



Reproductive ecology of the dioecious endemic *Erodium sibthorpiatum* subsp. *sibthorpiatum* in Türkiye

Dilek Oskay¹

Received: 18 January 2026 / Accepted: 14 April 2026 / Published online: 27 April 2026
© The Author(s) 2026

Abstract

Erodium sibthorpiatum subsp. *sibthorpiatum* (Geraniaceae) is an endemic taxon restricted to western Türkiye. Understanding its reproductive traits and the factors governing reproductive success is essential for elucidating its reproductive ecology. The pollen–ovule ratio was calculated as 3043, which falls within the expected range for dioecious species. Pollination is mediated by a diverse assemblage of insects, predominantly Diptera with licking–sucking mouthparts. Pollen viability and stigma receptivity were highly synchronized during anthesis, both peaking on the day of flower opening and declining gradually thereafter. Seed viability averaged 91%. Seeds collected in the same year exhibited high germination success (75–90%) at 10–20 °C, whereas seeds stored for approximately four years showed markedly reduced germination (10–36%) across the same temperature range. The capacity for seed formation per plant used as an indicator of population-level reproductive success was 33.3% in 2019 and 15.1% in 2022. Overall, the findings highlight several key reproductive characteristics of the subspecies and suggest that pollination may be a significant factor influencing reproductive success. Environmental conditions at the microhabitat level or annual climate variability may also have affected the decline. However, additional evidence is needed to clarify the nature and strength of this relationship.

Keywords *Erodium sibthorpiatum* subsp. *sibthorpiatum* · Endemic · Dioecy · Pollination · Reproductive ecology

Introduction

Flowering plants display remarkable diversity in sexual systems, ranging from hermaphroditic individuals bearing flowers with both male and female organs to strictly dioecious species in which the sexes occur on separate individuals, as well as numerous intermediate forms. Sexual system variation is a key determinant of genetic diversity, reproductive success, and long-term evolutionary dynamics within populations (Bawa 1980; Charlesworth and Wright 2001; Eppley and Pannell 2007; Queenborough et al. 2009; Goldberg et al. 2017) and, in some cases, across species (Vamosi and Vamosi 2005; Kay et al. 2006; Käfer et al. 2014; Sabath et al. 2016).

Understanding reproductive biology is particularly important for rare or geographically restricted species, as multiple factors including floral traits, pollination mechanisms, compatibility systems, and seed development collectively determine reproductive success (Marbaniang et al. 2018). Despite their significance, these aspects remain poorly documented for many endemic taxa.

Studies on some endemic and some geophyte species in Türkiye show that integrating assessments of flowering phenology, pollen–ovule ratios, self-pollination incompatibility indices, pollinator communities, and seed viability provides a comprehensive framework for understanding reproductive constraints in narrowly distributed taxa and prioritizing conservation efforts (Gücel and Seçmen 2008, 2009; Güvensen et al. 2013; Oskay 2017, 2020; Atasagun et al. 2018, 2021; Subaşı and Güvensen 2021; Yıldız et al. 2022; Yıldız et al. 2024; Aslay et al. 2023). These investigations underscore the value of reproductive biology studies for both evolutionary insights and conservation planning, particularly in regions with high endemism and fragmented populations.

The genus *Erodium* L'Hér., first described in *Hortus Kewensis* (Aiton 1789), comprises 159 taxa worldwide

Communicated by Ed Witkowski

✉ Dilek Oskay
dilek.oskay@cbu.edu.tr

¹ Faculty of Engineering and Natural Sciences, Department of Biology, Section of Botany, Manisa Celal Bayar University, Manisa, Türkiye

according to the World Flora Online Plant List, including 124 species. In Türkiye, 31 taxa of *Erodium* are recognized, 12 annual and 19 perennial, with 16 taxa endemic to the region (Davis 1967; Davis et al. 1988; Güner et al. 2000; Yıldırım and Doğru-Koca 2004; Özüdoğru and Özgişi 2025). All endemic taxa are perennial, and 11 are dioecious (Davis 1967; Davis et al. 1988; Özüdoğru and Özgişi 2025). Although dioecy occurs in 40–68% of land plant groups, only 5–6% of angiosperms roughly 15,600 species exhibit this sexual system (Villarreal and Renner 2013; Renner 2014; Walas et al. 2018). The relatively high number of endemic, dioecious *Erodium* taxa in Türkiye therefore represents an intriguing pattern in the context of angiosperm sexual system evolution.

Within this context, *Erodium sibthorpiatum* Boiss. subsp. *sibthorpiatum* is a dioecious, perennial, alpine species with a restricted distribution in Uludağ (2100–2440 m), located in northwestern Türkiye (Davis et al. 1988). *Erodium sibthorpiatum* is represented by geographically distinct subspecies with fragmented distributions. Among these, *E. sibthorpiatum* subsp. *sibthorpiatum* is a narrow endemic confined to Uludağ, whereas *Erodium sibthorpiatum* subsp. *vetteri* (Barbey & Fors.-Maj.) Davis (Davis 1967; Persson 1986) has been reported from Mount Kerkis on Samos Island (Greece). In addition, a third subspecies has recently been identified from Akdağ Mountain in western Türkiye (Oskay et al. 2026).

Its narrow range and susceptibility to environmental perturbations have led to its classification as Endangered (Ekim et al. 2000; Güleriyüz 2010). In addition to this core distribution, herbarium records from Sarıçalı Mountain (Ankara; 1700 m, 2011) and Günyarık-Kepen Village (Bilecik; 1000–1100 m, 2001) suggest that the taxon may also occur beyond its previously recognized range. However, one of these additional records emerged after a conservation assessment in which the subspecies was classified as Endangered, while the other is thought to have been disregarded because its distribution altitude is considerably lower than previously known and has not been confirmed.

Previous studies on this species have primarily focused on its micropropagation potential for *ex situ* conservation and provided baseline propagation protocols (Akin et al. 2014). However, key reproductive traits including sex ratio, reproductive efficiency, pollen–ovule ratios, stigma receptivity, pollinator identity, and seed viability remain understood. Because of these, aims of the present study were to: (1) quantify the sex ratio within the population; (2) evaluate reproductive traits, including the pollen–ovule ratio, pollen viability, and stigma receptivity; (3) identify the principal pollinators; (4) assess seed viability and germination capacity; and (5) evaluate reproductive efficiency and overall reproductive performance at the population level. Thus, by

conducting a comprehensive assessment of the reproductive biology of this endemic taxon, it will be possible to fill the knowledge gaps regarding the subspecies and generate fundamental data for its future conservation.

