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# Bees may drive the sexual reproduction of four sympatrically distributed cacti in a vanishing coastal Mediterranean ecosystem

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**Background.** Sympatric plant species that share pollinators may have similar mating systems because their floral traits are subject to comparable canalization imposed by pollinators. However, if each sympatric species bears specialized floral morphology, each species may attract different pollinators. Our study aims to describe the pollinator diversity and pollination systems of four taxa of *Eriosyce* that co-occur in an endangered coastal Mediterranean ecosystem in Central Chile. We took two approaches in our study: we assessed the composition and similarity of flower visitors among taxa, and we characterized the breeding systems to determine dependence on pollinators and self-compatibility.

**Methods.** We performed field observations to characterized pollinators during two consecutive years (2016-2017). Additionally, we performed pollination experiments to elucidate reproductive modes using three treatments: manual cross-pollination, automatic self-pollination, and control (unmanipulated individuals).

**Results.** We observed one bird species (Giant hummingbird *Patagona gigas* only visiting *E. subgibbosa*) and 14 bee species (13 natives plus *Apis mellifera*) visiting cacti of the genus *Eriosyce*. We observed variation in the similarity of intra-specific pollinator composition between years and among *Eriosyce* species within the same year. Individuals of *E. subgibbosa* were visited by less number of species (2016 = 4; 2017 = 2), while *E. chilensis* (2016 = 4; 2017 = 8), *E. chilensis* var. *albidiflora* (2016 = 7; 2017 = 4) and *E. curvispina* var. *mutabilis* (2016 = 7; 2017 = 6) were visited by a richest guild of visitors (up to 10 bee species each). Autonomous pollination was unfeasible in *E. chilensis*, which depend on bees to achieve their reproductive success. *Eriosyce subgibbosa*, visited mainly by the Giant hummingbird, depends on pollinators to achieve reproductive success. Both *E. chilensis* var. *albidiflora* and *E. curvispina* var. *mutabilis* were visited by a diverse assemblage of non-social native bees, showing some degree of autonomous pollination and self-compatibility.

**Discussion.** Pollinator diversity analyses showed considerable pollinator differences between the species with ornithophilous flowers (*E. subgibbosa*) and remain taxa which solely dependent on Apoidea species for pollen transfer. The high diversity of native bees among sympatric *Eriosyce* may be a caused by their microclimatic differences at spatial (differences among cacti microhabitats) and temporal levels (differences of climatic conditions between August to December when different *Eriosyce* species bloom). Our study contributes to unveiling the evolutionary mechanisms for pollinator partitioning of sympatric close-related plant species. Furthermore, it improves understanding of threatened species reproductive system and ecological interactions, especially to *E. chilensis* and *E. chilensis* var. *albidiflora*, whose

studied populations are the only known for these taxa.

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## 2 Introduction

3 Pollinator assemblages are among the main evolutionary driver of mating systems in plants  
4 (*Kalisz, Vogler & Hanley, 2004; Goodwillie, Kalisz & Eckert, 2005*). Animal pollination is a  
5 dynamic process that covaries both in spatial and temporal axis with abiotic and biotic factors  
6 (*Eckhart, 1992*). Indeed, several studies document differential pollinator composition, pollen  
7 flow, and pollination rates along geographical gradients (*Herlihy & Eckert, 2005*). These  
8 gradients generally covary with climatic factors, which in turn may impact the pollination  
9 process even at small geographical scales (*Kay & Sargent, 2009; Chalcoff, Aizen & Ezcurra,*  
10 *2012*). Little attention has been paid to the study of pollination of nearby species that co-occur  
11 sympatrically in specific geographic areas sharing the same bioclimatic variables and pollinator  
12 biota (but see *Marques et al., 2007; Ferreira et al., 2018*). In general, noticeable floral  
13 divergence (e.g., phenology and morphology among others) may contribute to differences in  
14 pollinator guild among evolutionarily closely related taxa that co-occur, and ultimately mediates  
15 a reduction in pollination niche overlap (*Cuautle & Thompson, 2010; Grossenbacher & Whittall,*  
16 *2012*).

17       Plants living in sympatry may largely share floral visitors, increasing the chances of  
18 heterospecific fertilization among co-specific plants, thus reducing reproductive output (*van der*  
19 *Niet, Johnson & Linder, 2006; Schlüter et al., 2009*). Under this scenario, when it is not possible  
20 to achieve pollinator partitioning, asexual reproduction strategies such as selfing can be  
21 promoted (*Eckert & Herlihy, 2004; Kalisz, Vogler & Hanley, 2004*). Selfing reduces seed  
22 production and can be exacerbated by anthropogenic disturbances such as biodiversity loss,  
23 invasive species, and climate change (*Kalisz, Vogler & Hanley, 2004; Young et al., 2016*). These  
24 drivers may disrupt pollination interactions and contribute to plant pollen limitation and  
25 population reproductive decay which in last term favor selfing strategies (*Kearns, Inouye &*

26 *Waser 1998; Knight et al., 2005; Hadley & Betts, 2009*). As a consequence, a rapid evolutionary  
27 change towards self-pollination and genetic self-compatibility can be found in plant taxa exposed  
28 to these stressors (*Vickery 2008; Roels & Kelly, 2011; Barrett & Harder, 2017*).

