



UNIVERSIDADE DE BRASÍLIA
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BOTÂNICA

Contribuição à conservação de *Chamaecrista fulgida* Barneby, uma espécie ameaçada do
Cerrado

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Cerrado

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RESUMO

O Cerrado é um dos biomas mais biodiversos e ameaçados do Brasil, porém há poucas informações detalhadas sobre as suas espécies de plantas ameaçadas. A espécie *Chamaecrista fulgida* Barneby, endêmica da Chapada dos Veadeiros, é atualmente classificada como criticamente em perigo (CR) B1ab(iii) pela IUCN, devido à distribuição restrita, fragmentação e perda de habitat por conversão agrícola. Este estudo teve como objetivo atualizar a distribuição geográfica da espécie e estimar sua Extensão de ocorrência (EOO) e Área de Ocupação (AOO) a partir dos novos registros; avaliar a estrutura populacional de quatro populações encontradas; definir os agentes de ameaça à espécie; reavaliar seu status de ameaça; e desenvolver protocolos de conservação *ex situ* a partir de experimentos de germinação de sementes. Foram realizadas expedições de campo para registro georreferenciado das populações e quantificação dos indivíduos por classes de diâmetro a altura do solo (DAS) e altura (Ht). A estimativa da perda de habitats foi realizada por meio da análise temporal de mapas de uso do solo disponibilizada pelo MAPBIOMAS, comparando a cobertura vegetal entre 1985 e 2023, permitindo quantificar modificações em áreas nativas para agricultura e outras alterações no uso do solo. Nos experimentos de germinação, as sementes foram submetidas a diferentes tratamentos de germinação (água quente, dessecação em sílica gel, dessecação + congelamento à -8°C) e monitoradas quanto à porcentagem de germinação, ao Índice de Velocidade de Germinação (IVG) e ao Tempo Médio de Germinação (TMG). O IVG indica a velocidade com que as sementes germinam, enquanto o TMG representa o tempo médio necessário para a germinação completa das sementes. A espécie apresentou EOO de 1.650,11 km² e AOO de 36 km². A análise temporal entre 1985 e 2023 indicou perda de 198,78 km² de vegetação nativa (11,32%) em sua EOO. Foram amostradas quatro populações, totalizando 157 indivíduos maduros, 86 juvenis e 31 plântulas, indicando recrutamento ativo e elevada abundância de indivíduos. Vestígios de fogo recente foram detectados em três populações, porém, as plantas demonstraram resiliência a esse distúrbio mediante a rebrota basal. Porém, a alta frequência do fogo pode comprometer a estrutura populacional, sendo este classificado como um dos principais agentes de ameaça à espécie. Testes de germinação mostraram dormência tegumentar (controle: $37,9 \pm 8,4\%$; IVG $2,83 \pm 0,81$; TMG $7,98 \pm 1,06$ dias), melhor quebrada com água quente ($54,0 \pm 11,5\%$ germinação; IVG $4,74 \pm 1,35$; TMG $4,67 \pm 1,47$ dias). A dessecação em sílica gel e o congelamento reduziram a germinação ($13,4 \pm 4,1\%$ e $24,9 \pm 16,8\%$, respectivamente) e

afetaram TMG e IVG. Esses resultados indicam que a água quente seria um método eficaz para superar a dormência tegumentar das sementes de *C. fulgida*. Apesar das sementes terem apresentado tolerância à dessecação e congelamento, houve redução nas taxas de germinação, sugerindo que testes adicionais são necessários para aprimorar o protocolo para conservação *ex situ* a longo prazo. Com base nos dados obtidos nesse trabalho, propõe-se a atualização do status de ameaça para em perigo (EN) B1ab(iii). Este trabalho fornece subsídios para aprimorar estratégias de conservação *in situ* e *ex situ*, contribuindo para a preservação de *C. fulgida* e da flora endêmica do Cerrado.

Palavras-chave: Biodiversidade, endemismo, germinação, lista vermelha, estrutura populacional

ABSTRACT

The Cerrado is one of the most biodiverse and threatened biomes in Brazil, yet there is limited detailed information on its threatened plant species. The species *Chamaecrista fulgida* Barneby, endemic to the Chapada dos Veadeiros, is currently classified as Critically Endangered (CR) B1ab(iii) by the IUCN due to its restricted distribution, fragmentation, and habitat loss caused by agricultural conversion. This study aimed to update the geographic distribution of the species and estimate its Extent of Occurrence (EOO) and Area of Occupancy (AOO) based on new records; assess the population structure of four identified populations; identify the main threats to the species; re-evaluate its conservation status; and develop ex situ conservation protocols through seed germination experiments. Field expeditions were conducted to georeference the populations and quantify individuals by diameter at soil height (DAS) and plant height (Ht). Habitat loss was estimated using land use maps provided by MAPBIOMAS, comparing vegetation cover between 1985 and 2023, allowing quantification of modifications from native areas to agriculture and other land-use changes. In the germination experiments, seeds were subjected to different treatments (hot water, silica gel desiccation, desiccation + freezing at -8°C) and monitored for germination percentage, Germination Velocity Index (IVG), and Mean Germination Time (TMG), with IVG indicating the speed at which seeds germinate and TMG representing the average time required for complete germination. The species showed an EOO of 1,650.11 km² and an AOO of 36 km², and temporal analysis between 1985 and 2023 indicated a loss of 198.78 km² of native vegetation (11.32%) within its EOO. Four populations were sampled, totaling 157 mature individuals, 86 juveniles, and 31 seedlings, indicating active recruitment and high individual abundance. Evidence of recent fires was detected in three populations; however, the plants demonstrated resilience through basal resprouting, although frequent fires may compromise population structure, being classified as one of the main threats to the species. Germination tests showed seed coat dormancy (control: $37.9 \pm 8.4\%$; IVG 2.83 ± 0.81 ; TMG 7.98 ± 1.06 days), which was best overcome with hot water treatment ($54.0 \pm 11.5\%$ germination; IVG 4.74 ± 1.35 ; TMG 4.67 ± 1.47 days), while silica gel desiccation and freezing reduced germination ($24.9 \pm 16.8\%$ and $13.4 \pm 4.1\%$, respectively) and affected both TMG and IVG. These results indicate that hot water is an effective method to overcome seed coat dormancy in *C. fulgida*. Although the seeds showed tolerance to desiccation and freezing,

germination rates were reduced, suggesting that additional tests are needed to improve long-term ex situ conservation protocols. Based on the data obtained in this study, an update of the species' threat status to Endangered (EN) B1ab(iii) is proposed. This work provides support for improving in situ and ex situ conservation strategies, contributing to the preservation of *C. fulgida* and the endemic flora of the Cerrado.

Keywords: Biodiversity, endemism, germination, Red List, population structure

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APRESENTAÇÃO

As transformações das paisagens naturais dos biomas são um problema de crescimento exponencial ao longo dos anos. Tal fato pode suceder em decorrência de alterações que ocorrem naturalmente no ambiente, porém, em sua grande maioria, são gerados a partir de intervenções antrópicas, como o crescimento desenfreado da agricultura, pecuária e urbanização, bem como o uso indevido do fogo (KLINK & MACHADO, 2005).

No Cerrado, em especial, o aumento das atividades antrópicas tem sido um dos principais causadores de perda de habitat e fragmentação de áreas naturais, a expansão agrícola em especial (COLMAN et al., 2024). Esse tipo de atividade tende a causar perturbações severas, que podem a longo prazo prejudicar diretamente espécies da fauna e flora do bioma. E, por se tratar de um bioma rico em biodiversidade e ser caracterizado por áreas de grande endemismo, tais perturbações podem ocasionar a extinção de espécies de ocorrência local, colocando assim em risco o equilíbrio do ecossistema, gerando prejuízos imensuráveis, visto que espécies raras e endêmicas tendem a ser mais vulneráveis às atividades antrópicas (MANES et al., 2021). Esse é um problema cada vez mais atual, visto que o Cerrado perdeu ao longo de 40 anos 28% de seu território natural, o que corresponde a uma perda de cerca de 40,5 milhões de hectares (MAPBIOMAS, 2025), um valor alarmante quando se almeja a conservação de áreas nativas.

Diante disso, promover estudos que busquem desenvolver estratégias de conservação de espécies raras e endêmicas é essencial para que se evite a extinção das mesmas (QAZI et al., 2025). Ademais, programas dedicados à conservação do meio ambiente são importantes para o desenvolvimento dessas estratégias, levando em consideração não somente as espécies-alvo, como também buscando um entendimento mais amplo sobre seus locais de ocorrência. Uma das principais iniciativas globais para espécies ameaçadas é a Lista Vermelha de Espécies Ameaçadas, criada pela IUCN - International Union for Conservation of Nature (IUCN 2024). Essa iniciativa busca catalogar globalmente espécies de fauna, flora e fungos, as classificando em níveis de ameaça, para que possam ser estudadas e manejadas de forma eficaz, visando reduzir significativamente os riscos de extinção. Outro exemplo, de âmbito nacional, são os PAN - Planos de Ação Nacional para a Conservação de Espécies Ameaçadas de Extinção, uma estratégia de gestão, desenvolvida em âmbito multidisciplinar, que busca por ordenar e definir uma lista de prioridades para promoção da conservação da biodiversidade, bem como dos seus habitats naturais (ICMBIO, 2025). Esse tipo de ação envolve não somente organizações

governamentais, como também a sociedade civil, trazendo informações e dados sobre seus estudos, promovendo a conscientização da comunidade a respeito da importância da conservação dos ambientes naturais, o que torna essas ações mais efetivas e transparentes.

Posto isso, a espécie *Chamaecrista fulgida* Barneby, endêmica da Chapada dos Veadeiros, adicionada à Lista Vermelha de Espécies Ameaçadas da IUCN em 2020 (FERNANDEZ et al., 2020) e na Lista Oficial de Espécies da Flora Brasileira Ameaçadas de Extinção (PORTARIA MMA Nº 148, 2022) com o status de Criticamente em Perigo (CR), foi escolhida como objeto do presente estudo. Os principais objetivos da pesquisa foram mapear suas populações conhecidas, buscar por possíveis novas populações, avaliar a estrutura populacional da espécie, analisar uma possível mudança em seu status de ameaça e desenvolver estratégias de manejo e conservação, tanto *in situ* quanto *ex situ*, para mitigar as ameaças à sua sobrevivência e promover sua sustentabilidade a longo prazo.

Para isso, a metodologia utilizada foi dividida em duas principais linhas. A primeira linha consistiu no levantamento de dados para aprimorar o conhecimento sobre a distribuição geográfica da espécie, a estrutura populacional e a perda de habitat ao longo do tempo, com a coleta inicial de informações em herbários e levantamentos em campo, onde foi feito um estudo de estrutura populacional e coleta de sementes. A segunda linha consistiu no desenvolvimento de um protocolo de conservação da espécie com base em experimentos de germinação de sementes, os quais avaliaram a sua capacidade de armazenamento em baixas temperaturas. Essas informações levantadas foram de extrema importância, não somente para um melhor entendimento da distribuição, estrutura populacional, germinação e propagação da espécie, como também para definir melhores estratégias para sua conservação a longo prazo.