Materials and methods

Study species and study site

Erodium sibthorpiatum subsp. *sibthorpiatum* forms growth habits ranging from a few lax rosettes to dense caespitose clusters. Stems are scarcely branched and typically bear one to two peduncles. Umbel inflorescences consist of 3–5 flowers in female plants and 6–11 flowers in male plants (Fig. 1). Flowers possess five sepals, five petals, five antipetalous nectaries, and five staminodes. Petals range from white to pale or pinkish lilac, often with distinct violet veins; pure white petals occur rarely. Sepals are translucent, with veins usually green and darker than the surrounding tissue. The fruit is a long-beaked schizocarp that separates into five mericarps; fruits measure 5.5–6.8 cm in length and mericarps 7–9 mm (Oskay and Yılmaz 2021).

The subspecies is endemic to Uludağ in Bursa, northwestern Türkiye. It occurs naturally in the alpine zone at 2100–2440 m elevation, predominantly in marble screes and limestone cliff crevices. Flowering takes place from June to August. Field studies were conducted on Uludağ between early June and late September in 2019 and 2022.

Sex ratio

Sex ratio was assessed using 50 randomly placed $1 \times 1 \text{ m}^2$ quadrats distributed across the study area. Within each quadrat, all individuals were recorded, including sexually mature male and female plants as well as immature (non-reproductive) individuals. However, only reproductively mature individuals were included in the calculation of sex ratio.

To calculate the surface area (cushion area) each plant occupied on the soil, the longest axis (length) and the vertical axis (width) were measured and recorded. Measurements were taken randomly from the individuals studied, and the minimum and maximum dimensions observed for each sex were recorded.

A total of 14 quadrats were sampled in 2019 and 36 in 2022, with the unequal sampling effort resulting from field-work constraints such as weather conditions and workload. As *E. sibthorpiatum* subsp. *sibthorpiatum* is a perennial species, this temporal imbalance in sampling is not expected to bias the overall estimation of sex ratio.

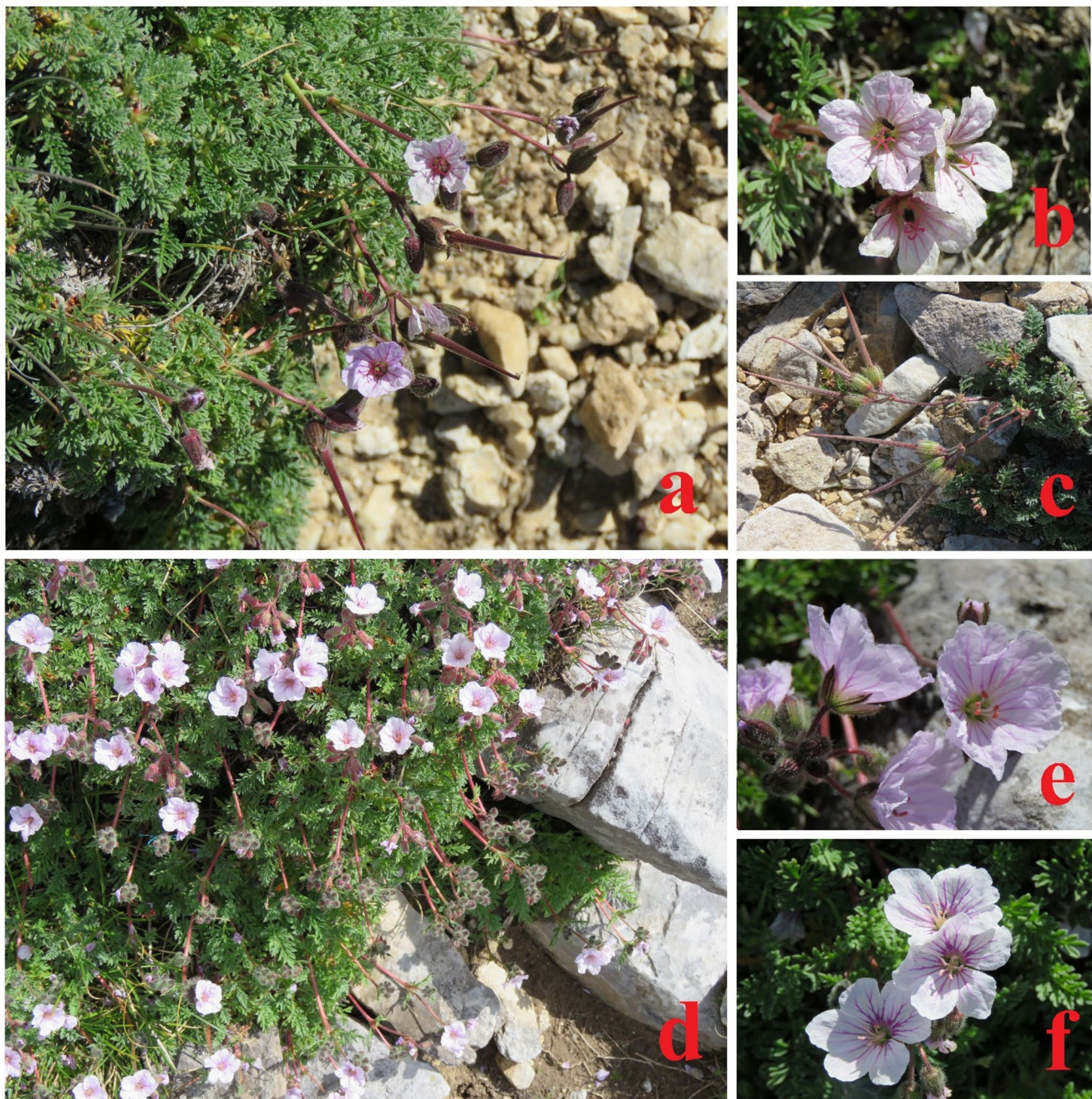


Fig. 1 *Erodium sibthorpiatum* subsp. *sibthorpiatum*, **a**: Female plant, **b**: Female flowers on female plants, **c**: Fruits on female plants, **d**: Male Plant, **e-f**: Male flowers on male plants

Pollen–ovule ratio

The pollen–ovule (P/O) ratio, widely used to infer reproductive systems (Cruden 1977, 2000), was calculated following the modification proposed by Alarcón et al. (2011) for dioecious *Erodium* species.