29         Among angiosperms, the species of the Cactaceae family present one of the most  
30 impressive evolutionarily labile reproductive systems (*Schlumpberger & Renner, 2012;*  
31 *Hernández-Hernández et al., 2014; Guerrero et al., 2019b*). Cacti have evolved conspicuous  
32 flowers that attract a wide range of animal pollinators, including vertebrates (e.g., bats,  
33 hummingbirds, passerine birds, lizards) and insects, such as moths and bees (*Guerrero et al.,*  
34 *2012*). Most cacti species have been characterized as self-incompatible and depend on biotic  
35 pollinators to achieve reproductive success (*Valiente-Banuet et al., 1997; McIntosh, 2002;*  
36 *Walter, 2008*). On large time scales, the complex interactions of cacti with their pollinators may  
37 have powered the diversification of the family (*Hernández-Hernández et al., 2014*). On smaller  
38 scales, studies have shown geographical co-variation between pollinator assemblages and floral  
39 morphology (*Schlumpberger et al., 2009; Walter, 2010*). Unfortunately, most cacti pollination  
40 studies have mainly focused on a single plant species (*Mandujano et al., 2010; Larrea-Alcázar*  
41 *& López, 2011; Egli & Giorgetta, 2015*), meanwhile the pollination processes of sympatric  
42 cacti have received less attention (but see *Fleming, Tuttle & Horner 1996; Egli & Giorgetta,*  
43 *2017; Ferreira et al. 2018*). Increasing knowledge of pollination in sympatric cacti species  
44 allows us to identify potential pollen limitation and reproductive isolation barriers as well as  
45 mechanisms of seed production, which are relevant issues to identify demographic bottlenecks of  
46 taxa, especially in those with high extinction risk.

47         In this study, we simultaneously focused on four sympatric *Eriosyce* taxa that overlap  
48 partially or wholly in their distribution inhabiting an endangered ecosystem in coastal central

49 Chile: Los Molles-Pichidangui Conservation Priority Zone (Guerrero et al., 2011; Fig. 1). Three  
50 of the studied taxa belong to *Eriosyce* sect. *Neoporteria* (Guerrero et al., 2019b): *E. chilensis*  
51 (Hildm. ex K.Schum.) Katt., *E. chilensis* var. *albidiflora* (F. Ritter) Katt. and *E. subgibbosa*  
52 (Haw.) Katt. The other cactus studied belongs to *Eriosyce* sect. *Horridocactus*: *Eriosyce*  
53 *curvispina* (Bertero ex Colla) Katt. var. *mutabilis* (F. Ritter) Katt. Observations on the  
54 reproductive process in some Chilean *Eriosyce* sect. *Neoporteria* species have suggested that  
55 they are mainly self-incompatible, although some degree of self-compatibility has been detected,  
56 with ornithophilous floral syndromes being mainly visited by hummingbirds (Ritter, 1980;  
57 Walter, 2008; Guerrero et al., 2012). Two of these taxa are narrow endemics (*E. chilensis* and *E.*  
58 *chilensis* var. *albidiflora*), which have reduced their distributional range through the effects of  
59 habitat loss in recent decades (Faundez et al., 2013). *Eriosyce subgibbosa* and *E. curvispina* var.  
60 *mutabilis* only overlap their ranges in the studied site (Fig. 1). Our study aims to describe the  
61 diversity of pollinator and reproductive systems of four cacti that co-occur in an endangered  
62 coastal Mediterranean ecosystem in central Chile. We took two approaches to our study. First,  
63 we assessed the composition and similarity of flower visitors during two consecutive years (2016  
64 and 2017). Then, we characterized the breeding system to determine dependence on pollinators  
65 and genetic compatibility after fertilization. Specifically, our study aims to contribute to  
66 understanding the fate of threatened species within an endangered ecosystem.