Dos resultados obtidos quanto à distribuição da espécie, que apresentava anteriormente somente três registros de ocorrência, foram encontrados três novos adicionais. Dos dez registros totais, quatro populações foram amostradas, a fim de melhor entender sua estrutura populacional, bem como identificar possíveis agentes de ameaça à espécie. Dos dez registros totais, três estão inseridos em Unidades de Conservação (UC), enquanto outros ocorrem em áreas de uso tradicional, como o Território Quilombola Kalunga, o que também pode contribuir para a conservação da espécie. Apesar de uma das áreas não estar dentro de uma UC, esta mostrou-se bem preservada, demonstrada pela abundância de indivíduos em diferentes estágios de crescimento no local. O compilado dessas informações foi de suma importância para reavaliar o

status de ameaça em que a espécie se encontra, atualmente CR. Após análise dos dados obtidos, levando em consideração os critérios de avaliação da IUCN, concluiu-se que a espécie pode ser reavaliada no status Em Perigo (EN), visto que sua extensão de ocorrência conhecida aumentou significativamente de 60,7 km² para 2.221 km².

Apesar de se tratar de uma espécie endêmica e com especificidade de habitat, ocorrendo em cerrado rupestre e apresentado preferência por afloramentos rochosos, a *C. fulgida* apresentou uma elevada abundância em suas populações (média de 98,5 indivíduos por hectare) com variação de densidade dos diferentes estágios de crescimento dentro das populações, o que indica ocorrência de reprodução e recrutamento adequados, importantes métricas para um bom estabelecimento e perpetuação da espécie em seus locais de ocorrência. A perda de habitat na extensão de ocorrência da espécie ao longo de 38 anos foi de 183,78 km² em EOO, correspondendo a aproximadamente 8,32% da área total e 1,08 km² em AOO, correspondendo a aproximadamente 2,72% de perda da área total, em sendo importante destacar que as áreas de ocupação não foram significativamente afetadas. Embora seja endêmica do Cerrado, sua ocorrência em cerrado rupestre reduz os riscos associados a atividades antrópicas, por se tratarem de locais pouco favoráveis para o cultivo ou criação de ruminantes.

Para os testes de germinação, foi observada uma dormência discreta em algumas sementes. Esse padrão é comum em representantes da família Leguminosae, incluindo diversas espécies endêmicas do Cerrado, representando uma estratégia evolutiva de propagação. A dormência garante que, durante longos períodos de estiagem, a viabilidade das sementes seja preservada, permitindo que a germinação ocorra somente após a chegada do próximo período chuvoso. Após os testes de quebra de dormência, a melhor resposta da espécie foi para o tratamento com água quente, com porcentagem de germinação média $\pm$ desvio padrão de $54,0 \pm 11,5\%$. Para o controle, obteve-se $37,9 \pm 8,4\%$ de média. Para as sementes secas em sílica gel por 9 dias a 7,0% de umidade, os resultados foram de taxas de germinação de $13,4 \pm 4,1\%$. O declínio da germinação e vigor após a secagem em sílica gel pode indicar uma baixa tolerância da semente ao processo de dessecação. Para as sementes submetidas à dessecação em sílica gel seguido de congelamento, a média de germinação foi de $24,9 \pm 16,8\%$, o que indica uma sensibilidade à secagem e ao armazenamento. Esses resultados ilustram a variabilidade germinativa quando as sementes de *C. fulgida* são submetidas a diferentes pré-tratamentos de germinação, destacando ainda que as sementes possuem dormência e, essa, é superada através do

choque térmico. Destaca-se ainda que as sementes aparentemente não toleram bem dessecação e congelamento, procedimentos padrão para depósito em banco genético. Portanto, são necessários ajustes no protocolo de dessecação e armazenamento, visando maior eficiência para conservação *ex situ* da espécie.

Dessa maneira, o presente estudo reúne relevantes informações sobre a distribuição geográfica, abundância populacional e comportamento germinativo de *Chamaecrista fulgida*, contribuindo para um melhor entendimento da espécie e fornecendo subsídios para ações de conservação do Cerrado. Os resultados do presente estudo são apresentados a seguir, na forma de artigo científico redigido em inglês.

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Contribution to the Conservation of *Chamaecrista fulgida* Barneby, a Threatened Cerrado Species

1. INTRODUCTION

Plant species play an important role in providing ecosystem services, such as climate regulation, maintenance of the hydrological cycle, and soil protection (PÖRTNER et al., 2023; MAHECHA et al., 2024). However, despite their ecological relevance, the conservation of plant species has been increasingly compromised by threats due to anthropogenic actions, resulting in the reduction of natural populations, loss of genetic diversity, and increased risk of extinction. Recent studies indicate that a large proportion of species are threatened with extinction globally, mainly due to habitat destruction and fragmentation, changes in land use, and other anthropogenic activities that directly affect natural structures (WWF, 2020).

In Brazil, a megadiverse country with exceptionally rich flora, approximately 20% of natural areas had already been lost by 1985 (MAPBIOMAS, 2023). Over the following 39 years, an additional 13% — equivalent to about 110 million hectares — disappeared by 2023, resulting in a total reduction of 33% of the country's natural areas. This loss of native vegetation has been concentrated mainly in the Amazon and the Cerrado, increasing pressure on biodiversity and reinforcing the urgency of conservation strategies to prevent the extinction of native species.

The Cerrado is considered the second largest Brazilian biome, covering about 21% of the national territory (KLINK & MACHADO, 2005), second only to the Amazon Rainforest, presenting different phytophysognomies that contribute to its great variety of species. Total annual rainfall varies between 800 and 2,000 mm, with seasonality marked by two well-defined seasons, dry and rainy, with the months of May to September marked by drought and November to March by precipitation, with April and October being the transition months (RIBEIRO & WALTER, 2008). It is also an important region for the conservation of the world's biodiversity (MYERS et al., 2000). This biome, in addition to being crucial for the conservation of global biodiversity, has the greatest diversity of flora globally among tropical savannas, with about 12,829 species of vascular plants, including approximately 4,500 endemic species (BFG, 2022).

Among the areas of endemism in the Cerrado, high altitude locations (>1000 m) and rocky soils stand out, which are home to a rich and unique flora (GIULIETTI & PIRANI, 1988).

Because it encompasses a wide diversity of phytophysiognomies, the Cerrado is classified as a mosaic of vegetation types (COUTINHO, 2006). The nutrient-poor soil characteristic of the biome is not an impediment to monoculture planting, where corrections with limestone and fertilizers are commonly made, contributing to agricultural planting and making the Cerrado the one of the last frontiers of agriculture in the world (BORLAUG, 2002). The suppression of native vegetation in the Cerrado between 1985 and 2023 was 38 million hectares, representing 27% of the total, with 9.7 million hectares, about 10% of the biome, being covered by secondary vegetation (MAPBIOMAS, 2025). The degradation of natural habitats in the Cerrado threatens the survival of numerous endemic species due to the increasing transformation of this biome into agricultural areas (STRASSBURG et al., 2017). This process has resulted in the occupation of Cerrado native areas by agriculture (47%) and pastures (54%) (ALENCAR et al., 2020).

The various transformations in the biome resulting from anthropogenic activities have serious environmental consequences, such as habitat loss and fragmentation, aquifer pollution, extinction of native species, and the proliferation of exotic species that compete with native species (KLINK & MACHADO, 2005). This damage directly affects the dynamics of vegetation regeneration and succession, altering ecological dynamics and threatening the survival of several endemic species. The reduction of native areas associated with unsustainable human activities has resulted in a high number of species at risk of extinction in the Cerrado (STRASSBURG et al., 2017).

Thus, to mitigate the damage caused by habitat loss and fragmentation in the biome, several conservation strategies have been implemented. For example, the National Action Plan for the Conservation of Endangered Flora in the Upper Tocantins Basin (PAN Bacia do Alto Tocantins), made official in 2023 and valid until 2028, is an important initiative in this process. This plan aims to expand, over five years, effective actions for the conservation of 98 threatened target species, the environment, and the ecosystem services provided in the biome, in partnership with civil society (VERDI & OLIVEIRA, 2025).

Among the most widely distributed families in the Cerrado, the Fabaceae family stands out, one of the largest among Angiosperms, with worldwide geographical distribution. This

family comprises about 650 genera and approximately 18,000 species, most of which have significant economic value (LPWG, 2017). In Brazil, there are about 200 genera and 1,500 species registered, representing a significant portion of the national flora (MORIM et al., 2024). The group includes species with arboreal, shrubby, and herbaceous characteristics, the latter being associated with agricultural crops of great economic potential, such as beans (*Phaseolus vulgaris*) and soybeans (*Glycine max*) (CARVALHO, 2021). Rare and endangered species are also common in the family, and these, in turn, are more vulnerable to habitat loss resulting mainly from anthropogenic activities, such as agricultural expansion, as well as misuse of fire (MARTINELLI, et al. 2014). Given this scenario, the genus *Chamaecrista* stands out as the second richest in the family in Brazil, with 268 species, of which 83% are endemic (MORIM et al., 2024), including 18 species classified as threatened to some degree (vulnerable, endangered, or critically endangered) (MMA, 2022). In addition, the genus has been the subject of recent research seeking to promote conservation strategies. PIZZARDO et al. (2024), for example, used *Chamaecrista* species endemic to rocky fields in an analysis that integrated geographic distribution and phylogenetic uniqueness, allowing for the development of more effective conservation strategies for the genus.

In this context, the species *Chamaecrista fulgida* Barneby stands out, a small tree or shrub endemic to Chapada dos Veadeiros in Goiás, described in 1999 (BARNEBY, 1999). It was evaluated and included in the IUCN Red List of Threatened Species (FERNANDEZ et al. 2020), as well as in the Official List of Brazilian Flora Species Threatened with Extinction (PORTARIA MMA Nº 148, 2022), being classified as Critically Endangered (CR). As it is an endemic species, restricted to a specific region of Goiás, there are few studies that address basic information about the species, noting that its with few collection records are also scarce (SPECIESLINK, 20264), there is an urgent need for studies on basic aspects of this species.

The species occurs in areas of rocky savanna, characterized by elevations above 800 meters, with nutrient-poor, acidic lithosols with low levels of organic matter, resulting from the decomposition of quartzites (PEREIRA & FERNANDES, 2022; RIBEIRO & WALTER, 2008). The vegetation of this phytophysognomy includes species typical of the Cerrado savannas, but it also stands out for the occurrence of restricted species due to the variety of local microhabitats, such as rocks, crevices, and fissures, where rare species may occur, including those threatened

with extinction (FERNANDES et al., 2020). Despite its endemism and rarity, studies on *C. fulgida* are still scarce, which hinders the development of effective conservation actions that could prevent its extinction. For example, the density of individuals in its populations is unknown, and there is no information on the long-term conservation potential of its seeds in *ex situ* collections.

Understanding the demographic dynamics of endangered species populations is essential for developing effective conservation strategies. These studies allow us to assess whether populations are declining or remaining stable, as well as to identify which stages of the species' life cycle require greater attention in order to optimize management actions and ensure their long-term survival and sustainability (BURMEIER & JENSEN, 2009; LEIMU, 2010). Analysis of plant population structure provides information on the number of individuals, ontogenetic stages (seedlings, juveniles, and adults), and their distribution in categories such as diameter and height classes (FELFILI et al., 2004). However, detailed population studies have never been conducted for the vast majority of threatened species, and risk assessments are mostly restricted to geographic distribution data (IUCN criterion B) due to the scarcity of demographic information on species (MACE et al., 2008; AUBUCHON-ELDER et al., 2022).

