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Exploring the diversity of sexual systems and pollination in Brazilian Cleomaceae species

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ABSTRACT

The family Cleomaceae is characterized by remarkable floral diversity and abundant nectar and pollen production. In this study, we evaluated the flower functional characteristics associated with the floral diversity of 10 Cleomaceae species, in addition to respectively seven and five accessions of *Tarenaya hassleriana* and *T. longicarpa*. The flowers were examined through five types of crosses and thus we determined the most efficient type of crossing based on the number of seeds. In addition, we evaluated pollen limitation, self-incompatibility, self-pollination and explored the possible effects on seed germination. The species can be grouped into three groups based on sexual systems: andromonoecious, hermaphroditism (chasmogamous and cleistogamous), and polygamomonoecious flowers. *Gynandropsis gynandra, T. aculeata, T. diffusa*, and *T. microcarpa* exhibited autogamy, whereas the other species did not. Overall, hand cross-pollination produced the highest seed yield, followed by open pollination. Self-pollination produced the lowest number of seeds, with no seed production noted in 16 accessions. Interestingly, the pollination type did not affect seed germination. Together, these results indicate the potential of the Cleomaceae family as a source of information regarding mechanisms involved in the determination of reproductive traits in plants.

1. Introduction

Cleomaceae Brecht. & J. Presl., a sister family of Brassicaceae Burnett, has been widely studied for its transitional C_3–C_4 and C_4 photosynthetic mechanisms (Marshall et al., 2007; Koteyeva et al., 2011; Reeves et al., 2018; Parma et al., 2022). The family is distributed worldwide, comprising 26 genera and 270 species (Bayat et al., 2018; Stevens, 2001; Soares-Neto et al., 2020, 2022). Cleomaceae can be easily distinguished from other plant families based on specific morphological traits, such as compound leaves (3–12 leaflets), zygomorphic flowers, six stamens, and dry fruits with longitudinal dehiscence (Stevens, 2001; Iltis et al., 2011). Members of this family are used as model plants in studies of floral evolution, C_4 photosynthesis, and comparative genomics and transcriptomics (Brown et al., 2005; Feodorova et al., 2010; Nozzolillo et al., 2010). Within Cleomaceae, closely related species may differ substantially in floral morphs and associated features (e.g., nectar and pollen). As such, while some species harbor only bisexual flowers (*Cleome violacea* L.; Cane, 2008; *C. viscosa* L.; Raju and Rani 2016), others bear male and bisexual flowers (*Gynandropsis gynandra* (L.) Briq.; Omondi et al., 2017; Zohoungbogbo et al., 2018; Raju and Rani 2016) and some even possess male, female and bisexual flowers on the same individual (*Tarenaya spinosa* (Jacq.) Raf.; Machado et al., 2006).

In Brazil, seven Cleomaceae genera (*Tarenaya* Raf., *Gynandropsis* (L.) Briq., *Melidiscus* Raf., *Cleoserrata* Iltis, *Dactylaena* Schrad. ex Schult. & Schult.f., *Haptocarpum* Ule, and *Physostemon* Mart.) comprising 34 species have been described (Flora of Brazil, 2022). Among these, *Tarenaya* is the most diverse genus, with 22 species, and 10 of these are endemic (Flora of Brazil, 2022). Despite their wide distribution throughout the Brazilian territory, the majority of the Cleomaceae species ($n = 21$) are documented from the Bahia state (Flora of Brazil, 2022). There have been few studies on Brazilian Cleomaceae species, and some are restricted to their tissue culture for pharmaceutical interests (Castro

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et al., 2014; Simões, 2006), regional floristic surveys (Akemi-borges and Pirani, 2017; Carneiro et al., 2018;) or with an emphasis on physiology, such as photosynthesis (Parma et al., 2022). In addition, many of these species are endemic (Flora of Brazil 2022), and they may become extinct without being studied (e.g., for *Haptocarpum*, there has been only one record corresponding to the type species, dated 1906, voucher B 100, 242,671).

Studies on the floral morphology and reproductive biology of Brazilian Cleomaceae species are scarce, although such studies have been conducted in Africa (Omondi et al., 2017; Zohoungbogbo et al., 2018), North America (Stout 1923; Cane, 2008; Higuera-Diaz et al., 2015; Schlessaman et al., 2020), and specific areas in Northeast Brazil (Machado et al., 2006). To this end, the present study furthers our understanding of the morphological diversity and reproductive biology of Cleomaceae species from different biomes. The observed diversity of Cleomaceae would provide important insights into pollination and floral function within this family. Further integration of morphological, phylogenetic, ecological, and population genetic studies may shed light on the specific mechanisms underlying the major evolutionary transitions between reproductive modes (Barrett, 2002). Here, we describe diversity in the functional aspects of the flowers of 10 Cleomaceae species, in addition to respectively seven and five accessions of

