophorinae, pollen and anther characters align the species in Chrestinae with Vernoniinae, which lack secondary heads. More recently, *Pithecoseris* was synonymized in *Chresta,* based on the herbaceous habit, lobed leaves, floret and secondary head morphology (Loeuille et al. 2014).

The first phylogenetic approach to look into Vernonieae in detail was published by Keeley et al. (2007), using two chloroplast regions (*ndh*F and *trn*L-F) and ribosomal ITS, but this work contains only one terminal of Chrestinae: *Chresta sphaerocephala* DC. This species emerged as sister to *Heterocypsela andersonii* H.Rob., although with low support, and in the parsimony analysis they are both sister to a clade containing two genera from Costa Rica and Venezuela, *Eirmocephala* H.Rob. and *Tephrothamnus* Sch.Bip., also with low support. This small clade is sister to *Vernonia* Schreb. and *Vernonanthura* H.Rob., and the Lychnophorinae appears as sister to this larger group, thus suggesting that Robinson's (1992) decision to remove *Chresta* from Lychnophorinae is supported by the molecular data as well.

In 2015, Loeuille et al. published an in-depth phylogeny of the American Vernonieae, focusing on the evolution of secondary heads on the group. This work used ITS, two chloroplast regions (*ndh*F and *trn*L-F), and a morphological matrix, including ten *Chresta* species, containing taxa from the four genera segregated by MacLeish (1984a, b, 1985a, b). Loeuille et al. (2015a) did not sample *Heterocypsela,* and *Stokesia* L'Hér., a monotypic genus from the Southern United States, emerges as sister group to *Chresta* in the parsimony analysis, without support and with a very long branch length. In this work, *Chresta* appears as monophyletic and composed of two clades: one with two species from the Caatinga, including the only species from *Pithecoseris*, and one Cerrado clade, which presents multiple polytomies. *Chresta* plus *Stokesia* emerge as sister to a larger clade formed by *Vernonia* and

Vernonanthura and the Lychnophorinae, but with low statistical support. Also, the composition of Chrestinae as proposed by Robinson (1992, 1999a), is shown as non-monophyletic, as *Soaresia velutina* Sch.Bip. groups with genera from Elephantopinae.

Although these two phylogenetic studies did not focus on *Chresta*, they both confirmed its monophyly and contributed partially to our understanding of the positon of the genus in the Vernonieae. Furthermore, the work by Loeuille et al. (2015a) recovered no support for the taxa segregated by MacLeish (1985a, b).

In its present circumscription, *Chresta* exhibits impressive morphological variation across its species (Figure 1). The genus encompasses plants with different habits, varying from biannual herbs to perennial shrubs and rosette herbs, three pollen types, *Vernonia* types A, C and F (Siniscalchi et al. 2017), four types of sweeping hairs on the style arms, distinct syncephalia (secondary head) morphologies (hemispherical with loosely joined capitula and spherical with tightly joined capitula) and growth patterns (determinate versus indeterminate), as well as differences in floret color (purple versus red).

This morphological diversity may reflect an intriguing evolutionary history, but without a better supported and resolved tree it is not possible to study this diversity in an evolutionary context. Although the work by Loeuille et al. (2015a) contained more than half of *Chresta*'s species, the resolution within the genus is low, with polytomies and low support, making the internal relationships doubtful. Also, the backbone of their phylogeny was unresolved, and without knowing which is the sister group to the genus it is not possible to reconstruct its evolutionary history with confidence.

Molecular studies in the Compositae family always have presented a challenge for scientists, as there is abundant polyploidization, both ancient and current, making the identification of orthologous nuclear markers challenging (Badouin et al. 2017, Mandel et al. 2017). The chloroplast genome (plastome) in the family has been shown to be highly conserved at the sequence level, reducing its usefulness in studies of closely related taxa (Shaw et al. 2005, Timmee et al. 2007).

More recently, the development of new sequencing methods provided new ways to tackle these issues, with one of the most promising being target enrichment or hybrid sequencing (HybSeq) (Weitemeier et al. 2014). In this approach, previously selected genomic regions are enriched during wet lab preparations, usually through hybridization with RNA probes, and the resulting enriched libraries are sequenced using a high-throughput method. This allows the sequencing of thousands of markers at once, including single or low copy nuclear genes. This method has been applied in Compositae since 2014, with promising results (Mandel et al. 2014, 2015, 2017), and constitutes the foundation for the present work.

Based on the hitherto known information about the phylogeny of *Chresta* and focusing on resolving some of the pending issues presented above, we carried out a phylogenetic study of *Chresta*, based on genomic methods, in order to: 1) understand the relationships within the genus, through analysis of molecular data from a virtually complete taxonomic sampling; 2) define the sister group to the genus; 3) understand the relationships of *Chresta* with other groups within Vernonieae, especially Lychnophorinae; and 4) use the phylogeny as a basis to address the morphological diversity in the group and its major evolutionary pathways, including ancestral area reconstructions. We also hope that this study will contribute to a better understanding of patterns of structural diversification in herbaceous and subshrubby lineages of the Cerrado and Caatinga biotas.

Outgroup choice and taxon sampling:

As Liabeae and Moquinieae have been shown to be the sister groups to Vernonieae in previous works (Keeley & Robinson 2009, Loeuille et al. 2015a), we chose as outgroups one taxon from Liabeae, *Munnozia gigantea,* and the only two representatives from tribe Moquinieae (*Moquinia racemosa* and *Pseudostifftia kingii*). As ingroup, 56 species representing 29 different genera from Vernonieae were included (4% of the species in the tribe). Taxa from 12 subtribes (from the 21 defined by Keeley and Robinson 2009) were included, of which nine occur in South America and three are distributed in Africa/Asia. The sampling was focused on groups that showed an uncertain relationship with *Chresta,* based on previous studies, especially Lychnophorinae, with 21 terminals (Keeley et al. 2007, Loeuille et al. 2015a). Within *Chresta,* 17 of the 18 recognized species were sampled, including all taxa that were previously placed on *Argyrovernonia*, *Glaziovianthus*, *Pithecoseris* and *Pycnocephalum.* The only taxon of *Chresta* we could not sample was *C. pinnatifida* (Philipson) H.Rob., because no collections were made since the 1880s and, although we visited the locality of the type specimen listed on Glaziou's memoirs (1909), the species was not found. Extraction from herbarium collections of this species was not attempted due to the old age of the materials and type specimen conditions. A list of sampled species and herbarium vouchers is presented in Table 1.

DNA extraction and sequencing:

Leaf samples were collected from live plants in the field and preserved on silica gel. Dried leaves were ground using a GenoGrinder 3000 (Spex® Sample Prep) and total DNA was extracted using E.Z.N.A.® SQ Plant DNA Kit from Omega Biotek, with addition of PVP and ascorbic acid to the first extraction buffer (10 mL SQ1 buffer, 100 mg PVP, 90 mg ascorbic acid). When necessary, the extracted DNA was cleaned with the E.Z.N.A.® Cycle Pure Kit from Omega Biotek to increase purity. Extracted samples were quantified using fluorometry (Qubit 3.0, ThermoFisher Scientific), diluted as necessary and sheared to a target size of 300 bp using a sonicator (Covaris S series or QSonica Q500), using 3.5 minutes of sonication, in 10-second intervals, at 20% amplitude as basis and doing additional cycles in case of suboptimal results on the first try. DNA fragmentation was verified through electrophoresis in 1% agarose gel.

The NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs Inc.) was used to prepare libraries, according to the manufacturer's instructions, using 15 cycles on the last amplification step. Final library concentrations and sizes were checked using Qubit and gel electrophoresis. Libraries were pooled in groups of four and target capture was performed using the MYbaits COS: Compositae/Asteraceae kit (MYbaits_Comp-COS-1Kv1, MYcroarray), using a 36-hour incubation time and 15 cycles on the last amplification step. Details on the targets and method can be found in Mandel et al. (2014). Quality checking with a Bioanalyzer instrument and sequencing were carried out at Macrogen Inc. (South Korea), in an Illumina HiSeq2500 device, in paired-end, high-throughput mode.

Sequence assembly and mapping:

Trimming of Illumina adaptors was carried out using Trimmomatic (Bolger et al. 2014) and reads were assembled into contigs using SPAdes (Bankevich et al. 2012), with kmer lengths of 21, 33, 55, 77, 99 and 127. The sequences were matched back to the original probes using the phyluce pipeline (Faircloth 2016), which generated individual alignments for each one of the original targeted regions. These alignments were then concatenated to generate two different matrices for phylogenetic analysis: one containing all loci recovered for all taxa (herewith called total matrix) and another containing only loci that were recovered for at least 75% of the taxa (called 75% matrix).

Phylogenetic analysis:

All analyses described were carried out with both matrices, total and 75%, containing invariable characters, using *Munnozia gigantea* as outgroup. The resulting trees are referred as total tree and 75% tree throughout the results and discussion. For parsimony analysis, the software TNT v.1.5 was used (Goloboff & Catalano 2016), using heuristic search with TBR swapping algorithm, for 1000 replicates, with ten trees hold at each replicate. Jackknife was calculated using 1000 replicates, with 100 search replications and 10 trees held in memory. Support was classified as high (95-100%), moderate (85%-94%) and low (> 84%).

Molecular evolution models were evaluated in jModelTest2 (Guindon & Gascuel 2003; Darriba et al. 2012), using the corrected Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC) to choose between models. The chosen model was GTR+I+G for both matrices and both information criteria. Maximum likelihood (ML) analyses were run on RAxML (Stamakis 2014) in the rapid bootstrapping mode, always using 1000 bootstraps and 25 threads.

The multi-species pseudocoalescence model was evaluated in Astral II (Mirarab et al. 2015), using gene trees generated from the individual locus matrices. Individual evolution models for each gene matrix were obtained with PartitionFinder v.1.1.0, in the RAxML version with rcluster search option and AICc criterion, with unlinked branch lengths (Stamakis 2006; Lanfear et al. 2012; Lanfear et al. 2014). Gene trees were obtained in RAxML, with 100 bootstraps for each matrix. Two different species trees were obtained from the gene trees: one using all recovered loci and other using only loci that were recovered for 75% of the taxa. Branch support was calculated using local posterior probabilities.

Character state reconstructions:

These analyses were performed including only *Chresta* and the closest outgroup, aiming to identify potential synapomorphies for each clade and to examine patterns of morphological evolution and homoplasy. They were carried out on Mesquite v.3.31 (Madison & Madison 2017), using ten different morphological and ecological traits. The total maximum likelihood tree was used as basis for reconstructions, also using the maximum likelihood reconstruction models. For characters with only two states, a likelihood ratio test (LTR) was used to test between evolution models with identical or different reversal and acquisition rates. Nine qualitative characters that have been previously used as basis to divide *Chresta* in other genera and that showed variation among species were chosen. Codification of the character states used in the reconstruction is presented in Table 2, and they are illustrated in Figures 1 and 6. Information about habit, underground system, soil type, leaf margin, synflorescence type, syncephalia morphology and floret color were obtained from observations carried out on the field and taken from herbarium specimens labels (mostly from HUEFS, NY, RB, SPF, UB, and US; acronyms according to Thiers 2018). Morphological concepts and terminology follow Harris

& Harris (2001), synflorescence and syncephalia morphology follow Endress (2010) and MacLeish (1984a), sweeping hair morphology also follow MacLeish (1984a). Pollen morphology follows data from Siniscalchi et al. (2017). Sweeping hair information was obtained from microscopy slides prepared with Hoyer's medium (Anderson 1954), using herbarium material as source for styles. Information about the outgroups, *Heterocypsela*, *Vernonanthura* and *Vernonia,* were obtained from the literature (e.g. Jones 1979, Robinson 1979, Robinson 1999a).

Ancestral areas reconstruction:

S-DIVA (Yu et al. 2010) was used to reconstruct possible ancestral areas, as implemented in RASP (Yu et al 2015), not allowing for extinction. The total ML tree was used as basis, distributions for Brazilian taxa followed the phytogeographical domains adopted by the Brazilian Flora Checklist (Forzza et al. 2012, BFG 2015). Taxa that do not occur in Brazil were classified in general areas, like "Africa" and "North America. A total of ten areas were used.

Results

Overview and general trends:

The sequencing generated approximately 902 million reads, and approximately 89 billion nucleotides (4 million to 33 million reads per sample). The total matrix contains 60 taxa and has an extension of 733,148 characters, including 709 of the markers contained in the probe set, with 74.9% missing data. The 75% matrix has 60 taxa as well, but the matrix length is of 107,613 characters, containing 89 loci and 34.9% missing data. Raw data and matrices will be deposited in Dryad Digital Repository.

Despite differences observed among analysis, some trends are remarkable in all obtained trees. These are:

- *Centrapalus pauciflorus* and *Stokesia laevis* change positions between each other, probably due to the incomplete sampling of African and North American taxa.
- *Chresta* is consistently monophyletic with high statistical support (support for total/75% trees: JK: 100%, 100%, ML bootstrap: 100%, 100%, local PP: 1, 1), and its sister group is a clade formed by *Heterocypsela andersonii* + *Vernonia* + *Vernonanthura* with high statistical support (JK: 99%, 100%, ML bootstrap: 100%, 100%, lpp: 1, 1).
- Within *Chresta, C. martii* is sister to all other species, which are grouped into two clades, one with rupiculous plants from the Caatinga and Atlantic Forest, and other with Cerrado species. Each of these clades is in turn divided in two clades each.
- For ease of discussion, the clades within *Chresta* are named as follows: Caatinga clade 1: (*C. filicifolia* (*C. pacourinoides, C. heteropappa*)*);* Caatinga clade 2: (*C. artemisiifolia* (*C. harleyi* (*C. hatschbachii, C. subverticillata*)))*;* Cerrado clade 1: (*C. angustifolia* (*C. souzae* (*C. speciosa, C. plantaginifolia*)))*;* Cerrado clade 2: (*C. curumbensis* (*C. pycnocephala* (*C. exsucca* (*C. scapigera, C. sphaerocephala*)))). Clade composition and geographical areas are displayed on Figure 2.
- The Vernoniinae sampled in our analyses were not recovered as a monophyletic group; instead they are split in two clades: *Vernonia* and *Vernonanthura* grouped with the Chrestinae, and *Cyrtocymura* as sister to Lepidaploinae (Fig. 3).

- *Stilpnopappus* and *Strophopappus,* which are also in the Lepidaploinae, emerge in a clade separate from *Lepidaploa* and *Lessingianthus*, being sister to Elephantopinae. *Soaresia* is included in the Elephantopinae, not in Chrestinae (Fig. 3).
- The two sampled species that belong to Dipterocypselinae always emerge in two different clades, *Heterocypsela* in a clade with Vernoniinae and *Allocephalus gamolepis* as sister-group of the Lychnophorinae, rendering Dipterocypselinae non-monophyletic.
- Some relationships and groupings in Lychnophorinae are stable in all analyses: *Allocephalus* and *Centratherum punctatum* always emerge in sequence in a position external to other Lychnophorinae. *Albertinia* and *Gorceixia* consistently group together, as well as *Eremanthus crotonoides* and *Hololepis pedunculata*. There is a clade formed by four species divided into two clades: *Paralychnophora harleyi + Maschalostachys mellosilvae* and *Chronopappus bifrons + Heterocoma ekmaniana.*
- Relationships within *Lychnophora, Eremanthus, Minasia* and *Piptolepis* vary depending on the dataset and analysis, likely due to the poor sampling of this diverse subtribe (only ~18 % of species sampled).

Maximum likelihood:

Both trees obtained in the ML analysis (Fig. 3, 4) present the same general topology, but with differences in overall support. The 75% tree shows less support than the total tree, with 10 nodes with ML bootstrap below 100%, while the total tree has only seven nodes below that. Also, the 75% tree has some increases in branch length, specially marked in *Stokesia laevis* and *Stilpnopappus tomentosus.*

The position of Moquinieae varies between datasets: outside Vernonieae in the total tree and inside Vernonieae in the 75% tree, but both relationships are well supported (bootstrap: 100%). The position of *Centrapalus pauciflorus* and *Stokesia laevis* differ between the two analysis, with their positions alternating, with very low support in the 75% tree (bootstrap: 2%).

The most important difference are the relative positions of Chrestinae+Vernoniinae, Lychnophorinae and Vernoniinae+Lepidaploinae, with higher support on the 75% tree.
While in the total analysis Lychnophorinae comes out as sister to a larger clade formed by Vernoniinae+Lepidaploinae and Chrestinae+Vernoniinae (bootstrap: 100%, 75%), in the 75% matrix these positions are inverted, with Lychnophorinae being sister to Vernoniinae+Chrestinae and Vernoniinae+Lepidaploinae being sister to both (bootstrap: 100%, 100%).

Internal relationships in the clades also present some disparities: in the Lychnophorinae clade, the position of *Lychnophora brunioides, Minasia pereirae* and *Piptolepis ericoides* vary between analyses, with the difference that *Lychnophora brunioides* and *Minasia pereirae* consistently remain outside the clade formed by *Lychnophora+Eremanthus,* while *Piptolepis* emerges as sister to the Bahian *Lychnophora* in the 75% tree.

Within *Chresta,* there is also variation regarding the relative positions of *C. exsucca, C. scapigera* and *C. sphaerocephala:* in the total tree, the two latter species are sister to each other, with 85% bootstrap, and *C. exsucca* is sister to both, with 100% support. In the 75% tree, the position of *C. exsucca* and *C. scapigera* is inverted, and the support for *C. exsucca* and *C. sphaerocephala* as sister groups is 96%. Also, three nodes within this clade present MLb support below 100%, although still high: 99%, 96%, 96%.

***Parsimony*:**

Both parsimony analyses generated only one most parsimonious tree (Fig. 5A, B). Jackknife values were overall good, with 13 nodes with support below 100% in the total tree and 10 nodes in the 75% tree. The total tree has a tree length of 418,215 steps, CI = 0.728 and RI = 0.684, and the 75% has a tree length of 90,890 steps, CI = 0.645 and RI = 0.730. The topology on both trees was overall very similar. Moquinieae shows the same pattern seen in the ML analysis, in the total matrix, Moquinieae comes out as sister group to the Vernonieae (JK: 100%), while in the 75% matrix this tribe comes out in a clade with African Vernonieae, although with low support (JK: 61%). In the total tree *Stokesia laevis* and *Centrapalus pauciflorus* come out in sequence, while in the 75% tree both taxa group together, again with low support (JK: 80%).

There is no change in the topology on the Vernoniinae + Lepidaploinae clade, but the internal topology in the Lychnophorinae presents large variation between analyses. The total analysis shows *Lychnophora brunioides, Minasia pereirae* and *Piptolepis ericoides* coming out in sequence as sister groups to the main *Eremanthus* + *Lychnophora* clade, with high support in these relations (JK: 100%, 97%, 95%). Also, in this analysis the three *Eremanthus* form a clade, although within *Lychnophora* (JK: 98%). In the 75% analysis, *Minasia pereirae* comes out as sister to *Eremanthus* + *Lychnophora* and both *Lychnophora brunioides* and *Piptolepis ericoides* merge with the clade formed by *L. haplopappa* sp. ined., *L. morii* and *L. salicifolia*, although without support (JK: 6%). In this analysis, *Eremanthus erythropappus* emerges as sister to a clade formed by *Eremanthus* and three other *Lychnophora* species.

There are changes in the topology and support within *Chresta* in these analyses too, similar to those seen in the ML analysis. The total analysis shows maximum support in all internal nodes in *Chresta*, except for one formed by *C. scapigera* and *C. sphaerocephala* (JK: 97%). In the 75% analysis, the relations in this clade are uncertain, with the node between *C. pycnocephala* and the rest showing very low support (JK: 35%) and with the positions of *C. exsucca* and *C. scapigera* changing between each other, resulting in very low support in the *C. exsucca* + *C. sphaerocephala* clade (JK: 17%).

Multispecies pseudocoalescence:

The topology obtained with the two different matrices using the multispecies pseudocoalescence method does not differ regarding the position of the large clades, but there is variation on the topology within clades (Fig. 5C, D). Local posterior probabilities values, however, are strongly affected by reducing the number of loci in the analysis, the total tree has 12 nodes with support below 1 and this number doubles in the 75% tree.

Both trees present the clade formed by Chrestinae+Vernoniinae as sister to Lychnophorinae *sensu lato,* with low support in both analysis (lpp total: 0.21, lpp 75%: 0.46), while Vernoniinae+Lepidaploinae emerges as sister to both of them (lpp: 1). Moquinieae emerges within Vernonieae, as sister to the African Vernonieae, again with low support in both trees (lpp total: 0.63, lpp 75%: 0.68). *Centrapalus* emerges before *Stokesia*, with good support (lpp: 1).

In *Chresta,* the position of the species in Cerrado clade 2 inverts, as seen in the other analysis, with *C. exsucca* and *C. scapigera* changing positions, although the support is higher in the total tree (lpp: 0.75 vs. 0.53), favoring *C. exsucca* as sister to *C. scapigera* + *C. sphaerocephala.* Also, in the 75% analysis, eight from the 17 nodes within *Chresta* have lower support (0.53<lpp<1), although all are above 0.8, except for the one in Cerrado clade 2 (lpp: 0.53).

Within Lychnophorinae there are more disparities, but the support is low in most nodes (0.35<lpp<1), regardless of the matrix used. In the total tree *Minasia, Lychnophora brunioides* and *Piptolepis* emerge in sequence before a core clade formed by *Lychnophora* and *Eremanthus*, while in the 75% tree only *Minasia* and *Lychnophora brunioides* emerge outside of this clade, while *Piptolepis* is sister to a smaller clade formed by *Eremanthus* and the Bahian *Lychnophora.* In both trees *Eremanthus erythropappus* is sister to the rest of the *Eremanthus* + Bahian *Lychnophora* clade.

Topology within Chresta*:*

All analysis show a highly consistent topology within *Chresta*, varying only in the relative position of *C. exsucca, C. scapigera* and *C. sphaerocephala* (Fig. 3 and 4). *Chresta martii,* a rupiculous species found in the Caatinga, emerges as sister to all other species, which are divided into two clades, one exclusively with Cerrado species and other combining all remaining rupiculous Caatinga species and the only species endemic to the Atlantic forest (chapter 4, Siniscalchi et al. 2016), with each of these two clades being further divided into two clades each.

In the large clade mostly composed by Caatinga plants, all taxa have rupiculous habit, but there are some differences between them. In Caatinga clade 1, *C. filicifolia* is endemic to quartzitic outcrops within the Atlantic Forest domain in eastern Minas Gerais and is sister to the clade formed by *C. pacourinoides* and *C. heteropappa,* both of which share very similar morphology and environmental preferences, growing over granitic outcrops (chapter 4, Siniscalchi et al. submitted). Caatinga clade 2 is composed of four species that share strong morphological and ecological features: all are exclusively rupiculous, usually occurring on quartzitic outcrops, with silver green lobate leaves and hemispherical syncephalia with loosely adjoined capitula with lilac flowers (Chapter 4, Siniscalchi et al. submitted).

In the Cerrado clade, the first clade is composed by *C. angustifolia, C. souzae, C. speciosa* and *C. plantaginifolia,* which are all subshrubs or rosette herbs with scapose, solitary syncephalia. These species seem to have specific environmental requirements, such as *C. angustifolia,* which only grows on river margins in highland rocky fields, and *C. souzae* that is found on very fertile and wet soils. The second Cerrado clade contains all species with globose syncephalia and erect and ramified synflorescences, with the exception of *C. curumbensis,* which is a rosette herb with solitary, hemispherical syncephalia, and *C. scapigera,* which is a rosette herb. Plants of this clade usually grown on latosol on different Cerrado physiognomies.

Character state reconstructions:

The trees generated from the reconstructions are found in Figures 7, 8 and 9. Regarding habit, subshrub with cauline leaves is the most common state, having emerged once and suffered two reversals and one reacquisition, and is the most probable ancestral state in *Chresta.* The habits (bi)annual herb, shrub with cauline leaves and rosette at stem tip each arose once within *Chresta;* the (bi)annual herb habit is also found in *Heterocypsela*. Rosette herb is found only in the Cerrado clades and is the most likely ancestral state for this larger clade (Fig. 7A).

The most likely ancestral state of the underground system in the genus is “slightly diffuse” (sparsely ramified and not thickened), also a putative synapomorphy for the whole genus, and is the predominant type in the rupiculous species, except for *C. heteropappa* and *C. pacourinoides*, which have thin, fasciculate systems, related to the fact that these species usually grow on accumulated plant matter upon rocks or in rock crevices. This state is also find in *C. angustifolia*, which usually inhabits sandy soil or rocks at river margins. The thickened root systems, such as diffuse and thickened, thickened and non-ramified, and moniliform, all occur in species of the Cerrado clade, with thickened and non-ramified being the most likely ancestral state in this clade (Fig. 7B).

The reconstruction of the "soil type" character indicates the ancestral state as being some kind of rock, as the probabilities are split into several states (Fig. 7C). The most likely origin of the Caatinga clade is related to quartzite outcrops, with a transition to crystalline outcrops in Caatinga clade 1. The preference for Cerrado latosol is found only in species of Cerrado clade 2, while components of Cerrado clade 1 may inhabit a variety of soil types. *C. angustifolia* and *C. souzae* are found in humid environments, the former usually close to water bodies, while the latter grows in dark and rich soils with high quartz content and that may stay slightly flooded during part of the year. *C. speciosa* and *C. plantaginifolia* are usually found on the transitional vegetation known as *cerrado rupestre* or in *campos limpos,* with high content of quartz and usually at higher elevations.

Although there are appropriate critics to the mapping of extrinsic characters in a phylogeny (e.g. Grandcolas et al. 2010), different groups of species in *Chresta* seem to be very specialized to contrasting types of substrate, strongly suggesting that each lineage harbors multiple physiological adaptations that could explain their restriction to narrowly delimited niches. Mapping the type of soil where different species grow works as a proxy to these apparent niches, which actually are intrinsic features of each lineage, thus allowing the reconstruction of this character.

The character "leaf margin" (Fig. 8A) has only two states and according to LRT, the symmetrical model better fits the data. Our definition of lobed include a wide range of morphologies, going from pinnatilobate to pinnatifid, and including deeply lobed and deeply dentate. The most likely ancestral state is lobed, and it is found in all rupiculous species. Leaves with entire margins are present in all Cerrado species.

With respect to the synflorescences, absence of syncephalia is found only in the outgroups. The likely ancestral state recovered for *Chresta,* and a putative synapomorphy for the genus, is a scapose and solitary syncephalium, and this is the only state found in Caatinga clade 2 and in Cerrado clade 1. Panicle of syncephalia emerged twice within *Chresta*, once in Caatinga clade 1 and once in Cerrado clade 2, where it suffered one reversal (Fig. 8B).

The most likely ancestral state of syncephalia morphology and putative synapomorphy recovered for *Chresta* is hemispherical, which is also the most widespread type, being found in all species of Cerrado clade 1 and Caatinga clade 2. Spherical syncephalia arose twice, in Caatinga clade 1 and Cerrado clade 2, being a putative synapomorphy of (*C. heteropappa,C. pacourinoides*) and (*C. pycnocephala(C. exsucca(C. scapigera,C.sphaerocephala*))) (Fig. 8C). Regarding syncephalia growth, the most likely ancestral state and putative synapomorphy is determinate growth, with indeterminate growth arising twice, once in *C. martii* and another in the clade (*C. heteropappa,C. pacourinoides*), also being a putative synapomorphy of this clade (Fig. 8D).

With respect to the character "Floret color", in *Chresta* species that have throats in a different color than the rest of the corolla, the color of the throat may spread to the base of the petals or to the tip of the floral tube, sometimes reaching extreme conditions, as seen in *C. souzae*, in which corolla lobes sometimes are almost completely white. The ancestral state recovered for the whole genus, and putative synapomorphy, is probably purple with white throat and this is found in both Caatinga clades and almost in all species of Cerrado clade 1. Red florets with yellow throats arose twice, once in each Cerrado clade, and purple florets with purple throats are found only in four species in Cerrado clade 2 (Fig. 9A).

Sweeping hair morphology (Fig. 9B) could not be accurately addressed for the outgroups. Within *Chresta,* acute hairs are found only in *C. martii,* clavate only in *C. curumbensis* and *C. speciosa* and lageniform in all species in Caatinga clade 1. The most likely ancestral state is subulate, which is also the most common feature.

Finally, pollen type F is found only in *C. martii* and type A probably arose twice, in *C. curumbensis* and *C. speciosa.* All other *Chresta* species have pollen of type C, and this is most likely the ancestral state for the genus (Fig. 9C).

Ancestral area reconstruction:

The S-DIVA reconstruction carried out in RASP (Fig. 10) indicates that the most likely ancestral area for *Chresta,* and also for the clade formed by *Chresta* and its sister group, *Heterocypsela+Vernonia+Vernonanthura,* is the Caatinga, while the internal node splitting into the two larger *Chresta* clades is probably related to the occupation of both the Cerrado and Caatinga at that step of the evolutionary history of the genus. There is one dispersal event indicated for the basal *Chresta* node, while the node joining *Chresta* and the outgroups indicate 2 dispersal events. The internal node in *Chresta* separating Cerrado and Caatinga groups indicate one vicariance event. There is one additional vicariance event in Caatinga clade 1, in the node separating *C. filicifolia* from *C. heteropappa* and *C. pacourinoides.*

There is also evidence that the clade (Lychnophorinae(Lepidaploinae+Elephantopinae, Vernoniinae+Chrestinae)) may have had its origin in the Cerrado, and this persists as the main domain where members of the Lychnophorinae occur. This node indicates a dispersal event, but this may be an artifact of the sparse sampling outside these clades.

Discussion

Agreement between datasets and analysis

The results obtained with different analyses was overall consistent and incongruences seem to be more related to the dataset used than to the type of analysis. Using a 75% matrix was a strategy to try to understand the effect that the high level of missing data, which is a frequent problem in studies based on multiple markers (Huang & Lacey Knowles 2016), might have over topology and support. The effect of missing data in phylogenetic analysis started being studied since fossils were included on them (Donoghue et al. 1989), and this is being increasingly discussed as larger datasets continue to appear. One view on the problem is that missing data does not influence as much if a sufficient amount of characters has been sampled (Wiens 2003, Wiens & Morrill 2011).

In our analysis, reducing the number of markers decreases overall support on the trees, especially on the ML and coalescence tree, while in the parsimony tree the effect is the opposite. The ML analysis, besides presenting lower support, presents three major changes in the position of the larger clades, with additional changes within the clades. In the coalescence analysis, the position of the major clades remains the same, although the number of nodes with low support doubles in the 75% tree. This possibly indicates that in these two analysis, the full dataset helps resolving internal nodes and gives more characters that support the relations established by the cleaner dataset. In the parsimony analysis, the tree obtained with the 75% dataset has a better supported backbone and less nodes with lower support, probably indicating a stronger negative effect of the missing data.

One interesting finding is that in all analyses ran with the 75% matrix, Moquinieae falls within Vernonieae, as sister to the African Vernonieae. Given that Moquinieae is strictly South American and that our analysis lacks several early diverging lineages of Vernonieae of diverse geographical locations, this may well be an artifact of reducing the number of loci on the analysis (Funk & Chan 2009, Loeuille et al. 2015a).

Geographical partitioning within Chresta

Chresta was previously included in two phylogenetic analysis of the Vernonieae; one focused in relationships within Vernonieae included only one *Chresta* terminal (Keeley et al. 2007) and another, having South American Vernonieae as target, but including ten species of the genus (Loeuille et al. 2015a). The separation of the genus into two geographically defined clades recovered here was already seen by Loeuille et al. (2015a), even if only two Caatinga species were sampled in this previous study.

Overall, the present study corroborates the monophyly of *Chresta,* including the merging of *Pithecoseris* within it (Loeuille et al. 2014), as the only species that composed this genus fall within *Chresta* (Fig. 2 and 3).

The position of *Chresta martii* as sister to all other species is a novel evidence, since that species was not included in previous analyses. However, the peculiar morphological features of *C. martii* give some clues about its distancing from the remaining species: it is the only species in the genus with pollen type F, the only one bearing aromatic leaves, and also its combination of hemispherical syncephalia with indeterminate growth of the inflorescence axis is pretty unique (Fig. 1A).

The clade including *C. pacourinoides, C. heteropappa* and *C. filicifolia* has as possible synapomorphy the presence of lageniform sweeping hairs on the back of the style arms, as this type of trichome is found only here. An interesting ecological implication that emerges with this clade is that *Chresta filicifolia* is known from only two locations within the Atlantic Forest phytogeographical domain, although it grows over rock outcrops within a semideciduous forest matrix (chapter 4, Siniscalchi et al. 2016), while *C. pacourinoides* and *C. heteropappa* are closely associated to granitic inselbergs and outcrops from the Caatinga, usually being found growing on the layers of vegetation on the base of those outcrops (chapter 4). Most of the known locations where *C. pacourinoides* and *C. heteropappa* are found belong in areas called *brejos de altitude,* which are fragmentary enclaves of seasonal semi deciduous montane forest found within the Caatinga, usually in areas between 500 to 1100 m a.s.l (Tabarelli & Santos 2004, chapter 3). The currently accepted hypothesis is that the *brejos* probably represent remnants of the incursion of the Atlantic Forest into the Caatinga that occurred due to the Pleistocenic climate changes (Tabarelli & Santos 2004).

This link between two species from *brejos de altitude* within the Caatinga and one species from the rock outcrops within the Atlantic Forest, with a fairly large gap among their distributions, could be achieved through an ancient dispersal event followed by allopatric speciation. On the other hand, it may indicate that there was a *Chresta* ancestral that once occurred throughout this continuum, and later became extinct, or that the distribution ranges of the current species were once much wider than their present ones. Additionally, the occurrence of *Chresta filicifolia,* a member of a genus mostly endemic to the Caatinga and Cerrado, on an "island" of open habitat (quartzitic outcrops) lying on a region mostly covered by Atlantic forests, may be the result of a vicariant event taking place after an expansion of the open "dry diagonal" towards the east during past dry, cooler climate periods, as also indicated in the ancestral area reconstruction, which indicates a vicariance event in this node. This latter scenario is supported by paleoecological evidence that since the early Holocene up to 5,500 years A.P., *campo cerrado* dominated on areas in eastern Minas Gerais which are nowadays covered by semideciduous forests (Behling 2002, de Oliveira *et al.* 2005).

The only *Chresta* species not sampled in the study is *C. pinnatifida,* a species collected a few times by Glaziou in the 1880's, but without further records. Glaziou's memoirs (1909: 381) indicate the samples were collected in the general area of Serra do Caraça, Minas Gerais, which lies in the limits between the Atlantic Forest and Cerrado, and initially identified as *Pithecoseris pacourinoides*. *Chresta pinnatifida* have features that approximate it from *C. pacourinoides,* such as the membranaceous dark green, pinnatilobate leaves, the branched synflorescence, and the long floret tubes, but other features, such as the hemispherical syncephalia and the stramineous, oblong to linear phyllaries, resemble those present in *C. filicifolia.* These morphological similarities with the species in this clade, together with the geographic locality, may indicate *C. pinnatifida* belongs in this clade and also may provide evidence in favor of the hypothesis that there once were more taxa belonging to *Chresta* present in this area.

The second Caatinga clade is composed of species that share strong morphological similarities and are geographically limited to the Espinhaço Range in Minas Gerais and Bahia and to the Chapada Diamantina massif in Bahia (Fig. 2), with specific ecological requirements: all these species are strictly rupiculous, only growing on quartzitic outcrops, which are usually surrounded by Caatinga vegetation, or transitions from Cerrado to Caatinga or *campo rupestre* to Caatinga vegetation. The group is morphologically homogeneous, and diagnostic characters are the presence of narrow pinnatilobate or pinnatifid leaves, covered by dense greyish-silver indument of T-shaped trichomes, solitary, hemispherical syncephalia, purple flowers with a white throat and type C pollen (Fig. 1D). Interestingly, *Chresta artemisiifolia* and *C. subverticillata* present evidence of indeterminate growth of the syncephalium axis, but not as markedly as seen in *C. martii,* and in only some of their syncephalia.

Despite widespread over a large area, *Chresta artemisiifolia, C. hatschbachii* and *C. subverticillata* are found at scattered locations, with few known populations. They all occur in a South-to-North pattern, with about 200 km of distance between populations of each species. *C. harleyi* is the most widely distributed, on the southernmost portion of the range, and is known from several populations located in an area that extend for ca. 200 km (Siniscalchi et al. submitted, chapter 4).

This interesting South-to-North pattern may either indicate the occurrence of speciation by isolation, with ancestral populations dispersing to new locations and posteriorly becoming isolated and speciating, especially because the plants are restricted to a very specific habitat that is fragmentary in its own, or the existence of an ancestral species that occupied a larger area that subsequently became fragmentary due to some vicariant event, such as changes in vegetation cover under past climatic fluctuations. (see Chapter 2)

The two Cerrrado clades present an overlapped distribution, although one is more restricted than the other (Fig. 2). Nevertheless, there are morphological and ecological traits that seem to delimit them well, with exception of the two red-flowered species, *Chresta curumbensis* and *C. speciosa.* Quite surprisingly, these two species do not form an exclusive clade, despite the extensive morphological similarities between them, with the acquisition of red flowers, along with a probable shift in pollination system and other features related to pollination, like pollen and sweeping hair type, occurring twice independently in *Chresta.* Red flowers are rare within the Vernonieae, occurring in only five genera besides *Chresta*: in South America some species of *Strophopappus* and *Mattfeldanthus,* and in the Old World, *Aedesia, Hoplophyllum* and some species of *Distephanus* that have orange flowers (MacLeish 1985a). The red corollas of *Chresta curumbensis* and *C. speciosa* also present a yellow band on the apex of the tube and base of petals (Fig. 1E). These two species are perennial herbs with well-developed underground systems, with rosette leaves and large scapose syncephalia, with the longest flowers in the genus (4-5 cm). Ecological preferences appear to differ between them: *C. speciosa* occurs in *cerrado* to *campo rupestre* transitions, usually on rocky, quartzitic soils, and flowering during the dry season (May to September), while *C. curumbensis* usually occurs in *cerrado* vegetation, on clay-rich latosol, usually growing in the understory, and flowering during the rainy season, from September to February (see Chapter 3).

These ecological preferences seem to be consistent with other species in these two clades as well. The first Cerrado clade (Fig. 2) is diagnosed by the presence of hemispherical, scapose and solitary syncephalia and purple florets with a white throat (Fig. 1F). Except for *Chresta angustifolia*, the other species within this clade are all rosette herbs. *C. plantaginifolia, C. souzae* and *C. speciosa* are usually found on rocky soils with high quartz content, usually in the *cerrado* to *campo rupestre* transition. *C. angustifolia* is found exclusively on sandy soils or rocks around perennial or intermittent water courses. *C. souzae* is endemic to the Chapada dos Veadeiros, in the state of Goiás, while *C. angustifolia* is mostly found in this area, with a few collections in the state of Tocantins. *C. speciosa* is found only in Goiás, while *C. plantaginifolia* is found on the states of Goiás, Distrito Federal and Minas Gerais. There is one collection of this species in the state of Paraná, presenting a large disjunction with the other known populations.

The second Cerrado clade (Fig. 2) is diagnosed by spherical syncephalia and entirely purple florets, without white bands in the throat (Fig. 1G, H, I), with the exception of *C. curumbensis.* Also, species of this clade show a preference for latosols, although eventually growing in more rocky or sandy soils, specially *C. exsucca* and *C. pycnocephala.* Also, the erect habit and ramified synflorescences prevail within this group, except for *C. curumbensis* and *C. scapigera,* which are rosette herbs with scapose syncephalia, although in *C. scapigera* the scape can produce some secondary branching. The most widely distributed species of the genus and type species, *C. sphaerocephala,* belongs in this clade (see Chapter 3).

Taxonomic history and agreement with the phylogeny

Species belonging to *Chresta* have been split into different genera during its taxonomic history. The distancing of *Chresta* species from *Eremanthus,* where they were placed for a long period (Baker 1873), was shown to be morphologically unsustainable in the 1980s (MacLeish 1984b, 1985a, 1985b; Robinson 1980), and subsequent phylogenies contributed to this view (Keeley et al. 2007, Loeuille et al. 2015a), as is now also confirmed by the present study.

MacLeish proposed the division of *Chresta* into five different genera: *Argyrovernonia*, with pinnatilobate leaves and silvery indument and pollen type F (1984a, 1984b), *Glaziovianthus*, with red flowers, deciduous pappus and type A pollen (1985a), *Chresta*, with coriaceous leaves, branched inflorescences and usually 3 florets per head, *Pycnocephalum,* with solitary syncephalia, with 5 to 25 heads per syncephalia and 4–12 florets per head (1985b) and *Pithecoseris*, with glabrate leaves, hundreds of heads per syncephalia, pollen type C and dimorphic cypselas (MacLeish 1984a). With the exception of *Pithecoseris,* which was monotypic, none of those genera proposed by MacLeish (1984a, b; 1985a, b) are recognized in the present work, since they were not recovered as monophyletic groups. *Argyrovernonia* contained *C. harleyi* and *C. martii*, which did not emerge in the same clade, with *C. martii* being the sister to all *Chresta* and *C. harleyi* being on Caatinga clade 2 (Fig. 2 and 3). *Pithecoseris* has been recognized as a separate genus since the 1830s (Candolle 1836, Baker 1873), but was recently synonymized into *Chresta,* based on morphological similarities and the results of a previous phylogeny (Loeuille et al. 2014, 2015a). *Glaziovianthus* contained the two red-flowered species, which were shown in the present study to be placed in the two different Cerrado clades. *Chresta* and *Pycnocephalum* roughly correspond to Cerrado clades 2 and 1, respectively, but both included only purple flowered species. *Chresta pinnatifida* was included in *Pycnocephalum,* but is more likely related to *C. pacourinoides*, due to its morphological affinities and habitat preferences as discussed in the previous session.

Relationships with Chresta and other Vernonieae

Relationships between *Chresta* and other Vernonieae were not very clear in previous phylogenies (Keeley et al. 2007 and Loeuille et al. 2015a) and still cannot be completely understood after the present study. In Keeley's work, the only *Chresta* terminal emerges as sister to *Heterocypsela*, and in one of the analysis both are sister to two other genera from South and Central America, with the clade formed by the four genera being sister to *Vernonia* and *Vernonanthura.*

Here we recovered that the sister group to *Chresta* is a clade with *Heterocypsela* as sister to *Vernonia* and *Vernonanthura* (Fig. 3). This clade has high support in all trees, but our sampling inside Vernoniinae is poor, and addition of more species could lead to different relations within this clade.

The relationship of the large clade formed by Chrestinae + Vernoniinae with the other Vernonieae varies depending on the analysis and dataset, although most trees agree with Chrestinae + Vernoniinae being sister to Lychnophorinae *sensu lato* (Fig. 5), although with low support. The exception is the ML analysis with the full dataset, which shows Chrestinae + Vernoniinae as sister to the clade formed by Lepidaploinae and Elephantopinae. In all other analysis, Lepidaploinae + Elephantopinae emerges as sister to (Chrestinae + Vernoniinae) + Lychnophorinae *sensu lato*. In Keeley's phylogeny (2007), the same relation of (Chrestinae + Vernoniinae) + Lychnophorinae *sensu lato* appears, with Lepidaploinae + Elephantopinae as sister to this larger clade, in agreement with most of the trees shown here. However, *Heterocypsela* does not group with *Vernonia* and *Vernoniinae,* as shown here, and this may be due to lack of sampling of Central America taxa.

In the parsimony analysis in Loeuille's work (2015a), *Stokesia,* a North American genus, appears as sister to all *Chresta,* although the support is low and with long branch length. *Chresta* and *Stokesia* emerge as sister to a clade formed by Lychnophorinae *sensu lato* and *Vernonia+Vernonanthura.* The bulk of Lepidaploinae groups with Elephantopinae and some other Vernoniinae, forming a clade sister to *Chresta* + (*Vernonia*+*Vernonanthura* + Lychnophorinae *sensu lato*). None of the trees recovered here support these relations, as we always recovered *Heterocypsela* and *Vernonia*+*Vernonanthura* as sister to *Chresta.*

When subtribe Chrestinae was created (Robinson 1992, 1999a), *Soaresia,* a monotypic genus from Central Brazil, was placed on it due to some morphological similarities, mainly the presence of secondary head and pollen type. However, in Loeuille's work (2015a), *Soaresia* emerges as sister to the Elephantopinae, with the same relation shown in all analysis presented here. *Soaresia* has morphological affinities to *Elephantopus,* such as the bristle-like awls that compose the pappus and the unbranched trichomes, further supporting its transference to subtribe Elephantopinae (Loeuille et al. 2015a).

Also, the analyses presented here do not support the monophyly of subtribe Dipterocypselinae. This subtribe was created to accommodate two monotypic genera that present dimorphic cypsela and a third monotypic genus without dimorphic cypsela (Keeley & Robinson 2009), with a fourth monotypic genus with dimorphic cypsela being added later (Bringel Jr. et al 2011). *Heterocypsela* and *Allocephalus* are both from Central Brazil, growing on limestone outcrops, while *Dipterocypsela* is found on northern Colombia, also on limestone outcrops (Blake 1945). *Manyonia* does not present fruit dimorphism, but the inflorescence structure and the pattern of the cells on the cypsela walls placed it close to *Heterocypsela* and *Dipterocypsela* (Robinson 1999b), even though this species in known only in Tanzania.

Due to the morphological singularities of these four genera, their placement within Vernonieae subtribes has always been putative at best (Blake 1945, Robinson 1999b), but our analysis indicates that Dipterocypselinae is not a monophyletic group, with *Heterocypsela* and *Allocephalus* falling in distant places in our trees. Without sampling *Dipterocypsela* and *Manyonia,* it is impossible to determine where in the tribe these two genera are placed and whether Dipterocypselinae indeed forms a monophyletic group in some reduced sense.

Another finding from our analysis is the non-monophyly of both Vernoniinae and Lepidaploinae. As sampled here, Lepidaploinae terminals emerged in two clades, one including *Cyrtocymura*, which is currently placed on Vernoniinae, and another sister to Elephantopinae. Vernoniinae terminals also emerged separated, with *Vernonia* and *Vernonanthura* being sister to *Chresta*, and *Cyrtocymura* grouping with the Lepidaploinae+Elephantopinae. These separations had already been shown in Loeuille's analysis (2015a), although with lower resolution and support. This probably indicates that more subtribes will have to be reevaluated as more inclusive analyses become available.

Regarding Lychnophorinae *sensu lato*, the relations uncovered here slightly differ from those seen in Loeuille et al. (2015b). However, these differences are difficult to interpret due to our low taxonomic sampling, which includes only a few representatives from each major clade within it. The monotypic *Allocephalus*, not included in previous phylogenies, here emerges as sister to the rest of Lychnophorinae. It displays various plesiomorphic features of Lychnophorinae: herbaceous habit (*Centratherum*), T-shaped trichomes (*Albertinia*, *Centratherum* etc.) and heads in dense glomerules (*Blanchetia*, *Gorceixia*). It shares with *Albertinia* a style with basal node (feature uncommon in Lychnophorinae) and especially, as noted by Bringel Jr. et al. (2011), an involucre with fused phyllaries.

This peculiar involucre sheds an interesting light on the origin of the unique alveolate receptacle of *Albertinia* which has been variously interpreted: Candolle (1836) assumed that *Albertinia* had one floret per capitulum and fused capitula as in *Eremanthus* and *Lychnophora*, but since Schultz-Bipontinus (1861, 1863), *Albertinia* capitula are interpreted as multiflowered and the receptacle surface with deep holes (alveolae) (Robinson 1999a, Loeuille et al. 2015a). More studies are clearly necessary but the position of *Allocephalus* as an early diverging lineage of Lychnophorinae calls to reevaluate the morphological interpretation of the "capitulum" of *Albertinia* and indicates further directions to study the evolution in syncephaly in Lychnophorinae.

The clade grouping *Chronopappus*, *Heterocoma*, *Maschalostachys* and *Paralychnophora* was also recovered by Loeuille et al. (2015b) but only in one analysis (Bayesian analysis without morphological data). However, it appeared as sister to the *Prestelia* Alliance clade (*Eremanthus crotonoides* + *Hololepis*) in that study, instead of sister to the derived Lychnophorinae genera, as seen in the present analysis. Similarly to previous phylogenies (Loeuille et al. 2015a, b), *Minasia*, *Lychnophorella*, *Piptolepis*, *Lychnophora* and *Eremanthus* are grouped in a large clade but internal relationships vary between the analysis.

Overall, our analyses agree in several parts with the previous phylogenies, confirming some trends seen before. The sister group to *Chresta* is consistently recovered in all analysis, but the overall position of *Chresta* in relation to Vernoniinae and Lychnophorinae remains doubtful, indicating the need of a more complete representation of these subtribes in order to clarify their position on the phylogeny.

Character state reconstruction

The character reconstructions unveiled interesting morphological and ecological trends in the genus. There is a close association between habit and underground system. Subshrubs with cauline leaves, which are common on the Caatinga species, always present slightly diffuse underground system, while in Cerrado clade 2, the habit "shrub with cauline leaves" is associated with the diffuse and thickened underground system, both being putative synapomorphy in this clade. Also, other types of thickened systems all occur in the Cerrado species, what is expected, as this is a fire-prone environment and this is a trend seen in several plant families and in other Asteraceae genera (Rizzini & Heringer 1961, Appezzato-da-Glória et al. 2008, Simon & Pennington 2012).

Plants with thickened and non-ramified underground system, such as xyllopodia, usually do not present clonal growth, but the diffuse thickened system seem in Cerrado clade 2 allows the underground spread of the same individual and also the appearance of new buds away from the main stem, with one single individual occupying a diameter of several meters (Appezzato-da-Glória et al. 2008). Field observations have shown that occasional fire may be beneficial for *Chresta* populations, as the cleaning of the surrounding grass allows dormant buds to grow and usually results in explosive flowering. This effect has been observed in *C. exsucca, C. speciosa* and *C. souzae*, and it is also common to find flowering herbarium specimens of *C. plantaginifolia* with recent fire damage.

Chresta speciosa presents a peculiar aerial habit and underground system. The leaves are parallel-veined and form a rosette on the tip of the stems, being also deciduous, usually drying out and falling when the floral scape is in development. Parallel-veined leaves are not common in Asteraceae, with only one other case known in Vernonieae, in *Lychnophora sellowii* (*=Lychnocephalus sellowii)*, which occurs in *campos rupestres*, which are sometimes affected by fire (Loeuille et al., submitted). Another notable exception is *Corymbium,* a genus from the Cape floristic region in South Africa, an area also fire-prone and that also presents quartz-rich soils (Nordenstam & Funk 2009), as the *cerrado rupestres* habitat where *C. speciosa* grows. This fact possibly indicates a case of convergent evolution due to ecological factors. The moniliform root system is not found in any other representative of the genus, but has been reported in other Asteraceae, like *Gyptis* (Appezzato-da-Glória et al. 2008).

The reconstruction of the character soil type evidences the preference that the genus presents for rocky environments. Half of the species grow directly over rocks, while other species, such as the ones in Cerrado clade 1, usually grow in quartz-rich soils. Many Vernonieae show preference for soils with unusual mineral content, hence the common name "ironweed" for many species of *Vernonia* (Keeley & Robinson 2009), and among the Brazilian Vernonieae the preference for quartz is notable, especially in subtribe Lychnophorinae, with several taxa endemic to the *campos rupestres* from central Brazil (Loeuille et al. 2015b).

Strongly related to the rupiculous habit are leaf traits. All species that present some kind of lobation on the margins, either in marked ways, like the pinnatisect leaves of *C. artemisiifolia,* or more discreetly, like in *C. harleyi,* where sometimes the leaves are deeply dentate, also are rupiculous. Lobed leaves may be advantageous in high irradiance conditions, increasing heat dissipation (Vogel 1968) and also improving water balance in drier conditions, due to decreased hydraulic resistance (Sisó et al. 2001). Rock outcrops, especially in the semi-arid Caatinga, are dry, hot and high-irradiance environments, so probably the lobed leaves played an important role during the expansion of the genus along this type of habitat.

Solitary, scapose syncephalia prevail in *Chresta*, with compound synflorescences arising twice, once in each clade. Compound synflorescences seem to be related to spherical syncephalia with tighly joined capitula, as the taxa that present branched synflorescences also are the only ones to present spherical syncephalia. The only difference is that the globose syncephalia on species of the Caatinga clade also present indeterminate growth of the axis, while those in the Cerrado have determinate growth.

Floret color, type of sweeping hair on the style arms and pollen type seem to be associated in the case of the red-flowered species, and may be related to the shift in pollinator present in these species (see Chapter 3). The red florets, with long corolla tubes and a long portion of the style projected above the anthers is indicative of hummingbird pollination, and there is field evidence that this is indeed the case in these species (chapter 3). The correlation of the change in pollen ornamentation and pollinator shift is unknown (Siniscalchi et al. 2017), but a change in sweeping hairs morphology would be expected, as their functionality might be decreased with different types of pollen ornamentation. *C. martii* also presents a different pollen type (F), and there is also a change on the type of sweeping hairs, although not as marked as the one in *C. speciosa* and *C. curumbensis.* Notwithstanding, all species in the Caatinga clade 1 present a different type of sweeping hair, and there is no change on pollen, with these three species presenting pollen type C as all other species.

Biogeographical background

The highest diversity of *Chresta* mostly within the Caatinga and Cerrado may be better understood in the light of the current knowledge about the history of these domains. There are several hypotheses for the formation of the "dry diagonal" on South America, with different processes influencing different environments. It is generally accepted that seasonally dry tropical forests (SDTFs), such as the Caatinga, were present on the continent somewhere along the Miocene–Pliocene, with possible expansions and contractions during the climatic fluctuations in the Pleistocene, that may have influenced in the higher diversification found in some areas (Pennington et al. 2004).

A hypothesis usually referred to as the "Pleistocenic Arc" postulated that most of the South American dry forest types were connected in the past, due to drier and colder climatic conditions during the Pleistocene (Prado & Gibbs 1993). Several studies have tested this possibility, either through floristic analyses (e.g. Prado 2000, Neves et al. 2015, Dryflor 2016) or using molecular data and niche modeling (e.g. Caetano et al. 2008, Vieira et al. 2015), with varying results. Although several species are indeed shared by several of the units that compose the dry diagonal, each one of these floristic units have very particular floras (Dryflor 2016), possibly indicating that other factors have been determining in the assembly of the SDTFs (Neves et al. 2015).

Another outstanding domain lying along the "dry diagonal" is the Cerrado, whose origin seems to be related to the decrease in atmospheric CO2 and subsequent expansion of the C4 grasslands and savannas worldwide in the late Miocene and Pliocene, around 8 million years ago (Beerling & Osborne 2006, Scheiter et al. 2012), with dated phylogenies showing a possible formation of the Cerrado around 10 to 5 m.y.a. (Simon et al. 2009). Niche evolution has been evoked as a major factor driving the diversification of plants within this domain, with taxa shifting from the surrounding environments, such as the Caatinga and Atlantic Forest, to the Cerrado, where they subsequently acquired structural traits related to fire-resistance, such as a geophytic habit and thick bark (Simon et al. 2009, Simon & Pennington 2012). A remarkable congruent pattern of biome shift has been also reported for the African savannas (Maurin et al. 2014).

The reconstruction analysis carried out here indicates that the *Chresta* ancestor probably originated somewhere in Northeastern Brazil, in the areas currently dominated by the Caatinga vegetation. Character state reconstructions suggest that this ancestor had a rupiculous habit, growing on rock outcrops, which are ancient parts of the Brazilian Shield that remain exposed throughout the Caatinga domain nowadays (Ross 1998). Thus, the initial diversification of *Chresta* occurred in the Caatinga area, and while one group stayed in this habitat, gradually expanding its range, another group colonized the adjacent Cerrado; our ancestral area reconstruction indicates the occurrence of a vicariance event in this node, possibly indicating that a more widely distributed *Chresta* ancestor started to differentiate due to the climatic changes brought on by the development of the savanna environment, originating two lineages adapted to different climates and vegetation.

Once in the Cerrado, one of the *Chresta* clades maintained a preference for rocky soils, while some species acquired adaptations to this fire-prone environment, such as a well-develop underground system associate to basal rosette leaves (Figures 7A, B). Similar structural transition to this geophytic habit related to a shift to the Cerrado has been already observed in other plant families, remarkably the Leguminosae (e.g. Simon et al. 2009). Moreover, it has been suggested that adaptation to drought and fire underlying the numerous evolutionary transitions into the savanna biome was easy to achieve, only requiring changes in gene regulation rather than structural mutation (Simon & Pennington 2012, Pennington & Hughes 2014). This may be the case in *Chresta,* since the contrasting features of species across distinct habitats are not so remarkably different in structural terms.

On the other hand, the ancestor to the larger clade in which *Chresta* is nested, containing also Vernoniinae, Lychnophorinae, Lepidaploinae and Elephantopinae, has a putative ancestral area in the

Cerrado, possibly indicating that the *Chresta* ancestor that was on the Caatinga region may have arose from a lineage inhabiting the Cerrado, through dispersal, as indicated by the reconstruction, thus making the colonization of this domain by *Chresta* a secondary re-colonization. This is an uncommon pattern, as it has been observed that endemic species from SDTFs are not usually replaced by migrants from elsewhere (Pennington et al. 2006), and that most lineages that diversified in the SDTFs seem to be confined to these biome, possibly limited to the rich calcareous soils usually found on these regions (Pennington & Hughes 2014). In a way, *Chresta* also seems to be limited to a very specific environment: the crystalline and quartzite outcrops that occur in Eastern and Northeastern Brazil. Particularly, the lineage inhabiting the quartzite outcrops within the Caatinga domain seems to have conserved this ecological niche and remained within a marked spatial range.

For the Lychnophorinae, which is mostly diversified on the *campos rupestres* on quartzitic soils along the Espinhaço Range, there is a hypothetic scenario of a colonization of the *campos rupestres* from ancestors inhabiting tropical seasonal forests, with a few members of the subtribe subsequently shifting to the cerrados (Loeuille et al. 2015b). Likewise, the lineage of *Chresta* that inhabits latosols or other non litholic soils in the cerrado s.s. seem to descend from ancestors of rocky substrates that acquired several morphological adaptations to the different environmental conditions presented by this new environment, as mentioned previously.

Nevertheless, the current uncertainties about the time of origin of the phytogeographical domains discussed here still limit the strength of the biogeographical hypothesis proposed for *Chresta*. The possibility of dry forest incursions on savanna areas during the Pleistocene, as well as the fact that forest physiognomies predate the Cerrado and Caatinga, suggest it is likely that the ancestor of *Chresta*, and even of the larger clade that also include Lychnophorinae and Lepidaploinae, dwelt in some kind of seasonal vegetation before differentiation into savannas and SDTFs, with the diversification and specialization of lineages within Vernonieae taking place contemporaneously with the gradual establishment of the physiognomies they were in. Unfortunately, the lack of a dated phylogeny of *Chresta* and related groups prevent a confident support for the previous biogeographic hypotheses.

In fact, even the time of origin and age of the Asteraceae are still in discussion, mostly due to the poor fossil record for the family. There is only one macrofossil unequivocally assigned Asteraceae, a capitulum of *Raiguenrayun cura*, which is probably close to Mutisioideae *s.l.*, but without certain placement in the family (Barreda et al. 2012). There are other fossil pollen records assigned to Asteraceae that have been used in dated phylogenetic studies, but most of them are concentrated in the early-divergent groups, such as Barnadesioideae and Mutisioideae (Panero & Crozier 2016).

Most of the divergence between the estimates of the possible age of the family stem from the exact placement of the fossils in the phylogeny. Barreda et al. (2015) estimated that Asteraceae originated on the Late Cretaceous period (ca. 89 Ma), based on pollen records of *Tubulifloridites lilliei* type A, while Panero & Crozier (2016) did not use this calibration point, due to the difficulty of assigning it exclusively to Asteraceae, and obtained an estimate of the divergence between Asteraceae and Calyceraceae around 69 Ma. Also, analysis with published Asteraceae genomes place a whole genome triplication event before the split of Cichorioideae and Asteroideae, dated 38-50 Ma (Badouin et al. 2017). Panero & Crozier's study (2016) is the only to provide age estimates for internal nodes within the family, placing Vernonieae in the early Miocene period (between 20 and 10 Ma).

Even though the lack of fossils close to Vernonieae prevent us from dating the phylogeny presented here, our ancestral area reconstruction for *Chresta* fit with the estimate given by Panero & Crozier (2016), as it would also fit the period when the Caatinga and Cerrado domains were being assembled.

Long distance dispersal is thought to be a strong factor driving Asteraceae diversification, aided by the development of the pappus in the cypsela. Vernonieae has likely arisen in Africa (Keeley et al. 2007), and presents abundant richness in South America, probably resulting from transoceanic dispersal. Likewise, the pattern depicted in our phylogeny, with North and Central American taxa (*Vernonia, Vernonanthura*) emerging as closely related to South American species (*Heterocypsela, Chresta*), indicate that other dispersal events may have taken place once the tribe was established in the New World. However, the presence of widespread taxa within Elephantopinae (e.g. *Elephantopus mollis*) and Lychnophorinae (e.g. *Centratherum punctatum*), may also indicate that the ancestors of those clades was actually widespread, going through multiple vicariance and speciation events through time.

Vernonieae is known for its plethora of monotypic genera and genera with few species, which make its taxonomy complicated and also impose an obstacle to well-sampled phylogenies (Keeley & Robinson 2009). However, this taxonomic pattern may either be the result of several extinction events occurring within the tribe, probably during expansion events as the ones mentioned above, or a consequence of several adaptative transitions generating specialized features that allowed these taxa to undergo successful domains shifts, colonizing a large array of contrasting environments. Further analyses are required in order to enlighten these questions.

While the lack of fossil records for Asteraceae will most likely remain an issue in future attempts to study the biogeography of the family, molecular resources may fill in the gaps of our knowledge. Studying the abundant polyploidization events that occurred in the family, through sequencing of genomes or transcriptomes, such as presented by Huang et al. (2016) and Badouin et al. (2017), may provide a workaround for the fossil record, allowing the estimation of diversification rates and even age estimates for these events, increasing our knowledge of the family. Thus, we expect that in the near future the morphological, ecological and spatial patterns observed in *Chresta* in the present work may contribute with valuable data for the reconstruction of the history of dynamics (expansions and retractions) that the Cerrado and Caatinga biotas may have undergone during the Tertiary and Quaternary.

Concluding Remarks

The HybSeq method used to obtain sequence data for phylogenetic reconstruction proved useful and powerful, allowing us to recover well-resolved and supported relationships within *Chresta* and Vernonieae as a whole. We consistently recovered the sister group of *Chresta* as the North and Central American taxa of Vernoniinae, together with *Heterocypsela*, but the relationships among *Chresta*, Lychnophorinae and Lepidaploinae are still unclear, mostly due to low support derived from poor sampling in these other two subtribes. The strong geographical partitioning of *Chresta* show remarkable spatial patterns, which in association to other results provided here may stimulate further analysis under evolutionary and biogeographical approaches, being especially useful for time-calibrated phylogenetic studies.

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Table 1. Voucher list of the samples used in the phylogeny.

Species name	**Location**	**Collector name**	**Herbarium**
Albertinia brasiliensis Spreng.	Brazil, Bahia, Caetité	C.M. Siniscalchi 466	SPF
Allocephalus gamolepis J.Bringel, J.N.Nakaj. & H.Rob.	Brazil, Goiás, Guarani de Goiás	J. Bringel Jr. 416	CEN
Baccharoides anthelmintica (L.) Moench	to be provided by V. Funk and S. Keeley		
Centrapalus pauciflorus (Willd.) H.Rob.	Greenhouse grown seed, USDA, PI 312852	J. Mandel 104	GA
Centratherum punctatum Cass.	Brazil, Goiás, Alto Paraíso	C.M. Siniscalchi 498	SPF
Chresta angustifolia Gardner	Brazil, Goiás, Alto Cavalcante	C.M. Siniscalchi 490	SPF
Chresta 'artemisiifolia' sp. ined.	Brazil, Bahia, Sento Sé	J. Siqueira-Filho 3671	HVASF
Chresta curumbensis (Philipson) H.Rob.	Brazil, Distrito Federal, Gama	C.M. Siniscalchi 573	SPF
Chresta exsucca DC.	Brazil, Goiás, Alto Paraíso	C.M. Siniscalchi 378	SPF
Chresta filicifolia Siniscalchi & Loeuille	Brazil, Minas Gerais, Conselheiro Pena	C.M. Siniscalchi 512	SPF
Chresta harleyi H.Rob.	Brazil, Bahia, Licínio de Almeida	C.M. Siniscalchi 459	SPF
Chresta hatschbachii H.Rob.	Brazil, Bahia, Oliveira dos Brejinhos	C.M. Siniscalchi 468	SPF
Chresta '*heteropappa*' sp. ined.	Brazil, Ceará, Uruburetama	C.M. Siniscalchi 614	SPF
Chresta martii (DC.) H.Rob.	Brazil, Bahia, Casa Nova	C.M. Siniscalchi 482	SPF
Chresta pacourinoides (Mart. ex DC.) Siniscalchi & Loeuille	Brazil, Bahia, Feira de Santana	B. Loeuille 351	SPF
Chresta plantaginifolia (Less.) Gardner	Brazil, Distrito Federal, Gama	C.M. Siniscalchi 573	SPF
Chresta pycnocephala DC.	Brazil, Minas Gerais, Itacambira	C.M. Siniscalchi 535	SPF
Chresta souzae H.Rob.	Brazil, Goiás, Alto Paraíso	C.M. Siniscalchi 571	SPF
Chresta scapigera (Less.) Gardner	Brazil, Minas Gerais, Rio Paranaíba	C.M. Siniscalchi 359	SPF
Chresta speciosa Gardner	Brazil, Goiás, Alto Paraíso	C.M. Siniscalchi 421	SPF
Chresta sphaerocephala DC.	Brazil, Distrito Federal, Planaltina	C.M. Siniscalchi 576	SPF
Chresta 'subverticillata' sp. ined.	Brazil, Bahia, Gentio do Ouro	C.M. Siniscalchi 634	SPF
Chronopappus bifrons (DC. ex Pers.) DC.	Brazil, Minas Gerais, Santo Antônio do Itambé	B. Loeuille 465	SPF
Cyrtocymura scorpioides (Lam.) H.Rob.	Brazil, São Paulo, São Paulo	C.M. Siniscalchi 644	SPF
Elephantopus mollis Kunth.	Brazil, São Paulo, São Paulo	C.M. Siniscalchi 643	SPF
Elephantopus tomentosus L.	USA, Tennessee, Shelby County	J. Mandel 121	MEM

Table 1. Voucher list of the samples used in the phylogeny. (continued)

Species name	**Location**	**Collector name**	**Herbarium**
Eremanthus auriculatus MacLeish & H.Schumach.	Brazil, Goiás, Alto Paraíso	B. Loeuille 845	SPF
Eremanthus crotonoides (DC.) Sch.Bip.	Brazil, Espírito Santo, Viana	Fraga 2347	MBM
Eremanthus erythropappus (DC.) MacLeish	Brazil, Minas Gerais, Santana do Riacho	B. Loeuille 861	SPF
Eremanthus incanus (Less.) Less.	Brazil, Bahia, Gentio do Ouro	C.M. Siniscalchi 627	SPF
Gorceixia decurrens Baker	Brazil, Minas Gerais, Leme do Prado	B. Loeuille 821	SPF
Gymnanthemum amygdalinum (Delile) Sch.Bip. ex Walp.	Africa: cultivated	Kew: 318-86-02802	
Heterocoma ekmaniana (Philipson) Loeuille, J.N.Nakaj. & Semir	Brazil, Goiás, Cavalcante, Chapada dos Veadeiros	C.M. Siniscalchi 494	SPF
Heterocypsela andersonii H.Rob.	Brazil, Minas Gerais, Januária	C.M. Siniscalchi 601	SPF
Hololepis pedunculata (DC. ex Pers.) DC.	Brazil, Minas Gerais, Rio Acima	C.M. Siniscalchi 588	SPF
Lepidaploa opposita A.Teles, Sobral & J.N.Nakaj.	Brazil, Minas Gerais, Alvarenga	C.M. Siniscalchi 508	SPF
Lessingianthus durus (Mart. ex DC.) H.Rob.	Brazil, Goiás, Cavalcante	C.M. Siniscalchi 495	SPF
Lessingianthus monocephalus (Gardner) H.Rob.	Brazil, Goiás, Alto Paraíso	C.M. Siniscalchi 579	SPF
Lychnophora brunioides Mart.	Brazil, Minas Gerais, Santo Antônio do Itambé	B. Loeuille 466	SPF
Lychnophora 'haplopappa' sp. ined.	Brazil, Minas Gerais, Alvarenga	B. Loeuille 902	SPF
Lychnophora hatschbachii (H.Rob.) Loeuille, Semir & Pirani	Brazil, Minas Gerais, Diamantina	B. Loeuille 613	SPF
Lychnophora morii H.Rob.	Brazil, Bahia, Palmeiras	B. Loeuille 658	SPF
Lychnophora salicifolia Mart.	Brazil, Bahia, Ibicoara	C.M. Siniscalchi 348	SPF
Lychnophorella leucodendron Loeuille, Semir & Pirani	Brazil, Bahia, Rio de Contas	B. Loeuille 670	SPF
Maschalostachys mellosilvae Loeuille & Roque	Brazil, Minas Gerais, Mato Verde	B. Loeuille 827	SPF
Minasia pereirae H.Rob.	Brazil, Minas Gerais, Santana do Riacho	B. Loeuille 862	SPF
Paralychnophora harleyi (H.Rob.) D.J.N.Hind	Brazil, Bahia, Mucugê	Ferreira 265	HUEFS
Piptolepis ericoides Sch.Bip.	Brazil, Minas Gerais, Santana do Riacho	B. Loeuille 866	SPF
Soaresia velutina Sch.Bip.	Brazil, Distrito Federal, Planaltina	C.M. Siniscalchi 577	SPF
Stilpnopappus tomentosus Mart. ex DC.	Brazil, Bahia, Rio de Contas, Chapada Diamantina	C.M. Siniscalchi 408	SPF

Table 1. Voucher list of the samples used in the phylogeny. (continued)

Species name	**Location**	**Collector name**	**Herbarium**
Stokesia laevis (Hill) Greene	USA, Louisiana, New Orleans (cultivated at Botanic Garden)	C.M. Siniscalchi 645	SPF
Strophopappus speciosus (Less.) R.Esteves	Brazil, Goiás, Cristalina	C.M. Siniscalchi 373	SPF
Vernonanthura patens (Kunth.) H.Rob.	Guatemala: Chimaltenango	S. Keeley 3297	
Vernonia gigantea (Walter) Trel. ex Branner & Coville	to be provided by V. Funk and S. Keeley		
Vernonia missurica Raf.	to be provided by V. Funk and S. Keeley		
Vernoniastrum ambiguum (Kotschy & Peyr.) H.Rob.	to be provided by V. Funk and S. Keeley		
Moquinia racemosa (Spreng.) DC.	Brazil, Minas Gerais, Olhos d'Água	C.M. Siniscalchi 536	SPF
Pseudostifftia kingii H.Rob.	Brazil, Bahia, Rio de Contas	C.M. Siniscalchi 353	SPF
Munnozia gigantea (Rusby) Rusby	Grown from seed by S. Keeley at CONN; Peru	M. Dilon s.n.	CONN

Table 2. Characters and states used for character state reconstruction.