Given this scenario, the International Union for Conservation of Nature (IUCN), created in 1948, plays a critical role in classifying species based on their risk of extinction, serving as an essential tool for prioritizing conservation actions (IUCN, 2022). These assessments are widely used and employed in the implementation of monitoring strategies (YOUNG et al., 2014; BROOKS et al., 2016). The criteria adopted by the IUCN are based on five main categories (A–E), which consider, among other factors, population decline, population size, risk of extinction, extent of occurrence (EOO), and area of occupancy (AOO). However, most global assessments are based on parameters related to geographic distribution, especially extent of occurrence, due to the scarcity of robust demographic data on the species being assessed (MACE et al., 2008).

There are different approaches to flora conservation, such as *in situ* conservation, which seeks to maintain species in their natural habitats, and *ex situ* conservation, which involves maintaining plants outside their places of origin (ASHTON, 1987; CONWAY, 1988). *Ex situ* conservation complements *in situ* conservation, since the storage of seed samples for later testing

and conservation in cold chambers contributes to a better understanding of their germination and propagation, with a view to their reintroduction into nature (HEYWOOD & IRIONDO, 2003). Seed storage is the most viable method for *ex situ* conservation, as it is economical and simple to perform. Proper storage allows the physical and, consequently, genetic structures of seeds to be preserved (IRIONDO & PÉREZ, 1999), promoting a higher success rate in germination and perpetuation of species.

Therefore, given the lack of specific studies on the species and considering its restricted distribution in areas that are undergoing constant loss of native vegetation cover, this study aims to contribute to the conservation of *C. fulgida*. More specifically, it aims to map known populations, identify new populations, analyze threat and habitat loss, evaluate the structure of selected populations, and develop management and conservation strategies, both *in situ* and *ex situ*, to reduce threats to its survival.

The main questions addressed in this study were: 1) What is the geographical distribution of the species? We expect that, although currently restricted to the Chapada dos Veadeiros region, new records will provide a more accurate understanding of the species' distribution. 2) What is the demographic structure of *C. fulgida* populations? It is expected that the analysis of the population structure will reveal viable populations with evidence of young individuals and recruitment. 3) What are the main threats to the species? Anthropogenic pressures are expected to be the main risk factor, as it is an endemic species of the Cerrado located in a region of growing agricultural expansion. 4) Do the seeds of the species have potential for *ex situ* conservation? As it belongs to the Fabaceae family, it is assumed that the seeds are orthodox, a physiological characteristic that favors long-term storage and expands the possibilities for *ex situ* conservation strategies.

2. METHODOLOGY

2.1 Study area

The study areas are located in Chapada dos Veadeiros, in the municipalities of Cavalcante and Alto Paraíso de Goiás, in the northeast of the state of Goiás. This region is recognized for its high biodiversity and endemism, which makes it important for the preservation of the Cerrado

biome (SOARES et al., 2025). In this region, there are federal, state, and municipal conservation units (UCs), as well as private reserves, which aim to protect and conserve the rich local biodiversity, including the endemic flora and fauna of the biome (LIMA & FRANCO, 2013). An example is the Chapada dos Veadeiros National Park, an important conservation unit created in 1961 by Decree No. 49,875 (BRASIL, 1961), currently covering an area of 240,586.56 hectares after a recent expansion (BRASIL, 2018).

The region's climate follows the typical pattern of the Cerrado biome and is classified as tropical Aw, according to KOPPEN's classification (1948). In terms of precipitation, with an annual average of 1,500 mm, ranging from 750 to 1,700 mm, there are two well-defined seasons, the wet season (November to March) and the dry season (May to September). The months of April and October are considered a transition between the two seasons. The average annual temperatures are around 22°C in the hottest months and around 19°C in the coldest months, according to data from the INMET (INMET, 2021) for the Alto Paraíso de Goiás climatological station.

2.2 Species studied

The species *C. fulgida* (Figure 1), defined as a small tree or shrub, is restricted to the municipalities of Cavalcante and Alto Paraíso de Goiás, located in the Chapada dos Veadeiros region (CNCFlora, 2024). It can be recognized by the growth habit and the resinous, lustrous dark green leaflets (Figure 1; BARNEBY). Due to its restricted distribution and the increasing deforestation and fragmentation of the Cerrado, the species was considered critically endangered (CR) B1ab(iii) with only three known records (IUCN, 2020; MMA, 2020).

This assessment was based exclusively on criterion B, which considers restricted geographic distribution combined with decline, fragmentation, or extreme fluctuations. Deforestation, although not directly measured, was mentioned as the main threats to the species' survival. At the time of its classification, its extent of occurrence (EOO) was only 60.792 km², while its area of occupancy (AOO) corresponded to 12 km², indicating high vulnerability (FERNANDEZ et al., 2020).

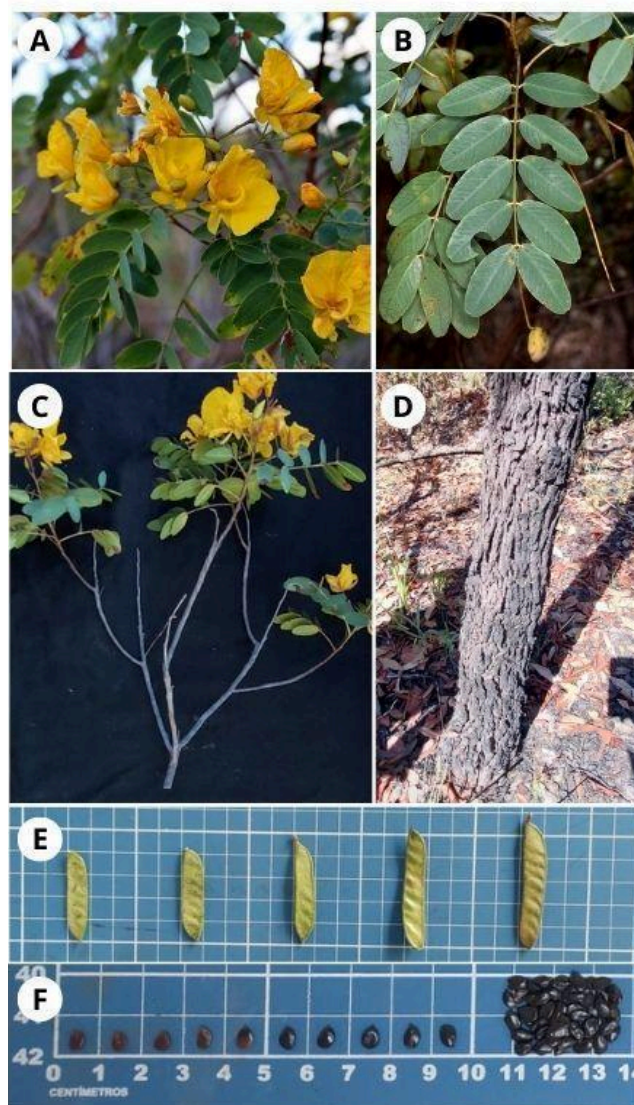


Figure 1 – *Chamaecrista fulgida*. (A) detail of flowers; (B) leaves with arrangement of leaflets; (C) fertile branch; (D) appearance of bark; (E) fruits; (F) seeds. Images: R. Arruda and M. Mercadante.

2.3 Mapping of *Chamaecrista fulgida* Populations

Mapping of areas where *C. fulgida* occurs was carried out using information obtained from virtual herbaria and specialized repositories, such as CRIA – Specieslink (2024), which provide data on specimens deposited in various national and international herbaria. From the labels on these samples, it was possible to obtain information on the known locations where the species has been recorded. In addition, field searches were conducted to identify possible new populations of the species in order to expand knowledge about its geographical distribution. Data collection was complemented by the use of georeferencing tools (GPS and GIS - Geographic

Information System) to map areas of occurrence and assist in the creation of a geospatial database.

2.4 Analysis of the Population Structure of *Chamaecrista fulgida*

Population data were collected from four populations between March and September 2025 through a census-type inventory, covering 100% of *C. fulgida* individuals present in the areas. The size of the sampling area varied among the inventoried populations and corresponded to patches of occurrence of the species delimited by habitat availability due to relief. These inventories made it possible to assess the abundance of the species in its habitat, as well as to identify the age structure of the individuals sampled. For each individual we recorded: location, which was recorded with geographical coordinates obtained by GPS (error 3-5 meters); diameter at ground level (DGL), measured with a digital caliper 15 cm above the ground; and height (Ht), using a telescopic ruler or tape measure. The individuals measured were marked with a numbered metal tag and classified into three different growth stages: seedling, when they were up to 15 cm tall; juvenile, when they were taller than 15 cm but showed no signs of reproductive development; and adult, when they showed signs of reproductive development.

2.5 Identification of threats to the species and habitat loss

As this is an endemic species that is on the red list of threatened species, it is necessary to identify the agents of threat. To this end, these were identified based on field observations, considering physical signs of stress, such as yellowed leaves, lesions on the bark, and the presence of dead individuals and xylophagous organisms. Risk factors such as fires, anthropogenic activities, invasive species, and local deforestation were also observed in the field. To investigate habitat loss within the species' geographic distribution we overlaid the EOO with land use data between 1985 and 2023 (the oldest and most recent data available) using the MAPBIOMAS science support platform (MAPBIOMAS, 2023a; 2023b). To estimate trends in natural vegetation loss and possible population loss of the species, the land cover classes found in MAPBIOMAS were divided into natural and anthropogenic (SCHINDLER et al., 2025). To assess the fire regime in the four surveyed populations, we used the MAPBIOMAS Fire Collection to calculate the Mean Fire Return Interval (MFRI). The MFRI was estimated based on the average number of fire events recorded across each 30 m pixel that comprised each sampled

population area from 1985-2023, dividing the total period analyzed, 38 years, by the number of fire occurrences recorded in each area. Thus, lower MFRI values indicate greater recurrence of fires, while higher values indicate a longer interval between fire events.

2.6 Update of Species Threat Status

Distribution data were used to reassess the threat status of *C. fulgida*. To conduct this reassessment, the demographic data collected, including population structure and density, as well as updated distribution and threat source data, were used in analyses based on criteria A, B, C, and D according to the Threat Classification Scheme (IUCN, 2022), such as population decline, extent of occurrence, small populations, very restricted distribution, and habitat loss and fragmentation. The calculation of the Area of Occupation (AOO) and Extent of Occurrence (EOO) was obtained using the GeoCat Program (BACHMAN et al., 2011), using a standard cell of 4 km² for the calculation of AOO and a convex polygon that encompasses all records of the species for EOO.

Criterion A was assessed based on indirect evidence of population decline, considering habitat loss and fragmentation in the areas where the species occurs. These data were obtained from the temporal analysis of land use and land cover (MAPBIOMAS, 2025), since historical population data are not available for the species. A generation length of 10 years was assumed (time from seed to reproductive adult), and the assessment was conducted over three generations (30 years), corresponding approximately to the time span covered by the MAPBIOMAS data which provides an indirect estimate of population reduction through habitat loss.

Criterion B was analyzed based on the known geographic distribution of the species. For this purpose, EOO and AOO were estimated from data obtained from surveys in herbaria and searches for new records of the species during field expeditions. Data on the degree of fragmentation and decline in extent or quality of habitat were also evaluated for this criterion, based on the loss of native vegetation based on the MAPBIOMAS historical series, as described above. The number of locations was defined according to the most serious plausible threat, following IUCN guidelines: occurrences were considered to belong to a single location when a threatening event could rapidly affect all individuals, and to multiple locations when such threats were unlikely to impact all occurrences simultaneously.