Inflorescences were collected from 10 male and 10 female individuals in 2022, from areas outside the permanent plots monitored in 2019 and 2022. One inflorescence

per individual was sampled at the bud stage and preserved in 70% ethanol, resulting in a total of 10 inflorescences per sex. In female flowers, ovule number per flower was determined under a stereomicroscope. In male flowers, pollen grains were counted from a single anther of the largest bud nearest to anthesis in each inflorescence under a light microscope.

Pollen production per flower was estimated by multiplying the number of stamens per flower by the average number

of pollen grains per anther. Pollen counts were based on at least 30 microscope fields per preparation, adjusted using the appropriate magnification factor. The P/O ratio was calculated using the formula:

$$(\text{No. of male individuals} \times \text{No. of male flowers per plant} \times \text{No. of pollen grains per flower}) /$$

$$(\text{No. of female individuals} \times \text{No. of female flowers per plant} \times \text{No. of ovules per flower}).$$

Population-level values were obtained from permanent plot observations and sex ratio counts. The logarithm of the final value was used for interpretation.

Pollination type

As a dioecious species, *E. sibthorpiatum* subsp. *sibthorpiatum* is obligatorily outcrossing and may rely on wind or biotic vectors. To assess potential wind-mediated pollination, four adult female individuals were enclosed in cages framed with mesh netting during the 2022 flowering season. The cages measured about $40 \times 40 \times 40$ cm³ in length, width, and depth that were designed to allow wind passage but prevented the entry of small pollinators. Although the number of individuals appears small, this process yielded a high number of flowers through repetition. Approximately 300–400 flowers were examined throughout the vegetative period, with each individual bearing at least 80 flowers. The caged female plants were checked weekly during the flowering period, and fruit formation was recorded at the end of the season to determine if wind pollination had occurred.

Pollinators

Pollinator observations were conducted during field studies in 2019 and 2022. The study was structured as a descriptive assessment of the diversity of flower visitors and plant-pollinator relationships in the population and did not include standardized observation periods or quantitative measurements of visitor frequency. Flower visitors observed during field studies were recorded to document species presence and interaction patterns, and representative individuals were collected and preserved in 70% ethanol for taxonomic identification.

Pollen viability

Pollen viability was assessed using lactophenol blue staining. Buds about to open were marked on inflorescences of 10 male plants. Inflorescences were harvested on the day of anthesis and placed in water-filled vases. Viability was tested on pollen sampled from flowers that were 1, 2, 3 days old. Pollen from unopened buds was also evaluated.

For each day, 3–4 microscope preparations were examined, with at least 20 fields of view per preparation. Viability percentages were calculated as the proportion of stained (viable) grains relative to total grains.

Stigma receptivity

Stigma receptivity was determined using enzymatic activity assays for peroxidase and catalase (Dafni and Maués 1998). Inflorescences on 12 female plants were marked, and individual buds within each inflorescence were color-labeled at the bud stage (Fig. 2). Each bud was monitored daily, and the day of anthesis was recorded. The day of opening was defined as day 1, and flowers were followed continuously until harvest, allowing precise determination of floral age based on direct daily field observations. After five consecutive days of monitoring, all marked inflorescences were harvested.

Flowers ($n=141$) were grouped according to their exact floral age (1–4 days old and older). Sepals and petals were removed, leaving only the pistils (Fig. 2). A separate set of buds ($n=11$) was similarly prepared. Stigmas were treated with Perex solution or 3% hydrogen peroxide. Color change and bubble formation were evaluated within identical conditions within standardized time intervals (1–4 min for peroxidase activity; 2–3 min for catalase activity). All assessments were performed by the same researcher to ensure scoring consistency. In addition, findings and observations regarding flower development stages were also noted during the period leading up to harvest.

Seed viability

Fruits were randomly collected from the population in both 2019 and 2022. Seed viability tests in 2022 included 33 seeds from the 2019 collection and 27 from the 2022 collection.

Seeds were soaked in distilled water for 24 h, after which seed coats were removed. Embryos were incubated in 0.1% 2,3,5-triphenyltetrazolium chloride solution for 24 h at room temperature. Embryos were then rinsed, and viability was scored based on staining intensity following Lakon (1949), AOSA (2000), and Rymer et al. (2002).

Germination

For germination tests were used total of 106 seeds. Seeds harvested in 2019 and 2022 were soaked in sterile water for 2 h before sowing and sown under various temperature conditions in Petri dishes or soil. Petri dishes provided controlled conditions, while natural soil represented semi-controlled conditions, similar to field conditions. For the 2019



Fig. 2 Images from the field and laboratory stages of stigmas prepared for stigma maturity testing

seeds, 10 seeds were placed at 10 °C, 9 seeds at 15 °C, 10 seeds were initially placed at 5 °C and later transferred to 15 °C after no germination was observed by day 9, and 11 seeds were sown at 20 °C. For the 2022 seeds, 10 seeds were sown at 5 °C, 15 seeds at 10 °C, 10 seeds at 15 °C, 15

seeds in soil at 15 °C, 4 seeds were initially placed at 5 °C and then transferred to 15 °C, 12 seeds at 20 °C, and 5 seeds in soil at 20 °C. Germination was recorded daily, and final germination percentages were calculated for each treatment.

Reproductive success

A permanent plot was established in 2019 on Uludağ, containing 15 male and 15 female individuals that were tagged and mapped. In both 2019 and 2022, the numbers of buds, open flowers, withered flowers, and fruits per plant were recorded during field visits. Fruit set was calculated as the percentage of flowers developing into fruits per female plant.

Seed number per fruit was determined from randomly selected fruits collected both within and immediately outside the permanent plot. As all samples belonged to the same population, this approach provided a more reliable estimate of the mean seed number per fruit.

Reproductive success (total seed production per plant) was calculated as the product of the mean seed number per fruit and the number of fruits per female individual. Theoretical maximum seed production was estimated based on ovule number per flower and total flower production. No pollination manipulation experiments were conducted; all values represent open-pollinated natural conditions.

Statistical analyses

Statistical significance between groups was determined using One-Way Analysis of Variance (ANOVA) followed by Tukey's Honestly Significant Difference (HSD) post-hoc test. Differences were considered significant at $p < 0.05$. To evaluate the temporal dynamics of phenotypic expression and reproductive output in two distinct phenological seasons (2019 and 2022); the Paired Samples t-test was conducted for parameters following a normal distribution while the non-parametric Wilcoxon Signed-Rank Test was used for data violating normality assumptions. In addition to p-values, Cohen's d was calculated to quantify the effect size, providing a standardized measure of the magnitude of biological change. Descriptive statistics were expressed as the mean $\pm$ standard deviation ($M \pm SD$).