67

## 68 **Materials & Methods**

69 **Study site and species.** We carried out our study in a coastal strip of central Chile between  
70 Pichidangui Bay (32°08' S; 71°32' W) and Los Molles Peninsula (32°14' S; 71°30' W) (Fig. 1).  
71 The area is the most important remnant of coastal Mediterranean-type scrubland (ca. 17.5 km<sup>2</sup> of

72 surface) dominated by sclerophyllous vegetation (*Luebert & Pliscoff, 2006; Alaniz, Galleguillos*  
73 *& Perez-Quezada 2016*), which is characterized by its high plant diversity, with 57% of  
74 flowering species endemic to central Chile (*Lund & Teillier, 2012*). Today, 17 plant taxa are  
75 recognized as having threatened status within this area (*Lund & Teillier, 2012*) due to  
76 anthropogenic pressures. The study was done in an area of public access (no permit required).

77 We studied all the species of the genus *Eriosyce* present in this area (4 taxa). The genus is  
78 composed of globular and sub-columnar species (Fig. 1). *Eriosyce chilensis* is a narrow endemic  
79 species distributed exclusively in this studied site occurring on rocky outcrops near the coast.  
80 The flowers of *E. chilensis* have tepals deflected outwards, suggesting bee pollination. This  
81 taxon has two taxonomic varieties. The nominal species *E. chilensis*, with flowers of pink tepals,  
82 occurs in all studied areas except in the northernmost portion (near Pichidangui) (Fig. 1). The  
83 second variety is *E. chilensis* var. *albidiflora*, which is a local endemic distributed in the  
84 northernmost distributional range of the species and has flowers with white tepals. *E. chilensis* is  
85 classified as Critically Endangered (*Faundez et al., 2013*). *Eriosyce subgibbosa* is a globular-  
86 elongated cactus distributed on the Pacific coastline along ca. 600 km between 31° S to 37° S,  
87 presenting pink to pale pink tepals which form a tubular flower, associated with ornithophilous  
88 pollination by hummingbirds (*Walter, 2008*). *Eriosyce subgibbosa* occurs in the northern and  
89 southern ranges of the studied site associated with coastal rocks (Fig. 1). These three taxa are  
90 very similar in their vegetative structures, but display more substantial differences regarding  
91 flower morphology; since *E. subgibbosa* have a rather tubular-shaped hypanthia in comparison  
92 with the funnellform flowers of *E. chilensis* and *E. chilensis* var. *albidiflora*. These latter species  
93 differ in the pigmentation of their flowers, as *E. chilensis* can be seen as pink by the human



94 vision while *E. chilensis* var. *albidiflora* white and yellowish. Details of diagnostic characters for  
95 determination of Chilean *Eriosyce* are available in Kattermann (1994).

96 Finally, *Eriosyce curvispina* var. *mutabilis* is a globular cactus distributed on the coast  
97 and in valleys with oceanic influence, and approximately 32° S latitude that has a hemicryptic  
98 habit, living semi-buried. In the studied site, *E. curvispina* var. *mutabilis* inhabits coastal  
99 terraces. This species has showy flowers with yellow to orange outward tepals, suggesting bee  
100 pollination (Fig. 1). This species differs from others because they have wide bodies compare to  
101 the height, bearing less and thicker spines.

102 **Flower visitors of *Eriosyce*.** We performed field observations to determine the main pollinators  
103 of each *Eriosyce* taxon during their flower anthesis peak: August (*E. subgibbosa*), October (*E.*  
104 *chilensis* and *E. chilensis* var. *albidiflora*) and November (*E. curvispina* var. *mutabilis*).

105 Observations for each taxon were performed during two consecutive years (2016 and 2017). We  
106 centered observations on individuals of each *Eriosyce* species which have clumped distribution  
107 forming groups. In the year 2016, we counted the number of individual per group and their  
108 opened flower number. Each group contains between 2 - 17 adult individuals (12.7 individuals  $\pm$   
109 1.5, mean  $\pm$  standard error) with 0.7 - 8 flowers per individual in each group (2.5  $\pm$  0.5 flowers  
110 per individuals). For each species, group of individuals were distanced least 20 m each other  
111 (Fig. 1). In 2017 we centered in the study the same groups. In spite that some species overlap  
112 their distribution (Fig. 1), *Eriosyce* species showed a marked difference in floral phenology.  
113 Therefore these did not overlap when bloomed. *Eriosyce* flowers still opened for a period of 4 - 6  
114 days.

115 For each species, we conducted observations between 8:00 and 20:00 on three  
116 consecutive days by four observers, except for *E. curvispina* var. *mutabilis* which we counted

117 with two observers. Time observation coincides with flower anthesis of each species, which once  
118 opened, still in this state. Previous observations after 20:00 hours led us to rule out the presence  
119 of nocturnal visitors. Specifically, our observations were performed in periods of 30 min for each  
120 group of individuals alternating among groups, covering 540 min of observation each day per  
121 observer. A total of 6,480 min of observation per species were performed (540 min x 4 observers  
122 x 3 consecutive days) except for *E. curvispina* var. *mutabilis* which accumulated 3,240 min of  
123 observation. When animals approached flowers, we recorded the time they entered the flowers  
124 and collected the animals when feasible. Since we do not evaluate the effectiveness of visitor  
125 animals as pollinators, we considered all the animals that entered the flowers and contacted  
126 reproductive parts (stigma and anthers) to be pollinators. The collected specimens pin-mounted  
127 and taken to the laboratory for later identification by Apoidea expert Dr. Luisa Ruz Pontificia  
128 Universidad Católica de Valparaíso (PUCV), Valparaíso, Chile. Brochure specimens will be  
129 deposited at Concepción University Entomology Collection (MZUC-UCCC), Chile.