Criterion C was assessed based on population size, more specifically, estimates of the total number of mature individuals. The global population size was estimated from field observations combined with extrapolation of sampled areas using satellite imagery. Estimates were based on the density of mature individuals per hectare recorded in field plots, which was then extrapolated to the total occupied area by each population. The extent of populations was defined through field observations of suitable habitat, including areas adjacent to the four sampled plots. We then multiplied the area of suitable habitat of each population by the corresponding number of mature individuals per hectare to obtain the overall population size (number of mature individuals).

Criterion D was assessed based on the estimated total number of mature individuals, using the same approach described above. The global population size was derived from field observations and extrapolation of sampled areas using satellite imagery, considering the density of mature individuals per hectare and the total area of occupied habitat. These estimates were used to determine whether the species meets the thresholds for restricted population size.^{2.7}

Seed collection and characterization

The fruits of *C. fulgida* were collected between 1 and 23 of September in 2025, from a population located in Chapada dos Veadeiros National Park, during the physiological ripening period of the fruits. Collected fruits were stored in the Embrapa Cenargen Seed Laboratory, where they were processed, sorted, and used in seed viability tests. From collection to testing, the seeds were kept in brown paper bags at room temperature.

After processing, the seeds underwent a sorting process, in which malformed or damaged seeds were separated from those in good physical condition. H

Next, the healthy seeds were counted, separated into batches, and stored in brown paper bags for viability testing. Seed mass was estimated by weighing a batch of 50 seeds using a Shimadzu AUY 220 analytical balance. The dimensions of the seeds were determined in a batch of 50 seeds using a digital caliper with a precision of 0.05 mm.

To assess the average mass and moisture content of the seeds, three aluminum molds were weighed beforehand on a precision scale. Next, 30 seeds were separated and distributed in batches of 10 units per mold. In each tray, the seeds were cut in half with pliers, weighed, and

then placed in an oven at 105°C for 24 hours. After this period, they were weighed again to determine the loss of mass. Moisture content was calculated on a wet basis using the following equation, according to standardized methodologies described by the International Seed Testing Association (2023) and the Association of Official Analytical Chemists (2019):

$$\text{Humidity (\%)} = \frac{(\text{initial weight} - \text{final weight})}{(\text{initial weight})} * 100$$

Next, the average moisture percentage of the three forms was calculated to obtain the final moisture content of the seeds after drying in an oven at 105 °C for 24 hours.

2.8 Germination and *ex situ* conservation tests

2.8.1 Tests to define a protocol for long-term seed conservation

Tests were conducted to compare the germination potential of seeds subjected to procedures aimed at *ex situ* conservation. Before conducting the experiments, the seeds were subjected to a disinfestation process in a 5% sodium hypochlorite solution for 5 minutes, followed by three washes with distilled water for another 5 minutes each, except for the hot water treatment, which did not require disinfestation. The tests included: 1) control; 2) hot water; 3) drying in silica 1 + hot water; 4) drying in silica + freezing at -8°C for three months + hot water. Each treatment consisted of four replicates of 30 seeds each in Petri dishes with a double layer of filter paper soaked in distilled water and stored in a BOD Lucadema model Luca 161/03 at a temperature of 30°C, with a 12-hour photoperiod. The dishes were sealed with parafilm to prevent the entry of external agents. The experiment was observed daily for 30 days, with the seeds counted as germinated or dead.

2.8.2 Tolerance to Desiccation and Freezing

Drying in silica gel is essential to gradually reduce the moisture content of the seeds, preserving their health and preventing damage during the freezing process. This step is important to assess the seeds' ability to withstand storage at low temperatures. For this type of storage, the ideal moisture content is between 6 and 7%, with silica drying being the most efficient method to achieve these levels without compromising the integrity of the seed cells.

For the silica drying process, three aluminum trays were used, which were previously weighed and then filled with seeds: 30 units in tray 1, 125 units in tray 2, and 125 units in tray 3. After distributing the seeds, each tray was weighed again to obtain the initial mass. The trays were then placed in a Gerbox box containing silica gel and sealed with plastic wrap to prevent moisture from entering. The masses were monitored daily until stabilization, indicating the correct moisture content for proper storage.

After the mass stabilized, the seeds from form 1 were cut in half, weighed again, and dried in an oven at 105°C for 24 hours to determine the moisture content. The seeds in form 2 were subjected to dormancy breaking treatment using hot water that was heated to 100 °C, then removed from the heat source; after being added to the seeds, the water gradually cooled over time. The seeds in form 3 were stored in aluminum foil, sealed in a sealer, labeled, and placed in a freezer at a temperature of –8 °C for a period of 90 days (3 months) to subsequently assess preliminary viability after storage.

To determine the moisture content of the seeds after drying in silica gel, the following formula was used to calculate the percentage of each form:

$$Humidity (\%) = \frac{(initial\ weight - final\ weight)}{(initial\ weight)} * 100$$

Next, the average moisture percentage of the three forms was calculated to obtain the final moisture content of the seeds after drying in an oven at 105°C for 24 hours.

2.8.3 Germination metrics

The following germination metrics were calculated for each treatment:

Germination percentage (G)

The germination percentage was calculated for each plate based on the data obtained within the 30-days period in which the seeds were observed using the following formula, according to the International Seed Testing Association (2023):

$$G = \left(\frac{N*100}{t} \right)$$

where,

G = % germination;

N = number of germinated seeds;

t = total time in days.

Germination percentages were calculated per experimental unit (plate), considering only intact seeds. Predated seeds were excluded from the analyses. Seeds that remained ungerminated at the end of the experimental period and showed no clear signs of deterioration were not classified as dead.

To evaluate possible differences in seed germination between the treatments tested, a one-way analysis of variance (ANOVA) was performed, considering the treatments as a fixed factor. The analyses were based on the values obtained per experimental unit (plate), adopting a significance level of 5% ($\alpha = 0.05$). Once statistical significance was verified, the means were submitted to Tukey's test to identify which treatments differed from each other.

Average Germination Time (AGT)

The following formula was used to calculate the average germination time, according to LABORIAU (1983)

$$TMG = \frac{\sum_{i=1}^k n_i * t_i}{\sum_{i=1}^k n_i}$$

where,

n_i = number of seeds germinated each day t_i ;

t_i = evaluation time in days from the start of the experiment to observation i ;

k = total number of counts performed;

$\sum n_i$ = total number of seeds germinated at the end of the experiment.

Germination Speed Index (GSI)

The following formula was used to analyze the germination speed index, according to MAGUIRE (1962):

$$IVG = \sum \frac{n_i}{t_i}$$

where,

n_i = number of seeds germinated in time period i ;

t_i = time in days after the start of the test.

3. RESULTS

3.1 Updated geographic mapping of the species

The records obtained from herbarium databases, as well as the new records made, were organized according to their location, voucher, and coordinates (Table 1). Thirteen records with geographic coordinates were used to create an updated distribution map of the species (Figure 2). The AOO and EOO parameters were 40 km² and 2,221 km² for Extent of Occurrence (EOO), respectively. It was observed that, of the ten total records, three are located in legally protected areas, including Chapada dos Veadeiros National Park and the Serra do Tombador and Renascer RPPNs.

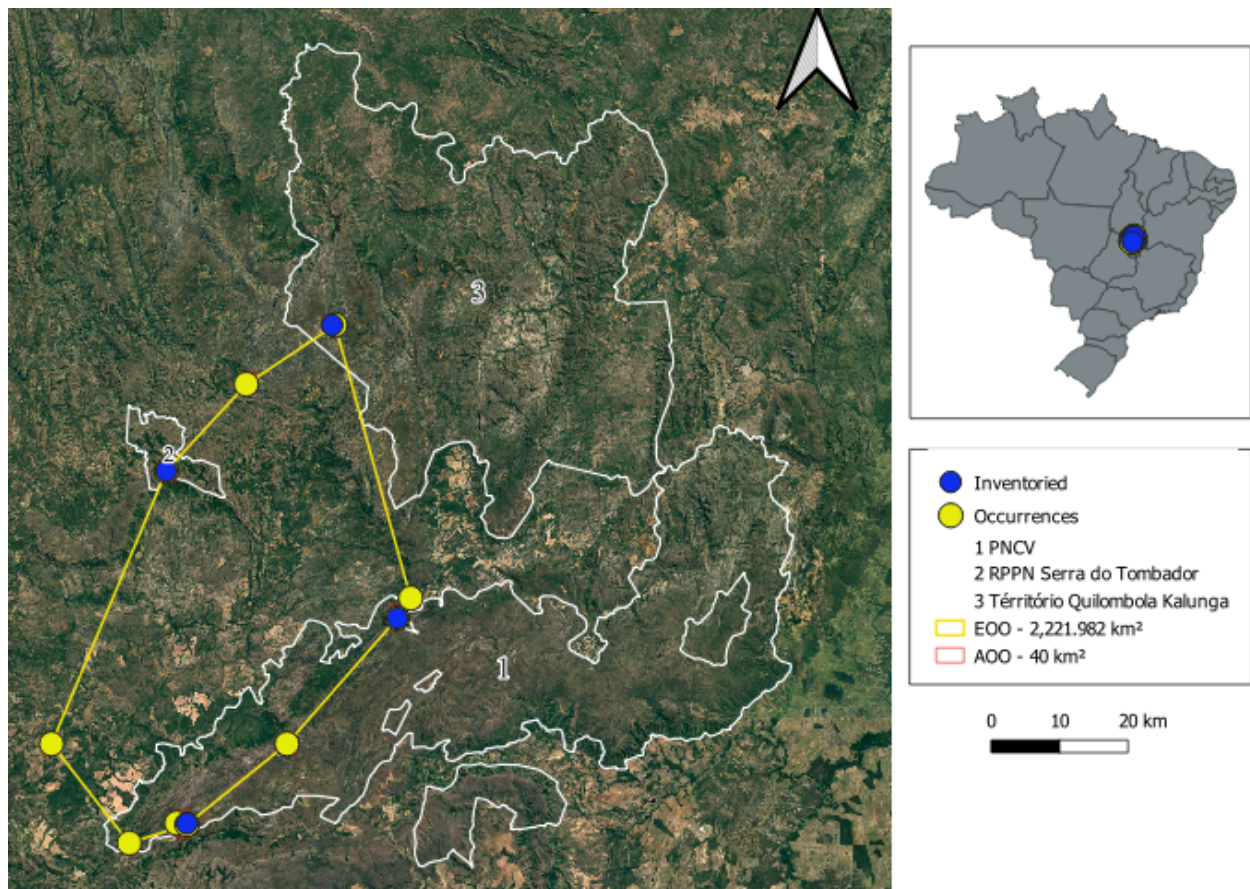


Figure 2 - Distribution map of *Chamaecrista fulgida*. The yellow dots represent herbarium records extracted from databases, and the blue dots represent locations where populations were inventoried in 2025. The white lines represent Conservation Units and Quilombola Territory: 1. Parque Nacional Chapada dos Veadeiros; 2. RPPN Serra do Tombador; 3. Território Kalunga .

Table 1 – Occurrence records of *Chamaecrista fulgida* based on herbarium specimens and recent records collected in this study.