Character	States
Habit	0 – (bi)annual herb (Fig. 1B) 1 – shrub 2 – basal rosette herb (Fig. 1F, H) 3 – shrub with cauline leaves (Fig. 1I) 4 – subshrub with cauline leaves (Fig. 1A) 5 – rosette at stem tip (Fig. 1E)
Underground system	0 – fasciculate 1 – slightly diffuse (Fig. 6A) 2 – diffuse/thickened (Fig. 6B) 3 – thickened/non-ramified (Fig. 6C) 4 – moniliform (Fig. 6D)
Soil type	0 – limestone 1 – various 2 – quartz/crystalline outcrops (Fig. 1C) 3 – cerrado latosol (Fig. 1H, I) 4 – river margins/sand 5 – wet/moist soils (Fig. 1F) 6 – dry, rocky soils with quartz (Fig. 1E) 7 – quartz outcrops (Fig. 1A) 8 – crystalline outcrops (Fig. 1B)
Leaf margins	0 – entire (Fig. 1E, F, H, I) 1 – lobed (Fig. 1A, B, C, D)
Synflorescence type	0 – no syncephalia 1 – scapose/solitary syncephalium (Fig. A, C, D, E, F, I) 2 – panicle of syncephalia (Fig. 1B, I)
Syncephalia morphology	0 – No syncephalia 1 – hemispherical (Fig. 1A, C, D, E, F) 2 – spherical (Fig. 1B, G, H, I)
Syncephalia growth	0 – No syncephalia 1 – determinate (Fig. 1C, D, E, F, G, H, I) 2 – indeterminate (Fig. 1A, B)
Floret color	0 – purple with purple throat (Fig. 1G, I) 1 – white 2 – purple with white throat (Fig. 1A, B, D, F) 3 – red with yellow throat (Fig. 1E)
Sweeping hair morphology	0 – acute (Fig. 6E) 1 – subulate (Fig. 6F) 2 – clavate (Fig. 6G) 3 – lageniform (Fig. 6H)
Pollen type	0 – type F (Fig. 6K) 1 – type A (Fig. 6I) 2 – type C (Fig. 6J)

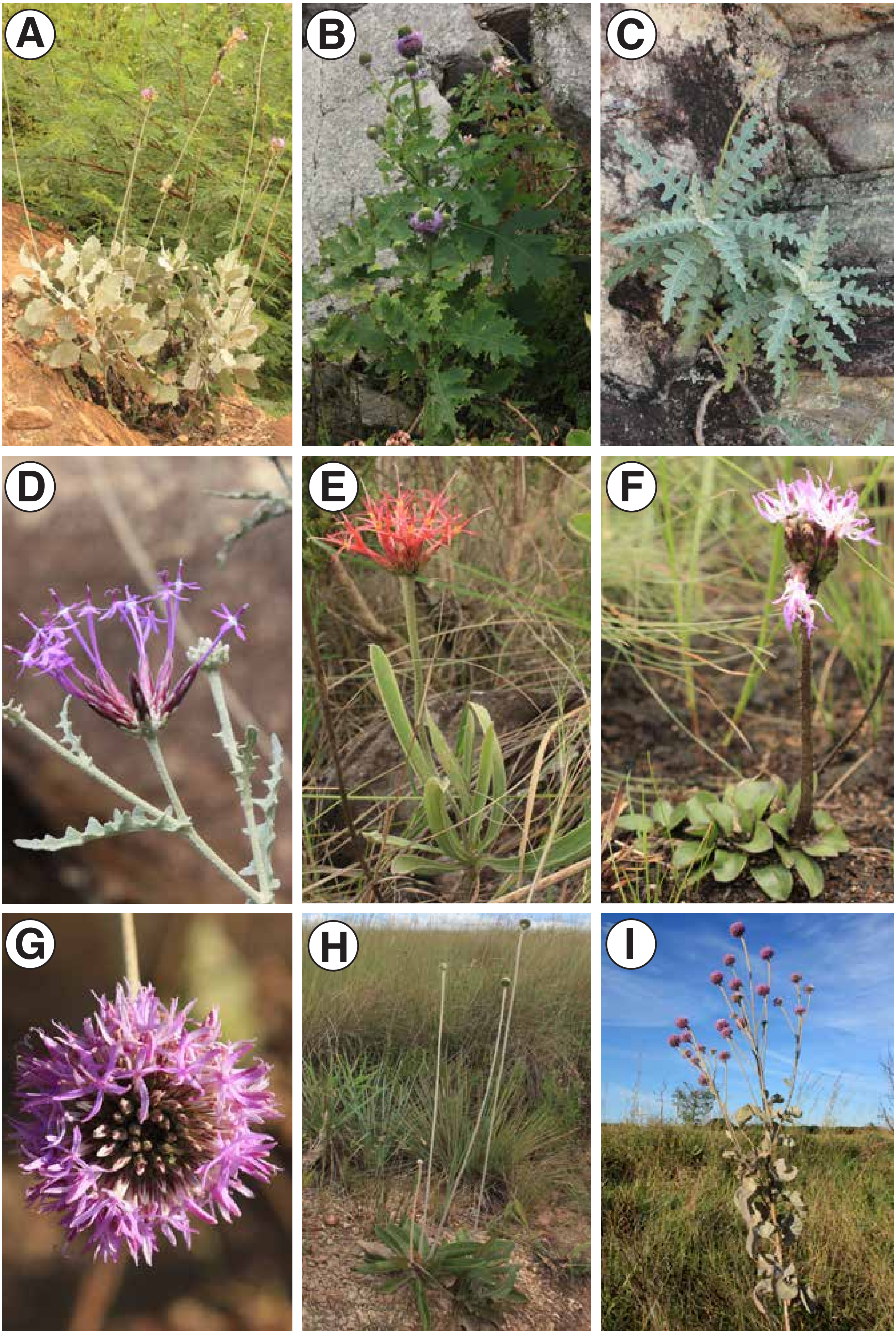

Figure 1. Diversity of *Chresta*. A. *C. martii*, habit. B. *C. pacourinoides,* habit. C. *C. filicifolia*, habit. D. *C. hatschbachii,* syncephalium. E. *C. speciosa*, habit and syncephalium. F. *C. souzae*, habit. G. *C. pycnocephala,* syncephalium. H. *C. scapigera*, habit. I. *C. sphaerocephala*, habit.

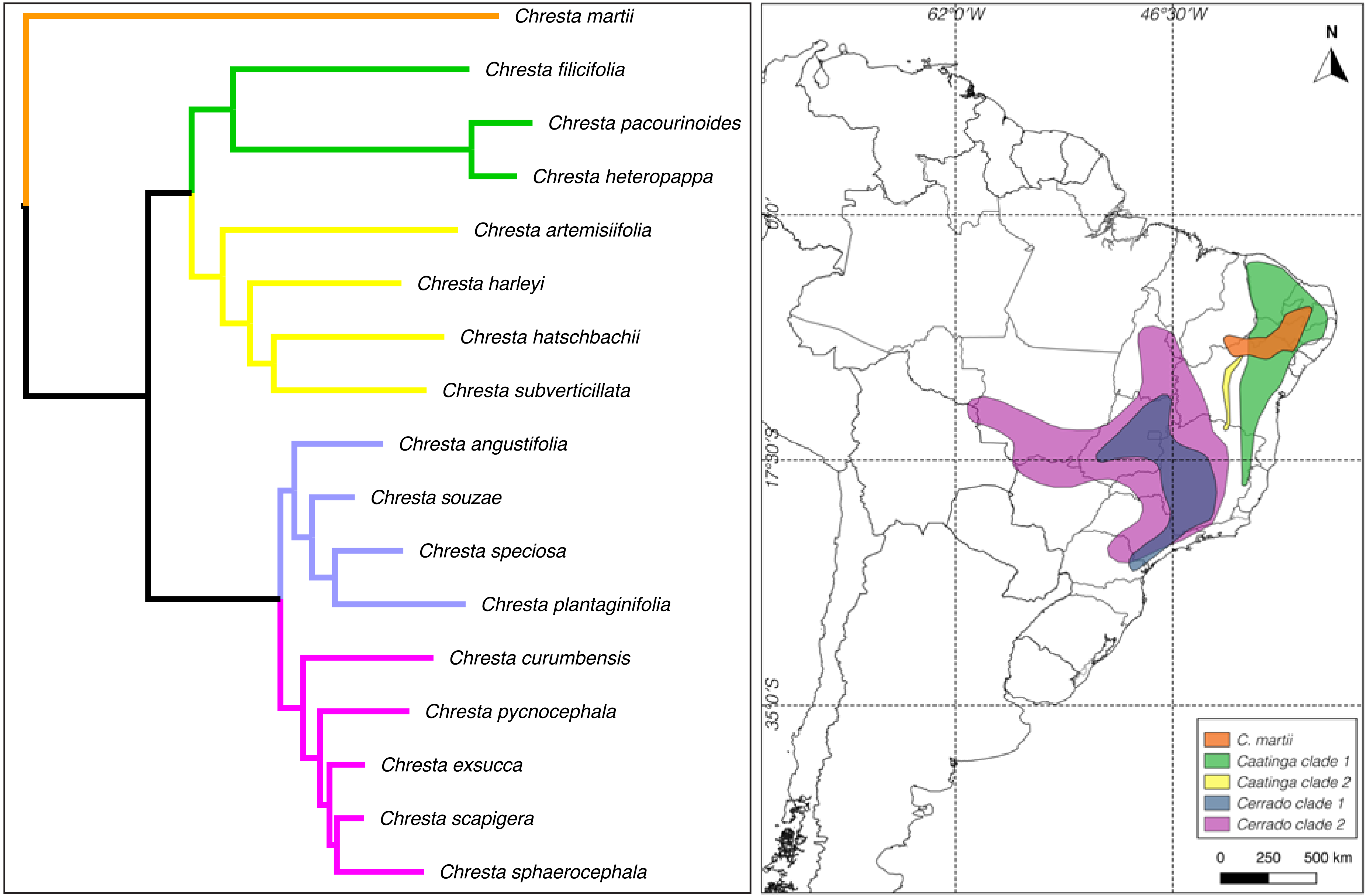

Figure 2. Geographic distribution of *Chresta* clades.

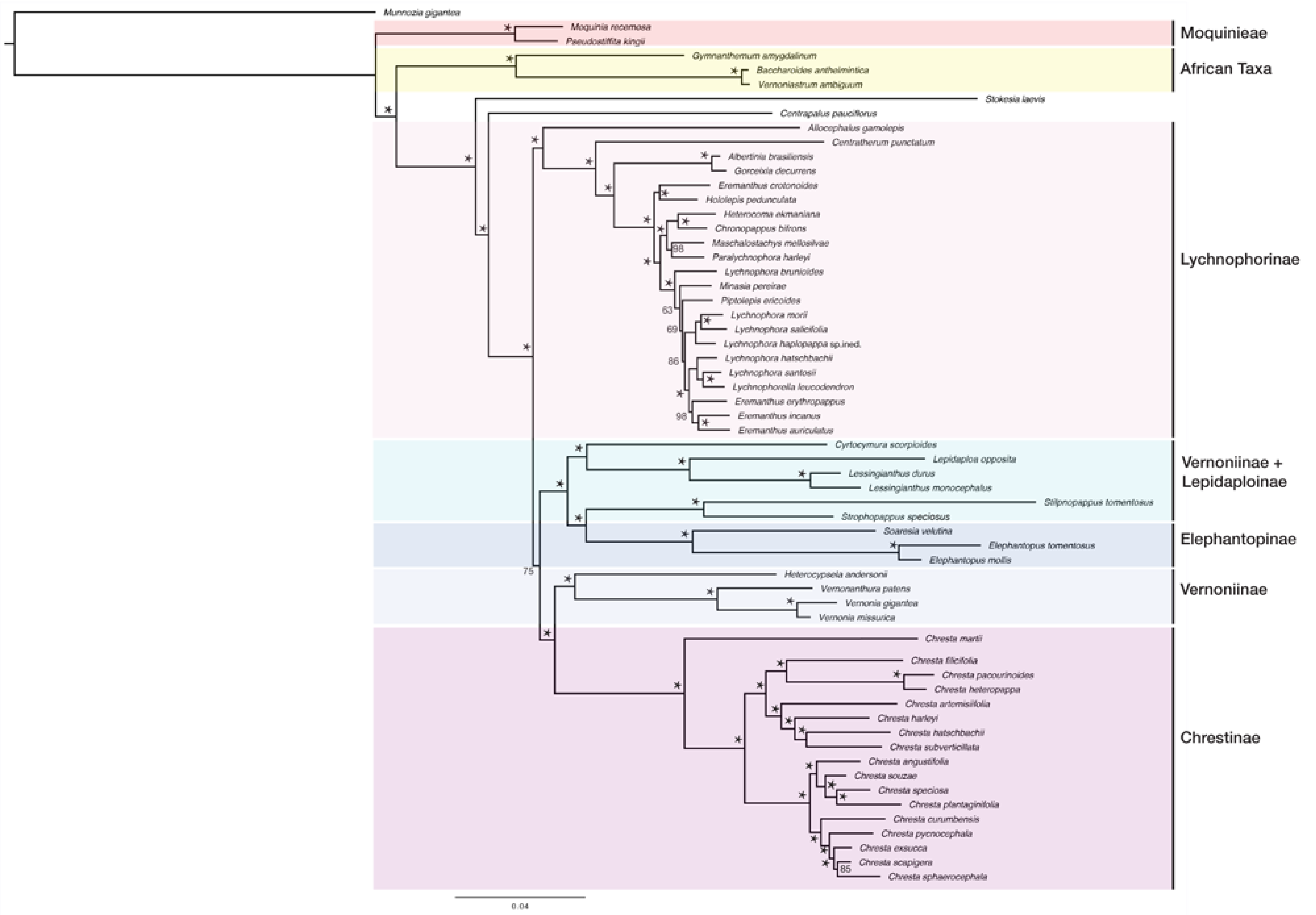

Figure 3. Maximum likelihood tree obtained with the total matrix (709 loci, 733,148 characters), with model GTR+G+I, with 1000 bootstrap replicates. Stars indicate bootstrap value of 100%. Subtribes are indicated on the right.

Figure 4. Maximum likelihood tree obtained with the 75% matrix (89 loci, 107,613 characters), with model GTR+G+I, with 1000 bootstrap replicates. Stars indicate bootstrap value of 100%. Subtribes are indicated on the right.

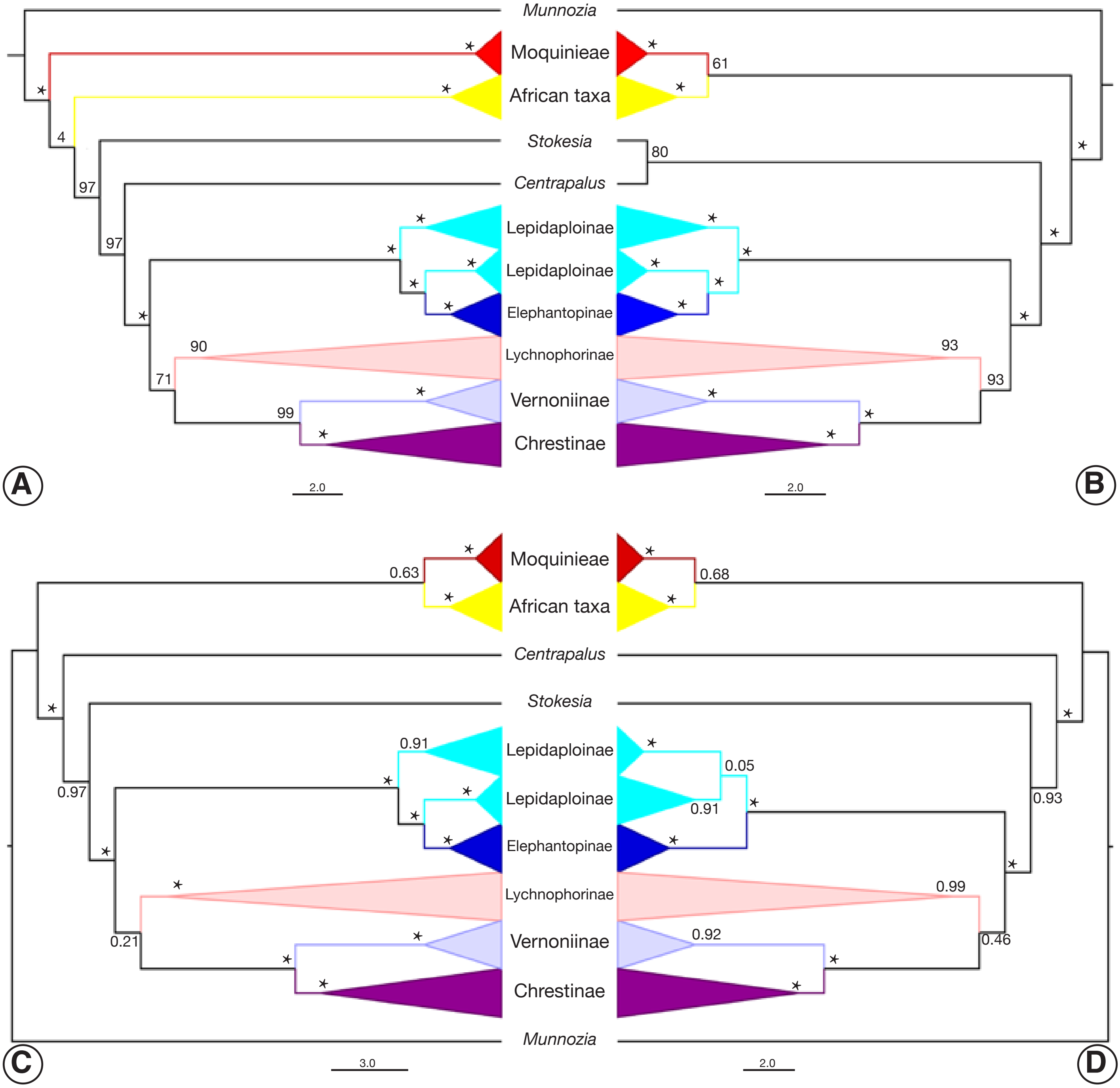

Figure 5. Comparison between trees obtained with different analysis methods. A. Total matrix, parsimony. B. 75% matrix, parsimony. C. Total matrix, pseudocoalescence. D. 75% matrix, pseudocoalescence. Stars indicate 100% jackknife support in parsimony analyses and local posterior probability of 1 in pseudocoalescence analyses.

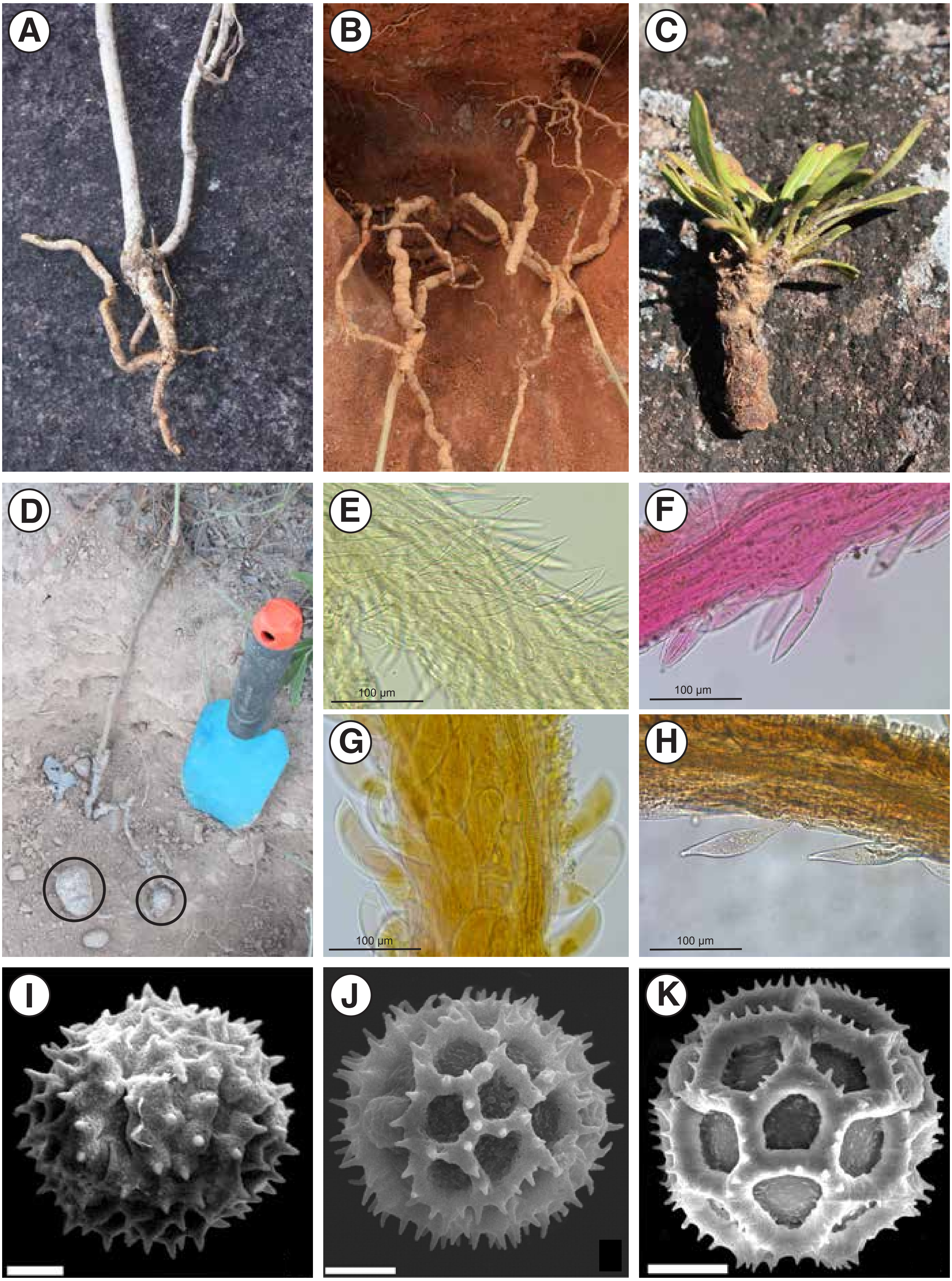

Figure 6. Characters states using for reconstruction. A–D. Underground system. A. Slightly diffuse (*C. filicifolia*). B. Diffuse/thickened (*C. pycnocephala*). C. Thickened/non-ramified (*C. souzae*). D. Moniliform (*C. speciosa*). E–H. Sweeping hair morphology. E. Acute (*C. martii*). F. Subulate (*C. angustifolia*). G. Clavate (*C. curumbensis*). H. Lageniform (*C. filicifolia*). I–K. Pollen types. I. Type A (*C. curumbensis*). J. Type C (*C. pycnocephala*). K. Type F (*C. martii*). Pollen pictures (I–K) taken from Siniscalchi et al. (2017). Picture D taken by B. Loeuille.

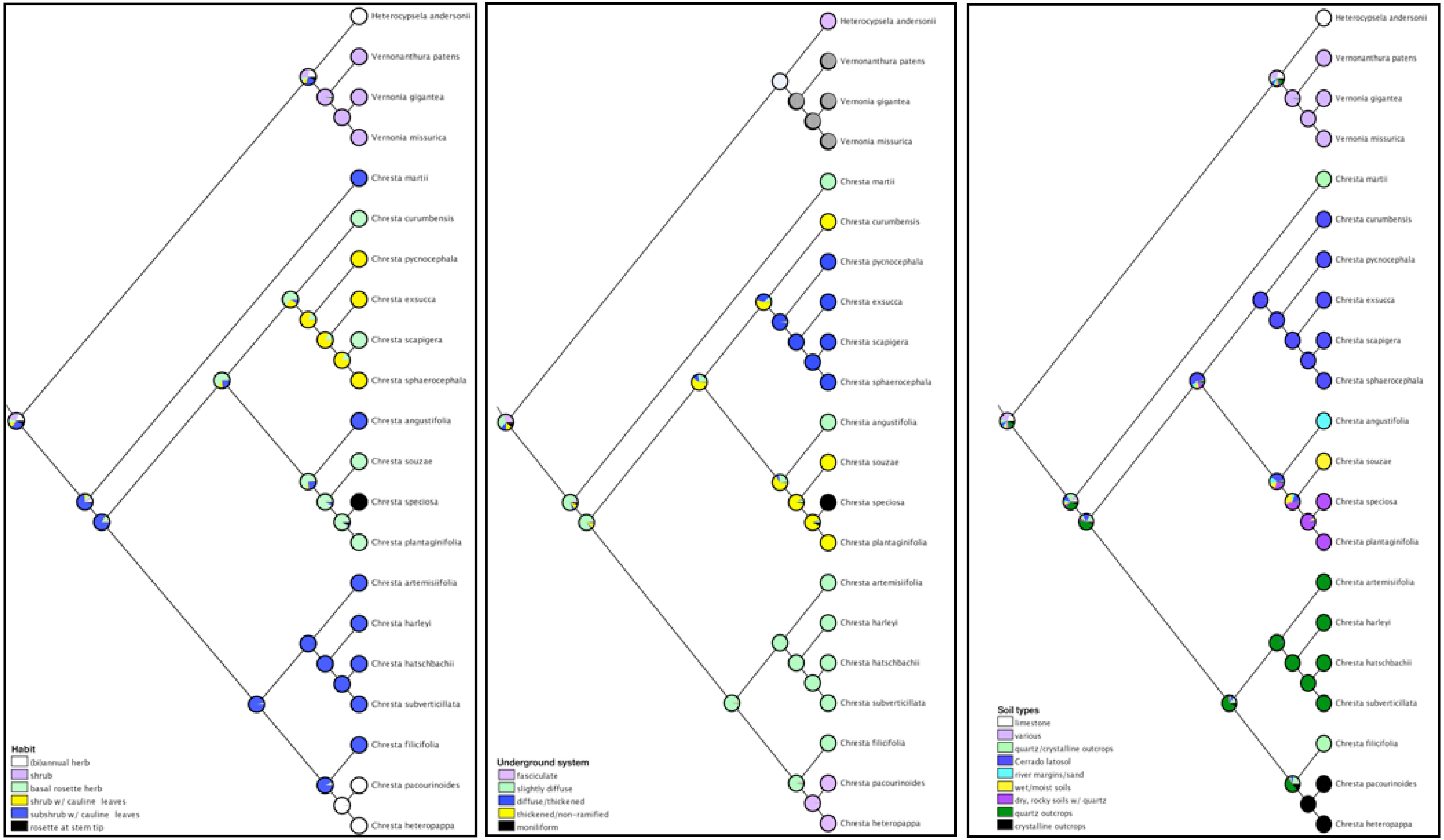

Figure 7. Character reconstructions based on the total ML tree. A. Habit. B. Underground system. C. Soil types.

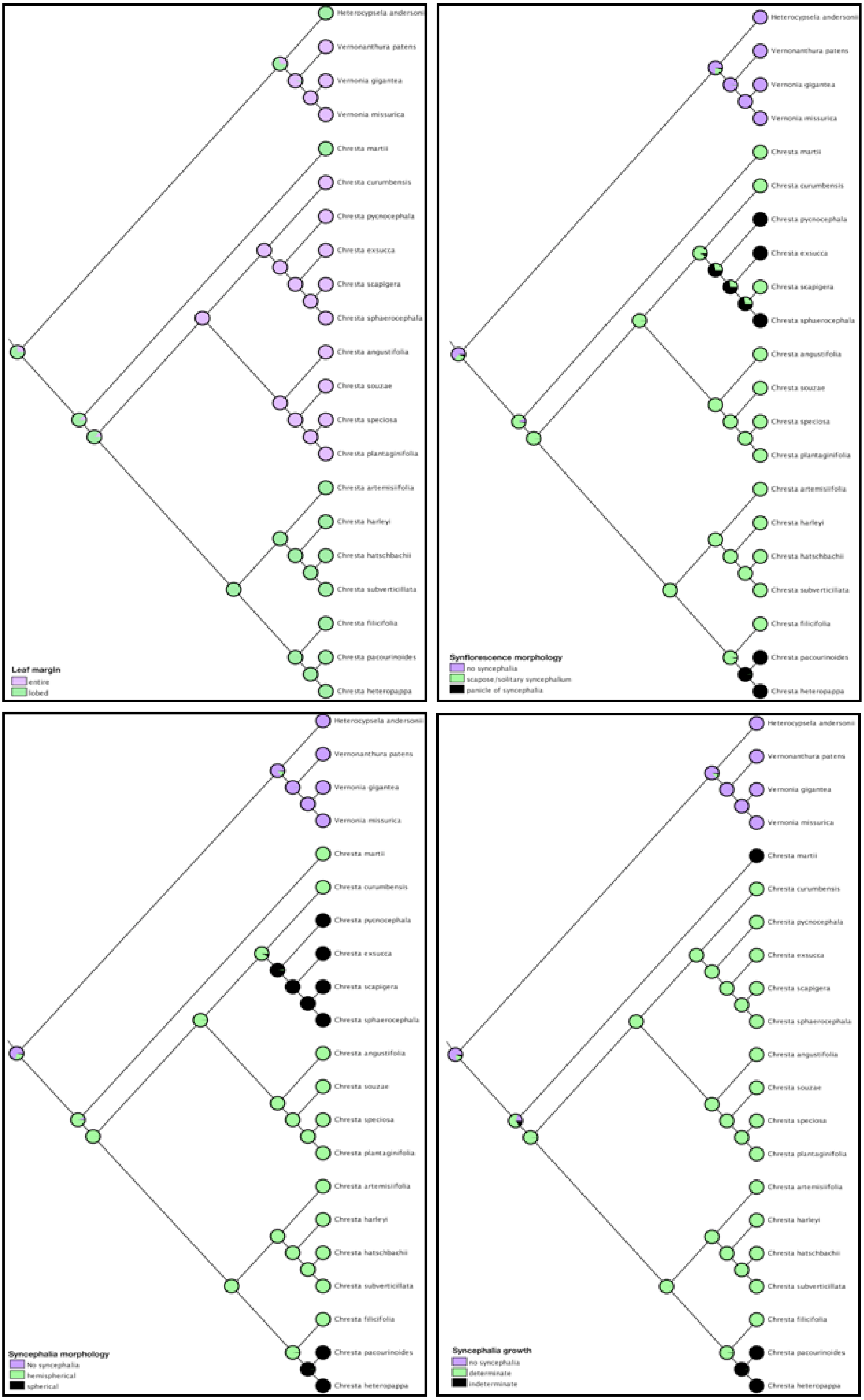

Figure 8. Character reconstructions based on the total ML tree. A. Leaf margin. B. Synflorescence morphology. C. Syncephalia morphology. D. Syncephalia growth.

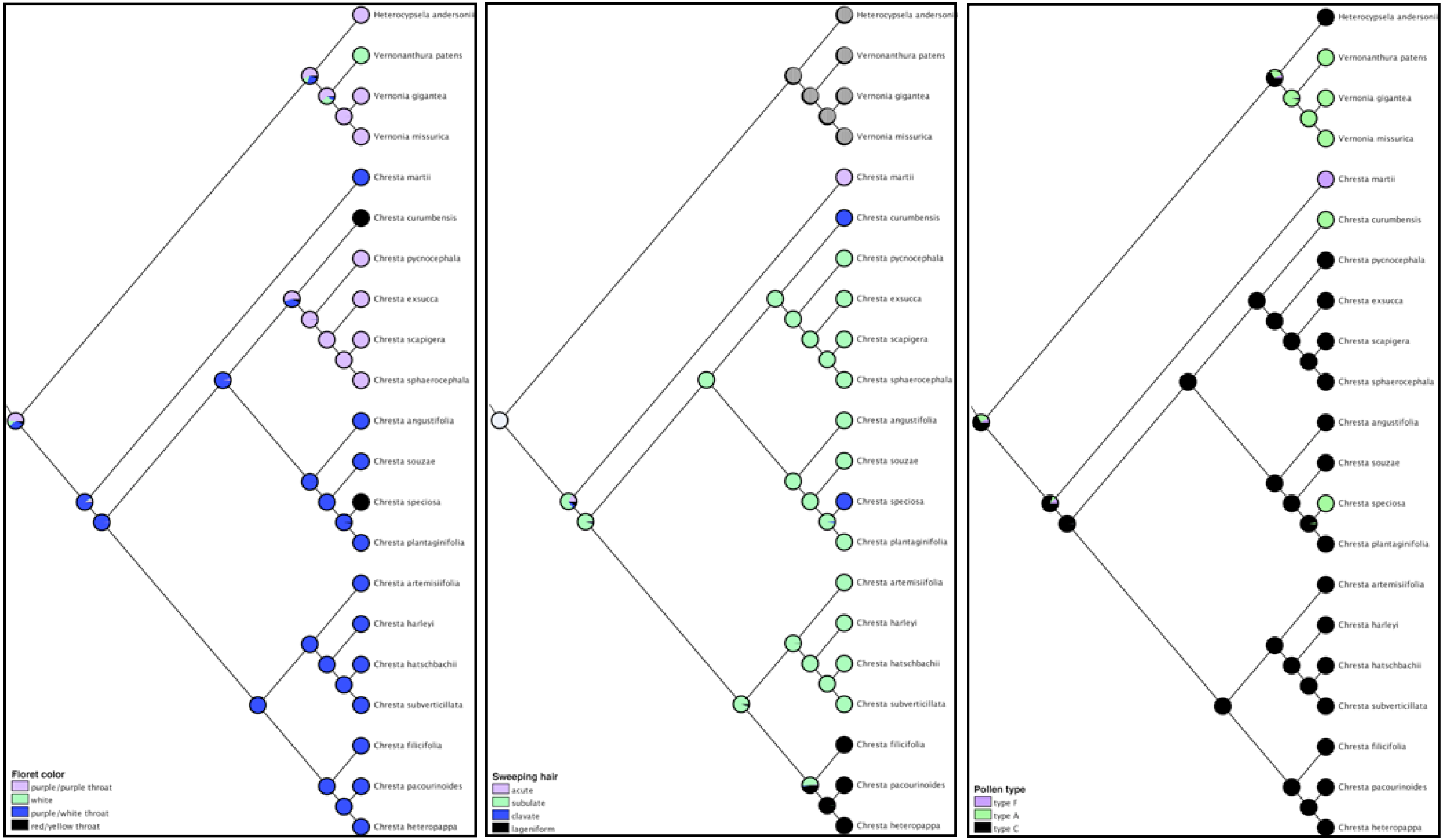

Figure 9. Character reconstructions based on the total ML tree. A. Floret color. B. Sweeping hair morphology. C. Pollen type.

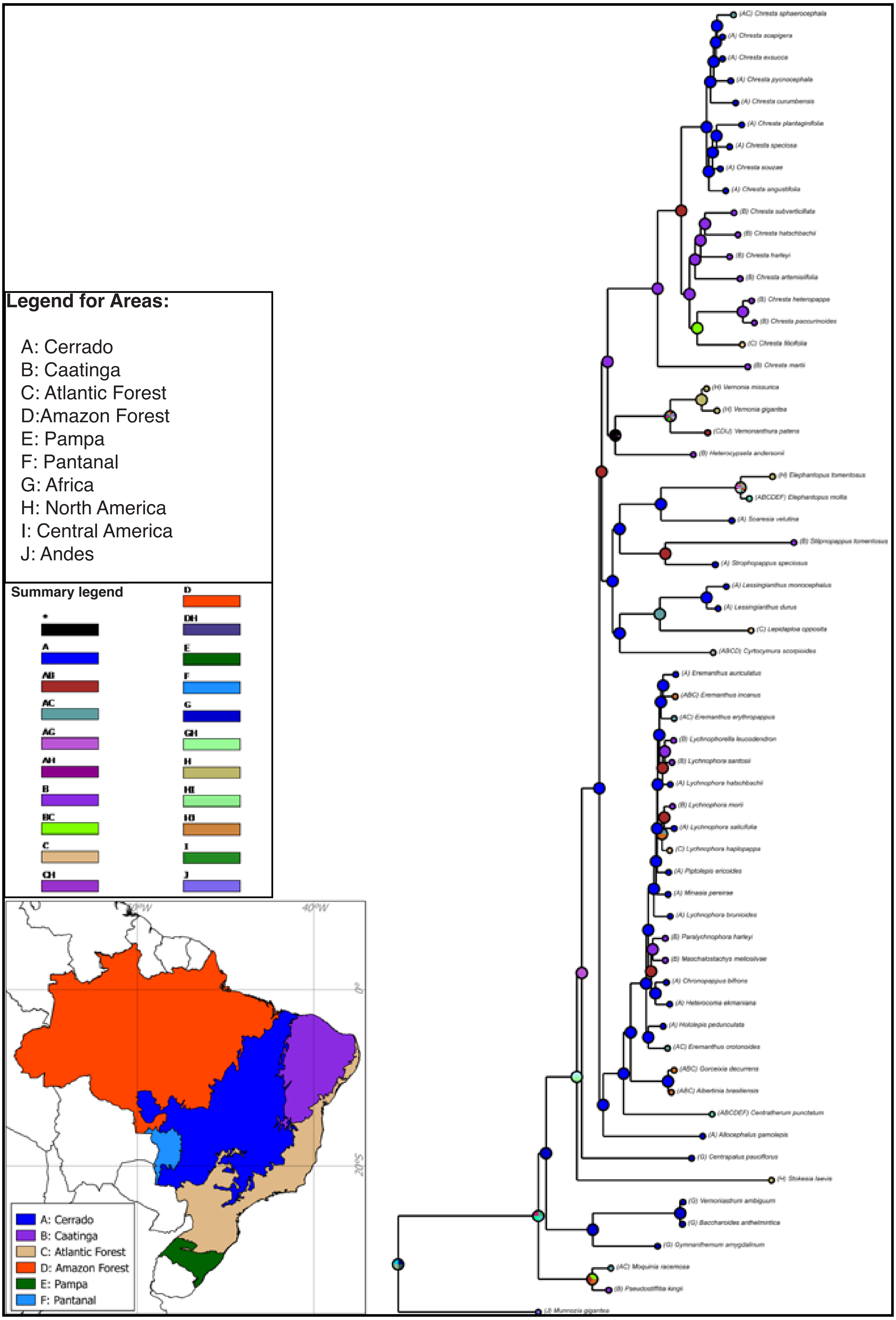

Figure 10. S-DIVA ancestral area reconstruction, based on the ML total tree.

Chapter 2

Population genetics of *Chresta*

Chapter 2
Part A

Using genomic data to develop SSR markers transferable among species of *Chresta* (Vernonieae, Asteraceae) from the Brazilian Caatinga

To be submitted to: Applications in Plant Sciences

Co-authored by: Benoit Loeuille, José R. Pirani and Jennifer R. Mandel

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Using genomic data to develop SSR markers transferable among species of *Chresta* (Vernonieae, Asteraceae) from the Brazilian Caatinga

Introduction

Chresta Vell. ex DC. (Asteraceae) is a genus with 18 species, endemic to Brazil, except for one species that also occurs in Bolivia (BFG 2015). *Chresta* species are distributed in the Cerrado, the savannah-like environment in central Brazil, and in the Caatinga, which is a diverse domain composed mainly of Seasonally Dry Tropical Forests in a semi-arid region in Northeastern Brazil.

The group of species that occur in the Caatinga is composed exclusively of rupiculous plants that have specific requirements of the type of rock where they grow. Usually these rock outcrops or inselbergs form islands that are surrounded by the forest matrix, naturally creating populations that are isolated from one another (Siniscalchi et al., submitted).

Chresta artemisiifolia Siniscalchi & Loeuille, *C. harleyi* H.Rob., *C. hatschbachii* H.Rob. and *C. subverticillata* Siniscalchi & Loeuille grow only over quartzitic outcrops and are restricted to the mountain ranges know as Espinhaço Septentrional and Chapada Diamantina, although there is no overlapping in the distribution range of each species, and they are sequentially distributed in a South to North pattern. *Chresta martii* (DC.) H.Rob. has a wider distribution, occurring in seven Brazilian states and following an East to West distribution, being found both in quartizitc crests and crystalline inselbergs; interestingly, there is some morphological differentiation between population on the extremes of the distribution (see Chapter 3).

The natural isolation of these populations and the presence of morphological variability throughout a geographical gradient raise interesting questions about the evolutionary processes that act on these species. Aiming to study this variation, we developed microsatellite markers using previously obtained genomic data. We used a dataset of approximately 700 conserved nuclear loci sequenced for 17 *Chresta* species to identify putative microsatellites and also an alignment of the whole chloroplast genome of seven species to identify possible microsatellite regions in this genome.

Methods and Results

Total DNA was extracted from dried leaves preserved in silica-gel of each *Chresta* species, using the E.Z.N.A.® SQ Plant DNA Kit from Omega Bio-Tek, with the addition of PVP and Ascorbic Acid to the first extraction buffer (10 mL SQ1 buffer, 100 mg PVP, 90 mg ascorbic acid). For *Chresta martii* extractions, one extra step was added, consisting of two washes with 1 mL of STE buffer (0.25 M sucrose, 0.03 M Tris, 0.05 M EDTA), followed by 10 minutes of centrifugation at 2,000 g, in order to remove mucilage (adapted from Shepherd & McLay 2011).

To obtain sequences from the targeted nuclear data, Illumina libraries were prepared with the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs Inc.), using 15 cycles on the last amplification step. Libraries were then pooled in groups of four and target capture was carried out using the COS Compositae MYbaits (Mandel et al. 2014) kit (Arbor Biosciences), according to the manufacturer's instruction and using a 36-hour incubation step. Sequencing was performed at Macrogen Inc. (South Korea), in an Illumina HiSeq2500 device, in paired-end, high-throughput mode.

The obtained reads were trimmed for quality using Trimmomatic (Bolger et al. 2014) and the assembly of reads into contigs was done using SPAdes (Bankevich et al. 2012), with kmer lengths of 21, 33, 55, 77, 99 and 127. The sequences were matched back to the original probes using the phyluce pipeline (Faircloth 2016), and individual alignments for all the recovered targeted loci were obtained.

We used three different approaches to select regions and markers. The identification of putative microsatellites was done using the plugin Phobos on Geneious (Mayer 2006–2010), searching for di- to pentanucleotides motifs, with a minimal length of 15 base pairs and allowing for imperfect motifs. All loci identified as having repeats were then visually analyzed, and those showing the longest repetitions or most promising regions were selected. Primers were developed using the Primer3 plugin in Geneious (Koressaar & Remm 2007, Untergasser et al. 2012) using the defaults of the program. The designed primers were synthesized at IDT, and a m13 sequence was added to the beginning of the forward primers following the method of Schuelke (2000).

The first dataset used was the individual alignments for each locus mapped by phyluce, which corresponded to *Arabidopsis* genes (see Mandel et al. 2014 for details). The consensus for each alignment was used in a blast search to GenBank, using blastn, to confirm the correspondence and also to identify in which part of the gene the repeat was found (i.e., putatively exonic or intronic). We gave preference to repeats located in introns or less conserved regions. This approach generated 28 markers.

To complement the analysis, the contigs assembled using SPAdes for *C. harleyi* and *C. pacourinoides* were also analyzed before running through the phyluce pipeline, to identify microsatellites out of the targeted regions. The most promising regions were also used in a blast search to GenBank, to confirm they did not correspond to other selected regions. This approach resulted in 18 additional regions.

To obtain chloroplast microsatellites, we used an alignment of seven whole chloroplast genome sequences, four obtained from a non-enriched sequencing and three from target-capture sequences, resulting in seven chloroplast markers. An overview of the selected regions and position of the repeats in the genes is found in Table S1 of the Supplementary Material.

After primers were synthetized, initial PCR tests were carried out using a basic recipe (1.5 µL 10X buffer, 0.5 µL MgCl2 25 nM, 0.2 µL DNTPs 20 nM (5 nM of each), 0.35 µL forward primer at 5 nM, 0.35 µL reverse primer at 20 nM, 0.35 µL of unlabeled m13 at 10 nM, 1.0 µL of Taq and 1.5 µL of DNA, composing a 15 µL reaction, with one individual of each species. A standard touch-down PCR protocol was used, using the following specifications: initial denaturation step of 3 min at 95ºC, then ten cycles with a 30 seconds denaturation step at 94ºC, followed by a 30 sec annealing step, with the temperature starting at 65ºC and decreasing one degree per cycle, then a 1 min extension step at 72ºC, followed for another 30 cycles with 30 sec denaturation at 94ºC, 30 sec annealing at 55ºC and 1 min extension at 72ºC, with a final 10 min extension period at 72ºC. Amplification was verified using agarose gel electrophoresis, and the primers that resulted in clear, single bands, with fragment size larger than 150 bp were selected for genotyping tests, totaling 28 markers.

These tests were carried out in five populations from three species: 2 populations each from *Chresta harleyi* and *C. martii*, totaling 23 individuals each, and one population of *C. hatschbachii*, with 14 individuals (voucher numbers available at Table S2 of the Supplementary Material). The DNA from these samples was extracted as specified above and the same PCR recipe was used, but with the addition of m13 labelled with 6-FAM, VIC or NED, using the same TD55-1min PCR protocol. Final

PCR products were combined into run plates with GeneScan 500 LIZ Size Standard (ThermoFisher Scientific, MA USA) and genotyping was carried out in an ABI 3130XL sequencer at the Molecular Resource Center at the University of Tennessee in Memphis. The sequenced fragments were analyzed on Gene Marker version 2.6.3 and genotypes were scored.

Thirteen of the nuclear markers were shown to be polymorphic among populations of a same species, their sequences, repeat motif and fragment size range are summarized in Table 1, and allele richness measures are presented in Table 2. Cross-amplification in other *Chresta* species is shown on Table 3. The other nine nuclear markers and all the chloroplast markers were polymorphic among species and are shown in Tables S3 and S4 in the Supplementary Material.

Conclusions

We developed 28 microsatellite markers, 22 nuclear and six plastidial, transferable among six *Chresta* species. From these 28, thirteen proved to present intra-specific variation, among populations of three different species, *C. harleyi, C. hatschbachii* and *C. martii*, while the remaining showed some variation among different species. Due to their variability and transferability, these markers will be useful in future genetic studies with species of *Chresta.*

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Table 1. Characteristics of 13 polymorphic nuclear microsatellite markers developed for *Chresta*.

Locus	**Primer sequence**	**T_a (°C)**	**Repeat motif**	**Allele size range (bp)**	**GenBank Accession**
NC2	F: AGGTTAAGGCACCTGCAACA R: GAGGTGGCTGCTGGAATTG	56.5	AG	180 – 200	XXXXXXXX
NC4	F: AGCACCAGTAGCGACGTAAC R: TGAACATCGCTTTTGTTTCTCA	53.1	AG	155 – 171	XXXXXXXX
NC13	F: ATCTCCTGCCCTTGGGTTTG R: GGCAGCTGAAATGTATGCCC	56.8	ACAT	193 – 243	XXXXXXXX
NC15	F: CCCACGAGGAGAYACGTTTG R: YAGCCAAAGCAAGATTYCCC	54.9	CGG	246 – 267	XXXXXXXX
NC16	F: AGCTCCCACCTGGTGTATGA R: AGGGGCTTGRAATTTTGGCTG	57.5	CA	187 – 204	XXXXXXXX
NC17	F: GCGTTGTTGGAATCGTCGAG R: CTTACCCAGACTCTCTGCCG	57.0	AT	308 – 329	XXXXXXXX
NC21	F: GGATGGCTTGGCTTTCCCTA R: CCAAAATTGGCCCTGCTCAT	56.3	TG/AT	362 – 448	XXXXXXXX
NC22	F: TACAAATGGGATGCTGCGGT R: AACCTGCATACACAAGCGGA	57.0	ACCA	200 – 272	XXXXXXXX
NC24	F: ACAAGGATTGTCCCTCTTGCA R: TTGATTGAAAACAGCCGCCT	55.5	TC	157 – 180	XXXXXXXX
NC26	F: ACAGGGAAAGGGTGTGTACC R: CAGAGCCCCTCCTCCTAGAA	57.8	TA/TGA	398 – 422	XXXXXXXX
NC27	F: CTGTYCGGCGRAGCACTGAT R: SACTGTCTTTGACAAATGCAG	52.2	CT	300 – 307	XXXXXXXX
NC29	F: KCTCTGATGGAGGAGACGAT R: GGATCTATTTCCCAAAAGAATTGTCA	53.6	AAG	262 – 273	XXXXXXXX
NC35	F: CCATCCACATGTCTGCCAGT R: ACGCACCCGATCGGAATATC	57.1	AGAT	395 – 419	XXXXXXXX

Note: T_a = annealing temperature.

Table 2. Genetic diversity of 13 nuclear microsatellite markers.

	C. harleyi		***C. harleyi***		***C. hatschbachii***		***C. martii***		***C. martii***	
	HMV (n=10)		**HLA2 (n=13)**		**HTOB (n=14)**		**MGJ2 (n=10)**		**MCN (n=13)**	
	A	**uH_e**	**A**	**uH_e**	**A**	**uH_e**	**A**	**uH_e**	**A**	**uH_e**
NC2	6	0.824	8	0.853	1	0	3	0.7	3	0.714
NC4	3	0.568	5	0.779	1	0	5	0.742	1	0
NC13	1	0	2	0.148	5	0.606	3	0.358	7	0.843
NC15	6	0.765	4	0.739	1	0	1	0	1	0
NC16	5	0.795	6	0.735	5	0.730	2	0.429	4	0.765
NC17	3	0.627	2	0.443	1	0	3	0.542	5	0.312
NC21	1	0	1	0	3	0.833	1	0	1	0
NC22	2	0.337	1	0	6	0.690	2	0.523	2	0.485
NC24	7	0.863	8	0.757	6	0.772	5	0.742	9	0.828
NC26	2	0.529	5	0.711	3	0.537	1	0	2	0.667
NC27	3	0.7	7	0.837	4	0.778	5	0.857	4	0.712
NC29	2	0.325	2	0.443	3	0.140	4	0.574	2	0.443
NC35	2	0.523	6	0.745	2	0.138	3	0.679	1	0

Note: A = number of alleles, uH_e = unbiased expected heterozygosity.

Table 3. Transferability of 13 microsatellite markers across six *Chresta* species.

Locus	*C. artemisiifolia*	*C. filicifolia*	*C. harleyi*	*C. hatschbachii*	*C. martii*	*C. subverticillata*
NC2	+	+	+	+	+	+
NC4	+	+	+	+	+	+
NC13	+	+	+	+	+	+
NC15	+	+	+	+	+	+
NC16	+	+	+	+	+	+
NC17	+	+	+	+	+	+
NC21	+	+	+	+	+	+
NC22	+	+	+	+	+	+
NC24	+	+	+	+	+	+
NC26	+	+	+	+	+	+
NC27	+	+	+	+	+	+
NC29	+	+	+	+	+	+
NC35	+	+	+	+	+	+

Note: positive transferability is showed as ‘+’.

Supplementary Material. Table S1. Putative regions where the microsatellite markers were found.

Locus name	Genomic region	Genomic region description	Possible location on genomic region
CL1	psbA	Photosystem II protein D1	Exon
CL2	atpA	ATP synthase CF1 alpha chain	Exon
CL3	psbD	Photosystem II protein D2	Exon
CL4	ndhK - ndhC	Intergenic region	Intergenic region
CL5	ycf2 - trnL	Intergenic region	Intergenic region
CL6	trnV-rrn16	Intergenic region	Intergenic region
NC1	At2g04790	PTB domain engulfment adapter	Unclear
NC2	At4g22300	Carboxylesterase	Possible intergenic space
NC3	At4g26530	Aldolase superfamily protein	Probably in exon
NC4	Unmapped	Possible intergenic space	Possible intergenic space
NC6	At5g65660	Hydroxyproline-rich glycoprotein family protein	Unclear
NC7		Malectin/receptor-like protein kinase family	Exon
NC8	At3g50690	Leucine-rich repeat (LRR) family protein	Unclear
NC9	At2g38360	Prenylated RAB acceptor 1.B4	Exon
NC10	At3g02360	6-phosphogluconate dehydrogenase family protein	Exon
NC12	At5g24930	Zinc finger CONSTANS-like protein	Exon
NC13	At4g02060	Microsome maintenance protein family	Possible intron
NC15	At4g27390	Transmembrance protein	Probably in exon
NC16	At5g02120	One helix protein, homologous to cyanobacterial high-light inducible proteins	Probably in intron
NC17	At2g01670	Nudix hydrolase homolog 17	Possible intergenic region
NC21	At3g59530	Calcium-dependent phosphotriesterase superfamily protein	Exon
NC22	At1g55000	Peptidoglycan-binding LysM domain-containing protein	Intergenic region
NC24	At2g26210	Ankyrin repeat family protein	Intron
NC26	At1g79040	Photosystem II subunit R	Intron
NC27	At4g09980	Methyltransferase MT-A70 family protein	Intron
NC29	At3g63510	FMN-linked oxidoreductases superfamily protein	Intron
NC35	Unknown	Unknown	Unknown
NC38	Unknown	Unknown	Unknown

Supplementary Material. Table S2. Voucher list of the populations sampled in this study.

Species	Population code	Locality (city, state, country)	Geographic coordinates	Voucher no.	Herbarium
Chresta artemisiifolia Siniscalchi & Loeuille	–	Sento Sé, Bahia, Brazil	10º04’11”S, 41º37’27”W	J. Siqueira Filho 3671	HVASF, SPF
Chresta filicifolia Siniscalchi & Loeuille	–	Conselheiro Pena, Minas Gerais, Brazil	19º15’03”S, 41º22’29”W	C.M. Siniscalchi 512	SPF
Chresta harleyi H.Rob.	HMV	Mato Verde, Minas Gerais, Brazil	15º23’19”S, 42º46’36”W	C.M. Siniscalchi 449	SPF
	HLA2	Licínio de Almeida, Bahia, Brazil	14º34’22”S, 42º31’27”W	C.M. Siniscalchi 460	SPF
	–	Urandi, Bahia, Brazil	14º44’46”S, 42º34’25”W	C.M. Siniscalchi 457	SPF
	–	Licínio de Almeida, Bahia, Brazil	14º34’38”S, 42º31’30”W	C.M. Siniscalchi 459	SPF
	–	Jacaraci, Bahia, Brazil	14º49’46”S, 42º26’06”W	C.M. Siniscalchi 462	SPF
	–	Caetité, Bahia, Brazil	14º15’24”S, 42º31’21”W	C.M. Siniscalchi 463	SPF
	–	Caetité, Bahia, Brazil	14º15’57”S, 42º31’45”W	C.M. Siniscalchi 464	SPF
Chresta hatschbachii H.Rob.	HTOB	Oliveira dos Brejinhos, Bahia, Brazil	12º22’19”S, 42º54’19”W	C.M. Siniscalchi 468	SPF
Chresta martii (DC.) H.Rob.	MJG2	Jaguarari, Bahia, Brazil	9º56’11”S, 40º15’49”W	C.M. Siniscalchi 474	SPF
	MCN	Casa Nova, Bahia, Brazil	9º22’52”S, 40º48’02”W	C.M. Siniscalchi 482	SPF
	–	Jaguarari, Bahia, Brazil	10º06’11”S, 40º13’47”W	C.M. Siniscalchi 473	SPF
	–	Sobradinho, Bahia, Brazil	9º28’54”S, 40º51’59”W	C.M. Siniscalchi 479	SPF
	–	Petrolina, Pernambuco, Brazil	9º21’41”S, 40º23’05”W	C.M. Siniscalchi 565	SPF
	–	Teixeira, Paraíba, Brazil	7º12’10”S, 37º15’30”W	C.M. Siniscalchi 635	SPF
	–	Santa Luzia, Paraíba, Brazil	6º53’05”S, 36º53’02”W	C.M. Siniscalchi 638	SPF
	–	Parelhas, Rio Grande do Norte, Brazil	6º42’09”S, 36º41’28”W	C.M. Siniscalchi 639	SPF
	–	São João do Sabugi, Rio Grande do Norte, Brazil	6º41’46”S, 37º09’47”W	C.M. Siniscalchi 641	SPF
	–	Campo Formoso, Bahia, Brazil	10º11’32”S, 41º04’45”W	J. Siqueira Filho 3537	HVASF, SPF
	–	Sento Sé, Bahia, Brazil	10º22’12”S, 41º44’58”W	J. Siqueira Filho 3657	HVASF, SPF
Chresta subverticillata Siniscalchi & Loeuille	–	Gentio do Ouro, Bahia, Brazil	11º11’31”S, 42º43’03”W	C.M. Siniscalchi 630	SPF
	–	Gentio do Ouro, Bahia, Brazil	11º05’48”S, 42º43’18”W	C.M. Siniscalchi 631	SPF
	–	Gentio do Ouro, Bahia, Brazil	11º06’26”S, 42º43’10”W	C.M. Siniscalchi 634	SPF

Supplementary Material. Table S3. Characteristics of 6 chloroplast markers and 9 nuclear markers developed for *Chresta*.

Locus	**Genome**	**Primer sequence**	**Ta (°C)**	**Repeat motif**	**Allele size range (bp)**	**GenBank Accession**
CL1	Chloroplast	F: AACCATGAGCGGCTACGATA R: TGGTAACCTCTAGTTTGATCAGGG	55.9	AAG	124	XXXXXXXX
CL2	Chloroplast	F: TTCCAGCCAATGATGACGCT R: ACCACCTCTTTCTCGACTTGAC	55.6	AAT	287 – 300	XXXXXXXX
CL3	Chloroplast	F: AAAGGGAGTGTGTGCGAGTT R: ATGCTGCGTCTGGACTTCAA	57.0	AAAG	293 – 298	XXXXXXXX
CL4	Chloroplast	F: ACTGATGGGGCCAACAAACA R: CGGCAGGGGGATTCTGAAAT	57.6	AAAT	248 – 254	XXXXXXXX
CL5	Chloroplast	F: GCGCGTGTGATACATGTTCC R: TAATGGCTGTAGACCCCCGA	57.7	AAT	340 – 343	XXXXXXXX
CL6	Chloroplast	F: AACGTGTCACAGCTTCCTCC R: GCTAGGTAAGCGCCCTGTA	56.6	AAAG	277 - 278	XXXXXXXX
NC1	Nuclear	F: GCCCAAGAGTTATCGCTAAAGC R: GCCGCCACGTAGACTTCATA	57.0	AAAT	164	XXXXXXXX
NC3	Nuclear	F: AGTGGGTGTGGGTGTAGGAT R: TGACGTGGAGCAATTGACGA	56.8	AGG	123	XXXXXXXX
NC6	Nuclear	F: ATAGGCTTTCCACTCGGCAC R: CGGCCAAACAGGAGCAAATC	57.1	AATTC	286 – 291	XXXXXXXX
NC7	Nuclear	F: CCCAATCACATGGTCACGGA R: TCAGGAACTCAAAGAAAATAACATCA	52.9	AAAAT	158 – 160	XXXXXXXX
NC8	Nuclear	F: GCCGTTGAAGTTGAGGAGGA R: GCCCACCAAGATCACCATCA	57.5	ATC	218 – 240	XXXXXXXX
NC9	Nuclear	F: CGACCGATCAGCATTCTCCA R: ATCACCAAGGGAGGATCGGA	57.8	ACG	241	XXXXXXXX
NC10	Nuclear	F: GGTGGCAACGAGTGGTATGA R: GTAAGCCTCAAAGGACCCTCC	57.4	AG	169	XXXXXXXX
NC12	Nuclear	F: GTAGAGCAGACTCCGCCTTC R: GGGGTTGGCGGAGTGAATAT	57.3	AC	209	XXXXXXXX
NC38	Nuclear	F: AGATCGAGGCGAAACCCAAG R: CACCAGTCTCAGTCGCAGTC	57.6	ACC	265 – 321	XXXXXXXX

Note: T_a = annealing temperature.

Supplementary Material. Table S4. Genetic diversity of 6 chloroplast markers and 9 nuclear markers developed for *Chresta*.

	C. artemisiifolia **(n=2)**		***C. filicifolia*** **(n=4)**		***C. harleyi*** **(n=28)**		***C. hatschbachii*** **(n=4)**		***C. martii*** **(n=22)**		***C. subverticillata*** **(n=12)**	
	A	**He**	**A**	**He**	**A**	**He**	**A**	**He**	**A**	**He**	**A**	He
NC1	1	0	1	0	2	0.082	1	0	2	0.089	2	0.356
NC3	1	0	1	0	1	0	1	0	1	0	1	0
NC6	1	0	1	0	1	0	1	0	3	0.545	1	0
NC7	0	0	1	0	2	0.045	1	0	1	0	2	0.485
NC8	1	0	1	0	1	0	1	0	6	0.630	1	0
NC9	1	0	1	0	1	0	1	0	1	0	1	0
NC10	1	0	1	0	2	0.268	1	0	4	0.694	1	0
NC12	1	0	1	0	2	0.216	1	0	3	0.330	1	0
NC38	2	0.500	5	0.893	7	0.803	1	0	10	0.859	3	0.750

Note: A = number of alleles, uH$_e$ = unbiased expected heterozygosity.

	C. artemisiifolia **(n=2)**		***C. filicifolia*** **(n=4)**		***C. harleyi*** **(n=28)**		***C. hatschbachii*** **(n=4)**		***C. martii*** **(n=22)**		***C. subverticillata*** **(n=12)**	
	A	**h**	**A**	**h**	**A**	**h**	**A**	**h**	**A**	**h**	**A**	**h**
CL1	1	0	1	0	1	0	1	0	1	0	1	0
CL2	1	0	1	0	1	0	1	0	4	0.633	1	0
CL3	1	0	1	0	1	0	1	0	1	0	1	0
CL4	1	0	1	0	1	0	1	0	2	0.514	1	0
CL5	1	0	1	0	1	0	1	0	2	0.189	1	0
CL6	1	0	1	0	1	0	1	0	1	0	1	0

Note: A = number of alleles, h = unbiased haploid diversity.

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Chapter 2
Part B

Population genetics of five rupiculous *Chresta* species (Asteraceae, Vernonieae) from the Brazilian Caatinga unveils different patterns for morphologically similar taxa

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Co-authored by: Benoit Loeuille, José R. Pirani and Jennifer R. Mandel

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Population genetics of five rupiculous *Chresta* species (Asteraceae, Vernonieae) from the Brazilian Caatinga unveils different patterns for morphologically similar taxa

Abstract

Measures of genetic diversity and population structure are fundamental when studying evolutionary processes in different species. In the present study, we use nuclear SSR markers to investigate the population genetics of five rupiculous species of *Chresta* that occur in the Brazilian Caatinga and present fragmentary populations. Eleven SSR markers previously developed for species of *Chresta* were used, and a total of 312 individuals from 27 populations were genotyped. Different species presented different patterns, *C. harleyi* showed medium levels of inbreeding and moderate population structuring; *C. martii* showed very high levels of inbreeding and structuring, with populations grouping in two geographically separate clusters, while *C. subverticillata* showed excess of heterozygotes. We discuss possible effects on the evolution of the group and also impacts on the conservation of these species.

Keywords: Chrestinae, Compositae, rock outcrops, microsatellites, genetic structure.

Introduction

The semi-arid Caatinga covers around 10% of the Brazilian territory, being surrounded by the Atlantic Forest, Amazon and Cerrado domains. Seasonally Dry Tropical Forests prevail in the Caatinga, but there are at least eleven different plant community types throughout the domain (Giulietti et al. 2004, Moro et al. 2014). Despite being one of the most threatened and least conserved phytogeographical domains in South America (Castelletti et al. 2003, Leal et al. 2005, Ribeiro et al. 2015), it is also one of the least well understood and studied, especially in terms of evolutionary and speciation processes.

While phylogeographical and population genetics studies are common with species from the Atlantic Forest and Cerrado (comprehensive review in Turchetto-Zolet et al. 2013), fewer have focused on Caatinga plants. Caetano et al. (2008) and Collevatti et al. (2012) investigated the genetic structure of widely distributed trees aiming to verify the Pleistocenic Arc hypothesis; Vieira et al. (2015) followed the same goal, but used a species of *Ficus* that occurs in limestone outcrops. There also two studies using Araceae species to investigate the relations of the humid forest remnants found within the Caatinga (brejos de altitude) with the Atlantic Forest and Amazon (Andrade et al. 2007, Andrade et al. 2008). Rock outcrop orchids have also been evaluated under these approaches, with species occurring on the *campos rupestres* (highland rocky fields) (Cruz et al. 2011) and in granitic inselbergs (Pinheiro et al. 2014).

Alternative good candidates to such studies are members of Asteraceae, which is the third richest family in Brazil, with ca. 2000 species, being the most numerous in two of the six phytogeographical domains (BFG 2015). The taxonomy and systematics of the family have been fairly well studied in the country and while the ecological importance of the family is undeniable, population level studies with endemic Asteraceae in Brazil are still rare. Interestingly, most of these studies focus on species endemic to *campos rupestres* (Jesus et al. 2001, Gomes et al. 2004, Freitas et al. 2008, Collevatti et al. 2009, Feres et al. 2009, Jesus et al. 2009), which are among the vegetation physiognomies found within the Cerrado and Caatinga domains. *Campos rupestres* are mostly restricted to the highest portions of the Espinhaço Range in central Brazil, usually on shallow, litholic soils around quartzitic outcrops, with high levels of endemic and micro-endemic plants (Fiaschi & Pirani 2009).

Asteraceae is also listed as the third richest family in the Caatinga, with 284 species (BFG 2015). Most of this diversity is concentrated on the *campos rupestres* located on Northern Minas Gerais, on the Espinhaço Range and on Southern Bahia on the Chapada Diamantina massif. Few species occur in other vegetation types and most of these are common, widespread species (Moro et al. 2014).

Among the Asteraceae of the Caatinga, *Chresta* Vell. ex DC. may be highlighted, as seven of its 18 species occur exclusively in rock outcrops within the Caatinga (see Chapter 3). Additionally, in sharp contrast to other Asteraceae, these species do not occur on typical *campos rupestres*, but are restrict to different types of rock where they occur. *Chresta martii* is found on quartzitc or granitic outcrops, along an East-West distribution. *Chresta artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* occur on quartzitic outcrops and transitional environments known as *caatinga rupestre* (Campos et al. 2017), distributed along the Espinhaço Septentrional and Chapada Diamantina massif, on a South-North pattern. *Chresta heteropappa* and *C. pacourinoides* occur on granitic outcrops and inselbergs associated to *brejos de altitude,* which are forest remnants found on top of low elevations within the Caatinga (Souza & Oliveira 2006) (Fig. 1).

These seven *Chresta* species have morphological features that correlate to the semi-arid climate where they are found, such as deeply lobed leaves, dense indument covering the whole plant and roots that penetrate on the rocks where they grow. Two of the species developed herbaceous and biannual habit, which allow them to dry out during the driest seasons (see Chapters 1 and 3).

Populations of these species are naturally isolated from each other, as the outcrops where they grow are usually surrounded by the prevalent dry forest matrix extending over non-rocky soils, or even by vegetation that has been degraded due to human action or pastures and grazing lands (Fig. 1B, C). This raises questions about how much contact these populations still have with each other and how the cohesion of each species is maintained, since low gene flow among them would imply stronger differentiation within each population due to drift or local adaptation, which might eventually lead to complete isolation and the formation of new species (Futuyma 1987, Morjan & Rieseberg 2004). Thus, it seems likely that geographic isolation may influence speciation processes in this kind of environment.

All these factors play a significant role in the development of conservation and management plans for threatened species. Species that present marked genetic structure and low gene flow among populations may require that larger areas and more populations are included in conservation units, in order to maintain the genetic diversity in the taxon (Moritz 1994, Byrne 2007). This is especially critical for species that inhabit in fragmented environments, like rock outcrops, and in regions that already suffer intense anthropogenic pressures, as is the Caatinga, where less than 2% of the original area is included in strict conservation areas (Ribeiro et al. 2015).

Therefore, in this study we chose five species of *Chresta* endemic to the Brazilian Caatinga as a model to study gene flow, population structure and speciation in the rocky environments found within this phytogeographical domain. Our main objectives are:

1. Use genetic diversity indexes to understand the similarities and differences among

populations of these five species;
2. Verify the levels of genetic structure in each species and estimate migration among populations, to investigate gene flow;
3. Investigate possible speciation mechanisms affecting rupiculous plants;
4. Assess possible impacts to the conservation of these species.

Methods

Study species

We chose five species of *Chresta* that occur on quartzitic outcrops on the Brazilian Caatinga (Fig. 1A) in order to compare the genetic structure and diversity between them. All species are perennial subshrubs that grow directly over rocks, with their roots penetrating in small crevices on the rocks (Fig. 1B-E, G, H). They all have lobed, silvery-green leaves, densely covered by trichomes, and purple florets that are grouped into secondary capitula (Fig. 1D-I). There is no information about chromosome numbers, ploidy or reproductive systems for these species, but our field observations have shown they are frequently visited by insects, especially bees and bumblebees.

Chresta martii is the sister species to all other species in the genus, including those that occur on the Cerrado (Siniscalchi et al. in prep., see Chapter 1). This species has a relatively wide but patchy distribution along the Caatinga, occurring both on quartzitic outrocps or crests or on crystalline outcrops with high quartz contents, in seven Brazilian states, following an East-West pattern. The populations group in three main areas: one in western Bahia, Pernambuco and Piauí, one in the Xingó area, in Sergipe, eastern Bahia and Alagoas, and the last in central Paraíba and Rio Grande do Norte.

Chresta artemisiifolia, C. harleyi, C. hatschbachii and *C. subverticillata* compose one of the clades in *C.* sect. *Rupicola* (Siniscalchi et al. in prep., see Chapter 3). The four species occur in the geological formation known as Espinhaço Septentrional or Serra Geral and also in the Northwestern portion of the Chapada Diamantina massif, in a South-North pattern (geological definitions follow Alkmim 2012). There is no distribution overlap among populations of these species: *Chresta harleyi* is found on the southernmost end, with several populations known over an area of ca. 200 km extending from northern Minas Gerais to southern Bahia. Approximately 200 km north of the latter area, *C. hatschbachii* occur, known from a single locality, and ca. 120 km North from this area lay the few known populations of *C. subverticillata*, which are restricted to the quartzitic formations around São Inácio locality, in the northwestern tip of the Chapada Diamantina massif. About 120 km Northeast from São Inácio the few known populations of *C. artemisiifolia* are found, also on the Chapada Diamantina massif, but on the northeastern end. There is an overlap in the distribution of *C. artemisiifolia* and *C. martii* (Fig. 1A), but we found no individuals with hybrid or intermediate phenotypes on the area of sympatry when analyzing herbarium specimens and in field observations, probably indicating they are reproductively isolated.

Due to the phylogenetic relationships in the genus, we analyzed *C. martii* separately from the other four species, and because of the interesting distribution pattern of the other species, with four of them located closely together, we analyzed the data from *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* together in the majority of the analysis, to investigate any evidence of gene flow among the four species.

Chresta martii was described by Candolle (1836) in *Stachyanthus* [=*Argyrovernonia*], a genus separate from *Chresta,* which then contained only Cerrado species. *C. harleyi* was described in the 1980s based on specimens collected in the previous decade (Robinson 1983). *Chresta hatschbachii* and *C. subverticillata* were usually identified as *C. harleyi* before being described as independent species. *Chresta hatschbachii* was described in the 2000s (Robinson 2005) and has been sparsely collected since then, while *C. subverticillata* was recently described (Siniscalchi et al. submitted, Chapter 4), but there are several records collected since the 1980s. *Chresta artemisiifolia* is the most recent addition to the genus, being completely unknown until 2010, likely due to the remoteness of its location (Chapter 4).

Regarding conservation status, none of the species are officially listed as threatened taxa, but some have been pointed as candidates for listing, due to very restricted occurrence and several environmental pressures (Siniscalchi et al. in prep., see Chapter 3, 4). A few populations of *C. martii, C. harleyi* and *C. artemisiifolia* are located within conservation units; however, most of the populations are not under any kind of management plan.

Sampled populations and areas

Herbarium specimens were used as starting points to define populations for collections, mainly based on collections from ALCB, CEPEC, HUEFS, HVASF, IPA, NY, PEUFR, R, RB, SPF, UFPE and US (acronyms follow Thiers 2018). Geographic coordinates were obtained from herbarium sheet labels and when collections were not georeferenced, the stated municipality was used as basis to search for propitious environments. Five field expeditions were undertaken from April 2014 to December 2015, on the states of Bahia, Minas Gerais, Paraíba, Pernambuco and Rio Grande do Norte. Populations were defined as all the individuals growing over one outcrop, as outcrops are usually inserted in a forest-like vegetation matrix over other types of soil, being naturally isolated from each other.

Due to the rupiculous nature of these plants, with roots penetrating directly among rock crevices, we considered each isolated individual as a unique genetic unit, as the existence of clonal individuals is very unlikely. In each population, at least five young leaves were collected from at least ten isolated individuals and the samples were preserved in silica-gel. One voucher of each population was prepared and deposited at the SPF herbarium, with duplicates distributed to other herbaria (mainly RB, UFPE and US).

A total of 312 individuals from 27 populations were collected (Table 1). Samples were obtained from eight populations of *C. harleyi*, three populations of *C. subverticillata,* which were isolated but very close to each other; and from a single population each of *C. artemisiifolia* and *C. hatschbachii.* Samples from 14 populations of *C. martii* were collected, from two geographic groupings: seven from Bahia and Pernambuco (Western group) and seven from Paraíba and Rio Grande do Norte (Eastern group).

Distance between populations of all species varied from 0.5 to 1493 km. The vegetation surrounding each outcrop varied from preserved forested areas to areas with high human influence, such as pastures or constructions.