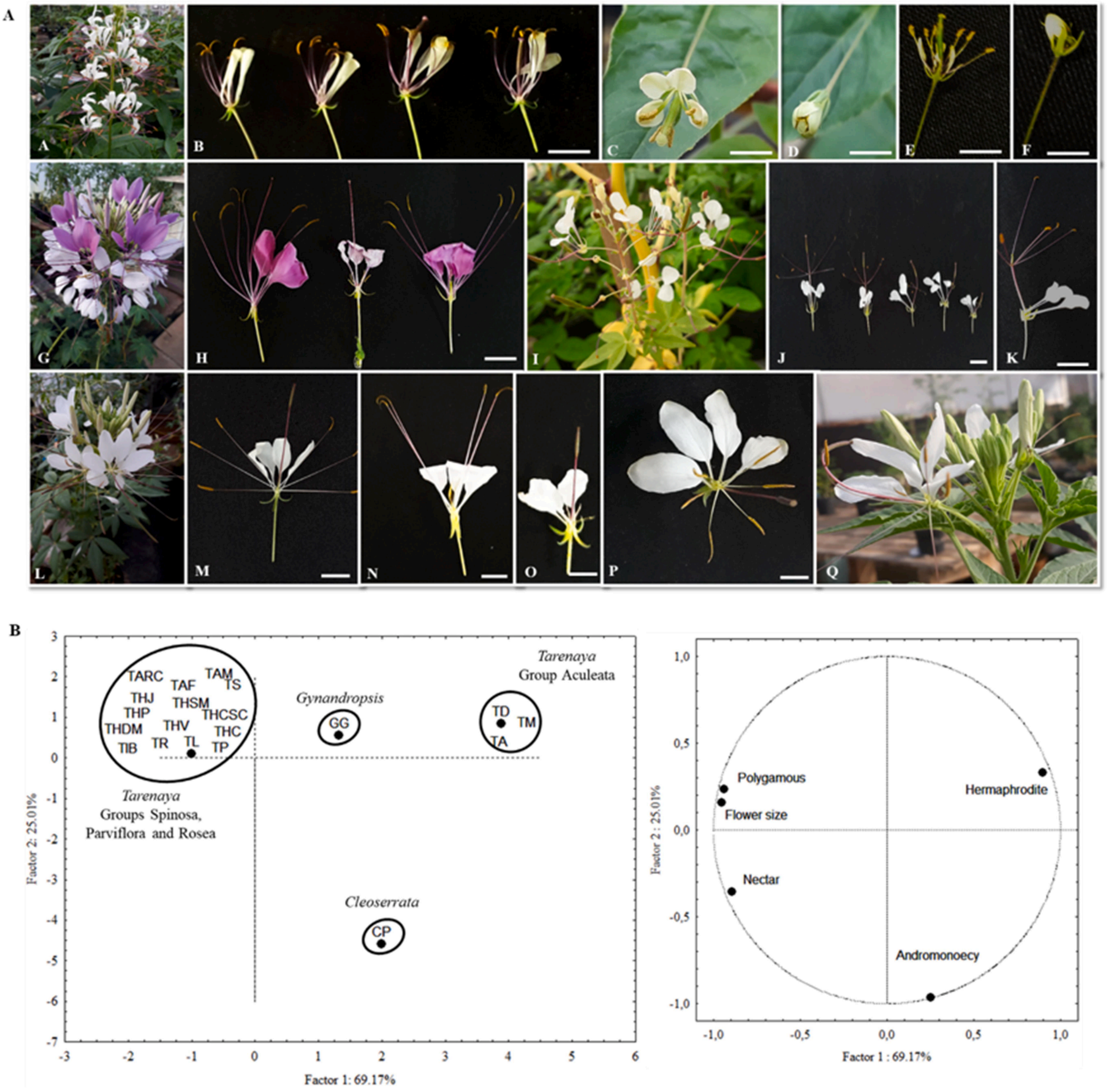

Fig. 1. Pictures of flowers and PCA based on floral features of Cleomaceae species. (**a**) - [Species name followed by Sexual Type] A-B *C. paludosa* (Andromonocy), A- inflorescence, B- male and bisexual flowers. C-D *T. diffusa* (Hermaphrodict), C- Chasmogam, D-Cleistogam, E-F *T. microcarpa* (Hermaphrodict), E- Chasmogam, F- Cleistogam, G-H *T. hassleriana* (THDM) (Polygamomonoecious), G-inflorescence, H-B- bisexual, female and male flowers. I-K *G. gynandra* (Polygamomonoecious), I- inflorescence, J- bisexual and female, K- male flowers. L-O *T. spinosa* (Polygamomonoecious) L-inflorescence, M- bisexual flower, N- male flowers, O- female flowers. P-Q *T. longicarpa*, P- Herkogamy. O- Dichogamy. Bars=1 cm. **(b) -** Principal component analysis based on floral characteristics such as sexual systems of the species (3), flower size and nectar production. Species (city and state of collection) - CP: *Cleoserrata paludosa* (Belém-PA); TA: *Tarenaya aculeata* (Feira de Santana-BA); TD: *T. diffusa* (Feira de Santana-BA); TM: *T. microcarpa* (Belém-PA).GG: *Gynandropsis gynandra* (Mossoró-RN); THC: *Tarenaya hassleriana* (Canaã-MG); THCS: *T. hassleriana* (Canoinhas-SC); THDM: *T. hassleriana* (Domingos Martins-ES); THJ: *T. hassleriana* (Joinville-SC); THP: *T. hassleriana* (Piau-MG); THS: *T. hassleriana* (São Miguel-MG); THV: *T. hassleriana* (Viçosa-MG); TL: *Tarenaya longicarpa* (Picos-PI); TR: *T. rosea* (Colatina-ES); TP: *T. parviflora* (Pombal-PB); TS: *T. spinosa* (Teresina-PI); TAF: *T. longicarpa* (Afrânio-PE); TAM: *T. longicarpa* (Manaus-AM); TARC: *T. longicarpa* (Arcoverde-PE); TIB: *T. longicarpa* (Ibimirim-PE).

T. hassleriana (Chodat) Iltis and *T. longicarpa* Soares Neto & Roalson, and discuss how and to what extent the observed diversity contributes to the fitness of this plant family in relation to insect pollinators (or lack thereof). Specifically, we performed five types of pollination to verify the type that was the most efficient for the seed set and explored the possible effects of these types on seed germination. In the present study, the potential of the Cleomaceae family, a model family to study the evolution of C_4 photosynthesis, to serve as a potential research model for developing varieties and hybrids with improved floral traits.

2. Material and methods

2.1. Taxon sampling

The botanical material of Cleomaceae was collected from different Brazilian states (Fig. 1A, Table 1S). The seeds of the 10 Cleomaceae species, seven *T. hassleriana* accessions, and five *T. longicarpa* accessions were collected. Different accessions, collections in different places, of the same species exhibit morphological, physiological and genetic diversity (e.g. *G. gynandra* - Reeves et al. (2018)). The species belong to the genera *Cleoserrata, Gynandropsis* and *Tarenaya*. The genus *Tarenaya* is very specious (ca. 40 species) and because it is morphologically very diverse, it is separated into small groups of species, which have also been shown to be monophyletic (Parma et al., 2022). These groups are recognized as Spinosa (or Spinosa I and II), Aculeata, Parviflora and Rosea. They receive the denomination of the species "type" representative of each cluster. In general, representatives of the Spinosa cluster are more robust (bushes, flowers and larger leaves) and Aculeata the most delicate, they are herbaceous and have fewer and smaller leaflets, as well as small to tiny flowers.

The seeds germinated in plastic containers (volume, 5 L) with Carolina Soil brand substrate. In this substrate, raw materials such as Sphagnum peat, known as Canadian and/or European peat, expanded perlite, expanded vermiculite, and roasted rice husks, among others, are used. The plants (10 replicates per species) were grown in a greenhouse under semi-controlled conditions (maximum photosynthetically active radiation of 1500 μmol photons m^{-2} s^{-1} and mean temperature of 30 ± 2 °C) and daily irrigation. Under these conditions, the plants remained in the vegetative phase (3–5 months). Following the emergence of the first flower bud, the plants, still in the vase, were randomly placed in the botanical garden of the Federal University of Viçosa for approximately five months, until the pollination tests ended and the plants set fruits.

2.2. Floral morphology

The morphometric data of 50 mature and fully open flowers of all types were collected. Specifically, petal, sepal, stamen, ovary, gynophore, and androgynophore length was measured using a pachymeter and ruler. For sexing, a total of 150 flowers were randomly collected per species on different days.

2.3. Reproductive biology

Fifty flowers per species and pollination type were exposed to the treatments, totaling 2500 tagged flowers. Reproductive success was evaluated based on the number of seeds produced through five different pollination types: (i) natural self-pollination (SP) in which the buds of each flower were covered with paper bags to avoid insect access; (ii) open pollination (OP) in which insects were allowed to pollinate the flowers; (iii) hand self-pollination (HP) in which the flowers were pollinated with their pollen; then the flowers were covered with paper bags to prevent cross-pollination; (iv) cross-pollination (CP) where two flowers from two different plants were hand-pollinated; the flowers have been emasculated; and (v) geitonogamy (G) where pollen from the same plant was collected and applied to a different flower previously emasculated.

Table 1

Summary of petals, sepals, stamens, androphorus, gynophore and ovary lengths of the studied Cleomaceae species - Group polygamomonoecious. F: Female flowers, M: Male flowers, B: Bisexual flowers. Number corresponds to the medium value of the size (cm) of the structures, followed by ± SE, $n = 50$.