Year	Municipality	Locality	Primary collector	Collector's number	Herbarium	Longitude	Latitude
1985	Alto Paraíso de Goiás	Chapada dos Veadeiros	ROMANIUC-NETO, S.	313	SP	-	-
1995	Alto Paraíso de Goiás	Brazil. Goiás: Alto Paraíso de Goiás, Chapada dos Veadeiros National Park, trail between the park headquarters and Rio Preto Canyon.	OLIVEIRA, F. C. A	402	IBGE	-47.808	-14.156
2013	Cavalcante	Chapada dos Veadeiros National Park	SOUZA, A. O.	829	UFG	-47.659	-14.049
2014	Cavalcante	Serra do Tombador Private Natural Heritage Reserve, 3.9 km southwest of the reserve headquarters.	SIMON, M. F.	2489	CEN	-47.822	-13.680
2014	Cavalcante	After Rio Preto, through Fazenda Bona Espero, in the vicinity of Morro Peito de Moça.	SOUZA, A. O.	1283	UFG	-47.872	-14.183
2016	Alto Paraíso de Goiás	Road behind the ICMBio lodging facility toward Rio Preto, approximately 200 m.	SOUZA, A. O.	1836	UFG	-47.794	-14.158
2017	Cavalcante	Renascer Private Natural Heritage Reserve, trail to Ponte de Pedra, Alto Paraíso de Goiás, Goiás, Brazil.	ANTAR, G. M.	1749	HUEFS	-47.492	-13.852
2019	Alto Paraíso de Goiás	Chapada dos Veadeiros region, Mr. Nilmar's farm, turning right approximately 11 km on the road from São Jorge to Colinas do Sul.	FARIA, J. E. Q.	9661	HEPH	-47.977	-14.049
2024	Cavalcante	Brazil. Goiás: Cavalcante, road to Prata Complex, 44 km north of the municipality.	SIMON, M. F.	4534	CEN	-47.594	-13.483
2024	Cavalcante	Brazil. Goiás: Cavalcante, São Félix Waterfall, ca. 70 km NW of the municipality, near the parking area.	SIMON, M. F.	4636	CEN	-47.714	-13.563
2025	Cavalcante	Brazil. Goiás: Road to the Complexo do Prata.	ARRUDA, R. O.	25	CEN, UB	-47.594	-13.483
2025	Cavalcante	Serra do Tombador Private Natural Heritage Reserve.	ARRUDA, R. O.	26	CEN, UB	-47.822	-13.680
2025	Cavalcante	Renascer Private Natural Heritage Reserve	ARRUDA, R. O.	27	CEN, UB	-47.510	-13.879
2025	Alto Paraíso de Goiás	Chapada dos Veadeiros National Park	ARRUDA, R. O.	28	CEN, UB	-47.659	-14.049

3.2 Population Inventories

The first surveyed population (Figure 3A) is located in Chapada dos Veadeiros National Park, near the ICMBio lodging area towards the Rio Preto (-47.794; -14.157), which had been previously recorded in a herbarium collection (Souza 1836). The physiognomy of the area is characteristic, with the presence of tree-shrub and subshrub-herbaceous individuals, shallow soil, and abundant rocky outcrops. In the sampled area of 0.33 hectares, 84 living individuals were identified, comprising approximately 255 individuals per hectare, consisting of 56 adults, 25 juveniles, and 3 seedlings. The mean diameter at ground level (DGL) was 44.9 mm, and the average height was 234 cm (Table 2). The largest individual presented a DGL of 155.1 mm, while the smallest had 2.65 mm. Additionally, the tallest individual reached 500 cm in height, whereas the shortest measured approximately 20 cm. The classification of adult individuals in this population was based on the remnants of reproductive structures from the last reproductive season. No signs of infestations or pests were observed; only two individuals had fallen but remained alive.

Table 2 – Density, dendrometric parameters, and fire incidence of *Chamaecrista fulgida* sampled in four surveyed areas. DGL (diameter at ground level) and height values are expressed as mean $\pm$ standard deviation. (PCV) Chapada dos Veadeiros National Park; (ECP) Road to the Complexo do Prata; (SRT) Serra do Tombador Private Natural Heritage Reserve (RPPN); (RNS) Renascer Private Natural Heritage Reserve (RPPN).

Area	Sampled area (ha)	Individuals / Adult individuals	DGL (mm)	Height (cm)	Density (ind/ha and adult ind./ha)	% Adult ind.	Mean Fire Return Interval (years)
PCV	0.33	84/53	47.0 $\pm$ 41.0	230 $\pm$ 130	255/170	67%	9,7
ECP	0.67	35/20	97.7 $\pm$ 44.2	340 $\pm$ 20	52/30	57%	5,3
SRT	2.73	72/30	60.9 $\pm$ 42.7	220 $\pm$ 120	28/11	40%	23,2
RNS	1.34	83/51	79.3 $\pm$ 51.3	230 $\pm$ 120	62/38	61%	>39

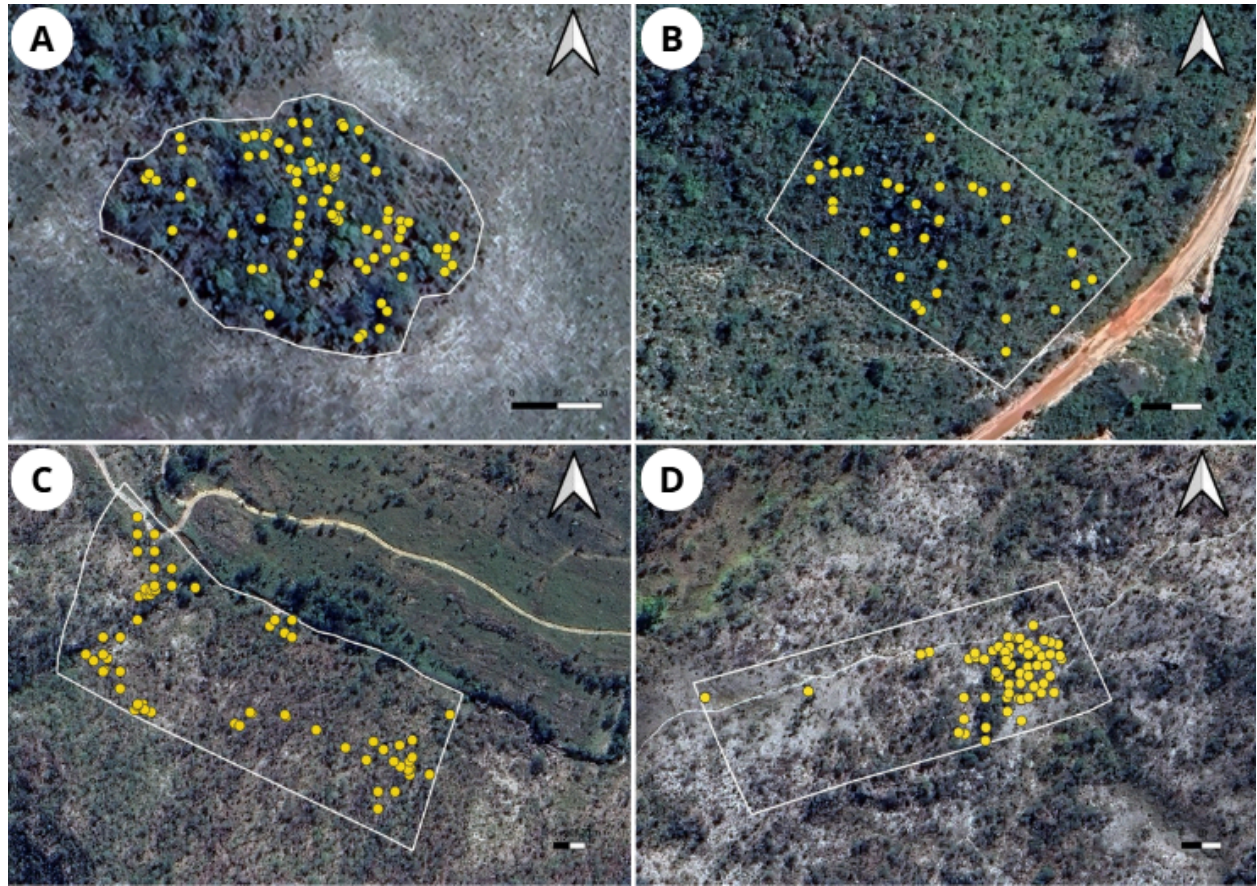


Figure 3 – Surveys of *C. fulgida* populations. (A) Chapada dos Veadeiros National Park; (B) Complexo do Prata; (C) Serra do Tombador Private Natural Heritage Reserve (RPPN); (D) Renascer Private Natural Heritage Reserve (RPPN).

The histograms (Figures 4A and 5A) indicated that the highest concentration of individuals ranged from 0 to 40 mm DGL, especially in the PNB and STB populations. Regarding height, intermediate values between 2 and 4 m were predominant.

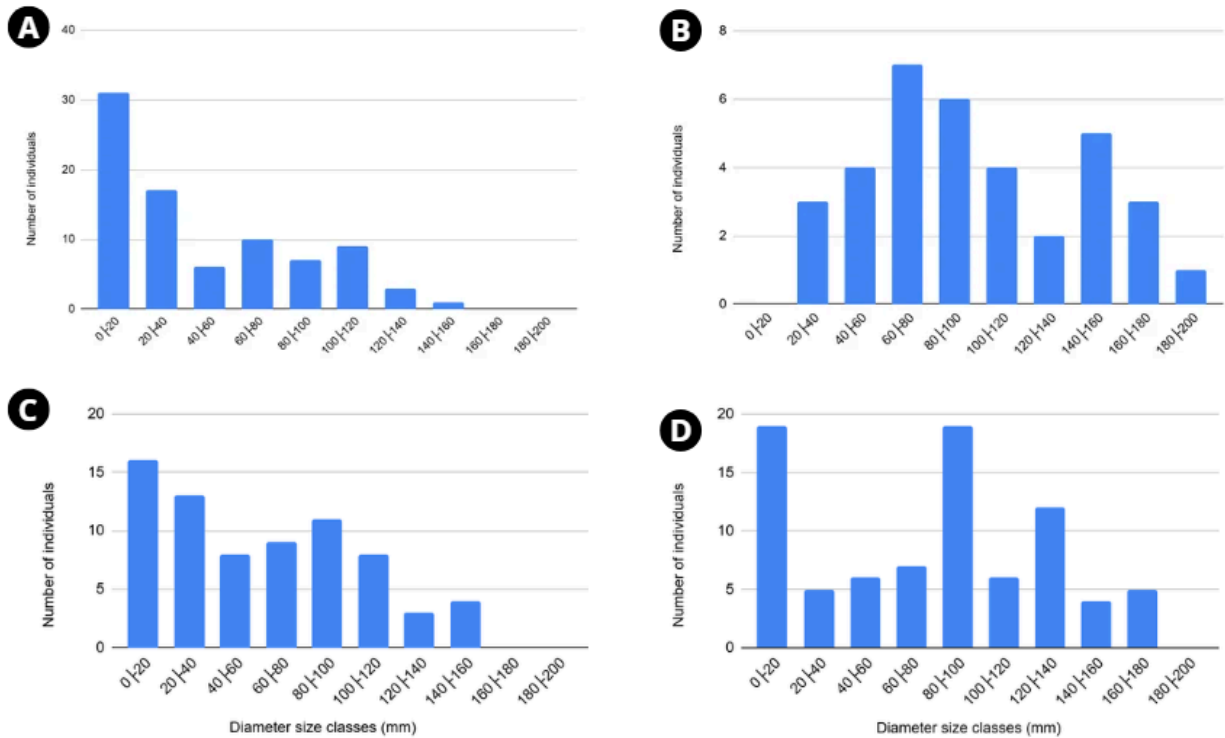


Figure 4 – Frequency of individuals of *C. fulgida* distributed in diameter at ground level (DGL) classes. Sampled areas: (A) Chapada dos Veadeiros National Park; (B) Complexo do Prata; (C) Serra do Tombador Private Natural Heritage Reserve (RPPN); (D) Renascer Private Natural Heritage Reserve (RPPN).