DNA extraction and genotyping

Extraction of DNA from the collected leaves was carried out with the E.Z.N.A.® SQ Plant DNA Kit from Omega Bio-Tek, with the addition of PVP and Ascorbic Acid to the first extraction buffer (10 mL SQ1 buffer, 100 mg PVP, 90 mg ascorbic acid). For *Chresta martii,* one initial extra step was added to remove mucilage, consisting of two washes with 1 mL of STE buffer (0.25 M sucrose, 0.03 M Tris, 0.05 M EDTA), followed by 10 minutes of centrifugation at 2,000 g, as adapted from Shepherd & McLay (2011).

The primers NC13, NC15, NC16, NC17, NC21, NC22, NC24, NC26, NC27, NC29 and NC35 developed by Siniscalchi et al. (Chapter 2, part A) were selected for this study. Amplification by PCR was carried out in a 15 µL volume, containing 1.5 µL 10X buffer, 0.75 µL MgCl2 25 nM, 0.2 µL dNTPs 20 nM (5 nM of each), 0.25 µL forward primer at 5 nM, 0.35 µL reverse primer at 20 nM, 0.35 µL of m13 labeled with 6-FAM, NED or VIC at 10 nM, 0.75 µL Taq diluted 1:3 and 1.5 µL of DNA; using a touch-down approach, with an initial denaturation step of 3 min at 95ºC, then ten cycles with a 30 sec denaturation step at 94ºC, followed by a 30 sec annealing step, with the temperature starting at 65ºC and decreasing one degree per cycle, then a 1 min extension step at 72ºC, followed for another 30 cycles with 30 sec denaturation at 94ºC, 30 sec annealing at 55ºC and 1 min extension at 72ºC, with a final 10 min extension period at 72ºC. PCR products were combined into run plates with GeneScan 500 LIZ Size Standard and genotyping was carried out in an ABI 3130XL sequencer at the Molecular Resource Center at the University of Tennessee in Memphis. Scoring of the fragments was carried out using GeneMarker version 2.6.3.

Genetic Diversity and Population Structuring

Populations were characterized for number of alleles and private alleles, allelic richness, observed and expected heterozygosity and inbreeding coefficient, using GenAlEex version 6.5 (Peakall & Smouse 2006, 2012).

Principal Component Analysis (PCO) was carried out using the standard genetic distance matrix calculated from the raw data on GenAlEx, for all populations of all species combined, all *C. martii* populations, *C. martii* populations from the Werstern and Eastern clusters separately, *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* combined and *C. harleyi* only.

The number of migrants (Nm) was estimated using the private alleles method in the online version of GENEPOP (Raymond & Rousset 1995, Rousset 2008). Estimates were calculated for all populations of a same species together (for *C. harleyi*, *C. martii* and *C. subverticillata*) and between populations of different species (for the *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata*). For *C. martii,* estimates were carried out with all populations together, between the pooled East and West populations, and among the populations in the East and West. Also, for the species where more than one population was available (*C. harleyi*, *C. martii* and *C. subverticillata*), pairwise estimates between all populations were also calculated. Deviation from Hardy-Weinberg equilibrium (HWE) was also tested in GenAlEx for each population and locus.

Isolation-by-distance (IBD) was evaluated using a Mantel test, with 1000 permutations, carried out with the pairwise Nei's unbiased genetic distance matrix and a geographic distance matrix, both obtained from the raw data fed into GenAlEx. The geographic distance matrix was calculated based on the geographic coordinates obtained during collection in each population. The same treatments used in the PCO were used for IBD testing.

Population structure was evaluated using analysis of molecular variance (AMOVA) to estimate F_{ST}. These analyses were run for *C. harleyi, C. martii* and *C. subverticillata* individually. *Chresta* *artemisifolia* and *C. hatschbachii* were not included because only one population of each was available. Pairwise F_{ST} between populations of each species was also evaluated and compared to geographic distance.

The Bayesian assignment algorithm implemented in STRUCTURE v. 2.3.4 (Pritchard et al. 2000) was used to assign individuals to genetic clusters (K). Five replications were run for each K, with a 200,000-iteration burn-in period and one million posterior iterations. The total results of each run were loaded in Structure Harvester (Earl & von Holdt 2012), and the best K for each run was determined using the Evanno method (Evanno 2005). Permutation of the results of each run carried out for the best K was performed on CLUMPP (Jakobsson & Rosenberg 2007), and distruct was used to draw plots showing the results (Rosenberg 2004).

Several different runs were carried out, in order to investigate macro and micro-structuring. First, we ran *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* together (K = 1 – 13) and all *C. martii* populations together (K = 1 – 14). Then we ran only *C. harleyi* (K = 1 – 8) and *C. artemisiifolia, C. hatschbachii* and *C. subverticillata* without *C. harleyi* (K = 1 – 5), and the two geographic groups in *C. martii* separately, Bahia and Pernambuco (K = 1 – 7) and Paraíba and Rio Grande do Norte (K = 1 – 7). Maps were prepared in QGIS v.2.18, using the 'popq' file produced by CLUMPP as basis for the pie charts.

Neighbor-joining (NJ) trees were constructed on POPTREE2 (Takezaki et al. 2010), using the Cavalli-Sforza & Edwards distance method (1967), with 1000 bootstraps. Two trees were prepared, one with all populations of *C. martii* and other with all populations of *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata*.

Results

Genetic diversity

From the 12 tested markers, 11 were polymorphic in *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata,* with one (NC38) being dropped due to unreliable amplification across populations, and 10 were polymorphic in *C. martii*, with two being dropped due to excessive missing data (NC26 and NC38).

Mean number of alleles per population, in all species and combining all loci (Table 2), varied from 1.3 to 4.545, but were higher in *C. harleyi,* varying from 2.727 to 4.545, than in *C. martii*, with a range from 1.3 to 3.6. Observed heterozygosity was generally lower than the expected, except for two populations of *C. subverticillata.* Fixation indexes were usually high, varying from 0.117 to 0.409 in *C. harleyi,* from 0.311 to 0.810 in *C. martii*, and being 0.609 in *C. artemisiifolia* and 0.441 in *C. hatschbachii.* Again, *C. subverticillata* shows a different trend, with two populations showing negative fixation indexes, ranging from -0.050 to 0.179.

Private alleles were common in all markers, but the species where they occurred varied. *Chresta martii* is the species with the highest value, with 60 private alleles across all markers, followed by *C. harleyi,* with 51 private alleles. The number of shared alleles across all species varied from two in marker NC15 to thirteen in NC24 (Fig. 2).

Regarding HWE, all populations showed departure from equilibrium for one loci or more (Fig. 3). For *C. harleyi,* NC27 was the loci that was in equilibrium for most populations, while for *C.*

subverticillata, this was NC22. For *C. martii,* NC13 was the loci that was predominantly in equilibrium. NC15 and NC29 departed from equilibrium in most populations (19 populations out of 27). All loci were monomorphic in at least one population, except for NC24, which was polymorphic in all populations. NC21 was monomorphic for 17 of 27 populations.

The estimates of number of migrants were overall below 1, which is considered the minimal value to avoid predominance of effect from genetic drift; the only species with an overall number higher than 1 was *C. harleyi* (Table 3). Gene flow between the four studied species was very low (all below 0.4), indicating that populations of each species are largely reproductively isolated from each other. Regarding *C. martii,* the migrant estimate between the East and West population groups was low, but the indexes for the populations within each group are also low and very similar to the overall pattern.

The pairwise calculations within populations of each species showed a clear trend: the number of migrants between populations is inversely proportional to the geographic distance between these populations (Table 4, 5). However, two population comparisons showed Nm>1, and there were cases where the geographic correlation was not followed, such as in *C. martii,* where the two populations that were closest together also were the ones with one of the lowest migrant number. The average pairwise migrant number was 0.4426174 for *C. harleyi,* 0.3173784 for *C. martii* and 0.81431 for *C. subverticillata.*

Population structure

For the first STRUCTURE run containing all populations of *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* combined, the DeltaK method supported the presence of eight genetically distinct clusters (K=8), with six of the clusters being found almost exclusively in *C. harleyi,* one of the clusters being predominant in *C. artemisiifolia* and *C. hatschbachii,* and one being found in *C. subverticillata* (Fig. 4A).

The analysis run only with *C. harleyi* found six clusters (K=6), largely consistent to the results from the complete analysis (Fig. 4B). Although the clusters are fairly mixed within each population, there seems to be a predominance of a certain genetic cluster in distinct geographic locations, with closer populations being more similar to each other (Fig. 5A).

The analysis with *C. artemisiifolia, C. hatschbachii* and *C. subverticillata* found two clusters (K=2), separating *C. artemisiifolia* and *C. hatschbachii* from *C. subverticillata,* although there is one individual in *C. hatschbachii* that groups with *C. subverticillata* (Fig. 4C). The second-best probability in this analysis is four clusters (K=4), separating the three species and showing further structure within *C. subverticillata* populations, with population 2 being more different from the other two (Fig. 4D).

The analysis with all *C. martii* populations together presents two clusters (K=2), with a strong geographical correlation, one cluster composed by all populations from Bahia and Pernambuco, on the westernmost part of the distribution, and the other containing populations from Paraíba and Rio Grande do Norte, more to the East (Fig. 6A). It is noteworthy that some individuals of the second cluster show strong affinities with the first cluster, while the opposite is not seen.

Further analysis of each of the *C. martii* clusters individually reveals finer structuring. The analysis with Paraíba and Rio Grande do Norte populations showed three genetic clusters (K=3), with all clusters being represented in all populations, but with the two populations further apart from the main population group showing a predominance of different clusters (Fig. 6B).

The group of populations from Bahia and Pernambuco shows two genetic clusters (K=2), where two populations from Jaguarari are strongly different from the other five (Fig. 6C). The second-best K on this analysis was four (K=4) and, in this case there is a more marked geographical separation, with the three northern populations being similar to each other, containing two major clusters, the two westernmost populations belonging to an almost exclusive genetic cluster, and the two Jaguarari populations belonging to a fourth cluster, with some influence from the clusters from the Northern populations (Fig. 6D, 7B).

The plots obtained from the PCO reflect the results from the STRUCTURE analysis (Fig. 8). The analysis of *C. harleyi* populations shows distinct overlapping groups, with some scattered individuals (Fig. 8A), while the analysis with *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* together shows all populations of *C. harleyi* grouped together and overlapping, with some scattered individuals, and a strong separation of *C. artemisiifolia* and *C. hatschbachii*, while the three populations of *C. subverticillata* group together (Fig. 8B). The PCO plot for *C. martii* shows a slight separation of the two geographic groups (eastern versus western), but with a strong overlap among individuals (Fig. 8C). Separating the two geographic clusters and running two individual analysis provided better definition of the grouping, with most populations segregating and forming groups, especially the populations from the western group (Fig. 8D, E).

F_{ST} values as estimated by AMOVA are very high in *C. martii,* high in *C. harleyi* and very low in *C. subverticillata* (Table 3). It was not possible to calculate F_{ST} for *C. artemisiifolia* and *C. hatschbachii* because only one population of each was sampled. Comparison of pairwise F_{ST} and geographical distance shows a positive correlation, with F_{ST} increasing with distance (Fig. 9). Pairwise F_{ST} and pairwise migration estimation did not seem to be correlated.

The trees obtained with Neighbor Joining showed a pattern similar to that discussed above. On the tree with *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* (Fig. 10A), all populations of *C. harleyi* grouped together, mostly following a geographical pattern, while *C. artemisiifolia* and *C. hatschbachii* were close together, and the three populations of *C. subverticillata* grouped together in a long branch. In the *C. martii* tree (Fig. 10B), the two geographical groups are separate and the distance between the two groups is larger than the distance between each population. The Bahia and Pernambuco populations formed a grouping in agreement with their geographical locations, while this does not happen with the group of populations from Paraíba and Rio Grande do Norte.

Isolation-by-distance

Results from Mantel test correlating genetic and geographic distance varied among different scenarios. The test carried out with *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* together (Fig. 11A) showed strong evidence of isolation by distance ($R^2 = 0.8274$, $p < 0.001$); however, the test carried out with *C. harleyi* only (Fig. 11B) showed a weak correlation that was not significant ($R^2 = 0.0643$, $p < 0.144$), while the test with *C. artemisiifolia, C. hatschbachii* and *C. subverticillata* (Fig. 11C) showed a correlation almost as strong as the one with all species ($R^2 = 0.8007$, $p < 0.059$).

Chresta martii showed a somewhat strong correlation ($R^2 = 0.2508$, $p < 0.002$) in the analysis with all populations (Fig. 11D), but when the two geographic clusters were separated (eastern versus western), only the populations from Bahia and Pernambuco showed evidence of isolation by distance ($R^2 = 0.4032$, $p < 0.002$) (Fig. 11E), while the populations from Paraíba and Rio Grande do Norte showed no correlation ($R^2 = 0.0035$, $p < 0.496$) (Fig. 10F).

Discussion

Our comparison of the results of the population genetics analyses of five rupiculous species of *Chresta* from the Brazilian Caatinga revealed contrasting patterns among them. Given the peculiar habitat and distribution of the study species, with geographically isolated populations, we expected some level of geographical structuring and inbreeding and due to the morphological and environmental similarities among species, we expected similar results for all species, but our analyses indicate they likely have differing population genetic histories.

Chresta harleyi was overall more allele-rich than the other species, and harbored moderate to high inbreeding coefficients (Table 2), even though there is a strong departure from HWE in all but one populations. The F_{ST} value estimated from AMOVA (0.191, Table 6) is moderate and in the range expected for outcrossing plant species (Morjan & Rieserberg 2004). Also, it is similar to values found for other Asteraceae, such as several species of *Helianthus* (Mandel et al. 2013), *Achillea millefolium* (López-Vinyallonga et al. 2015) and *Wunderlichia mirabilis* (Feres et al. 2009), which has similar environmental requirements to *C. harleyi.*

Two of the three *C. subverticillata* populations sampled here present excess of heterozygotes and negative fixation indexes, and also very low F_{ST} (Table 2 and 6). The range of this species is very narrow, and the three sampled populations are very close to each other (Fig. 5B). This may imply that these three populations are actually a meta-population, without geographical barriers limiting gene flow among them. Also, the region where this species is found is largely composed of continuous quatzitic outcrops and massifs, probably contributing to a less patchy occupation of the landscape and maintenance of gene flow among populations, as there is available substrate for seeds to germinate and stablish new individuals in-between the populations sampled by us.

Chresta artemisiifolia and *C. hatschbachii* had only one population of each sampled, as they are also narrow endemics, however, they are slightly different in their genetic diversity. *Chresta artemisiifolia* presents a high inbreeding coefficient (0.609), while the one in *C. hatschbachii* is closer to the values in *C. harleyi* (0.449). With the few known collections of *C. hatschbachii*, it is possible to calculate its extent of occurrence as being 31 km^2, while for *C. artemisiifolia,* this value is around 143 km^2 (see Chapter 3). The closeness among *C. hatschbachii* populations may allow some current gene flow among them; however, this was not captured here, as only one population was sampled. In *C. artemisiifolia*, as the known but unsampled, populations are farther away from each other, they may also be more genetically isolated from each other. The strong departure from HWE found on all populations is a reflection of the high fixation indexes, as well as the large number of monomorphic loci in some populations, indicating fixation of the alleles in these populations, a reflection of the effect of genetic drift or inbreeding (Fig. 3).

Chresta martii populations present higher fixation indexes and lower number of alleles per loci, indicating a marked heterozygote deficit. Also, the F_{ST} value found for *C. martii* is higher than for the other species (0.308, Table 6), and within the range usually found in plants that present mixed mating systems (Morjan & Rieseberg 2004).

Field observations have shown that individual plants of *Chresta* are frequently visited by insects, most of the time bees and bumblebees. The presence of secondary order capitula (syncephalia), when at any given time there are flowers in both male and female phases, and the fact that insects usually move around the whole structure while visiting may contribute to self-pollination (Fig. 1F).

Plants in Asteraceae are considered to be mostly sporophytic self-incompatible, with diploid control of pollen incompatibility, providing a very selective system (Crawford et al. 2009). Nevertheless, there are several factors that can result in pseudo-self-compatibility, allowing incompatible crosses to set seed, and this seems to be the case in some self-incompatible Asteraceae, such as *Senecio squalidus* (Brennan et al. 2003). This process is considered important in species that suffered bottlenecks or in founder populations, such as those colonizing oceanic islands, where it has been observed there is an over-representation of self-compatible Asteraceae (Grossenbacher et al. 2017).

A controlled-pollination study with *Chresta sphaerocephala* (Barbosa 1997) demonstrated self-compatibility in this species, which belongs to the Cerrado clade (Siniscalchi et al.in prep., see Chapter 1). However, studies about mating systems in the Caatinga species of *Chresta* still need to be carried out. Given the isolation observed between populations of *C. martii,* a propitious situation for pseudo-self-compatibility is likely to arise. Another possibility that might explain the high structuring found in *C. martii* is the occurrence of bi-parental inbreeding (crossing between relatives), which produces effects similar to selfing in the genetic diversity indexes in a given population (Coutellec-Vreto et al. 1997, Griffin & Eckert 2003). The combination of geographically isolated populations with pollinators that forage in small areas could definitely contribute to that, but it is difficult to distinguish the effects of both processes without dedicated experiments (e.g. Griffin & Eckert 2003, transplantation of plants among different populations reduce the apparent level of inbreeding by ~30%).

Population genetic studies of rupiculous plants from the Caatinga are scarce, while a few studies carried out with plants from highland rocky fields (*campos rupestres*), which also present fragmented and isolated populations, found high F_{IS} values and evidence of inbreeding in different families, such as Eriocaulaceae (Pereira et al. 2007), Leguminosae (Conceição et al. 2008), Orchidaceae (Borba et al. 2001), and Velloziaceae (Franceschinelli et al. 2006). There is evidence of self-compatibility in some of the species analyzed in these studies, but small population sizes also seem to be a factor favoring genetic drift over selection.

High F_{ST} values have also been found in two other studies with Brazilian Vernonieae that have strict environmental requirements as well, *Lychnophora ericoides* Mart., a shrub that usually occurs on quartzitic soils (Collevatti et al. 2009), and *Proteopsis argentea* Mart. & Zucc. ex Schultz-Bipontinus, which is found on highland rocky fields that usually present quartzitic outcrops and soils (Jesus et al. 2001). There is evidence of self-compatibility for *L. ericoides* (Avelino 2005), but so far, no information is available for *P. argentea.* Another study with several species of *Minasia*, another Brazilian Vernonieae, showed low to high F_{ST} values for different species (Jesus et al. 2009). These studies used different markers than our study; the *Lychnophora* study used ITS and chloroplast sequences and the *Proteopsis* and *Minasia* studies used allozymes, so a direct comparison among the values should be taken with caution.

The STRUCTURE results also show different trends across different species. The analysis with *Chresta harleyi* shows a high number of clusters (K=6), and when plotted geographically (Fig. 5A), it is clear that even though all clusters are present in all populations, there is always one cluster that is more prevalent in each population, and populations close to each other usually present the same prevalent cluster. Despite the marked structuring in this species, the fact that all clusters are shared indicates that although the gene flow is usually low between populations, it is still enough to make populations more similar to each other. It is possible that there may have been past connections or shorter distance among *C. harleyi* populations, allowing more gene flow, and subsequent reduction of the spatial connections and gene flow probably led to population differentiation. The Tertiary and/or Quaternary climatic fluctuations are often indicated as a possible factor influencing range expansion and contraction of dry biomes as the SDTFs, since the colder and drier conditions found during the Last Glacial Maximum may have been benefited this type of vegetation (e.g. Prado & Gibbs 1993, Prado 2000, Pennington et al. 2003, 2006). Open formations on habitats occurring within the same region, as the rock outcrops where *Chresta* species dwell, may have also be expanded in area during those same cooler and dryer periods.

The populations of *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* are located on the higher parts of different geological formations. *Chresta harleyi* and *C. hatschbachii* are both found on the massif known as Espinhaço Septentrional, while *C. subverticillata* and *C. artemisiifolia* occur in different parts of the Chapada Diamantina massif, which has two arms: *C. subverticillata* inhabits on the western arm, while *C. artemisiifolia* on the eastern one. The lower lands laying between the Espinhaço Septentrional and Chapada Diamantina massifs, and between both arms of Chapada Diamantina, may act as a barrier to the dispersal and foundation of new populations of these species, especially where outcrops are not present. However, *C. harleyi* and *C. hatschbachii* are both on the same massif and still seem to be consistently isolated from each other, with very low estimated number of migrants, indicating that there may be other, less perceptible barriers that influence gene flow between them.

On the other hand, *C. martii* presents a very marked genetic and geographic structure, with two clusters (K=2), corresponding to the two main sampled areas, with further structuring within each cluster (Fig. 6, 7). This strong separation may indicate that these population groups have been isolated for a longer period, and this is also expressed in the morphology of these groups. The populations on the western group present the typical morphological features of the species (the type specimen was collected on the surroundings of Juazeiro, Bahia): these plants are robust subshrubs, profusely ramified at the base, with wide leaves with relatively small dentation (Fig. 1H). The plants found on the eastern group, however, are smaller and more delicate, each bearing few stems and smaller and narrower leaves that are usually grouped on the tip of the stems and have marked dentate margins (Fig. 1I).

Chresta martii seem to be less limited by environmental factors, as it is also found growing on granitic outcrops, especially on the eastern end of the species range (Fig. 1B,C). However, among the three known geographical groupings (Bahia+Piauí+Pernambuco, Xingó and Paraíba+Rio Grande do Norte), is the geological formation known as Planalto da Borborema, which corresponds to several elevated areas that occupy a portion of the Northeast region in Brazil, located on the ecotone between the semi-arid Caatinga and the tropical Atlantic Forest (Hasui 2012). Borborema is a heterogeneous formation, with large portions rich in sandstone, which is a substrate not favoring the establishment of *C. martii,* and with some other areas where the climate is more humid and mild than the one prevailing where the species is usually found. Thus, it seems likely that this geological formation may represent a barrier for this species, impairing the establishment of populations halfway through the current geographical groupings and stopping gene flow among them. Curiously, plants collected on the Xingó region are morphologically similar to the plants found on Paraíba and Rio Grande do Norte, even though the populations from Bahia and Pernambuco are closer to this region (Fig. 1A).

The two geographic clusters recovered in *C. martii* also present different patterns between each other (Fig. 7A, B). In the eastern group (Fig. 7A), there is a fairly equal representation of the three genetic clusters found on the STRUCTURE analysis in all populations, except two (MTX and MSJS) that show predominance of one of the clusters. Contrary to the expected, population MSJS presents some of the highest pairwise estimates of migrants (Table 5). The picture on the western geographic group is quite different, with strong geographical separation of each cluster (Fig. 7B). The northernmost populations share two main clusters, while the two populations in Jaguarari (MJG1 and MJG2) present predominantly one cluster and the other two populations, which are located on the end of the Chapada Diamantina massif share another predominant cluster, different from the others. The Jaguarari populations show the lowest pairwise migration estimates in this group of populations, but the other populations present fairly similar migration estimates.

The results from the NJ trees are in agreement to those found on STRUCTURE. The tree obtained for *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* follows this pattern (Fig. 10A): *C. harleyi* populations are mostly geographically cohesive, but the fact they share the same genetic clusters in different proportions could explain the short distances between each population in the tree. The distance between *C. harleyi* and the other species is large, but between *C. artemisiifolia* and *C. hatschbachii* is small, and the same is seen in the K=2 STRUCTURE plot (Fig. 4C), where these two species belong to the same genetic cluster. The branch between these two species and *C. subverticillata* is also long and it is interesting that populations 1 and 3 group together, with these being the ones presenting heterozygote excess (negative fixation indexes).

Chresta martii also is divided into two geographical groups (Fig. 10B), and the western group is more geographically cohesive, similar to the plot found with K=4 in STRUCTURE (Fig. 6D), while the populations in the Eastern group are more similar among each other, therefore presenting less geographic structure. Also, the distance among populations in the eastern side is slightly smaller, showing they are more similar to each other than the ones on the western grouping. The two populations from Jaguarari are very distant from the other western populations, a trend also seen on the STRUCTURE plots (Fig. 6C, D).

Geographical distance among populations may be a factor influencing gene flow among populations, as these species require insect pollinators and wind dispersal. The whole distribution of *C. harleyi* is restricted to an extent of occurrence of around 2,000 km^2, while *C. martii* extends over 145,000 km^2 (Siniscalchi et al. in prep., see Chapter 3). All populations of *C. harleyi* are close together, distributed continuously over a portion of the Espinhaço Septentrional, while *C. martii* is distributed in three main clusters that are more than 300 km away from each other. The results of the STRUCTURE analysis have shown that all *C. harleyi* populations share six genetic clusters, with different predominant cluster in each population, while *C. martii* populations strongly split into two clusters that are geographically apart.

Our field observations indicate that all these species seem to be pollinated by large solitary bees that usually have a wider flight range than regular bees, reaching up to 20 km for *Xylocopa*, which usually forages around 6 to 10 km from the nest (Roubik 1989). Therefore, populations of a species that is restricted to a smaller area may receive pollen from other populations, like *C. harleyi,* where the longest distance between our sampled populations are 128 km, with several spatially intermediate populations that could serve as "stepping stones" for the visiting bees. On the other hand, the two geographic clusters of *C. martii* populations that we analyzed are more than 500 km apart, while the populations within each cluster are within the same range found for *C. harleyi.* It is very unlikely that bees are able to overcome the distance between each geographic cluster in *C. martii,* which then can be effectively reproductively isolated.

The strict environmental requirements of these species may have a bearing on seed dispersal. The pappus from the five species of *Chresta* studied here is composed of setose elements distributed in multiple series, features that do not favor dispersal, contrary to species that present plumose pappus. There are no records of these species collected in other types of soil than rock outcrops, so it is possible that seeds deposited on the latosol that surrounds each outcrop would not generate new individuals. In regions where outcrops are near to each other, there are more chances for seeds to set, while in regions where outcrops are farther apart, seed flow may be restricted among a few populations that are closer to each other. Interestingly, the Northeast region of Brazil has several areas with high wind potential, predominantly with a southeast to northwest direction (Amarante et al. 2001), and these overlap with the distribution of the study species, therefore, it is possible that most of the gene flow between more distant populations is due to seed dispersal instead of pollen dispersal.

Genetic data at a population level allow us to understand micro-evolutionary processes that act in the populations to maintain or disrupt species cohesion (Morjan & Rieseberg 2004). Our analysis reveals an interesting picture of rupiculous species that are naturally fragmentary. Our data corroborates previous studies conducted with plants presenting similar ecology (e.g., Barbará et al. 2007), high levels of inbreeding, low gene flow among populations and marked genetic structure, indicating that probably the Caatinga populations of *Chresta* are isolated from each other and being affected by the effects of genetic drift, which is the expected result given the ecology of the study species. However, our data also suggest that these processes act with different strengths upon each species, and this seems to be mostly related to the geographical distance among populations. For example, while *C. harleyi* presents moderate to high population structure and inbreeding, *C. martii* shows extreme values, possibly indicating distinct degrees of influence of the micro-evolutionary processes on each species.

Even though there are no dated phylogenies available for *Chresta*, the current phylogenetic hypothesis (Siniscalchi et al. in prep., see Chapter 1) shows that *C. martii* is the earliest-divergent clade within the genus, while the other four species studied here belong in an internal clade in the topology. This may indicate an older history for *C. martii*, which has led to the current strong isolation observed between its eastern and western population groups. Climatic fluctuations may have provided more favorable conditions for the expansion of the species range in the past, with subsequent changes leading to a contraction on the range, resulting in the geographic clusters seen today. Another hypothesis is that the species has had more time to disperse and successfully establish on suitable habitats. The presence of strong winds year-round in the Caatinga may have favored this process.

Despite the overall morphological similarity among *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata,* they are well differentiated genetically, as seen in the STRUCTURE plots (Fig. 4). The fact that *C. harleyi* is widespread and genetically diverse, while the remaining three are narrow endemics and less diverse may indicate a scenario where the ancestral species of the whole clade was located around the current area of *C. harleyi.* This putative ancestor could then have acted as a source of propagules spreading to new areas, some of them being successful founders that underwent posterior speciation in geographically distinct groups. These new groups would be less genetically diverse due to low initial diversity and the effects of drift in these small founder populations. The results from the Mantel tests show some support to this hypothesis, as there is strong evidence of isolation-by-distance between populations of these four species (Fig. 11A).

Our results also raise interesting reflections about taxonomic history. Despite their geographical proximity and morphological similarities, *C. harleyi* and *C. hatschbachii* were promptly recognized and described as different species (Robinson 1983, 2005). Specimens currently belonging to *C. subverticillata* were often identified as *C. harleyi*, and their recognition as a distinct species was not acknowledged until recently (Siniscalchi et al. submitted), probably due to lack of dedicated studies in the genus. *C. martii* was described more than a century ago, based on specimens presenting the "western" morphology. Specimens presenting the "eastern" morphology have been collected since the 1960s (Chapter 3), and despite the marked differences, no attempts to divide the taxa in two occurred so far. The genetic data show that the geographic clusters of *C. martii* are as genetically differentiated as the species that compose the other clade studied here, showing discrepancies that taxonomic treatments sometimes bear and it is quite likely that a more careful examination of the morphological features of additional specimens collected throughout the whole range of *C. martii* may provide support for taxonomic changes, such as recognition of two geographically and genetically delimited taxa, even though with very similar general morphology.

Our results also have strong implications for the conservation of rupiculous species of *Chresta*. The Caatinga is one of the least protected phytogeographical domains in Brazil, with less than 2% of their original area being included in conservation units (Leal et al. 2005). Very few populations of *Chresta* species that occur on the Caatinga are included in conservation units, with none of the narrow endemics, such as *C. artemisiifolia, C. hatschbachii* and *C. subverticillata,* being protected.

Due to their rupiculous nature, populations of *Chresta* are usually spared when the lands surrounding the outcrops are deforested to be used in agriculture or to be converted in pastures, as the outcrops are largely left untouched. The most direct risks to the outcrop plants are being grazed by goats and sheep, which are frequently raised as free-range livestock by the local inhabitants (Leal et al. 2003), or being destroyed by illegal fires used to clean areas that will be planted. However, the removal of the original vegetation can have deep impact on the populations of pollinators that visit *Chresta,* as they depend on the native vegetation for shelter and additional food sources; loss of pollinators could reduce even further the gene flow among populations, contributing to the high inbreeding levels already seen today.

The high levels of geographical and genetic structuring observed in the *Chresta* species studied here also have implications in the number of populations that would need to be included in conservation units in order to maintain the genetic diversity of these species. Using *Chresta martii* as an example, populations in both extremes of the distribution would need to be held under protection to capture an adequate level of genetic diversity. Regarding *C. harleyi,* target populations for conservation would be those in Caetité (HCA1, HCA2) and Licínio de Almeida (HLA1, HLA2), as they contain a good representation of allele diversity in this species (Fig. 5A). The elevated areas in the Espinhaço Septentrional and Chapada Diamantina massifs are of great economic interest due to the different mineral products found in the area and their high wind potential, with several wind farms being implemented in the last decade (Chapter 4), posing a direct threat to the populations on these areas, as the wind turbines are usually installed in higher elevations and rocky areas, directly impacting local flora.

Inselbergs, which are usually defined as monolithic crystalline outcrops that occur around the world, have long been described as 'terrestrial islands', due to their natural isolation from the surrounding vegetation (Porembski & Barthlott 2000). Inselbergs provide different environmental conditions from those found around them, and their flora is usually diverse and adapted to these conditions, and due to their natural isolation and fragmentation, it has been a target for population genetics studies (e.g. Sarthou et al. 2000, Barbará et al. 2007, Millar et al. 2013, Lavor et al. 2014, Pinheiro et al. 2014). Smaller rock outcrops with different compositions, on the other hand, have not been given as much attention, with few studies focusing on understanding genetic characteristics of their flora and how they compare with those found on inselbergs. In this study, we have demonstrated that even small quartzite outcrops may provide enough environmental differentiation to work as a terrestrial island, with populations showing high genetic structure and inbreeding, as usually found on inselbergs.

We hope our results will contribute to improve the general knowledge about rupiculous plants, and will stimulate new studies with other plant species occurring on quartzitic rock outcrops. Such studies may provide additional evidence for a better knowledge on the micro-evolutionary processes that are underway in plants with fragmented populations along the peculiar type of environmental conditions found on neotropical inselbergs and rocky substrates as a whole.

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Table 1. Voucher list: sampled populations of *Chresta* species for the molecular analyses.

Population code	**Locality (city, state, country)**	**Geographic coordinates**	**Voucher no.**	**Herbarium**
Chresta artemisiifolia Siniscalchi & Loeuille				
ASS	Sento Sé, Bahia, Brazil	10°04’11”S, 41°37’27”W	J. Siqueira Filho 3671	HVASF, SPF
Chresta harleyi H.Rob.				
HMV	Mato Verde, Minas Gerais, Brazil	15°23’19”S, 42°46’36”W	Siniscalchi 449	SPF
HUR	Urandi, Bahia, Brazil	14°44’46”S, 42°34’25”W	Siniscalchi 457	SPF
HLA1	Licínio de Almeida, Bahia, Brazil	14°34’38”S, 42°31’30”W	Siniscalchi 459	SPF
HLA2	Licínio de Almeida, Bahia, Brazil	14°34’22”S, 42°31’27”W	Siniscalchi 460	SPF
HJC	Jacaraci, Bahia, Brazil	14°49’46”S, 42°26’06”W	Siniscalchi 462	SPF
HCA1	Caetité, Bahia, Brazil	14°15’24”S, 42°31’21”W	Siniscalchi 463	SPF
HCA2	Caetité, Bahia, Brazil	14°15’57”S, 42°31’45”W	Siniscalchi 464	SPF
Chresta hatschbachii H.Rob.				
HTOB	Oliveira dos Brejinhos, Bahia, Brazil	12°22’19”S, 42°54’19”W	Siniscalchi 468	SPF
Chresta martii (DC.) H.Rob.				
MJG1	Jaguarari, Bahia, Brazil	10°06’11”S, 40°13’47”W	Siniscalchi 473	SPF
MJG2	Jaguarari, Bahia, Brazil	9°56’11”S, 40°15’49”W	Siniscalchi 474	SPF
MSO	Sobradinho, Bahia, Brazil	9°28’54”S, 40°51’59”W	Siniscalchi 479	SPF
MCN	Casa Nova, Bahia, Brazil	9°22’52”S, 40°48’02”W	Siniscalchi 482	SPF
MPE	Petrolina, Pernambuco, Brazil	9°21’41”S, 40°23’05”W	Siniscalchi 565	SPF
MTX	Teixeira, Paraíba, Brazil	7°12’10”S, 37°15’30”W	Siniscalchi 635	SPF
MSL1	Santa Luzia, Paraíba, Brazil	6°54’59”S, 36°51’15”W	Siniscalchi 637	SPF
MSL2	Santa Luzia, Paraíba, Brazil	6°53’05”S, 36°53’02”W	Siniscalchi 638	SPF
MPR1	Parelhas, Rio Grande do Norte, Brazil	6°42’09”S, 36°41’28”W	Siniscalchi 639	SPF
MPR2	Parelhas, Rio Grande do Norte, Brazil	6°42’10”S, 36°38’57”W	Siniscalchi 640	SPF
MSJS	São João do Sabugi, Rio Grande do Norte, Brazil	6°41’46”S, 37°09’47”W	Siniscalchi 641	SPF
MSJE	Jardim do Seridó, Rio Grande do Norte, Brazil	6°36'49"S, 36°46'29"W	Siniscalchi 642	SPF
MCF	Campo Formoso, Bahia, Brazil	10°11’32”S, 41°04’45”W	J. Siqueira Filho 3537	HVASF, SPF
MSS	Sento Sé, Bahia, Brazil	10°22’12”S, 41°44’58”W	J. Siqueira Filho 3657	HVASF, SPF
Chresta subverticillata Siniscalchi & Loeuille				
SBGO1	Gentio do Ouro, Bahia, Brazil	11°11’31”S, 42°43’03”W	Siniscalchi 630	SPF
SBGO2	Gentio do Ouro, Bahia, Brazil	11°05’48”S, 42°43’18”W	Siniscalchi 631	SPF
SBGO3	Gentio do Ouro, Bahia, Brazil	11°06’26”S, 42°43’10”W	Siniscalchi 634	SPF

Table 2. Measures of genetic diversity in *Chresta*, containing 11 polymorphic SSRs loci for *Chresta artemisiifolia* (ASS), *C. harleyi* (HMV to HRER), *C. hatschbachii* (HTOB) and *C. subverticillata* (SBGO1 to 3) and for 10 polymorphic loci for C. martii (MJG1 to MSS).

Population	N	Mean N (SE)	A (SE)	Ho (SE)	UHe (SE)	F (SE)
ASS	18	10.273 (2.179)	1.909 (0.530)	0.106 (0.072)	0.201 (0.085)	0.609 (0.162)
HMV	10	9.273 (0.195)	3.091 (0.610)	0.383 (0.093)	0.497 (0.091)	0.117 (0.160)
HUR	12	10.545 (0.474)	3.000 (0.674)	0.319 (0.086)	0.423 (0.092)	0.250 (0.077)
HLA1	11	9.727 (0.273)	3.909 (0.579)	0.329 (0.074)	0.596 (0.066)	0.409 (0.141)
HLA2	13	12.909 (0.091)	4.000 (0.763)	0.369 (0.094)	0.505 (0.097)	0.269 (0.145)
HJC	13	12.091(0.163)	3.273 (0.675)	0.364 (0.098)	0.432 (0.090)	0.170 (0.089)
HCA1	12	9.727 (0.384)	3.636 (0.664)	0.244 (0.074)	0.474 (0.093)	0.376 (0.142)
HCA2	13	11.364 (0.310)	4.545 (0.835)	0.298 (0.074)	0.504 (0.100)	0.309 (0.122)
HRER	12	6.818 (1.189)	2.727 (0.557)	0.328 (0.105)	0.456 (0.103)	0.209 (0.187)
HTOB	14	11.273 (1.244)	3.545 (0.545)	0.271 (0.089)	0.475 (0.101)	0.441 (0.125)
SBGO1	6	6 (0)	2.455 (0.207)	0.530 (0.129)	0.470 (0.067)	-0.150 (0.213)
SBGO2	10	9 (0.447)	3.091 (0.368)	0.413 (0.111)	0.537 (0.072)	0.179 (0.179)
SBGO3	12	10.455 (0.511)	3.182 (0.569)	0.532 (0.118)	0.465 (0.076)	-0.050 (0-180)
MJG1	16	12.1 (1.169)	3.6 (0.6)	0.297 (0.095)	0.511 (0.089)	0.341 (0.148)
MJG2	10	7.8 (0.854)	2.9 (0.458)	0.293 (0.105)	0.470 (0.091)	0.387 (0.162)
MSO	12	9.8 (0.854)	3.2 (0.646)	0.195 (0.076)	0.479 (0.088)	0.519 (0.149)
MCN	13	10.6 (0.777)	3.6 (0.872)	0.221 (0.093)	0.439 (0.110)	0.478 (0.153)
MPE	8	7.8 (0.133)	2.5 (0.453)	0.141 (0.058)	0.370 (0.1)	0.461 (0.163)
MTX	8	7.3 (0.3)	1.3 (0.153)	0.086 (0.086)	0.102 (0.057)	0.417 (0.320)
MSL1	12	4.8 (0.786)	1.4 (0.163)	0.038 (0.038)	0.176 (0.076)	0.810 (0.120)
MSL2	11	10.6 (0.163)	2.3 (0.367)	0.098 (0.045)	0.343 (0.086)	0.717 (0.096)
MPR1	12	6.2 (0.8)	1.9 (0.407)	0.094 (0.066)	0.261 (0.109)	0.594 (0.156)
MPR2	12	8.6 (0.306)	2.3 (0.3)	0.079 (0.031)	0.304 (0.071)	0.582 (0.145)
MSJS	12	10.1 (0.163)	1.9 (0.482)	0.091 (0.044)	0.182 (0.078)	0.311 (0.148)
MSJE	16	12.7 (0.597)	2.3 (0.423)	0.045 (0.026)	0.265 (0.089)	0.668 (0.132)
MCF	8	4.1 (0.526)	2.4 (0.267)	0.190 (0.063)	0.455 (0.074)	0.427 (0.173)
MSS	18	10.6 (1.688)	3.8 (0.917)	0.237 (0.109)	0.522 (0.100)	0.455 (0.218)

Table 3. Estimates of number of migrants within populations of the same species and between all populations of different species of *Chresta* analyzed.

Populations	**Nm**
All *C. harleyi* populations	1.38903
All *C. subverticillata* pops	0.769797
C. artemisiifolia – C. hatschbachii	0.129334
C. artemisiifolia – C. subvercillata (all pops)	0.317725
C. artemisiifolia – C. harleyi (all pops)	0.183848
C. harleyi (all pops) – *C. hatschbachii*	0.261429
C. harleyi (all pops) – *C. subverticillata* (all pops)	0.245556
C. hatschbachii – C. subverticillata (all pops)	0.204668
All *C. martii* populations	0.526536
East populations vs. West populations	0.488996
All West populations	0.44543
All East populations	0.44155

Table 4. Pairwise migrant estimates between all populations of *Chresta harleyi* and *C. subverticillata*.

Populations	Nm	Distance (km)
Chresta harleyi populations		
HCA1 – HCA2	1.24483	1.247
HCA1 – HJC	0.510401	64.382
HCA1 – HLA1	0.556222	35.645
HCA1 – HLA2	0.583843	35.15
HCA1 – HMV	0.562766	128.798
HCA1 – HRER	0.369918	89.683
HCA1 – HUR	0.606998	54.701
HCA2 – HJC	0.447863	63.485
HCA2 – HLA1	0.596628	34.628
HCA2 – HLA2	0.537332	34.135
HCA2 – HMV	0.517093	127.650
HCA2 – HRER	0.438734	88.610
HCA2 – HUR	0.407912	53.618
HJC – HLA1	0.432595	29.669
HJC – HLA2	0.534683	30.108
HJC – HMV	0.252524	72.189
HJC – HRER	0.407818	30.378
HJC – HUR	0.305135	17.548
HLA1 – HLA2	0.621046	0.502
HLA1 – HMV	0.366099	94.185
HLA1 – HRER	0.259321	54.202
HLA1 – HUR	0.389431	19.494
HLA2 – HMV	0.200104	94.684
HLA2 – HRER	0.260512	54.704
HLA2 – HUR	0.173619	19.994
HMV – HRER	0.423698	41.566
HMV – HUR	0.184796	74.695
HRER – HUR	0.201366	35.005
Chresta subverticillata populations		
SBGO1 – SBGO2	0.559002	10.604
SBGO1 – SBGO3	1.1809	9.423
SBGO2 – SBGO3	0.703028	1.198

Table 5. Pairwise migrant estimates between all populations of *Chresta martii*.

Populations	Nm	Distance (km)	Populations	Nm	Distance (km)
West populations			East populations		
MCF - MCN	0.487398	95.218	MJSE – MPR1	0.430315	13.538
MCF – MJG1	0.226201	93.504	MSJE – MPR2	0.3402	17.061
MCF – MJG2	0.212863	93.712	MSJE – MSJS	0.380363	43.842
MCF – MPE	0.212073	119.689	MSJE – MSL1	0.116945	34.786
MCF – MSO	0.47332	82.377	MSJE – MSL2	0.253226	32.459
MCF – MSS1	0.341343	75.952	MSJE – MTX	0.192743	84.497
MCN – MJG1	0.23432	101.774	MPR1 – MPR2	0.384788	4.632
MCN – MJG2	0.270626	85.304	MPR1 – MSJS	0.643985	52.124
MCN – MPE	0.388382	45.674	MPR1 – MSL1	0.101711	29.829
MCN – MSO	0.767477	13.311	MPR1 – MSL2	0.413717	29.387
MCN – MSS1	0.329103	151.314	MPR1 – MTX	0.288023	83.751
MJG1 – MJG2	0.310001	18.9	MPR2 – MSJS	0.506589	56.757
MJG1 – MPE	0.191413	84.201	MPR2 –MSL1	0.187031	32.810
MJG1 – MSO	0.237062	98.189	MPR2 – MSL2	0.596658	32.878
MJG1 – MSS1	0.168066	168.924	MPR2 – MTX	0.221539	87.247
MJG2 – MPE	0.236536	65.301	MSJS – MSL1	0.195032	41.989
MJG2 – MSO	0.184905	83.194	MSJS – MSL2	0.552449	37.282
MJG2 – MSS1	0.223908	169.626	MSJS – MTX	0.568081	57.312
MPE – MSO	0.274835	54.503	MSL1 – MSL2	0.192882	4.813
MPE – MSS1	0.19898	186.894	MSL1 – MTX	0.0714378	54.802
MSO – MSS1	0.226011	138.246	MSL2 – MTX	0.497355	54.390

Table 6. F_{ST} as estimated by AMOVA.

Species	F_{ST}	p	Within population	Among populations	F_{ST} per locus range
C. harleyi	0.191	0.001	81%	19%	0.076 – 0.370
C. martii	0.308	0.001	69%	31%	0.137 – 0.619
C. subverticillata	0.076	0.001	92%	8%	-0.053 – 0.286

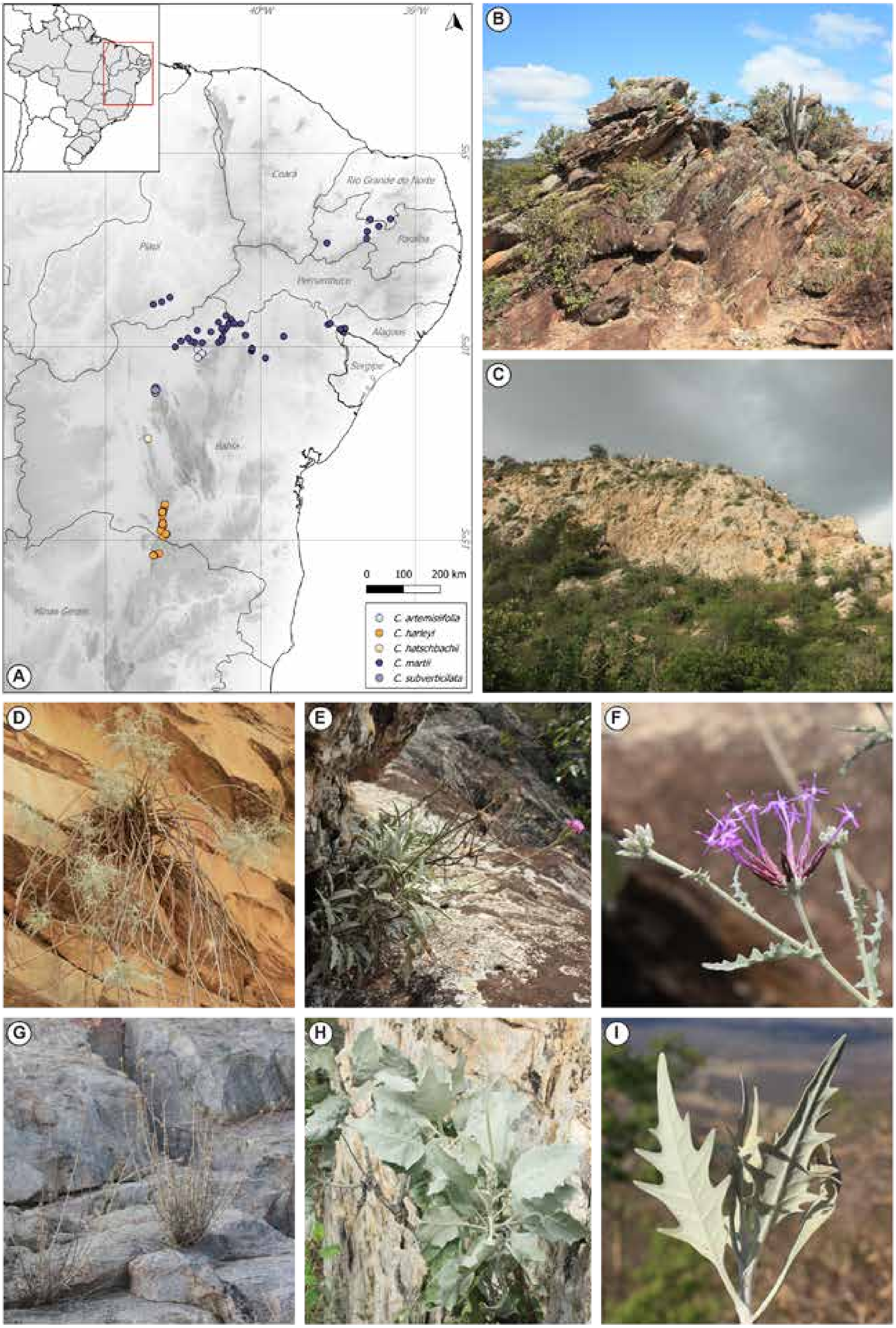

Figure 1. A. Distribution map of *Chresta artemisiifolia*, *C. harleyi, C. hatschbchii, C. martii* and *C. subverticillata*. B. Quartzitic outcrop where *C. harleyi* population HLA1 occurs. C. Quartzitic crest where *C. martii* population MJG1 occurs. D. *C. artemisiifolia*, habit. E. *C. harleyi*, habit. F. *C. hatschbachii,* secondary head and florets. G. *C. subverticillata*, habit and environment. H. *C. martii*, Western morphology. I. *C. martii*, Eastern morphology.

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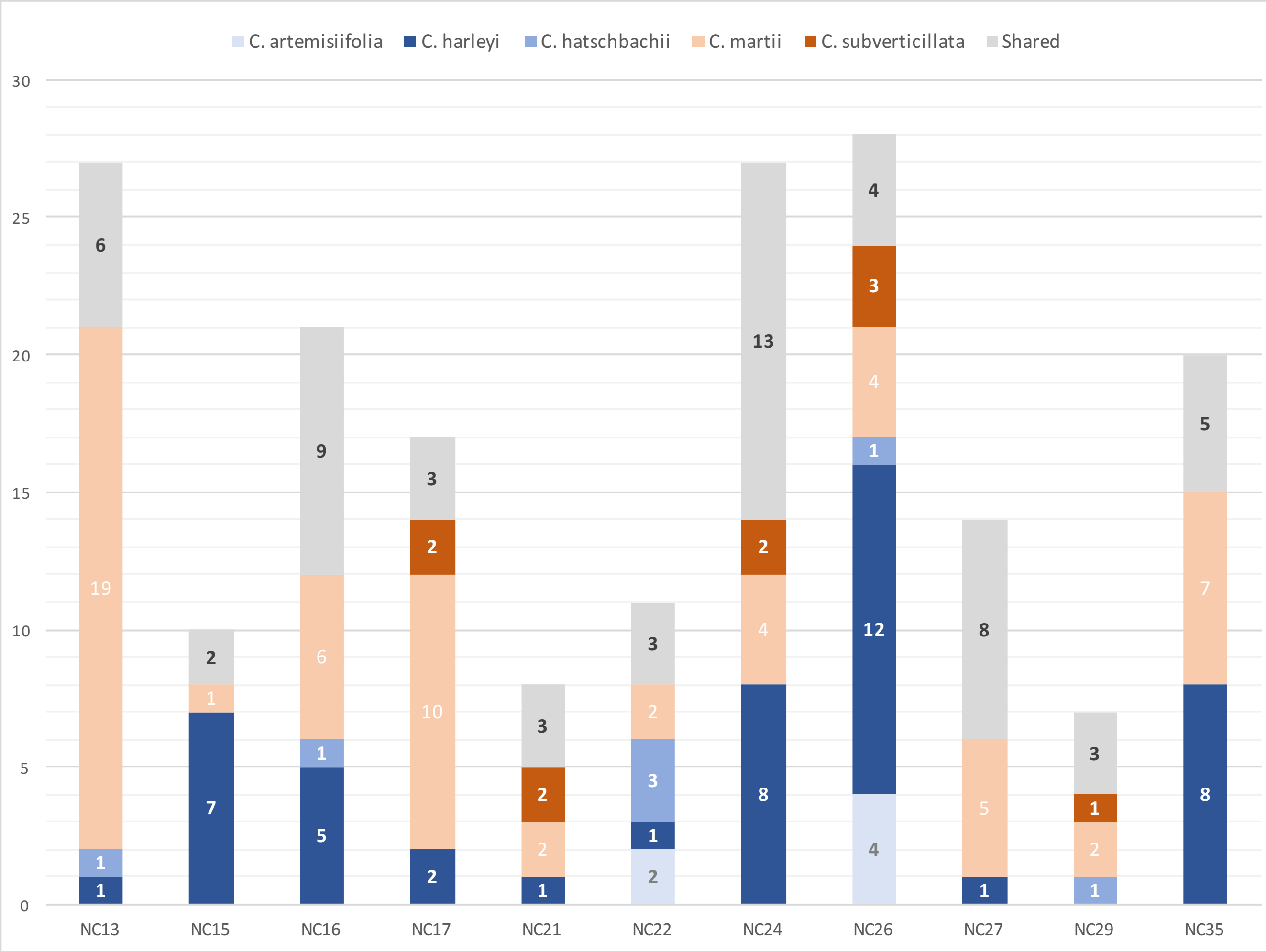

Figure 2. Graph showing private alleles per locus per species.

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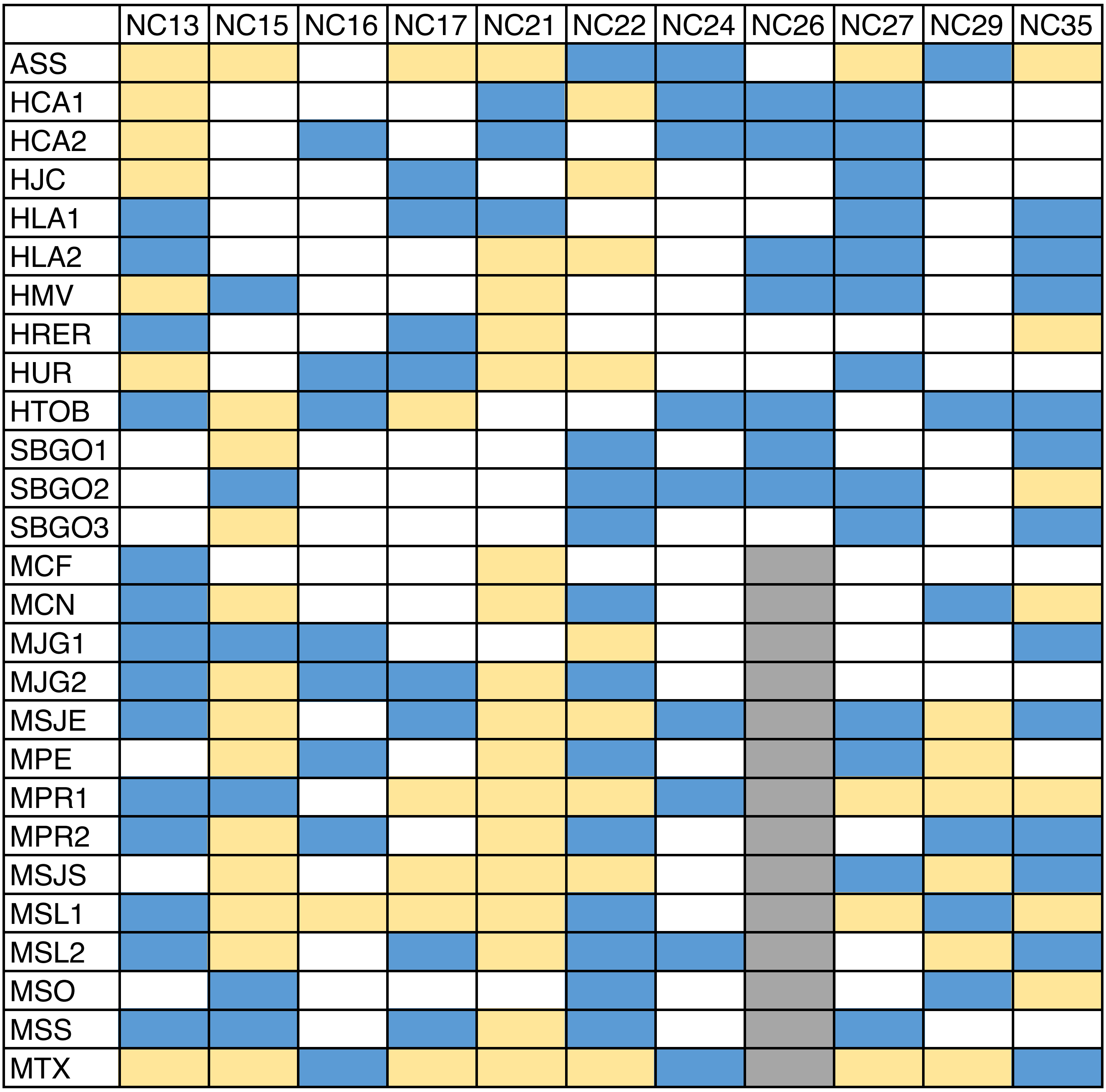


	NC13	NC15	NC16	NC17	NC21	NC22	NC24	NC26	NC27	NC29	NC35
ASS	🟨	🟨	⬜	🟨	🟨	🟦	🟦	⬜	🟨	🟦	🟨
HCA1	🟨	⬜	⬜	⬜	🟦	🟨	🟦	🟦	🟦	⬜	⬜
HCA2	🟨	⬜	🟦	⬜	🟦	⬜	🟦	🟦	🟦	⬜	⬜
HJC	🟨	⬜	⬜	🟦	⬜	🟨	⬜	⬜	🟦	⬜	⬜
HLA1	🟦	⬜	⬜	🟦	🟦	⬜	⬜	⬜	🟦	⬜	🟦
HLA2	🟦	⬜	⬜	⬜	🟨	🟨	⬜	🟦	🟦	⬜	🟦
HMV	🟨	🟦	⬜	⬜	🟨	⬜	⬜	🟦	🟦	⬜	🟦
HRER	🟦	⬜	⬜	🟦	🟨	⬜	⬜	⬜	⬜	⬜	🟨
HUR	🟨	⬜	🟦	🟦	🟨	🟨	⬜	⬜	🟦	⬜	⬜
HTOB	🟦	🟨	🟦	🟨	⬜	⬜	🟦	🟦	⬜	🟦	🟦
SBGO1	⬜	🟨	⬜	⬜	⬜	🟦	⬜	🟦	⬜	⬜	🟦
SBGO2	⬜	🟦	⬜	⬜	⬜	🟦	🟦	🟦	🟦	⬜	🟨
SBGO3	⬜	🟨	⬜	⬜	⬜	🟦	⬜	⬜	🟦	⬜	🟦
MCF	🟦	⬜	⬜	⬜	🟨	⬜	⬜	▨	⬜	⬜	⬜
MCN	🟦	🟨	⬜	⬜	🟨	🟦	⬜	▨	⬜	🟦	🟨
MJG1	🟦	🟦	🟦	⬜	⬜	🟨	⬜	▨	⬜	⬜	🟦
MJG2	🟦	🟨	🟦	🟦	🟨	🟦	⬜	▨	⬜	⬜	⬜
MSJE	🟦	🟨	⬜	🟦	🟨	🟨	🟦	▨	🟦	🟨	🟦
MPE	⬜	🟨	🟦	⬜	🟨	🟦	⬜	▨	🟦	🟨	⬜
MPR1	🟦	🟦	⬜	🟨	🟨	🟨	🟦	▨	🟨	🟨	🟨
MPR2	🟦	🟨	🟦	⬜	🟨	🟦	⬜	▨	⬜	🟦	🟦
MSJS	⬜	🟨	⬜	🟨	🟨	🟨	⬜	▨	🟦	🟨	🟦
MSL1	🟦	🟨	🟨	🟨	🟨	🟦	⬜	▨	🟨	🟦	🟨
MSL2	🟦	🟨	⬜	🟦	🟨	🟦	🟦	▨	⬜	🟨	🟦
MSO	⬜	🟦	⬜	⬜	⬜	🟦	⬜	▨	⬜	🟦	🟨
MSS	🟦	🟦	⬜	🟦	🟨	🟦	⬜	▨	🟦	⬜	⬜
MTX	🟨	🟨	🟦	🟨	🟨	🟨	🟦	▨	🟨	🟨	🟦

Figure 3. Hardy-Weinberg equilibrium test for all populations and all loci. Blue cells indicate that the locus is in equilibrium at P<0.05, yellow cells indicate that the locus was monomorphic is that population, white cells are populations out of equilibrium. Grey cells indicate the locus was not tested in that population.

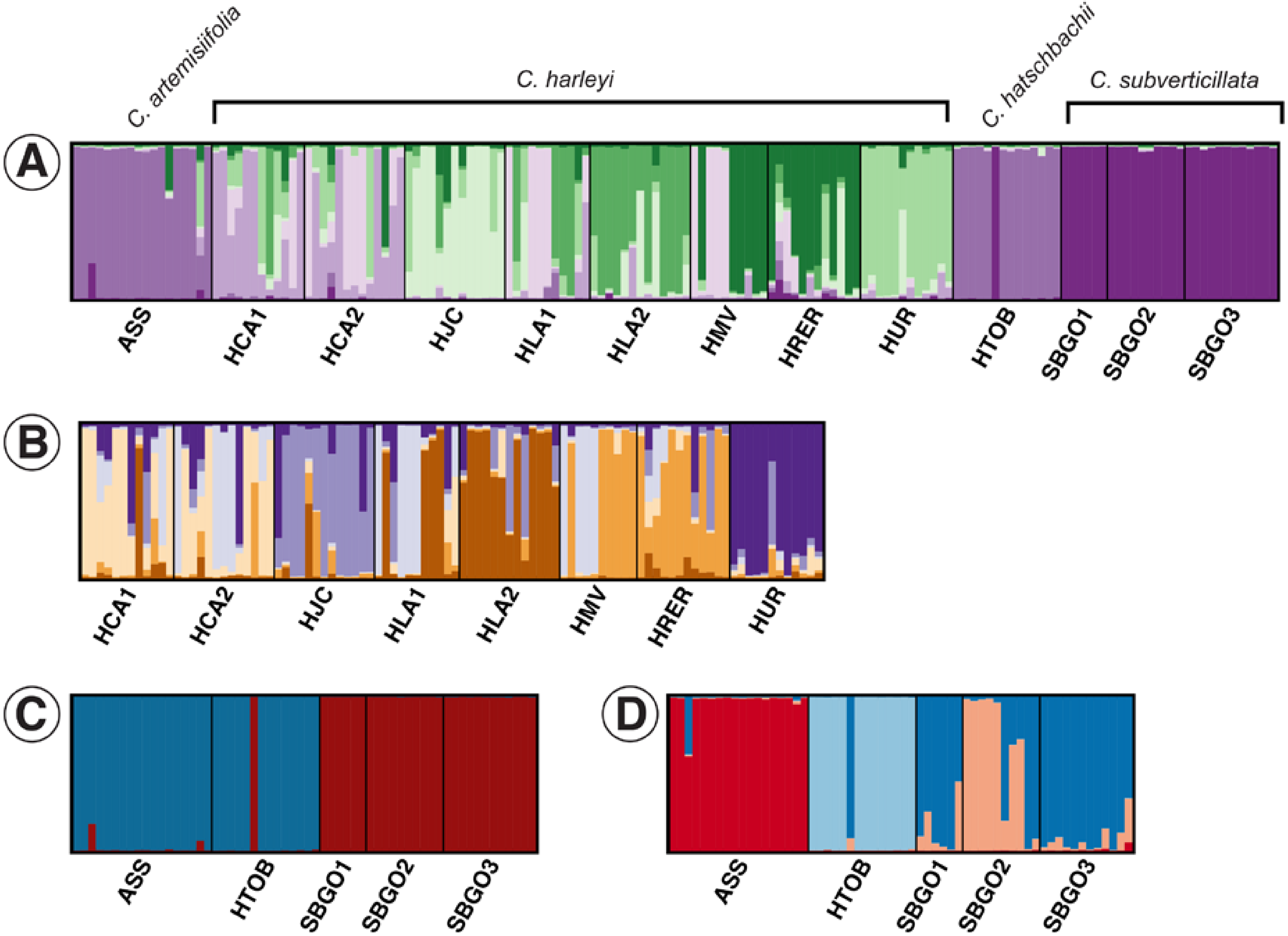
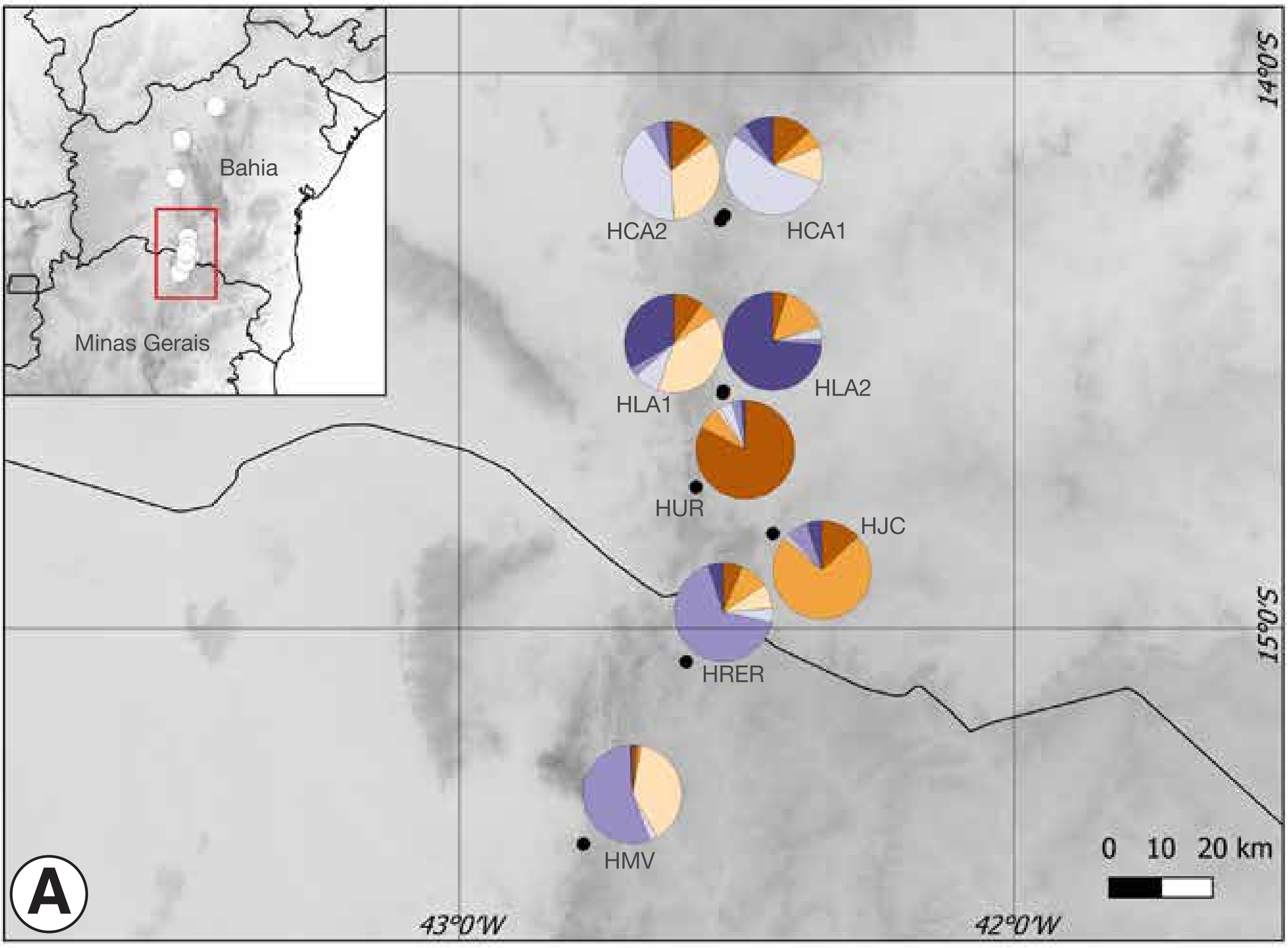


Figure 4. STRUCTURE plots. A. All populations of *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata*, K=8. B. All populations of *C. harleyi*, K=6. C. Populations of *C. artemisiifolia, C. hatschbachii* and *C. subverticillata*, K=2. D. Populations of *C. artemisiifolia, C. hatschbachii* and *C. subverticillata*, K=4.



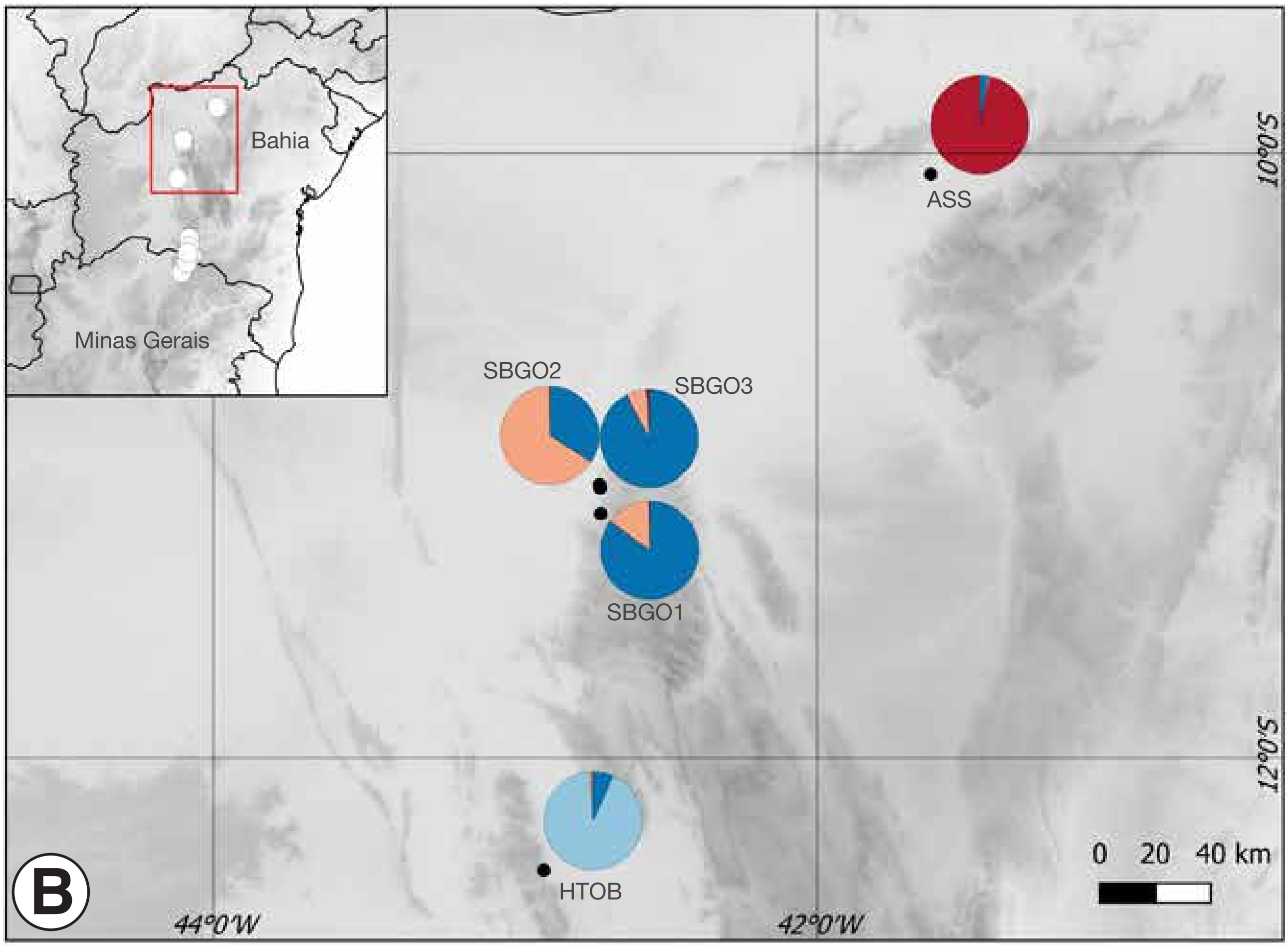


Figure 5. Maps showing the proportion of each cluster from STRUCTURE analysis in each population. A. All populations of *C. harleyi* (K=6). B. Populations of *C. artemisiifolia* (ASS), *C. hatschbachii* (HTOB) and *C. subverticillata* (SBGO) (K=4).