Results

Sex ratio

Plant density within $1 \times 1 \text{ m}^2$ quadrats ranged from 1 to 11 individuals, with a mean of approximately 4 individuals per quadrat. Counts included sexually mature male and female individuals, as well as immature plants that had not yet reached reproductive maturity.

The female-to-male ratio was 0.80 in 14 quadrats sampled in 2019 and 0.78 in 36 quadrats sampled in 2022. The

overall mean sex ratio across all 50 quadrats was 0.79, indicating a slight male bias in the population.

Plant size varied between sexes. In female plants, observed cushion area ranged from $5 \times 5 \text{ cm}$ to $55 \times 45 \text{ cm}$, whereas in male plants they ranged from $5 \times 8 \text{ cm}$ to $46 \times 30 \text{ cm}$.

Pollen–ovule ratio

In male flowers, pollen grains were counted from a single anther of the largest bud nearest to anthesis in each inflorescence under a light microscope. Pollen counts per microscopic field of view ranged from 7.31 to 9.63, with a mean of 8.84 ± 0.72 . Using the microscopic vision factor (68), estimated pollen production per anther ranged from approximately 497 to 655 grains (601 ± 49). Considering five stamens per flower, estimated pollen production per flower ranged from approximately 2485 to 3275 grains (3005 ± 110), indicating moderate variation among sampled flowers.

Female flowers contained five ovules per flower, consistent across all sampled individuals. Inflorescences of female individuals were typically single or double, bearing 3–5 flowers, whereas inflorescences of male individuals were double or triple, containing 6–11 flowers. Thus, at least four male flowers were produced per female flower. Incorporating the population sex ratio (0.79) into Cruden's formula yielded a P/O ratio of 3043 ($\log_{10} \text{P/O} = 3.48$).

Pollination type

Female plants enclosed in cages produced abundant flowers (≥ 80 per individual, totaling approximately 300–400 flowers), which were monitored weekly throughout the flowering season (Fig. 3). Despite this, no fruit set was recorded by the end of the season.

Pollinators

Field observations revealed that *E. sibthorpiatum* subsp. *sibthorpiatum* is pollinated by a range of entomophilous taxa. Male flowers provided both pollen and nectar, whereas female flowers offered nectar only. Insects visiting male flowers became heavily dusted with pollen and subsequently transferred pollen to female stigmas when foraging for nectar.

Pollinator taxa were identified to at least the family level (Fig. 4). Among the visitors were Diptera (Syrphidae: *Eristalis tenax* (Linnaeus, 1758), *Scaeva pyrastris* (Linnaeus, 1758); Tephritidae: *Dioxya picciola* (Bigot, 1857); Empididae: *Empis* sp., *Hilara* sp.; Simuliidae: *Simulium* sp.; Muscidae; Sarcophagidae), Hymenoptera (Halictidae:

Fig. 3 Female individuals placed in wire-mesh-framed cages to assess their wind pollination potential



Halictus ligatus (Say, 1837); Apidae: *Apis mellifera* (Linnaeus, 1758), Orthoptera (Tettigoniidae), and Lepidoptera (Oecophoridae: *Esperia sulphurella* (Fabricius, 1775); Pieridae: *Pieris rapae* (Linnaeus, 1758); Sphingidae: *Macroglossum stellatarum* (Linnaeus, 1758), (GBIF, 2026).

Pollen viability

Pollen viability rate was highest on day 1 ($91.95 \pm 3.86\%$), and decreased slightly over the next days (day 2: $85.83 \pm 9.84\%$; day 3: $80.77 \pm 3.83\%$). These differences were not statistically significant ($p=0.188$). However, due to the high variance on day 2 ($SD=9.84$), the post-hoc analysis confirmed a statistically significant reduction in pollen viability between the first and last day of the experiment ($p=0.0236$). These results suggest that, a clear time-dependent negative influence the pollen viability of the studied days.

Stigma receptivity

As shown in Table 1, stigma receptivity was assessed using both hydrogen peroxide (catalase) and peroxidase tests, and supported by field observations. Buds (-1 day) generally showed low receptivity in both tests. In fact, stigmas of buds one day before opening showed higher activity compared to younger buds. Flowers aged 1 and 2 days showed the highest receptivity levels, while older flowers (3–4 days and older) retained functional receptivity, but a gradual decrease in the rate of strong reaction was observed.

Field observations supported laboratory results. Fertilized flowers closed within one day, while unfertilized flowers remained open for up to four days. Fertilized flowers exhibited rapid morphological changes associated with early fruit development. The observations also showed that enzymatic activity in the stigmas of newly fertilized flowers was lower than in flowers of the same age group.

Seed viability

Seed viability was high in both years. Of 33 seeds collected in 2019 and tested in 2022, 30 were viable (91%). Of 27 seeds collected and tested in 2022, 25 were viable (93%).

Germination

Germination of seeds from different harvest years was assessed under a range of temperature conditions and environments, with results expressed in Table 2 as percentages. Germination generally was beginning on day 3, peaking between days 6–8, and continuing until day 20. For the 5°C and $5 \rightarrow 15^\circ\text{C}$ treatments, 2022 seeds germinated at 5°C with a rate of 50%, while a portion was transferred to 15°C , resulting in a 75% germination rate. In comparison, 2019 seeds were initially kept at 5°C and, after no germination was observed by day 9, transferred to 15°C , achieving a 50% germination rate.