	Polygamomonoecious					
	Gynandropsis gynandra (GG)			*Tarenaya hassleriana* (THC)		
	F	M	B	F	M	B
Petals	1.12 ± 0.02^{B}	1.26 ± 0.02^{A}	1.58 ± 0.02^{A}	2.76 ± 0.01^{B}	3.13 ± 0.06^{A}	3.21 ± 0.04^{A}
Sepals	0.67 ± 0.09^{A}	0.67 ± 0.03^{A}	0.69 ± 0.03^{A}	0.96 ± 0.01^{A}	0.95 ± 0.01^{A}	0.90 ± 0.01^{A}
Stamens	0.23 ± 0.03^{B}	1.34 ± 0.02^{A}	1.59 ± 0.01^{A}	1.69 ± 0.05^{B}	5.35 ± 0.18^{A}	4.76 ± 0.10^{A}
Androphorus	0.66 ± 0.03^{B}	1.47 ± 0.02^{A}	1.25 ± 0.01^{A}	–	–	–
Gynophore	0.50 ± 0.02^{A}	0.23 ± 0.05^{B}	0.57 ± 0.04^{A}	3.03 ± 0.52^{B}	0.55 ± 0.05^{C}	4.51 ± 0.05^{A}
Ovary	0.52 ± 0.03^{A}	0.52 ± 0.01^{A}	0.53 ± 0.01^{A}	1.06 ± 0.04^{A}	0.51 ± 0.01^{B}	0.94 ± 0.01^{A}
	Tarenaya hassleriana (THCS)			*Tarenaya hassleriana* (TDM)		
	F	M	B	F	M	B
Petals	2.76 ± 0.08^{B}	3.00 ± 0.03^{A}	3.03 ± 0.02^{A}	2.98 ± 0.18^{B}	3.70 ± 0.10^{A}	3.66 ± 0.06^{A}
Sepals	0.95 ± 0.01^{A}	0.93 ± 0.01^{A}	0.95 ± 0.01^{A}	0.9 ± 0.01^{A}	0.93 ± 0.01^{A}	0.96 ± 0.01^{A}
Stamens	1.69 ± 0.05^{C}	5.15 ± 0.09^{A}	4.45 ± 0.19^{B}	0.80 ± 0.01^{B}	5.16 ± 0.09^{A}	5.03 ± 0.01^{A}
Gynophore	3.03 ± 0.52^{B}	0.94 ± 0.08^{C}	4.25 ± 0.16^{A}	2.90 ± 0.01^{A}	0.37 ± 0.19^{B}	3.42 ± 0.01^{A}
Ovary	1.06 ± 0.04^{A}	0.53 ± 0.03^{B}	1.04 ± 0.01^{A}	1.00 ± 0.01^{A}	0.62 ± 0.01^{B}	0.96 ± 0.01^{A}
	Tarenaya hassleriana (THJ)			*Tarenaya hassleriana* (THP)		
	F	M	B	F	M	B
Petals	2.25 ± 0.01^{B}	2.89 ± 0.05^{A}	2.95 ± 0.08^{A}	2.50 ± 0.01^{B}	3.16 ± 0.02^{A}	3.43 ± 0.03^{A}
Sepals	1.01 ± 0.01^{A}	1.10 ± 0.01^{A}	0.91 ± 0.01^{A}	0.99 ± 0.01^{A}	0.95 ± 0.01^{A}	0.99 ± 0.01^{A}
Stamens	0.91 ± 0.04^{C}	5.39 ± 0.24^{A}	4.38 ± 0.16^{B}	0.77 ± 0.04^{C}	5.75 ± 0.06^{B}	6.38 ± 0.03^{A}
Gynophore	3.10 ± 0.08^{B}	0.75 ± 0.07^{C}	4.96 ± 0.19^{A}	3.00 ± 0.05^{B}	0.10 ± 0.01^{C}	4.51 ± 0.03^{A}
Ovary	1.05 ± 0.02^{A}	0.48 ± 0.01^{B}	1.17 ± 0.01^{A}	1.00 ± 0.01^{A}	0.30 ± 0.01^{B}	1.00 ± 0.01^{A}
	Tarenaya hassleriana (THSM)			*Tarenaya hassleriana* (THV)		
	F	M	B	F	M	B
Petals	2.32 ± 0.02^{A}	2.98 ± 0.05^{A}	2.87 ± 0.05^{A}	2.33 ± 0.01^{B}	3.02 ± 0.04^{A}	2.85 ± 0.05^{A}
Sepals	0.81 ± 0.01^{A}	0.89 ± 0.01^{A}	0.90 ± 0.01^{A}	0.84 ± 0.02^{A}	0.92 ± 0.01^{A}	0.90 ± 0.01^{A}
Stamens	0.59 ± 0.05^{C}	5.35 ± 0.20^{A}	4.62 ± 0.27^{B}	0.63 ± 0.03^{C}	5.51 ± 0.19^{A}	4.38 ± 0.25^{B}
Gynophore	3.22 ± 0.03^{B}	0.52 ± 0.06^{C}	4.32 ± 0.14^{A}	3.23 ± 0.02^{B}	0.55 ± 0.07^{C}	4.26 ± 0.12^{A}
Ovary	1.02 ± 0.07^{A}	0.38 ± 0.01^{B}	0.94 ± 0.02^{A}	1.07 ± 0.04^{A}	0.40 ± 0.02^{B}	0.95 ± 0.01^{A}

GG: *Gynandropsis gynandra* (Mossoró-RN); THC: *Tarenaya hassleriana* (Canaã-MG); THCS: *T. hassleriana* (Canoinhas-SC); THDM: *T. hassleriana* (Domingos Martins-ES); THJ: *T. hassleriana* (Joinville-SC); THP: *T. hassleriana* (Piau-MG); THS: *T. hassleriana* (São Miguel-MG); THV: *T. hassleriana* (Viçosa-MG). Letters indicate significant groupings according to Tukey's Test.

Through the characterization of the flowers produced by 10 individuals of each species, we classified the type of sexual systems of each species according to Sakai and Weller (1999).

Additionally, we evaluated pollen limitation (PL), as described by Larson and Barrett 2000, using the following formula: PL = 1 − (OP/CP). A PL value exceeding 0.66 indicates high reproductive efficiency of the species resulting from the pollen flow promoted by pollinators (Zapata and Arroyo, 1978), while a value close to or below 0.25 indicates pollen limitation (Sobrevila and Arroyo, 1982). Values exceeding 1.00 are noted when the fruit set through open pollination is greater than that through cross-pollination, indicating highly efficient pollinators.

We also calculated the self-incompatibility index (ISI) using the following formula: ISI = HP/CP. The ISI is the ratio between the number of fruits formed through self-pollination and that through cross-pollination. As such, the ISI values below 0.25 indicates self-incompatibility (Bullock, 1985). Values between 0.25 and 0.75 indicate self-incompatibility with some level of self-compatibility; and values exceeding 0.75 indicate self-compatibility (Lloyd and Schoen, 1992). Finally, we calculated the self-pollination index (AI) using the following formula: AI = A/CP (Lloyd and Schoen, 1992). In 10 flowers that were previously bagged at the bud stage, nectar volume was measured as described by Machado et al. (2006).

2.4. *Seed germination test*

Mature fruits (approximately 45 days after anthesis) produced from each tagged flower were harvested. Then, the seeds were removed from the fruit and allowed to dry at room temperature (~19 °C) for 5–7 days, in which the number of seeds per fruit/species was counted and the seeds weighed. After this process, seeds of the same species that underwent the same treatment were mixed in the same package. Subsequently, the seeds were sown in the same commercial substrate described above for the germination tests. Fifty seeds per species per treatment were used, which were separated into five pots with 10 seeds each. These pots were placed on a tray, randomly, and covered with plastic film. Finally, approximately 200–250 seeds per species were tested. For species that did not produce seeds by self-pollination, 200 seeds were tested, and for species in which self-pollination was observed, 250 seeds were tested. The seeds were maintained in a growth chamber at the day/night temperature of 25 °C/19 °C under a 16:8 h photoperiod (~200 μmol photons $m^{-2} \cdot s^{-1}$) and 60% relative humidity. The germinated seeds were counted weekly. As required, the substrate was moistened to maintain suitable conditions for germination.

2.5. *Statistical analysis*

Descriptive statistics including mean and standard error were calculated for all quantitative traits (sepals length, petals length, stamens length, gynophore length, androphore length, ovary length, and floral bud length). Data of species or treatment (HP, G, CP, OP, and A) were normally distributed and we used the analysis of variance ($P < 0.05$), and the means were subjected to Tukey's test (number of seeds per treatment in each species, seeds germinated per treatment in each species, amount of nectar produced per species). Moreover, the main component analysis (PCA) was used to evaluate the interrelationship/grouping among the different species, taking into account data from the sexual systems of the species (3), flower size, and nectar production. All statistical analyzes were performed using Statistica (version 8.0) and R.3.2.0 software (R Core Development Team 2015).