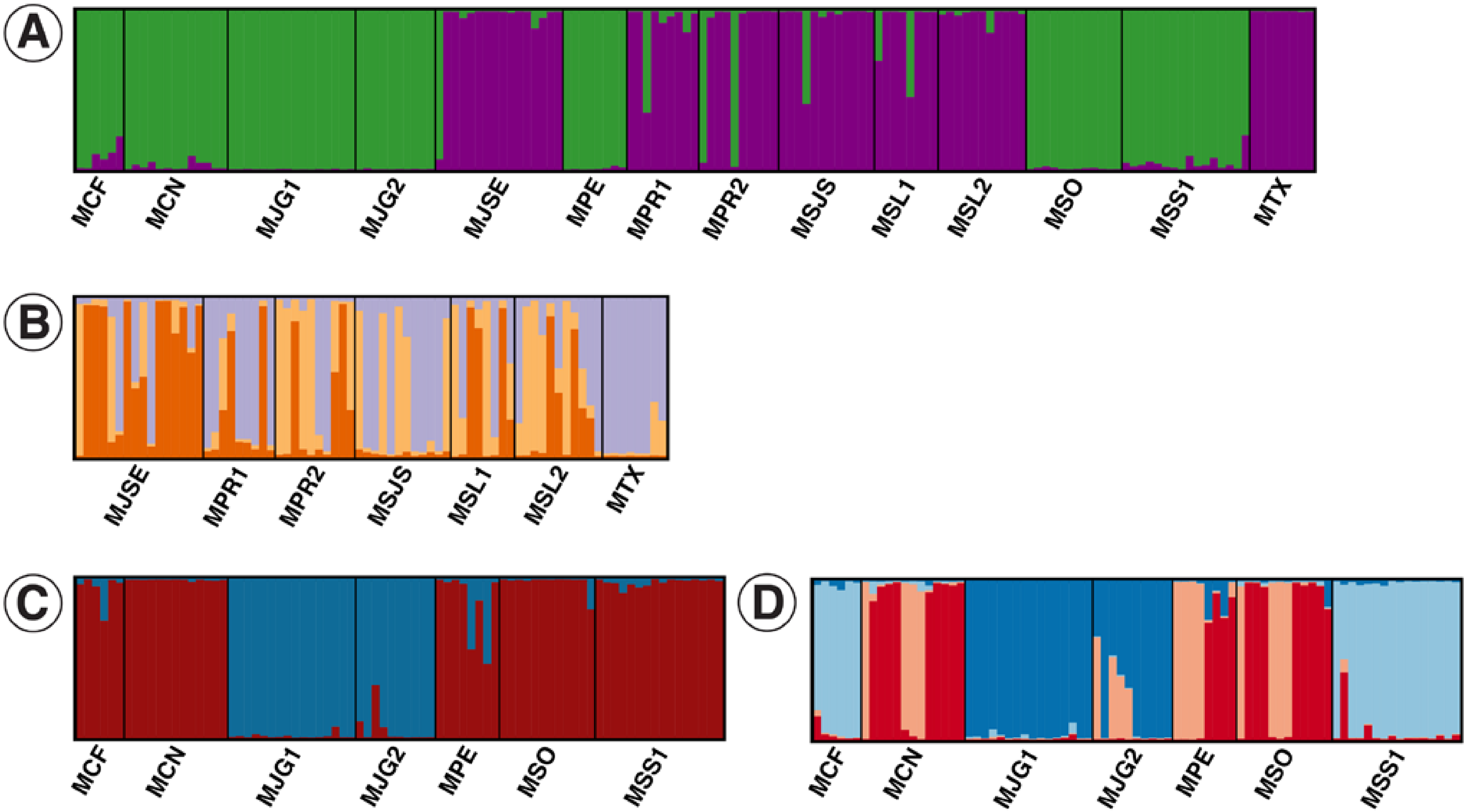


Figure 6. STRUCTURE plots. A. All *C. martii* populations. B. All Eastern (Paraíba + Rio Grande do Norte) populations (K=3). C. All Western (Bahia + Pernambuco) populations (K=2). D. All Western (Bahia + Pernambuco) populations (K=4).

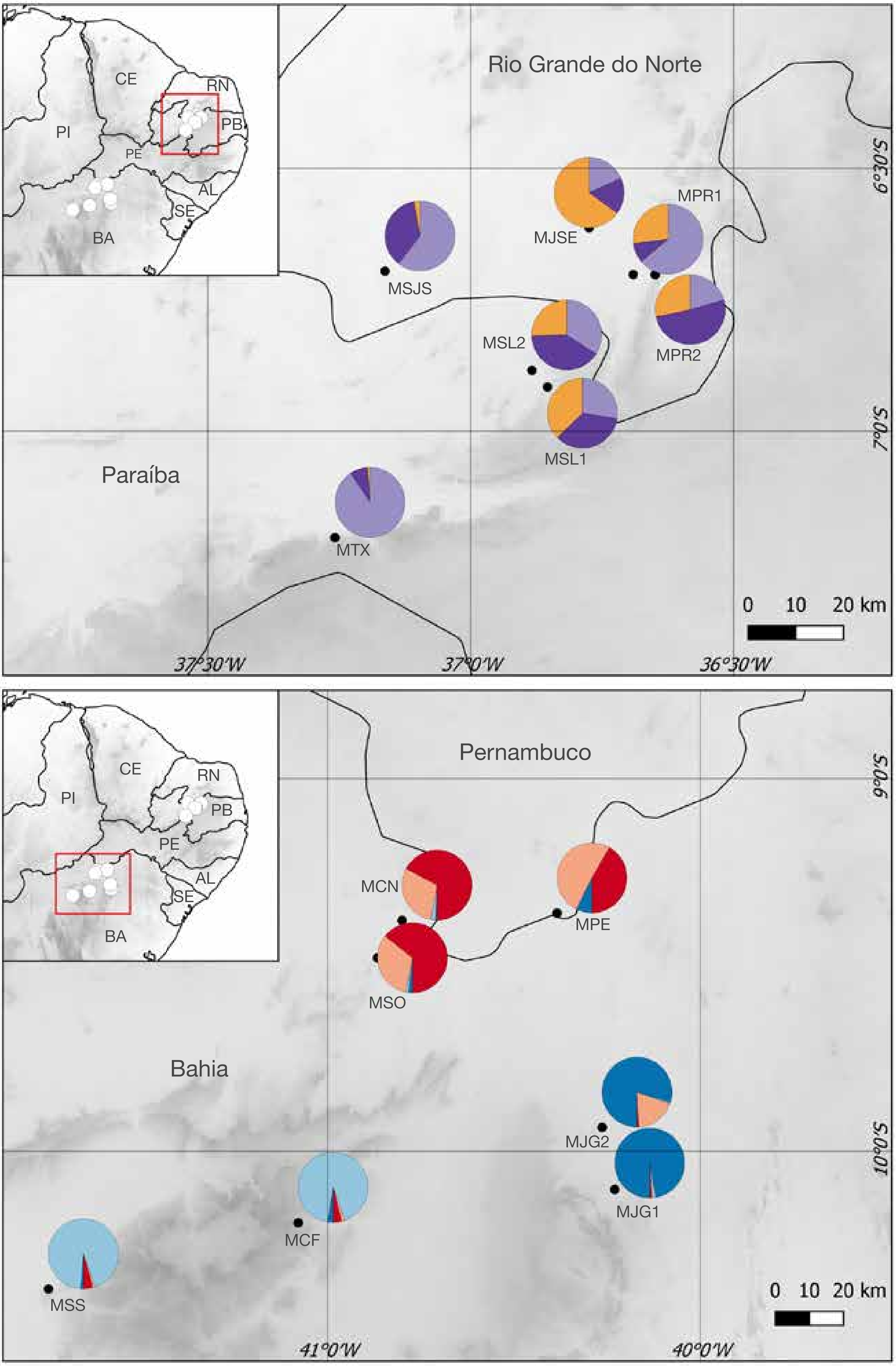


Figure 7. Maps showing the proportion of each cluster from STRUCTURE analysis in each population of *C. martii*. A. All Eastern (Paraíba + Rio Grande do Norte) populations (K=3). B. All Western (Bahia + Pernambuco) populations (K=4).

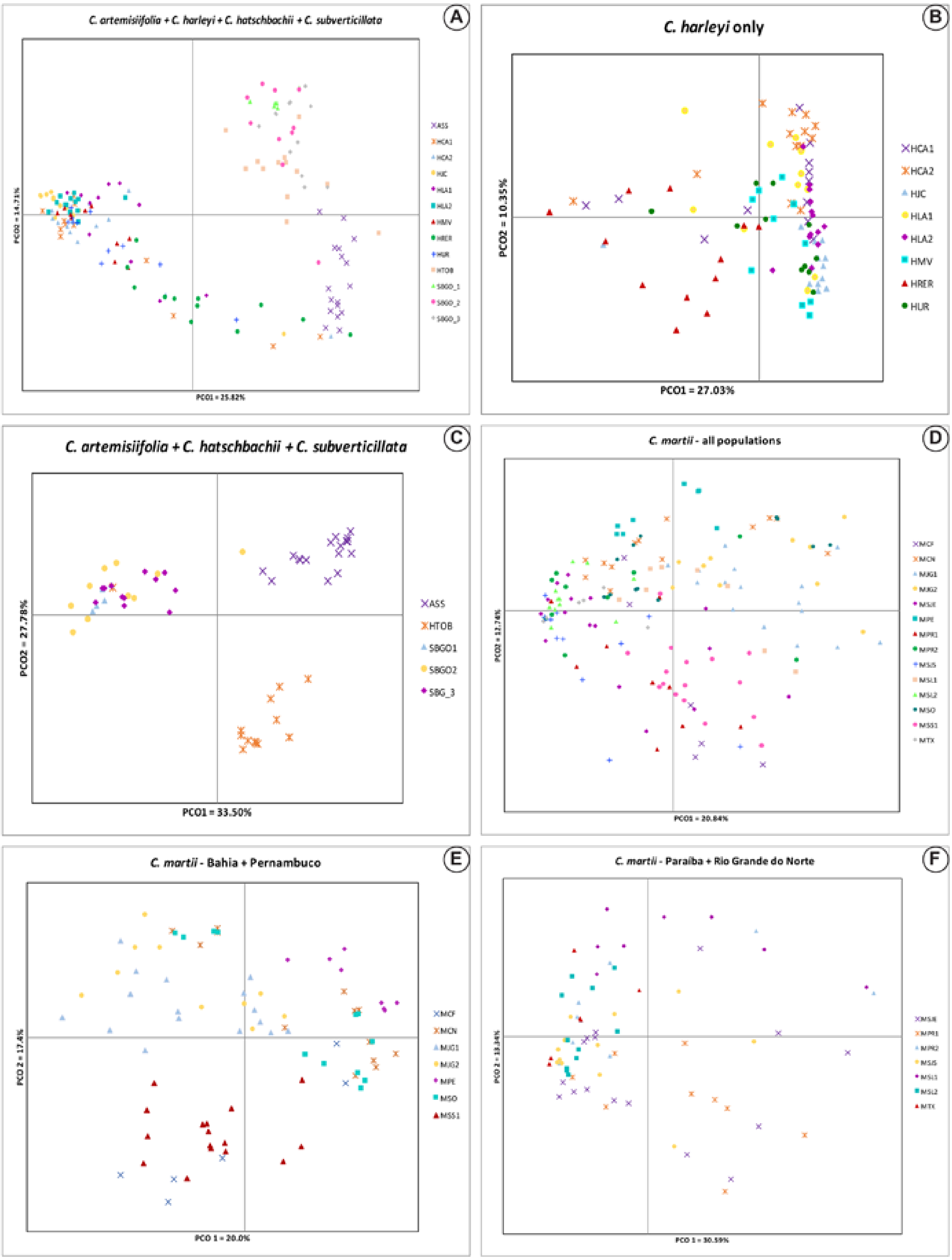


Figure 8. Principal Component Analysis plots. A. All populations of *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* (PCO1 = 25.82%, PCO2 = 14.71%). B. Only *C. harleyi* populations (PCO1 = 27.03%, PCO2 = 10.35%). C. *C. artemisiifolia, C. hatschbachii* and *C. subverticillata* populations (PCO1 = 33.50%, PCO2 = 27.78%). D. All *C. martii* populations (PCO1 = 20.84%, PCO2 = 12.74%). E. Only western *C. martii* populations (PCO1 = 20.0%, PCO2 = 17.4%). F. Only eastern *C. martii* populations (PCO1 = 30.59%, PCO2 = 13.34%).

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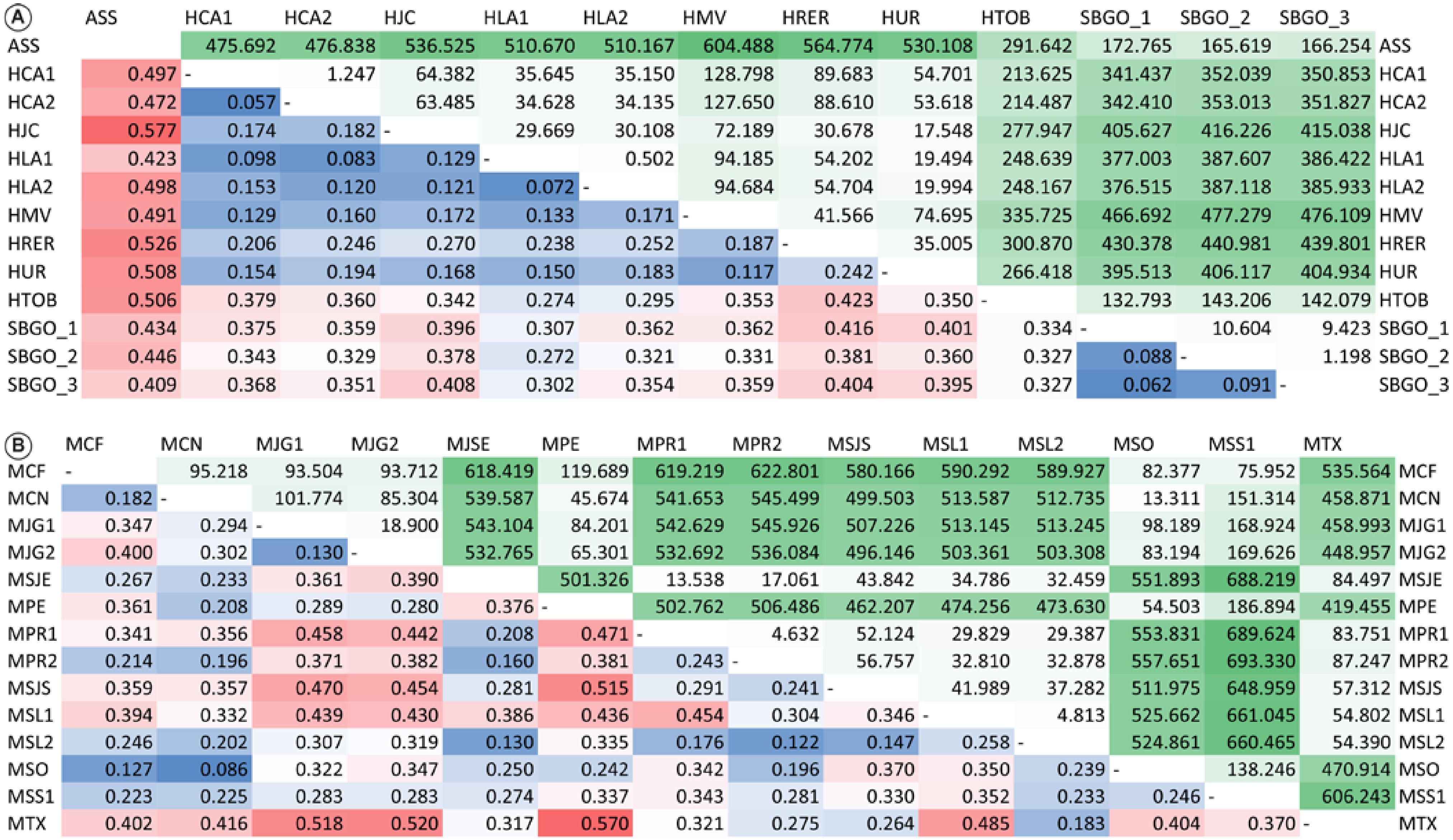

Ⓐ	ASS	HCA1	HCA2	HJC	HLA1	HLA2	HMV	HRER	HUR	HTOB	SBGO_1	SBGO_2	SBGO_3	
ASS		475.692	476.838	536.525	510.670	510.167	604.488	564.774	530.108	291.642	172.765	165.619	166.254	ASS
HCA1	0.497	-	1.247	64.382	35.645	35.150	128.798	89.683	54.701	213.625	341.437	352.039	350.853	HCA1
HCA2	0.472	0.057	-	63.485	34.628	34.135	127.650	88.610	53.618	214.487	342.410	353.013	351.827	HCA2
HJC	0.577	0.174	0.182	-	29.669	30.108	72.189	30.678	17.548	277.947	405.627	416.226	415.038	HJC
HLA1	0.423	0.098	0.083	0.129	-	0.502	94.185	54.202	19.494	248.639	377.003	387.607	386.422	HLA1
HLA2	0.498	0.153	0.120	0.121	0.072	-	94.684	54.704	19.994	248.167	376.515	387.118	385.933	HLA2
HMV	0.491	0.129	0.160	0.172	0.133	0.171	-	41.566	74.695	335.725	466.692	477.279	476.109	HMV
HRER	0.526	0.206	0.246	0.270	0.238	0.252	0.187	-	35.005	300.870	430.378	440.981	439.801	HRER
HUR	0.508	0.154	0.194	0.168	0.150	0.183	0.117	0.242	-	266.418	395.513	406.117	404.934	HUR
HTOB	0.506	0.379	0.360	0.342	0.274	0.295	0.353	0.423	0.350	-	132.793	143.206	142.079	HTOB
SBGO_1	0.434	0.375	0.359	0.396	0.307	0.362	0.362	0.416	0.401	0.334	-	10.604	9.423	SBGO_1
SBGO_2	0.446	0.343	0.329	0.378	0.272	0.321	0.331	0.381	0.360	0.327	0.088	-	1.198	SBGO_2
SBGO_3	0.409	0.368	0.351	0.408	0.302	0.354	0.359	0.404	0.395	0.327	0.062	0.091	-	SBGO_3

Ⓑ	MCF	MCN	MJG1	MJG2	MJSE	MPE	MPR1	MPR2	MSJS	MSL1	MSL2	MSO	MSS1	MTX	
MCF	-	95.218	93.504	93.712	618.419	119.689	619.219	622.801	580.166	590.292	589.927	82.377	75.952	535.564	MCF
MCN	0.182	-	101.774	85.304	539.587	45.674	541.653	545.499	499.503	513.587	512.735	13.311	151.314	458.871	MCN
MJG1	0.347	0.294	-	18.900	543.104	84.201	542.629	545.926	507.226	513.145	513.245	98.189	168.924	458.993	MJG1
MJG2	0.400	0.302	0.130	-	532.765	65.301	532.692	536.084	496.146	503.361	503.308	83.194	169.626	448.957	MJG2
MSJE	0.267	0.233	0.361	0.390		501.326	13.538	17.061	43.842	34.786	32.459	551.893	688.219	84.497	MSJE
MPE	0.361	0.208	0.289	0.280	0.376	-	502.762	506.486	462.207	474.256	473.630	54.503	186.894	419.455	MPE
MPR1	0.341	0.356	0.458	0.442	0.208	0.471	-	4.632	52.124	29.829	29.387	553.831	689.624	83.751	MPR1
MPR2	0.214	0.196	0.371	0.382	0.160	0.381	0.243	-	56.757	32.810	32.878	557.651	693.330	87.247	MPR2
MSJS	0.359	0.357	0.470	0.454	0.281	0.515	0.291	0.241	-	41.989	37.282	511.975	648.959	57.312	MSJS
MSL1	0.394	0.332	0.439	0.430	0.386	0.436	0.454	0.304	0.346	-	4.813	525.662	661.045	54.802	MSL1
MSL2	0.246	0.202	0.307	0.319	0.130	0.335	0.176	0.122	0.147	0.258	-	524.861	660.465	54.390	MSL2
MSO	0.127	0.086	0.322	0.347	0.250	0.242	0.342	0.196	0.370	0.350	0.239	-	138.246	470.914	MSO
MSS1	0.223	0.225	0.283	0.283	0.274	0.337	0.343	0.281	0.330	0.352	0.233	0.246	-	606.243	MSS1
MTX	0.402	0.416	0.518	0.520	0.317	0.570	0.321	0.275	0.264	0.485	0.183	0.404	0.370	-	MTX

Figure 9. Pairwise F_{ST} vs. geographic distance (km). A. All populations of *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata*. B. All populations of *C. martii.*

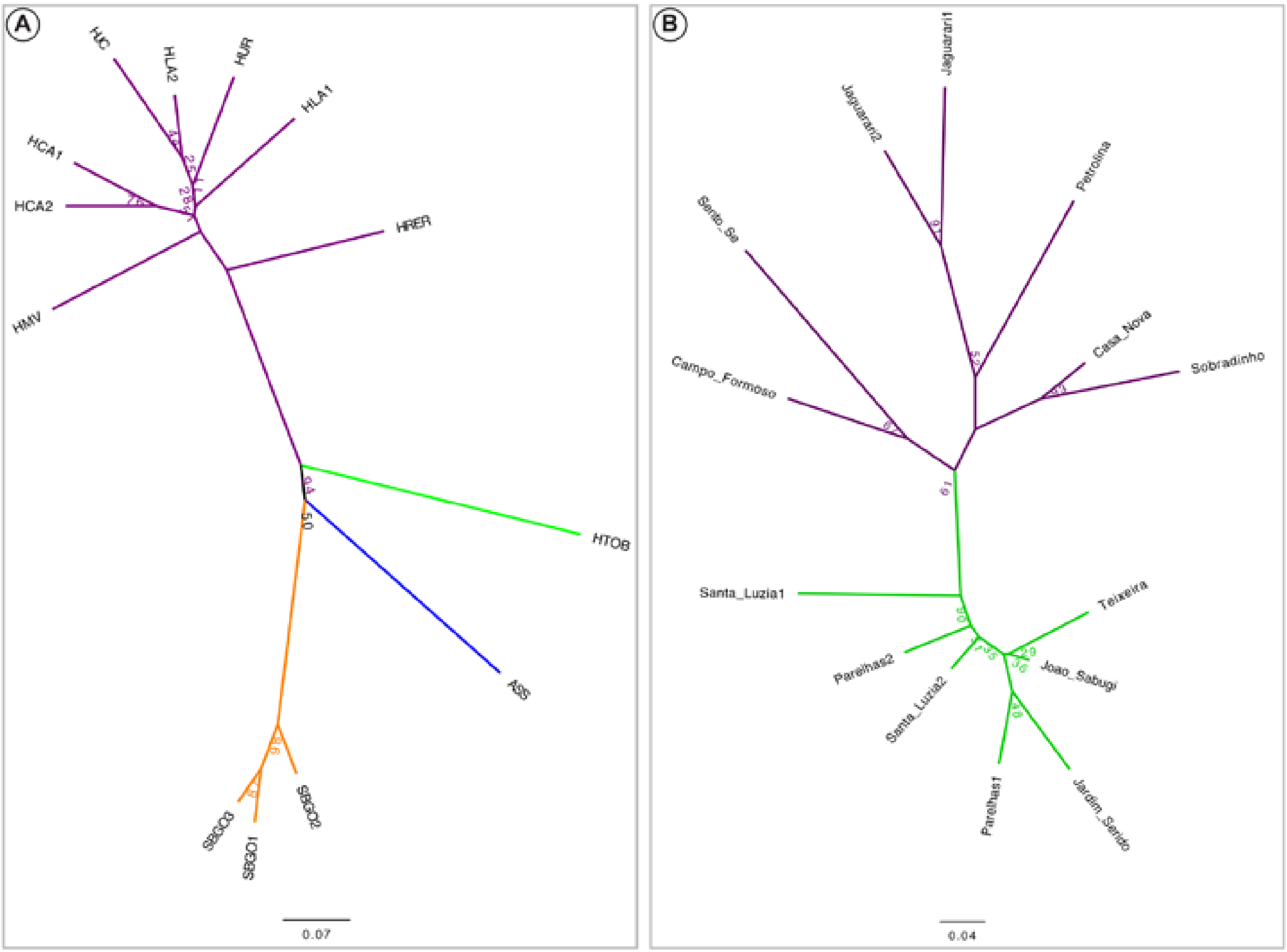

Figure 10. Neighbor-joining trees. A. All populations of *C. artemisiifolia* (blue), *C. harleyi* (purple), *C. hatschbachii* (green) and *C. subverticillata* (orange). B. All populations of *C. martii*, divided in western populations (purple) and eastern populations (green).

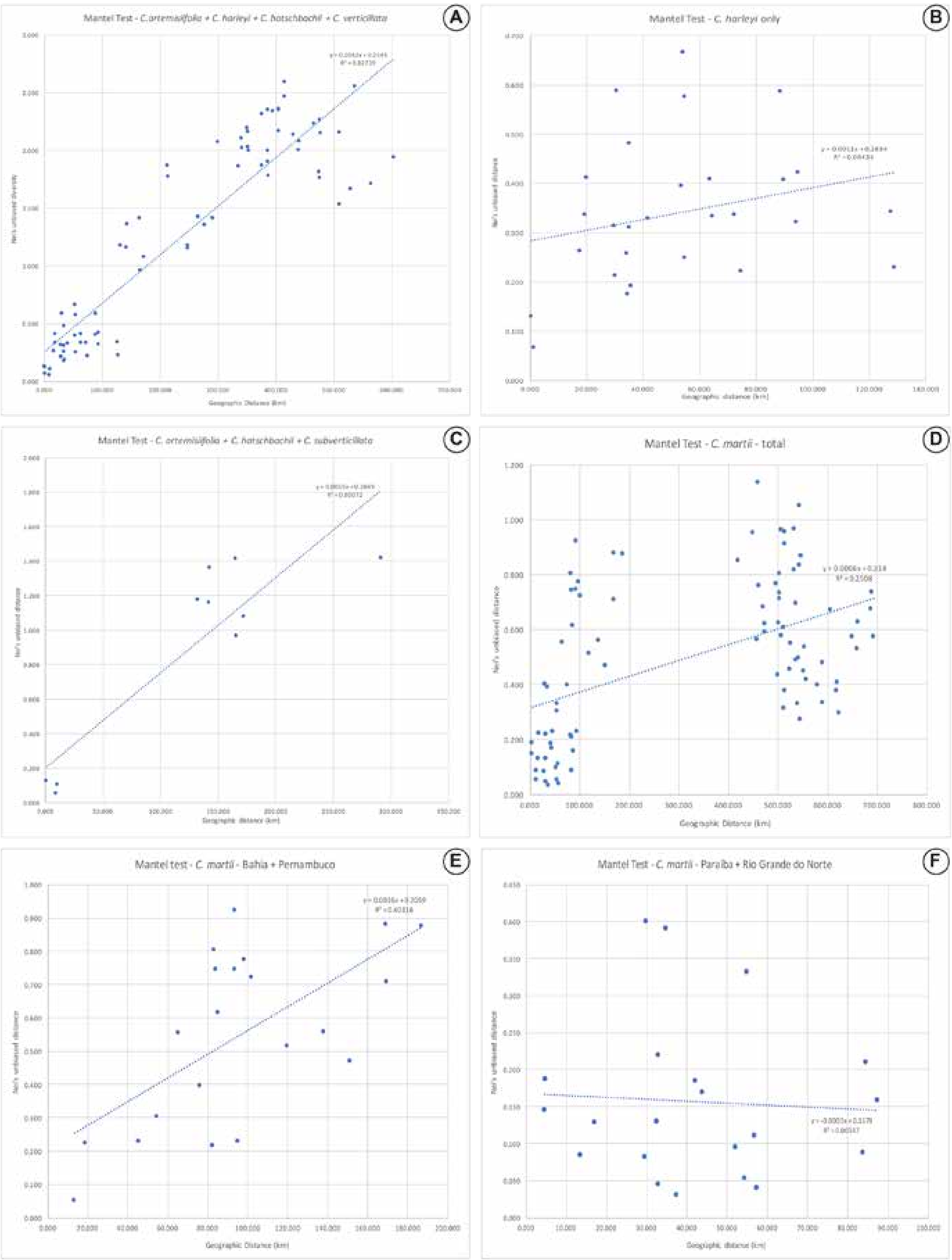

Figure 11. Mantel test plots. A. All populations of *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata* (y=0.0042x + 0.245, R^2=0.82739). B. All populations of *C. harleyi* (y=0.0011x + 0.2834, R^2=0.06434). C. All populations of *C. artemisiifolia, C. hatschbachii* and *C. subverticillata* (y=0.0055x+0.2049, R^2=0.80072). D. All *C. martii* populations (y=0.0006x+0.314, R^2=0.2508). E. All western *C. martii* populations (y=0.0036x+0.2059, R^2=0.40316). F. All eastern *C. martii* populations (y=0.0003x+0.1679, R^2=0.00347).

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Chapter 3

Synopsis of *Chresta* Vell. ex DC. (Vernonieae, Asteraceae) and a new phylogenetic infrageneric classification

To be submitted to: Phytotaxa

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Synopsis of Chresta Vell. ex DC. (Vernonieae, Asteraceae) and a new phylogenetic infrageneric classification

Abstract

Based on the results of a phylogenetic analysis, we propose a new infrageneric classification for *Chresta*, and present a synopsis of the genus. *Chresta* contains eighteen species divided into two subgenera and three sections, with one subgenera and correspondent section being monotypic. The remaining two sections are geographically defined, one containing the Caatinga and Atlantic Forest species and the other containing all Cerrado species. We present a key for all subgenera, sections and species, as well as descriptions for the new infrageneric categories. For each species, we present synonyms, a short description of main characteristics, comments on distribution and phenology, assessment of the conservation status, list of selected examined materials, illustrations and photographs and distribution maps.

Key words: taxonomy, Compositae, Chrestinae, sections.

Introduction

Chresta is composed by 18 species of herbs, subshrubs and shrubs that are mostly restricted to rock outcrops in the Cerrado and Caatinga domains, with only one species occurring in the Atlantic Forest. One of the most striking features of the genus is the presence of syncephalia, or secondary heads, which display several different morphologies. Some species, like *Chresta sphaerocephala* and *C. scapigera*, are widespread and commonly seen in Cerrado environments, and others, like *C. souzae* and *C. hatschbachii,* are narrow endemics and restricted to very specific habitats. The genus has a convoluted history that intermingles to that of several other South American Vernonieae groups, like the subtribe Lychnophorinae. Previous taxonomic treatments placed the species currently belonging to *Chesta* in different genera, such as *Eremanthus* (Baker 1873), *Argyrovernonia* (MacLeish 1984a), *Pycnocephalum* and *Glaziovianthus* (Mac Leish 1985a, 1985b), until they were united in *Chresta* (Robinson 1992, 1999). In the light of a new phylogenetic hypothesis for the genus (Siniscalchi et al. in prep., see Chapter 1), and taking into account that new species and synonyms were published since the last revisions (Robinson 2005, Roque et al. 2008, Loeuille et al. 2014, Siniscalchi et al. 2016), here we present a synopsis of the genus and propose a new phylogeny-based infrageneric classification.

Material & Methods

The study was based on the analysis of approximately 1,300 herbarium records belonging to *Chresta,* from 66 different herbarium collections. The specimens were seen in person by one of the authors and when only digital images of specimens were seen, those are indicated with 'e!'.

General morphology follows Harris & Harris (2001), inflorescence morphology follows Endress (2010), with additional terms taken from MacLeish (1984b); receptacle and pappus characters follow Small (1919), and microcharacters follow Robinson (1999).

All photographs were taken by the first author, unless specified, and maps were produced in QGIS v.2.18, using the geographic coordinates stated on herbarium records. In case of specimens not presenting coordinates, those were referenced using the municipality coordinate (centroid) or trying to approximate to landmarks or addresses, using Google Earth. Geological and geomorphological data were taken from Alkmim (2012), classification of vegetation types follow Forzza et al. (2012). Evaluation of conservation status was carried out using the GeoCAT Tool (Bachman et al. 2011) for Area of Occupancy (AOO) and Extent of Occurrence (EOO) analysis, using the IUCN default of 2 km. Conservation status was further evaluated using the IUCN manual (IUCN 2012).

Historical Background

Chresta was initially described by Velloso in *Flora Fluminensis*, with only two plates depicting *C. cordata* and *C. lanceolata*; however, due to the effective date of publication of Velloso's work being only 1881 (Carauta 1973), the valid publication of the name is ascribed to Candolle in the Prodromus (1836). Candolle accepted only *C. cordata* in his treatment, and changed its name to *C. sphaerocephala.* The illustration of *C. lanceolata* presented by Velloso shows features that could correspond to a number of different Vernonieae species and thus the binomial has been left aside in posterior works (e.g. Candolle 1836, MacLeish 1985b).

Candolle also included two other species in *Chresta*, *C. exsucca* and *C. pycnocephala,* indicating specimens from the Martius herbarium previously annotated as *Vernonia exsucca* and *Vernonia pycnocephala,* names that had not been published before.

In 1831, in the fourth dissertation regarding plants of the Berlin herbarium, Lessing treated the whole tribe Vernonieae, and created six sections in genus *Vernonia*, including *V.* sect. *Pycnocephalum,* composed by species with capitula grouped in terminal glomerules. Several species that now belong in *Lychnophora* were placed in that section, together with two species later considered to belong to *Chresta, V. scapigera* and *V. plantaginifolia.* In 1836, Candolle raised *V.* sect. *Pycnocephalum* to genus *Pycnocephalus,* keeping only those two species on it and transferring the remaining ones to *Lychnophora,* while describing also a new species, *P. spathulaefolium,* based on a specimen from Martius herbarium.

Also in the Prodromus (1836), Candolle presented treatments for two monospecific genera: *Pithecoseris* and *Stachyanthus,* both based on plants collected in the Caatinga, with secondary inflorescences and lobed leaves.

In 1842, after his travels around Brazil, Gardner described three new species of *Chresta* and proposed to reduce *Pycnocephalum* to a section of *Chresta,* on the basis that the difference between ramose and scapose habit and the position of the bracts would not be enough to distinguish them, and because one of the new species he was publishing presented intermediate characters between both groups. Gardner's work presented *Chresta* divided into *C.* sect. *Euchresta* and *C.* sect. *Pycnocephalum,* containing nine species in total, lacking any considerations regarding *Stachyanthus* and *Pithecoseris.* In 1846, the same author described *C. intermedia,* considering it an intermediate form between *C. sphaerocephala* and *C. pycnocephala.*

In the Compositae monograph for *Flora Brasiliensis* (1873), Baker grouped several genera of plants with secondary heads in *Eremanthus,* including all *Chresta* and the single *Stachyanthus* species, albeit in different sections. He recognized *Eremanthus* sect. *Chresta,* containing all species with a corymb of glomerules, *E.* sect. *Pycnocephalum,* containing species with solitary syncephalium, and *E.* sect. *Stachyanthus,* containing only *E. martii*. Nevertheless, *Pithecoseris* remained as a stand-alone genus in this treatment. Species circumscriptions were also modified by Baker: *Chresta spathulaefolia* was synonymized under *C. plantaginifolia; C. alpestris,* previously described by Gardner, was transferred to *Vernonia alpestris; C. intermedia* was synonymized as a variety of *E. sphaerocephalus;* and *Prestelia eriopus* was included in *E.* sect. *Pycnocephalum,* as *E. eriopus.* In the same year, the Compositae treatment in Genera Plantarum was published (Bentham 1873), and *Chresta* was not recognized as a genus, only *Eremanthus* and *Pithecoseris.*

After that, there were no further treatments for the whole genus for almost a century. In 1896, Taubert described *Eremanthus rivularis* and *E. harmsianus,* based on specimens collected by Ule on the state of Goiás, Central Brazil. He distinguished *E. rivularis* from *E. angustifolius* by the shape of the phyllaries and pointed out that *E. harmsianus* was somewhat similar to *E. speciosus.*

In 1938, Philipson described two species based on materials collected by Glaziou in Brazil, *E. curumbensis* and *E. pinnatifidus.* He cited as type specimen for *E. pinnatifidus* the material deposited at Kew, which did not have the original label by Glaziou, as he mentions the locality as being Rio de Janeiro, while the specimens deposited at P carry the original label showing Minas Gerais as the collection site, with one of the sheets also containing the locality Serra do Inficcionado, which is located at Serra do Caraça. No further records of this species are known and it might be extinct, or in fact it could have been collected somewhere else, since several Glaziou's collections lack confidence of geographical data (e.g. Wurdack 1970).

Barroso described a new genus in 1947, *Glaziovianthus,* including a single species, *G. purpureus.* She chose as type specimen a material collected by Helmut Sick in Goiás state. In 1960, Barroso described *Eremanthus imbricatus*, indicating that it belonged in *E.* sect. *Chresta* and was closely related to *E. pycnocephalum,* but not mentioning the similarity of the new species to *E. exsuccus.* She also cited a collection made by Glaziou where he had annotated the name *Eremanthus labordeii*, suggesting it was a new species, which was never published. In 1976, Zardini transferred *E. harmsianus* to *Glaziovianthus,* proposing the combination *G. harmsianus*.

In 1980, Robinson resurrected *Chresta,* placing on it some of the herbaceous species previously contained in *Eremanthus*, taking into account their difference in habit and in the presence of papillae on the inner surface of the base of the corolla lobes, as well as their lophorate pollen grains. He considered eleven species in *Chresta*, containing taxa previously placed in *Pycnocephalum, Stachyanthus* and *Glaziovianthus.* He provided some synonyms and also described a new species, *C. souzae*, based on material collected by Howard Irwin at Chapada dos Veadeiros in Goiás state. In 1983, Robinson described *Chresta harleyi,* based on materials from Bahia, northeastern Brazil, that were distributed as *C. martii,* noting that both species were closely related.

Also in the 1980s, MacLeish produced a revision of *Eremanthus,* in which she proposed several changes to its circumscription, along with correlate genera. Instead of keeping the herbaceous species together in *Chresta,* as did Robinson, she split them in four different genera.

In 1984, MacLeish described *Argyrovernonia* to replace *Stachyanthus* DC., because this was a latter homonym in the Icacinaceae, and transferred *C. martii* to it. In 1985, she published full taxonomic treatments for *Chresta, Pycnocephalum* and *Glaziovianthus.*

In MacLeish's works, *Chresta* contains four species with coriaceous leaves, corymbs of glomerules or solitary glomerules with more than 20 heads per glomerule and heads with usually three florets, while *Pynocephalum* has three species with membranaceous leaves, solitary glomerules or corymb of glomerules, with less than 25 heads per glomerule and heads with 4-12 florets. The two species in *Glaziovianthus* are distinguished by the solitary glomerules with red flowers, deciduous pappus and pollen type A.

MacLeish also transferred *C. eriopus* back to *Prestelia,* synonymized *C. souzae* into *Pycnocephalum plantaginifolium* and transferred *C. harleyi* to *Argyrovernonia harleyi.* Regarding the names in *Glaziovianthus,* she combined *G. purpureus* and *Eremanthus curumbensis* in *G. curumbensis,* and *Eremanthus speciosus* and *Glaziovianthus harmsianus* in *G. speciosus.*

In 1992, Robinson described the genus *Minasia* and transferred *Chresta alpestris* to it, which was first described by Gardner (1842) and later considered as *Vernonia alpestris* by Baker (1873). Also, Robinson then proposed that *Chresta* and *Soaresia* did not belong in the Lychnophorinae, due to the lophate pollen and non-sclerified anther appendage cells, but left both as unplaced genera within Vernonieae.

In 1999, in his revision of the American taxa of Vernonieae, Robinson recommended the use of *Chresta* in its wider interpretation, including the genera segregated by MacLeish, stating that the characteristics used to separate these taxa violate close relationships. He created subtribe Chrestinae, containing *Chresta* with 12 species plus *Pithecoseris* and *Soaresia,* both monospecific. The characters that separate Chrestinae from Lychnophorinae, in which these genera had been placed before, were the presence of glaucolides, lophate pollen and anther appendages lacking thick-walled cells.

In the 2000s two new species were added to *Chresta: C. hatschbachii*, with strong affinities to *C. harleyi,* described by Robinson in 2005, and *C. amplexifolia,* published by Roque *et al.* in 2008. In 2014, Loeuille et al. synonymized *Pithecoseris pacourinoides* into *Chresta,* based either on morphological characters that approximate the two genera and on results of a molecular and morphological phylogeny. *Chresta amplexifolia* was also synonymized into *C. pacourinoides,* as the specimen used to describe the former was a young individual of the latter. In 2016, a species endemic to the Atlantic Forest domain was published, *C. filicifolia* (Siniscalchi et al. 2016).

In 2015, a molecular and morphologic phylogeny was published (Loeuille *et al.* 2015), showing that, in order to be monophyletic, *Chresta* should be used in the wider sense as proposed by Robinson, since the other generic divisions did not emerge as monophyletic groups. This work also showed that *Soaresia* should belong with the Elephantopinae, not with Chrestinae.

In the light of a new phylogenetic hypothesis based on hundreds of molecular markers (Siniscalchi et al. in prep., see chapter 1), the monophyly of *Chresta* sensu lato is highly supported, as are its subordinated clades. Hence, we reviewed the taxonomy of the group and provided novel phylogenetic classification and synopsis.

Proposed infrageneric classification

Chresta Vell. ex DC.
- *C.* subg. *Argyrovernonia* Siniscalchi, Loeuille & Pirani
  - *C.* sect. *Argyrovernonia* Siniscalchi, Loeuille & Pirani
    - *C. martii* (DC.) H.Rob.
- *C.* subg. *Chresta*
  - *C.* sect. *Rupicola* Siniscalchi, Loeuille & Pirani
    - *C. pacourinoides* (Mart. ex DC.) Siniscalchi & Loeuille
    - *C. heteropappa* Siniscalchi & Loeuille
    - *C. filicifolia* Siniscalchi & Loeuille
    - *C. pinnatifida* (Philipson) H.Rob.
    - *C. harleyi* H.Rob.
    - *C. artemisiifolia* Siniscalchi & Loeuille
    - *C. subverticillata* Siniscalchi & Loeuille
    - *C. hatschbachii* H.Rob.
  - *C.* sect. *Chresta*
    - *C. angustifolia* Gardner
    - *C. souzae* H.Rob.
    - *C. plantaginifolia* (Less.) Gardner
    - *C. speciosa* Gardner
    - *C. curumbensis* (Philipson) H.Rob.
    - *C. pycnocephala* DC.
    - *C. exsucca* DC.
    - *C. scapigera* (Less.) Gardner
    - *C. sphaerocephala* DC.

Composition and characteristics of infrageneric divisions

Our recent phylogenetic study (Siniscalchi et al.in prep., see Chapter 1) support the classification of *Chresta* in two subgenera and three sections (Fig. 1). These groupings are based on the clades recovered with molecular data, but they are also clearly recognized on morphological grounds.

Chresta subg. *Argyrovernonia* sect. *Argyrovernonia* contains only *Chresta martii*, which appears as sister to all other species in the phylogeny. Main features that characterize this group are the presence of aromatic leaves, hemispherical syncephalia with marked indeterminate growth, and pollen type F. *Chresta* subg. *Chresta* contains all other species, which in turn are divided in two sections: *C.* sect. *Rupicola* and *C.* sect. *Chresta.*

Chresta sect. *Rupicola* corresponds to the "Caatinga clade" (Siniscalchi et al.in prep., see Chapter 1), containing all species that grow directly on rocks, most of them on the Caatinga. The rupiculous species are grouped in two clades. The first contains two sister species that occur in forest remnants (*brejos de altitude)* or more humid vegetation on top of granitic inselbergs found within that dry domain, *C. heteropappa* and *C. pacourinoides,* plus the single species found on rock outcrops in the Atlantic Forest, *C. filicifolia*. The putative synapomorphy of this clade is the presence of lageniform sweeping hairs on the back side of the style arms.

The second "Caatinga clade" composing *C.* sect. *Rupicola* contains *C. artemisiifolia, C. harleyi, C. hatschbachii* and *C. subverticillata.* It is morphologically homogeneous and some of its diagnostic features are the narrow pinnatilobate or pinnatisect leaves, covered by dense greyish-silver indument of T-shaped trichomes, the solitary, hemispherical syncephalia, purple flowers with a white throat and type C pollen. *C. artemisiifolia* and *C. subverticillata* also present evidence of indeterminate growth of the syncephalium axis, but not as markedly as seen in *C. martii,* and in only some of the syncephalia. They are distributed on Espinhaço Septentrional in northern Minas Gerais and Bahia and in the northwestern and northeastern ends of Chapada Diamantina massif.

Chresta sect. *Chresta* contains all species from the Cerrado and it is also divided into two clades. The first one contains four species provided with scapose, solitary syncephalia with rosette leaves, except for *C. angustifolia,* which has cauline leaves. Except for *C. speciosa,* which has red florets with a yellow band on the tip of the corolla tube, all other species present purple florets with white throats. The species of this clade have a somewhat more restricted distribution, often related to specific environmental conditions; *C. souzae* that occurs in wet, dark soils with high quartz content, *C. angustifolia* grows on the margins of intermittent rivers and waterfalls, in sand banks and rocks, and *C. plantaginifolia* and *C. speciosa*, which occur in dry, rocky soils with high quartz content.

The second Cerrado clade is composed mostly by species with coriaceous leaves and compound synflorescences, with spherical syncephalia. The exceptions are *C. curumbensis,* which has subcarnose leaves and solitary, hemispheric syncephalium, and *C. scapigera,* which presents solitary, spherical syncephalium on top of a very long scape that sometimes develop discreet lateral branching. The five species in this clade usually grow on the red latosols widespread throughout the Cerrado, and except for *C. curumbensis,* all species have a diffuse, thickened underground system.

Comparative morphology

Habit and branching

Most of the species of *Chresta* are perennial herbs, subshrubs and shrubs, with only *C. pacourinoides* and *C. heteropappa* being annual or biannual herbs, unbranched and with cauline leaves, sprouting from a fasciculate underground system. All species in the first Caatinga clade, plus *C. filicifolia,* are erect subshrubs ramified at the base, with a slightly diffuse underground system that penetrates in crevices and cracks in the rocks where they grow. Five of the Cerrado species present rosettes, but they do not form a monophyletic group. *C. speciosa* presents a variation of the rosette habit, where the rosettes emerge from the tip of the stems, not from the base. All rosette species produce thickened underground systems, such as diffuse and thickened, non-ramified and thickened and moniliform. The remaining Cerrado species are erect shrubs, usually ramified at the base, with cauline leaves and diffuse and a thickened underground system.

Leaves

The phyllotaxy in the genus is always alternate, even in the rosetted species. Leaves from Caatinga and Cerrado species are easily distinguishable, all Caatinga species (*C.* sect. *Argyrovernonia* and *C.* sect. *Rupicola*) have more or less deeply pinnatilobate leaves, while the Cerrado species (*C.* sect. *Chresta*) have leaves with entire margins, or slightly serrulated or dentate.

Chresta artemisiifolia, C. harleyi, C. hatschbachii, C. martii and *C. subverticilata* have silvery green leaves, with dense indumentum composed of T-shaped hairs. *C. martii* has petiolate, subcarnose leaves that are aromatic, usually distributed along the stems, except for some populations on the eastern end of the distribution where the leaves are more crowded on the top of very short stems. *C. harleyi* and *C. artemisiifolia* have petiolate leaves spaced along the stem, but mostly crowded on the tip of the axes. *C. hatschbachii* has subsessile leaves equally distributed along the stem and *C. subverticillata* has minute leaves that grow on very short branches, appearing to be leaf whorls. *C. pacourinoides* and *C. heteropappa* have green and coarse leaves, with multicellular setae; *C. filicifolia* is in the same clade, but has leaves more similar to the other Caatinga group, with dense indumentum with T-shaped hairs and more crowded on the top of the stems.

The Cerrado species (*C.* sect. *Chresta*) can either present membranaceous, soft leaves, or strongly coriaceous leaves; these characters correlate loosely with the two Cerrado clades. Species in the first Cerrado clade present soft leaves with sparse indumentum, except for *C. speciosa,* which can present dense whitish indumentum, especially in the younger leaves. *C. angustifolia* and *C. souzae* have thicker, subcarnose leaves and are mostly glabrous, but *C. angustifolia* has narrow leaves that are evenly distributed along the stem, while *C. souzae* has leaves in variable shapes, sometimes being very small. *C. plantaginifolia* has leaves that can become coriaceous with age, and the indumentum is denser in younger leaves.

Species in the second Cerrado clade have coriaceous leaves, which are more or less greyish-green and distributed along the stem, the only exception being *C. curumbensis,* which has sub-carnose, bright green leaves with reddish venation and margins. *C. exsucca* and *C. scapigera* present dense venation and abundance of fibers, while *C. pycnocephala* has subcoriaceous, silvery green leaves with obscure venation. *C. scapigera* presents a basal rosette, while *C. exsucca* and *C. pycnocephala* have sessile, cauline leaves that usually stay in an upright position, almost adpressed to the stems. *C. sphaerocephala* has petiolate leaves with denser indumentum that can vary depending on the age and environmental conditions and usually older leaves are curved backwards and the margins are undulated. Most species within this clade present leaves with pinnate camptodromous to brochydodromous venation, the exceptions being *C. exsucca,* which has actinodromous venation, with 3 to 7 primary veins, and *C. speciosa*, which has parallel veins.

Synflorescences

There are two main patterns of synflorescences in *Chresta*: solitary syncephalium in an unbranched scape or panicles of syncephalia. It is not possible to define groups based on this feature, but some remarkable patterns are observed. In the Caatinga species, the clade formed by *C. harleyi* and its correlates all present solitary syncephalia, as well as *C. martii*. The other clade presents two species with compound synflorescences, *C. pacourinoides* and *C. heteropappa,* while *C. filicifolia* presents solitary syncephalia in unbranched scapes.

In the Cerrado groups, all species on the first clade present solitary syncephalia and most of the species in the second group are provided with compound synflorescences. The exceptions in that second group are *C. curumbensis,* which has solitary syncephalia, and *C. scapigera*, which produces a very long scape (usually more than one meter long) that not uncommonly is branched and presents some lateral syncephalia.

Species bearing panicles of syncephalia are usually provided with leaves at the base of the synflorescence branches, and those are similar to the vegetative leaves, but smaller. The species with solitary syncephalia usually present bracts on the base of the scape, and those usually are small and different from the leaves.

Syncephalia and capitula

There are two basic types of syncephalia in *Chresta:* the hemispherical type, where the capitula are loosely joined at their bases, and the spherical type, with the capitula tightly joined together and somewhat emerged in a tissue matrix. Additionally, the axis of the syncephalia may present determinate and indeterminate growth. Marked indeterminate growth occurs only in *C. martii,* with loosely joined capitula, and *C. pacourinoides,* with spherical syncephalia. *C. heteropappa* and *C. subverticillata* sometimes produce slightly elongated axes.

The more predominant type of syncephalia is the hemispherical, which occurs in *C.* sect. *Argyrovernonia*, almost all species from *C.* sect. *Rupicola*, and in half of the species from *C.* sect. *Chresta.* Nevertheless, spherical syncephalia arose twice in the genus, once in *C.* sect. *Rupicola* and once in *C.* sect. *Chresta.*

Capitula in the genus are usually cylindrical or obconic in shape and the involucres are always multiseriate, usually with 4 to 7 series of imbricate phyllaries. The outermost series of phyllaries usually are smaller, and the size gradually increases towards the innermost series. Most of the species present membranaceous, green phyllaries, which in some taxa have purplish or reddish margins and tips. Phyllaries are usually elliptic, lanceolate or oblong, the apex acute, most of the times bearing a mucron or arista.

Each capitulum can present from 3 to 12 florets, and although each species has a more or less fixed number, we found that this character does not have any significance in distinguishing among clades. The receptacles are usually flat, foveolate, glabrous and without palea.

Florets

The florets are always bisexual and fertile, with five petals, usually ranging from 1 to 5 cm in length. Petal margins may be thickened or not, and the tips of the petals can have simple or glandular trichomes, or combinations of both, although these are rare. Except for two red-flowered species, all others have purple florets, although these can be divided into two groups: purple flowers with a white throat and purple flowers with purple throat. The white throat is observed in all Caatinga species, and in one of the Cerrado clades, and sometimes the white color can spread to the base of the petals, as usually seen in *C. souzae*. Florets with purple throat are found only in Cerrado species with spherical syncephalia. The two red-flowered species, *C. curumbensis* and *C. speciosa,* present a yellow band on the apical portion of the corolla tube, sometimes spreading to the base of the petals.

The petals are spread out in a 90°-angle with the tube, with the style and anther ring straight up. The portion of the style that projects above the anther ring is usually very minute, with exception of the two red-flowered species and *C. pacourinoides,* which produce very long styles, with the portion above the anthers almost as long as the corolla tube. Style arms are strongly curved backwards, as with most Vernonieae.

Reproductive biology

As in most Vernonieae species presenting syncephalia, the maturation of the individual florets on the capitula is sequential. The maturation starts from the base of the syncephalium upwards, with each capitulum blooming a new floret each day. The pollen presentation phase occurs first and lasts for a few hours, after which the stigmatic surfaces are exposed.

Due to the characteristics of the florets and their purple color, it is expected that most species are pollinated by insects, especially bees and bumblebees. Field observations by the first author have shown that visiting insects move around the syncephalia, what would lead to the spread of pollen of the syncephalium over itself, probably suggesting the presence of some self-incompatibility mechanism.

Nevertheless, our field observations have shown that the red-flowered species are mostly visited by hummingbirds. In the two red-flowered species, the styles are greatly projected above the anthers, which seems to be a common feature in bird-pollinated Asteraceae (Vogel 2015). *C. pacourinoides* presents the same morphology and there is evidence of hummingbird visitation on it as well (Las Casas 2009).

Cypsela

Most species on the genus have monomorphic cypselas, but *C. heteropappa* and *C. pacourinoides* present dimorphic cypselas. Cypselas in *Chresta* are usually cylindrical to turbinate, with ca. 20 inconspicuous ribs, and vary from brown to brownish-black. The twin-hair indument is usually dense, covering the whole cypsela body. The twin-hairs are usually long and straight, with both cells with slightly unequal size, joined together throughout their length, being separated only at the tip. Notable exceptions are *C. martii,* in which both cells are separated at the base, with unequal lengths, and *C. heteropappa* and *C. pacourinoides*, where one of the cypsela types is glabrous and the other present short and stiff twin-hairs.

Putative interspecific hybrids

Observations of individuals of natural populations during field work, associated to the analysis of herbarium specimens revealed two possible cases of interspecific hybrids in *Chresta*, one between *C. pycnocephala* and *C. scapigera* and another between *C. exsucca* and *C. sphaerocephala.* In both cases, the populations present a wide spectrum of distinct morphologies, that do not appear to be parts of a gradual cline along some kind of environmental condition; instead they possibly indicate back-crossing of the hybrids with the putative parents.

The possible *C. pycnocephala* × *scapigera* population is located in western Minas Gerais and populations of both parents were found nearby the hybrid population. Collected individuals showed difference in habit, from plants with basal rosette to plants with cauline leaves grouped in a rosette-like structure to true cauline leaves. Leaf morphology and size also varied, with different combinations of textures (coriaceous to subcoriaceous), shapes (from the large, obovate leaves of *C. scapigera* to the narrower, elliptical leaves of *C. pycnocephala*). The size and degree of branching also varied among individuals, going from the scapose syncephalium of *C. scapigera* to variable branched panicles (Fig.

2A).

The second putative hybrid population, *C. exsucca* × *C. sphaerocephala*, was identified in Distrito Federal, around Planaltina. As with the first populations, there is a variety of differently morphologies, but most plants are shrubs with few ramifications at the base, long and flexuose stems and panicle of syncephalia. The main difference between individuals is leaf shape and size, varying from the large leaves curved backwards typical of *C. sphaerocephala* to the smaller, rigid leaves of *C. exsucca.* The venation varies, going from the actinodromous venation seen in *C. exsucca* to pinnate, with different numbers of primary veins and the position from where they diverge (Fig. 2B).

There are a few other records identified as *C. pycenocephala* that were collected in the same general location of these possible hybrids [Hatschbach et al. 59295 (HUEFS, MBM, NY, US) Loeuille et al. 272 (SPF), Ulhoa 19 (SPF), Vichnewski 35203 (UEC)] and these specimens show some of the features of the hybrid individuals, such as the actinodromous venation, the variability in size and the striated stems. It is likely that these records actually belong to hybrids and not to *C. pycnocephala*, as all other populations of this species occur only in Minas Gerais and present an overall different, less variable, morphology.

Taxonomic treatment

Chresta Vellozo ex Candolle (1836: 85). Type (designated by Robinson (1980: 91):—*C. sphaerocephala* DC.
Chresta Vellozo (1829: t. 150, 151), nom. inval.
Vernonia sect. *Pycnocephalum* Lessing (1829: 630). *Pycnocephalum* (Lessing) Candolle (1836: 83). Type (designated by MacLeish (1985b: 461):—*P. plantaginifolium* (Less.) DC. [≡ *Chresta plantaginifolia* (Less.) Gardner]
Pithecoseris Martius ex Candolle (1836: 84). Type:—*P. pacourinoides* Mart. ex DC. [≡ *Chresta pacourinoides* (Mart. ex DC.) Siniscalchi & Loeuille]
Glaziovianthus Barroso (1947: 114). Type:—*G. purpureus* Barroso [= *Chresta curumbensis* (Philipson) H.Rob.]
Stachyanthus Candolle (1836: 84), nom. rej. vs. *Argyrovernonia* MacLeish (1984: 106). Type:—*A. martii* (DC.) MacLeish [≡ *Chresta martii* (DC.) H.Rob.]

Herbs, subshrubs, shrubs, erect, branched or not at the base, up to 2.5 m tall, perennial or (bi) annual, with thickened and ramified underground systems, or fasciculate roots (these subterete or moniliform). Indumentum composed by T-shaped trichomes, trichome pedicel with 1–7 cells. Leaves alternate, cauline or in basal rosettes or fasciculate on the tip of the stems, coriaceous, sub-coriaceous, membranaceous or sub-carnose, sessile or petiolate; petioles 1–40 mm compr.; leaf blades 0.5–50 cm length, 0.2–25 cm wide, linear, oblanceolate, obovate or elliptic, base cuneate, cordate, decurrent to acuminate, apex acute to acuminate, rounded or emarginate, margin entire, pinnatilobate, pinnatifid, pinnatisect, dentate, deeply dentate, denticulate or biserrate, adaxial and abaxial surfaces glabrate to tomentose; venation pinnate camptodromous or brochidodromous or actinodromous or parallel, primary vein usually prominent, secondary and tertiary veins obscure to profusely reticulate. Synflorescence scapose or paniculate, with slender branches, usually covered by indumentum, frequently engorged on the base of the syncephalia, sometimes hollow, 4–150 cm tall.

Secondary bracts on the base of the syncephalia or on the base of the synflorescence branch. Solitary syncephalia or in panicles composed from 2 to 10 syncephalia. Syncephalia 11-120 mm high, 12-60 mm wide, hemispherical, with capitula loosely joined by the base, or spherical, with tightly joined capitula. Capitula 5–200 per syncephalium, homogamous, discoid, sessile; involucre cylindrical to obconic, 3–25 mm high, 2–12 mm wide, with 4–7 series of imbricate phyllaries; phyllaries with membranaceous margins, apex acute, mucronate or aristate, external surface glabrous to tomentulose. Florets 3–13 per capitulum, corollas 8–45 mm high, purple with purple or white throat, with white spots sometimes spreading to the base of the corolla lobes, or red with yellow throat; corolla lobes with acute to acuminate apex, with 2 marginal veins. Anthers with base sagitate to acuminate or obtuse, appendages with non-sclerified cells; styles 3–40 mm long., style arms 3–6 mm length, long and convolute, pubescent, sweeping hairs acute, clavate, lageniform or subulate. Pollen *Vernonia*-type A, C or F. Cypselas monomorphic or dimorphic, cylindrical to cylindrical-turbinate to turbinate, 1.0–11.5 mm tall, glandular to sericeous, 10-20-ribbed; carpopodium prominent, symmetrical to asymmetrical, 0.15–0.5 mm tall. Pappus persistent, sometimes caducous, 2–4 series of stramineous to whitish elements, setose to paleaceous, serrulate.

Key to subgenera, sections and species:

1. Leaves aromatic, covered by a dense indumentum of T-shaped trichomes; solitary scapose syncephalia, composed of capitula attached to the axis only by the base; syncephalia axis presenting indeterminate growth ……..........…… ***Chresta*** **subg.** ***Argyrovernonia*** **sect.** ***Argyrovernonia (C. martii)***

1'. Leaves non-aromatic, glabrous to covered by dense indumentum of T-shaped trichomes; scapose or branched synflorescence, composed of capitula joined only by the base (hemispheric) or tightly joined (spherical), if hemispheric, not presenting indeterminate growth …………..…… ***Chresta*** **subg.** ***Chresta***

  2. Rupiculous plants; leaves with deeply lobate, dentate, pinnatilobate or pinnatifid margins, membranaceous, dark green, coarse, with few trichomes and multicellular setae or silvery-green, with dense indumentum of T-shaped trichomes; occurring in the Caatinga or Atlantic Forest ……. ………..……..……….……..…………..……..……………..………….... ***Chresta*** **sect.** ***Rupicola***

    3. Leaves membranaceous, dark green, glabrate to glabrous, turning dark when dried; spherical syncephalia with tightly joined capitula, presenting indeterminate growth ….. …………..……... **4**

      4. Style length ca. 5.5 mm, with no more than 2 mm exserted above the anthers; pappus elements paleaceous-setose …..…………..……..……………..……..……….. ***C. heteropappa***

      4'. Style length ca. 10 mm, exserted part of the style almost as long as the corolla tube; pappus elements barbelate-setose ……..……………..……..…………..……..………….. ***C. pacourinoides***

    3'. Leaves membranaceous to subcarnose, greyish-green, usually silvery-gray when dried, covered by dense indumentum of T-shaped trichomes; hemispherical syncephalia with capitula joined only at the base ……….…..…………..……..…………..……..…………..…..………..….... **5**

      5. Leaves distinctly petiolate ………..…………..……..…………..……..…………..…..…..……. **6**

        6. Leaves silvery-grey on dried material; scape 12-28 cm long; capitula 10-30 per syncephalium; flowers 7-8 flowers per capitulum, carpopodium ca. 0.15 mm length ……. ……….……..……….……..…………..…..…………..……..…………..… ***C. harleyi***

        6'. Leaves brown on dried material, scape 21-40 cm long; capitula 8-22 per syncephalium; 8

flowers per capitulum, carpopodium 0.3-0.5 mm length ………………………… ***C. filicifolia***

5’. Leaves sessile or subsessile ……………………………………………………………… **7**

7. Leaves minute, usually less than 4 cm long, grouped on the tip of very short branches, resembling whorls of leaves; scape 6-25 cm long, syncephalia sometimes presenting slight indeterminate growth, resembling spikes ……………………………………… ***C. subverticillata***

7’. Leaves 2-7 cm long, evenly distributed on the stems; scape 1-4.5 cm long; syncephalium not presenting indeterminate growth ……………………………………… ***C. hatschbachii***

2’. Plants not rupiculous; leaves with margins entire or minutely dentate, serrate or bi-dentate, membranaceous, subcarnose or coriaceous, usually glabrate or covered by denser indumentum when young; occurring in the Cerrado ……………………………………… ***Chresta* sect. *Chresta***

8. Hemispherical syncephalia, with capitula joined only at the base ……………………………… **9**

9. Florets red with yellow throat and band on the tip of the corolla tube; fruits with deciduous pappus ……………………………………………………………………………… **10**

10. Herbs with a basal rosette of subcarnose leaves, bright green, with reddish venation and margins, in the live plant and turning dark-brown or black on dried specimens; venation pinnate; bracts on the base of the scape absent ……………………………… ***C. curumbensis***

10’. Herbs presenting fascicles on the tip of the stems; leaves sub-coriaceous, light-green to grayish-green in the live plants, turning dull green in dried specimens, usually drying and falling off during flowering; venation parallel; filiform bracts minute present on the base of the scape …………………………………………………………………… ***C. speciosa***

9’. Florets purple with white throat or white band or spots on the tip of the corolla tube or base of corolla lobes; pappus persistent, not deciduous……………………………………… **11**

11. Subshrub with cauline leaves, these lanceolate, narrow, glabrous to glabrate; growing on river margins ………………………………………………………………… ***C. angustifolia***

11’. Herbs with basal rosettes of leaves ……………………………………………………… **12**

12. Plants usually growing on moist, dark soil with high content of quartz; leaves minute, elliptic or obovate, glabrate or with erect trichomes; venation usually obscure and not salient; indumentum, florets usually presenting the basal half of the corolla lobes white; restricted to Chapada dos Veadeiros, Goiás ……………………………………… ***C. souzae***

12’. Plants growing on dry soils with high quartz content; leaves long, oblong, narrow-elliptic or spatulate, glabrate to tomentose; venation marked and salient; florets usually only with the throat white; found in Minas Gerais, Distrito Federal, Goiás and Paraná ……………………………………………………………………… ***C. plantaginifolia***

9’. Spherical syncephalia, with tightly joined capitula ……………………………………… **13**

13. Herbs with a basal rosette of leaves; scape usually unbranched, reaching up to 1.5 m ……………………………………………………………………………… ***C. scapigera***

13’. Erect shrub with a branched synflorescence ……………………………………………… **14**

14. Leaves petiolate, usually with the blade strongly curved backward ……………………………………………………………………………………… ***C. sphaerocephala***

14’. Leaves sessile, usually adpressed to stems, the blade flat and erect ……………………… **15**

15. Leaves coriaceous, decreasing in size from the base up; venation actinodromous, with marked midrib and 2-4 lateral primary veins, tertiary veins strongly reticulate,

…..…………….…….…….…….…….…………….…….…….………….….. ***C. exsucca***

15’. Leaves sub-coriaceous, not markedly decreasing in size from the base up; venation pinnate, midrib salient on the abaxial face, secondary and tertiary veins usually obscure and not strongly reticulate ………………….…….…….…..... ***C. pycnocephala***

I. *Chresta* subg. *Argyrovernonia* Siniscalchi, Loeuille & Pirani, *subg. nov.* Type:—*C. martii* (DC.) H.Rob.

Erect subshrubs, ramified at the base, with diffuse, not thickened underground system, with cauline, aromatic leaves, these sometimes crowded at the tips of the stem axes. Leaves petiolate, semi-fleshy, aromatic, covered with a dense indumentum composed of T-shaped trichomes, margins deeply dentate or serrate. Syncephalia solitary, scapose, hemispherical, presenting indeterminate growth. Florets purple with white throat; style arms covered by acute sweeping hairs; pollen type F. Cypselas monomorphic, cylindrical, tomentose, twin-hairs presenting uneven cells separate at the base, carpopodium symmetrical, pappus persistent.

A single monospecific section is recognized in this subgenus.

I.I. *Chresta* sect. *Argyrovernonia* Siniscalchi, Loeuille & Pirani, *sect. nov.* Type:—*C. martii* (DC.) H.Rob.

A monospecific section, diagnosed by the same characters as the subgenus.

I.I.1. Chresta martii (Candolle) Robinson (1980: 91). *Stachyanthus martii* Candolle (1836: 84). *Eremanthus martii* (Candolle) Baker (1873: 167). *Argyrovernonia martii* (Candolle) MacLeish (1984: 106). Type—BRAZIL. Habitat in siccis sylvis aestu aphyllis ad Joazeiro; Provinciae Bahia et in Prov. Piauhiensi. April 1819. *Martius 2354* [Holotype: M e! (29462); isotypes: G e! (464241), M e! (3 sheets, 29461, 29463, 111202), P e! (683146)].

Erect subshrubs, ramified at the base, with cauline leaves, these sometimes crowded at the tips of the stem axes. Leaves petiolate, semi-fleshy, aromatic, covered with a dense indumentum composed of T-shaped trichomes, margins deeply dentate or serrate. Syncephalia solitary, scapose, hemispherical, presenting indeterminate growth. Florets purple with white throat; style arms covered by acute sweeping hairs; pollen type F.

Illustrations:—Baker (1873: Tab. 47), Fig. 3A-E, Fig. 9A-G.
Distribution and habitat:—*Chresta martii* is a rupiculous species from the Caatinga, growing on quartzitic outcrops and granitic rocks with high quartz content, being widespread throughout Alagoas, Bahia, Paraíba, Pernambuco, Piauí, Rio Grande do Norte and Sergipe states in Northeastern Brazil, forming four main clusters of populations: one in Northern Bahia and Southern Pernambuco, another in Piauí, at Serra da Capivara, a third one in the Xingó region and another on central Paraíba and Rio Grande do Norte (Fig. 17A). Populations on the outcrops are usually dense, sometimes reaching

hundreds of individuals.

Phenology:—The species has a long flowering period, due to the indeterminate growth of the syncephalia, and flowering and fruiting specimens have been found year-round.

Conservation Status:—The EOO is 145,747.192 km^2 and the AOO is 264 km^2, classifying the species as Least Concern and Endangered, respectively. Even though the species has narrow environmental requirements and is restricted to a small region (Fig. 17A), it has been collected constantly over the last century throughout its distribution, suggesting the number of populations has not decreased. Field observations have shown that it endures severe changes to its habitat, like introduction of grazing animals, like goats, changes on the vegetation around and over the outcrops and extreme drought and heat. Therefore, we classify it as Least Concern (LC).

Comments:— A remarkable feature of this species are the hemispherical syncephalia that have indeterminate growth. These secondary heads present a pyramidal shape when young, but grow out to resemble densely packed spikes. Flowering occurs from bottom to top, and it is not unusual to find syncephalia bearing heads that already dispersed fruits on the bottom while the top is still flowering or in bud. Plants from this species endure extreme climatic conditions, with intense heat and prolonged droughts. The presence of *Vernonia*-type F pollen may be an adaptation to these conditions (Siniscalchi et al. 2017).

There are marked morphological differences between plants from the Eastern and Western sites of the distribution. The plants on the westernmost part, including the type specimen, are robust subshrubs, densely ramified at the base, reaching up to 1 m in height, with wide leaves with deeply dented margins (Fig. 9B, C, D), while the plants on the easternmost part of the distribution are usually smaller, less ramified, with the leaves crowded on the tip of the stems, with narrower blades (Fig. 9E, F, G), however, plants from both areas share similar reproductive aspects. The aromatic leaves are used as a traditional medicine for gastric ailments along its distribution, with its gastroprotective effect being proved in mice (Silva et al. 2013).