At 10°C and 15°C , germination rates for 2022 seeds were 87% and 90%, respectively, whereas 2019 seeds



Fig. 4 Pollinators of *Erodium sibthorpiatum* subsp. *sibthorpiatum*; **a**, **b**: *Eristalis tenax*, **c**: *Scaeva pyrastris*, **d**: *Dioxyna picciola*, **e**: *Empis* sp., **f**: *Hilara* sp., **g**: *Simulium* sp., **h**, **i**: Muscidae, **j**: Sarcophagidae,

l: *Halictus ligatus*, **m**: *Apis mellifera*, **n**: Tettigonidae, **k**: *Esperia sulphurella*, **o**: *Pieris rapae*, **p**: *Macroglossum stellatarum*

Table 1 Summary of changes in stigma receptiveness according to flower age with H_2O_2 and Perex application

Flower Age	H_2O_2 (Catalase Reaction)	Perex (Peroxidase Reaction)	Overall Activity
-1 Day (Bud)	3 (+)	3 (+), 5 (++)	Low (+)
1-Day-Old	2 (+), 3 (++) , 13 (+++)	2 (++) , 3 (+++), 13 (++++)	Very High (++++)
2-Day-Old	1 (+), 8 (++) , 9 (+++)	1 (+), 2 (++) , 7 (+++), 8 (++++)	Very High (++++)
3-Day-Old	3 (+), 11 (++) , 7 (+++)	1 (++) , 10 (+++), 8 (++++)	High (+++)
4-Day and Older	8 (+), 5 (++) , 1 (+++)	2 (+), 11 (++) , 2 (+++)	Medium (++)

The numbers presented in the table represent the number of stigmas examined for enzymatic reactions. For H_2O_2 (catalase reaction), activity levels are defined as: + (low, few bubbles), ++ (medium, moderate bubbles), and +++ (high, many to very dense bubbles). For Perex (peroxidase reaction), activity levels are classified as follows: 50–100 ppm (+, low), 100–200 ppm (++, medium), 200–300 ppm (+++, high), and 300–500 ppm (++++, very high)

Table 2 Germination rates (%) of the seeds in different harvest years and different environments

Year/ Temperature (°C)	5	10	15	15 (Soil)	5→15	20	20 (Soil)
2019	-	10	11	-	50	36	-
2022	50	87	90	73	75	75	60

Germination rates was given as %. 5→15°C indicates seeds initially kept at 5°C and then transferred to 15°C.” The “-” sign indicates that no planting was done from the seeds harvested in 2019 for that temperature

Table 3 Parameters determining the reproductive success of female individuals in the permanent sample area

Reproductive Parameters	2019	2022	<i>p</i> -value	Cohen’s <i>d</i>	Significance
Stem Number	26.1±23.7	23.4±25.0	=0.55	0.11	NS
Estimated Number of Buds	104.5±94.9	92.3±101.2	=0.50	0.13	NS
Number of Fruits	43.3±40.4	26.3±31.1	=0.044	0.47	Significant
Withered Flower Count	30.3±30.7	33.9±29.1	=0.63	0.12	NS
Fruit Set (%)	41.6±13.3	24.9±19.0	<0.001	1.02	Significant
Potential Number of Seeds	522.7±474.5	468.0±500.6	=0.55	0.11	NS
Estimated Number of Seeds Produced	173.1±161.5	78.8±93.4	=0.008	0.71	Significant
Reproductive Success (%)	33.3±10.9	15.1±11.4	<0.001	1.64	Significant

Significant differences between groups was determined with One-way ANOVA, Tukey’s HSD, $p < 0.05$. For temporal comparisons, the Paired-Samples *t*-test was applied for normally distributed data, and the Wilcoxon Signed-Rank Test for non-parametric data. Cohen’s *d* values represent the effect size of biological changes. Values were expressed as $M \pm SD$. NS: not significant ($p > 0.05$)

showed substantially lower rates of 10% and 11%. Under soil conditions at 15 °C, only 2022 seeds were tested, achieving a germination rate of 73%.

At 20 °C, germination reached 75% for 2022 seeds and 36% for 2019 seeds in Petri dish conditions, while in soil, only 2022 seeds were evaluated, showing a 60% germination rate. These results indicate that seeds harvested in 2022 consistently exhibited higher germination rates under all tested conditions compared to those harvested in 2019.

Reproductive success

Reproductive parameters for each female individual in the permanent sample area are presented in Table 3. The reproductive performance of the 2019 and 2022 data reveals a systematic and statistically significant contraction in the reproductive vigor of the population. Statistical tests revealed that stem number 26.13 ± 23.72 in 2019 to 23.40 ± 25.03 in 2022 ($p = 0.55$) and bud number 104.5 ± 94.9 in 2019 to 92.3 ± 101.2 in 2022 ($p = 0.50$) did not differ significantly between years, confirming stable vegetative and reproductive potential. In contrast, significant reductions were observed for fruit number ($p = 0.044$) and seed production ($p = 0.008$). The average fruit-setting capacity declined from $41.6 \pm 13.3\%$ to $24.9 \pm 19.0\%$, and seed-setting capacity from 4 to 3 seeds per fruit. Across all individuals, the most dramatic finding of this study is the reproductive success (%), which declined from 33.3 ± 10.87 in 2019 to 15.1 ± 11.36 in 2022 ($p < 0.001$).

Discussion

The reproductive biology of *E. sibthorpiatum* subsp. *sibthorpiatum*, a strictly dioecious taxon reliant on obligate external fertilisation, provides valuable insights into the ecological and evolutionary forces shaping reproductive performance in alpine habitats. Although pollen/ovule (P/O) ratios are commonly used to infer mating systems (Cruden 1977), in dioecious taxa their primary utility lies in assessing whether pollen production adequately supports outcrossing. The P/O ratio calculated for *E. sibthorpiatum* subsp. *sibthorpiatum* (3005 ± 110) falls well within the range reported for perennial dioecious *Erodium* species (903–3624; Alarcón et al. 2011), indicating that pollen availability is unlikely to constrain reproduction.

Marked sexual dimorphism in floral display further supports this conclusion. Male individuals consistently produce larger inflorescences with more flowers (6–11) compared with female plants (3–5), reflecting typical patterns in dioecious taxa in which sexual selection favours increased male floral display (Eckhart 1991; Matsuhisa and Ushimaru 2015). This enhanced male floral output likely contributes to the high P/O ratio (3.48) and may facilitate efficient pollen dispersal.

Floral morphology of *E. sibthorpiatum* subsp. *sibthorpiatum* including relatively large corollas, petal-base trichomes, dark venation, and nectaries located between petals corresponds closely with recognised pollinator-guidance traits in the Geraniaceae (Kevan 1978; Kay 1979; Aldasoro et al. 2000). The consistent inclination of flowering stems observed in natural populations may represent an adaptive response to strong alpine winds, potentially improving both

mechanical stability and pollinator visibility, as suggested for alpine *Geranium* species (Wang et al. 2014). However, this hypothesis was not directly tested in the present study.