The analyzed species exhibit diverse growth habits (herb or shrub) and flowers of different colors (white or pale pink to purple) (Fig. 1A). All species exhibited nocturnal anthesis and flower opening, beginning at sunset. At the beginning of anthesis, nectar was secreted through a disk-shaped nectary. Large flowers observed in Spinosa I, Spinosa II, *Cleoserrata*, Parviflora, and Rosea groups, produced abundant nectar at the corolla base (~20–60 μL per flower, collected at up to three times within the same night; Fig. 1S). Meanwhile, small flowers (Aculeata and *Gynandropsis*) produced no more than 5 μL of nectar per flower. In general, each inflorescence produced approximately five opened flowers daily, which could be of different sexes. Moreover, each inflorescence produced up to 900 μL of nectar (in large flowers) per day. Of note, a fully developed individual of the shrubby species branched profusely, bearing up to 10 racemes per plant.

3. Results

3.1. *Diversity within Cleomaceae*

Morphological characteristics, such as flower size, nectar production together with the sexual systems of the species, and the production of pollen were used to perform a principal component analysis (PCA) (Fig. 1B, Table 2S). The results showed the formation of four groups: (i) Spinosa + Parviflora + Rosea, (ii) Aculeata, (iii) Cleoserrata and (iv) Gynandropsis. The first two axes used for species separation explain 94.18% of the variation. The contribution of each variable is shown in table 2S. Accordingly, the groupings were performed basically by the type of sexual systems of the species (next topic) (Fig. 1B). It is worth mentioning that the result of the grouping of species by PCA, despite having been carried out with morphological data, is in accordance with the molecular phylogeny based on five molecular markers and three genomes (mitochondrial, chloroplast and nuclear; Parma et al., 2022). Thus, it can be concluded that the formed groups are the closest phylogenetically as well.

3.2. *Sexual systems in Cleomaceae species*

Through the characterization of the flowers produced by 10 individuals of each species or access, it was possible to organize the 10 species in three groups of sexual systems: (i) andromonoecy, (ii) hermaphroditic and, (iii) polygamomonoecious (Tables 1-3). Most of the species were allocated as polygamomonoecious species (Table 1), which means that there are individuals able to produce all three types of flowers (bisexual, male and, female). However, it is worth mentioning that some polygamomonoecious species have unisexual female individuals and individuals with all three floral morphs (Table 2). The species categorized are *G. gynandra* (GG) - cluster *Gynandropsis* - and *T. hassleriana* accesses THC, THCSC, THDM, THJ, THP, THSM and, THV, which belong to the cluster Spinosa II. In this group, female flowers are smaller than both male and bisexual flowers, noticeable by the length of the petals (Table 1). Stamens also have different lengths when compared to the sexual types of flowers. Occasionally, staminodes were observed in all the three sexual types, even though, they were always present in female flowers.

In polygamomonoecious species *T. parviflora* (Kunth) Iltis (TP), *T. rosea* (Vahl ex DC.) Soares Neto & Roalson (TR), *T. longicarpa* (TL, TAF, TIB, TARC, and TAM), and *T. spinosa* (TS) we occasionally observed individuals that produced only female flowers (Table 2). Female unisexual subjects appeared at a frequency of 20–50%. Thus, for example, the species *T. rosea* and *T. parviflora* presented 20% of female unisexual individuals, *T. spinosa* 30% and the different accessions of *T. longicarpa* differed a little, TAM with 30%, TL and TIB with 40%, TAF and TARC with 50%. In this group, the female flowers of the female plants were smaller than those of the polygamomonoecious plants (Table 2). The petals, sepals, stamens/staminodes, gynophores, and ovaries was shorter in the female plants (Table 2). Of note, the polygamomonoecious, which comprised 16 species, variation among and within species in the proportions of the three types of flowers is summarized in Fig. 2S. Overall, these species produced more bisexual and male flowers, although the occurrence of female flowers was observed (Fig. 2S).

Cleoserrata paludosa (Willd. ex Eichler) Iltis ex Soares Neto & Roalson alone was classified as andromonoecious, which comprised accessions that produced bisexual and male flowers on the same plant (Table 3). The flowers were small, without evident differences between the male and bisexual flowers, except in the length of the gynophores and ovaries (Table 3).

Finally, the bisexual species *T. aculeata* (L.) Soares Neto & Roalson (TA), *T. microcarpa* (Ule) Soares Neto & Roalson (TM), and *T. diffusa* (Banks ex DC.) Soares Neto & Roalson (TD) produced only hermaphroditic flowers. Nevertheless, these flowers could be chasmogamous (open flower for cross-pollination) or cleistogamous (non-opening

Table 2

Summary statistics for mean values and standard deviations for petals, sepals, stamens, gynophore and ovary length in accesses of Cleomaceae species – Group Polygamomonoecious (unisexual female individual and individual with the three floral morphs - female, male and bisexual). F: Female flowers, M: Male flowers, B: Bisexual flowers. Number corresponds to the medium value of the size (cm) of the structures, followed by ± SE, $n = 50$.