The second surveyed population (Figure 3B) is located along the Road to Complexo do Prata (-13.484, -47.594), an area with a physiognomy similar to that of PCV. In the sampled area of 0.67 hectares, 35 living individuals were recorded, approximately 52 individuals per hectare (Table 2), consisting of 20 adults, 14 juveniles, and 1 seedling. The mean DGL was 97.70 mm, and the average height was 341 cm. The largest sampled individual had a DGL of 185.34 mm, while the smallest measured 24.06 mm; heights ranged from 160 to 533 cm. The histograms (Figures 4B and 5B) showed that the most frequent DGL class ranged from 60 to 100 mm, and the most common heights were between 300 and 350 cm. The observed values indicate the predominance of adult individuals, although individuals at different growth stages were also present (Figure 3B).

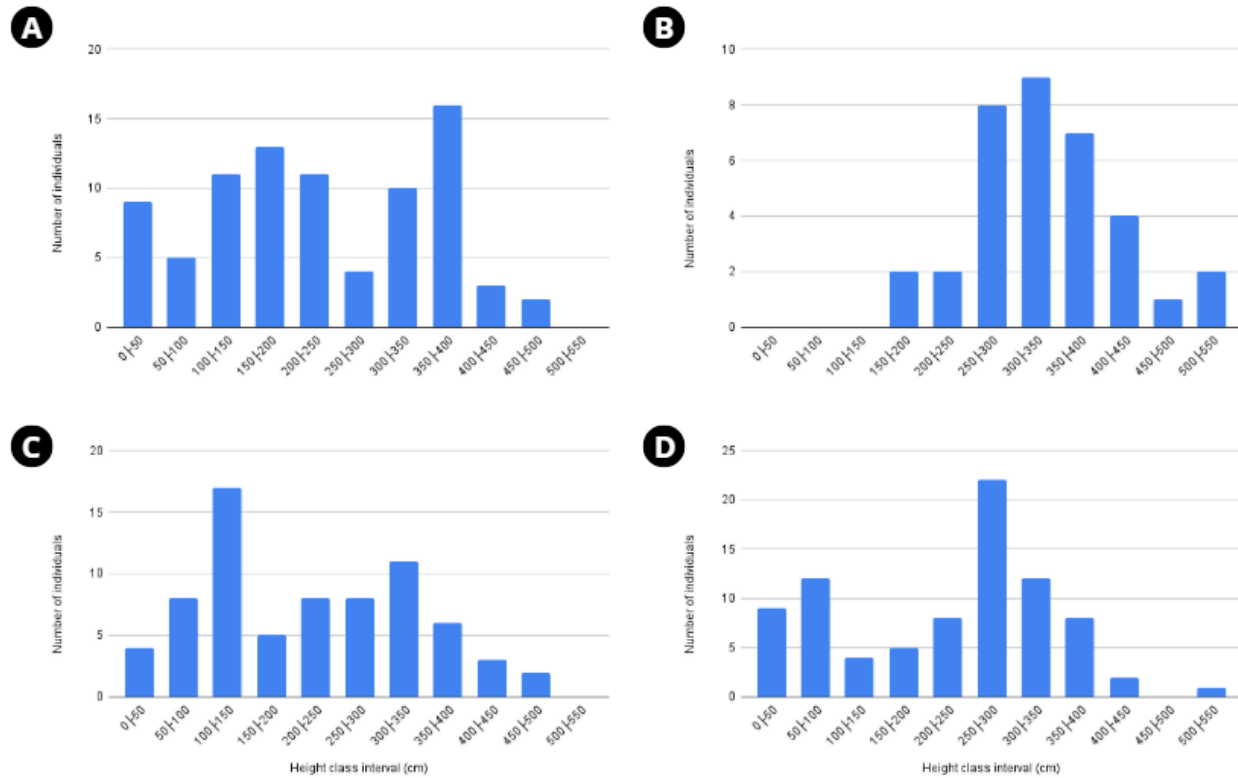


Figure 5 – Frequency of individuals of *C. fulgida* distributed in height classes. Sampled areas: (A) Chapada dos Veadeiros National Park; (B) Complexo do Prata; (C) Serra do Tombador Private Natural Heritage Reserve (RPPN); (D) Renascer Private Natural Heritage Reserve (RPPN).

The third surveyed locality (Figure 3C) is located in the Serra do Tombador Private Natural Heritage Reserve (RPPN) (-47.822; -13.681), which previously had only a single known collection record (Simon 2489; Table 1; Figure 2). In the sampled area of 2.73 hectares, 72 living individuals were identified, approximately 28 per hectare (Table 2), including 30 adults, 35 juveniles, and 7 seedlings. The mean DGL was 60.91 mm, and the average height was 221 cm. The largest individual recorded had a DGL of 152.35 mm, and the smallest 1.15 mm. In terms of height, individuals ranged from approximately 30 to 500 cm. At the time of the survey, in late July, adult individuals exhibited initial flowering, with no fruits present. During sampling, recent controlled burning had occurred in the area, conducted by the reserve managers about 50 days prior, aiming to reduce dry biomass accumulation and prevent larger-scale fires. In some individuals, particularly seedlings and juveniles, leaf color changes were observed from green to intense red, as well as basal resprouting following the death of aerial parts caused by fire (Figure 6).



Figure 6 – Fire disturbance response in *C. fulgida* 50 days after a controlled burn in the Serra do Tombador Private Natural Heritage Reserve (RPPN). (A) Aerial part of an adult individual showing dead leaves; (B) Juvenile individual showing death of main shoot and signs of basal resprouting (green leaves at plant base).

The most common DGL class was 0 to 40 mm, and heights averaged 100 to 150 cm (Figures 4C and 5C). The highest frequencies were concentrated in the smallest diameter and height classes. This pattern suggests that the evaluated population is predominantly composed of younger individuals. This demographic profile may reflect ecological characteristics, such as active vegetation regeneration or the predominance of individuals in early growth stages.

Finally, the fourth surveyed area (Figure 3D) is located in the Renascer Private Natural Heritage Reserve (RPPN), which had only one previous record (Antar, 1749), at the locality known as “Trilha para a Ponte de Pedra” (-47.492, -13.485). In the sampled area of 1.34

hectares, 83 living individuals were recorded, approximately 62 per hectare (Table 2), including 51 adults, 12 juveniles, and 20 seedlings. The mean DGL was 79.25 mm, and the average height was 226 cm. The largest individual had a DGL of 176.10 mm, while the smallest measured 2.92 mm. Heights ranged from 550 cm for the tallest individual to 20 cm for the shortest. No pressure vectors were observed acting on the population at the time of the survey, despite the area being used for ecotourism. Adult individuals exhibited initial flowering, with no signs of fruiting.

The histograms (Figures 4D and 5D) showed that the most frequent DGL classes were around 20 mm and also between 80 and 100 mm. The highest concentration of heights was observed in individuals approximately 250 to 300 cm tall. For DGL and height, a heterogeneous distribution across classes was observed, reinforcing the presence of individuals at different growth stages.

4.2 Identification of Threat Agents and Habitat Loss

Based on field observations in the four surveyed populations, no signs of natural threats such as herbivory or xylophagy were detected. Recent fire occurrence was noted in the populations of Complexo do Prata, Serra do Tombador RPPN, and Chapada dos Veadeiros National Park, with the first two being more noticeable. No evidence of fire was observed in the Renascer RPPN population, likely due to its geographically remote location.

In the Chapada dos Veadeiros National Park area, where complete burning occurred on four occasions during the analyzed period, the MFRI was 9.7 years, indicating an intermediate fire frequency. In the Road to Complexo do Prata area, 45 fire events were recorded, with a pixel-weighted mean of 7.3 events and an MFRI of 5.3 years. In the Serra do Tombador RPPN, six events were recorded, with a pixel-weighted mean of 1.7 events and an MFRI of 23.2 years. Finally, in the Renascer RPPN area, no fire events were recorded over the past 39 years.

Based on temporal land use and land cover analysis using the MAPBIOMAS platform, a loss of 183.78 km² of native vegetation was observed within the species' extent of occurrence (EOO) over 38 years, corresponding to approximately 8.32% of the total area (Figure 7). Despite this significant loss of native vegetation in the EOO, the reduction directly affected only 1.08

km² of the occurrence area, corresponding to approximately 2.72% of the AOO , since most populations are located at the edges of the EOO, where habitat loss was negligible.

Habitat quality varied according to land use and cover data, with areas of occupancy (AOO) for the species remaining relatively unaffected. The main threats to the species, according to the IUCN Threats Classification Scheme, are: 1.3 Tourism and recreation areas; 2.1 Annual and perennial non-timber crops; 2.3 Livestock farming and ranching; and 7.1 Fires and fire suppression. Major threats are directly associated with habitat loss, which was replaced mostly by planted pastures and annual crops.

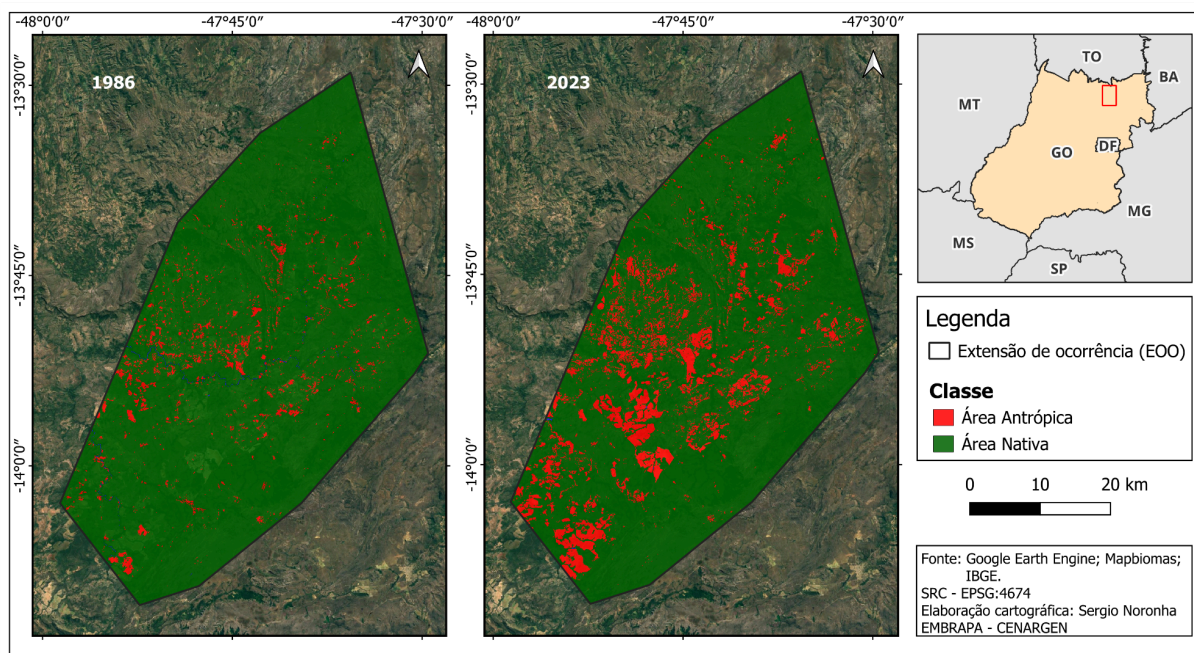


Figure 7 – Map comparing land use and land cover within the extent of occurrence (EOO) of *C. fulgida* between 1986 and 2023.