Nectar is produced by both male and female flowers, with male flowers providing the additional reward of pollen. The presence of nectar droplets on the nectaries, a feature known to benefit short-tongued insects (Link 1994), aligns with the predominance of Diptera especially Syrphidae within the pollinator assemblage. Dipteran pollination is recognised as particularly effective in *Erodium* (Fiz et al. 2008) and tends to increase under cold, alpine conditions (Elberling and Olesen 1999; Tiusanen et al. 2016). The observations of the current study reveal a relatively diverse pollinator assemblage, including at least 15 taxa from Diptera, Hymenoptera, and Lepidoptera, contributing to reproductive success. While reproductive success values (33.3% in 2019; 15.1% in 2022) indicate that visitation rates and/or pollen transfer efficiency could influence seed set, direct experimental assessment of pollinator effectiveness would help clarify the mechanisms underlying these patterns.

Flower longevity patterns reveal additional adaptive responses to alpine pollination constraints. Female flowers remain open for up to five days when unfertilised but senesce rapidly upon pollination, thereby reducing unnecessary resource expenditure and discouraging visitation to flowers with diminished reproductive value. This phenology mirrors that of alpine taxa where prolonged flower lifespan compensates for limited pollinator activity, but rapid post-fertilisation closure prevents resource waste (Trunschke and Stöcklin 2017). The observed receptive period also aligns with the 3–4-day window typically reported for dioecious species (Matsuhisa and Ushimaru 2015).

Fruit development in *E. sibthorpiatum* subsp. *sibthorpiatum* is highly sensitive to early pollination events. Although fruits contain five mericarps, seed set per fruit varies widely, and the fertilisation of even a single ovule is sufficient to initiate schizocarp formation. This pattern mirrors results from *Erodium paularense* Fern. Gonz. & Izco, in which low seed/fruit ratios were attributed to fruit development triggered by minimal fertilisation (Gonzales-Benito et al. 1995). Thus, final seed number likely reflects the quantity of pollen deposited on stigmas and the behavioural patterns of pollinators.

Synchrony between pollen viability and stigma receptivity an essential prerequisite for successful reproduction in dioecious systems that was found to be high in this species. Pollen viability was slightly lower on days 2 and 3 compared to day 1, but these differences were not statistically significant ($p=0.188$). However, the reduction in pollen viability between the first and last day of the experiment statistically significant ($p=0.0236$). In addition, the results indicating that early male function remains largely effective

throughout the flowering period. Given that both traits last approximately 4 days, overlap is expected in a large population with many simultaneously flowering individuals. Although some variation in flowering onset and floral longevity among individuals may influence the degree of functional overlap, the substantial synchrony observed suggests that temporal mismatch between male and female functions is unlikely to represent a major constraint on reproductive success in this population. In contrast, in hermaphroditic species or populations with staggered male and female flowering, such overlap may be more restricted and ecologically significant. Similar assessments have been widely applied in many plant species (Gücel and Seçmen 2008, 2009; Güvensen et al. 2013; Atasagun and Aksoy 2018; Atasagun et al. 2021; Subaşı and Güvensen 2021; Jadhav et al. 2025; Buxton et al. 2025).

Seed viability tests revealed consistently high values (91–93%); however, germination success differed substantially between seed cohorts, with older seeds (harvested 2019) showing much lower germination (10–36%) than freshly collected seeds (harvested 2022) (75–90%) at 10–20 °C. These results may indicate limited long-term seed persistence. Germination occurs primarily at 10–15 °C, corresponding to late May–early June in the species' natural alpine habitat. Comparisons with *E. somanum*, a alpine like congener that germinates in autumn (Oskay 2017, 2020), highlight the role of phenological adaptation in determining germination timing and underscore the importance of matching germination strategy to environmental constraints when planning in situ conservation.

A marked decline in reproductive success was observed between 2019 and 2022, despite no significant differences in stem and bud numbers. This indicates that while the plants maintained similar levels of vegetative and reproductive potential, the transition from bud to fruit and seed production was substantially reduced in 2022. Such a pattern suggests that external factors, rather than intrinsic plant capacity, limited reproductive success. Possible explanations include reduced pollination efficiency, changes in pollinator abundance or activity, or unfavorable environmental conditions during flowering and fruit set. The strong decrease in seed production further supports the idea that reproductive limitation occurred at later developmental stages.

Conclusions

Overall, *E. sibthorpiatum* subsp. *sibthorpiatum* exhibits its reproductive traits consistent with adaptation to alpine environments, including synchronized reproductive phenology, flexible flower longevity, and reliance on cold-tolerant dipteran pollinators. While pollen availability appears

sufficient, the extent to which pollination limits reproductive output remains uncertain, and direct experimental assessment of pollinator effectiveness would help clarify these patterns. Climatic variability may influence pollinator activity and vegetative growth, potentially constraining reproductive output. Further multi-year monitoring and experimental assessment of pollinator effectiveness and climatic sensitivity will be necessary to clarify the ecological mechanisms governing reproduction in this dioecious alpine species.

Acknowledgements I would like thank to Prof. Dr. Özer Yılmaz and Merve Özerkan for their valuable help with the fieldwork. I am also deeply grateful to Prof. Dr. Sinan Anlaş, Prof. Dr. Ferhat Altunsoy, and Assoc. Prof. Dr. Yakup Şenyüz for their valuable assistance in the identification of insect specimens. Finally, I would like to thank TUBITAK (Project Number: 118Z662) for their financial support of this project.

Author contributions All aspects of the work, including study conception and design, methodology, data acquisition, analysis, and manuscript preparation, were carried out by the author.

Funding Open access funding provided by the Scientific and Technological Research Council of Türkiye (TÜBİTAK).

Data availability All data generated or analysed during this study are included in this article.

Declarations

Competing interests The author declare no competing interests.