	Polygamomonoecious							
	Tarenaya longicarpa (TL)				*Tarenaya rosea* (TR)			
	Female	Polygamomonoecious			Female	Polygamomonoecious		
	F	F	M	B	F	F	M	B
Petals	1.90 ± 0.01^{C}	2.50 ± 0.09^{B}	2.65 ± 0.07^{A}	2.78 ± 0.05^{A}	1.69 ± 0.01^{B}	1.92 ± 0.01^{B}	3.25 ± 0.01^{A}	2.81 ± 0.08^{A}
Sepals	0.79 ± 0.01^{B}	0.89 ± 0.01^{A}	0.86 ± 0.001^{A}	0.94 ± 0.01^{A}	0.97 ± 0.01^{A}	0.80 ± 0.01^{B}	1.10 ± 0.09^{A}	0.96 ± 0.01^{A}
Stamens	0.45 ± 0.01^{C}	0.95 ± 0.04^{B}	5.00 ± 0.01^{A}	5.03 ± 0.11^{A}	0.36 ± 0.02^{C}	0.55 ± 0.02^{C}	6.25 ± 0.01^{A}	4.60 ± 0.01^{B}
Gynophore	2.40 ± 0.02^{B}	3.03 ± 0.04^{A}	0.82 ± 0.01^{C}	3.14 ± 0.02^{A}	1.64 ± 0.05^{C}	2.19 ± 0.04^{B}	0.40 ± 0.01^{D}	4.32 ± 0.01^{A}
Ovary	0.92 ± 0.01^{A}	1.00 ± 0.02^{A}	0.47 ± 0.01^{B}	0.94 ± 0.02^{A}	0.99 ± 0.01^{A}	0.94 ± 0.08^{A}	1.10 ± 0.01^{A}	1.09 ± 0.01^{A}
	Tarenaya parviflora (TP)				*Tarenaya spinosa* (TS)			
	Female	Polygamomonoecious			Female	Polygamomonoecious		
	F	F	M	B	F	F	M	B
Petals	1.93 ± 0.01^{C}	2.50 ± 0.06^{B}	2.92 ± 0.02^{A}	2.99 ± 0.01^{A}	1.69 ± 0.01^{C}	2.50 ± 0.01^{B}	2.95 ± 0.01^{A}	3.11 ± 0.01^{A}
Sepals	0.81 ± 0.01^{B}	0.91 ± 0.03^{A}	1.04 ± 0.01^{A}	1.09 ± 0.01^{A}	0.80 ± 0.01^{B}	0.98 ± 0.01^{A}	0.83 ± 0.01^{B}	0.87 ± 0.01^{B}
Stamens	0.47 ± 0.03^{D}	0.95 ± 0.01^{C}	5.34 ± 0.05^{A}	2.12 ± 0.03^{B}	0.36 ± 0.02^{C}	0.95 ± 0.01^{C}	5.87 ± 0.17^{A}	4.64 ± 0.34^{B}
Gynophore	2.38 ± 0.01^{B}	3.03 ± 0.08^{A}	0.15 ± 0.01^{C}	3.29 ± 0.04^{A}	1.64 ± 0.01^{B}	3.03 ± 0.05^{A}	1.37 ± 0.08^{B}	3.30 ± 0.15^{A}
Ovary	0.95 ± 0.01^{A}	1.00 ± 0.01^{A}	0.25 ± 0.01^{B}	0.91 ± 0.01^{A}	0.99 ± 0.01^{A}	1.00 ± 0.01^{A}	0.67 ± 0.01^{B}	1.03 ± 0.02^{A}
	Tarenaya longicarpa (TAF)				*Tarenaya longicarpa* (TAM)			
	Female	Polygamomonoecious			Female	Polygamomonoecious		
	F	F	M	B	F	F	M	B
Petals	1.90 ± 0.09^{C}	2.18 ± 0.10^{B}	2.93 ± 0.05^{A}	3.01 ± 0.07^{A}	1.80 ± 0.01^{B}	2.14 ± 0.02^{A}	2.21 ± 0.09^{A}	2.30 ± 0.27^{A}
Sepals	0.89 ± 0.04^{A}	0.99 ± 0.02^{A}	0.89 ± 0.01^{A}	0.85 ± 0.02^{A}	1.02 ± 0.01^{A}	1.00 ± 0.01^{A}	0.91 ± 0.01^{A}	0.94 ± 0.01^{A}
Stamens	0.45 ± 0.04^{B}	0.61 ± 0.05^{B}	5.70 ± 0.17^{A}	5.32 ± 0.31^{A}	0.61 ± 0.06^{C}	1.01 ± 0.02^{B}	2.81 ± 0.02^{A}	2.79 ± 0.27^{A}
Gynophore	2.40 ± 0.12^{B}	2.71 ± 0.21^{B}	0.45 ± 0.05^{C}	5.26 ± 0.12^{A}	2.84 ± 0.03^{B}	2.94 ± 0.05^{B}	0.62 ± 0.07^{C}	4.08 ± 0.03^{A}
Ovary	0.92 ± 0.01^{A}	0.97 ± 0.02^{A}	0.57 ± 0.03^{B}	1.07 ± 0.01^{A}	1.14 ± 0.02^{A}	1.09 ± 0.01^{A}	0.58 ± 0.01^{B}	1.05 ± 0.01^{A}
	Tarenaya longicarpa (TARC)				*Tarenaya longicarpa* (TIB)			
	Female	Polygamomonoecious			Female	Polygamomonoecious		
	F	F	M	B	F	F	M	B
Petals	1.00 ± 0.01^{C}	1.70 ± 0.02^{B}	2.24 ± 0.06^{A}	2.30 ± 0.04^{A}	1.00 ± 0.01^{C}	1.70 ± 0.02^{B}	2.24 ± 0.06^{A}	2.30 ± 0.04^{A}
Sepals	0.90 ± 0.01^{A}	0.95 ± 0.01^{A}	0.79 ± 0.01^{B}	0.93 ± 0.03^{A}	0.90 ± 0.01^{A}	0.95 ± 0.01^{A}	0.79 ± 0.03^{B}	0.93 ± 0.01^{A}
Stamens	1.00 ± 0.01^{C}	0.95 ± 0.03^{C}	3.07 ± 0.06^{A}	2.38 ± 0.04^{B}	1.00 ± 0.01^{C}	0.95 ± 0.03^{C}	3.07 ± 0.18^{A}	2.38 ± 0.17^{B}
Gynophore	1.20 ± 0.01^{B}	1.20 ± 0.01^{B}	0.23 ± 0.11^{C}	2.63 ± 0.04^{A}	1.20 ± 0.01^{B}	1.20 ± 0.01^{B}	2.63 ± 0.11^{A}	0.23 ± 0.04^{C}
Ovary	0.60 ± 0.01^{A}	0.62 ± 0.18^{A}	0.35 ± 0.01^{B}	0.60 ± 0.01^{A}	0.60 ± 0.01^{A}	0.62 ± 0.01^{A}	0.30 ± 0.01^{B}	0.60 ± 0.01^{A}

TL: *Tarenaya longicarpa* (Picos-PI); TR: *T. rosea* (Colatina-ES); TP: *T. parviflora* (Pombal-PB); TS: *T. spinosa* (Teresina-PI); TAF: *T. longicarpa* (Afrânio-PE); TAM: *T. longicarpa* (Manaus-AM); TARC: *T. longicarpa* (Arcoverde-PE); TIB: *T. longicarpa* (Ibimirim-PE). Letters indicate significant groupings according to Tukey's Test.

Table 3

Summary statistics for mean values and standard deviations for petals, sepals, stamens, gynophore, ovary and floral bud length in accesses of Cleomaceae species – Group Andromonoecy and Hermaphroditic. M: Male flowers, B: Bisexual flowers. Cleis: cleistogamous, Casm: chasmogamous flowers. Number corresponds to the medium value of the size (cm) of the structures, followed by ± SE, $n = 50$.

	Andromonoecy		Hermaphroditic					
	Cleoserrata paludosa (CP)		*Tarenaya aculeata* (TA)		*Tarenaya diffusa*(TD)		*Tarenaya microcarpa* (TM)	
	M	H	Cleis	Casm	Cleis	Casm	Cleis	Casm
Petals	2.00 ± 0.01^{A}	1.96 ± 0.01^{A}	–	1.25 ± 0.01	–	1.20 ± 0.01	–	1.23 ± 0.01
Sepals	0.41 ± 0.01^{A}	0.50 ± 0.01^{A}	–	0.40 ± 0.01	–	0.40 ± 0.01	–	0.41 ± 0.01
Stamens	1.93 ± 0.01^{A}	1.95 ± 0.01^{A}	–	0.69 ± 0.01	–	0.67 ± 0.01	–	0.66 ± 0.01
Gynophore	0.1 ± 0.01^{B}	0.62 ± 0.01^{A}	–	0.35 ± 0.01	–	0.32 ± 0.01	–	0.33 ± 0.01
Ovary	0.4 ± 0.01^{B}	0.84 ± 0.01^{A}	–	0.45 ± 0.01	–	0.44 ± 0.01	–	0.44 ± 0.01
Floral bud	0.00^{A}	0.00^{A}	0.52 ± 0.01	0	0.41 ± 0.01	–	0.47 ± 0.01	–

CP: *Cleoserrata paludosa* (Belém-PA); TA: *Tarenaya aculeata* (Feira de Santana-BA); TD: *T. diffusa* (Feira de Santana-BA); TM: *T. microcarpa* (Belém-PA). Letters indicate significant groupings according to Tukey's Test.

flowers for self-pollination) (Fig. 3S, Table 3).

3.3. Effects of pollination type on fruit set

Hand cross-pollination produced the highest seed yield, followed by open pollination (Figs. 2 and 3). Conversely, natural self-pollination (autogamy) produced the lowest seed yield ($n = 18$), with no seed production in 10 species (Figs. 2 and 3). Through natural self-pollination, TD produced the highest number of seeds. Through cross-pollination, TA, THC, THV, THP, THCSC, THSM, TL, TR, TS, TAF, TAM, and TIB produced the highest number of seeds (Figs. 2 and 3). *Gynandropsis gynandra* was the only species that produced the highest number of seeds through open pollination. In contrast, THDM and TP produced more seeds per silique through geitonogamy. *Cleoserrata paludosa*, TM, and THJ produced more seeds through cross- and open pollination, although there were no significant differences between these treatments. *T. longicarpa* (Arcoverde-PE; TARC) produced more seeds through cross-pollination, open pollination, and geitonogamy (Figs. 2 and 3).