4.3 Update of Threat Status

Based on the data obtained in this study, including occurrence records, the study of population structure in four populations, assessment of threat agents, and analysis of land use and land cover, updated information was gathered regarding *C. fulgida*. The integration of these data with the IUCN assessment criteria allowed for a reassessment of its threat status, previously

defined in 2020 based on an EOO of less than 50 km² and indications of ongoing population decline. Based on 13 occurrence records, the extent of occurrence (EOO) was estimated here at 2,221.982 km², while the area of occupancy (AOO) totaled 40 km².

Under Criterion A, a population reduction associated with habitat loss was observed; however, the reductions of 8.32% in the EOO and 2.72% in the AOO do not meet the minimum 30% threshold required for classification as Endangered (EN).

Considering Criterion B1, the species falls under the Endangered (EN) category due to an EOO of less than 5,000 km². Five locations were considered, defined based on the spatial distribution of population nuclei and the likelihood that distinct threatening events could affect each set of occurrences independently. One location comprises records within Chapada dos Veadeiros Park, while the other four correspond to the localities along the road to Complexo do Prata, the road to Colinas, São Félix, and the Serra do Tombador Private Natural Heritage Reserve (RPPN), respectively. Accordingly, the species meets subcriterion “a” with a number of locations ≤ 5 . Furthermore, due to observed anthropogenic threats in part of the nuclei, particularly associated with habitat loss and high fire occurrence, a continuous decline in habitat quality was inferred, meeting subcriterion “b(iii)”. This results in the classification EN B1ab(iii).

Regarding Criterion C, a total of 274 individuals were sampled across the four populations, comprising 157 adults, 86 juveniles, and 31 seedlings. High abundance of individuals was also observed in the field outside the sampled areas, including extensive patches of suitable habitat for the species. Based on extrapolation of sampled areas using field observations and satellite image analyses, the estimated areas of suitable habitat within each population were: PCV (7.56 ha), ECP (15.38 ha), SRT (23.29 ha), and RNS (70.40 ha). Therefore, the number of mature individuals was estimated by multiplying the density of individuals in each area by the respective extent of suitable habitat, resulting in 1,285.2 individuals for PCV, 461.4 for ECP, 256.19 for SRT, and 2,675.2 for RNS. Thus, the global number of mature individuals is estimated at approximately 4,678. It should be noted that this estimate is based on density extrapolation within each area, assuming a relatively homogeneous distribution of the species across the considered areas.

Based on the same data on the number of mature individuals, Criterion D was also not met, as the recorded number of individuals exceeds the thresholds established for categorizing species with very small or restricted populations.

Thus, based on all evaluated parameters, *C. fulgida* is classified as Endangered (EN) B1ab(iii).

4.4 Seed Characterization

Fruits of *C. fulgida* collected in September 2025 produce in general five to eight seeds per fruit (Figure 1E). Collected fruits were subjected to a screening process, resulting in approximately 1,500 seeds, of which 1,150 were classified as healthy (66.7%) and 350 seeds classified as malformed and/or predated (23.3%). After measuring a batch of 30 seeds, the following results were obtained: mean length of 5.0 ± 0.4 mm, mean width of 3.5 ± 0.3 mm, and mean thickness of 1.3 ± 0.2 mm (Figure 1F).

The average seed mass of a batch of 30 seeds under normal storage conditions was 0.167 g. After drying in an oven at 105°C for 24 hours, the mean mass was 0.150 g, indicating a mean seed moisture content of 10.3%.

4.4.1 Tolerance to Desiccation and Freezing

The drying process in silica gel to reach the desired moisture levels for storage lasted a total of 9 days. The mean mass of seeds dried in silica gel was 0.175 ± 0.015 g, and after 24 hours in an oven at 105°C the mean mass was 0.163 ± 0.015 g. The average moisture content of the silica-dried seeds was $7.0 \pm 0.6\%$, considered within the ideal range for freezing without damaging the seeds' cellular tissues.

4.4.2 Germination

Germination at 30 days differed significantly among treatments (ANOVA, $F_{3,11} = 9.71$, $p = 0.0016$). The hot water treatment showed the highest germination percentage ($54.0 \pm 11.5\%$), whereas silica gel showed the lowest values ($13.4 \pm 4.1\%$). Control ($37.9 \pm 8.4\%$) and frozen storage ($24.9 \pm 16.8\%$) showed intermediate values and did not differ statistically from each other or from the hot water and silica gel treatments (Figure 8; Table 3).

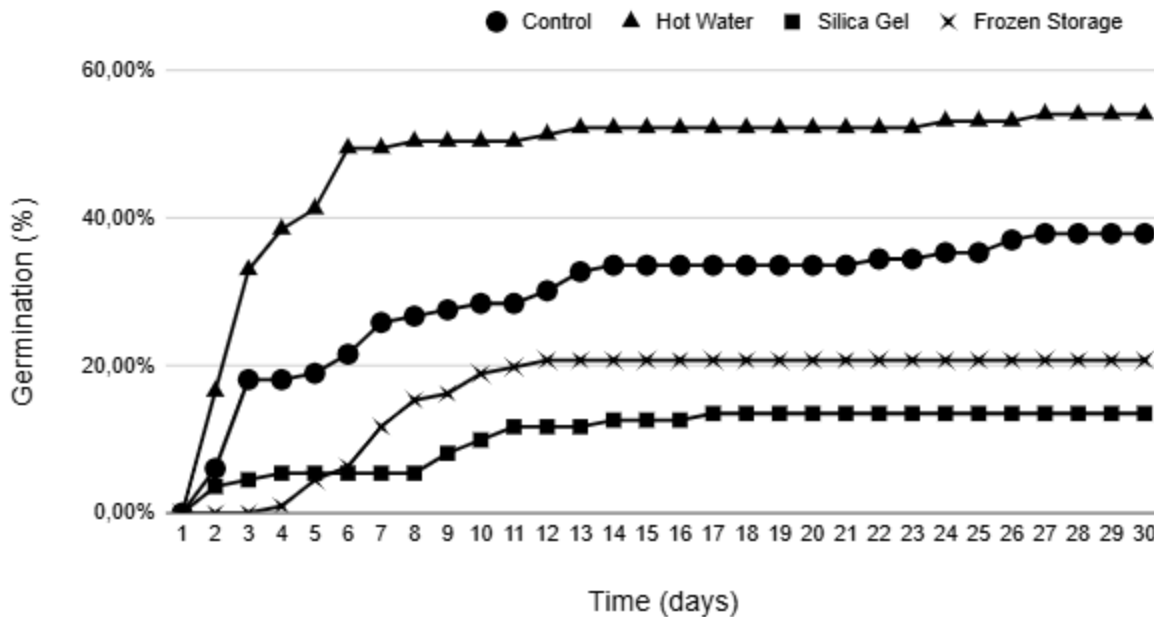


Figure 8 – Cumulative germination percentage (40 days) of *C. fulgida* seeds under control, hot water treatment, drying, and drying + freezing at -8°C.

Table 3 – Seed traits and germination tests of *Chamaecrista fulgida*, including germination speed index (GSI), mean germination time (MGT), and germination percentage (G, %) under the following treatments: control, hot water, silica gel-dried seeds, and dried seeds with storage (*ex situ* conservation). Cumulative germination (40 days) corresponds to germination observed after dormancy breaking at 30 days.

Tratament	Germination (30 dias)	MGT (30 dias)	GSI (30 dias)
Control	37.9 ± 8.4 ab	8.0 ± 1.1 a	2.7 ± 0.8 b
Hot Water	54.0 ± 11.5 a	4.7 ± 1.5 b	4.7 ± 1.4 a
Silica Gel	13.4 ± 4.1 c	8.04 ± 1.6 a	0.9 ± 0.5 b
Frozen Storage	24.9 ± 16.8 bc	7.4 ± 0.8 ab	0.8 ± 0.6 b

4.4.3 Mean Germination Time (MGT)

Mean germination time (MGT) differed significantly among treatments (ANOVA, $F_{3,11} = 6.01$; $p = 0.011$). The hot water treatment exhibited the shortest MGT (4.7 ± 1.5 days), differing significantly from the silica gel treatment (8.0 ± 1.6 days) and the control (8.0 ± 1.1 days). The freezing treatment showed an intermediate value (7.4 ± 0.7 days), not differing significantly from the other treatments (Tukey, $p < 0.05$; Table 3).

4.4.4 Germination Speed Index (GSI)

Germination speed index (GSI) differed significantly among treatments (ANOVA, $F_{3,12} = 18.08$; $p < 0.001$). The hot water treatment exhibited the highest GSI (4.7 ± 1.4), differing significantly from the control (2.7 ± 0.8), silica gel (0.86 ± 0.47), and freezing (0.8 ± 0.6) treatments. No statistical difference was observed among these latter three treatments (Tukey, $p < 0.05$; Table 3).

5. DISCUSSION

5.1 Geographic Mapping and Population Structure

Mapping the populations was fundamental to expand the known distribution area of the species, contributing to increased available knowledge and supporting conservation strategies for *C. fulgida*. Compared to FERNANDEZ & MORAES (2020) data, which recorded only three occurrences and classified the species as Critically Endangered (CR), the new surveys and recent botanical explorations resulted in a significant expansion of its distribution, with the recording of new populations. Notably, many of the recorded populations are located within protected areas. These results reinforce the relevance of protected areas for species conservation, as *C. fulgida* shows high habitat specificity, being associated with environments containing rocky outcrops and high altitude. . Despite its endemic occurrence, the species exhibits high local abundance, indicating that although restricted to this habitat type, it forms numerous populations. The expansion of the distribution area observed in this study follows a recurring pattern for Cerrado species, initially known from few records, whose distributions are frequently underestimated due to the scarcity of systematic surveys, especially in remote or difficult-to-access areas (SCHINDLER et al., 2025). Furthermore, knowledge about species in Chapada dos Veadeiros is still largely based on isolated floristic surveys, which may result in misidentifications and the use of outdated nomenclature, compromising understanding of the species' true distribution (SOARES et al., 2025).

Demographic analysis of the different *C. fulgida* populations found in northeastern Goiás revealed a small number of locations with habitat specificity, but locally well-established and abundant populations. Population surveys indicated significant variations in diameter and height

of individuals, contributing to a better understanding of the species' population structure in different sites. In addition, individual tagging and georeferencing of individuals will enable long-term population monitoring, allowing evaluation of growth patterns, reproductive development, and mortality, providing support for more robust analyses of population dynamics.

The population in Chapada dos Veadeiros National Park, corresponding to the type locality of *C. fulgida*, was impacted by fires in 2023. The high presence of seedlings and regenerating individuals demonstrates the species' resilience to disturbances, a trait often observed in endemic Cerrado species (HOFFMANN et al., 2012). The predominance of recently regenerating individuals, along with the presence of mature individuals, suggests a demographic profile supporting continuous recruitment, indicating good reproductive performance of the population.