Use of AI tools Language polishing of several paragraphs was supported using OpenAI's ChatGPT. The tool was used only to improve readability and grammar; it did not generate scientific ideas, data, or interpretations. All revisions were manually checked and validated by the author.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Aiton W (1789) Hortus kewensis: Diadelphia-cryptogamia (Vol. 3). Nicol, London
- Akın B, Kocaçalışkan İ, Güleriyüz G (2014) Micropropagation of *Erodium sibthorpiatum* subsp. *sibthorpiatum*, an endemic threatened species of Uludağ Mountain. Turk J Bot 38:148–155. <https://doi.org/10.3906/bot-1304-24>
- Alarcón ML, Roquet C, Aldasoro JJ (2011) Evolution of pollen/ovule ratios and breeding system in *Erodium* (Geraniaceae). Syst Bot 36:1–16. <https://doi.org/10.1600/036364411X583637>
- Aldasoro JJ, Aedo C, Navarro C (2000) Insect attracting structures on *Erodium* petals (Geraniaceae). Plant Biol 2:471–481. <https://doi.org/10.1055/s-2000-5960>
- AOSA (2000) Tetrazolium Testing Handbook. Contribution No. 29. Association of Official Seed Analysts, Washington, DC
- Aslay M, Yıldız F, Kaya O, Bitá-Nicolae C (2023) Reproductive biology and pollination ecology of *Fritillaria michailovskyi* Fomin (Liliaceae), endemic to East Anatolia (Turkey). Diversity 15:414. <https://doi.org/10.3390/d15030414>
- Atasagun B, Aksoy A (2018) Autecology and conservation biology of *Centaurea amaena* (Asteraceae). J Anim Plant Sci 28:208–214
- Atasagun B, Aksoy A, Güllü IB, Albayrak S (2021) Reproductive Biology of *Astragalus argaeus* (Fabaceae), a critically endangered endemic species. Acad Bras Cienc 93:e20201613. <https://doi.org/10.1590/0001-37652021020201613>
- Bawa KS (1980) Evolution of dioecy in flowering plants. Annu Rev Ecol Syst 11:15–39
- Buxton MN, Gaskett AC, Lord JM, Pattenmore DE (2025) New evidence that blueberry (*Vaccinium* spp.) has floral traits that enable pollination at night. Arthropod-Plant Interact 19:1–15. <https://doi.org/10.1007/s11829-025-10135-3>
- Charlesworth D, Wright SI (2001) Breeding systems and genome evolution. Curr Opin Genet Dev 11:685–690. [https://doi.org/10.1016/S0959-437X\(00\)00254-9](https://doi.org/10.1016/S0959-437X(00)00254-9)
- Cruden RW (1977) Pollen ovule ratios: a conservative indicator of breeding systems in flowering plants. Evol 31:32–46. <https://doi.org/10.2307/2407542>
- Cruden RW (2000) Pollen grains: why so many? Plant Syst Evol 222:143–165. <https://doi.org/10.1007/BF00984100>
- Dafni A, Maues MM (1998) A rapid and simple procedure to determine stigma receptivity. Sex Plant Reprod 11:177–180. <https://doi.org/10.1007/s004970050138>
- Davis PH (1967) *Erodium* L'Hérit. In: Davis PH (ed) Flora of Turkey and the East Aegean Islands, vol 2. Edinburgh University, Edinburgh, pp 475–487
- Davis PH, Mill RR, Tan K (1988) *Erodium* L'Hérit. In: Davis PH, Mill RR, Tan K (eds) Flora of Turkey and the East Aegean Islands. Suppl. I, vol 10. Edinburgh University, Edinburgh, pp 105–106
- Eckhart VM (1991) The effects of floral display on pollinator visitation vary among populations of *Phacelia linearis* (Hydrophyllaceae). Evol Ecol 5:370–384. <https://doi.org/10.1007/BF02214154>
- Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z, Adıgüzel N (2000) Türkiye Bitkileri Kırmızı Kitabı (Red Data Book of Turkish Plants). Türkiye Tabiatını Koruma Derneği ve Van Yüzüncü Yıl Üniversitesi, Ankara
- Elberling H, Olesen JM (1999) The structure of a high latitude plant-flower visitor system: the dominance of flies. Ecography 22:314–323. <https://doi.org/10.1111/j.1600-0587.1999.tb00507.x>
- Eppley SM, Pannell JR (2007) Sexual systems and measures of occupancy and abundance in an annual plant: testing the metapopulation model. Am Nat 169:20–28. <https://doi.org/10.1086/509944>
- Fiz O, Vargas P, Alarcón ML, Aedo C, García JL, Aldasoro JJ (2008) Phylogeny and historical biogeography of Geraniaceae in relation to climate changes and pollination ecology. Syst Bot 33:326–342. <https://doi.org/10.1600/036364408784571482>
- GBIF. org (2026) Global biodiversity information facility. Available at <https://www.gbif.org> Accessed 01 April 2026
- Goldberg EE, Otto SP, Vamossi JC, Mayrose I, Sabath N, Ming R, Ashman TL (2017) Macroevolutionary synthesis of flowering plant sexual systems. Evolution 71:898–912. <https://doi.org/10.1111/evo.13181>