Dichogamy is when anthers dehisce and stigmas become receptive at different times (Pattemore 2017), being the protandry - gynoecia matured after androecia - observed in all species, except in the Aculeata cluster in which this strategy to avoid self-fertilization was not observed (adichogamy). In some of the observations, we found that the gynoecium matured up to 12 h after the androecium (the gynoecium was covered by a petal). However, most of the time the difference in maturation between the organs was up to an hour. In addition, most of the Cleomaceae species exhibited herkogamy (Fig. 1A). Both, styles

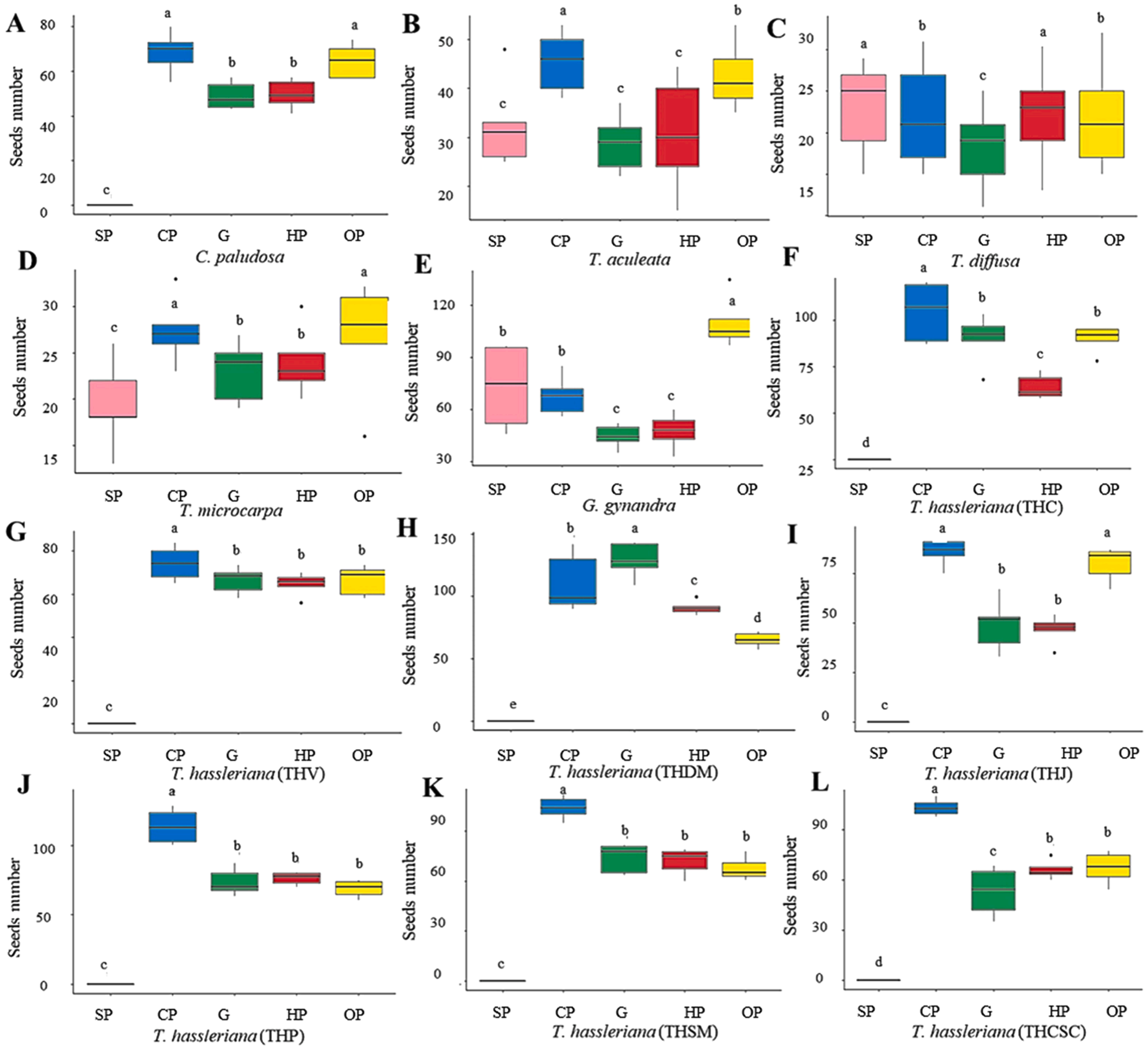

Fig. 2. Mature seeds per fruit according to the pollination treatments. Letters above individual box-scatter indicate significant groupings according to Tukey's Test, $n = 50$. The median is indicated by solid lines in each box, data dispersion is represented by the interquartile range, followed by standard error and outliers. Natural self-pollination (SP); Open pollination (OP); Hand self-pollination (HP); Cross-pollination (CP); Geitonogamy (G).

extending beyond stamens and stamens extending beyond styles were observed. Notably, all species were genetically self-compatible (ISI $>$ 0.3) (Fig. 4S); therefore, various types of mating systems may occur given the lack of pollen limitation (PL $>$ 0.20). However, only GG, TA, TD, and TM exhibited autogamy (AI $>$ 0.7), while the rest of the species exhibited AI values below 0.7 (Fig. 4S), indicating very little or no autogamous self-pollination.

3.4. *Seed germination*

Species in the Spinosa I cluster exhibited the highest germination rate (~65%), followed by those in *Gynandropsis* and *Cleoserrata* (both 38%), Spinosa II (35%), and Aculeata (25%) (Fig. 5S). In addition, the seeds of Spinosa I species germinated faster and were more homogeneous (synchrony of seed germinated) than those of the other species. Overall, for some species, there were no significant differences in germination rate among the different treatments (Table 4). Specifically, there were no significant differences in the seed germination of TA, TD, and TM (~25%) among the five pollination treatments. Likewise, there were no significant differences in the seed germination of TARC (57%), TR (65%), THCS (37%), THS (32%), and TAM (75%) among the five pollination treatments, except in autogamy, under which no seeds were produced (Table 4).

4. Discussion

4.1. *Cleomaceae exhibits remarkable diversity in floral functions and reward production*

Herkogamy reduces the interference between the sexual parts and lowers the deposition of self-pollen on the stigma (Pattemore, 2017), thereby minimizing the conflict between sexes in bisexual flowers (Barrett, 2002). Cleomaceae species also exhibit several floral morphs (Kers 1993; Raju and Rani 2016). In the present study, we observed both functionally male short-gynoecium and functionally bisexual medium and long-gynoecium floral types (Fig. 1), consistent with the findings in *G. gynandra* reported by Raju and Rani (2016) and Zohoungbogbo et al. (2018). In this study, we observed that flowers differ between individual plants of a species in heights at which the stamens and style are positioned (Fig. 1A). The exception for those traits was observed in the Aculeata cluster.