The population surveyed along the road to Complexo do Prata showed high fire occurrence according to MapBiomas Fire data, with portions of the area burning between five and ten times over the last 39 years and a mean fire return interval of 5.3 years. No signs of recent recruitment of young individuals were observed within the sampled unit, which might indicate that high fire frequency may be impacting recruitment. Despite a lower number of individuals compared to other localities, most being adults, the population shows no evidence of reduction or major threats. Moreover, numerous individuals were observed in areas adjacent to the sampled region, reinforcing its high local abundance.

In Serra do Tombador RPPN, results again highlighted the high abundance of the species, associated with the simultaneous presence of regenerating and mature individuals. The demographic profile, predominantly composed of younger individuals, reflects active recruitment in the population. In this area, an Integrated Fire Management strategy was adopted by reserve managers to reduce dry biomass accumulation and consequently minimize the risk of larger wildfires. The occurrence of a managed fire 50 days before our sampling enabled the observation of a rapid response of regenerating individuals to disturbance, with signs of basal resprouting in seedlings after death of the stem. These observations suggest that the species exhibits some resilience to fire and the capacity for rapid regeneration. However, frequent fire events may become detrimental to individuals, potentially increasing mortality within the

population, particularly in young individuals.

Despite the presence of basal resprouting in fire-affected individuals, aboveground biomass loss was observed, which may compromise natural plant growth by inducing individual physiological stress due to the use of internal reserves for resprouting (HERINGER & JACQUES, 2001), and also collective stress through alterations in nutrient and water availability in the soil, which can affect plant-soil interactions (BAUR et al., 2024). Other studies indicate that savanna vegetation is fire-resilient due to adaptive traits, but this resilience depends on fire frequency and intensity, as frequent fires can reduce biomass and alter floristic composition (SILVA et al., 2011).

Finally, in the Renascer RPPN population, the coexistence of different age classes and high individual density was again observed. The concentration of heights between 250 and 300 cm suggests a predominance of individuals in an intermediate growth stage, while the distribution of values above and below this range indicates the presence of young and mature individuals. This result can be interpreted as reflecting variations associated with different growth stages and environmental factors, also evidenced by the diameter distribution, with the highest frequencies around 20 mm for early stages and 80 mm for adult individuals. This pattern indicates continuous population growth, with active recruitment and maintenance of different growth stages.

5.2 Identification of Threat Agents

The absence of evident signs of herbivory and xylophagy in the studied areas may be related to local environmental conditions, including altitude. Studies conducted on *Chamaecrista linearifolia* indicate that herbivory levels can vary along altitudinal gradients (MADEIRA et al., 1998), suggesting that environmental factors may influence plant-herbivore interactions within the genus. Although fire occurrence was recorded, the species exhibited significant resistance, demonstrating resilience to this disturbance with signs of basal resprouting days after fire incidence. This regenerative capacity is frequently observed in Cerrado species, whose adaptive mechanisms allow them to tolerate fire events (SOUCHIE et al., 2017). However, despite this initial positive response, aboveground biomass loss was observed in affected individuals, which may compromise plant growth and survival.

Furthermore, frequent disturbance events may increase mortality of smaller individuals, affecting population recruitment and dynamics. In the Chapada dos Veadeiros region especially, records of anthropogenic fires have increased significantly, representing one of the main degradation agents in the local biome, exemplified by the largest fire in Chapada dos Veadeiros National Park in 2017, which affected approximately 65,000 hectares, corresponding to 25% of the park's total area (MORIYAMA, 2018).

Finally, differences in fire regimes observed across the four studied areas may influence the structure and population dynamics, as well as the recruitment of new individuals of *C. fulgida*. One evidence is the low proportion of juvenile individuals in the population with highest fire frequency (ECP).

5.3 Update of Threat Status

The new data obtained in this study enabled the application of IUCN criteria A, B, C, and D. Habitat loss estimates obtained from land-use maps were useful for applying criterion A as an indirect measure of population reduction, as well as for assessing habitat area and quality (criterion B). Updated information on geographic distribution contributed to improved estimates EOO and AOO, as per criterion B. Moreover, sample-based results regarding population size and the number of mature individuals were important for evaluating criterion C, as well as for identifying small or declining populations, in accordance with criterion D.

Based on new occurrence records and an improved understanding of the population structure of *C. fulgida*, a preliminary reassessment of its conservation status was conducted following the IUCN Red List criteria (IUCN, 2024). The significant increase in the extent of occurrence (EOO), currently estimated at approximately 2,221 km², indicates that the species no longer qualifies as Critically Endangered (CR) and now fits under criterion B1 for the Endangered (EN) category, which includes species with EOO less than 5,000 km². The updated AOO and EOO values represent a substantial increase compared to the 12 km² AOO and 60.792 km² EOO reported in the previous assessment (FERNANDEZ & MORAES, 2020).

Within this classification, the species remains under subcriterion “a” due to the restricted distribution of populations, with the number of locations ≤ 5 . Subcriterion “b(iii)” remains part of

the species' threat status classification, considering the ongoing decline in habitat quality, particularly in areas affected by deforestation or frequent fire events. Accordingly, the most appropriate and recommended classification for the species is EN B1ab(iii). Even if a potential downlisting in the threat category is considered in the future, it is essential to maintain *in situ* conservation efforts, as well as to promote close monitoring of populations to ensure the species' long-term persistence.

5.4 Fruit collection and reproductive output

Field observations regarding fruit production in *C. fulgida* individuals in PNC indicates that the species' reproductive output is capable of generating hundreds of viable seeds units per individual. However, the high proportion of malformed or aborted seeds (23.3% in a batch of 1,500 seeds) suggests the occurrence of reproductive limitations that may reduce recruitment potential.

High rates of seed abortion and inviability may reflect environmental disturbances and constraints in the plants' reproductive process. In a study on stress-induced abortion in *Arabidopsis*, SUN et al. (2004) observed that environmental stresses, such as drought or other abiotic factors, can induce ovule and seed abortion during reproductive development. Furthermore, pollination limitation, due to low pollinator visitation or habitat fragmentation, has also been associated with reductions in seed quantity and increased reproductive failures, indicating that pollen availability directly influences the formation of viable seeds, as reported by WALSH et al. (2014) in a study on pollination limitation and seed predation in orchids. Finally, observations in tropical species reveal that high rates of seed abortion may represent a common biological response in populations under disturbance or resource limitation, negatively impacting recruitment potential (CUSTÓDIO et al., 2014).

5.5 Germination and *ex situ* Conservation

Understanding the biological and reproductive patterns of the species is fundamental to support conservation strategies suited to its phenological characteristics, especially considering that a large proportion of threatened species in Brazil are still not represented in germplasm

banks and lack information on germination behavior and storage (SILVEIRA et al., 2018). Germination tests, therefore, constitute indispensable tools to evaluate propagation potential and ex situ conservation.

The seed coat dormancy observed in *C. fulgida* is common in Fabaceae, in which seed coat impermeability prevents imbibition and delays germination (GRUS, 1990; BEWLEY, 1997). The higher germinability achieved through hot water immersion confirms the presence of physical dormancy and demonstrates that heat promotes seed coat softening without causing embryonic damage, a result similar to that observed in other species of the genus, such as *Chamaecrista flexuosa* (BECHARA et al., 2007), which showed germination rates around 73%, and *Chamaecrista debilis* (ARAÚJO et al., 2017), with approximately 40% germination. These differences suggest that germination percentage may act as a limiting factor for species distribution, as *C. flexuosa* is widely distributed and commonly found, whereas *C. debilis* is endemic, indicating that lower germination rates may contribute to more restricted occurrence patterns. The increase in germination under the heat treatment, compared to the control, reinforces the role of heat as an efficient stimulus for overcoming physical dormancy. The shorter mean germination time (MGT) observed in the hot water treatment indicates greater speed and uniformity of germination, a pattern described for legumes subjected to thermal stimuli, as reported by ZANON (1992) when testing different temperatures for breaking seed coat dormancy in *Mimosa flocculosa*. Partial germination in the control suggests the coexistence of dormant and non-dormant seeds, an adaptive strategy common in savanna environments subject to variable water availability (FERREIRA & BORGHETTI, 2004). The positive response to heat simulates natural conditions associated with fire events, an important ecological factor in breaking physical dormancy in species with impermeable seed coats and a common disturbance in the Cerrado biome (AULD & O'CONNELL, 1991; ZIRONDI et al., 2019; DAIBES et al., 2019).

On the other hand, drying in silica gel reduced both germination percentage and speed, indicating seed sensitivity to water loss. Although orthodox seeds generally tolerate desiccation, excessive drying can cause cellular damage, reduced vigor, and compromised viability (MASETTO & SCALON, 2014; DRESCH et al., 2016; REED et al., 2022; RUSDY, 2017).

Freezing after desiccation did not compensate for these effects, maintaining germination similar to the control but with reduced vigor.

Values for the germination speed index (GSI) corroborate this pattern, as seeds subjected to hot water exhibited the highest germination speed, while seeds subjected to desiccation and freezing showed a significant reduction in vigor, a parameter directly associated with seedling establishment potential (MAGUIRE, 1962). Seeds that germinate more rapidly tend to have a higher probability of establishment, particularly in seasonal environments subject to disturbances and resource scarcity (FERREIRA & BORGHETTI, 2004).

Overall, the results obtained for the treatments in *C. fulgida* are consistent with the behavior described for tropical savanna species (ZANON, 1992; BECHARA et al., 2007; MASETTO & SCALON, 2014), in which different stimuli for breaking dormancy result in varied germination responses, reflecting adaptive strategies for germination under different environmental conditions. The predominance of dormancy in Fabaceae seeds contributes to seedling emergence under favorable conditions for development, increasing survival chances (JAGANATHAN & BIDDICK, 2021). Finally, the hot water treatment proved partially efficient in promoting rapid and uniform germination, while desiccation and low-temperature storage negatively affected vigor, providing relevant information for the development of conservation and restoration protocols in the Cerrado.

6. CONCLUSION

The results observed in the present study provided important information on *C. fulgida*, helping to fill knowledge gaps regarding its geographic distribution, population structure, and seed germination behaviour and storage capacity for long-term ex situ conservation.

These findings, including an expanded geographic range and analyses of trends in habitat loss, support the proposal to redefine the species' extinction risk conservation status from CR B1ab(iii) to EN B1ab(iii).

Regarding population studies, it was observed that despite its habitat specificity, the species showed high local abundance, with individuals present at different growth stages. Although the species responds rapidly to fire, with signs of basal resprouting in young

individuals affected by recent fires, frequent disturbance events may cause structural damage to populations. Despite an inferred reduction in the level of threat, continuous monitoring of populations remains essential. In addition, it is crucial to develop studies aimed at establishing in situ conservation strategies, as well as to implement public policies to ensure the species' protection, in order to guarantee its long-term viability.

Experimental seed germination data revealed seed coat dormancy in *C. fulgida*, which can be broken by heat. Despite a reduction in seed vigor, freezing may represent a promising strategy for seed conservation. Although a reduction in seed vigor was observed, freezing showed potential as a short-term conservation strategy. However, further studies exploring different desiccation levels and storage methods are necessary before confirming its suitability for long-term conservation. Additional germination tests using new protocols may improve conservation efficiency for this species.

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