Selected specimens examined:—BRAZIL. **ALAGOAS:** Piranhas, Xingó, Margem do rio São Francisco, 4.VII.1999, *J.A. de Assis 560* (HRB, IPA, MAC). **BAHIA:** Casa Nova, Morro do Cruzeiro, próximo a Barragem de Sobradinho, 575 m, 9°24’80”S, 40°48’39”W, 13.V.1999, *F. França et al. 2906* (CEN, HUEFS, SPF); Jaguarari, crista quartzítica em morro no km 84 da rodovia Senhor do Bonfim-Juazeiro (BR-407), 521 m, 10º06’11”S, 40º13’47”W, 5.V.2014, *C.M. Siniscalchi & K. Beraldo 473* (SPF); Juazeiro, Ilha do Fogo, 2.VI.1913, *J.N. Rose & F.G. Russell 19766* (NY, US); Paulo Afonso, Serra do Umbuzeiro, subida da Serra do Umbuzeiro, 29.III.2012, *F. Hurbarth et al. 266* (ALCB); Riachão do Jacuípe, Serrote Branco, 9°42’10”S, 40°23’20”W, 26.III.2000, *N.G. Jesus et al. 868* (ALCB, CEN, HUEFS, SPF, RB); Sento Sé, ca. 20 km de Sento Sé. Fazenda Magnesita, 420 m, 9°51’50”S, 41°47’59”W, 14.V.1999, *F. França et al. 2912* (CEN, HUEFS, SPF); Sobradinho, morro isolado dentro da área do Assentamento Nova Esperança, 348 m, 9º30’25”S, 40º48’02”W, 6.V.2014, *C.M. Siniscalchi & K. Beraldo 477* (SPF); Uauá, Serra do Jerônimo, Reserva Biológica Serra dos Papagaios, 9°44’S, 39°24’W, 23.X.2006, *M. Oliveira 2605* (MOSS, UFP, IPA). **PARAÍBA**: Cacimbas, estrada de terra entre Passagem e Desterro, 500 m, 7º10’46”S, 37º03’44”W, 3.XII.2015, *C.M. Siniscalchi & I. Gomes 636* (SPF); Santa Luzia, rodovia BR-230, ca. 10 km do centro de Santa Luzia, 348 m, 6º54’59”S, 36º51’15”W, 3.XII.2015, *C.M. Siniscalchi & I. Gomes 637* (SPF); Teixeira, Pedra do Tendó, Rodovia PB-262, ca. 5 km a norte do centro do município, 742 m, 7º12’10.25”S, 37º15’30.05”W, 2.XII.2015, *C.M. Siniscalchi & I. Gomes 635* (SPF). **PERNAMBUCO:** Petrolina, Ilha do Fogo, Rio São Francisco, entre as cidades de Petrolina (Pernambuco) e Juazeiro (Bahia), 20.I.1970, *P. Carauta & D. Vital 975* (RB, GA); Petrolina, Serrote do Urubu, estrada vicinal acessada pela Av. Pedrinhas, aproximadamente 14 km do centro de Petrolina, 370 m, 9º21’41.46”S, 40º23’05,92”W, 27.X.2014, *C.M. Siniscalchi 565*

(SPF). **PIAUÍ:** Coronel José Dias, Parque Nacional da Serra da Capivara, 416 m, 8°50'41"S, 42°33'22"W, 10.III.2005, *L.P. Queiroz et al. 10083* (HUEFS, ALCB); São Raimundo Nonato, descida para variante da Serra Vermelha, 601 m, 8°51'2.8"S, 42°42'26.7"W, 25.IV.2007, *M.F.A. Lucena & E.S. Silva 1715* (HVASF, RB). **RIO GRANDE DO NORTE:** Acari, Sítio Talhado, nas proximidades do km 17 da BR427, sentido Currais Novos-Acari, a ca. 3 km da rodovia, serra após casas do sítio, 378 m, 6°19'53"S, 36°37'29"W, 26.II.2011, *A.A. Roque et al. 898* (HUEFS, EAC); Jardim do Seridó, margens do rio Seridó, próximo à ponte Zé de Bastos, 231 m, 6°36'49.04"S, 36°46'29.58"W, 5.XII.2015, *C.M. Siniscalchi & I. Gomes 642* (SPF); Parelhas, açude da cidade, 313 m, 6°42'10"S, 36°38'57"W, 4.XII.2015, *C.M. Siniscalchi & I. Gomes 640* (SPF); São João do Sabugi, Serra Mulungu, 250 m, 6°42'30"S, 37°10'00"W, 11.VI.1980, *O.F. de Oliveira et al. 1021* (MOSS). **SERGIPE:** Canindé de São Francisco, Fazenda Poço Verde, Mata ciliar rio temporário, 178 m, 9°33'26"S, 37°56'24"W, 26.X.2005, *D. Coelho 828* (UFP, IPA).

II. *Chresta* subg. *Chresta,* Type:—*C. sphaerocephala* DC.

Herbs, subshrubs, shrubs, erect, branched or not at the base, perennial or (bi)annual, with slender to thickened and ramified underground systems, or fasciculate roots. Leaves cauline or in basal rosettes or fasciculate on the tip of the stems. Synflorescence scapose or paniculate, solitary syncephalia or in panicles composed from 2 to 10 syncephalia. Syncephalia hemispherical, with capitula loosely joined by the base, or spherical, with tightly joined capitula. Florets purple with purple or white throat, with white spots sometimes spreading to the base of the corolla lobes, or red with yellow throat; sweeping hairs clavate, lageniform or subulate. Pollen *Vernonia*-type A or C. Cypselas monomorphic or dimorphic, cylindrical to cylindrical-turbinate to turbinate, twin-hairs with even cells, usually united thought their length; carpopodium symmetrical to asymmetrical. Pappus persistent, sometimes caducous.

Chresta subg. *Chresta* contains all species except for *C. martii.* The two sections recognized within this subgenus correspond to the *Caatinga* clade (*C.* sect. *Rupicola*) and to the *Cerrado* clade (*C.* sect. *Chresta*), two lineages highly supported in the phylogenetic study (Fig. 1, see Siniscalchi et al. in prep., Chapter 1).

II.I. *Chresta* sect. *Rupicola* Siniscalchi, Loeuille & Pirani, *sect. nov.* Type:—*C. harleyi* H.Rob.

Herbs or subshrubs, erect, branched or not at the base, perennial or (bi)annual, with ramified, slender underground systems or fasciculate roots. Leaves cauline, sessile or petiolate; margin pinnatilobate, pinnatifid, pinnatisect, deeply dentate. Synflorescence scapose or paniculate. Syncephalia hemispherical, with determinate growth, or spherical, with indeterminate growth. Florets purple with white throat; sweeping hairs lageniform or subulate. Pollen *Vernonia*-type C. Cypselas monomorphic or dimorphic.

Chresta sect. *Rupicola* corresponds to the "Caatinga clade" (Fig. 1, and also Siniscalchi et al. in prep., Chapter 1), containing all species that grow directly on rocks, most of them on the Caatinga, northeastern Brazil.

II.I.1. Chresta artemisiifolia Siniscalchi & Loeuille (2018: XXX). Type—BRAZIL. Bahia, Sento Sé, Nascente na Grota dos Prazeres, no paredão rochoso, bioma Caatinga, fitofisionomia Savana Estépica, 437 m, 10º04'11.60" S, 41º37'27.60" W, 18 December 2015, *J.A. Siqueira Filho., E.D.S. Almeida, E.M.B. Nunes, T.T. Bezerra, C.M. Siniscalchi 3671* [holotype HVASF! (xxxx); Isotypes: RB! (xxxx), SPF! (xxxx), US! (xxxx)].

Erect subshrubs, ramified at the base, with cauline leaves. Leaves sessile or subsessile, covered with dense indumentum composed of T-shaped trichomes, pinnatisect. Syncephalia solitary, scapose, hemispherical. Florets purple with white throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Siniscalchi et al. in prep., see Chapter 4 (Fig. 1, 2); Fig. 11A–C.

Distribution and habitat:—*Chresta artemisiifolia* is known from a few collections from a region known as Boqueirão da Onça, in the municipality of Sento Sé, in the Caatinga domain, Bahia (Fig. 17B). It grows on quartzitic outcrops close to seasonal water courses.

Phenology:—Flowering and fruiting specimens were collected on February, June, July and December.

Conservation Status:—As the species is known only from the few collections cited in the protologue, we keep its status as EN B1ab(iii)+2ab(iii); C2a(i).

Comments:—The pinnatisect leaves are the most striking feature of this species. It shares overall similarities to the other species in the same clade, such as *C. harleyi, C. hatschbachii* and *C. subverticillata*, such as syncephalia and florets morphology and cypsela features. Its distribution overlaps with *C. martii*, with at least one mixed population known. There are no known hybrids or intermediate forms.

Specimens examined:—BRAZIL. Bahia: Sento Sé, Parque Nacional do Boqueirão da Onça, Olho d'água, afloramento rochoso, 526.97 m, 10º10'27.19" S, 41º30'27.52" W, 1.II.2010, *J.A. Siqueira Filho et al. 2343* (HVASF!); localidade Prazeres, região de água corrente na época chuvosa, 541 m, 10º10'21.22" S, 41º30'35.23" W, 19.VII.2011, *V.M. Cotarelli et al. 890* (HVASF!); Brejo da Brázida, Grota de Santana, 492 m, 10º16'37.40" S, 41º37'05.30" W, 13.VI.2012, *N.M.P. Braga & C.R.S. Oliveira 71* (HVASF!, SPF!).

II.I.2. Chresta filicifolia Siniscalchi & Loeuille (2016: 81). Type—BRAZIL. Minas Gerais: Conselheiro Pena, Parque Estadual de Sete Salões, trilha para o Córrego da Onça, afloramentos rochosos quartzíticos em área de floresta estacional semidecidual, 513 m, 19º15'03"S; 41º22'29"W, 5 August 2014, *C.M. Siniscalchi, B. Loeuille & C.T. Oliveira 512* [Holotype: SPF!; isotypes: K!, MBM!, R!, RB!, SP!, US!].

Erect subshrubs, ramified at the base, with cauline leaves, these sometimes crowded on the tips of the stem axes. Leaves petiolate, pinnatilobate, covered with a dense indumentum composed of T-shaped trichomes. Syncephalia solitary, scapose, hemispherical. Florets purple with white throat; style arms covered by lageniform sweeping hairs; pollen type C.

Illustrations:—Siniscalchi et al. 2016 (Fig. 1, 2), Fig. 10H, I

Distribution and habitat:—The species is known only from the region of Sete Salões state park, in the eastern part of state of Minas Gerais (Fig. 17A), growing on quartzitic outcrops, and to our knowledge, no other collections were carried out in the area after the original description.

Phenology:—Flowering and fruiting specimens were collected on May and August.

Conservation Status:—*Chresta filicifolia* was initially evaluated as Data Deficient, however, given the small EOO and AOO (0 km^2 and 4 km^2, respectively), plus the lack of new collections, we update the conservation status to EN B1ab(iii) + 2ab(iii).

Comments:—This species bears similarities to *Chresta harleyi* and related species, but phylogenetic analysis have shown it to be more closely related to *C. pacourinoides* and *C. heteropappa*. This fact indicates a strong connection between the Atlantic Forest domain and the forest remnants known as *brejos de altitude* that occur on the Caatinga (Siniscalchi et al. submitted), as previous floristic and biogeographical works have already suggested (e.g. Souza & Oliveira 2006). A common feature of these three species is the lageniform sweeping hairs on the style arms (Siniscalchi et al. in prep., see Chapter 1). The leaves in the dried specimens usually show a brownish color that is not found in the remaining species of this section. The carpopodium of the cypselas are among the largest in the genus.

Specimens examined:—BRAZIL. Minas Gerais: Conselheiro Pena, Parque Estadual de Sete Salões, área com Floresta Estacional Semidecidual e com palmeira (*Attalea* sp.) e cactos, nas margens do córrego da Onça, além de afloramentos rochosos no entorno, 19º15'09.4"S; 041º23'37.1"W, 7.V.2006, *A. Salino, T.E. Almeida & G. Heringer 10883* (BHCB!, RB!).

II.I.3. Chresta harleyi Robinson (1983: 385). *Argyrovernonia harleyi* (Robinson) MacLeish (1985: 469). Type—BRAZIL. Bahia: Serra Geral de Caetité, 1.5 km S of Brejinho das Ametistas. Sandstones rocks with scrub and dense low woodland on undisturbed summit, 14º09'S, 42º29'W, 900 m, 11 April 1980, *R.M. Harley et al. 21228* [Holotype: CEPEC! (25738), isotypes: HUEFS! (photograph from K, 66446), K e! (69363), NY! (39250), RB! (363075), U e! (4311), US! (3 sheets, 2901191, 2918154, 2927772)].

Erect subshrubs, ramified at the base, with cauline leaves, these sometimes crowded on the tips of the stem axes. Leaves petiolate, covered with a dense indumentum composed of T-shaped trichomes, margins lobate or dentate. Syncephalia solitary, scapose, hemispherical. Florets purple with white throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Robinson (1983: 387), Fig. 4A–C; 11D–G

Distribution and habitat:—*Chresta harleyi* is distributed on the southern portion of Serra Geral, also known as Espinhaço Septentrional, in northern Minas Gerais and southern Bahia, occupying an area of approximately 200 km in diameter (Fig. 17B). The species is closely associated to quartzitic outcrops and the environment known as *caatinga rupestre* (Campos et al. 2016), growing directly on rock crevices.

Phenology:—Flowering and fruiting specimens were collected from November to August.

Conservation Status:—*Chresta harleyi* has an EOO of 2,419 km^2 and AOO of 76 km^2, placing it as Endangered. None of the known populations is included in protected areas and there has been a growth of wind-farms in many of the areas around populations, which can possibly increase the threat over this species, as many of the outcrops where it occurs are in elevated areas with high wind potential. Therefore, we classify it as EN B1b(iii,iv)+2b(iii,iv).

Comments:— *Chresta harleyi* is the most widespread taxa in Caatinga clade 2, presenting some morphological variation between populations, specially in leaf size and depth of the lobes on the leaf margins. This variation, however, is not strictly correlated to specific areas and populations, with different morphologies being registered within the same areas. *C. harleyi* is usually sparsely ramified on the base, and the leaves are mostly concentrated towards the upper portion of the stems. Herbarium specimens of *C. subverticillata* are often identified as *C. harleyi*, as the former was only recently described (Siniscalchi et al., submitted); however, the distribution of *C. harleyi* does not go further North than Caetité, and the distributions of the four species on Caatinga clade 2 do not overlap.

Selected specimens examined:—BRAZIL. **BAHIA:** Caetité, Brejinho das Ametistas, 2 km a WS da sede do povoado, 900 m, 15.IV.1983, *A.M. de Carvalho et al. 1776* (HUEFS, US, CEPEC, RB); Caetité, em afloramentos rochosos, próximo a cidade, 13º31'23"S, 41º57'31"W, 24.VII.2007, *S.C. Ferreira et al. 326* (HUEFS); Jacaraci, Montanhas quartzíticas próximas à cidade, 970 m, 14°50'03"S, 42°25'29"W, 1.V.2009, *R. Mello-Silva et al. 3211* (SPF); Licínio de Almeida, Serra do Salto, estrada de terra para Caetité, base da Serra do Salto, 761 m, 14°32'15"S, 42°31'51"W, 3.XI.2006, *R.F. Souza-Silva et al. 238* (HUEFS); ibid., Serra Geral, Garimpo dos Areiões, 702 m, 14º37'42"S, 42º30'39"W, 14.VII.2011, *N. Roque et al. 3130* (ALCB); ibid., Riacho Fundo, 849 m, 14º35'11"S, 42º32'25"W, 10.V.2012, *N. Roque et al. 3480* (ALCB); Urandi, Rod. Licínio de Almeida/ Urandi 15,4 km, 1020 m, 14°44'50"S, 42°34'27"W, 31.III.2001, *J.G. Jardim et al. 3344* (HUEFS, NY, CEPEC). **MINAS GERAIS:** Mato Verde, Serra do Mandaçaia, na estrada para Montezuma, 1100 m, 15°23'03"S, 42°46'27"W, 17.III.1994, *C.M. Sakuragui et al. CFCR15165* (K, SPF); Monte Azul, 14.III.1995, *G. Hatschbach & J.M. Silva 61879* (MBM, US); Rio Pardo de Minas, à 12 km de Mato Verde na estrada para Sto. Antônio do Retiro, 900-1000 m, 15º23'S, 42º45'W, 9.XI.1988, *R.M. Harley et al. 25522* (CEPEC); Santo Antônio do Retiro, Serra do Pau d'Arco, 15°20'S, 42°37'W, 3.IX.2001, *M.F. Vasconcelos 13* (BHCB).

II.I.4. Chresta hatschbachii Robinson (2005: 83). Type—BRAZIL. Bahia: Oliveira dos Brejinhos, Estrada Cana Brava a Chapadão de Cima, próximo ao alto da Serra Geral, campo rupestre, afloramentos rochosos e no solo arenoso junto às rochas, 16 March 1998, *G. Hatschbach et al. 67804* [holotype: MBM e! (221988), isotypes: ASU (12937), BHCB (44608), CEPEC! (80974), CESJ (31895), ESA! (60868), FLOR! (30034), GGMS! (17755), HCF e! (2095), HEPH! (17281), HUEFS! (39776), ICN (159560), INPA e! (217140), K e! (1092361), LL! (208821), MO (100228359), NY! (781574), RB! (2 sheets, 377177), SPF!, UPCB (34714), US! (3363422)].

Erect subshrubs, profusely ramified at the base, with cauline leaves. Leaves sessile to subsessile, covered with a dense indumentum composed of T-shaped trichomes, margins lobate. Syncephalia solitary, scapose, hemispherical. Florets purple with white throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Robinson (2005: 201), Fig. 4D–G, 12A–C.

Distribution and habitat:—The records of this species are few, always from the quartzitic outcrops on the mountains surrounding Oliveira dos Brejinhos in Bahia, an area located on the northern end of Serra Geral (Fig. 17B). The populations of *C. hatschbachii* are located approximately 200 km North of the northernmost populations of *C. harleyi*.

Phenology:—Flowering and fruiting specimens were collected in March to May and November.

Conservation Status:—*Chresta hatschbachii* has an EEO of 31.691 km^2 and AOO of 12 km^2, what would make it Critically Endangered and Endangered, respectively. All the known populations seem to have ranges near to each other, at least according to herbarium labels, and none of them are found in protected areas, being easily accessible from roads. Therefore, we categorize it as CR B1a,b(iii,iv).

Comments:—*Chresta hatschbachii* is morphologically close to *C. harleyi*, with the main differences being the growth form of the plants, which are profusely ramified on the base, with slender stems, the dimensions of the leaves, which are distributed throughout the stems, while in *C. harleyi* they tend to group on the tips of the stems, the lack of petiole or presence of a very reduced petiole and the round lobes on the leaf margins. The capitula have 5 or 6 flowers and the cypselas are slightly smaller than those of *C. harleyi.*

Specimens examined:—BRAZIL. **BAHIA:** Oliveira dos Brejinhos, Estrada Oliveira dos Brejinhos/Água Quente, ca. 6 km a partir de Oliveira dos Brejinhos, 15.IV.1999, *A.M. Amorim et al. 2867* (CEPEC); Oliveira dos Brejinhos, Serra da Água Quente, a 7 km da cidade, 707 m, 12º20'23"S, 42º54'31"W, 15.XI.2012, *R.C. Forzza et al. 7468* (RB); Oliveira dos Brejinhos, morro atrás da cidade, entre rochas, 711 m, 12°20.51'S, 42°54.34'W, 2.II.2008, *B. Loeuille et al. 377* (HAW, K, SPF); Oliveira dos Brejinhos, Estrada para Chapada de Cima, ca. 6 km de Oliveira dos Brejinhos, 827 m, 12º22'19"S, 42º54'19"W, 3.V.2014, *C.M. Siniscalchi et al. 468* (SPF, MEM?,).

II.I.5. Chresta heteropappa Siniscalchi & Loeuille (2018: XXX). Type—BRAZIL. Ceará: Uruburetama, Estrada do centro de Uruburetama para bairro da Água Sumida, 03º37'44.01"S, 39º31'45.02"W, 209 m, 13 August 2015, *C.M. Siniscalchi & A. Frazão 614* [Holotype: SPF! (xxxxxx), isotype: K! (xxxxxx), RB! (xxxxxx), US! (xxxxxx)].

Erect herbs, with cauline leaves, decreasing in size towards the apex. Leaves sessile, membranaceous, glabrate, margins biserrate, base auriculate. Panicle of syncephalia, which are spherical, sometimes presenting indeterminate growth. Florets purple with white throat; style arms covered by lageniform sweeping hairs; pollen type C.

Illustrations:—Siniscalchi et al. (2018: Fig. 1, 2), Fig. 10F, G.
Distribution and habitat:—*Chresta heteropappa* is known from a few collections, all from granitic outcrops on forest remnants known as *brejos de altitude* in the Caatinga. It seems to be restricted to the Northern portion of Ceará, northeastern Brazil, mainly on the Uruburetama massif, but there is also one collection from Maranguape (Fig. 17A). The distribution of *C. heteropappa* overlaps with *C. pacourinoides* in the region of Maranguape.
Phenology:—Flowering and fruiting specimens were collected in March to May and November.
Conservation Status:— The status remains as in the recent protologue: EN B1ab(iii,iv)+ B2ab(iii,iv).
Comments:—*Chresta heteropappa* is morphologically similar to *C. pacourinoides*, the main differences being the length of the part of the style projected above the anthers (1-1.25 mm in *C. heteropappa* vs. up to 1 cm) and type of pappus element, paleaceous-setose, which is different from all remaining species in the genus. Although some syncephalia present indeterminate growth, it is not as marked as seen in *C. pacourinoides.*

Specimens examined—BRAZIL: Ceará: Uruburetama, Estrada do centro de Uruburetama para bairro da Água Sumida, 03º37'48.50"S, 39º31'59.93"W, 220 m, 13.VII.2015, *C.M. Siniscalchi & A. Frazão 615* (SPF); Maranguape, Serra de Maranguape, Pico da Rajada, 13.VII.2017, *M.L. Bazante, N.K. Luna & L.J. Leitão 892* (UFP); Itapipoca, Maciço de Uruburetama, Pico de Itacoatiara, área de floresta subperenifólia tropical plúvio-nebular (mata úmida) com afloramentos graníticos e ilhas de vegetação, 03º34'27"S, 39º35'22"W, 870 m, 1.7VII.2017, *F.D.S. Santos 560* (HUVA, UFP).

II.I.6. Chresta pacourinoides (Martius ex Candolle) Siniscalchi & Loeuille (2014: 6). *Pithecoseris pacourinoides* Martius ex Candolle (1836: 84). Type—BRAZIL. Habitat in campis, Provinciae Pernambucanae, 1819, *Martius 2529 (550)* [Holotype: M e! (M0029492), Isotypes: G e! (464242), P

(4318252)].

Chresta amplexifolia Dematteis, Roque & Miranda (2008: 588). Type—BRAZIL. Bahia, Ipuaçu, Monte Alto, 12º13'55"S, 39º4'35"W, 12 August 2003, *A.C. Pereira et al. 15* [Holotype: HUEFS! (77811), isotypes: ALCB! (80262), K e! (768728), CTES].
Pithecoseris pacourinoides var. *capitata* Glaziou (1909: 381), *nom. nud.*

Erect herbs, with sessile, cauline leaves, decreasing in size towards the apex. Leaves membranaceous, glabrate, margins deeply dentate to pinnatilobate, base auriculate. Panicle of syncephalia, which are spherical, presenting indeterminate growth. Florets purple with white throat; style arms covered by lageniform sweeping hairs; pollen type C.

Illustrations:—Baker (1873: Tab. 36), Fig. 4F–H, Fig. 10A–E.
Distribution and habitat:—*Chresta pacourinoides* is widely distributed throughout the Caatinga, in the states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí and Rio de Grande do Norte (Fig. 17A). It is found growing on granitic outcrops or inselbergs, usually on pockets of accumulated organic matter, or on the shallow soil around the outcrops. Most areas where the species is found belong to *brejos de altitude*, as is the case of *C. heteropappa;* however, *C. pacourinoides* is also found in drier areas.
Phenology:—Flowering and fruiting specimens were collected during the whole year, except February and April.
Conservation Status:—The EEO of *C. pacourinoides* is 610,721.818 km^2, indicating Least Concern, and the AOO is 404 km^2, indicating Endangered. Several populations are found within National Parks and other conservation areas, such as PARNA Chapada Diamantina, PARNA Serra das Confusões, PARNA Sete Cidades and PARNA Catimbau. The main threats to the species is the removal of vegetation layers for agriculture, as soils on regions of *brejos de altitude* are fertile and the climatic conditions are milder. However, as the plants grow on rock outcrops, sometimes they remain in place, even though the vegetation around them has been disturbed or destroyed. Therefore, we classify the species as Least Concern.
Comments:—The morphological variation of this species is remarkable, with plant height varying from 15 cm to 2 m. It is common to find plants of several sizes flowering, and this seems to be related to where the plant is growing; plants on the base of inselbergs, where available soil is deeper, tend to grow taller, while plants growing on the rock surface towards the top are gradually smaller. The indeterminate growth of the syncephalia is striking, sometimes reaching more than 10 cm in length; nevertheless, not all syncephalia develop this type of growth, and some remain small and spherical until the end of flowering. The synflorescence branches are hollow and engorged at the base of the syncephalia. This species is annual or biannual, drying out and dying after flowering. Like *C. heteropappa, C. pacourinoides* also presents dimorphic cypselas.
Selected specimens examined:—BRAZIL. **ALAGOAS:** Maravilha, no topo da Serra da Caiçara, 9º13'02"S, 37º16'05"W, 15.IX.2000, *R.P. Lyra-Lemos 5032* (RB); Quebrângulo, REBIO Pedra Talhada, mata próxima a Pedra Talhada, 9º15'04"S, 36º25'41"W, 11.XI.2011, *D. Araújo et al. 1815* (UFP, NY). **BAHIA:** Feira de Santana, João Durval Carneiro, platô mais alto, antigo distrito de Ipuaçu, Inselberg Monte Alto, 12°13'55"S, 39°04'35"W, 12.VIII.2003, *A.C. Pereira et al. 15* (HUEFS, ALCB); Palmeiras, Morro do Pai Inácio, km 224 da rodovia BR242, 1040 m, 12º30'S, 41º27'W, 19.XII.1981, *G.P. Lewis*

et al. 892 (CEPEC); Sento Sé, Serra Branca, 622 m, 10º00'1.9"S, 41º52'45"W, 11.VIII.2006, *J.A. Siqueira-Filho et al. 1664* (HVASF). **CEARÁ:** Alcântaras, Rodovia CE-241, ca. 10 km a oeste de Alcântaras, 515 m, 3º36'31.59"S, 40º35'36.67"W, 12.VIII.2015, *C.M. Siniscalchi & A. Frazão 611* (SPF); General Sampaio, RPPN Francy Nunes, 1.VI.2008, *M.F. Moro et al. 459* (EAC); Meruoca, Sítio Santo Antônio, 801 m, 3º34'46.96"S, 40º29'53.71"W, 13.VIII.2015, *C.M. Siniscalchi & A. Frazão 613* (SPF); Quixadá, Açude Cedro, estrada do Cedro Velho, ramal da estrada principal que leva à entrada do açude, 201 m, 4º58'32.53"S, 39º03'11.08"W, 8.VIII.2015, *C.M. Siniscalchi & A. Frazão 606* (SPF); Santana do Cariri, Chapada do Araripe, Pontal de Santa Cruz, 733 m, 7º12'38.34"S, 39º44'00,50"W, 9.VIII.2015, *C.M. Siniscalchi & A. Frazão 607* (SPF). **PARAÍBA:** Areia, on the Serra da Borborema, 20.IX.1945, *J.M. Vasconcellos 2246* (US); Maturéia, Pico do Jabre, 1225 m, 7°15'96"S, 37°23'03"W, 11.VII.2007, *M.F. Agra et al. 6976* (JPB). **PERNAMBUCO:** Arcoverde, Serra das Varas, Mata do João Rosa, 8º26'03"S, 37º00'06"W, 14.IX.2006, *A. Bocage et al. s.n.* (IPA); Buíque, Vale do Catimbau, trilha da Pedra da Concha, 23.I.2006, *A. Bocage et al. 1065* (IPA); São Joaquim do Monte, inselbergue Pedra do Guariba, 685 m, 8º22'55"S, 35º50'38.2"W, 20.VII.2007, *P. Gomes* 479 (RB, UFP); Sertânia, Brejo Velho, Serra Jabitacá, Sítio Bolandeira, 1058 m, 7º58'32.9"S, 37º20'57.7"W, 3.VIII.2010, *A.P. Fontana et al. 7077* (HVASF). **PIAUÍ:** Caracol, PARNA Serra das Confusões, Caracol, área próxima da entrada do parque, 535 m, 8º53'25"S, 43º7'55" W, 18.VII.2011, *E. Melo et al. 10203* (HUEFS); Monsenhor Gil, Fazenda Saquinho, 5º33'45"S, 42º37'02"W, 5.VI.1999, *R. Barros et al. 223* (PEUFR). **RIO GRANDE DO NORTE:** Felipe Guerra, Sítio do Boqueirão, 5°36'01"S, 37°41'02"W, 2.VII.2007, *R.C. Oliveira et al. 1924* (MOSS).

II.I.7. Chresta pinnatifida (Phillipson) Robinson (1995: 384). *Eremanthus pinnatifidus* Philipson (1938: 299). *Pycnocephalum pinnatifidum* (Philipson) MacLeish (1985: 464). Type—BRAZIL. 1882, Minas. *Glaziou 14033* [Holotype: K e! (K000497164), Isotype: C e! (10007812), P e! (683148)].

Erect herbs, with cauline, petiolate, membranaceous leaves, margins pinnatilobate. Panicle of hemispherical syncephalia. Florets purple.

Distribution and habitat:—Although the original labels, seen in the specimen at P, state the collection locality only as "Minas Gerais", Glaziou's memoirs (1909) list the two specimens belonging to this taxa as being collected at Serra do Caraça and Serra do Inficcionado, the latter being one of the mountains found at Serra do Caraça. The area has been extensively explored by botanists along two centuries, but the species was never found again there, raising a suspicion that the locality cited on the labels is probably incorrect, or even that the species is extinct. The labels found on the K and C specimens state the locality as Rio de Janeiro, but this is due to the original handwritten labels being removed and replaced by typed ones.

Phenology:—The few know specimens were collected between June and August.

Conservation Status:—Given the uncertainty of the location of this species, we classify it as Data Deficient (DD).

Comments:—*Chresta pinnatifida* bears similarities to *C. filicifolia, C. heteropappa* and *C. pacourinoides.* The pinnatilobate leaves resemble those of *C. filicifolia,* as well as the characteristics of the syncephalia, while the branched inflorescences approximate it to *C. heteropappa* and *C. pacourinoides.* The corolla tubes are also usually longer than 1 cm, and this is also seen in *C. pacourinoides. C. filicifolia, C. heteropappa* and *C. pacourinoides* are all found in areas with forest remnants, growing over rocks, and many of their characteristics are not found in other *Chresta* species; it is likely that *C. pinnatifida* has similar environmental requirements to those three species.

Specimens examined:—BRAZIL. Minas Gerais. Serra do Inficcionado, 8.VI.1882, *Glaziou 12812* (P e!); without location, *Glaziou 13813* (P e!).

II.I.8. Chresta subverticillata Siniscalchi & Loeuille (2018: XXX). Type—BRAZIL. Bahia: Gentio do Ouro, Distrito de Santo Inácio, Afloramentos rochosos na entrada da vila, em frente a casa antiga abandonada, 11°06’26.33”S, 42°43’10.50”W, 560 m, 8 November 2015, *C.M. Siniscalchi & J. Vidal 634* [Holotype: SPF! (xxxxxx), isotype: K! (xxxxxx), RB! (xxxxxx), US! (xxxxxx)].

Erect subshrubs, profusely ramified at the base. Leaves sessile, apparently whorled (alternate but tightly grouped on very short branches), covered with dense indumentum composed of T-shaped trichomes, margins serrulate or denticulate to minimally denticulate. Syncephalia solitary, scapose, hemispherical. Florets purple with white throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Siniscalchi et al. (2018: Fig. 4, 5), Fig. 12D–G.
Distribution and habitat:—*Chresta subverticillata* occurs on the quartzitic outcrops around the district of Santo Inácio, in the municipality of Gentio do Ouro in Bahia, northeastern Brazil, within the Caatinga domain (Fig. 17B).
Phenology:—Flowering and fruiting specimens were collected from February to November.
Conservation Status:—We maintain the status as EN B1ab(iii)+2ab(iii), as indicated in the original publication.
Comments:—The most distinguishing feature of this species is the position of the leaves, which are group into very short branches, resembling whorls of leaves, a feature not found in any other species of the genus.
Specimens examined:—**BRAZIL. BAHIA**: Gentio do Ouro, Distrito de Santo Inácio, on rocky hillside called Pedra da Mulher just south of town, 11°07’S, 42°44’W, ca. 500-600 m, 25.II.1977, *R.M. Harley 19029* (CEPEC, NY, RB, US); ibid., área muito seca, última chuva em dezembro de 1989, 11°7’S, 42°44’W, ca. 500 m, 5.X.1990, *A. Freire-Fierro et al. 1782* (SPF)*;* ibid., área muito seca, última chuva em dezembro de 1989, 11°7’S, 42°44’W, ca. 500 m, 5.X.1990, *A. Freire-Fierro et al. 1787* (SPF)*;* ibid., Caminho para Santo Inácio, 11°03’28”S, 42°42’37”W, 680 m, 24 June 1996, *M.L. Guedes et al. 2998* (ALCB, CEPEC, HUEFS, SPF); ibid., ca. 24 km S de Xique-Xique, na Estrada para Santo Inácio, 16.VI.1994, *L.P. de Queiroz & N.S. Nascimento 3958* (HUEFS); ibid., 11°06’S, 42°40’W, 19.VI.1998, *J. Santino de Assis 210* (RB); ibid., Vale das Pedras (CASF), 11°06’40”S, 42°43’17”W, 14.IV.2000, *S.S. Lima s.n.* (ALCB); ibid., dunas vicariantes, 11°06’40”S, 42°43’17”W, 2.VI.2000, *S.S. Lima s.n.* (ALCB); ibid., ramal para a cachoeira, 11°5’48”S, 42°43’18”W, 536 m, 20.VII.2000, *M.M. da Silva et al. 466* (HUEFS); ibid., Serra de Sapé, 11°11’23”S, 42°43’07.40”W, 624 m, 26.V.2009, *J.A. Siqueira-Filho et al. 2060* (HVASF, RB); ibid., Serra do Sapê, Estrada de Gentio do Ouro para Santo Inácio, 11°11’31.75”S, 42°43’03.02”W, 622 m, 7.XI.2015, *C.M. Siniscalchi & J. Vidal 630* (SPF); ibid., Estrada para a cachoeira, acesso pela Estrada que liga a rodovia BA-330 a Santo Inácio, 11°05’48”S, 42°43’18”W, 532 m, 7.XI.2015, *C.M. Siniscalchi & J. Vidal 631* (SPF).

II.II. ***Chresta*** **sect.** ***Chresta,*** Type:—*C. sphaerocephala* DC.

Subshrubs or shrubs, erect, branched or not at the base, perennial, with thickened underground systems, ramified or not, lacking fasciculate roots. Leaves cauline or in rosettes, sessile or petiolate; margin entire, denticulate or biserrate. Synflorescence scapose or paniculate. Syncephalia hemispherical or spherical, with determinate growth. Florets purple with purple or white throat or red with yellow throat; sweeping hairs clavate or subulate. Pollen *Vernonia*-type A or C. Cypselas monomorphic.

Chresta sect. *Chresta* corresponds to the *Cerrado clade* (Fig. 1, and Siniscalchi et al. in prep., see Chapter 1), containing all species distributed in the Brazilian Cerrado.

II.II.1. Chresta angustifolia Gardner (1842: 240). *Eremanthus angustifolius* (Gardner) Baker (1873: 170). *Pycnocephalum angustifolium* (Gardner) MacLeish (1985a: 462). Type:—BRAZIL. Goiás: shady places at Arrayas, April 1841, *G. Gardner 3802* [holotype: BM; isotypes: B, E e! (417131), F! (2 sheets, 1012051, 1013164), G e! (1 sheet 223227, 2 sheets 223278), K e! (2 sheets, 677890, 677891), NY! (163362), P e! (3 sheets, 683130, 683131, 683132), S e! (S-R-1100), W e! (42790, 117754? – annotated as 4819)].

Eremanthus rivularis Taubert (1896: 453-454). Type:—BRAZIL. Goiás: região do Maranhão superior, September 1892, *E. Ule 26 (=2962)* [holotype: HBG e! (506543); isotypes: P e! (2 sheets, 683165, 683167), F! (as photograph from P, 971118), GH!, R! 4 sheets (15498)].

Erect subshrubs, profusely ramified at the base, with cauline leaves, these sessile, glabrous, margins entire, sometimes revolute. Syncephalia solitary, scapose, hemispherical. Florets purple with white throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Gardner (1842: Tab. VIII), Fig. 4A–D, 13A–D.
Distribution and habitat:—*Chresta angustifolia* occurs in Central Brazil, mostly in the state of Goiás, but a few registers are found also in Tocantins, with the typical locality, near Arraias, being currently in the second state (Fig. 18A), while it belonged to Goiás by the time of the collection of the type. It forms dense populations on the sandy and boggy soil along the margins of seasonal streams on *campos rupestres* (highland rocky fields), which usually are found on quartzite areas and also among rocks near to waterfalls. It seems to be mostly restricted to the massif that forms the Chapada dos Veadeiros, which extends from Northern Goiás to Tocantins.
Phenology:—Fertile specimens have been collected from July to February, with the majority of registers from August to November.
Conservation Status:—The EOO is 9,100 km2 (Vulnerable) and the AOO is 76 km2 (Endangered). Part of the registers of the species is within the area of Chapada dos Veadeiros National Park, but the region as a whole suffers with illegal fires, cattle ranching and mining interests. Therefore, we classify it as VU B1ab(iii).
Comments:—*Chresta angustifolia* has a very limited habitat, always in shady places, linked to seasonal streams that are abundant in the region, and also on the rocks and sand banks near to waterfalls. It usually flowers in the end of the dry season, while the water levels are still low in its habitat. There are not many registers from Tocantins, but this is probably due to insufficient collections in that state. The lanceolate, deep green leaves, sometimes bearing purplish or reddish margins and primary vein, are unique in the genus.
Specimens examined:—BRAZIL. **GOIÁS:** Alto Paraíso de Goiás, Chapada dos Veadeiros, 20 October 1965, *H.S. Irwin, R.*

Souza & R. Reis dos Santos 9356 (NY, UB, US, TEX); 23.X.1965, *A.P. Duarte 9456* (RB, GA); 28.IX.1975, *G. Hatschbach & R. Kummrow 37243* (ALCB, HUEFS, MBM, SPF, US); 10.X.1979, *E.P. Heringer et al. 2291* (IBGE, RB); 14 October 1980, *N.F.F. MacLeish et al. 735* (GA, GH, UB, US, TEX, F, NY); 15.X.1980, *N.F.F. MacLeish et al. 740* (GA, US); 7.IX.1994, *M.L. Fonseca & T.S. Filgueiras 99* (IBGE, RB, US); 16 August 1995, R. *Marquete et al. 2308* (IBGE, UEC, US); 10.XI.1996, *R.C. Mendonça et al. 2671* (IBGE, US); 12.XI.1996, *B.A.S. Pereira & D. Alvarenga 3202* (IBGE, US); 9.X.2004, E. *Chaves 58* (UB); 17 November 2005, *T.B. Cavalcanti, G.P. Silva & S.A.T. Graham 3614* (CEN); 20.VII.2007, *B. Loeuille et al. 291* (SPF). Alto Paraíso de Goiás, Parque Nacional Chapada dos Veadeiros, 6.II.1987, *J.R. Pirani et al. 1669* (SPF); 16.VIII.1995, *R. Marquete et al. 2308* (IBGE, RB); 27.IX.1995, *M.L. Fonseca, M.P. Neto & E. Cardoso 620* (IBGE, RB, US); 11.IX.1996, *R.C. Mendonça et al. 2713* (IBGE, RB); 12.IX.1996, *R.C. Mendonça et al. 2799* (IBGE, RB); 22 October 1996, *R. Marquete et al. 2746* (IBGE, RB).

II.II.2. Chresta curumbensis Philipson (Robinson) (1980: 91). *Eremanthus curumbensis* Philipson (1938: 298). *Glaziovianthus curumbensis* (Philipson) MacLeish (1985a: 349). Type:—BRAZIL. Goiás: valley of the Rio Curumba in the plains between the mountains, 1896, *A.F.M. Glaziou 21645* [holotype K e! (497165); isotypes: BR e! (531842), C e! (10007399), F! (974949), G e! (223679) NY! (168378)].

Glaziovianthus purpureus Barroso (1947: 114). Type:—BRAZIL. Goiás: Aragarças, 20 September 1946, *H. Sick B.28* [holotype: RB! (57317)].

Basal rosette herb. Leaves sessile, sub-carnose, margins biserrate, veins reddish. Syncephalia solitary, scapose, hemispherical. Florets red with yellow throat; style arms covered by clavate sweeping hairs; pollen type A.

Illustrations:—MacLeish (1985: 350 Fig. 2), Fig. 7A–D, 15A–C.
Distribution and habitat:—*Chresta curumbensis* occurs in the states of Goiás and Mato Groso and in the Federal District, Central-Western Brazil (Fig. 19A). It is found in Cerrado physiognomies on red latosol, predominance of grasses, usually in shadowed places.
Phenology:—Fertile specimens have been collected from July to December, with a few collections from March to May; the flowering seems to be linked to the rainy season in the area.
Conservation Status:—The EOO is 137,307.853 km^2 (Least Concern) and the AOO is 340 km^2 (Endangered). The species has been frequently collected in the past decades, and there are populations included in conservation areas in the three states where it occurs. The main threats to the species are those common to cerrado species: habitat degradation due to agriculture and illegal fires. Therefore, we keep the conservation status as Least Concern.
Comments:—*Chresta curumbensis* is one of the two red-flowered species in the genus. Although both species are very similar in the reproductive parts, they did not emerge as sister species in the phylogeny (Siniscalchi et al. in prep., see Chapter 1), and can be easily distinguished by the vegetative parts. *C. curumbensis* has large, semi-fleshy light-green leaves, with the larger veins and margins frequently presenting a dark red shade; the leaves persist during flowering and turn dark brown or blackish in the dry material. Also, it is usually found flowering in the rainy season, from September to January, opposite to *C. speciosa,* which flowers during the dry season. Together with *C. speciosa,* it has been placed in *Glaziovianthus* by MacLeish (1985a), based on flower color, pollen morphology (*Vernonia*

type A) and deciduous pappus; however, phylogenetic analysis has shown *Glaziovianthus* to be non-monophyletic, and possibly the similarities between both species derive from convergent evolution. The pollen type is different than most species of *Chresta* (type A vs. type C), and probably there is a shift to hummingbird pollination, as observed with *C. speciosa*.

Selected specimens examined:—BRAZIL. DISTRITO FEDERAL: Brasília, Chapada da Contagem, ca. 25 km NE of Brasília, 1000 m, 7.IX.1965, *H.S. Irwin et al. 8033* (GA, NY, SP, US, TEX); Brasília, área do Jardim Botânico de Brasília, 1025 m, 15º52'S, 47º51'W, 26.IX.2006, *R.G. Chacon & M. Oliveira 96* (HEPH); Brasília, área do Cristo Redentor, 1010 m, 15º52'50"S, 47º52'52"W, .XI.2002, *R. Rodrigues-da-Silva & A.P.P.G. Melo 704* (HEPH); Brasília, DF-100, 8 km após a ponte sobre o córrego Extrema, 15º47'05"S, 47º23'36"W, 7.XI.2002, *A.A. Santos et al. 1592* (CEN, UEC); Brasília, Parque Ecológico do Rasgado, 1085 m, 15º50'21"S, 47º28'00"W, 28.X.2003, *M. Aparecida da Silva & D. Alvarenga 5554* (IBGE); Gama, Parque Municipal do Gama, "Prainha", 1090 m, 16º02'S 48º03'W, 2.IX.2001, *M. Aparecida da Silva et al. 6005* (US, IBGE, UB); **GOIÁS:** Alexania, Estrada do posto Medalhão ao povoado de Igrejinha, 16º11'26"S, 48º23'03"W, 7.XI.2005, *A.A. Santos & G.A. Moreira 2702* (CEN); Alto Paraíso de Goiás, Parque Nacional da Chapada dos Veadeiros, 1477 m, 14º09'65"S 47º47'50"W, 26.IX.1995, *M.L. Fonseca & M.P. Neto 562* (US, IBGE, RB); Cavalcante, Estrada balsa "Porto dos paulistas" (rio Tocantins) para HU, cerca de 32 km da balsa, 760 m, 13º22'52"S, 48º00'00"W, 9.XI.2000, *G. Pereira-Silva et al. 4389* (CEN, UEC); Cocalzinho de Goiás, morro na entrada da cidade, 1130 m, 15º44'47.9"S, 48º50'23.9"W, 23.X.2003, *M.L. Fonseca et al. 5030* (IBGE, US); Cristalina, Serra dos Cristais, 75 km N. of Cristalina, on road to Brasília, 6.XI.1965, *H.S. Irwin et al. 10018* (NY, UB, TEX); Luziânia, Estrada da Fazenda Alagado II, 885 m, 16º14'54" S, 48º09'40"W, 4.XI.2002, *G. Pereira-Silva et al. 6718* (CEN, UEC); Niquelândia, Fazenda Engenho, ca. 11 km de Niquêlandia em direção a Dois Irmãos, 640 m, 14°36'42"S, 48°29'05"W, 3.X.1997, *M.L. Fonseca et al. 1615* (IBGE, UEC, US); Pirenópolis, Serra dos Pirineus, 7.XII.1987, *L.P. Morellato et al. 10939* (UEC); **MATO GROSSO:** Barra do Garças, Serra Azul, ca. 5 km de Barra do Garças, estrada para Cristo Redentor, 550 m, 15º53'S, 52º15'W, 13.VII.1994, *R.F. Viera et al. 1994* (CEN).

II.II.3. Chresta exsucca Candolle (1836: 85). *Eremanthus exsuccus* (Candolle) Baker (1873: 166). Type:—BRAZIL. Minas Gerais: montosis Minarum, *Martius s.n.* [holotype: M e! (29453); isotypes: G e! (464237)].

Eremanthus labordeii Glaziou (1909: 380), *nom. nud.*
Eremanthus imbricatus Barroso (1962: 6). Type:—BRAZIL. Distrito Federal: Brasilândia, 25 July 1961, *Macedo 4* [Holotype: RB! (110389)].

Erect shrubs, ramified at the base, with cauline leaves, these sessile, coriaceous , glabrate, pilose when young, margins entire. Panicle of spherical syncephalia. Florets purple with purple throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Barroso (1960: 8), Fig. 8F–I, 15D–G.
Distribution and habitat:—The type specimen collected by Martius is the only known collection from Minas Gerais and it is uncertain whether it was in fact collected there or in Goiás. The collection is undated and Martius travelled between these two states in 1818, and it is possible this plant was collected somewhere in Goiás not yet acknowledged as so. *C. exsucca* is usually found in several types of Cerrado physiognomies, especially on rocky soils, where it can form dense populations, especially due to the potential of vegetative spread through the underground system. The distribution forms an

arc from Tocantins, passing through Goiás and Distrito Federal, where most of the populations are, and then extending westward to Mato Grosso, Mato Grosso do Sul and Rondônia (Fig. 19A). This is the only species in the genus that also occurs outside Brazil, with a few collections in Bolívia, in areas covered with Cerrado vegetation relatively close to Brazil.

Phenology:—Flowering and fruiting specimens were found from January to October, with the majority of records being from June to August.

Conservation Status:—The EOO is 1,223,400.146 km2, corresponding to Least Concern, and the AOO is 228 km2, indicating Endangered. There are several populations included in conservation units (PARNA Chapada dos Guimarães and PARNA Chapada dos Veadeiros), including the populations found in Bolívia (Parque Nacional Noel Kempff). However, the unprotected populations are threatened by the agricultural expansion ongoing in Brazil, which is decimating Cerrado areas, and also by illegal fires. As the other requisites for classifying it as Vulnerable or Endangered are not met, we classify it as Least Concern.

Comments:—*Chresta exsucca* is recognized by the erect habit, with plants usually reaching around 1.5 m, ramified at the base, and by the coriaceous, rigid leaves, with abundant reticulate tertiary veins. Due to the spreading, thickened and diffuse underground system, the occurrence of several clones of the same plant in the same area is common, and sometimes they form dense populations. The leaves are adpressed to the stems, erect, and usually decrease in size from the base upwards. In some plants the basal leaves seem to form a rosette, but they are actually cauline leaves with a wider angle from the stem. The plants seem to benefit from occasional fires, with intense sprouting and flowering after a few months. *C. pycnocephala* is morphologically close to *C. exsucca,* but the two species can be distinguished by the usually obscure venation and lack of reticulate tertiary veins in *C. pycnocephala* and also by the distribution, *C. pycnocephala* being restricted to Minas Gerais.

Selected specimens examined:— **BOLÍVIA. SANTA CRUZ**: José Miguel de Velasco, Parque Nacional Noel Kempff, Parcela II, Pampa Flor de Oro, 200 m, 13°33'39"S, 61°01'07"W, 10.V.1994, *E. Gutiérrez et al. 1365* (MO, US); ibid., Meseta de Caparuch by trail from top of escarpment to Campamiento Huanchaca 2, 750 m, 30.VII.2000, *J.R.I. Wood et al. 16539* (K, SPF). **BRASIL. DISTRITO FEDERAL:** Brasília, Chapada da Contagem, ca. 15 km E of Brasília, 18.VIII.1964, *H.S. Irwin & T.R. Soderstrom 5278* (NY, UB); Parque Nacional de Brasília, 15º44'15"S, 47º56'49"W, 29.VI.2006, *C.A. Faria et al. 111* (CEN); Bacia do Rio Preto, à margem da DF-252, 1550 m, 15°43'73"S, 47°36'99"W, 29.VI.1999, *K. Calago 212* (CEN). **GOIÁS:** Chapada dos Veadeiros, Hilly campo ca. 16 km S of Alto Paraíso (formerly Veadeiros), 1000 m, 20.III.1969, *H.S. Irwin et al. 24758* (NY, UB, F); Alto Paraíso de Goiás, RPPN Cara Preta, rodovia GO-118, a ca. 12 km de Alto Paraíso, 1502 m, 14º00'30.54"S, 47º31'25.95"W, 20.VII.2013, *C.M. Siniscalchi et al. 375* (SPF); Alto Paraíso de Goiás, Morro da Baleia, estrada de Alto Paraíso para São Jorge, morro mais baixo no lado oposto da estrada ao Morro da Baleia, 1258 m, 14º08'13.71"S, 47º40'22.35"W, 20.VII.2013, *C.M. Siniscalchi et al. 378* (SPF); Goiás Velho, Fazenda das Esmeraldas, perto do córrego Paciência e GO164, ca. 5.5 km NE de Goiás Velho, 6.II.1980, *J.H. Kirkbride et al. 3235* (UB); Pirenópolis, Serra dos Pireneus, Estrada Pirenópolis – Cocalzinho, 23.VII.2007, *B. Loeuille et al. 303* (SPF). **MARANHÃO:** Balsas, Agrovila de Carli, Lote 23 area de proteção ambiental, 506 m, 8°37'20"S, 46°44'21"W, 4.VII.1998, *R.C. Oliveira et al. 1223* (UB, HEPH). **MATO GROSSO:** Chapada dos Guimarães, Chapada dos Guimarães, Fazenda Morro dos Lajes, between Buriti and Água Fria, 14,6 km N of Buriti, 600 m, 14.VII.1984, *S.A. Mori et al. 16797* (NY, US, TEX, F, CEPEC). **MATO GROSSO DO SUL:** Corumbá, Reserva Acurizal, Serra do Amolar, borda oeste do Pantanal, 17º54'27"S, 57º34'08"W, 10.V.2003, *A. Pott et al. 11217* (CGMS). **RONDÔNIA**: Serra Ricardo Franco, Fazendo Ouro Verde, 14°01'S, 60°05'W, 1.V.1977, *C.A. Bantel S. & Silva s.n.* (RB). **TOCANTINS:** Dianópolis, Serra do Ouro, Fazenda Santa Cruz, 800 m, 15.VII.1963, *A.L. Costa s.n.* (ALCB);

Itacajá, 8°19'S, 47°05'W, 19.III.1978, *W.N. Fonseca 108* (RB).

II.II.4. Chresta plantaginifolia (Lessing) Gardner (1942: 241). *Vernonia plantaginifolia* Lessing (1829: 251). *Pycnocephalum plantaginifolium* (Lessing) Candolle (1836: 83). *Eremanthus plantaginifolius* (Lessing) Baker (1873: 168). Type—BRAZIL. *Sellow s.n.* [Lectotype: P e! (683168) designated by MacLeish (1985: 462)].

Pycnocephalum spathulaefolium Candolle (1836: 83). *Chresta spathulaefolia* (Candolle) Gardner (1842: 241). Type—BRAZIL. Minas Gerais, aridis desertis versus Chapada de Parano, *Martius s.n.* [Holotype: M e! (29457), P e! (683169) [fragment])

Rosette herbs. Leaves sessile, glabrate to pilose, margins entire. Syncephalia solitary, scapose, hemispherical. Florets purple with white throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Fig. 6F–H, 14A–B.

Distribution and habitat:—*Chresta plantaginifolia* is found on *campos limpos* and other open Cerrado physiognomies, usually associated to soils with high quartz content, in the states of Minas Gerais, Goiás, Distrito Federal (Central Brazil) and with one disjunct population in Paraná, Southern Brazil (Fig. 18B).

Phenology:—Most of the flowering and fruiting specimens were collected between September and December, but there a few samples collected in January, February and May. Flowering seems to be associated with fire, with several specimens showing signs of recent fire damage.

Conservation Status:—The EOO is 657,653.538 km2 (Least Concern) and the AOO is 164 km2 (Endangered). The species is abundant within Parque Nacional da Serra da Canastra, and has been collected in other conservation units, such as Jardim Botânico de Brasília. As with other Cerrado species, it might be threatened by the agricultural business and by frequent illegal fires, but currently it does not fit any category, so we classify it as Least Concern.

Comments:—*Chresta plantaginifolia* presents variable morphology, especially regarding leaf shape and size, and this fact led to the description of *C. spathulaefolia* by Gardner. MacLeish synonymized *C. souzae* into *C. plantaginifolia* (1985b), considering it to be a local variation found at Chapada dos Veadeiros; however, both species present different ecological preferences and marked morphological differences as well. MacLeish also tried to associate these synonyms with specimens that present fire damage or not, and while this is a reasonable explanation for the observed morphological variation, the analysis of additional herbarium specimens have shown her attempts to be incorrect. At Serra da Canastra, in Minas Gerais, there is a morphological variant that was thought to be a new species (Nakajima 2000), but in the present work we decided to keep it as *C. plantaginifolia*, due to the widespread variation seen throughout the distribution of this taxon. The specimens collected at Paraná show a leaf morphology similar to *C. souzae;* however, it has not been recollected and we do not know details about its habitat, so we decided to keep it in *C. plantaginifolia*, for the same reasons mentioned above. The main difference between *C. plantaginifolia* and *C. souzae* is the habitat, with the first usually growing on dry cerrado soils, and with the latter is found on dark and moist soils, with abundance of organic matter, usually close to several species of Xyridaceae and Eriocaulaceae. *C. plantaginifolia* usually presents marked venation on the leaves, those usually being long and thin, about half the size of the scape. It is likely that *C. plantaginifolia* is actually a species complex, given the morphological variations and disrupted distribution, which may be even more confused by the action of fire. Morphological and molecular studies at a population level could help distinguishing potential cryptic species.

Selected specimens examined:—BRAZIL. DISTRITO FEDERAL: Brasília, EEJBB, Área do Cristo Redentor, 1010 m, 15º52'50"S, 47º52'52"W, XI.2002, *E. Rodrigues-da-Silva & A.P.P.G. Melo 714* (HEPH); Gama, elevações após a cidade, acesso pela estrada à direita na bifurcação do cemitério, 812 m, 15º59'17.68"S, 48º05'45.95"W, 10.III.2015, *C.M. Siniscalchi 575* (SPF). **GOIÁS:** Alto Paraíso, Parque Nacional Chapada dos Veadeiros, 23 km de Alto Paraíso em direção a Teresina de Goiás, 1410 m, 13°56'39"S, 47°29'38"W, 13.XI.1996, *M. Aparecida da Silva & A.J.V. Santos 3237* (IBGE, US); Caiapônia, Serra do Caiapó, ca. 33 km S. of Caiapônia on road to Jataí, 17°12'S, 51°47'W, 21.X.1964, *H.S. Irwin & T.R. Soderstrom 7104* (MBM, NY, US, BRIT). **MINAS GERAIS:** Capitólio, Represa de Furnas, Estrada depois do Paraíso Perdido, 967 m, 20°36'44"S, 46°19'53"W, 25.X.2006, *J.N. Nakajima et al. 4240* (HUFU, SPF); Jaboticatubas, Serra do Cipó, km 110, 23.XI.1978, *J. Semir 8665* (UEC); Joaquim Felício, Serra do Calixto, 6.X.1995, *F.R.S. Pires et al. 512* (CESJ, SPF); Lima Duarte, Parque Estadual da Serra do Ibitipoca, Trilha do Monjolinho para as três Pontinhas, 1600 m, 21°42'S, 43°53'W, 24.XI.2004, *R.C. Forzza et al. 3680* (RB); Sacramento, Parque Nacional da Serra da Canastra, próximo à portaria de Sacramento, 67 km de Jaguarê, 1.X.1999, *F.N. Costa et al. 105* (SPF); São Roque de Minas, Parque Nacional da Serra da Canastra, próximo ao córrego dos Passageiros, 9.XII.1994, *J.N. Nakajima & R. Romero 713* (HUFU, US, R); ibid., estrada São Roque - Sacramento, próximo à Torre de observação, 24.IX.1996, *J.N. Nakajima & R. Romero 2107* (HUFU, SPF). **PARANÁ:** Almirante Tamandaré, Rodovia dos Minérios, Rio Barigui, 9.II.1982, *G. Hatschbach 44568* (MBM, NY, F, US, UEC).

II.II.5. Chresta pycnocephala Candolle (1836: 85). *Eremanthus pycnocephalus* (Candolle) Baker (1873: 166). Type—BRAZIL. Minas Gerais, campis deserti inter Min. Nov. et F.S. Francisci, *Martius s.n.* [holotype: M e! (M0029455)].

Chresta pycnocephala Candolle var. *halimodendron* Candolle (1836: 85). Type—BRAZIL. Minas Gerais, prope Reacho de Fogo et ad fl. S. Franc. Prope Salgado, *Martius s.n.* [holotype: M e! (M0029454), isotypes: G-DC e! (G00464282)].

Erect shrubs, ramified at the base, with cauline leaves, these sessile, sub-coriaceous, glabrate, margins entire. Panicle of spherical syncephalia. Florets purple with purple throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Fig. 7E–G, 16A–C.

Distribution and habitat:—*Chresta pycnocephala* is restricted to central Minas Gerais, practically along the Espinhaço Range (Fig. 19A). There are collections from Goiás and Distrito Federal identified as this taxon, but close examination of herbarium sheets and one population in the field has shown them to be very variable in morphology and more likely to be hybrids between *C. exsucca* and *C. sphaerocephala,* which are both found in that region. The species is usually found on rocky Cerrado latosol, usually in locations with sparse and open vegetation.

Phenology:—Most flowering and fruiting specimens were collected between June and October, but

there are a few collections in February.
Conservation Status:—The EOO is 41,269.511 km^2 (Near Threatened) and the AOO is 68 km^2 (Endangered). Most of the known populations of the species are not included in conservation units and as most Cerrado species, it is threatened by agricultural expansion, especially *Eucalyptus* farms that are in expansion on the northern part of Minas Gerais. Therefore, we classify the species as Near Threatened (NT).
Comments:—*Chresta pycnocephala* bears similarities to *C. exsucca,* especially regarding habit and the position of the leaves, which are adpressed to the stems. The leaves of *C. pycnocephala,* however, do not present the reticulate tertiary venation seen in *C. exsucca*, and they are also narrower and longer than the ones in that species. The putative hybrid populations present intermediate morphology, sometimes presenting reticulate venation, or petiolate leaves or even larger secondary heads, similar to those of *C. sphaerocephala.*
Selected pecimens examined:—BRAZIL. MINAS GERAIS: Bocaiúva, Rodovia Bocaiúva - Diamantina, descida ao Rio Jequitinhonha, 23.VII.1998, *G. Hatschbach et al. 68115* (HUEFS, MBM, SPF, UB, US, TEX, ESA, FLOR); Botumirim, trilha para o Cruzeiro, na entrada da cidade, pelo acesso a partir de Cristália, 16°51.26’ S, 43°00.46’ W, 7.VII.2001, *P. Fiaschi et al. 841* (K, SPF); Buenópolis, Serra do Cabral, 27.VII.1976, *P. Davis et al. 2358* (UEC); Francisco Sá, ca. 10 km NE of Francisco Sá, road to Salinas, 12.II.1969, *H.S. Irwin et al. 23164* (NY, RB); Grão Mogol, Barracão, Rodovia para Botumirim, km 3-5, 22.VIII.2002, *G. Hatschbach et al. 73722* (ALCB, HUEFS, MBM, SPF, CGMS, ESA, RB); Itacambira, rodovia MG-308, km 53, de Juramento para Itacambira, 963 m, 16º57’40.8”S, 43º32’59.7”W, 1.IX.2014, *C.M. Siniscalchi et al. 522* (SPF); Rio Paranaíba, estrada de terra de acesso à garagem de ônibus, 1048 m, 19º11’23”S, 46º14’28”W, 21.VI.2013, *C.M. Siniscalchi et al. 371* (SPF).

II.II.6. Chresta scapigera (Lessing) Gardner (1842: 241). *Vernonia scapigera* Lessing (1829: 250). *Pycnocephalum scapigerum* (Lessing) Candolle (1836: 83). *Eremanthus scapigerus* (Lessing) Baker (1873: 168). Type—BRAZIL. *Sellow s.n.* [Lectotype: B e! (B100094097) (designated by MacLeish (1985: 466), isolectotype: P e! (P00683156), K e! (K000955285)].

Rosette herbs. Leaves sessile, coriaceous, glabrate to pilose, margins entire to serrulate. Syncephalia solitary, scapose, spherical, scape sometimes presenting lateral branching. Florets purple with purple throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Fig. 8A–D, 16D–F.
Distribution and habitat:—*Chresta scapigera* is found in several open Cerrado physiognomies, on red latosols, rocky or not, in the Distrito Federal, Minas Gerais and Goiás (Fig. 19B). It usually forms small populations and it is sometimes found in disturbed environments.
Phenology:—Flowering and fruiting specimens have been collected in all months.
Conservation Status:—The EOO is 325,539.515 km2 (Least Concern) and the AOO is 332 km2 (Endangered). There are populations including in Conservation Units, such as PARNA Serra da Canastra, and the species has been constantly collected in the last decades. The main threats to the species are those common to other Cerrado species, such as agricultural expansion, illegal fires and grazing. Therefore we classify the species as Least Concern.
Comments:—The species is morphologically variable, mostly regarding leaf shape, which varies from

obovate, ovate, round, and leaf apex that can be round, acute and acuminate. The margins are entire, but can be serrulate to denticulate, especially on the apex. The length of the scape also varies from 30 cm to more than 1.5 m, and sometimes present small lateral branches, with smaller syncephalia or only a few capitula joined together. The syncephalia sometimes show evidence of third order grouping, with more than one syncephalia joined together.

Selected specimens examined:—BRAZIL. DISTRITO FEDERAL: Brasília, Parque do Gama, Margem do Rio Corumbá, 13.VII.1964, *A.P. Duarte & A. Mattos 8275* (NY, RB, UB, F); Brasília, ao sul da cabeça do Córrego Palmeiras, 1000 m, 16°02'S, 47°39'W, 24.VIII.1981, *J.H. Kirkbride 4380* (UB Brasília, Jardim Botânico, área próxima a CAESB, estrada de acesso a QI 17, 1025 m, 15°32'S, 47°51'W, 25.VIII.2000, *T. Regis et al. 47* (UB, HEPH); Brasília, Fazenda Água Limpa, Área de Proteção ambiental do Gama - Cabeça de Veado, 1155 m, 15°58'15"S, 47°57'19"W, 25.IX.2002, *R.C. Mendonça & D. Alvarenga 5098* (IBGE); Catetinho, lado esquerdo da estrada de ferro do Centro-Oeste próximo ao Catetinho, 11.V.1988, *M. Aparecida da Silva et al. 648* (UEC, IBGE, SP, SPF). **GOIÁS:** Caldas Novas, beira da estrada a 2 km da torre número 220, 540 m, 17°57'84"S, 48°31'88"W, 27.VIII.1997, *H.G.P. Santos et al. 499* (CEN, SPF, UEC); Catalão, Distrito de Pires Belo, BR 050, km 237, em direção a Cristalina, 870 m, 17°51'63"S, 47°46'92"W, 6.II.2002, *R. Schütz Rodrigues et al. 1387* (UEC); Cristalina, 110 km South from Brasília towards Cristallina, 17.X.1980, *N.F.F. MacLeish et al. 750* (GA, RB, TEX, MO, CEPEC); Luziânia, 1,5 km à oeste da ponte do rio Alagado em direção a BR-060, 870 m, 16°12'01"S, 48°11'08"W, 6.XI.2002, *G. Pereira-Silva et al. 6862* (CEN); Niquelândia, estrada que sai da rodovia Uruaçu-Niquelândia (cerca de 60 km de Uruaçu) em direção a Barro Alto, 600 m, 14°32'22' S, 48°41'52' W, 15.VII.2000, *V.C. Souza et al. 23956* (ESA, SPF). **MINAS GERAIS:** Delfinópolis, estrada para Gurita, 816 m, 20°16'52"S, 46°52'16"W, 14.V.2003, *R.A. Pacheco et al. 556* (HUFU); Lagoa Dourada, 8 km de Lagoa Dourada em direção a Entre Rios de Minas, Fazenda do Bom Retiro, 20°51'41"S, 44°03'31"W, 8.III.1995, *V.C. Souza et al. 7974* (ESA, SPF); Rio Paranaíba, estrada de terra de acesso à garagem de ônibus, 1048 m, 19º11'23"S, 46º14'28"W, 20.VI.2013, *C.M. Siniscalchi et al. 359* (SPF); São Roque de Minas, Parque Nacional da Serra da Canastra, estrada para Cachoeira dos Rolinhos, 1124 m, 20º10'12,20"S, 46º33'52.61"W, 27.III.2014, *C.M. Siniscalchi et al. 438* (SPF). **SÃO PAULO:** Franca, In campis pr. Franca, VII.1834, *L. Riedel & B. Lushnatt 2410* (NY, US); Pedregulho, Estreito, Usina do Estreito, 720 m, 20°09'28"S, 47°16.38"W, 24.V.2003, *D. Sasaki & A.B. Junqueira 525* (HUFU, SPF, UEC).

II.II.7. Chresta souzae Robinson (1980: 92). Type—BRAZIL. Goiás: Chapada dos Veadeiros, ca. 12 km NW of Veadeiros, wet campo. 14ºS, 47ºW, 1200 m, *H.S. Irwin, R. Souza & R. Reis dos Santos 9286* [Holotype: UB! (1518); isotypes: NY! (163364), US! (2 sheets, 2818642, 2964537)].

Rosette herbs. Leaves sessile, subcarnose, glabrous to pilose, with long trichomes, margins entire. Syncephalia solitary, scapose, hemispherical. Florets purple with white throat, the withe spots spreading to the base of the corolla lobes; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Fig. 6A–E, 14C-H.

Distribution and habitat:—*Chresta souzae* has been registered only on the region of Chapada dos Veadeiros, in Goiás, Central Brazil (Fig. 18A). It is found on dark, peat soils, with high content of quartz, which became partially flooded during the rainy season, usually growing along with several Xyridaceae and Eriocaulaceae.

Phenology:—Most of the flowering and fruiting specimens were collected from September to December, corresponding to the rainy season, but there are collections on June too.

Conservation Status:—*Chresta souzae* is listed in the Brazilian Red List (Martinelli & Moraes 2013), being evaluated as Endangered. The Red List states that the species also occurs in the Federal District, but during this study we did not find specimens collected in this state; probably the specimens used for preparation of the list belong to *Chresta plantaginifolia.* In our evaluation, the species has EEO of 709.118 km^2, against the 2362.36 km^2 stated in the list, and AOO of 36.000 km^2, both placing it as Endangered. Thus, our evaluation is EN B1ab(iii)+2ab(iii).

Comments:—There has been some confusion about the identity of *C. souzae* in relation to *C. plantaginifolia*, especially since they have been synonymized before. *C. souzae* usually presents obscure venation on the leaves, which are usually semi-fleshy and glabrous, sometimes presenting long simple trichomes, while *C. plantaginifolia* has marked venation on the leaves that are coriaceous and glabrate. *C. souzae* is restricted to dark, wet soils that present high content of quartz in Chapada dos Veadeiros, in the state of Goiás, while *C. plantaginifolia* is found on drier cerrado latosols, which can present high quartz content as well, usually in open Cerrado physiognomies, presenting a wider distribution range. Flowering in *C. souzae* can be stimulated by fire, and the species presents a thickened underground system, although flowering specimens have been observed in locations without recent fire effect.

Selected specimens examined:—BRAZIL. GOIÁS: Rod. GO-327, 2-5 km Oeste de Alto Paraíso, 1200 m, 15.X.1990, *G. Hatschbach et al. 54601* (MBM, US); 14 km de Alto Paraíso/Colinas, campo limpo queimado recentemente, 24.XI.1994, *M. Aparecida da Silva & F.C.A. Oliveira 2421* (IBGE, US, RB); Buriti Sozinho, ca. 8 km de Alto Paraíso para São Jorge, 14º9’78”S, 47º36’79”W, 27.IX.1997, *C. Munhoz et al. 539* (HEPH); GO-118 de Alto Paraíso a Teresina de Goiás, 15.4 km N de Alto Paraíso, 14º00’55”S, 47º31’35”W, 11.VI.2011, *J.B. Bringel & H.J.C. Moreira 823* (CEN, UB); Rodovia GO 118, km 184, em direção a Teresina de Goiás, 1500 m, 14º00’00.86”S, 47º31’07.3”W, 1.XII.2013, *C.M. Siniscalchi & C.T. Oliveira 425* (SPF)*;* Rodovia GO-118, a ca. 12 km de Alto Paraíso, a ca. 200 m da estrada, 1502 m, 14º00’30.54”S, 47º31’25.95”W, 3.XII.2014, *C.M. Siniscalchi 570* (SPF)*;* Rodovia GO-239, entre Alto Paraíso e São Jorge, estrada de terra na entrada da Escola Bona Espero, ca. 13 km do centro de Alto Paraíso, 1155 m, 14º09’47.88”S, 47º37’49.71”W, 3.XII.2014, *C.M. Siniscalchi 571* (SPF)*;* Alto Paraíso, Parque Nacional Chapada dos Veadeiros, 1477 m, 15°44.14’ S, 47°54.54’ W, 25.IX.1995, *M.L. Fonseca & F.C.A. Oliveira 551* (IBGE, US, RB); 23 km de Alto Paraíso em direção a Teresina de Goiás, 1410 m, 13º56’39”S, 47º29’38”W, 13.XI.1996, *M. Aparecida da Silva & A.J.V. Santos 3237* (IBGE, US); Cavalcante, caminho para cachoeira do rio Prata, ca. 39 km de Cavalcante, 11.VI.2011, *J.B. Bringel & H.J.C. Moreira 838* (CEN, UB); Estrada para Colinas do Sul, ca. 1,5 km de Cavalcante, próximo ao Morro da Cruz, 809 m, 13º48’37”S, 47º27’19”W, 26.IX.2011, *R.C. Forzza et al. 6770* (RB); Teresina de Goiás, Chapada dos Veadeiros, Rodovia GO 118, km 196, 1460 m, 13º56’01.33”S, 47º26’28.71”W, 1.XII.2013, *C.M. Siniscalchi & C.T. Oliveira 428* (SPF).

II.II.8. Chresta speciosa Gardner (1842: 240). *Eremanthus speciosus* (Gardner) Baker (1873: 169). *Glazioviantһus speciosus* (Gardner) MacLeish (1985a: 351). Type—BRAZIL. Goiás, dry campos near Villa de Arrayas, *G. Gardner 3801* [Holotype: BM, isotype: BM].

Eremanthus harmsianus Taubert (1896: 453). *Glaziovianthus harmsianus* (Taubert) Zardini (1976: 240). Type—BRAZIL. Goiás, Serra dos Pyreneos, *Ule 2984* [Holotype: HBG e! (HBG506544), isotype: P e! (P00683160, annotated as 488), R! (146981)].

Herbs with rosettes on tip of stems. Leaves sessile, with parallel venation, glabrous to pilose, margins entire or denticulate close to the apex. Syncephalia solitary, scapose, hemispherical. Florets red with yellow throat; style arms covered by clavate sweeping hairs; pollen type A.

Illustrations:—Gardner (1842: Tab. IX), Fig. 5E–J, 13E–H.

Distribution and habitat:—*Chresta speciosa* is found in Goiás, with a few records from Tocantins, Central Brazil (Fig. 18B). It grows mostly on rocky soils and open environments, such as *cerrado rupestre*, and close to small outcrops.

Phenology:—Flowering and fruiting specimens were collected from May to December, but most of the collections are from May to August, which usually corresponds to the dry season in the region. Although fire is not a requirement for flowering in this species, populations that suffer its effect usually present very intense flowering after a few months.