Dichogamy, as pointed out by Stout (1928), was observed mainly in

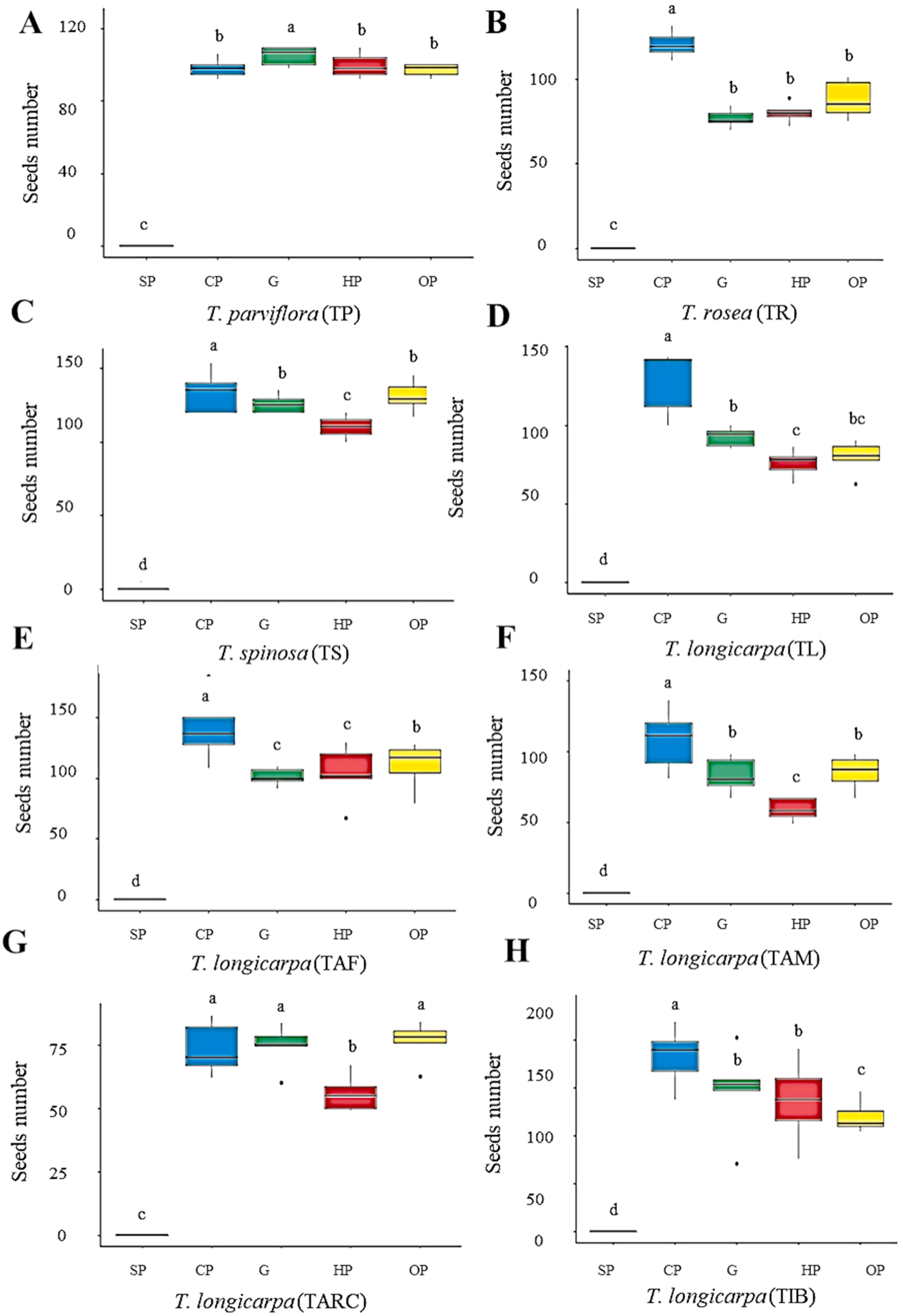

Fig. 3. Mature seeds per fruit by pollination treatments for Cleomaceae species. Letters above individual box-scatter indicate significant groupings according to Tukey's Test, $n = 50$. The median is indicated by solid lines in each box, data dispersion is represented by the interquartile range, followed by standard error and outliers. Natural self-pollination (SP); Open pollination (OP); Hand self-pollination (HP); Cross-pollination (CP); Geitonogamy (G).

Table 4

Germinated seeds by pollination treatments for Cleomaceae species. Mean of treatments followed by letters indicate significant groupings according to Tukey's Test, $n = 5$. Natural self-pollination (SP); Open pollination (OP); Hand self-pollination (HP); Cross-pollination (CP); Geitonogamy (G).

SPECIES	TYPES OF POLLINATION				
	HP	G	CP	OP	SP
Cleoserrata paludosa (CP)	3^{A}	4^{A}	3.4^{A}	5^{A}	0^{A}
Gynandropsis gynandra (GG)	2.4^{B}	3^{B}	4.4^{AB}	5.6^{A}	3.2^{AB}
Tarenaya aculeta (TA)	2^{A}	2.8^{A}	2.6^{A}	3.4^{A}	2.2^{A}
Tarenaya diffusa (TD)	2^{A}	2.6^{A}	2.4^{A}	2.4^{A}	1.8^{A}
Tarenaya hassleriana (THC)	4^{A}	3.6^{A}	2.6^{A}	4.2^{A}	0^{B}
Tarenaya hassleriana (THCS)	3^{A}	4.2^{A}	3.2^{A}	4.4^{A}	0^{B}
Tarenaya hassleriana (THDM)	3.8^{BC}	2.6^{C}	6.6^{A}	6^{AB}	0^{D}
Tarenaya hassleriana (THJ)	2.4^{AB}	2.6^{A}	2.4^{AB}	4.2^{A}	0^{B}
Tarenaya hassleriana (THP)	3^{A}	4.2^{A}	2^{AB}	4.2^{A}	0^{B}
Tarenaya hassleriana (THS)	2^{AB}	3.2^{AB}	3.8^{A}	3.8^{A}	0^{B}
Tarenaya hassleriana (THV)	3^{A}	3.4^{A}	1.4^{AB}	3^{A}	0^{B}
Tarenaya longicarpa (TAF)	2.8^{B}	7.2^{A}	9^{A}	8.6^{A}	0^{C}
Tarenaya longicarpa (TAM)	8.4^{A}	7^{A}	6.4^{A}	8.4^{A}	0^{B}
Tarenaya longicarpa (TARC)	6^{AB}	5.2^{AB}	4.2^{B}	7.4^{A}	0^{C}
Tarenaya longicarpa (TIB)	2.8^{B}	3.6^{B}	8.6^{A}	8.2^{A}	0^{C}
Tarenaya longicarpa (TL)	8.6^{A}	4.8^{B}	6.6^{AB}	5.8^{B}	0^{C}
Tarenaya microcarpa (TM)	2^{A}	2.6^{A}	2.6^{A}	2.6^{A}	2.4^{A}
Tarenaya parviflora (TP)	8.4^{A}	6.6^{AB}	4.6^{AB}	8^{A}	0^{C}
Tarenaya rosea (TR)	6^{A}	5.6^{A}	7.2^{A}	7^{A}	0^{B}
Tarenaya spinosa (TS)	4^{B}	4.6^{B}	4.4^{B}	8.2^{A}	0^{C}

polygamous species. Herkogamy and dichogamy are strategies that hinder self-pollination. In this way, these strategies increase genetic variability, what may provide a strong evolutionary potential (Pattemore 2017). However, plants that have these strategies are often dependent on pollinating agents. In contrast, the Aculeata group does not have strategies to avoid self-pollination. This group is formed by species that guarantee seed production and, therefore, are pollinator-independent. In this way, they are able to preserve the most adapted genotypes. Notwithstanding, their descendants are organisms with reduced genetic variability and may exhibit few adaptations to changes in environmental conditions.

Thus, in addition to studies on the reproductive biology and floral morphometrics, the frequency of specific floral morphs in the populations of heterostylous species must be explored to understand the adaptive meaning of this polymorphism (Matias et al., 2016). In the present study, for instance, *G. gynandra* possessed 27% female, 10% male, and 63% of bisexual flowers (Fig. 1S), contrary to previous observations by Zohoungbogbo et al. (2018) in the same species but in plants from other population (70% male and 30% bisexual flowers).

Andromonoecy, a sexual system in which all individuals have both bisexual and male flowers, despite few records for the family, have already been found for the genera *Cleome* L., *Cleomella* DC., *Polanisia* Raf., and *Tarenaya* Raf. (Schlessman et al., 2020), and *Gynandropsis* (Zohoungbogbo et al., 2018). However, it should be noted that in *Gynandropsis* (monotype genus – *G. gynandra*) we observed the species as polygamomonoecious as previously described (Raju and Rani, 2016). The hermaphrodite species produce only bisexual flowers (i.e. *C. viscosa*), thus we verified this trait only in herbaceous species (*T. aculeata, T. diffusa* and *T. microcarpa*). This result is in line with the findings of Wang et al. (2020), who observed that in herbaceous species hermaphroditism prevails compared to woody species.