- Gonzales-Benito E, Martin C, Iriondo JM (1995) Autecology and conservation of *Erodium paularense*. Biol Conserv 72:55–60. [https://doi.org/10.1016/0006-3207\(94\)00063-V](https://doi.org/10.1016/0006-3207(94)00063-V)
- Gücel S, Seçmen Ö (2008) Reproductive biology of subalpin endemic *Minuartia nifensis* MC Neill (Caryophyllaceae) from West Anatolia, Turkey. Biol Divers Conserv 1:66–74
- Gücel S, Seçmen Ö (2009) Conservation biology of *Asperula daphneola* (Rubiaceae) in Western Turkey. Turkish J Bot 33:257–262. <https://doi.org/10.3906/bot-0806-7>
- Güleryüz G (2010) Uludağ (Bursa). In: Özhatay N, Byfield A, Atay S (eds) Important plant areas in Turkey: 122 Key Turkish Botanical Sites. WWF Turkey, İstanbul, pp 77–79
- Güner A, Özhatay N, Ekim T, Başer KHC (2000) *Erodium* L'Hérit. In: Güner A, Özhatay N, Ekim T, Başer KHC (eds) Flora of Turkey and East Aegean Islands. Suppl. II), vol 11. Edinburgh University, Edinburgh, pp 74–75
- Güvensen A, Seçmen Ö, Şenol SG (2013) Heterostyly in *Linum aretioides*. Turk J Bot 37:122–129. <https://doi.org/10.3906/bot-1202-43>
- Jadhav P, Dhumal SS, Boraiah KM et.al (2025) Floral and pollination biology of dragon fruit reveals strategies for enhancing productivity through pollination management and reproductive window extension. Sci Rep 15:37296. <https://doi.org/10.1038/s41598-025-21168-2>
- Käfer J, de Boer HJ, Mousset S, Kool A, Dufaÿ M, Marais GA (2014) Dioecy is associated with higher diversification rates in flowering plants. J Evol Biol 27:1478–1490. <https://doi.org/10.1111/jeb.12385>
- Kay QON (1979) Ultraviolet photography of the colours and patterns of flowers. Watsonia 12:339–340
- Kay KMC, Voelckel JY, Yang KM, Hufford DD, Kaska S, Hodges A (2006) Floral characters and species diversification. In: Harder LD, Barrett SCH (eds) Ecology and evolution of flowers. Oxford University Press, Oxford, pp 311–325
- Kevan PG (1978) Floral coloration, its colorimetric analysis and significance in anthecology. In: Richards AJ (ed) The pollination of flowers by insects. Academic, London, pp 51–78
- Lakon G (1949) The topographical tetrazolium method for determining the germinating capacity of seeds. Plant Physiol 24:389–394. <https://doi.org/10.1104/pp.24.3.389>
- Link DA (1994) The nectaries of Geraniaceae. In: Vorster P (ed) Proceedings of the International Geraniaceae Symposium. University of Stellenbosch, Stellenbosch, South Africa, pp. 215–225
- Marbaniang EJ, Venugopal N, Verma S, Raina R, Khajuria A, Gautam K (2018) Floral biology and embryological studies are important for conservation of threatened plants having reproductive bottlenecks: a case study of *Illicium griffithii* Hook. f. Thomson Curr Sci 114:576–587. <https://doi.org/10.18520/cs/v114/i03/576-587>
- Matsuhisa S, Ushimaru A (2015) Sexual dimorphism in floral longevity and flowering synchrony in relation to pollination and mating success in three dioecious *Ilex* species. Am J Bot 102:1187–1197. <https://doi.org/10.3732/ajb.1500073>
- Oskay D (2017) Reproductive biology of the critically endangered endemic plant *Erodium somanum* in Turkey. Turk J Bot 41:171–179. <https://doi.org/10.3906/bot-1603-9>
- Oskay D (2020) Conservation essays and phenology of critically endangered endemic plant *Erodium somanum*. Celal Bayar Univ J Sci 16:237–243. <https://doi.org/10.18466/cbayarfe.690831>
- Oskay D, Yılmaz Ö (2021) *Erodium olympicum* Gemici & Leblebici (Geraniaceae)'un Taksonomik Durumu. Bagbahce 8:61–69. <https://doi.org/10.35163/bagbahce.880625>
- Oskay D, Özdöl T, Pirhan AF, Yıldırım H (2026) *Erodium sibthorpium* subsp. *akdagensis* (Geraniaceae), a new subspecies from Türkiye. Phytotaxa 752:51–60. <https://doi.org/10.11646/phytotaxa.752.1.3>
- Özudoğru B, Özgişi K (2025) *Erodium hakangurii* (Geraniaceae), a new species from inner Anatolia (Turkey). Phytotaxa 684:265–274. <https://doi.org/10.11646/phytotaxa.684.2.9>
- Persson J (1986) *Erodium* L'Hérit. In: Strid A (ed) Mountain flora of Greece, vol 1. Cambridge University Press, Cambridge, pp 549–552
- Queenborough SA, Mazer SJ, Vamasi SM, Garwood NC, Valencia R, Freckleton RP (2009) Seed mass, abundance and breeding system among tropical forest species: do dioecious species exhibit compensatory reproduction or abundances? J Ecol 97:555–566. <https://doi.org/10.1111/j.1365-2745.2009.01485.x>
- Renner SS (2014) The relative and absolute frequencies of angiosperm sexual systems: dioecy, monoecy, gynodioecy, and an updated online database. Am Bot 101:1588–1596. <https://doi.org/10.3732/ajb.1400196>
- Rymer PD, Morris EC, Richardson BJ (2002) Breeding system and population genetics of the vulnerable plant *Dillwynia tenuifolia* (Fabaceae). Austral Ecol 27:241–248. <https://doi.org/10.1046/j.1442-9993.2002.01174.x>
- Sabath N, Goldberg EE, Glick L et al (2016) Dioecy does not consistently accelerate or slow lineage diversification across multiple genera of angiosperms. New Phytol 209:1290–1300. <https://doi.org/10.1111/nph.13696>
- Subaşı Ü, Güvensen A (2021) Reproductive and pollination biology of the critically endangered endemic *Campanula vardariana* in Western Anatolia (Turkey). Plant Ecol Evol 154:49–55. <https://doi.org/10.5091/plecevo.2021.1676>
- Tiusanen M, Hebert PDN, Schmidt NM, Roslin T (2016) One fly to rule them all—muscid flies are the key pollinators in the Arctic. Proc Biol Sci 283(1839):20161271. <https://doi.org/10.1098/rspb.2016.1271>
- Trunschke J, Stöcklin J (2017) Plasticity of flower longevity in alpine plants is increased in populations from high elevation compared to low elevation populations. Alp Bot 127:41–51. <https://doi.org/10.1007/s00035-016-0176-4>
- Vamasi JC, Vamasi SM (2005) Present day risk of extinction may exacerbate the lower species richness of dioecious clades. Divers Distrib 11:25–32. <https://doi.org/10.1111/j.1366-9516.2005.00119.x>
- Villarreal JC, Renner SS (2013) Correlates of monoecy and dioecy in hornworts, the apparent sister group to vascular plants. BMC Evol Biol 13:239. <https://doi.org/10.1186/1471-2148-13-239>
- Walas L, Mandryk W, Thomas PA, Tyralla-Wieruck Z, Iszkulo G (2018) Sexual systems in gymnosperms: a review. Basic Appl Ecol 31:1–9. <https://doi.org/10.1016/j.baec.2018.05.009>
- Wang H, Xiao CL, Gituru RW, Xiong Z, Yu D, Guo YH, Yang CF (2014) Change of floral orientation affects pollinator diversity and their relative importance in an alpine plant with generalized pollination system, *Geranium refractum* (Geraniaceae). Plant Ecol 215:1211–1219. <https://doi.org/10.1007/s11258-014-0379-y>
- World Flora Online (WFO), A Project of the World Flora Online Consortium (2012) Available at <http://www.worldfloraonline.org/taxo n/wfo-0000677010> Accessed 20 December 2025
- Yıldırım Ş, Doğru-Koca A (2004) A new species from Turkey, *Erodium aytacii* Yıldırım & A. Doğru-Koca (Geraniaceae). Ot Sist Bot Dergisi 11:1–6
- Yıldız F, Aslay M, Kandemir A, Kaya O (2022) Reproductive biology of *Fritillaria aurea* Schott (Liliaceae), a rare species endemic to Turkey. Diversity 14:1052. <https://doi.org/10.3390/d14121052>
- Yıldız F, Aslay M, Kaya O (2024) Pollination strategies and reproductive biology of *Fritillaria imperialis* L. (Liliaceae): insights from Erzincan. Türkiye Divers 16:455. <https://doi.org/10.3390/d16080455>