Conservation Status:—The EOO is 73,082.781 km^2 (Least Concern) and AOO is 188 km^2 (Endangered). There are populations in conservation units, such as PARNA Chapada dos Veadeiros and Parque Estadual da Serra dos Pirineus, therefore, we classify it as Least Concern.

Comments:—*Chresta speciosa* has several peculiar morphological features, such as the parallel venation, the leaves that dry and fall down during flowering, and the moniliform underground system. Those characteristics also separate it from *C. curumbensis,* the other red-flowered species in the genus, which presents perennial, semi-fleshy leaves with pinnate venation.

Plants of this species frequently present ants foraging over the scape and syncephalia, due to the presence of an extra-floral nectary on the outside part of the corolla tube, above the phyllaries. The ants usually do not go above the corolla lobes, not coming into contact with the anthers or style, probably indicating they are not involved in the pollination in this species, which usually is carried out by hummingbirds. The ants may play a protection role in the plant, but more studies are necessary to confirm this hypothesis.

Selected specimens examined:—BRAZIL. GOIÁS: Alto Paraíso, Chapada dos Veadeiros, 43 km north of Alto Paraíso towards Cavalcante, 14.X.1980, *N.F.F MacLeish et al. 738* (GA, F, RB, US); ibid., 6 km south of Alto Paraíso towards Brasília, 16.X.1980, *N.F.F MacLeish et al. 745* (GA, GH, NY, RB); Alto Paraíso, Parque Nacional da Chapada dos Veadeiros, trilha entre a sede do parque e o Cânion do Rio Preto, descida para o Cânion, 14º09’25”S, 47º47’42”W, 16.VIII.1995, *R.C. Mendonça et al. 2260* (IBGE, RB, US); ibid., trilha para as cachoeiras de 80 e 120 metros, 14º10’29”S, 47º48’03”W, 10.IX.1996, *R.C. Mendonça et al. 2614* (IBGE, RB); Alto Paraíso de Goiás, rodovia GO-118, a ca. 7 km de Alto Paraíso, 1125 m, 14º04’15.55”S, 47º30’29.56”W, 20.VII.2013, *C.M. Siniscalchi et al. 376* (SPF); Alto Paraíso de Goiás, Morro da Baleia, Estrada de Alto Paraíso para São Jorge, morro mais baixo no lado oposto da estrada ao Morro da Baleia, 1258 m, 14º08’13.71”S, 47º40’22.35”W, 20.VII.2013, *C.M. Siniscalchi et al. 377* (SPF); Alto Paraíso de Goiás, Chapada dos Veadeiros, Rodovia GO-239, entre Alto Paraíso e São Jorge, estrada de terra na entrda da Escola Bona Espero, ca. 13 km do centro de Alto Paraíso, 1155 m, 14º09’47.88”S, 47º37’49.71”W, 28.VIII.2015, *C.M. Siniscalchi 619* (SPF); Cavalcante, Balsa de São Félix (Lago Serra da Mesa), 365 m, 13º31’44.2”S, 48º02’43.6”W, 1.IX.2005, *R.C. Mendonça et al. 6008* (IBGE); Cavalcante, Reserva Particular do Patrimônio Natural Serra do Tom, 13º40’01”S, 47º48’04”W, 2.XI.2011, *C.B.R. Munhoz et al. 7956* (IBGE); Cocalzinho de Goiás, BR070, sentido Águas Lindas de Goiás-Cocalzinho, km 27 à esquerda, 15º45’26”S, 48º26’13”W, 22.III.2001, *A.A. Santos & A. Amaral-Santos 913* (CEN); Goiás Velho, Serra Dourada, 8.IX.1976, *P.; Gibbs et al. 2792* (UEC, F, NY); Niquelândia, CNT, final da estrada, lado direito da Mina, 14º20’12”S, 48º23’54”W, 8.VIII.1995, *R. Marquete et al. 2215* (RB, US); Niquelândia, ca. de 7 km da cidade em direção a Colinas de Goiás, 14°27.03’ S, 48°19.37’ W, 28.V.1996, *F.C.A. Oliveira et al. 585* (IBGE); Pirenópolis, Estrada para a Serra dos Pireneus 15º49’77”S, 48º54’05”W, 19.VI.1998, *R. Romero et al. 5571* (UEC); Pirenópolis, Serra de Pirenópolis, Morro da antena, 975 m, 15º49’49”S, 48º58’8”W, 18.VII.2007, *R.F. Monteiro et al. 95* (RB). **TOCANTINS:** Palmeirópolis, Estrada Palmeirópolis-Balsa do Coronel, ca. de 3 km após a entrada, 340 m, 13º01'37"S, 48º12'07"W, 13.VII.2007, *G. Pereira-Silva et al. 11992* (CEN); Paraná, Central de britagem próximo ao eixo da barragem, 350 m, 12º48'23"S, 48º13'47"W, 23.I.2008, *G. Pereira-Silva & G.A. Moreira 12857* (CEN).

II.II.8. Chresta sphaerocephala Candolle (1836: 85). *Eremanthus sphaerocephalus* (Candolle) Baker (1873: 167). Type—BRAZIL. Minas Gerais, Tejuco, 1833, *Vauthier 294* [Lectotype: G-DC e! (G00464238) designated by MacLeish (1985b: 466), isolectotype: G e! (G00223276), GH e! (GH00004790)].

Chresta intermedia Gardner (1846: 236). *Eremanthus sphaerocephalus* Candolle (Baker) var. *intermedia* (Gardner) Baker (1873: 167). Type—BRAZIL. Minas Gerais, near Formigas, July 1840, *Gardner 4818* [Holotype: BM e! (BM001009180), isotype: K e! (2 sheets, K000677881, K000677882)].
Chresta cordata Vellozo (1831 [1827]: Tab. 150). Type:—BRAZIL. Campis apricis mediterraneis transalpinis inter gramina, *Vellozo s.n.* [Lectotype: as figure, Fl. Flum. Ic. 8: t. 150, 1831, designated by MacLeish (1985b: 467).

Erect shrubs, sparsely ramified at the base, with petiolate cauline leaves, these coriaceous, usually curved backwards, pubescent to glabrate, margins entire, sometimes sinuate. Panicle of spherical syncephalia. Florets purple with purple throat; style arms covered by subulate sweeping hairs; pollen type C.

Illustrations:—Baker (1873: Tab. 46). Fig. 8J-L, 16G–I.
Distribution and habitat:—*Chresta sphaerocephala* is the most widely distributed species of *Chresta,* occurring in Bahia, Distrito Federal, Goiás, Mato Grosso do Sul, Minas Gerais, Paraná and São Paulo, Brazil (Fig. 19B) . It is also the one with the highest number of records, a reflection of its quite common occurrence throughout most Cerrado environments.
Phenology:—Flowering and fruiting specimens have been found in all months, but most of the records are from May to September.
Conservation Status:—The EOO is 965,507.038 km^2 (Least Concern) and the AOO is 628 km^2 (Vulnerable). The high number of records indicate the species is commonly found, and there are collections from several conservation units. Also, it is not uncommon to find the species in somewhat disturbed environments, however, the species suffers the same pressures of the other Cerrado species. We classify it as Least Concern (LC).
Comments:—*Chresta sphaerocephala* forms dense populations, sometimes through re-sprouting from the underground system. The species is easily identified by the large, conspicuously petiolate, conduplicate, silvery grey leaves, and is a common component of the flora in several Cerrado physiognomies. The species has been target of several studies, such as root anatomy (Appezzato-da-Glória et al. 2008), secondary biochemistry (Bohlmann et al. 1982) and pharmacological effects (Costa et al. 2015).
Selected specimens examined:—BRAZIL. BAHIA: Barreiras, drainage of the Rio Corrente, near Rio Piau, ca. 150 km SW of Barreiras, 850 m, 14.IV.1966, *H.S. Irwin et al. 14825* (NY, UB, TEX). **DISTRITO FEDERAL:** Brasília, Rio Torto, ca. 10 km N of Brasília, 1000 m, 8.VII.1966, *H.S. Irwin et al. 18067* (NY, US, MO, BRIT); ibid., Reserva Ecológica do IBGE, 1100 m, 15°57'05"S, 47°52'10"W, 20.VI.2001, *M. Aparecida da Silva 5052* (IBGE, RB). **GOIÁS:** Campo Alegre de Goiás, BR-050,

5-8 km S do trevo para Ipameri, 11.VI.1993, *G. Hatschbach et al. 59270* (MBM, NY, MO, US); Ipameri, BR-050, populações ao longo da estrada, 951 m, 17º30'22.12"S, 47º49'25,80"W, 19.VII.2013, *C.M. Siniscalchi et al. 372* (SPF)*;* Pirenópolis, Parque Estadual da Serra dos Pireneus, estrada lateral a direita da estrada Pirenópolis-Cocalzinho para Fazenda Capitão do Mato, 22.VII.2007, *B. Loeuille et al. 299* (SPF). **MATO GROSSO DO SUL:** Camapuã, Córrego São Domingos, Rodovia BR-060 entre Camapuã e Chapadão do Sul, 27.VI.2001, *A. Pott et al. 9175* (HMS, HUFU, CGMS). **MINAS GERAIS:** Caeté, IX.1874, *M. Motta s.n.* (R); Caxambú, Campo alto, 1223 m, 13.VII.1954, *A.P. Duarte 3848* (RB, UB, UEC, US); Datas, Morro do Coco, 1300 m, 18°26'S, 43°41'W, 17.VII.1987, *R. Mello-Silva & J.R. Pirani 11018* (BHCB, SPF); Grão Mogol, Estrada para Botumirim, Morro do Chapéu, entre Mombucas e Boa Vista do Bananal, 1200 m, 22.VII.1985, *G. Martinelli et al. 11267* (RB); Joaquim Felício, Serra do Cabral, ca. 15 km de Joaquim Felício, camino a Varzea da Palma, 1180 m, 17°42'S, 44°17'W, 20.V.1990, *M.M. Arbo et al. 4577* (CTES, MBM, SPF); Sacramento, Parque Nacional da Serra da Canastra, Guarita de Sacramento, 9.V.1995, *R. Romero et al. 2145* (HUFU); São Gonçalo do Rio Preto, Parque Estadual do Rio Preto, 11.VI.1999, *A. Salino 4793* (BHCB, US). **PARANÁ:** Jaguaraíva, Parque Estadual do Cerrado, 17.VIII.1996, *A.C. Cervi & E.P. Santos 6150* (UPCB); Sengés, Rio Cajuru, 15.VI.1971, *G. Hatschbach 26718* (MBM, NY, US). **SÃO PAULO:** Itapeva, Rodovia Itararé-Itapevi, próximo ao Rio Verde, 24°06'S, 49°13'W, 5.VI.1994, *V.C. Souza et al. 6219* (ALCB, MBM, SPF, ESA); Mogi Guaçu, Reserva Biológica da Fazenda Campininha, 23.VI.1980, *W. Mantovani 810* (SP, NY); Pedregulho, Parque Estadual das Furnas do Bom Jesus, Cerrado em encosta próxima ao encontro dos córregos Pedregulho e Bom Jesus, 900 m, 20°11'50"S, 47°25'10"W, 21.VI.2003, *D. Sasaki et al. 578* (SP, SPF, SPSF); Pirassununga, Cerrado de Emas, 22°02'S, 47°30'W, 8.IX.1994, *S. Aragaki & M. Batalha 112* (SP).

Names excluded from *Chresta*

1. *Chresta lanceolata* Vellozo (1831[1827]: Tab. 151) = Doubtful identity.
2. *Chresta alpestris* Gardner (1842: 239) = *Minasia alpestris* (Gardner) Robinson (1992: 650).
3. *Chresta eriopus* (Schultz Bipontinus ex Baker) Robinson (1980: 91) = *Prestelia eriopus* Schultz Bipontinus (1864: 73).

Numerical list of accepted species

1. *Chresta angustifolia*
2. *Chresta artemisiifolia*
3. *Chresta curumbensis*
4. *Chresta exsucca*
5. *Chresta filicifolia*
6. *Chresta harleyi*
7. *Chresta hatschbachii*
8. *Chresta heteropappa*
9. *Chresta martii*
10. *Chresta pacourinoides*
11. *Chresta pinnatifida*
12. *Chresta plantaginifolia*
13. *Chresta pycnocephala*
14. *Chresta scapigera*

15. *Chresta souzae*
16. *Chresta speciosa*
17. *Chresta sphaerocephala*
18. *Chresta subverticillata*

Index to scientific names in *Chresta* with accepted names (in bold), followed by its number, and synonyms.

Argyrovernonia harleyi (H.Rob.) MacLeish ≡ **C. harleyi**
A. martii (DC.) MacLeish ≡ **C. martii**
C. amplexifolia Dematt., Roque & Miranda Gonç. = **C. pacourinoides**
Chresta angustifolia Gardner (1)
C. artemisiifolia Siniscalchi & Loeuille (2)
C. cordata Vell. = **C. sphaerocephala**
C. curumbensis Philipson (H.Rob.) (3)
C. exsucca DC. (4)
C. filicifolia Siniscalchi & Loeuille (5)
C. harleyi H.Rob. (6)
C. hatschbachii H.Rob. (7)
C. heteropappa Siniscalchi & Loeuille (8)
C. intermedia Gardner = **C. sphaerocephala**
C. martii (DC.) H.Rob. (9)
C. pacourinoides (Mart. ex DC.) Siniscalchi & Loeuille (10)
C. pinnatifida (Phillipson) H.Rob. (11)
C. plantaginifolia (Less.) Gardner (12)
C. pycnocephala DC. (13)
C. pycnocephala DC. var. *halimodendron* = **C. pycnocephala**
C. scapigera (Less.) Gardner (14)
C. souzae H.Rob. (15)
C. spathulaefolia (DC.) Gardner = **C. plantaginifolia**
C. speciosa Gardner (16)
C. sphaerocephala DC. (17)
Eremanthus angustifolius (Gardner) Baker ≡ **C. angustifolia**
E. curumbensis Philipson ≡ **C. curumbensis**
E. exsuccus (DC.) Baker ≡ **C. exsucca**
E. harmsianus Taub. = **C. speciosa**
E. imbricatus Barroso = **C. exsucca**
E. labordeii Glaziou, *nom. nud.* = **C. exsucca**
E. martii (DC.) Baker = **C. martii**
E. pinnatifidus Philipson ≡ **C. pinnatifida**
E. plantaginifolius ≡ **C. plantaginifolia**
E. pycnocephalus (DC.) Baker ≡ **C. pycnocephala**

E. rivularis Taub. = **C. angustifolia**
E. scapigerus (Less.) Baker ≡ **C. scapigera**
E. speciosus (Gardner) Baker ≡ **C. speciosa**
E. sphaerocephalus (DC.) Baker ≡ **C. sphaerocephala**
Eremanthus sphaerocephalus DC. (Baker) var. *intermedia* (Gardner) Baker = **C. sphaerocephala**
Glaziovianthus curumbensis (Philipson) MacLeish ≡ **C. curumbensis**
G. purpureus Barroso = **C. curumbensis**
G. speciosus (Gardner) MacLeish ≡ **C. speciosa**
G. harmsianus (Taub.) Zardini = **C. speciosa**
Pithecoseris pacourinoides Mart. ex DC. ≡ **C. pacourinoides**
Pithecoseris pacourinoides var. *capitata* Glaziou, *nom. nud.* **= C. pacourinoides**
Pycnocephalum angustifolium (Gardner) MacLeish ≡ **C. angustifolia**
P. pinnatifidum (Philipson) MacLeish ≡ **C. pinnatifida**
P. plantaginifolium (Less.) Candolle ≡ **C. plantaginifolia**
P. spathulaefolium DC. = **C. plantaginifolia**
P. scapigerum (Lessing) DC. ≡ **C. scapigera**
Stachyanthus martii DC. ≡ **C. martii**
Vernonia plantaginifolia Less. ≡ **C. plantaginifolia**
Vernonia scapigera Less. ≡ **C. scapigera**

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Abrahum, M.A. 6 (4); Agra, M.F. 1736 (9); 1742 (9); 2518 (9); 5899 (10); 6976 (10); Alves, H. 245 (17); Alves, J.L.H. 7 (17); Alves, M.A. 164 (17); Amaral, A.G. 20 (17); 178 (17); 840 (17); 1479 (17); 1656 (17); 1724 (17); 1780 (17); 1924 (17); Amaral, M.C.E. 97 (14); Amburgo Alves, J.L. s.n. (9); Amorim, A.M. 2867 (7); Anderson, W.R. 10059 (14); 35611 (12); Andrade, A.G. 399 (14); 1083 (14); Andrade, I.M. 3087 (10); Andrade, J.B. 2182 (17); Angélica, M. s.n. (10); Aparecida da Silva, M. 648 (14); 748 (17); 1037 (3); 1146 (14); 1404 (17); 1480 (17); 1731 (17); 2421 (15); 2644 (17); 2652 (14); 3237 (15); 5052 (17); 5554 (3); 6005 (3); 6171 (17); 6567 (17); 7386 (3); 7702 (14); 5063A (17); 2077 (16); 6249 (16); Aragaki, S. 104 (17); 112 (17); Arantes, A.A. s.n. (14); Araújo, D. 1815 (10); Araujo, F.S. s.n. (10); Arbo, M.M. 4577 (17); 7417 (10); Arlindo 3 (17); Armando, M. 12 (17);
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(10); Castro, J.F. 5 (14); Cavalcanti, F.S. s.n. (10); Cavalcanti, M.F. 167 (10); Cavalcanti, T.B. 505 (17); 695 (16); 987 (16); 1665 (16); 1685 (14); 1776 (3); 1795 (3); 3614 (1); CFCR10326 (12); Caxambu, M.G. 4783 (14); Cerati, T.M. 227 (17); Cervi, A.C. 6150 (17); Cesar, H.L. 284 (17); 722 (17); Cezzare, C.H.G. 377 (3); Chacon, R.G. 96 (3); 703 (3); 739 (3); Chaves, E. 58 (1); Chiappeta, A. s.n. (10); Claussen, P. 1121 (17); s.n. (17); Coelho, D.M. 538 (9); 828 (9); 371 (9); 487 (9); Conceição, M.C.A. 107 (17); 153 (17); Coradin, L. 2396 (17); 4222 (17); 5987 (9); 6176 (10); 7550 (4); Cordeiro, I. CFCR10009 (17); Cordeiro, L.M. 523 (9); Cordovil, S.P. 21 (3); Correia, C. 328 (9); Costa, A.L. s.n. (4); s.n. (4); s.n. (4); s.n. (9); s.n. (10); s.n. (10); Costa, E.A. 14 (17); Costa, F.N. 105 (12); Costa, R.C. da s.n. (10); Cotarelli, V.M. 890 (2); 954 (9); 1540 (9); Croat, T.B. 53598 (17); Cunha, C.N. 28 (4).

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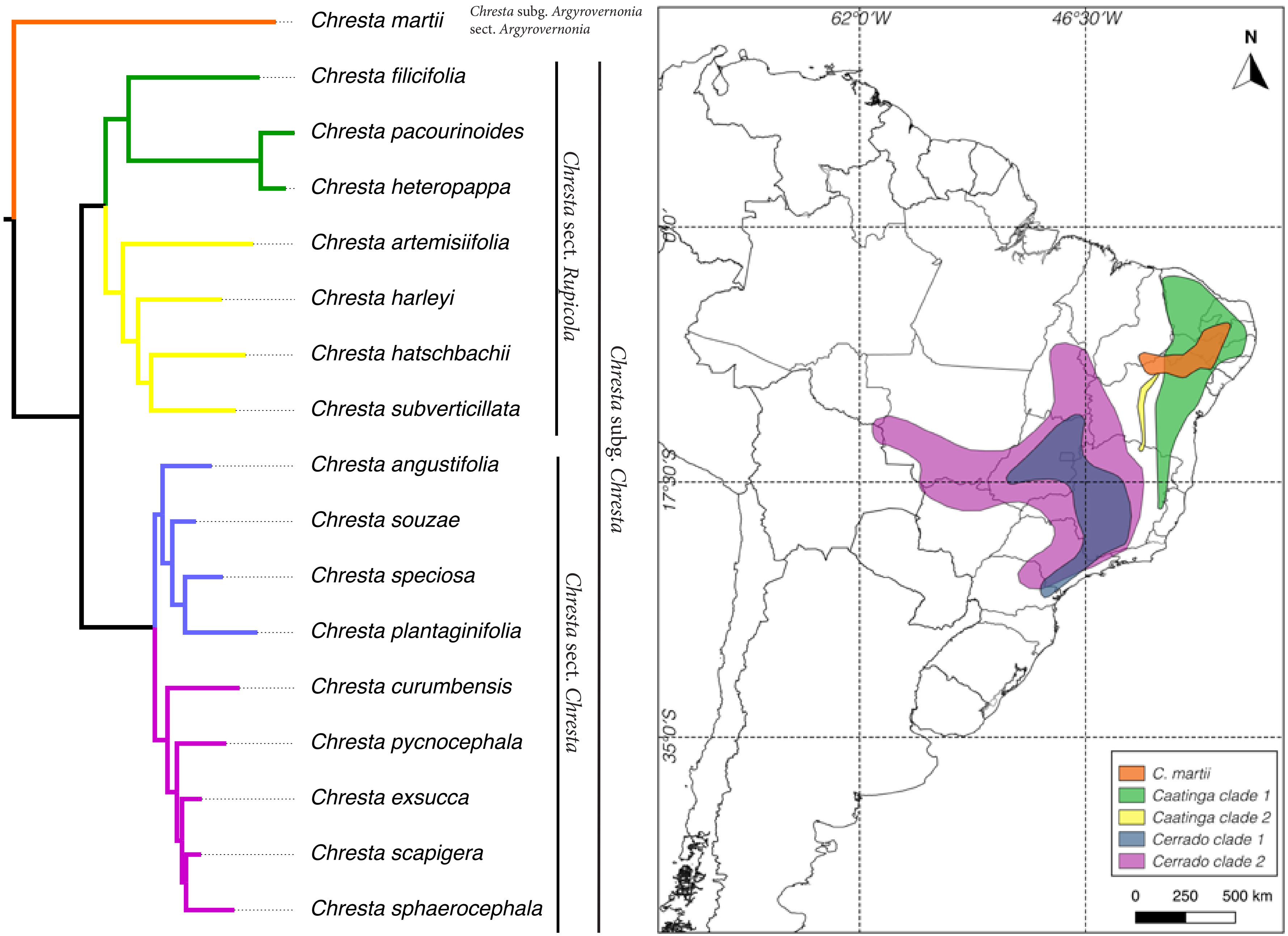

Figure 1. Phylogenetic relations in *Chresta* (based on a maximum likelihood tree run with 709 loci and 1000 bootstraps), showing the proposed infrageneric classification and map showing the distribution of each clade.

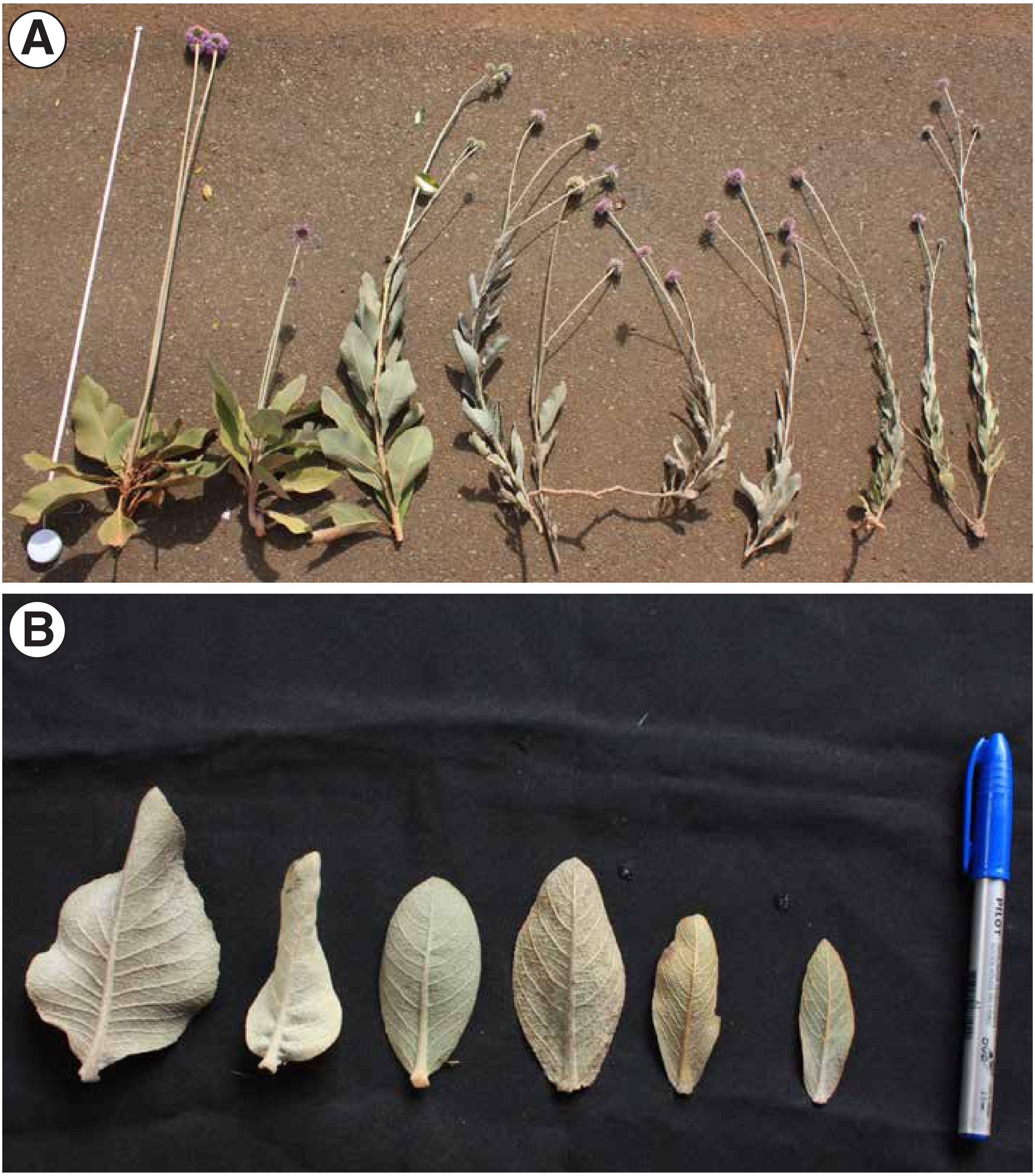

Figure 2. Putative interspecific hybrids of *Chresta*. A. *C. scapigera* × *pycnocephala*, individuals with different morphologies, on the left *C. scapigera* and *C. pycnocephala* on the right. B. *C. exsucca* × *sphaerocephala*, different leaf morphologies collected from several individuals in a population.

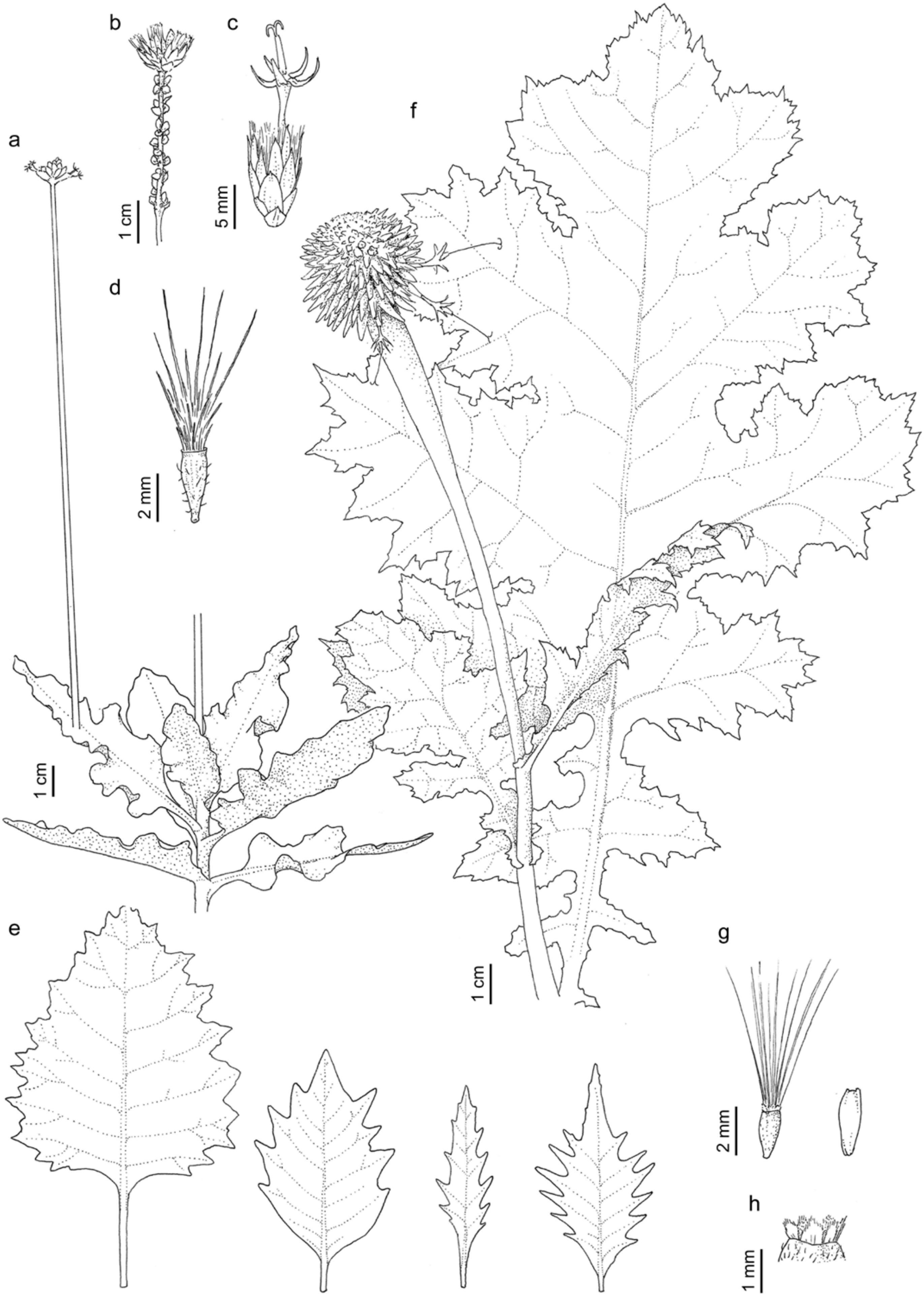

Figure 3. *Chresta martii.* A. Habit and synflorescence. B. Syncephalium showing marked indeterminate growth. C. Capitulum with open floret. D. Cypsela. E. Different leaf shapes found in different populations. *Chresta pacourinoides.* F. Leaf and syncephalia. G. Cypsela type I (left) and type II (right). H. Outer pappus series of cypsela type I. Illustration by M.T. Kubo.

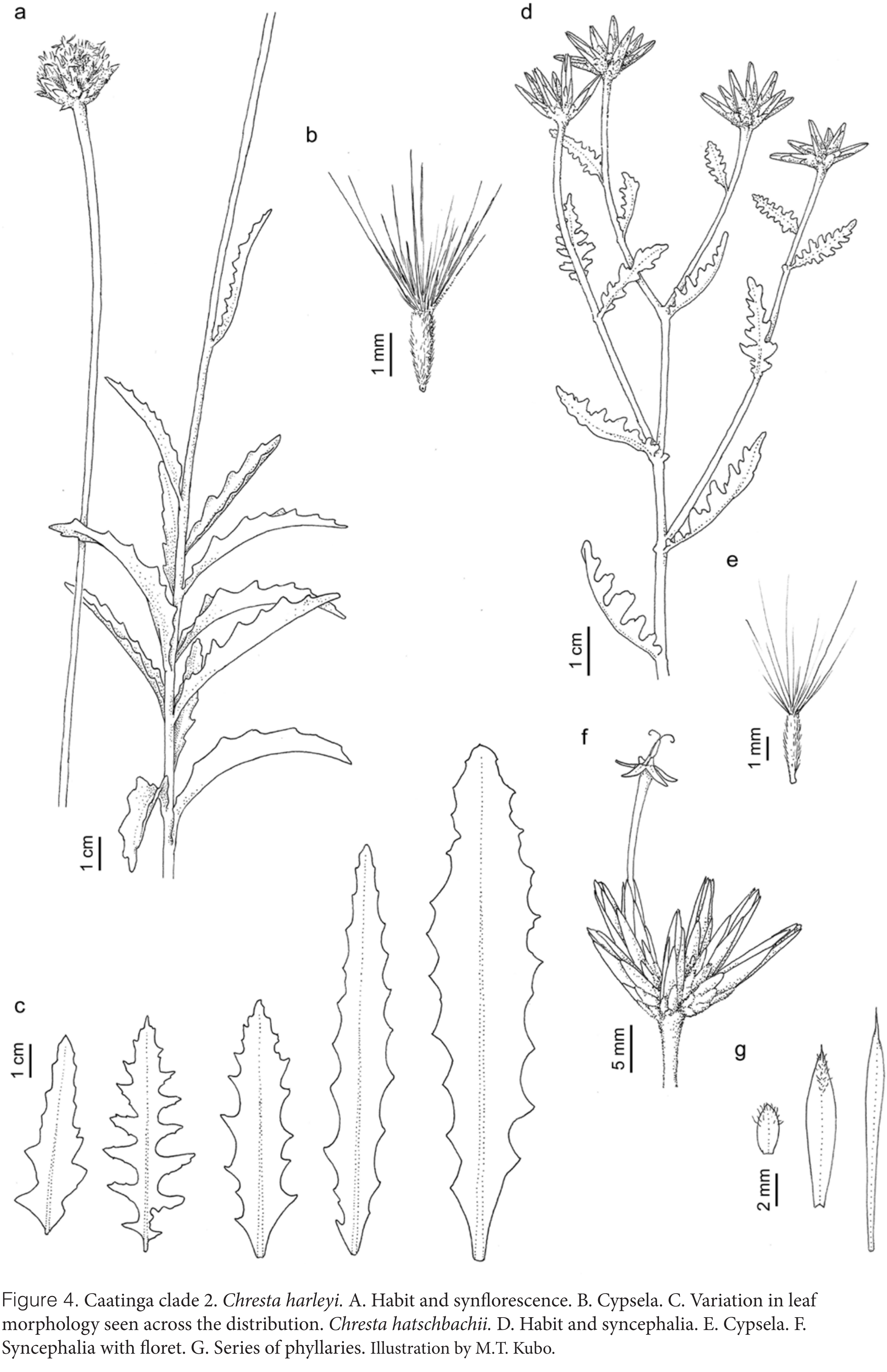

Figure 4. Caatinga clade 2. *Chresta harleyi.* A. Habit and synflorescence. B. Cypsela. C. Variation in leaf morphology seen across the distribution. *Chresta hatschbachii.* D. Habit and syncephalia. E. Cypsela. F. Syncephalia with floret. G. Series of phyllaries. Illustration by M.T. Kubo.

Figure 5. Cerrado clade 1. *Chresta angustifolia.* A. Habit and synflorescence. B. Leaf, adaxial surface. C. Capitulum with floret. D. Cypsela. *Chresta speciosa.* E. Habit and syncephalia. F. Capitulum with floret. G. Cypsela. H. Detail of the secondary bracts of the syncephalia. I. Leaf, adaxial surface. J. Detail of parallel venation. Illustration by M.T. Kubo.

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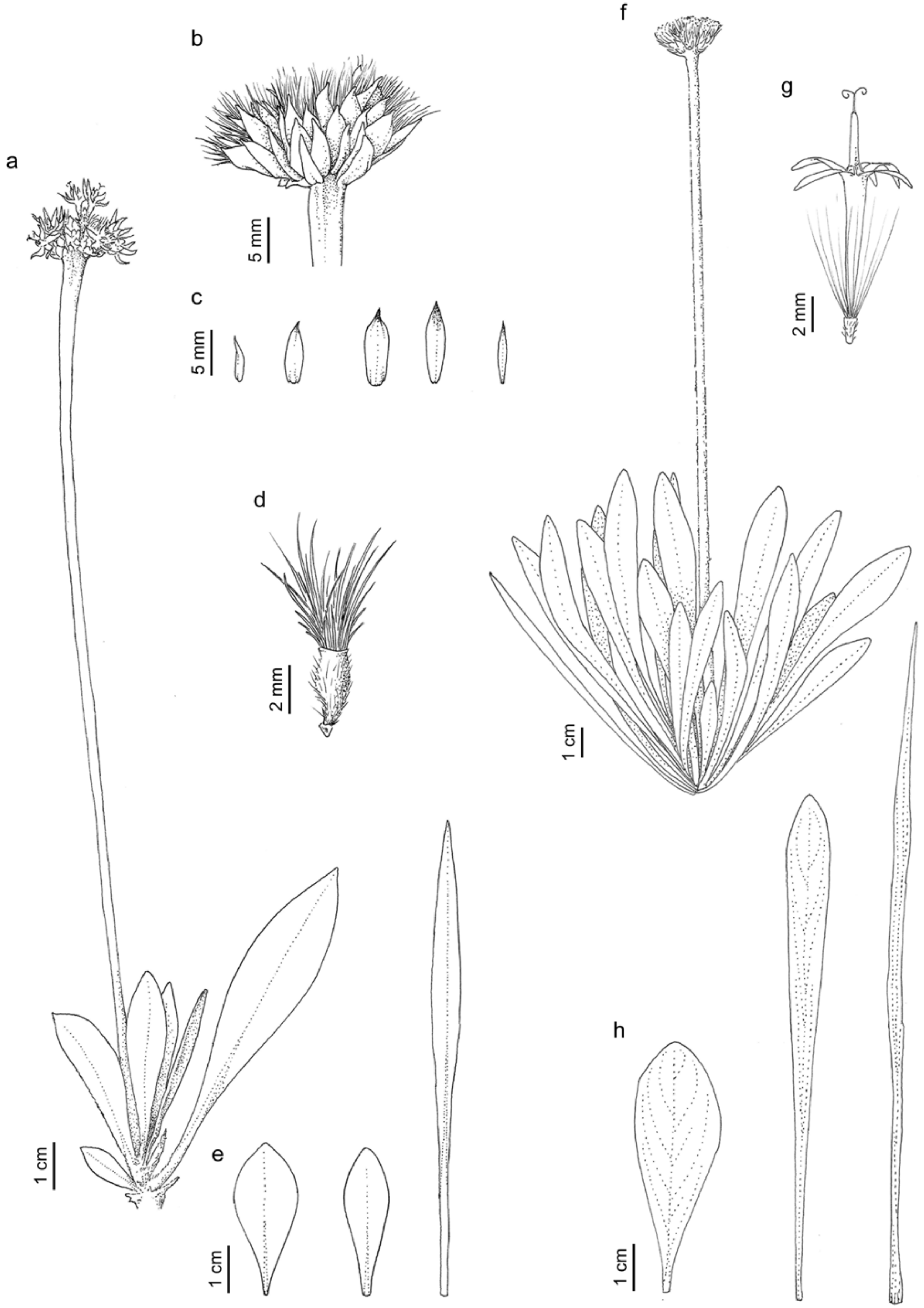

Figure 6. Cerrado clade 1. *Chresta souzae.* A. Habit and synflorescence. B. Syncephalium. C. Series of phyllaries. D. Cypsela. E. Different leaf morphologies. *Chresta plantaginifolia.* F. Habit and synflorescence. G. Floret with cypsela and pappus. H. Different leaf morphologies. Illustration by M.T. Kubo.

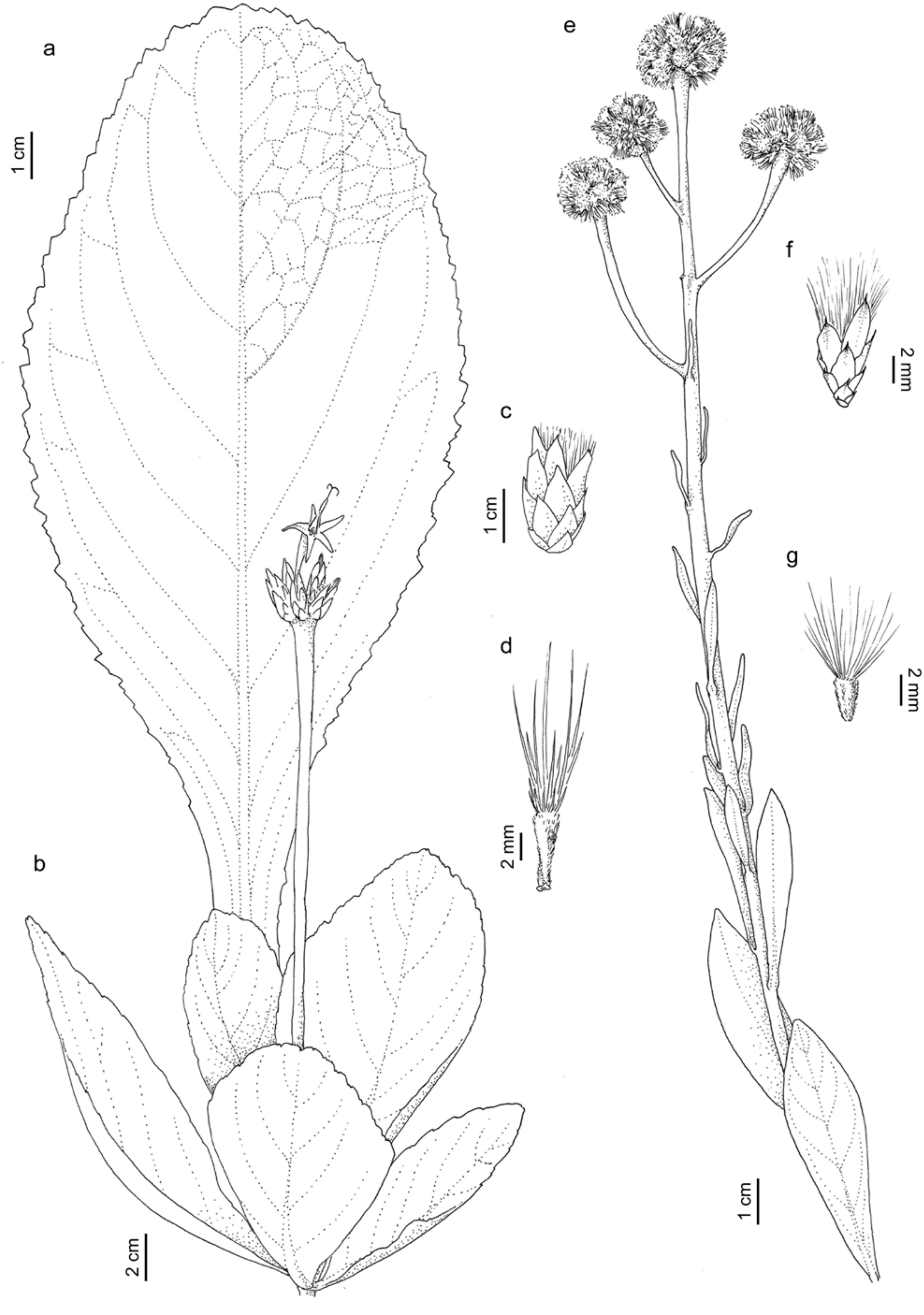

Figure 7. Cerrado clade 2. *Chresta curumbensis.* A. Leaf, adaxial surface. B. Habit and Synflorescence. C. Capitulum. D. Cypsela. *Chresta pynocephala.* E. Habit and synflorescence. F. Capitulum. G. Cypsela. Illustration by M.T. Kubo.

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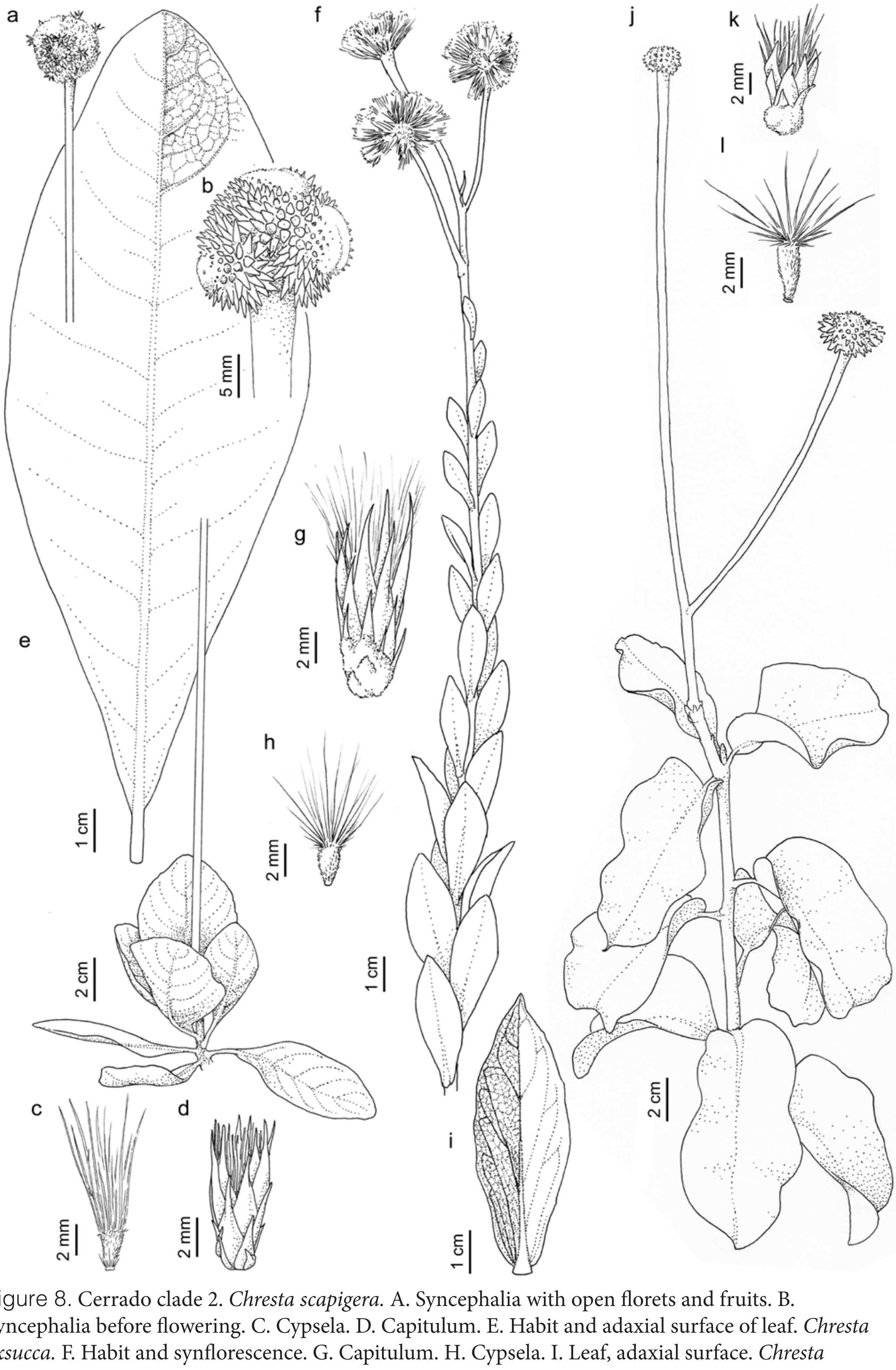

Figure 8. Cerrado clade 2. *Chresta scapigera.* A. Syncephalia with open florets and fruits. B. Syncephalia before flowering. C. Cypsela. D. Capitulum. E. Habit and adaxial surface of leaf. *Chresta exsucca.* F. Habit and synflorescence. G. Capitulum. H. Cypsela. I. Leaf, adaxial surface. *Chresta sphaerocephala.* J. Habit and synflorescence. K. Capitulum. L. Cypsela. Illustration by M.T. Kubo.

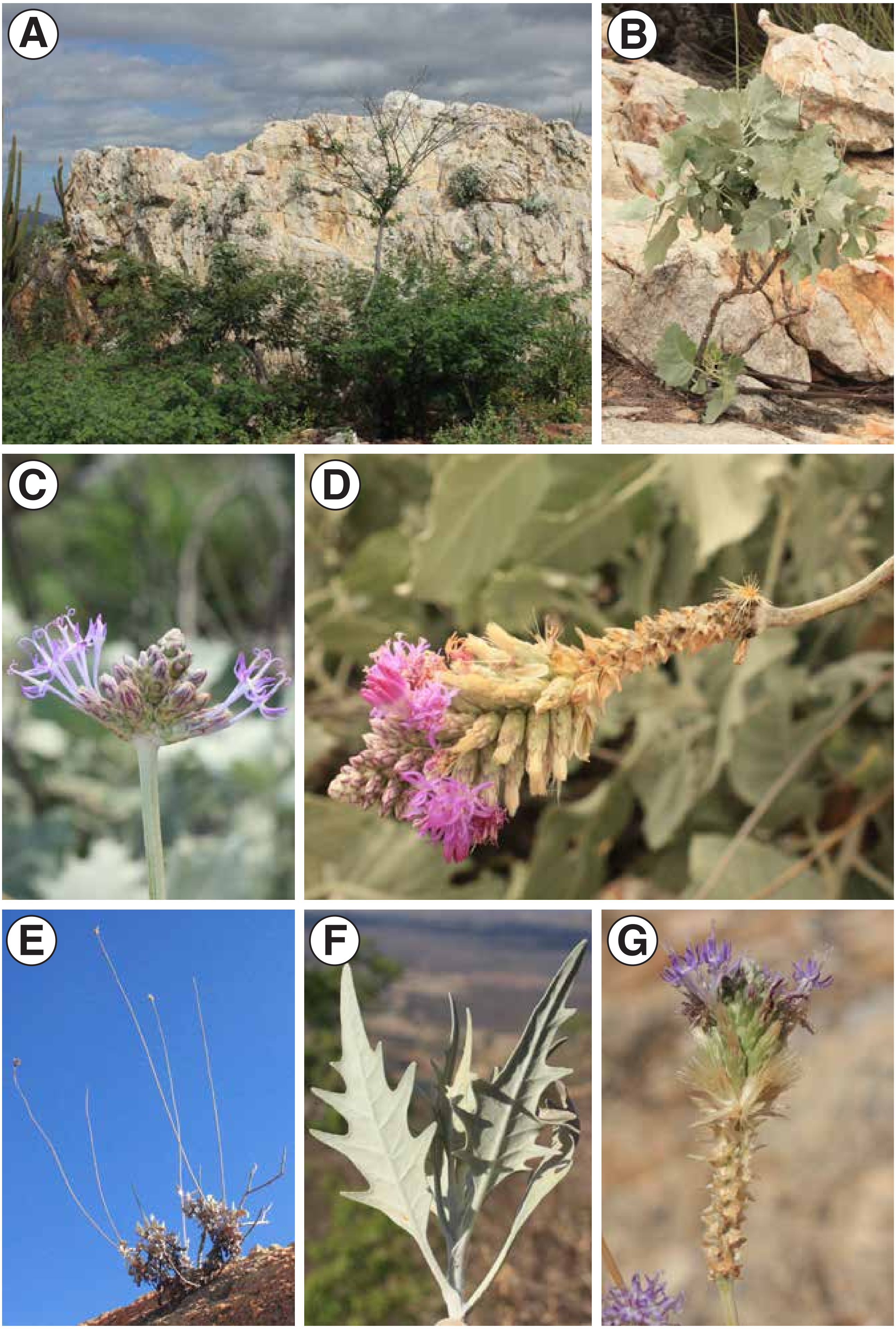

Figure 9. *Chresta martii.* A. Habitat. B. Habit (plant from Bahia). C. Young syncephalium. D. Old syncephalium showing indeterminate growth. E. Habit (plant from Paraíba). F. Leaves (plant from Paraíba. G. Old syncephalium.

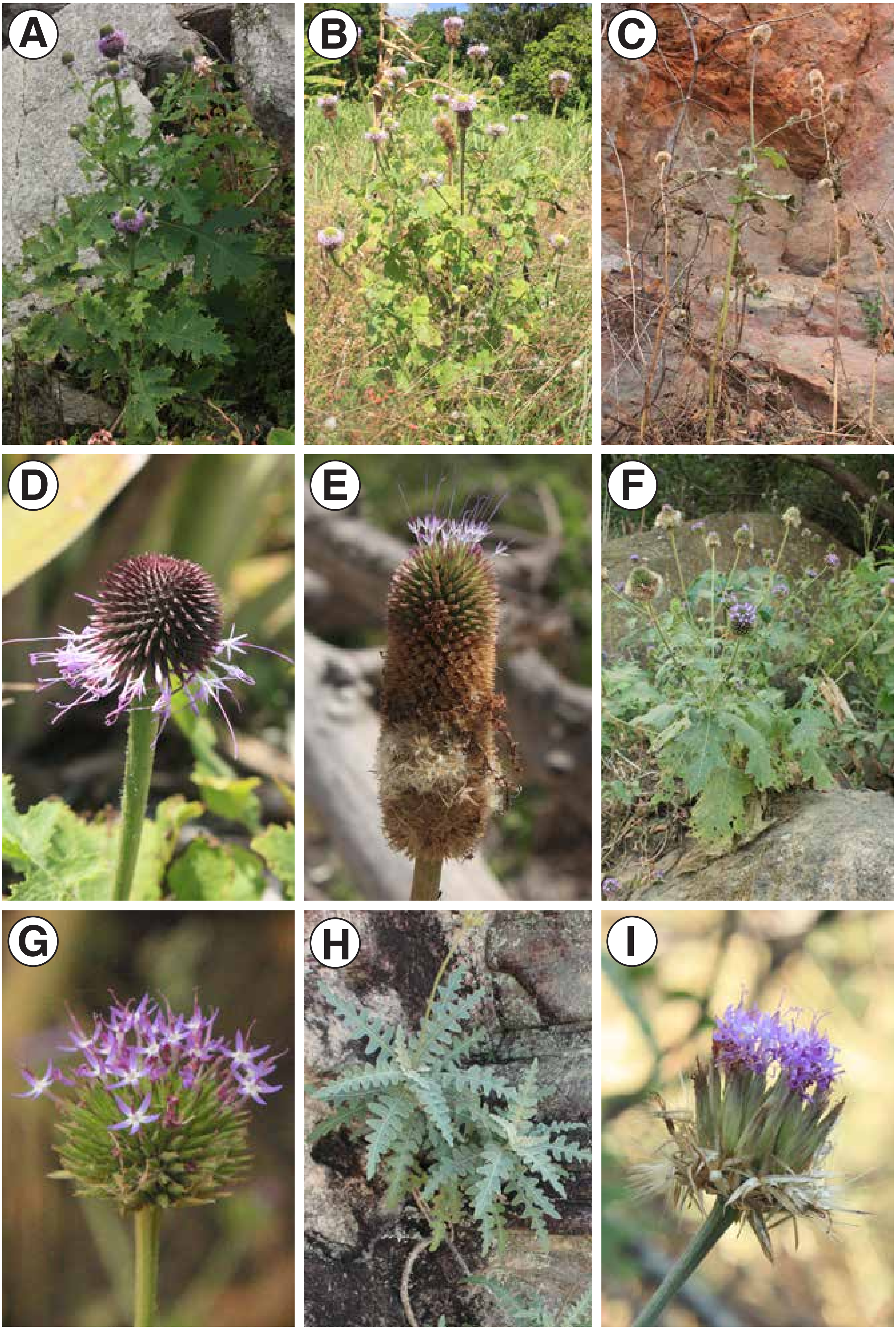

Figure 10. Caatinga clade 1. *Chresta pacourinoides.* A. Habitat in rock outcrop. B. Habitat in open vegetation. C. Plants drying out after flowering. D. Young syncephalium. E. Old syncephalium showing indeterminate growth. *Chresta heteropappa.* F. Habit. G. Syncephalium. *Chresta filicifolia.* H. Habitat and leaves. I. Syncephalium.

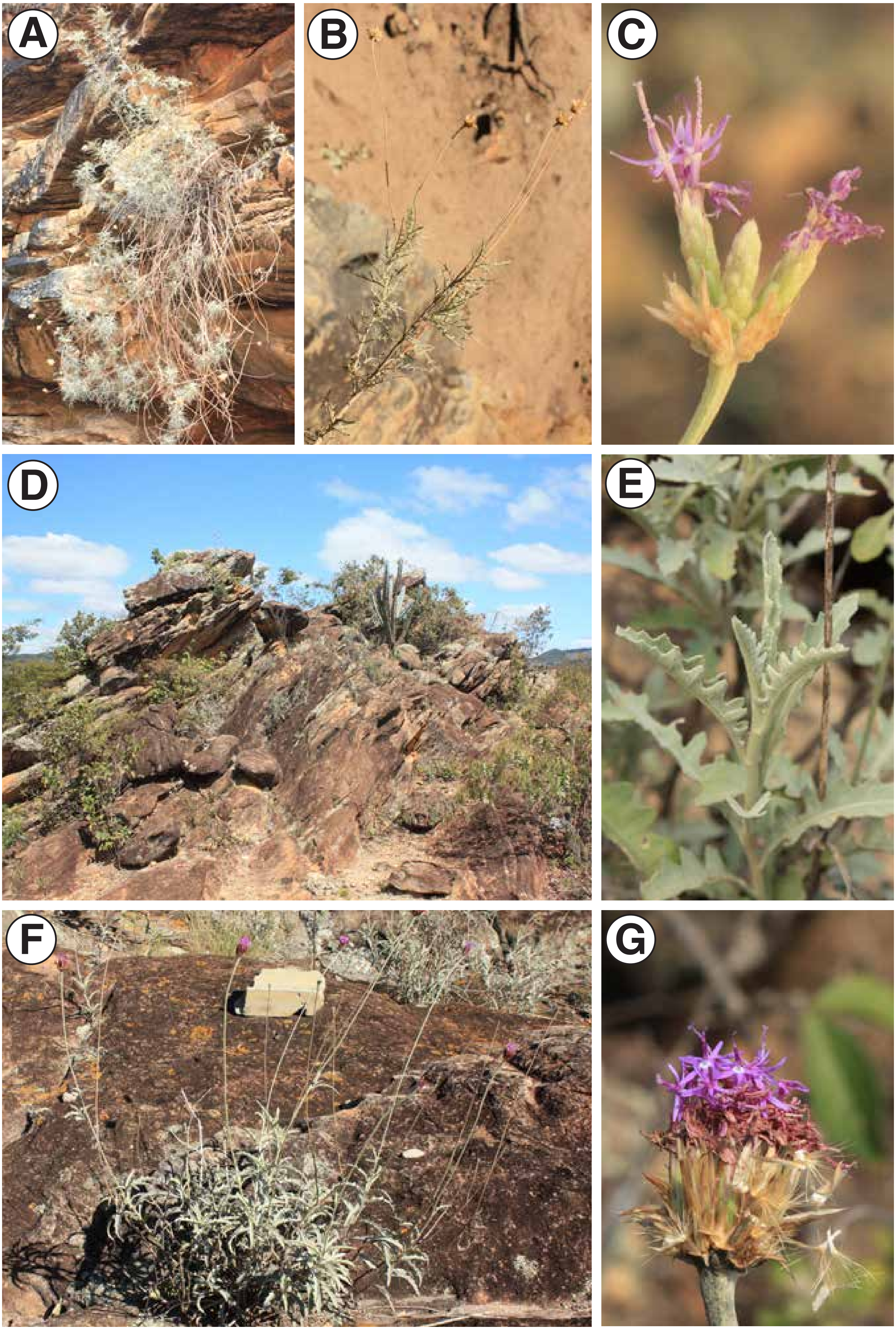

Figure 11. Caatinga clade 2. *Chresta artemisiifolia.* A. Habitat in rock outcrop. B. Leaves. C. Syncephalium. *Chresta harleyi.* D. Habitat in rock outcrop. E. Leaves. F. Habit. G. Syncephalium.

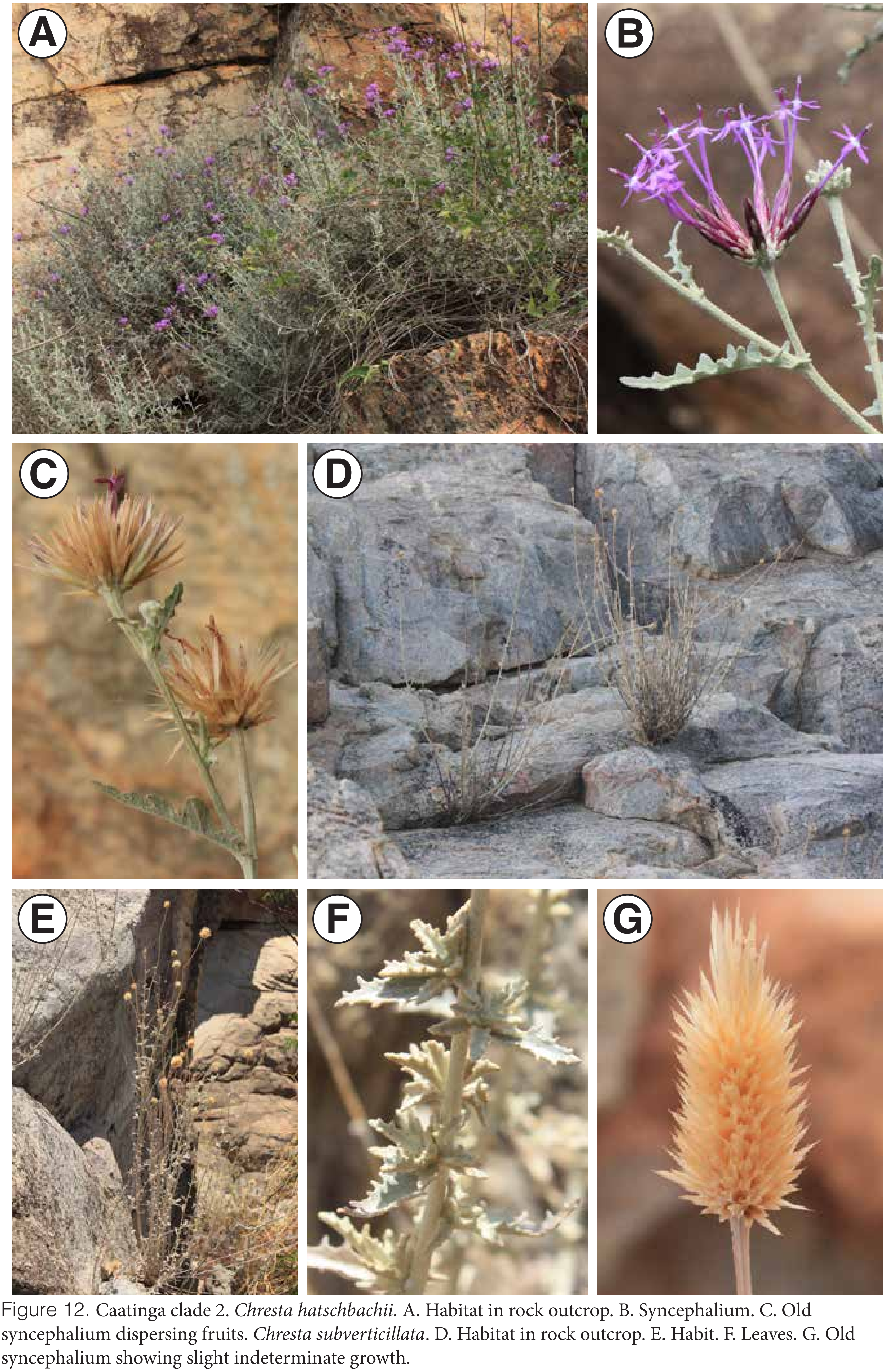

Figure 12. Caatinga clade 2. *Chresta hatschbachii*. A. Habitat in rock outcrop. B. Syncephalium. C. Old syncephalium dispersing fruits. *Chresta subverticillata*. D. Habitat in rock outcrop. E. Habit. F. Leaves. G. Old syncephalium showing slight indeterminate growth.

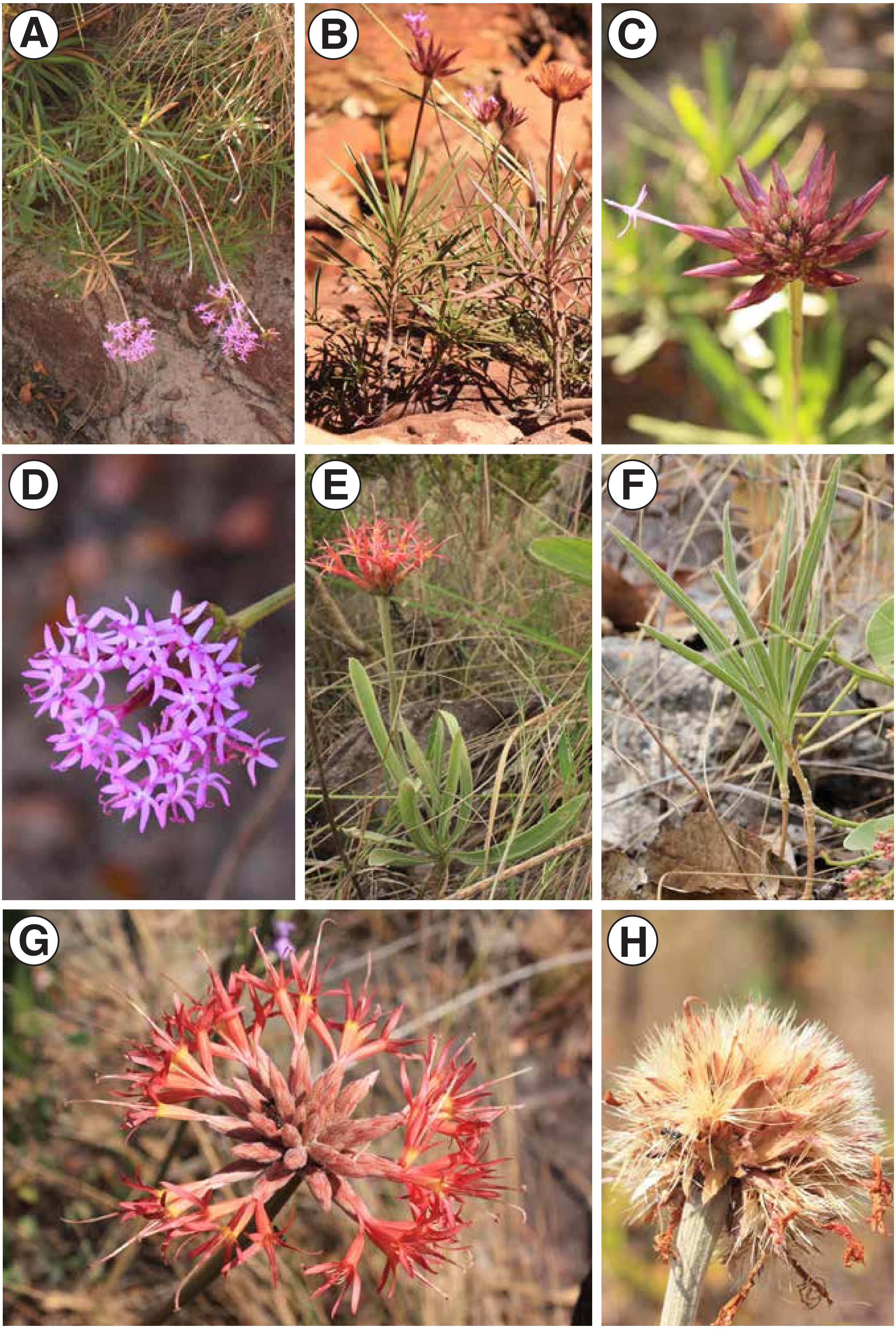

Figure 13. Cerrado clade 1. *Chresta angustifolia.* A. Habitat in sand bank. B. Habit. C. Syncephalium. D. Syncephalium with florets. *Chresta speciosa.* E. Habit with syncephalium. F. Leaves. G. Syncephalium in bloom. H. Old syncephalium dispersing fruits.

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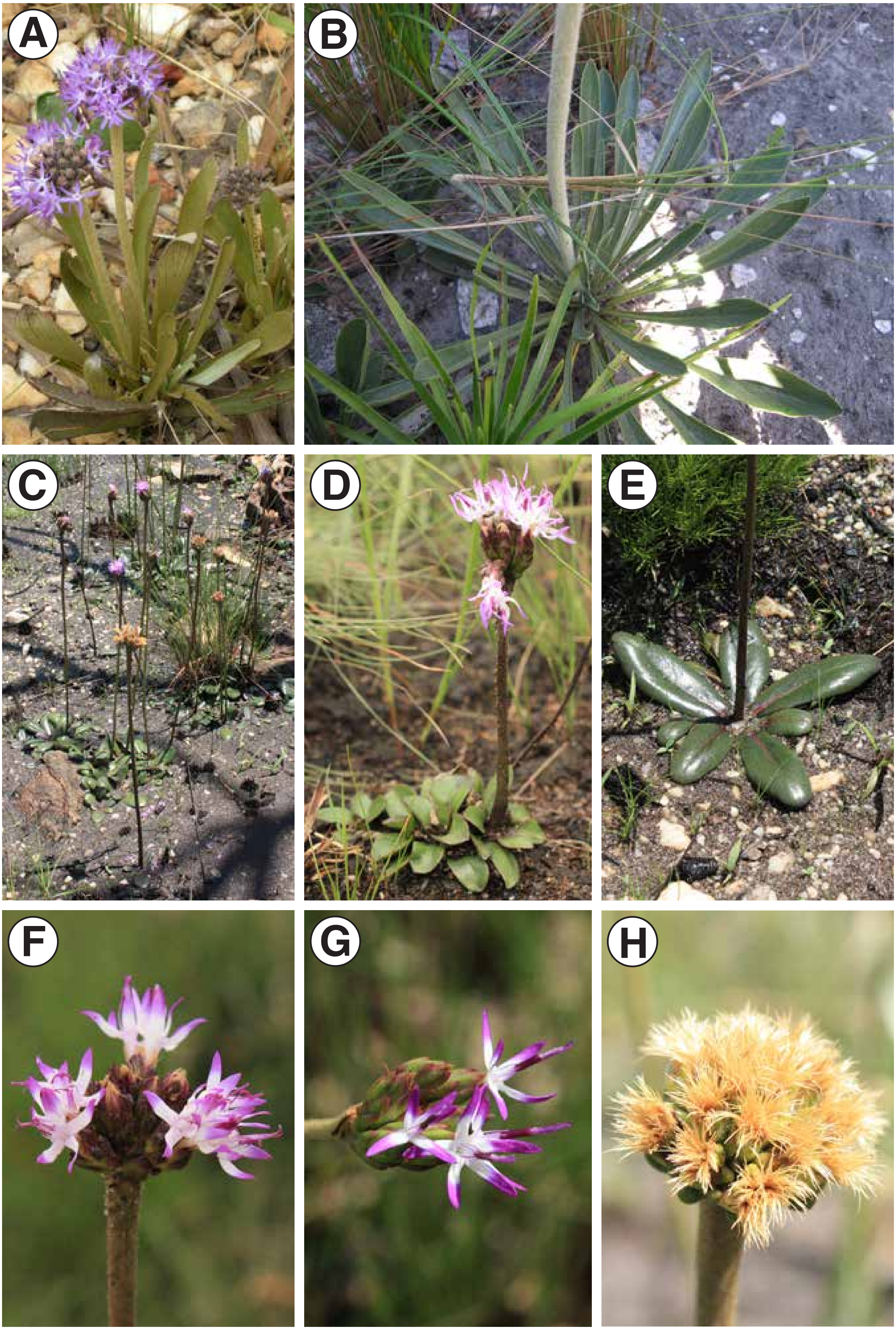

Figure 14. Cerrado clade 1. *Chresta plantaginifolia*. A. Plant flowering. B. Rosette of leaves. (pictures by B. Loeuille). *Chresta souzae.* C. Habit. D. Whole plant flowering. E. Rosette of leaves. F. Syncephalium. G. Florets. H. Old syncephalium dispersing fruits.