The genetic and environmental factors conferring the observed plasticity in sex expression in Cleomaceae are still incipient. Nonetheless, in addition to the endogenous factors, such as hormones (e.g., autonomous gibberellin pathways), environmental factors, such as photoperiod, vernalization, temperature, drought, and salinity, exogenous applied hormones and chemicals, and pathogenic microbes influence sex expression in many species (Aryal et al., 2014; Banks, 2008; Chuck et al., 2007; Lai et al., 2018; Pawełkowicz et al., 2019; She et al., 2009). In this sense, it was observed in *Tarenaya spinosa*, that pistil development is stimulated by the presence of mature leaves and mineral nutrition, which, in turn, has its development inhibited by the increase of auxin in the seeds of young fruits (Jong and Bruinsma 1974a,b,c). However, in the species *Cleome iberidella* Welw. Ex Oliv., nutritive factors and their interaction with growth substances, petal development is more sensitive to carbohydrate and nitrogen deficiencies than pistil growth, which, in turn, is favored by low pH values (Jong et al., 1974). Therefore, given the variability in factors that regulate and influence floral formation, the elucidation of the molecular mechanisms underlying sex determination represents an enormous challenge in plant biology (Pawełkowicz et al., 2019). Of note, environmental factors affect the sex of flowers as well as the timing of flowering (Kozlov et al., 2020). Therefore, mechanisms underlying flower formation warrant further exploration to aid the development of novel techniques of sex manipulation, particularly for plants closely related with Cleomaceae species. Previous studies in this regard were very broad, analyzing the genomics and transcriptomics of flower buds and flowers and exploring the links among the related genes (Durand and Durand, 1991).

4.2. Cleomaceae employs various pollination strategies

Cleomaceae species benefit from both self- and cross-pollination to ensure reproductive success (Raju and Rani 2016). However, hand cross-pollination and open pollination resulted in the highest seed yield in most of the Cleomaceae species studied (Figs. 2 and 3). This finding reflects the importance of pollinators in plant reproductive mechanisms, enabling gene flow between individuals and increasing heterozygosity. Cross-pollination improved crop quality and yield, in addition to enhancing seed and fruit set (Vinícius-Silva et al., 2017), as well affects fruit color, acidity, firmness and shelf life (Dung et al., 2021). Meanwhile, as observed in the Aculeata and Gynandropsis clusters (Fig. 2), regardless of the genetic benefits of outcrossing, a significant number of angiosperm species (around 20%) have evolved to be predominantly autogamous (Barrett, 2002). However, this trait may be highlighted as an advantage in adverse environments with low frequency of floral visitors, as anticipated in the face of current climate crisis. Therefore, Cleomaceae is a key model to understand the various pollination strategies, given that the populations of pollinators are currently dwindling (Hallmann et al., 2017; Marshman et al., 2019) and at least 75% of plants still dependent on pollinators (Klein et al., 2007; Kremen, 2018).

Furthermore, the type of pollination did not affect seed germination (Table 4). Interestingly, however, the recorded germination rates (of the untreated seeds) of majority of the species were different from the values reported previously. For instance, for *G. gynandra*, we recorded the germination rate of 38%, whereas Zohoungbogbo et al. (2018) and Ekpong (2009) have recorded the germination rate of only 25% and 17%, respectively. Overall, the germination rate remained low in *Gynandropsis, Cleoserrata*, Spinosa II, and Aculeata, which may be explained by the fact that the mature seeds of Cleomaceae species enter a period of inactivity or extremely low metabolic activity (dormancy). This fact/characteristic has already been verified in *G. gynandra* (Ekpong, 2009; Zohoungbogbo et al., 2018), *T. spinosa* (Castro et al., 2014), *T. hassleriana, Polanisia dodecandra* (Gomez Raboteaux and Anderson, 2010), *Cleome lutea*, and *C. serrulata* (Cane, 2008) and *C. viscosa* (Raju and Rani 2016). Nonetheless, the higher germination rates observed in these previous studies were achieved through additional treatments, such as prolonged storage at specific temperatures; application of GA_3 or KNO_3; and leaching, pre-chilling, soaking, and pre-heating at specific temperatures (e.g., vernalization). In addition, the seeds of some Cleomaceae (e.g., *T. hassleriana*) bear thick coats, which delay germination. In such species, scarification, which involves mechanical or chemical processes to soften the seed coat, can improve the germination rate.

Moreover, although no pollen incompatibility was detected in the crosses of the studied species, the overall low germination rate of Cleomaceae species may be attributed to the disruption of the pollen, embryo sac, embryo, and endosperm development, which may result in seed abortion (Shao et al., 2020). Normal viable seeds cannot form during pollination, fertilization, and embryo development (Shao et al., 2020). Thus, a systematic study of these processes may provide a theoretical basis and reference for exploring the mechanism of seed abortion and establishing an efficient assisted-breeding system.

4.3. Perspectives on Cleomaceae

The species studied here reflect the remarkable diversity of the Cleomaceae family in terms of morphology (Patchell et al., 2011), photosynthetic mechanisms (Feodorova et al., 2010; Marshall et al., 2007; Voznesenskaya et al., 2007), genomics (Inda et al., 2008), pollination (Cane, 2008; Machado et al., 2006; Zohoungbogbo et al., 2018), and geographic distribution (Bayat et al., 2018). Accordingly, this family has been proposed as a model thanks to its several peculiarities and its close phylogenetic relationship with *Arabidopsis thaliana* of the Brassicaceae family (Iltis et al., 2011), from which Cleomaceae has diverged relatively recently (~35 mya.) (Schranz and Mitchell-olds, 2006).

Most of the species here studied, in addition to bats, are pollinated by bees (e.g., Machado et al., 2006), which benefit both from the large amounts of pollen (proteins) and nectar (sugars) produced. The abundance and diversity of pollinators, particularly bees, have declined over the past several decades (Aizen et al., 2009; Hallmann et al., 2017), eliciting cascading effects on the food webs and jeopardizing the ecosystem services (Hallmann et al., 2017). In this regard, the creation of flower-rich habitats, such as hedgerows, field borders, or cover crops, has been proposed to conserve bee populations and enhance crop pollination (Williams et al., 2015; Wratten et al., 2012). Nonetheless, whether these habitats actually increase the number of pollinators required for targeted crop pollination (Sidhu and Joshi, 2016) and whether flowers in these habitats compete with the crop to interfere with crop pollination (Bostanian et al., 2013) remain unclear. Hence, to help address the needs of agriculture and pollinator preservation, the development of crop varieties with specific nectar or nectar-related traits (as observed in Cleomaceae species) for attracting and retaining pollinating insects is an appealing strategy (Prasifka et al., 2018). Thus, Cleomaceae (which is phylogenetically close to Brassicaceae) has emerged as a promising model to devise strategies for improving crop yield (through promoting cross-pollination) and floral reward production (pollen and nectar) for pollinators, particularly bees. Furthermore, the various types of sexual systems observed in the family (i.e. Schlessman et al., 2020) should provide interesting new insights into the evolution of sexual systems in angiosperms.

5. Conclusions

Cleomaceae species have different types of sexual systems, essentially characterized by three types: andromonoecy, bisexual plants and polygamomonoecious. In addition, through the various pollination tests, the perception of a need for pollinators was confirmed. The majority of species had the higher yield in number of seeds when cross pollinated. Evolutionary features, such as herkogamy, dichogamy and unisexual flowers, to avoid self-pollinated were present in some of them. All of that lead us to a co-evolution perspective through the Cleomaceae family. Furthermore, it should be noted that the floral function diversity and production of rewards observed are important characteristics at times of pollinators population decline, since the larger part of angiosperms is pollinated by animals, especially bees. In this sense, Cleomaceae family expressed interesting tools to attract pollinators, which could be very useful in cultivated species if we are able to identify and characterize the genetic factors involved in these traits. However, further studies are needed to verify the viability of introducing these factors in plant breeding programs.

Author contribution statement

DFP and ANN designed the research; DFP performed most of the research with the support of MGMVV, KFS, and SBM; WLA, AZ, APMW, and ANN provided technical, logistic, and financial support; DFP and ANN analyzed the data; DFP and ANN wrote the article with input from all the others.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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