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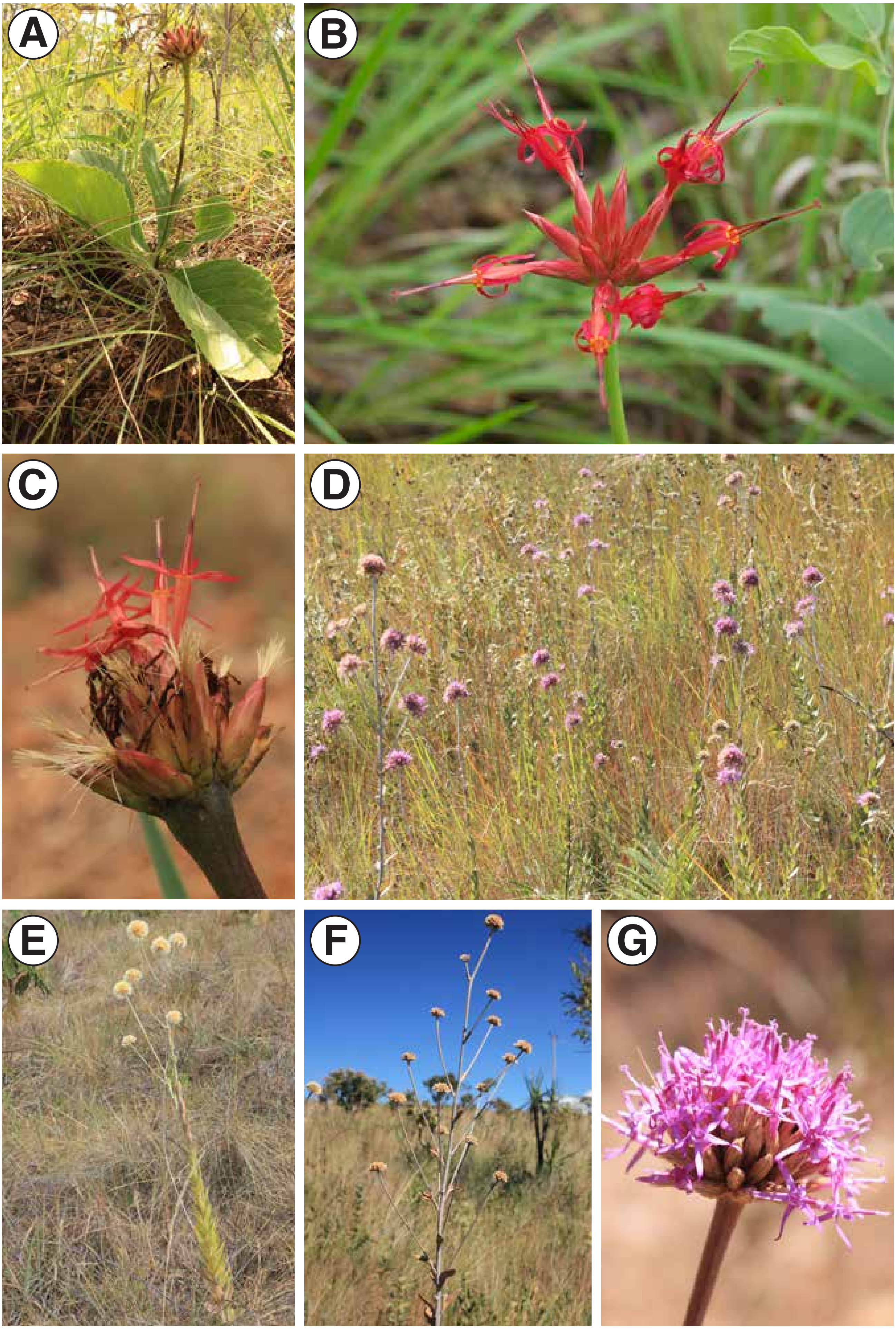

Figure 15. Cerrado clade 2. *Chresta curumbensis.* A. Habit. B. Syncephalium (picture by M.F. Devecchi). C. Old syncephalium. *Chresta exsucca.* D. Habitat with plants flowering. E. Habit and old synflorescence. F. Panicle of syncephalia. G. Syncephalium.

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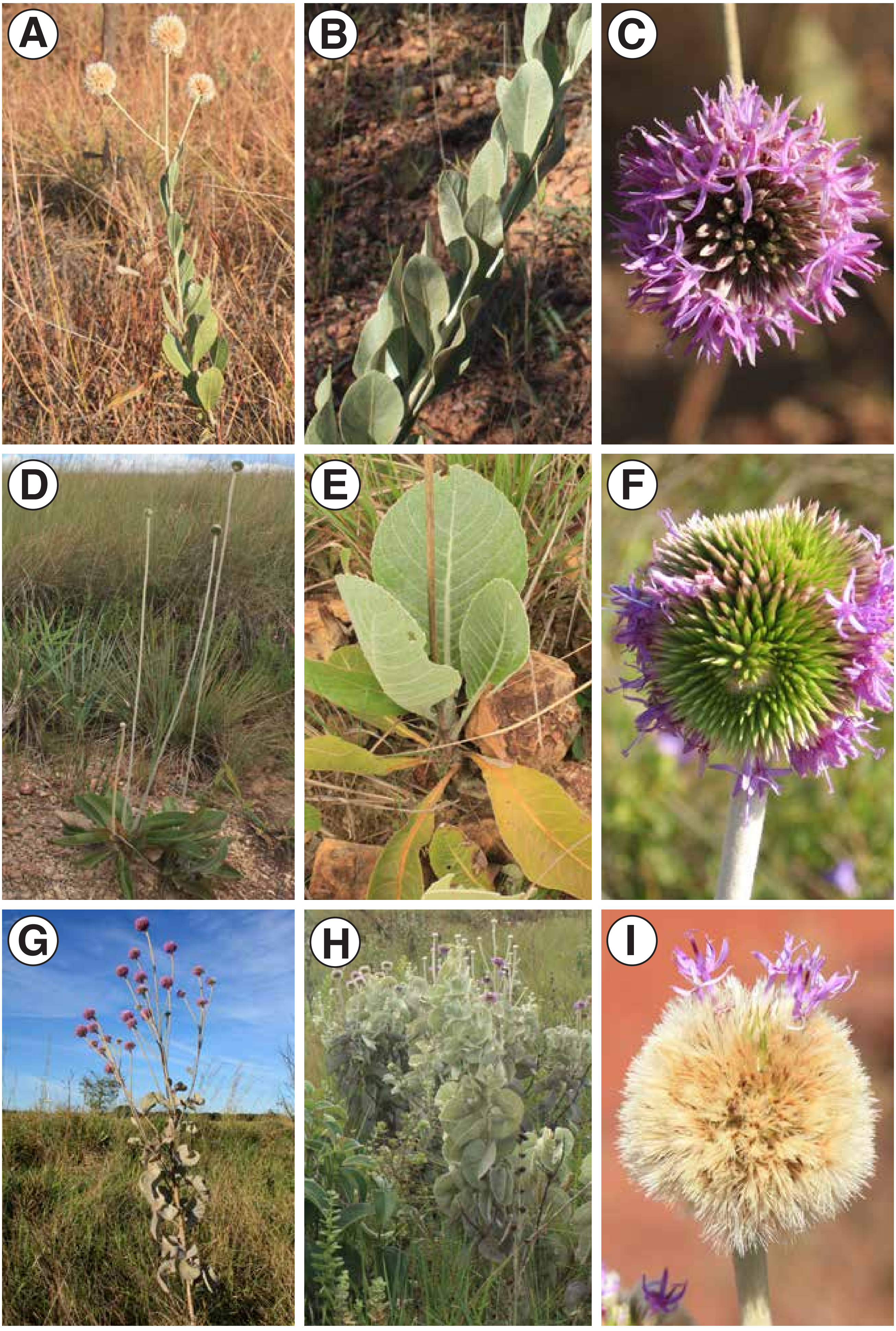

Figure 16. Cerrado clade 2. *Chresta pycnocephala.* A. Habit and old synflorescence. B. Leaves. C. Syncephalium. *Chresta scapigera.* D. Habit. E. Rosette of leaves. F. Syncephalium starting to flower. *Chresta sphaerocephala.* G. Habit with panicle of syncephalia. H. Habit. I. Syncephalium with fruits.

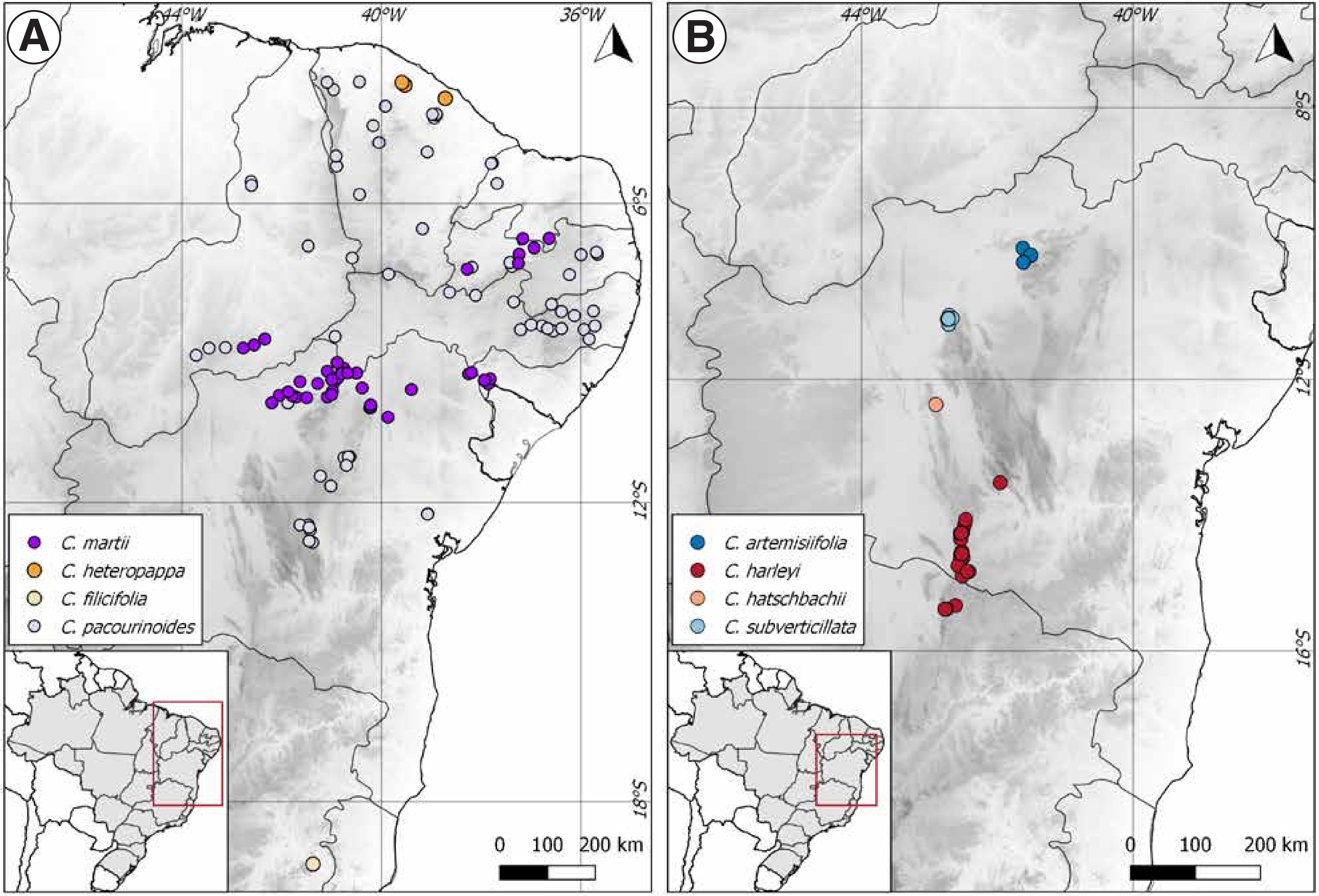

Figure 17. Distribution map of Caatinga and Atlantic Forest species. A. *Chresta martii, C. heteropappa, C. filicifolia, C. pacourinoides*. B. *C. artemisiifolia, C. harleyi, C. hatschbachii, C. subverticillata.*

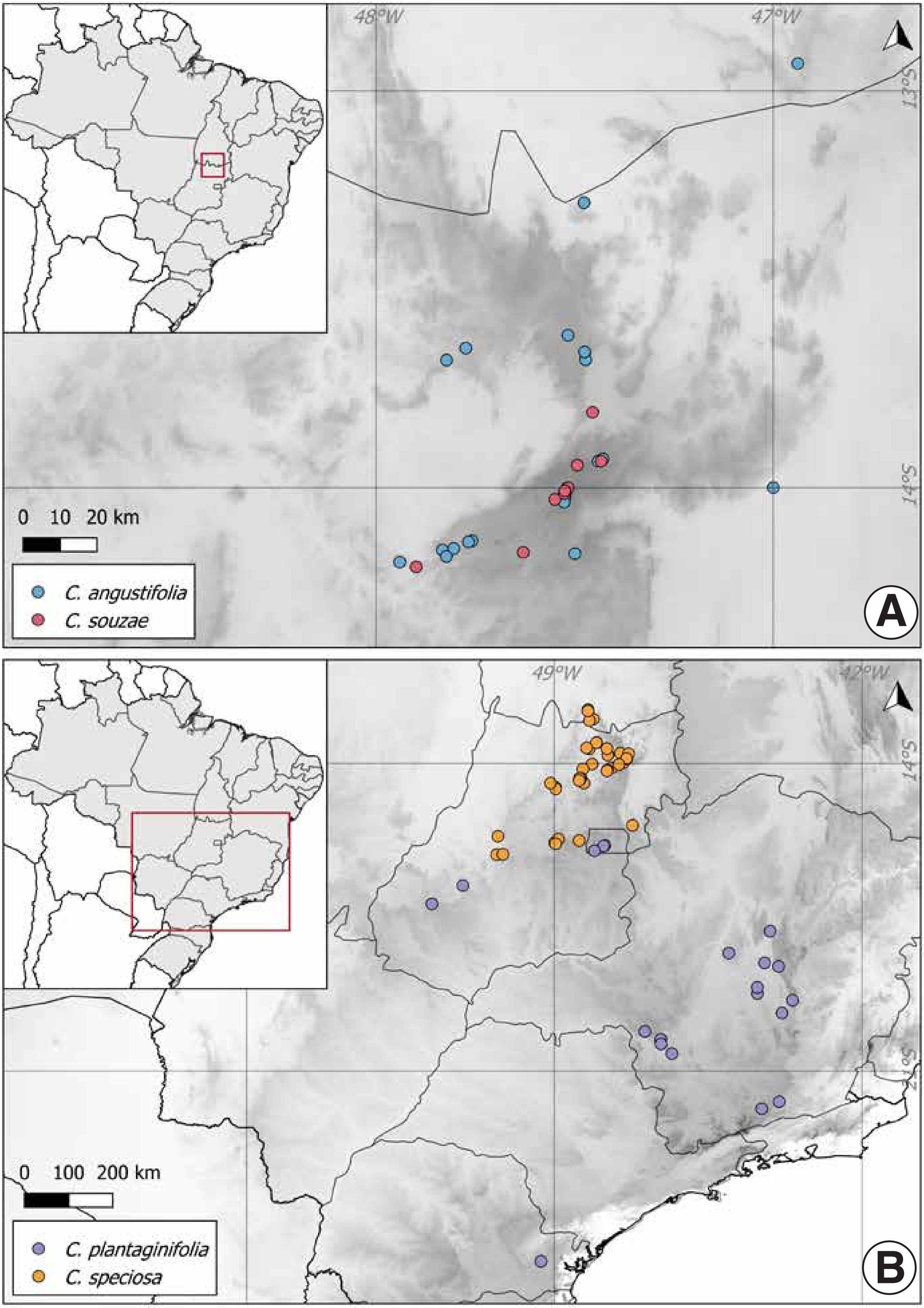

Figure 18. Distribution map of species from Cerrado clade 1. A. *Chresta angustifolia, C. souzae*. B. *C. plantaginifolia, C. speciosa.*

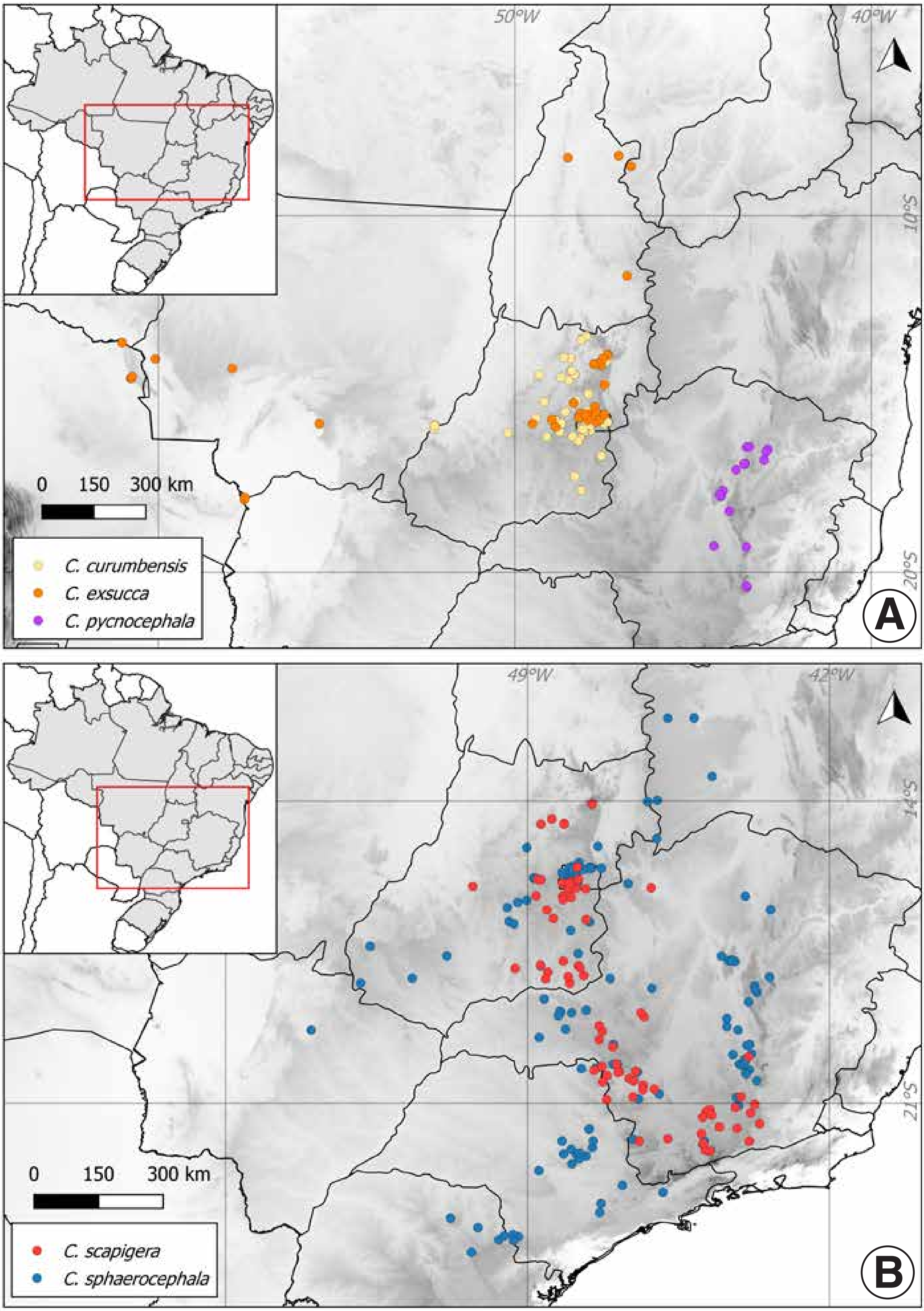

Figure 19. Distribution map of species from Cerrado clade 2. A. *Chresta curumbensis, C. exsucca, C. pycnocephala*. B. *C. scapigera, C. sphaerocephala*.

Chapter 4

New species of *Chresta* Vell. ex DC. (Vernonieae, Asteraceae)

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Chapter 4
Part A

A new species of *Chresta* (Vernonieae, Asteraceae) endemic to the Mata Atlântica Domain, Brazil

Co-authored by: Benoit Loeuille and José R. Pirani

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A new species of *Chresta* (Vernonieae, Asteraceae) endemic to the Mata Atlântica Domain, Brazil

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Abstract

Chresta filicifolia is described and illustrated. It is a perennial herb known only from open vegetation on top of small rock outcrops at the Rio Doce Valley in Eastern Minas Gerais, Southeastern Brazil. This is the first record of a species of *Chresta* endemic to a phytogeographic domain other than the *Cerrado* and *Caatinga*, since those outcrops are included in the *Mata Atlântica* Domain (Atlantic Forest). The new species is closely related to *C. harleyi* and *C. hatschbachii,* but differs from these by its leaves, which are larger and more deeply lobed than those of the other two species, and also by its florets and cypselae, which are both larger in *Chresta filicifolia.* Moreover, the two other species are allopatric from *C. filicifolia*, being restricted to the *Caatinga* Domain. The southernmost distribution of *C. harleyi* lies more than 500 km away from the locality where the new species is found, making this discovery unexpected, and raising interesting biogeographical questions, which are briefly addressed here.

Resumo

Chresta filicifolia é descrita e ilustrada. Trata-se de uma erva perene conhecida apenas de áreas campestres sobre alguns afloramentos rochosos do Vale do Rio Doce, Leste de Minas Gerais, Sudeste do Brasil. Este é o primeiro registro de uma espécie de *Chresta* endêmica de um domínio fitogeográfico que não o Cerrado e a Caatinga, uma vez que seu habitat está inserido no Domínio da Mata Atlântica. A espécie é proximamente relacionada a *C. harleyi* e *C. hatschbachii,* mas difere dessas por ter folhas maiores e mais profundamente lobadas e flores e cipselas maiores. Além disso, as duas outras espécies têm distribuição alopátrica à da *C. filicifolia*, pois são restritas ao Domínio das Caatingas. O limite meridional conhecido da distribuição de *C. harleyi* situa-se a mais de 500 km a norte da área de ocorrência da nova espécie, o que torna esta descoberta ainda mais surpreendente e desperta questões biogeográficas relevantes, que são brevemente abordadas neste trabalho.

Key words: Compositae, Chrestinae, Cerrado, rock outcrops, *campo rupestre*, endemism, Atlantic Forest Domain, Rio Doce

Introduction

Chresta Velloso ex Candolle (1836: 85) is a genus of the tribe Vernonieae, with 14 species currently accepted (Dematteis & Siniscalchi 2014). Although there is a taxonomic revision (MacLeish 1985), this work considers *Chresta* as being a small genus, with only four species, and splits the remaining species into another three genera, namely *Argyrovernonia* MacLeish (1984: 106)*, Glaziovianthus* Barroso (1947: 113–115) and *Pycnocephalum* (Lessing 1831: 630) Candolle (1836: 83). Robinson (1999) proposed that all species should be treated under *Chresta,* based on morphological and chemical affinities. A recent phylogenetic analysis of the American Vernonieae (Loeuille *et al*. 2015) has confirmed the monophyly of *Chresta sensu lato* as considered nowadays.

Species of *Chresta* are herbs or subshrubs, rosulate or caulescent, with simple or T-shaped hairs, alternate leaves, with entire or lobed margins. The capitula are aggregated into syncephalia, second-order inflorescences, which can vary in number of capitula per syncephalia and in number of florets per capitula. Syncephalia in the genus are spherical, with closely adjoined capitula, or hemispherical, forming a looser aggregation. Following the common pattern in Vernonieae, corollas are usually purple to lilac, with a white throat or a white band in the apical portion of the corolla

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tube and base of petals; nevertheless, two red-flowered species are known in the genus, *Chresta curumbensis* (Philipson 1938: 298) Robinson (1980: 91) and *C. speciosa* (Gardner 1842: 240).

Chresta is mostly endemic to Brazil, with only one species, *Chresta exsucca* Candolle (1836: 85), also occurring in Bolivia. Eight species are restricted to the Brazilian *Cerrado,* with differential distribution among its phytophysiognomies, such as *campos rupestres* (literally rocky fields), *cerrado s.s.* (savanna woodlands), woodlands and others. Two of the most widespread species of the genus, *Chresta scapigera* (Lessing 1829: 250–251) Gardner (1842: 241) and *Chresta sphaerocephala* Candolle (1836: 85), occur in the *Cerrado* and also in the *Mata Atlântica* Domain [Atlantic Forest], mainly in areas covered by *cerrado* vegetation or that suffered anthropogenous action. The remaining four species are mostly restricted to rock outcrops and their surroundings in the Brazilian *Caatinga* (Dematteis & Siniscalchi 2014).

In the course of a phylogenetic and revisionary study of the genus, we found a new species from Eastern Minas Gerais, in the Rio Doce valley, a rather unexpected location for the genus for several reasons, specially its geological formation, its vegetation type and its position outside the *Cerrado* and *Caatinga* Domains, where the genus is known to be restricted to so far.

The Rio Doce Valley is included in the *Mata Atlântica* phytogeographic domain and despite having a mostly sedimentary cover, there are several granitic and quartzitic formations (Nalini Júnior *et al*. 2005). This proposed new species was found in the Sete Salões State Park, which covers two quartzitic ridges, Serra da Onça and Serra do Boiadeiro, some carstic caves—from which the park's name derives—and tributaries of Rio Doce (Baeta & Mattos 2007). Hardly any botanical collections have been made in the park area since its establishment in 1998, and its floristic composition remains largely unknown. Its location, on the middle course of Rio Doce, and its vegetation, combining semideciduous forest remnants and open formations on top of quartzitic rock outcrops, make this area interesting for botanical studies. Particularly, this local rocky habitat seems to be one of the easternmost patches of *campo rupestre* or *cerrado*, away from the *Cerrado* Domain, and the presence there of a species belonging to plant genera mostly restricted to the *Cerrado* and *Caatinga* is certainly of very special interest. Besides describing the new species, and comparing it to two closely related species, we here also aim to address the bearing of its restricted occurrence for some phytogeographic controversies, namely those discussed by authors such as Alves *et al.* (2007) and Vasconcelos (2011).

Material & Methods

Morphological features of the specimens were analyzed with a 10–60 × magnification stereomicroscope. Measurements of only fully mature structures were made using a digital caliper rule and optical graticule attached to the microscope. Parts of the corolla lobes, style arms, cypsela and carpopodium were mounted on a microscope slide with Hoyer's solution (Anderson 1954) for microcharacter analysis, which was performed in a Leica DM 4000B microscope. Photomicrographs were taken with a Leica DFC 425 camera. Terminology follows Hickey (1973) for leaf shape and Harris & Harris (2001) for general morphology.

Assessment of conservation status was performed using the GeoCAT Tool (Bachman *et al*. 2011), with the IUCN default for Area of Occupancy (AOO) analysis. The distribution map was produced in QGIS version 2.6.1 (Quantum GIS Development Team 2012). Geographic coordinates came from herbarium specimens; specimens lacking exact reference of the location received the coordinate relative to the municipality of collection.

Chresta filicifolia Siniscalchi & Loeuille, **sp. nov.** (Fig. 1)

Species Chrestae harleyi *et item* C. hatschbachii *simile sed foliis longioribus latioribusque profunde lobatis, flosculis majoribus, cypselis longioribus et carpopodiis conspicuis asymmetricis differt.*

Type:—BRASIL. Minas Gerais, Conselheiro Pena, Parque Estadual de Sete Salões, trilha para o Córrego da Onça, afloramentos rochosos quartzíticos em área de floresta estacional semidecidual, 513 m, 19º15'03"S; 41º22'29" W, 5 August 2014, *C.M. Siniscalchi, B. Loeuille & C.T. Oliveira 512* (holotype SPF!; isotypes to be distributed to K!, MBM!, R!, RB!, SP!, US!).

Description:—Perennial herb from a stout branched woody rootstock, up to 1.0 m, base branched; stems 6–23 cm tall, striate, brown-yellowish, with conspicuous leaf scars. Leaves forming a rosette at top of stem, simple, petiolate, petiole

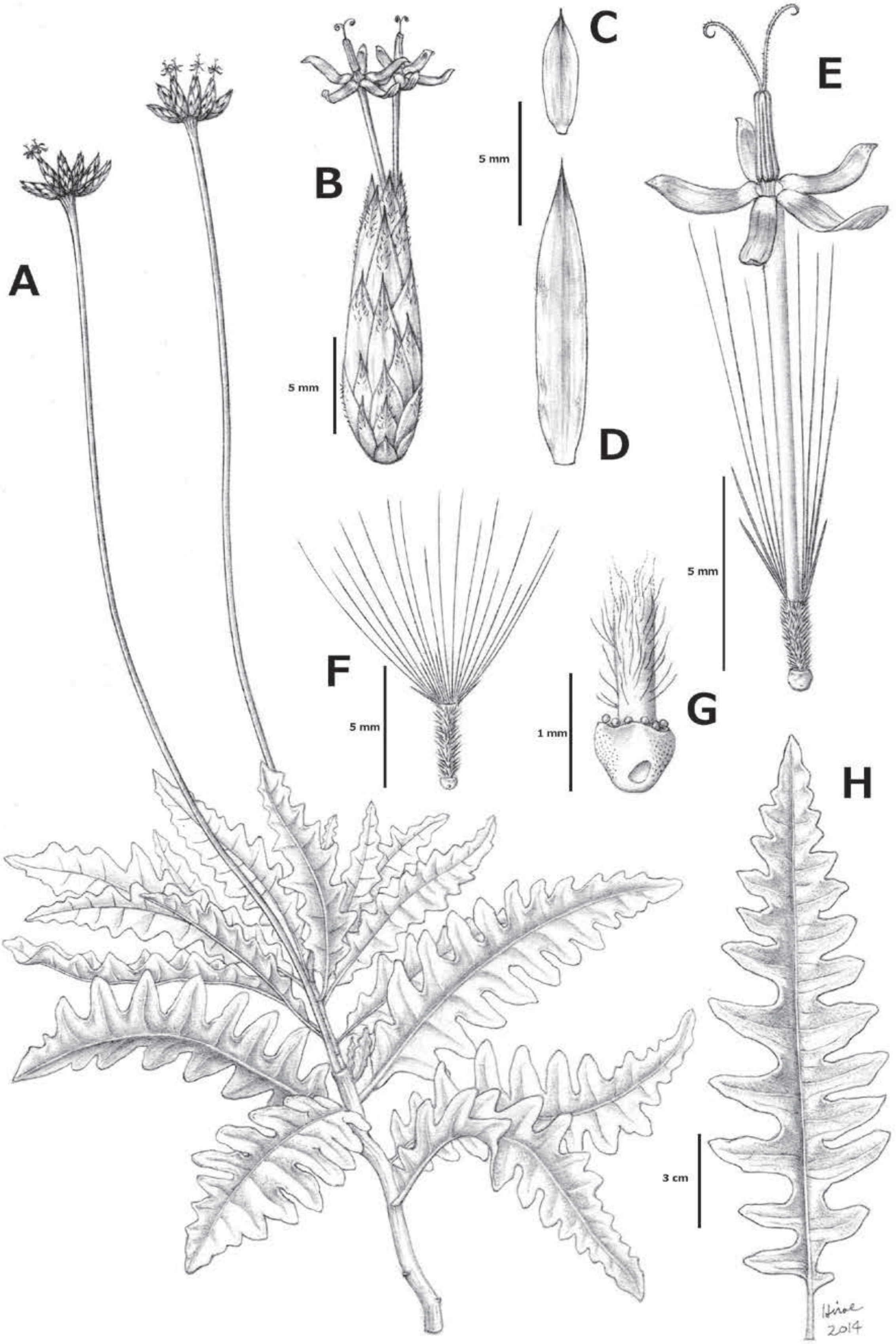

FIGURE 1. *Chresta filicifolia.* A. Habit. B. Capitulum with open corollas. C. Outer phyllary. D. Inner phyllary. E. Open corolla, with style arms not totally exerted. F. Cypsela. G. Detail of carpopodium. H. Adaxial surface of leaf. Drawing by Hiroe Sasaki.

0.4–2.1 cm long, densely whitish tomentose, hairs T-shaped, blade 4.2–19.1 × 1.8–4.4 cm, pinatilobate to pinatifid, margins with 4 to 8 oblong to deltoid lobes on each side, sinuses reaching from midway to midvein, upper blade shallowly lobed with dissimilar lobes, apex acute, base attenuate, membranaceous, upper surface grayish to green (brown when dried), glandular-punctate, pubescent, hairs appressed, T-shaped, lower surface glandular-punctate, densely sericeous, hairs appressed, T-shaped, midrib prominent beneath, with denser pubescence above, venation pinnate, lateral veins ascending, slightly conspicuous. Inflorescence scapose, 21.1–40.4 cm long, canescent, hairs T-shaped; syncephalia solitary, 2.1–1.9 cm tall, 2.4–3.9 cm diam., hemispherical, with capitula slightly adpressed at base; secondary bracts 1.3–2.0 mm, linear, villous. Capitula 8–22 per syncephalium, homogamous, discoid, sessile; involucre cylindrical, 14.5–16 × 3.4–3.5 mm; phyllaries 5- or 6-seriate, outer phyllaries narrowly elliptic to very narrowly elliptic, 3.6–6.0 × 1.1–1.3 mm, apex aristate, pilose, stramineous with purple apical portion, margins membranaceous, inner phyllaries narrowly oblong to linear, 11.5–14.9 ×1.2–2.0 mm, apex aristate, glabrous except pilose apical portion, stramineous, apical portion purple, margins membranaceous; receptacle flat, glabrous, slightly fimbrillate. Florets 8, bisexual, fertile; corollas actinomorphic, 5-lobed, tube 8.0–9.0 × 0.35–0.5 mm, glandular-punctate, lilac, throat 0.4–2.4 × 0.4–0.6 mm, white, lobes 3.5–3.7 × 0.8 mm, spreading, lilac, whitish at base, apex acute, glandular, stipitate glands, margins thick; apical anther appendages obtuse, cell walls not thickened, basal anther appendages auriculate; style 13.5–14.0 mm, style shaft glabrous throughout except for pubescent distal portion (ca. 0.3 mm beneath style arms), style base glabrous, lacking basal node, with nectariferous disc 0.7–0.8 mm long, style arms 3.8–4.0 mm long, short-pubescent outside throughout, hairs lageniform. Cypselae 3.0–4.5 × 0.9–1.2 mm, ca. 20-ribbed, slightly obconic, densely sericeous, twin-hairs with cells fused to near tip, glandular-punctate; carpopodium 0.3–0.5 mm long, 0.6–0.7 mm diam., conspicuous, asymmetrical, yellowish, glandular towards apex; pappus triseriate, white to stramineous, straight, serrulate, persistent, outer series 0.81–0.87 mm long, linear, paleaceous, median series 1.5–5 mm long, lanceolate, paleaceous, inner series 10.1–12.0 mm long, setose, apices slightly tapering.

Distribution and habitat:—*Chresta filicifolia* is known from two records from open vegetation on top of quartzitic outcrops surrounded by *cerrado* and semideciduous forest in Eastern Minas Gerais, southeastern Brazil. All collections were made inside the Sete Salões State Park, on Serra das Onças, at elevations between 400 and 600 m (Fig. 2).

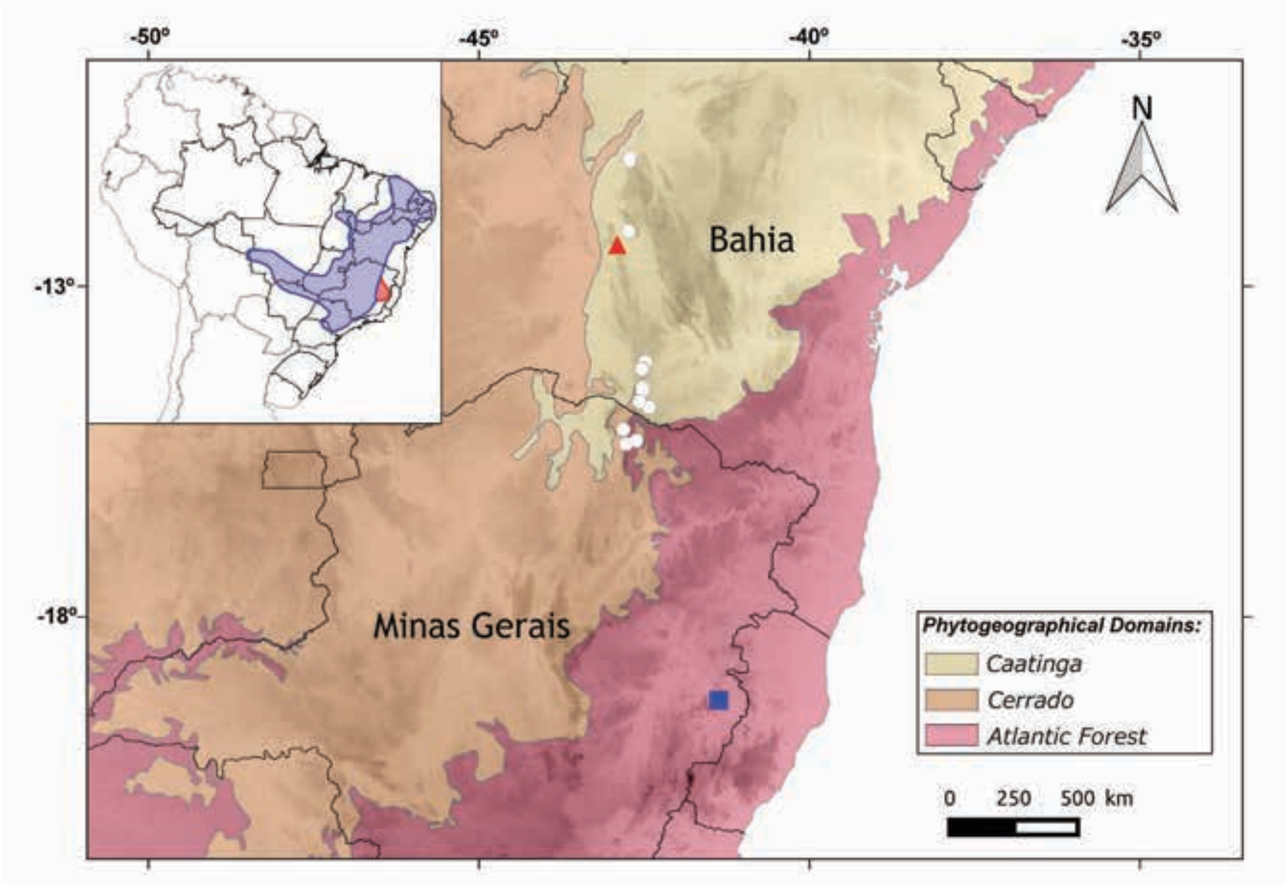

FIGURE 2. Currently known distribution of *Chresta filicifolia* and its closest taxa (blue square: *C. filicifolia;* white circles: *C. harleyi;* red triangle: *C. hatschbachii)*. Upper right corner: previously known distribution of *Chresta* (blue shading), with expansion represented by the new species (red solid shading).

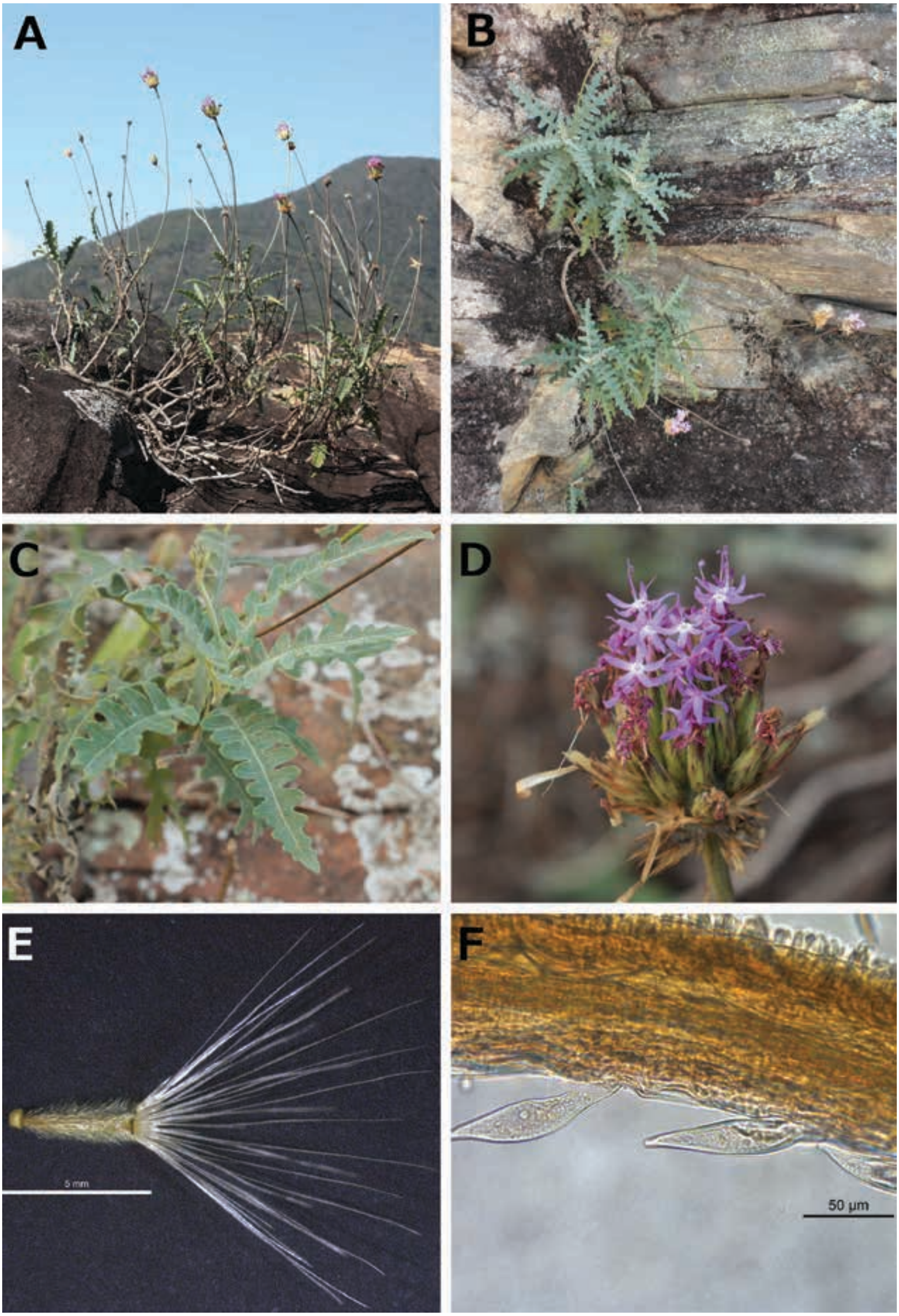

FIGURE 3. Morphology of *Chresta filicifolia.* A, B. Habit. C. Detail of leaves. D. Inflorescence. E. Cypsela. F. Detail of lageniform hairs on the outside of style arms. All pictures by the first author.

Flowering and Fruiting:—Flowering and fruiting specimens were found in May and August (Fig. 3).

Conservation status:—According to results obtained through GeoCAT analysis (EOO = 0 km^2; AOO = 4 km^2; using a cell size of 2 km^2), the species is classified as Critically Endangered. Nevertheless, the only two known collections of the species may not allow a confident evaluation of its conservation status. Thus, we suggest this species should be classified as Data Deficient (DD).

Etymology:—The epithet *filicifolia* refers to the leaves of this species, which are deeply lobed and reminiscent of those of a fern (Fig. 1 H, 3 C).

Additional collection examined (paratypes):—BRAZIL. Minas Gerais: Conselheiro Pena, Parque Estadual de Sete Salões, área com Floresta Estacional Semidecidual e com palmeira (*Attalea* sp.) e cactus, nas margens do córrego da Onça, além de afloramentos rochosos no entorno, 19º15’09.4”S; 041º23’37.1”W, 7 May 2006, *A. Salino, T.E. Almeida & G. Heringer 10883* (BHCB!, RB!).

Discussion

Chresta filicifolia is related to *C. harleyi* Robinson (1983: 385) and *C. hatschbachii* Robinson (2005: 83–84), differing from those by longer, larger and more deeply lobed leaves, and also by its larger florets and larger cypselae. In overall size, habit and habitat, the three species are quite similar; they are all perennial, erect herbs, of ca. 1 m tall, growing on quartzite rock outcrops. The distribution of the leaves on the stem varies among them: while in *C. hatschbachii* they are distributed throughout the stem, in *C. harleyi* and *C. filicifolia* they are mostly grouped at the apex of the stem, sometimes appearing rosettiform. The leaves show great variation in size, color, petiole size and lobe shape among the three taxa (Fig. 4 A, B, C). Although the leaf size overlaps among them, in general *C. filicifolia* has the longest leaves, from 4 to 19 cm, which in addition present a distinct brown color when dry, not usually seen on the other two species. Leaf lobes are the most distinctive characteristic: in *C. harleyi* they are triangular, with acute apices and with sinuses not reaching the midrib, while in *C. hatschbachii* and *C. filicifolia* the lobes are usually oblong or widely deltoid, with round apices, deeply reaching the midrib (Table 1).

TABLE 1. Comparison of key characters of *Chresta filicifolia*, *C. harleyi* and *C. hatschbachii*. Source material: *C. harleyi*—Siniscalchi *et al.* 459, 460, 462, 463, 464 (SPF); *C. hatschbachii*—Siniscalchi *et al.* 468 (SPF), Hatschbach 67804 (HEPH, HUEFS, SPF).

Character	*C. filicifolia*	*C. harleyi*	*C. hatschbachii*
Leaf size (length × width, cm)	4.2–19.1 × 1.8–4.4	4.5–12 × 0.6–1.9	2–7 × 0.9–1.7
Leaf lobe shape	deltoid, round apices	triangular, deltoid, acute apices	oblong, round apices
Petiole length (cm)	0.4–2.1	0.2–1.0	leaves sessile or petiole to 0.5
Inflorescence length (cm)	21.1–40.4	12–38	1–4.5
Number of capitula per syncephalium	8–22	10–30	7–14
Number of florets per capitulum	8	7 or 8	5 or 6
Cypsela length (mm)	3.0–4.5	2.6–3.5	2–2.8
Pappus color	white to pale stramineous	stramineous	white
Carpopodium size (length × diam., mm)	0.3–0.5 × 0.6–0.7	0.15 × 0.5	0.15 × 0.4

Although the inflorescences seem to be quite similar among the three species, their length is distinctive: *C. filicifolia* has the longest, from 20 to 40 cm long, and *C. hatschbachii* the shortest, up to 4 cm long. The cypselae show some variation in size, with *C. filicifolia* presenting the largest ones, and in the carpopodium, which is three to four times longer in *C. filicifolia* than in the two other species (Fig. 4 D, E, F).

The three species also display contrasting geographic distributions. *Chresta harleyi* is found from Northern Minas Gerais into central Bahia, over hills known as Serra Geral, which are a sector of the Espinhaço Range included in the *Caatinga* phytogeographic Domain. *Chresta hatschbachii* is known from only one location in Central Bahia, in an area included within *C. harleyi*’s distribution. Thus, while both former species are found only in the *Caatinga* Domain, especially in Bahia, *C. filicifolia* is known from Eastern Minas Gerais, completely allopatric from them. Additionally, it should be pointed that the southernmost distribution range of *C. harleyi* lies more than 500 km away from the locality where the new species is found (Fig. 2). This fact raises a question on how these closely related species came to achieve such a large disjunction gap. Phylogenetic and phylogeographic studies which are underway by the authors will certainly help understanding the processes underlying this intriguing geographic pattern.

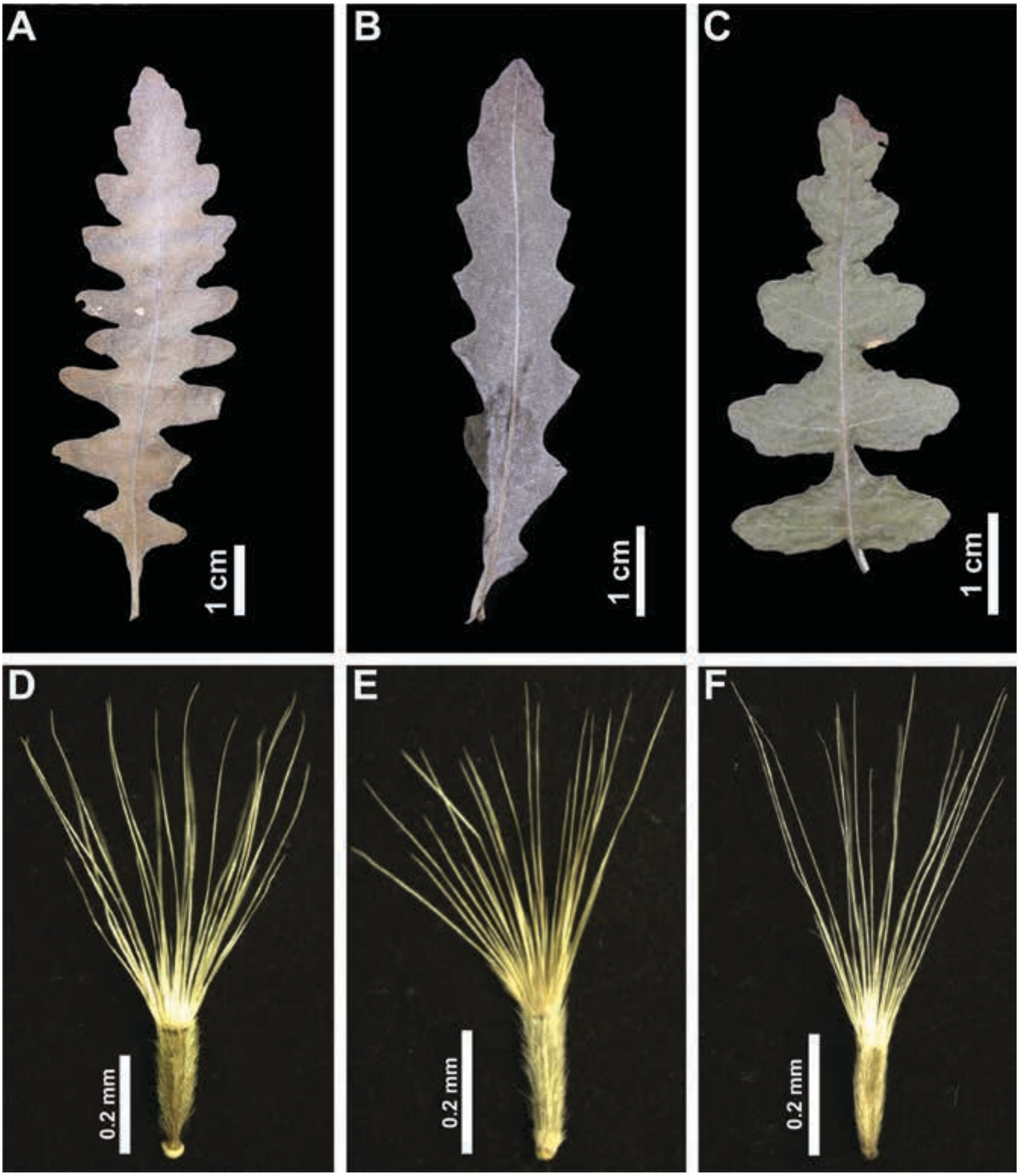

FIGURE 4. *Chresta filicifolia.* A. Leaf. D. Cypsela; *C. harleyi*. B. Leaf. E. Cypsela; *C. hatschbachii.* C. Leaf, F. Cypsela.

The occurrence of *C. filicifolia* on quartzitic outcrops in the *Mata Atlântica* Domain may also have a bearing on the recognition of these localities as disjunct, easternmost open sandy or rocky habitats where some *cerrado* and *campo rupestre* species may occur. It is known that the quartzitic substrate under these open formations (as well as in the Sete Salões area) sharply contrasts with the granitic-gneissic bedrock of the mountains of the *Mata Atlântica* Domain, covered by vegetation known as *campos de altitude* (e.g. Safford 2007, Vasconcelos 2011). These *campos de altitude* occur at elevations of 1800–2000 m, in a cool-humid climate (Safford 2007). Even though the phytophysiognomy and floristic assemblage of the much lower Sete Salões Park outcrops are yet to be studied, we may suggest the importance of the evidence provided by this new endemic *Chresta.* The presence in that low, rocky, open area of a member of a genus mostly endemic to the *Caatinga* and *Cerrado* may probably stand as a botanical evidence supporting the proposal of vegetation classification presented in the beginning of this discussion. Additionally, its presence in a "*cerrado* island" area lying in a region mostly covered by forests (*Mata Atlântica* Domain) raises the fundamental biogeographic questions: is such a disjunct pattern likely to be the result of an old long-distance dispersal event followed by allopatric speciation? Or is it the result of a vicariant event taking place after an expansion of the *Cerrado* area towards the east during past dry, cooler climate periods? This latter scenario is supported by paleoecological evidence that since the early Holocene up to 5,500 years A.P. *campo cerrado* dominated on areas in eastern Minas Gerais which are nowadays covered by semideciduous forests (e.g. Behling 2002, de Oliveira *et al.* 2005).

Thus, biogeographical studies in *Chresta* and other floristic elements from that region in Minas Gerais may contribute with valuable data for the reconstruction of the history of the dynamics (expansions and contractions) that several plant formations in the *Mata Atlântica* and *Cerrado* domains may have undergone in the Tertiary and Quaternary.

Acknowledgments

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Chapter 4
Part B

Two New Rupicolous Species of *Chresta* (Asteraceae, Vernonieae) from the Brazilian Caatinga

Co-authored by: Benoit Loeuille and José R. Pirani

Submitted to Systematic Botany

Two New Rupicolous Species of Chresta (Asteraceae, Vernonieae) from the Brazilian Caatinga

Abstract—Two new rupicolous and endangered species from the Caatinga are described and illustrated. *Chresta heteropappa* is restricted to the *brejos de altitude* (montane forests) from Northeastern Ceará, growing on granite inselbergs. *Chresta subverticillata* occurs on Northern Bahia, inhabiting quartzitic outcrops on the Chapada Diamantina mountain range. Relationships of the two new taxa to other *Chresta* species are discussed, and a key for all species occurring on the Caatinga domain is presented, as well as maps and illustrations, including field pictures.
Keywords—Compositae, Chrestinae, inselbergs, rock outcrops, Seasonally Dry Tropical Forest.

Resumo—Duas novas espécies ameaçadas da Caatinga são descritas e ilustradas. *Chresta heteropappa* é restrita aos brejos de altitude do nordeste do Ceará, crescendo sobre inselbergues graníticos. *Chresta subverticillata* ocorre no norte da Bahia, sobre afloramentos rochosos quartzíticos no maciço da Chapada Diamantina. Relações desses dois táxons com outras espécies de *Chresta* são discutidas e uma chave para as espécies que ocorrem na Caatinga é apresentada, assim com mapas e ilustrações, incluindo fotos de populações naturais.
Palavras-chave—Compositae, Chrestinae, inselbergues, afloramentos rochosos, Florestas Estacionais Semi-deciduais.

The Caatinga is very peculiar when compared to the other phytogeographic domains in Brazil: it is one of the largest semiarid regions in the continent, characterized by low and erratic rainfall and strong seasonality, accompanied by a very distinct vegetation composed mainly of drought-resistant plants, usually classified as a seasonally dry tropical forest or SDTF (Leal et al. 2005).

SDTFs are scattered throughout Central and South America, but they form a very heterogeneous group, with each different area presenting its own particular flora (DRYFLOR 2016). As a whole, they are very threatened by agricultural expansion, high human population density and conversion to pasture, with few protected areas. The Brazilian Caatinga is a stark example, with only 1.2% of its area being included in protected areas and with less than 10% of its original area remaining unchanged (Leal et al. 2005, Santos et al. 2011).

The Caatinga is a heterogeneous domain, with at least eleven different community types (Moro et al. 2014), besides transitional areas where the flora intermixes with those from the neighboring domains, the Cerrado and Atlantic Forest. Most of these physiognomies are marked by the presence of several woody Leguminosae, Euphorbiaceae and Cactaceae, besides other families traditionally associated with drier environments (Fiaschi and Pirani 2009). In sharp contrast with the Cerrado, another domain with strong seasonality, where the presence of Asteraceae is remarkable, plants from this family are more sparsely distributed in the Caatinga.

The Brazilian Flora Group (2015) cites Asteraceae as the third most diverse family in the Caatinga, with 284 species, while the catalogue by Moro et al. (2014) mentions 67 species of Asteraceae. This large difference is mainly because BFG considers taxa that occur in *campos rupestres* (highland rocky fields), which are restricted to a portion of the Caatinga, as occurring in this phytogeographical domain, while Moro et al. excluded this transitional physiognomy from their analysis, as it is usually considered as belonging to the Cerrado domain. In fact, from the 284 Caatinga species listed in the Brazilian checklist, 159 are listed as occurring in *campos rupestres* and 44 are listed as occurring in caatinga stricto sensu (BFG 2015).

Most of the *campos rupestres* in the Caatinga are concentrated in Chapada Diamantina and Serra Geral, from Northern Minas Gerais to Bahia, resulting in a high concentration of species in a relatively small area. Floristic surveys in the Chapada Diamantina region, like those from Pico das Almas (Harley 1995), Catolés (Zappi et al. 2003) and Mucugê (Harley and Simmons 1986), all have Compositae as one of the richest families. However, other Caatinga environments are less known and it is unclear how Compositae figures in these other formations.

One of the less explored physiognomies in the Caatinga is the inselberg and rock outcrop flora. Moro et al. (2014) list ten works that focused on the flora in these environments, most of them targeting the granitic inselbergs that are abundant throughout this domain. Studies on the flora of quartzitic outcrops are even rarer, as those are usually smaller and scattered over wider areas; our bibliographical survey revealed only one study that explicitly mentions quartzitic formations (Campos et al. 2016).

Within the Caatinga domain, *Chresta* Vell. ex DC. is one of the Asteraceae genera that is found outside the *campos rupestres*, although it is strongly associated to rocky environments. From the four species of *Chresta* found in the Caatinga, three are associated to quartzitic outcrops and one is usually found on granitic inselbergs, all growing directly on top of the rocks or in pockets of plant matter found in small cracks and crevices.

Chresta harleyi H.Rob and *C. hatschbachii* H.Rob. are restricted to the montane area known as Espinhaço Septentrional, a sliver of mountain ranges formed of meta-volcanic and meta-sedimentary rocks, with an abundance of quartz and other mineral deposits, that connects the bulk of the southern Espinhaço Range on Minas Gerais to the northern Chapada Diamantina on Bahia (Knauer 2007, Saadi 1995).Those two species are found on quartzitic rock outcrops, usually in a type of vegetation known as *caatinga rupestre* or rocky caatinga, a transitional vegetation between Cerrado *s. str*. and Caatinga (Campos et al. 2017).

Chresta martii (DC.) H.Rob. follows an East to West distribution pattern, being found in northern Bahia, Sergipe and Alagoas states, on the Xingó region and also in the states of Paraíba, Pernambuco, Piauí and Rio Grande do Norte. While it is usually found on quartzitic outcrops or crests that emerge over hills, in Paraíba and Rio Grande do Norte the species also occurs on crystalline outcrops (pers. obs., Lucena et al. 2015).

Chresta pacourinoides (Mart. ex DC.) Siniscalchi & Loeuille, on the other hand, is found mainly on granitic inselbergs or outcrops. Besides being associated to this particular type of rock formation, it also seems related to the Atlantic Forests remnants found on the Caatinga domain, known as "*brejos de altitude*", usually found at higher elevations and under more humid conditions. Differently from other *Chresta,* this species is an annual or biannual herb, drying up after the flowering season. Nevertheless, individuals may reach impressive size, sometimes attaining 2 m in height, especially when growing on the base of inselbergs or in deep pockets of soil upon rocks.

We are aware of 17 floristic studies in the Caatinga that cite an occurrence of *Chresta* (Table 1). The most cited species is *Chresta pacourinoides,* reflecting the larger number of studies carried out in crystalline formations, also, it is worth noting that some of the studies did not specify the type of environment in which each species was observed or collected.

In most studies Asteraceae contributes with 5% to 6% to the total of collected species, and in two studies it contributes with more than 10% of the species, one of these being a study carried out in a quartzitic area, an environment that usually presents higher number of Asteraceae. Three studies dealing exclusively with the family show a much higher number of species than in the general ones, possibly as a reflection of focused collection efforts.

During herbarium and field work towards a taxonomic revision of *Chresta*, two new taxa came to our attention. The first is known from several specimens that have been repeatedly collected along the last decades, but which have always been identified as *C. harleyi.* The other species is known only by a few, recently collected specimens, found in small herbaria. These two species are herein described and illustrated, and their relationships are discussed. Maps and photographs are also presented, along with a key for all species of *Chresta* that occur in the Caatinga.

MATERIAL AND METHODS

Morphological characters of the specimens were analyzed with a 10-60 × magnification stereomicroscope. Mature structures were measured using a digital caliper rule and optical ruler attached to the microscope. Parts of corolla lobes, style arms, cypsela and carpopodium were mounted in a microscope slide with Hoyer's solution

(Anderson 1954), for analysis of microcharacters, which was performed in a Leica DM 4000B microscope. Photomicrographs were taken with a Leica DFC 425 camera. Terminology follows Hickey (1973) for leaf shape, Endress (2010) for inflorescence morphology and Harris and Harris (2001) for general morphology. Pollen classification was based on Siniscalchi et al. (2017) and pappus elements were classified according to Small (1919).

Assessment of conservation status was performed using the GeoCAT Tool (Bachman et al. 2011) for Area of Occupancy (AOO) and Extent of Occurrence (EOO) analysis, using the IUCN default of 2 km. Conservation status was further evaluated using the IUCN manual (IUCN 2012). Distribution maps were produced in QGIS version 2.18 (Quantum GIS Development Team 2012). Geographic coordinates came from herbarium specimens; specimens lacking exact reference of the location received the coordinate relative to the municipality of collection.

Taxonomic Treatment

Chresta heteropappa Siniscalchi & Loeuille, sp. nov.—Type: Brazil. Ceará: Uruburetama, Estrada do centro de Uruburetama para bairro da Água Sumida, 03°37’44.01”S, 39°31’45.02”W, 209 m, 13 August 2015, *C.M. Siniscalchi & A. Frazão 614* (Holotype: SPF!, isotypes to be distributed to: K!, RB!, US!).

Species Chrestae pacourinoidi *simile, sed syncephalis tenuioribus, stylis brevioribus (ca. 5.5 mm, non ca. 10 mm) et pappi seriebus exterioribus paleaceis-setaceis (non barbellatis-setaceis) differt.*

Herb, non-ramified at the base, up to 2 m, originating from a ramified, slender underground system; stem striate, green, turning yellowish when dry, minutely pilose with T-shaped hairs, abundant glandular trichomes and sparse setae composed by 8 to 10 cells. Leaves alternate, caulinar, simple, sessile; blade 6–34.5 × (2.9)5.5–17.8 cm, pinnatilobate to pinnatisect, light green, membranaceous, few T-shaped hairs on the adaxial side, with glandular trichomes and simple, multicellular conical trichomes on the margins, pubescent with very small T-shaped hairs (less than 0.2 mm tall) and abundant globose glandular trichomes on the abaxial side, midrib prominent adaxially, pilose and with multicellular setae, canaliculate abaxially, venation semicraspedrodomous, margins biserrate, apex acute, base auriculate. Inflorescence panicle of syncephalia, minutely pilose with T-shaped hairs, scape slighlty engorged beneath the syncephalium. Syncephalia 0.8–2.4 cm tall, 1.4–2.6 cm diam., spherical, with tightly joined capitula; secondary bracts 4–5 mm, subulate, apex acuminate, pubescent to villose, with T-shaped hairs and small, yellow glandular trichomes. Capitula 10-80 per syncephalium, homogamous, discoid, sessile; involucre cylindrical; phyllaries 4- or 5-seriate, outer phyllaries narrowly subulate, 4–5 mm, apex aristate, pubescent with a high concentration of glandular trichomes on the median part of the blade, stramineous, margins membranaceous, ciliate, mid phyllaries elliptical, 5–8 mm, apex acuminate, pubescent, with glandular trichomes, stramineous with a dark spot on the bottom half, margins membranaceous, ciliate, inner phyllaries narrowly elliptical, 9–10 mm, apex acute to acuminate, pubescent with predominance of glandular trichomes, stramineous, sometimes with a dark spot, margins membranaceous, ciliate; receptacle flat, glabrous. Florets 4, bisexual, fertile; corollas actinomorphic, 5-lobed, tube 2.8–3.0 mm, without glands, lilac, throat 0.25–0.75 mm, white, lobes 2–2.5 × 0.4–0.5 mm, spreading, lilac, whitish at base, apex rounded to acute, with globular glands and simple trichomes concentrated at the apex, margins not thickened; apical anther appendages acute, cell walls not thickened, basal anther appendages rounded; style ca. 5.5 mm, style shaft glabrous, except for ca. 0.3 mm beneath the arms, style base glabrous, lacking basal node, with nectariferous disc 0.4 mm long, style arms 1.5 mm long, with 1–1.25 mm exposed above the anthers, pubescent outside throughout, hairs lageniform. Pollen grains ca. 50 μm diam., sub-spheroidal, echinolophate, *Chresta*-type II. Cypselae in two types, type 1: 1.9–2.3 mm × 0.7–0.8 mm, light brown, ca. 20-ribbed, cylindrical to turbinate, pubescent, with very short, rigid twin-hairs with cells fused up to 2/3 of the length; carpopodium 0.1 mm long × 0.5 mm diam., symmetrical, whitish;

pappus biseriate, whitish, straight, barbelate, outer series persistent, 0.4–0.5 mm long, paleaceo-setose, inner series deciduous, 7–8 mm long, barbelate-setose; type 2: 2.2–2.4 mm × 0.7–0.9 mm, dark brown, ca. 20-ribbed, turbinate, glabrescent to glabrous; carpopodium highly reduced, whitish; pappus biseriate, whitish, straight, barbelate, outer series persistent, 0.5–1.5 mm long, paleaceo-setose, bristles fused together up to 2/3 of the length, inner series caducous, 6–7 mm long, barbelate-setose. Figures 1, 2.

Distribution and habitat—*Chresta heteropappa* seems to be restricted to the Northern portion of state of Ceará, in two crystalline massifs, Maciço de Uruburetama and Serra de Maranguape (Fig. 3), both belonging to the same geological domain, composed by ancient shields and massifs (Souza and Oliveira 2006, Lima et al. 2015). These massifs are considered Atlantic Forest enclaves in the Caatinga domain, presenting very different climatic and vegetation conditions than those found on the surrounding areas; both areas have a rainy season that lasts from January to June/July, with total annual precipitation above 1000 mm and were originally covered by Pluvio-Nebular Tropical Subperennial Forest. *C. heteropappa* grows over exposed rocks on pockets of soil or around rock outcrops, usually close to denser forest-like vegetation.

Etymology—The name of the species comes from the characteristics of the outer pappus, which is different from all other *Chresta* species, being composed by paleaceous-setose elements.

Flowering and fruiting—Specimens with fruits and flowers were found in July and August, nevertheless, due to the temporal nature of syncephalia maturation, the flowering and fruiting can extend for several months, as seen in other *Chresta* species.

Conservation status—*Chresta heteropappa* has an EOO of 187.165 km^2 and AOO of 12.000 km^2, resulting in a classification as endangered in criteria B1 and B2. There are less than five known populations and both areas where the species is found are used for agricultural purposes, specially cultivation of bananas done without conservation concerns, with large portions of these massifs already presenting degraded vegetation (Souza and Oliveira 2006), what indicates an expect decline in habitat quality, possibly leading to lost populations. Thus, the full conservation status is EN B1ab(iii,iv)+ B2ab(iii,iv).

Additional specimens examined—BRAZIL: Ceará: Uruburetama, Estrada do centro de Uruburetama para bairro da Água Sumida, 03°37’48.50”S, 39°31’59.93”W, 220 m, 13 Aug 2015, *C.M. Siniscalchi & A. Frazão 615* (SPF); Maranguape, Serra de Maranguape, Pico da Rajada, 13 Jul 2017, *M.L. Bazante, N.K. Luna & L.J. Leitão 892* (UFP); Itapipoca, Maciço de Uruburetama, Pico de Itacoatiara, área de floresta subperenifólia tropical plúvio-nebular (mata úmida) com afloramentos graníticos e ilhas de vegetação, 03°34’27”S, 39°35’22”W, 870 m, 17 Jul 2017, *F.D.S. Santos 560* (HUVA, UFP).

Taxonomy notes—*Chresta heteropappa* is closely related to *C. pacourinoides*, being very similar morphologically and in their environmental requirements. The mains characteristics distinguishing both species are the robustness of the synflorescence, the size of the syncephalia, the style length and how much of it is extended above the anthers (Fig. 2D,E), as well as the distinct types of cypsela and pappus (Table 2).

Style length and type of pappus elements are the stronger feature separating both species. *Chresta heteropappa* follows the more common pattern seen in the whole genus, where only a small portion of the style is projected above the anthers, while *C. pacourinoides* has a long portion (almost up to 1 cm) of the style projected (Fig. 2D,E). Curiously, the other two *Chresta* species that present a projection like this are *C. curumbensis* and *C. speciosa,* both presenting red flowers and being pollinated by hummingbirds (Siniscalchi et al. 2017). The projection of the style above the anthers is a recurrent characteristic in bird-pollinated Compositae, usually associated to larger capitula and red corollas (Vogel 2015); despite presenting purple corollas, there is evidence of hummingbird visitation on *C. pacourinoides* (Las Casas 2009). It is possible that the difference in style length causes a divergence in pollinators between the two species, contributing for their reproductive isolation.

The presence of paleaceous-setose pappus elements (Fig. 1I,J) is also a very distinctive feature, differentiating *C. heteropappa* from all other *Chresta* species, which present setose or barbelate-setose elements. The occurrence of two different types of cypsela is registered for *C. pacourinoides* as well, and there are at least three other genera in Vernonieae that present this feature (Robinson 1999). In a previous phylogenetic work (Keeley et al. 2007), *Heterocypsela andersonii* H.Rob., a rupicolous species from central Brazil that also has two types of cypsela, emerges as sister to the only *Chresta* species sampled.

Regarding the distribution of both species, *Chresta pacourinoides* has a wide distribution (Fig. 3), with collections in the states of Alagoas, Bahia, Ceará, Paraíba, Pernambuco, Piauí and Rio Grande do Norte, usually associated with the montane forest remnants called *brejos de altitude* and granitic inselbergs (see Souza and Oliveira 2006 for a complete map of all *brejos*). *C. heteropappa,* however, is currently known only from the Northeastern portion of Ceará, in the same type of environment (Fig. 2A).

All available collections from the Uruburetama massif present the morphological characters that define *C. heteropappa.* There are older collections from the second region where *C. heteropappa* was found, on Maranguape, that are currently identified as *C. pacourinoides* (Lima 55-2409, RB and Martins & Nunes s.n., EAC). We have studied photographs of these specimens, but it is not possible to verify style length or cypsela characteristics based on the available pictures. Therefore, there is the possibility that the two species overlap on Serra de Maranguape. We have not identified other materials collected in different parts of the state of Ceará that match the character set that defines *C. heteropappa.*

Chresta subverticillata Siniscalchi & Loeuille, sp. nov.—Type: Brazil. Bahia: Gentio do Ouro, Distrito de Santo Inácio, Afloramentos rochosos na entrada da vila, em frente a casa antiga abandonada, 11º06'26.33"S, 42º43'10.50"W, 560 m, 8 November 2015, *C.M. Siniscalchi & J. Vidal 634* (Holotype: SPF!, isotypes to be distributed to: K!, RB!, US!).

Species Chrestae harleyo *et* C. hatschbachio *simile, sed foliis minutis brevissimis ramis verticillatim aggregatis differt.*

Description—Perennial herb, ramified at the base, up to 0.5 m; stem 14–50 cm tall, striate, silvery-white, with densely panose indumentum composed of T-shaped hairs. Leaves alternate, grouped on very short branches, resembling whorls, simple, sessile; blade 0.6–3.8 (5.1) × 0.1–1.0 cm, narrowly linear to linear, narrowly lanceolate, elliptical to obovate or spatulate, silvery-white, panose with T-shaped hairs on both sides, with white glandular punctuations on the adaxial surface, no punctuations on the abaxial surface, midrib prominent abaxially on the proximal part, inconspicuous adaxially, venation pinnate, hyphodromous, margins entire, serrulate or denticulate to minimally denticulate, apex acute, rounded or acuminate, base attenuate. Inflorescence scapose, scape 6–25 cm long, panose, with T-shaped hairs, glabrescent, striated and stramineous in older inflorescences, engorged beneath the syncephalium. Syncephalia solitary, 1.1–2.5 cm tall, 0.7–2.5 cm diam., hemispherical, with capitula adpressed at base; secondary bracts 1.0–2.5 mm, subulate to ovate, apex acuminate, panose, T-shaped hairs. Capitula 10-57 per syncephalium, homogamous, discoid, sessile; involucre cylindrical; phyllaries 5- or 6-seriate, outer phyllaries ovate, 2.2–4.5 × 1.0–1.2 mm, apex acuminate, spinescent, pilose on the center of the blade, T-shaped hairs, with white glandular punctuations, whitish-yellow to stramineous, margins membranaceous, mid phyllaries elliptical, 4.3–6.2 ×1.2–1.8 mm, apex acuminate, spinescent, pilose on the center of the blade, T-shaped hairs, with white glandular punctuations, whitish, margins membranaceous, inner phyllaries elliptical, 7.8–12 ×1.5 mm, apex acuminate, spinescent, pilose on the top half portion, with white glandular punctuations, whitish-yellow to stramineous, margins membranaceous; receptacle flat, glabrous, slightly fimbrillate. Florets 5–6, bisexual, fertile; corollas actinomorphic, 5-lobed, tube 6.0–7.0 × 0.35–0.5 mm, with sparse digitiform glands, lilac, throat 1.5–1.8 × 0.4 mm, white, lobes 1.8–2.1 × 0.8–1.0 mm, spreading, lilac, whitish at base, apex acuminate, with abundant globular glands at the apex, margins slightly thickened; apical anther appendages sagitate, rounded, cell walls not thickened, basal anther appendages calcarate, acute; style

12.0 mm, style shaft glabrous, style base glabrous, lacking basal node, with nectariferous disc 0.03 mm long, style arms 1.0–1.3 mm long, short-pubescent outside throughout, hairs lageniform. Pollen grains ca. 50 µm diam., sub-spheroidal, echinolophate, *Chresta*-type II. Cypselae 3.2 × 0.7 mm, ca. 20-ribbed, cylindrical, pilose, twin-hairs with cells fused up to near to tip, with digitiform glands on the junction between carpopodium and cypsela; carpopodium 0.2 mm long, 0.2 mm diam., conspicuous, asymmetrical, golden-brown, glandular on the apex; pappus triseriate, whitish, straight, setose, serrulate, persistent, outer series 0.5–0.6 mm long, median series 1.3–2.1 mm long, inner series 5–7 mm long. Figures 4, 5.

Distribution and habitat—All collections of *Chresta subverticillata* come from the region of the district of Santo Inácio, which belongs to the municipality of Gentio do Ouro, on northwestern Bahia (Fig. 6). Most of the collections mentions that plants were growing over rock outcrops, which are abundant in the region. The area surrounding the district is formed almost exclusively by quartzitic massifs, intermingled with sand patches and dunes. The area is located in the northwestern end of the Chapada Diamantina mountain range, close to the end of Espinhaço Septentrional and the São Francisco river. According to Rocha et al. (2004), the local flora is composed mainly by typically sand-dweller Caatinga species, several of them endemic from the dunes.

Etymology—The species is named *subverticillata* because of the insertion of its leaves, which are grouped in very short branches, resembling whorls of leaves.

Flowering and fruiting— Flowering and fruiting plants were collected from February to November, which probably indicates a long flowering period not necessarily linked to the rainy or dry season.

Conservation status—*Chresta subverticillata* has an Area of Occupancy (AOO) of 13.463 km2 and an Extent of Occurrence (EOO) of 54.058 km2, placing the species as Critically Endangered and Endangered, respectively, according to criteria B1 and B2. However, the populations seem to be numerous and stable, as they have been collected several times since the 70's. On the other hand, none of the known populations are within conservation areas, nor there are plans to create conservation areas on the region, which could lead to increasing decline in quality of habitat and consequently cause populations to go extinct. Therefore, the species is classified as EN B1ab(iii)+2ab(iii).

Additional specimens examined—BRAZIL: Bahia, Gentio do Ouro, Distrito de Santo Inácio, on rocky hillside called Pedra da Mulher just south of town, 11°07'S, 42°44'W, ca. 500-600 m, 25 February 1977, *R.M. Harley 19029* (CEPEC, NY, RB, US); ibid., área muito seca, última chuva em dezembro de 1989, 11°7'S, 42°44'W, ca. 500 m, 5 October 1990, *A. Freire-Fierro et al. 1782* (SPF)*;* ibid., área muito seca, última chuva em dezembro de 1989, 11°7'S, 42°44'W, ca. 500 m, 5 October 1990, *A. Freire-Fierro et al. 1787* (SPF)*;* ibid., Caminho para Santo Inácio, 11°03'28"S, 42°42'37"W, 680 m, 24 June 1996, *M.L. Guedes et al. 2998* (ALCB, CEPEC, HUEFS, SPF); ibid., ca. 24 km S de Xique-Xique, na Estrada para Santo Inácio, 16 June 1994, *L.P. de Queiroz & N.S. Nascimento 3958* (HUEFS); ibid., 11°06'S, 42°40'W, 19 June 1998, *J. Santino de Assis 210* (RB); ibid., Vale das Pedras (CASF), 11°06'40"S, 42°43'17"W, 14 April 2000, *S.S. Lima s.n.* (ALCB); ibid., dunas vicariantes, 11°06'40"S, 42°43'17"W, 2 June 2000, *S.S. Lima s.n.* (ALCB); ibid., ramal para a cachoeira, 11°5'48"S, 42°43'18"W, 536 m, 20 July 2000, *M.M. da Silva et al. 466* (HUEFS); ibid., Serra de Sapé, 11°11'23"S, 42°43'07.40"W, 624 m, 26 May 2009, *J.A. Siqueira-Filho et al. 2060* (HVASF, RB); ibid., Serra do Sapê, Estrada de Gentio do Ouro para Santo Inácio, 11°11'31.75"S, 42°43'03.02"W, 622 m, 7 November 2015, *C.M. Siniscalchi & J. Vidal 630* (SPF); ibid., Estrada para a cachoeira, acesso pela Estrada que liga a rodovia BA-330 a Santo Inácio, 11°05'48"S, 42°43'18"W, 532 m, 7 November 2015, *C.M. Siniscalchi & J. Vidal 631* (SPF).

Taxonomic notes—Several registers of *Chresta subverticillata* have been gathered since the 1970s, but the specimens have been identified as *C. harleyi.* However, these populations are disjunct from the distribution of *C. harleyi* (Fig. 6) and also show clear morphological differences from the latter and from other related species. *Chresta subverticillata, C. harleyi, C. hatschbachii* and *C. martii* are all perennial herbs, ramified at the base, with leaves covered by dense panose indument composed by T-shaped hairs and hemispheric syncephalia with capitula loosely joined at the base, with this new species being morphologically related to *C. harleyi* and *C. hatschbachii.*

Besides having the leaves grouped in very short branches, *C. subverticillata* is distinct from *C. harleyi* and *C. hatschbachii* by the length and shape of the leaves (Table 3), even if *C. subverticillata* has a wide range of leaf shapes and different types of margin serration (Fig. 4L).

The syncephalia in *C. subverticillata* sometimes present indeterminate growth, and in this case, they resemble dense spikes (Fig. 5F); different from that observed in *C. martii,* while indeterminate growth has never been observed in *C. harleyi* and *C. hatschbachii.*

Microcharacters also place *C. subverticillata* closer to *C. harleyi* and *C. hatschbachii,* with similarities in pollen type and morphology of the twin-hairs on the cypsela (Table 3). Also, *C. subverticillata* presents conspicuous glands on the junction of the carpopodium with the body of the cypsela (Fig. 5H) that are not present in the other species.

Chresta harleyi, C. hatschbachii occur in the mountain range known as Espinhaço Septentrional, which connects the southern portion of the Espinhaço Range with Chapada Diamantina, and *C. subverticillata* occurs on the end of the Chapada Diamantina range closer to Espinhaço Septentrional, the three species are spread out in a South to North pattern. *C. harleyi* has the southernmost distribution, with a range that extends for about 200 km from northern Minas Gerais to southern Bahia. The few known populations of *C. hatschbachii* are restricted to the municipality of Oliveira dos Brejinhos, about 200 km North of the northernmost populations of *C. harleyi,* and all populations of *C. subverticillata* are about 120 km North of *C. hatschbachii.*

It is noteworthy that there is no overlapping in the distribution of these three species, and our study of several of these populations revealed no individuals of different species intermixed in the same populations nor intermediate forms. This fact raises interesting questions about speciation processes in rupicolous plants, with two main hypotheses being suggested.

One possible scenario would be that the whole mountain range was first colonized by an ancestral species that then speciated in loco*,* with the possibility of intermediate populations going extinct during the process. The second possibility would be that one of the extremes of the mountain range was first colonized and then several long-distance dispersal events led to the colonization of different areas with posterior speciation. These two scenarios have been explored in some phylogeographical studies with trees from the Caatinga (Caetano et al. 2008, Collevatii et al. 2012, Vieira et al. 2015), one of them with exclusive occurrence in limestone outcrops, but with contradictory results. As far as we know, no studies testing these hypotheses were carried out with herbaceous plants from the Caatinga, leaving an open question about possible evolutionary processes in this group.

IDENTIFICATION KEY FOR SPECIES OF *CHRESTA* FROM THE CAATINGA

1. Leaves membranaceous, dark green, glabrate to glabrous, turning dark when dried; spherical syncephalia with tightly joined capitula, presenting indeterminate growth 2

    2. Style length ca. 5.5 mm, with no more than 2 mm exserted above the anthers; pappus elements paleaceous-setose .... *C. heteropappa*

    2. Style length ca. 10 mm, exserted part of the style almost as long as the corolla tube; pappus elements barbelate-setose .... *C. pacourinoides*

1. Leaves membranaceous to subcarnose, greyish-green, usually silvery-gray when dried, covered by dense indument of T-shaped trichomes; hemispherical syncephalia with capitula joined only at the base 3

    3. Leaves aromatic, usually grouped on the tip of the stems; syncephalia presenting indeterminate growth when older, younger syncephalia with pyramidal shape .... *C. martii*

    3. Leaves not aromatic, evenly distributed along the stems or sometimes grouping on the tip of the stems;

syncephalia not usually presenting indeterminate growth or pyramidal shape 4

4. Leaves minute, usually less than 4 cm long, grouped on the tip of very short branches, resembling whorls of leaves; syncephalia sometimes presenting slight indeterminate growth, resembling spikes .... *C. subverticillata*

4. Leaves usually longer than 4 cm, evenly distributed along the stems or sometimes grouping on the tip of the stems 5

5. Leaves petiolate, with triangular, deltoid or acute apices; scape 12-28 cm long; capitula 10-30 per syncephalium; flowers 7-8 flowers per capitulum .... *C. harleyi*

5. Leaves sessile or subpetiolate, with oblong, rounded apices; scape 1-4.5 cm long; capitula 7-14 per syncephalium; flowers 5-6 per capitulum .... *C. hatschbachii*

Concluding Remarks

The two new species herein described are morphologically related to the group of rupicolous species from the Caatinga, *Chresta subverticillata* being closer to the species that grow over quartzitic outcrops, and *C. heteropappa* being closer to *C. pacourinoides.*

The abundance of rocky environments amidst the Caatinga domain, varying on type of rock, climatic conditions and surrounding vegetation, creates a range of microhabitats that may have favored high levels of speciation and diversification. Many of these habitats are still underexplored, floristically unknown and threatened. As the present work shows, these sites may contain a diverse biota whose components have not yet been sampled or remain undescribed in smaller herbaria. Studies leading to a better knowledge of the flora present on these formations may uncover additional new or rare species, and can provide the necessary knowledge to propose new conservation areas.

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TABLE 1. Published floristic studies carried out in the Caatinga containing at least one occurrence of *Chresta*.

Author and publication	**No. Asteraceae species/Total no. species**	***Chresta* species recorded**	**City or location, state**	**Type of environment where *Chresta* was collected**
Lucena et al. 2015	4/101 (~ 4%)	*C. martii*	Patos, PB	Crystalline inselberg
Amorim and Bautista 2016	52/52 (100%)	*C. martii*	Raso da Catarina, BA	Ecoregion (imprecise location)
Campos et al. 2017	95/811 (~ 12%)	*C. harleyi*	Licínio de Almeida, BA	Caatinga rupestre
Fevereiro and Fevereiro 1980	2/26 (~ 8%)	*C. pacourinoides*	Remígio, PB	Crystalline inselberg
Araújo et al. 2008	4/77 (~ 5%)	*C. pacourinoides*	Quixadá, CE	Crystalline inselberg
Agra et al. 2004	18/315 (~ 6%)	*C. pacourinoides*	Maturéia, PB	Crystalline formation
Barbosa et al. 2004	14/309 (~ 5%)	*C. pacourinoides*	Areia, PB	Imprecise
Gomes and Alves 2009	8/201 (~ 4%)	*C. pacourinoides*	Bezerros, PE	Crystalline inselberg
Pereira et al. 2009	56/56 (100%)	*C. pacourinoides*	Parque Nacional do Catimbau, PE	Imprecise
Gomes and Alves 2010	17/211 (~ 8%)	*C. pacourinoides*	Caruaru, PE	Crystalline inselberg in forest remnant
Mendes and Castro 2010	1/136 (~ 0.7%)	*C. pacourinoides*	São José do Piauí, PI	Sandstones, shales and silts (imprecise location)
Araújo et al. 2011	21/419 (~ 5%)	*C. pacourinoides*	Crateús, CE	Deciduous forest in latosol
Gomes et al. 2011	3/125 (~ 2%)	*C. pacourinoides*	Venturosa, PE	Granite rock outcrop
Tölke et al. 2011	12/97 (~ 12%)	*C. pacourinoides*	Puxinanã, PB	Granite inselberg
Oliveira et al. 2013	14/247 (~ 6%)	*C. pacourinoides*	Around Apodi-Mossoró river, RN	Limestone outcrop
Moura and Roque 2014	80/80 (100%)	*C. pacourinoides*	Jacobina, BA	Transition caatiga – caatinga rupestre
Staudt et al. 2017	119/119 (100%)	*C. pacourinoides*	Morro do Chapéu, BA	Caatinga

TABLE 2. Comparison between *C. heteropappa* and *C. pacourinoides*

	C. heteropappa	***C. pacourinoides***
Inflorescence type	Panicle of syncephalia	Panicle of syncephalia
Inflorescence branches	Slender, slightly engorged on the base of syncephalia, sometimes hollow on wider branches	Thick, hollow, markedly engorged on the base of the syncephalia
Syncephalia dimensions	Around 2.5 cm in height	Up to 15 cm in height
Syncephalia growth	Not always present, subtle	Marked indeterminate growth
Portion of the style exserted above anthers	1 – 1.25 mm	Up to 10 mm
Pollen type	Type II	Type II
Shape of pappus elements	Paleaceous-setose	Setose

TABLE 3. Comparison among *C. subverticillata* and related species

	C. subverticillata	***C. harleyi***	***C. hatschbachii***	***C. martii***
Leaf position on stem	Grouped in very short branches	Distributed along the stem, sometimes grouping on the tip of the stems	Evenly distributed on the stems	Frequently grouping on the tip of the stems
Leaf length (cm)	> 4	4 – 12	2 – 7	< 6
Leaf shape	Not fully pinnatilobate, different shapes and leaf margins	Pinnatilobate, with deltoid lobes	Pinnatilobate, with oblong lobes	Lobed, pinnatilobate, deeply dented
Syncephalia growth	Sometimes indeterminate, resembling a dense spike	Determinate	Determinate	Marked indeterminate in older specimens, pyramidal shape when young
Pollen type	Type II	Type II	Type II	Type I
Glands on the carpopodium/cypsela junction	Present, conspicuous	Not present	Not present	Not present
Twin-hairs	Long cells united almost to the tips, with slightly different sizes	Long cells united almost to the tips, with slightly different sizes	Long cells united almost to the tips, with slightly different sizes	Separated at the base with unequal lengths

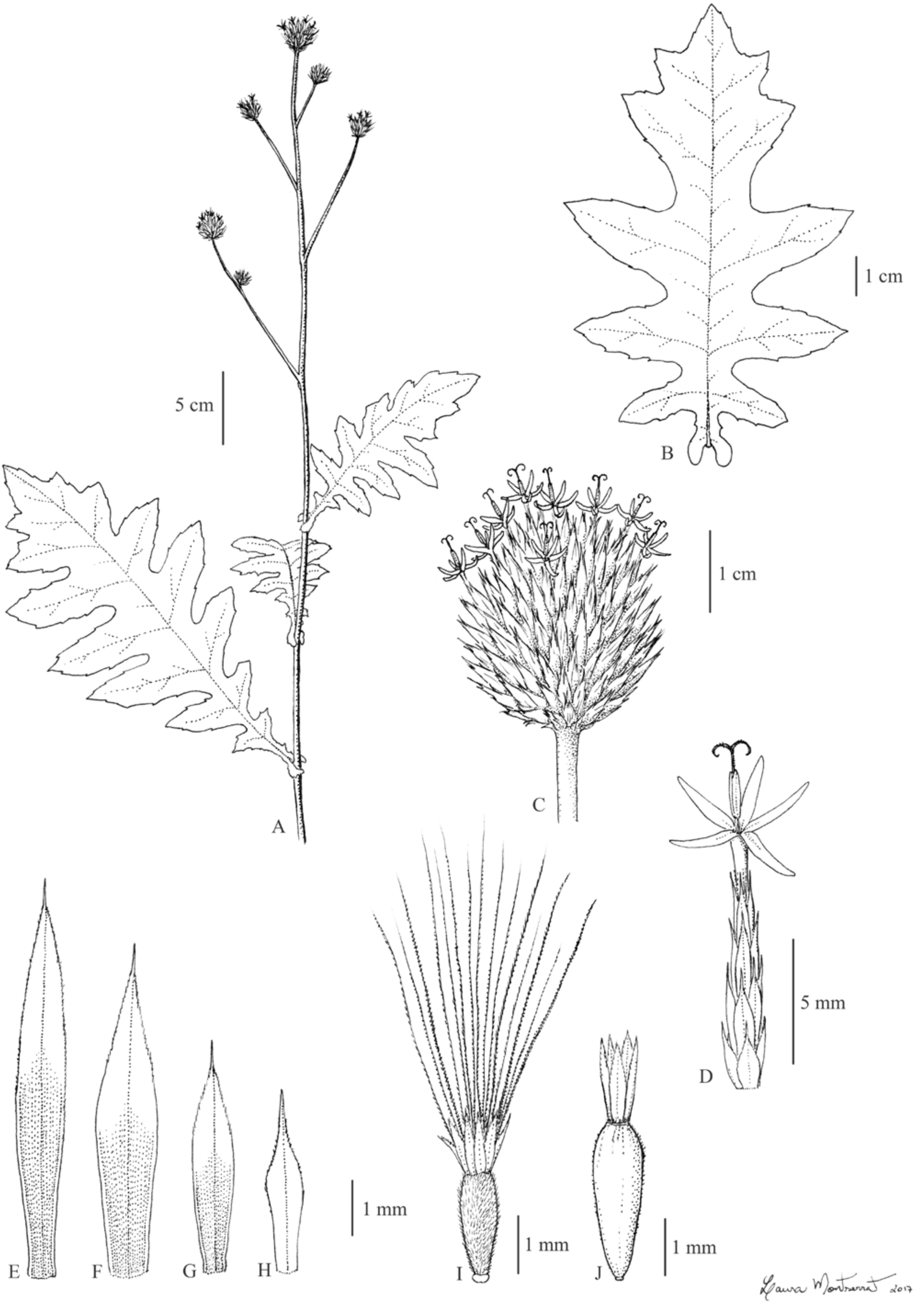

FIG. 1. *Chresta heteropappa.* A. Habit and synflorescence. B. Adaxial surface of the leaf. C. Syncephalium and florets. D. Capitulum with florets. E. Inner phyllary. F. Intermediate phyllary. G. Intermediate phyllary. H. Outer phyllary. I. Cypsela type 1. J. Cypsela type 2. Drawing by Laura Montserrat.

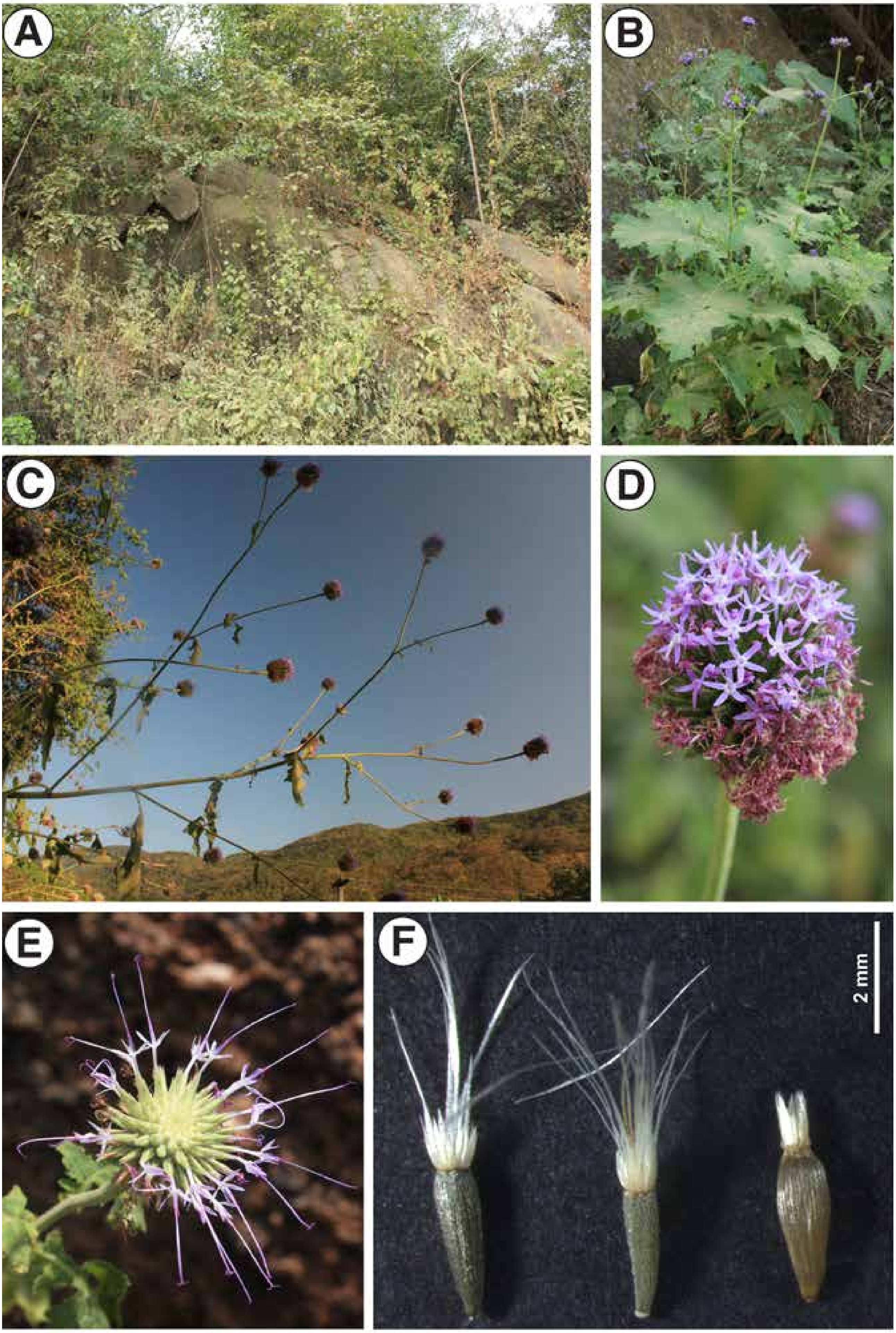

FIG. 2. *Chresta heteropappa*. A. Environment where the type specimen was collected. B. Habit of a small individual. C. Synflorescence of a larger individual. D. Syncephalium. E. Syncephalium of C. pacourinoides. F. Cypselas, type 1: two on the left, type 2: one on the right.

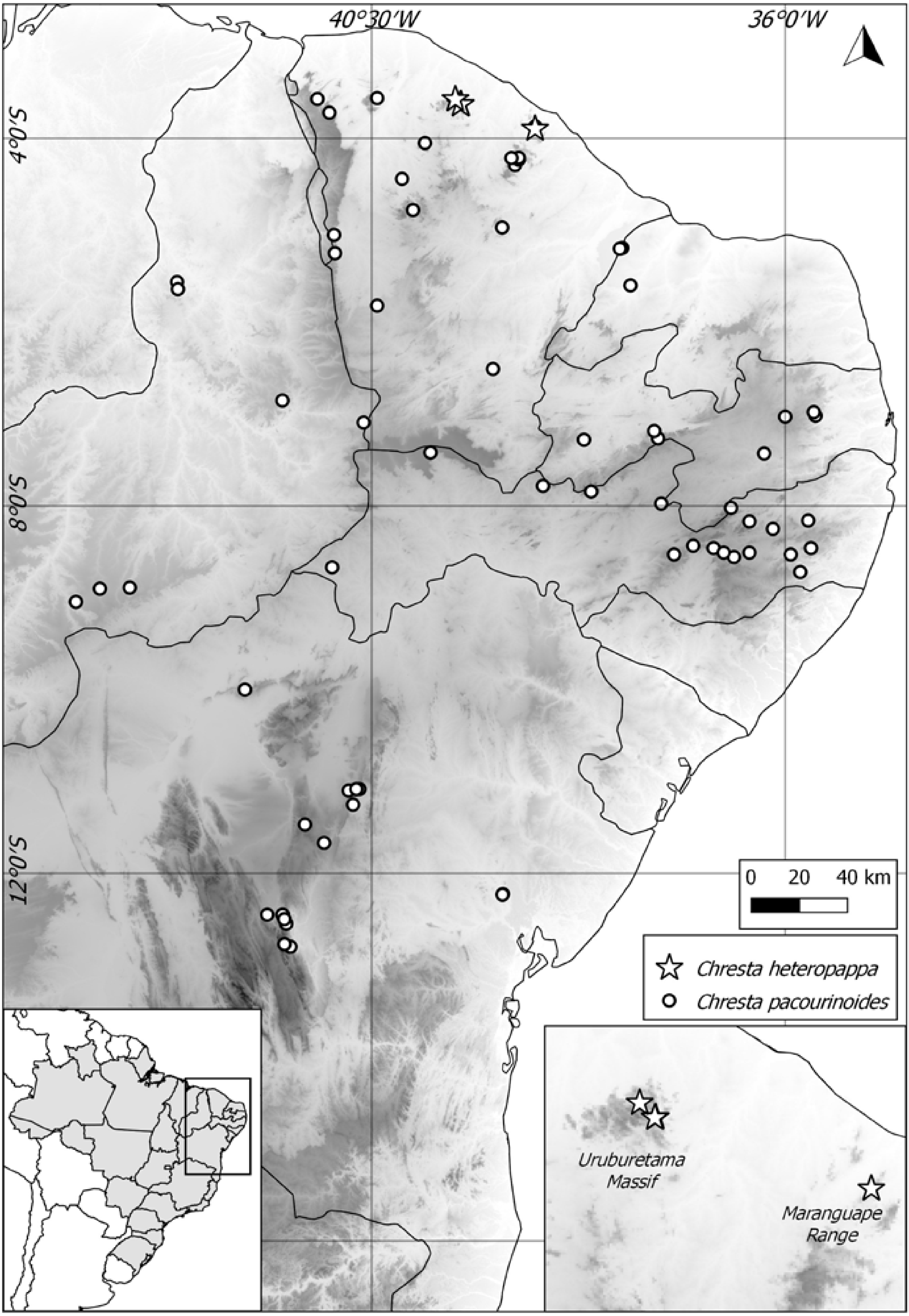

FIG. 3. Map showing the distribution of *C. heteropappa* and C. *pacourinoides.*

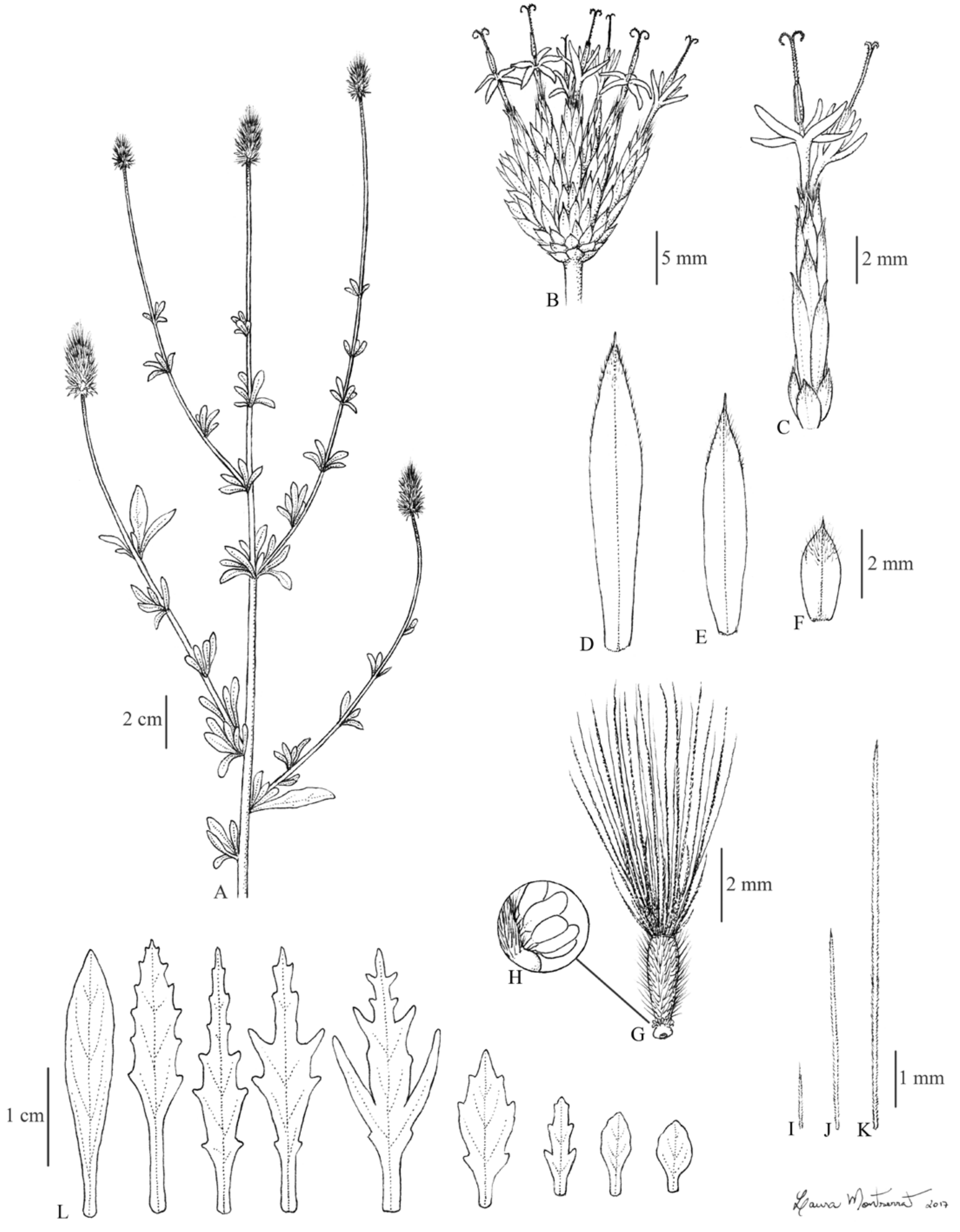

FIG. 4. *Chresta subverticillata.* A. Habit. B. Syncephalia with open florets. C. Capitulum with florets. D. Inner phyllary. E. Intermediate phyllary. F. outer phyllary. G. Cypsela. H. Detail of the glands on the top of the carpopodium. I. Outer pappus bristle. J. Intermediate pappus bristle. K. Inner pappus bristle. L. Range of leaf shapes found in the examined material. Drawing by Laura Montserrat.

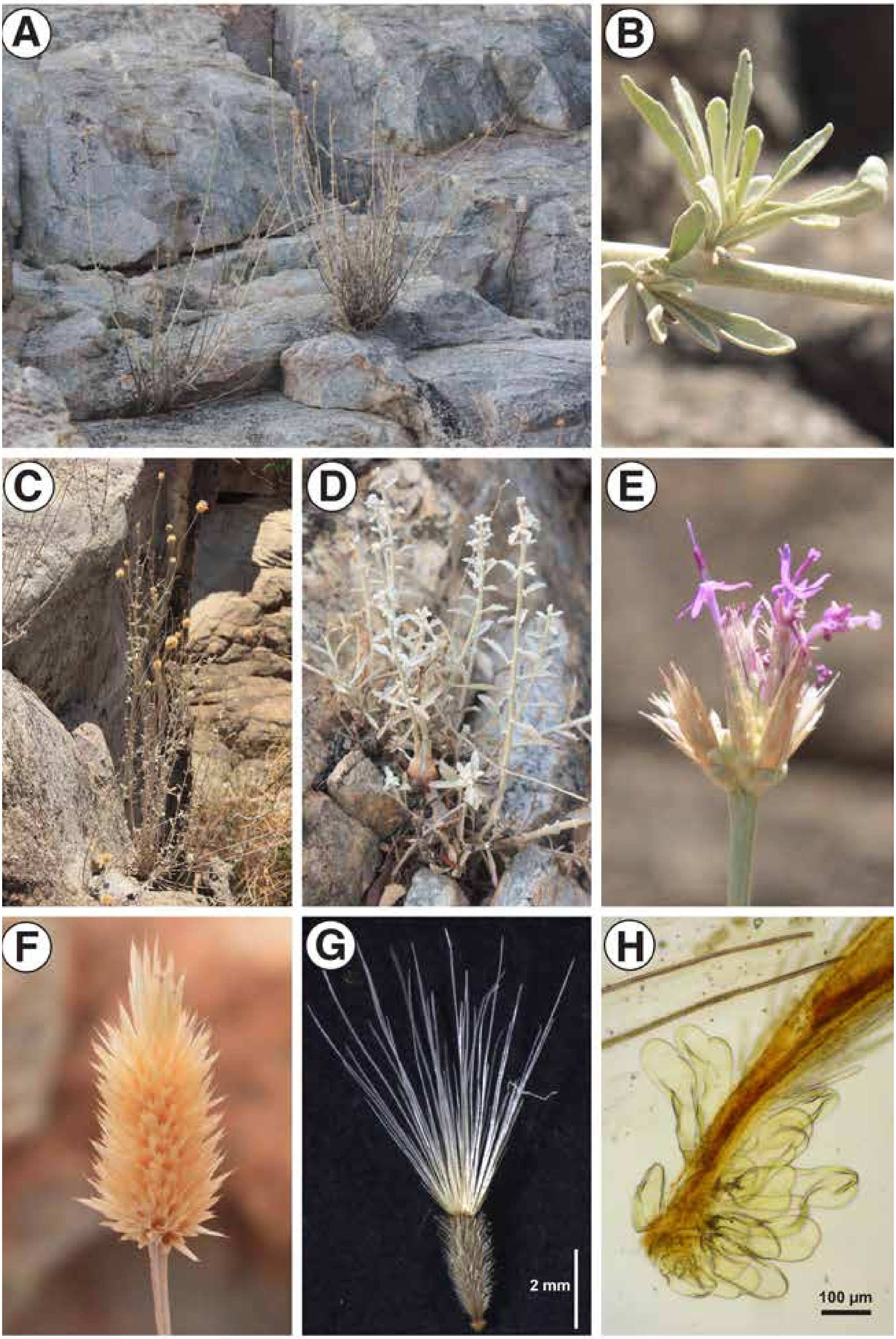

FIG. 5. *Chresta subverticillata.* A. Habit and environment. B. Detail of the very short branches and leaves. C. Older plant showing older inflorescences. D. Younger plants showing different leaf shapes. E. Young syncephalium and florets. F. Older syncephalium showing indeterminate growth. G. Cypsela. H. Detail of the glands on the top of the carpopodium and base of the cypsela.

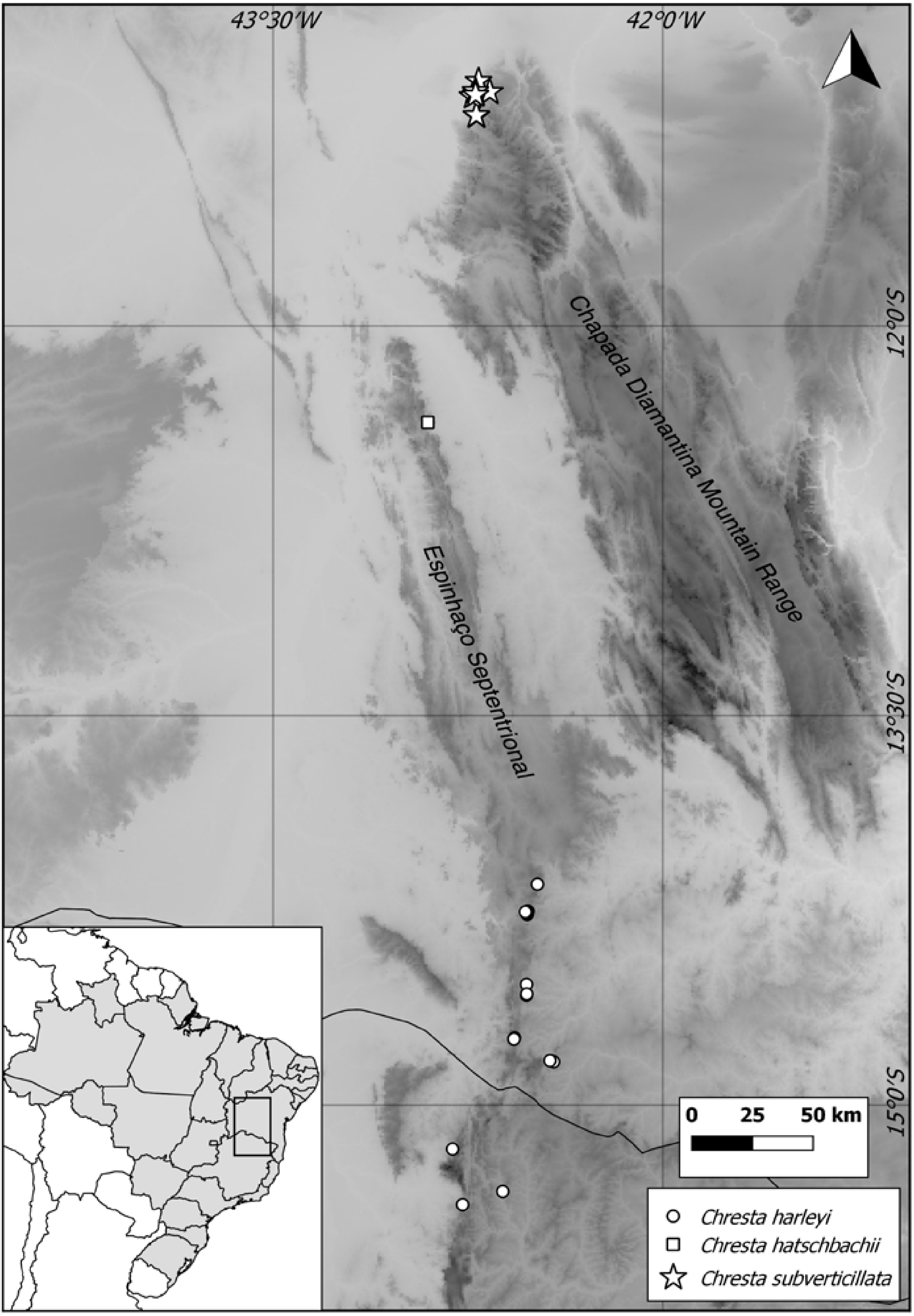

FIG. 6. Distribution map of *C. subverticillata* and correlated species, *C. harleyi* and *C. hatschbachii.*

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Chapter 4
Part C

Chresta artemisiifolia (Vernonieae, Asteraceae), a new species from a threatened area in the Brazilian Caatinga

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To be submitted to Phytotaxa

Chresta artemisiifolia (Vernonieae, Asteraceae), a new species from a threatened area in the Brazilian Caatinga

Abstract

Chresta artemisiifolia, a new species from northern Bahia, is described and illustrated. This species occurs on rock outcrops amidst the dry Caatinga dominium, and is morphologically related with *C. harleyi, C. hatschbachii* and *C. martii.* It is endemic to a region known as Boqueirão da Onça, where a national park has been proposed but not created so far. We hope that the description of this taxon contributes to the efforts in the preservation of this area.

Resumo

Chresta artemisiifolia, uma nova espécie do norte da Bahia, é descrita e ilustrada. Essa espécie ocorre em afloramentos rochosos no domínio da Caatinga e é morfologicamente relacionada com *C. harleyi, C. hatschbachii* e *C. martii.* Ela é endêmica de uma região conhecida Boqueirão da Onça, onde um parque nacional foi proposto, mas ainda não criado. Esperamos que a descrição desse táxon contribua para os esforços de preservação dessa área.

Keywords: Compositae, taxonomy, seasonally dry tropical forest, national park, Boqueirão da Onça.

Introduction

The Caatinga is one of the most threatened phytogeographical domains in Brazil. Despite the harsh semi-arid conditions, its rich soils are widely used for agricultural purposes and there is wide mining activity exploring several geological resources, such as quartz and granite, as well as production of coal from native trees. More recently, several areas in the Caatinga are being prospected by wind energy companies interested in installing new parks in the area (Leal et al. 2005). Conservative estimates state that ca. 30% of the Caatinga area is dedicated to agricultural activity and less than 7% of the area is included in conservation units, with 1.3% being areas of integral protection (Castelletti et al. 2003, Maciel 2010).

The need to create new conservation units in the Caatinga has been affirmed during the last few decades by a number of researchers, non-governmental organizations and governmental bodies; yet with little effect (Santos et al. 2011). One case that represents this failure to protect this unique domain is the creation of Parque Nacional do Boqueirão da Onça. The area known as Boqueirão da Onça (jaguar's ravine, in a loose translation) encompasses five municipalities in Northern Bahia, close to the border with state of Piauí, with most of its area in the municipality of Sento Sé. The local flora and fauna are rich in endemic and rare species, including the critically endangered jaguar (*Panthera onca* Linnaeus 1758). The area also has numerous caves and archeological sites, including Toca da Boa Vista, the longest cave in the Southern hemisphere, besides being sparsely populated (Fukuda et al. 2010, de Paula et al. 2012).

The first proposal to create a national park in the region dates from 2002 and included an area of almost 1 million hectares, however it was heavily criticized and subsequently cancelled. In 2006, some research expeditions were carried out and in 2008 public consultations were made in municipalities that would be affected by the implementation of the park and it was decided that the area of the park would have to be reduced to accommodate traditional communities. In 2010, further analysis by the Environment ministry and ICMBio reached the conclusion to reduce the proposed area for the National Park to ca. 750,000 ha and also create a Natural Monument of ca. 165,000 ha (Fukuda et al. 2010).

Nevertheless, the creation of this mosaic of conservation units stalled until July 2017, when the State Government of Bahia authorized the creation of the mosaic of conservation units, including a national park with ca. 350,000 ha and an Environmental Protection Area with ca. 500,000 ha, the second one containing an exception area on it (Bragança 2017, and several other media outlets). This proposal still has to run its course at the Ministry of the Environment and the Federal Government, and it may long before the conservation units are effectively decreed. Although this proposal creates the largest protected area in the Caatinga dominium, it still represents a remarkable decrease from the original proposal. The fact that most of the area is destined to be an Environmental Protection Area (Área de Proteção Ambiental – APA) is preoccupying, as this category does not foreseen expropriation of inhabitants and allows some types of use of the land, including commercial use.

Currently, the main threat to the region is the installation of wind farms for generation of energy. Despite being considered a clean and renewable energy, their construction in preserved areas can have great environmental impact. Previous studies have shown that the turbines present a high risk of death by collision for birds and bats, especially if placed on migration routes and the service road network that has to be built around them may cause habitat disruption due to fractioning of areas, affecting small mammals and reptiles and causing mortality by vehicles (Kuvlesky et al. 2007). Also, the opening of roads and installation of turbines deforests the affected areas, with unknown impact to the plant populations on those areas.

The atlas of wind potential in Brazil (Amarante et al. 2011) shows the Northeastern region as the one with highest potential for energy production, and some of the areas with highest potential are contained within Boqueirão da Onça, with eight wind farms already installed in Sento Sé. The creation of the APA will still allow the construction of wind farms, with stricter environmental requirements, but it is a real possibility that these farms will generate more impact in this threatened region, which may hinder conservation efforts.

Among the peculiar plant species of the Caatinga, four endemic species of *Chresta* Velloso ex Candolle (1836: 85) are known, while the remaining 11 species occurr in the Cerrado and Atlantic Forest dominia. Plants belonging to *Chresta* are herbs or subshrubs, with capitula aggregated in secondary heads or syncephalia and the flowers are purple, except for two red-flowered species. The species of *Chresta* from the Caatinga are rupiculous plants with lobed leaves and purple flowers with white throats, and two species, *C. martii* (Candolle 1836: 84) Robinson (1980: 91) and *C. pacourinoides* (Martius ex Candolle 1836: 84) Siniscalchi & Loeuille (2014: 8), have syncephalia with indeterminate growth.

In the course of a revisionary study of the genus, three specimens collected in the Boqueirão da Onça region that did not belong with any of the known species of the genus were found on the HVASF herbarium. At first it was thought these specimens were a local variation of *Chresta martii*, which also occurs on the region, but further morphological studies and new field observations in one of the known variant populations made it clear these individuals represent an undescribed species. Therefore, this species is herein described and its affinities discussed.

Material and Methods

Morphological features of the specimens were analyzed using 10–60 × magnification stereomicroscope. Measurements of mature structures were made with a digital caliper rule and optical graticule attached to the microscope. Microcharacters were evaluated by mounting parts of the corolla lobes, style arms, cypsela and carpopodium on a microscope slide with Hoyer's solution (Anderson 1954) and analyzing the slides in a Leica DM 4000B microscope. Photomicrographs were taken with a Leica DFC 425 camera. Terminology follows Harris & Harris (2001) for general morphology and Siniscalchi *et al.* (2017) for pollen grain morphology. Assessment of conservation status was performed using the GeoCAT Tool (Bachman *et al.* 2011), with the default for Area of Occupancy (AOO), to obtain AOO and Extent of Occurrence (EOO), and the full assessment was based on the IUCN manual (IUCN 2012). The distribution map was produced in QGIS version 2.18 (Quantum GIS Development Team 2017) with geographic coordinates obtained from herbarium specimens.

Chresta artemisiifolia Siniscalchi & Loeuille, **sp. nov.** (Figure 1, 2)

Species Chrestae martii *simile, sed foliis laminis constrictis secus costas et nervos secundarios (non expansis), scapo longiore (5–11.5 cm, non 30–60 cm), syncephalo crescentia determinata (non indeterminata) et apice phyllariorum acuto (non obtuso) differ.*

Type:—BRAZIL. Bahia, Sento Sé, Nascente na Grota dos Prazeres, no paredão rochoso, bioma Caatinga, fitofisionomia Savana Estépica, 437 m, 10°04'11.60" S, 41°37'27.60" W, 18 December 2015, *J.A. Siqueira Filho., E.D.S. Almeida, E.M.B. Nunes, T.T. Bezerra, C.M. Siniscalchi 3671* (holotype HVASF!; isotypes to be distributed to RB!, SPF!, US!).

Description:—Perennial herb with a branched rootstock, ramified at the base, up to 0.5 m; stem 15–20 cm tall, striate, brownish-red, villose with simple minute hairs and glandular trichomes, glabrescent at the base. Leaves alternate, mostly concentrated at the apex of stems, simple, sessile or subsessile; overall contour of the leaves 1.4–8.2 × 0.6–3.2 cm, pinnatisect blade restricted to 0.1–0.3 cm around primary and secondary veins and sometimes tertiary veins, overall contour of the leaf ovate, greyish-green, villose with short-armed T-shaped hairs intermixed with abundant white glandular hairs on both sides, midrib prominent on both sides, venation pinnate, margins entire, apex acute, base attenuate. Inflorescence scapose, 5–11.5 cm long, scape villose with short-armed T-shaped hairs intermixed with abundant white glandular hairs, glabrescent in older inflorescences, light brownish-orange, engorged beneath the syncephalium, with evident striation. Syncephalia solitary, 1.2–1.8 cm tall, 0.8–1.5 cm diam., hemispherical, with capitula adpressed at base; secondary bracts 1.0–1.3 × 1 mm, subulate to ovate, villose with short-armed T-shaped hairs intermixed with abundant white glandular hairs. Capitula 9-30 per syncephalium, homogamous, discoid, sessile; involucre cylindrical; phyllaries 5- or 6-seriate, involucre 7–8 × 1.5–2 mm, outer phyllaries widely ovate, 0.8–1.6 × 0.6 mm, apex rounded, spinescent, completely villose or with indumentum concentrated on the upper half, glandular trichomes concentrated on the upper half of the blade, greenish-white on the center, purplish on the margins, margins membranaceous, mid phyllaries widely elliptical to obovate, 3.8–4.3 × 1–1.6 mm, apex rounded to widely acute, spinescent, pilose on the upper half of the blade, with white glandular trichomes, greenish-white on the center, purplish on the margins, margins membranaceous, inner phyllaries narrowly obovate to elliptical, 7–8.5 ×1–1.3 mm, apex acute to acuminate, spinescent, pilose on the upper half of the blade, with white glandular trichomes, greenish-white on the center, purplish on the margins, margins membranaceous; receptacle flat, glabrous, foveolate. Florets 6, bisexual, fertile; corollas actinomorphic, lilac, 5-lobed, tube 4.9–5.2 mm, , throat 0.4–0.55 mm, with sparse glandular trichomes, lobes 2–2.5 × 0.3–0.4 mm, spreading, apex acute, with abundant globular glandular trichomes and simple unicellular trichomes at the apex, margins not thickened; apical anther appendages rounded, sagittate, cell walls not thickened, basal anther appendages calcarate, acute; style 10–11 mm, style shaft glabrous except for upper part right below arms, lilac trichomes, style base glabrous, lacking basal node, with nectariferous disc 0.4 mm long, style arms 3 mm long, pilose outside throughout, hairs acute, subulate. Pollen grains ca. 30 μm diam., oblate-spheroidal to spheroidal, echinolophate, *Chresta*-type II. Cypselae 2.0–2.2 × 0.6–0.8 mm, ca. 20-ribbed, turbinate, sericeous, brownish, twin-hairs with cells fused up to near to tip, with long digitiform glands on the junction between carpopodium and cypsela; carpopodium 0.1 mm long, 0.3 mm diam., conspicuous, ring-shaped, asymmetrical on the top, brownish-yellow, glandular on the apex, cell walls thickened; pappus triseriate, whitish to stramineous, straight, serrulate-setose, persistent, outer series 0.4–0.6 mm long, lanceolate, paleaceous, apex acuminate, median series 1.3–2.6 mm long, linear, paleaceous, apex long acuminate, inner series 4.9–6 mm long, lanceolate, paleaceous, apex rounded.

Distribution and habitat:—*Chresta artemisiifolia* is a rupicolous plant, growing over quartzitc rock outcrops or grottos, usually close to water sources or in places with intermittent water courses, at elevations between 400 and 600 m a.s.l. Three of the known records are from within the proposed area of Boqueirão da Onça national park, along the border with the Environmental Protection Area, including the type specimen, and the remaining record is completely out of the proposed protected areas (Figure 3). At least in one of the known populations (Grota dos Prazeres) the new species grows sympatrically with *Chresta martii*, however, no intermediate individuals or signs of hybridization between the two species were found there.

Flowering and fruiting:—Flowering and fruiting specimens were collected in February, June, July and December, indicating a long flowering period not necessarily linked to the rainy season (October to April), as more usually seen with Caatinga plants.

Conservation status:—*Chresta artemisiifolia* has an extent of occurrence of 143.547 km^2 and area of occupancy of 15.977 km^2, pointing the species as Endangered (EN) under subcriteria B1 and B2, with the additional factors that the species is found in less than 5 locations, with a projected decline in both EOO and AOO and habitat quality in case the region ends up not being part of a conservation unit. Moreover, the know total population has less than 2,500 individuals, with each subpopulation presenting ca. 100 individuals. Therefore, the conservation status is EN B1ab(iii)+2ab(iii); C2a(i).

Etymology:—The epithet *artemisiifolia* refers to the leaves with constricted leaf blades that follow the venation, similar to the leaves of *Artemisia,* Asteraceae (Figure 2C, D).

Additional specimens examined (paratypes):—BRAZIL: Bahia: Sento Sé, Parque Nacional do Boqueirão da Onça, Olho d'água, afloramento rochoso, 526.97 m, 10°10'27.19" S, 41°30'27.52" W, 1 February 2010, *J.A. Siqueira Filho et al. 2343* (HVASF!); localidade Prazeres, região de água corrente na época chuvosa, 541 m, 10°10'21.22" S, 41°30'35.23" W, 19 July 2011, *V.M. Cotarelli et al. 890* (HVASF!); Brejo da Brázida, Grota de Santana, 492 m, 10°16'37.40" S, 41°37'05.30" W, 13 June 2012, *N.M.P. Braga & C.R.S. Oliveira 71* (HVASF!, SPF!).

Discussion

Chresta artemisiifolia is morphologically related to three other Caatinga species: *C. harleyi* Robinson (1983: 385), *C. hatschbachii* Robinson (2005: 83) and *C. martii*. Plants in this group of species are perennial herbs that grow in rock crevices or directly upon rocks, mostly on quartzitc outcrops. They also present lobed leaves with a thick silvery indumentum and scapose inflorescences with solitary hemispherical syncephalium with loosely joined capitula.

In this group, *C. martii* has the wider distribution, extending in a more or less continuous line from the East (Paraíba and Rio Grande do Norte states), to the West (on Piauí state), while *C. harleyi* extends South-North following the Espinhaço Range and Serra Geral on Minas Gerais and Bahia. *C. hatschbachii* is known from one population on the Northern end of *C. harleyi's* distribution (Figure 3). The new species is found on the region where *C. harleyi* and *C. martii* ranges meet, close to the northernmost populations of *C. harleyi* and to the westernmost populations of *C. martii* in Piauí.

Analysis of herbarium collections have shown that *C. martii* exhibits some morphological variation, with plants from Piauí and Bahia being tall, sturdy herbs with wide, deeply dentate leaves with many teeth on the margins, while on the other end of the distribution plants are generally smaller and more delicate, with narrower leaves, usually with only three to five teeth on the margins on each side. This variation led to the initial conclusion that *C. artemisiifolia* was just a local variant of *C. martii*, especially because both are found in sympatry on at least one location.

However, besides the obvious difference in leaf shape (Figure 2D) there are other differences between them and in some aspects the new species seems to be more closely related to *C. harleyi* and *C. hatschbachii*.

C. martii has aromatic leaves and syncephalia that present marked indeterminate growth, almost resembling a spike, a combination of characters lacking in any other species. The new species displays non-aromatic leaves and the shape of the syncephalia is similar to those in *C. harleyi* and *C. hatschbachii,* with loosely joined capitula and determinate growth. The scapes in *C. martii* are also much longer, between 30-60 cm, and engorged on the base of the syncephalia, differently from *C. artemisiifolia*. The phyllaries in the new species are narrow, with an acute apex, similar to those in *C. harleyi* and *C. hatschbachii,* while *C. martii* has wider phyllaries with rounded apex. Regarding microcharacters, the twin-hairs on *C. artemisiifolia* are very similar to those in *C. harleyi* and *C. hatschbachii,* long and adjoined up to the top, with almost equal lengths, while in *C. martii* the twin-hairs are separated at the base with very unequal lengths. Also, the new species present a very unusual combination of glandular and simple trichomes on the tip of the corolla lobes, while usually these two types are not found together. Also, *C. martii* has *Chresta*-type I pollen, while *C. artemisiifolia* has type II pollen, as found on the rest of the Caatinga species (Siniscalchi *et al.* 2017).

A better knowledge about the flora of the Caatinga is still needed in order to assess areas that should be prioritized for conservation. *Chresta artemisiifolia* is endemic to the Boqueirão da Onça, a region that seems to be particularly rich in endemism. Several rare and endangered bird species have been recorded in the area (Schunck *et al.* 2012), as well as 26 species of ferns and lycophytes (Braga 2013). A list of the angiosperms that occur in the region is still ongoing, but there is an estimate of ca. 900 species (Siqueira Filho, unpubl. data), and at least one more endemic plant species has been described recently, *Comanthera borbae* Pereira & Giulietti (2016:26–27).

Analyses of degraded areas using satellite images have shown that from 2000 to 2009 the percentage of forested areas in the proposed area of the park fell from 89% to 65%, with most of the loss being in the margins of the park and in areas close to water bodies (Ramos *et al.* 2010). There are no more recent estimates about the loss of vegetation in the proposed area, but it is not hard to imagine that the degradation has continued in the past years. We hope that describing this new, endangered, narrowly endemic species will help the efforts into making this area a national park, aiming to preserve not only *Chresta artemisiifolia,* but all the biodiversity found on the region.

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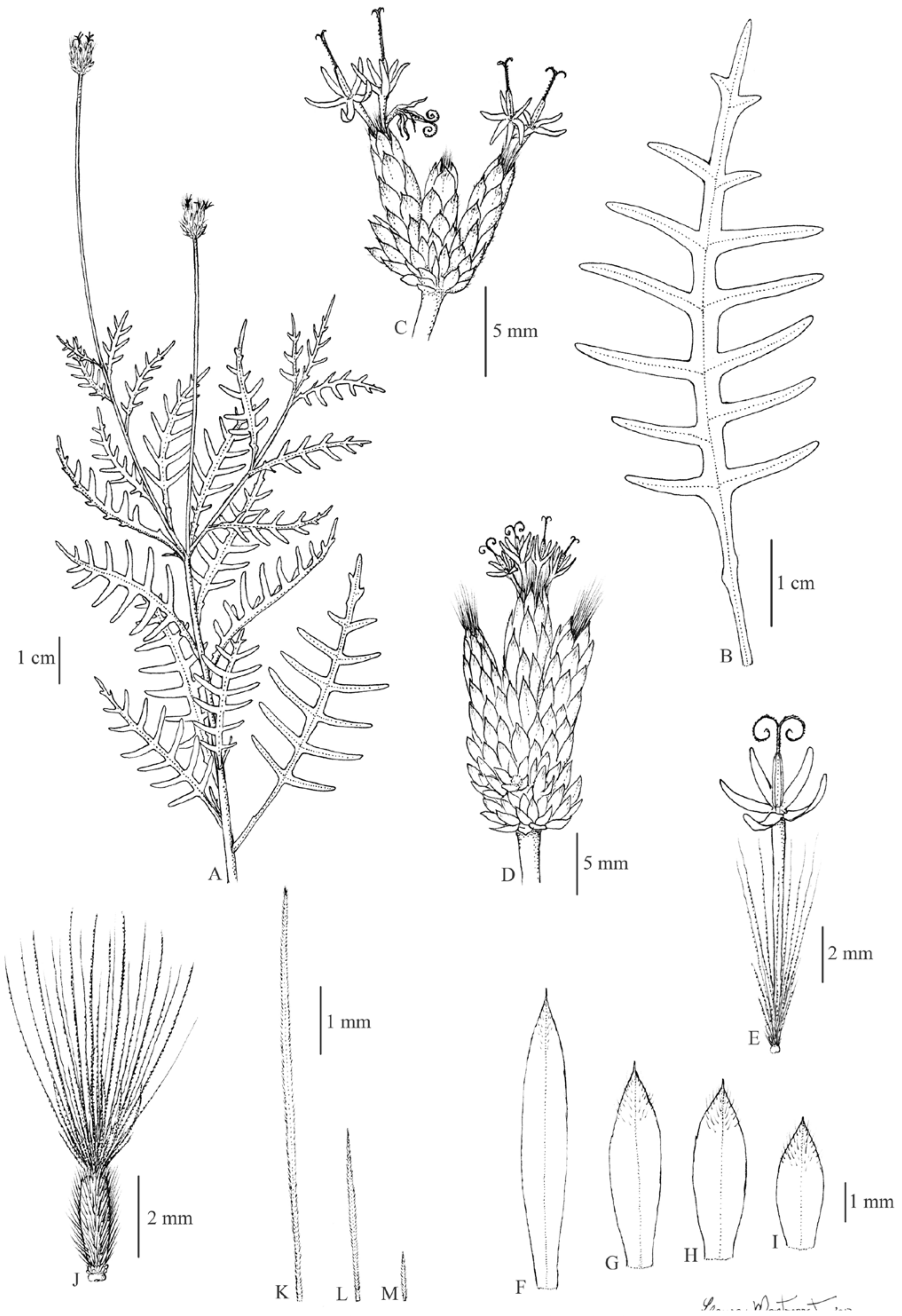

Figure 1: *Chresta artemisiifolia.* A. Habit with inflorescences. B. Adaxial surface of leaf. C–D. Syncephalia. E. Floret with pappus. F–I. Phyllaries from most internal to most external. K–M: Pappus series, from most internal to most external. J. Cypsela.

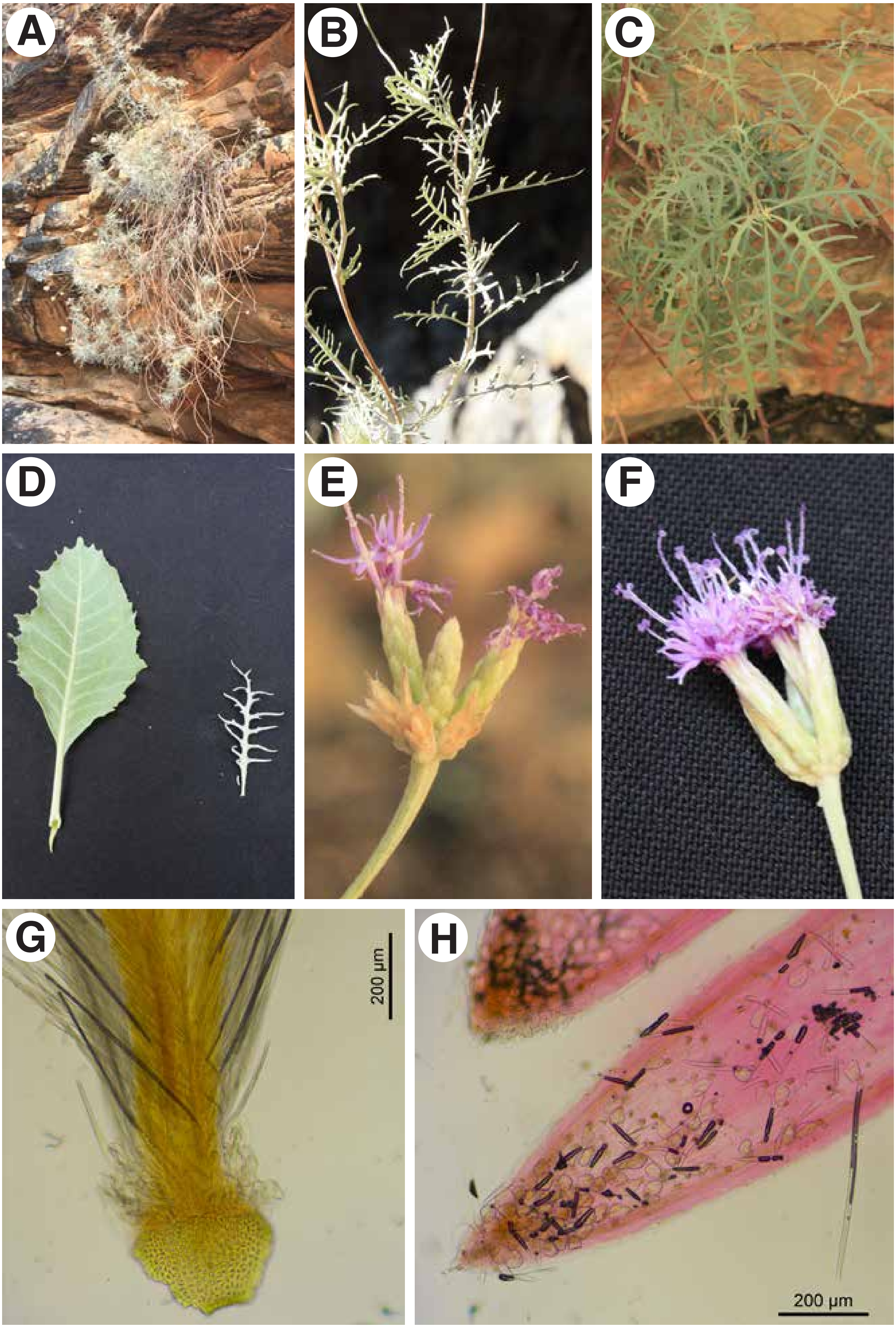

Figure 2: *Chresta artemisiifolia.* A. Habit. B–C. Details of branches showing the leaves with constricted blades. D. Comparison between leaves of C. martii (left) and C. artemisiifolia (right). E–F. Syncephalia with open florets. G. Microscopic section showing the cells of the carpopodium wall, the glands above the carpopodium and the twin-hairs. H. Microscopic section showing the tips of the petals, with short, simple trichomes and globular glandular trichomes.

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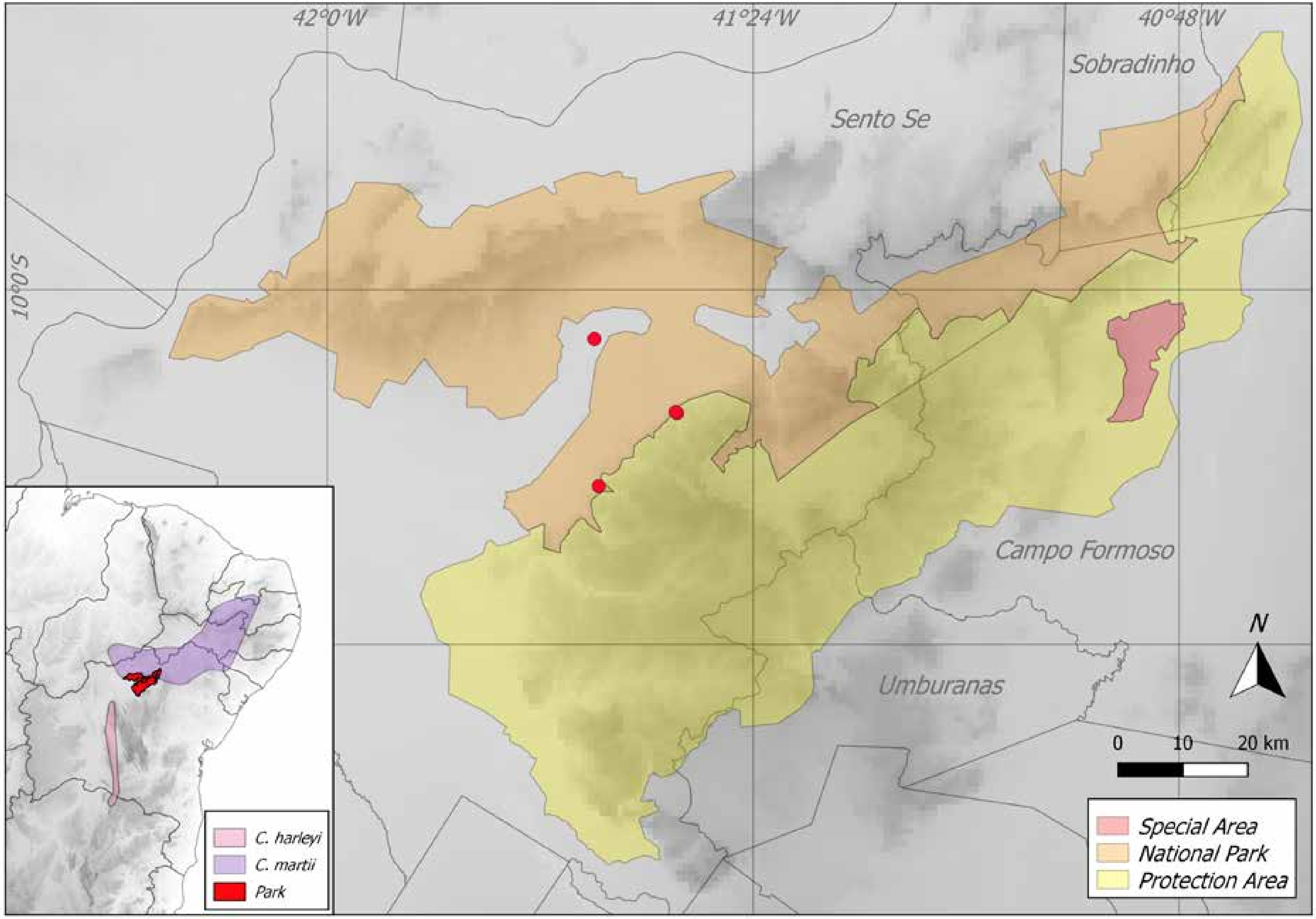

Figure 3: Known distribution of *Chresta artemisiifolia* in relation to the proposed area of the mosaic of conservation units in Boqueirão da Onça. The red circles represent the records of *C. artemisiifolia.* The detail on the bottom left represents the distribution of *C. harleyi* and *C. martii* in relation to the proposed area of the park. The known population of *C. hatschbachii* is included in the shadowing representing *C. harleyi.*

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Final Conclusions and List of Publications

Final Conclusions

In the last decades, phylogenetic studies have been used to study the evolution of morphological characters and biogeographical history of different groups, greatly increasing the knowledge about the current biodiversity and the evolutionary processes that acted on them. As one of the richest families of Angiosperms, Asteraceae is an important target for these studies, as the understanding of how this diversity came to be can also increase our knowledge about the diverse environments where they occur, and about how shifts in morphology can boost diversification.

The tribe Vernonieae is very diverse in Brazil, especially in drier environments, like the Cerrado, *campos rupestres*, rock outcrops and in the Caatinga. *Chresta* is a remarkable member of the tribe, found on the Caatinga, Cerrado and Mata Atlântica, presenting from widely distributed species like *C. scapigera* and *C. sphaerocephala,* to narrow endemics like *C. hatschbachii* and *C. souzae.* The morphological variety found in the group, expressed in different combinations of characters like leaf morphology, pattern of synflorescence branching, syncephalium morphology and growth, floret color and pollen type, has led to different interpretations of its taxonomy, with the species being kept in different genera (e.g. Candolle 1836, MacLeish 1984, 1985a, b) or united in the same genus (e.g. Gardner 1842, Baker 1873, Robinson 1999). The relationships of *Chresta* to other Vernonieae genera has also been a point of discussion, with the genus being grouped in the Lychnophorinae (e.g. Baker 1873) or as part of *Vernonia* (Lessing 1831) or even as a separate tribe (e.g. Robinson 1999), due to the unusual combination of characters like syncephalia, typical of Lychnophorinae, with the pollen type more commonly found in other Vernonieae. Previous phylogenies did not elucidate the position of *Chresta* in Vernonieae with certainty, due to a lack of resolution and support (e.g. Keeley et al. 2007, Loeuille 2015).

With the present study, we obtained a well resolved and supported phylogeny of *Chresta*, using Phylogenomics methods that provide hundreds of markers (Mandel et al. 2014), and used the phylogeny as a basis to study the evolution of the characters and biogeography in the genus. We verified that all taxonomic treatments that separated *Chresta* species in multiple genera relied in non-monophyletic groupings, thus supporting *Chresta* as a single unit. Also, most of the characters used to divide these genera, like the ramification of the synflorescence or floret color, actually arose multiple times in the phylogeny or suffered reversals. Also, we recovered a biogeographical history depicting an early diversification of *Chresta* in the Caatinga area with posterior expansion into the adjacent Cerrado.

We also used five rupiculous, isolated and fragmentary species of *Chresta* from the Caatinga to study microevolutionary processes that may be underway in their populations, aiming to delineate general patterns of diversification. We found that although these species share several similarities in morphology and environmental requirements, they differ in their genetics, varying in the degree of isolation between populations and intensity of effects of genetic drift and inbreeding. These results have implications on conservation strategies for these species, as they may require different strategies to be protected.

In order to summarize the knowledge obtained during these studies, we also present an illustrated synopsis of the genus, proposing a new infrageneric classification based on the clades obtained in the phylogeny. We propose a wide circumscription for *Chresta,* containing eighteen species divided into two subgenera and three sections, defined by molecular synapomorphies and morphological characteristics.

With this study, we hope to contribute to the general knowledge of Asteraceae in Brazil and also to the understanding of the evolution and history of the plant biota of the phytogeographical domains that compose the Brazilian landscape. We also expect that our results will serve as a model for the incorporation of new methods and approaches to phylogenetic studies.

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