130         Based on flower visits, we built a plant x visitor matrix for each year. We estimated  
131 pollinator richness using randomization of matrices ( $N = 9999$  randomizations) based on swap  
132 models that find  $2 \times 2$  submatrices that can be swapped holding totals of columns (pollinators)  
133 and rows (plant individuals) (“swap count” algorithm, *Gotelli & Entsminger, 2001; Gotelli &*  
134 *Entsminger, 2003*). Additionally, we estimated the similarity of pollinator assemblages among  
135 *Eriosyce* cacti, both between years within each taxon and among taxa within each year, using the  
136 Bray-Curtis index (*Bray & Curtis, 1957*), which bounds between 0 (two samples share all the  
137 species) and 1 (two sites do not share any species). Since the Bray-Curtis index (*BC*) is a  
138 dissimilarity index, we reported similarity as  $1 - BC$ . All these analyses were performed using  
139 library *vegan* for R (R Core Team, 2018).

140 **Dependence from pollinators and breeding system.** We marked and bagged flower buds of  
141 each cacti species during their bloom peak: *E. subgibbosa* in August 2017 (N = 70 individuals);  
142 *E. chilensis* var. *albidiflora* (N = 32 individuals) and *E. chilensis* var. *chilensis* (N = 55  
143 individuals) in October 2016; and *E. curvispina* var. *mutabilis* (N = 75 individuals) in November  
144 2017. We used a plastic silk fabric (200 x 200 mm) to bag the flowers, which prevented animals  
145 from entering the flowers. We monitored individuals daily, and as anthesis proceeded, we  
146 randomly assigned one of the following treatments to each individual: (i) manual cross-  
147 pollination, where flowers were pollinated with the pollen of individuals located at least 5 m  
148 away from focal individuals; (ii) automatic self-pollination, where flowers were kept bagged;  
149 and (iii) the control, a group of plants whose bags were removed on anthesis day and whose  
150 flowers were unmanipulated (following *Eckert et al.*, 2010). For manual cross-pollination, donor  
151 individuals were two-day-old flowers. Only one flower was treated per individual plant. Thus,  
152 because the use of more than one pollinated flower may generate resource limitation to make  
153 offspring (*Charnov, 1979*), which may alter seed production among individuals with a different  
154 number of treated flowers. Before performing this experiment, we assessed the stigma receptivity  
155 by using the bubble production method, immersing the stigmas in a 3% hydrogen peroxide  
156 solution and observing bubble production within 2-3 min. The solution bubbling indicates the  
157 activity of stigmatic peroxidases and hence, the receptivity of the stigma (*Arnbruster et al.*,  
158 2002). The receptivity of stigmas was assayed in 1- to 2-day-old flowers (N = 10 flowers of  
159 different individuals per species), and we observed receptivity in 100% of flowers. All taxa of  
160 *Eriosyce* included in this study showed stigma receptivity even early to anthesis (we assessed the  
161 receptivity of some stigmas in floral buds). Therefore, *Eriosyce* species may be considered as  
162 protogynous. At the time of fruit maturation (ca. 4 weeks after the initiation of treatments), we

163 collected fruits obtained from tagged individuals and counted the seed production in each. We  
164 reported the proportion of fruits produced per total flowers treated (for each species) and  
165 assessed the differences in seed number among treatments for each plant species using a  
166 Kruskal-Wallis analysis (Sokal & Rohlf, 1995). In cases of significant differences, we performed  
167 a Nemenyi *a posteriori* test (Zar, 1996).

168

## 169 **Results**

170 For the studied *Eriosyce* species, we observed 16 taxa of floral visitors during two consecutive  
171 years (2016 = 199 visits, 2017 = 303 visits; Table 1; Appendix 1). Pollinators belonged to two  
172 main groups: Insects, represented exclusively by the superfamily Apoidea (Hymenoptera) and  
173 birds; specifically, the giant hummingbird *Patagona gigas* (Trochilidae: Apodiformes)  
174 (Appendix 1). The composition and total richness of floral visitors varied between consecutive  
175 years (Table 1 and 2). All taxa presented some level of inter-annual variation in composition and  
176 richness, but we detected significant dissimilarity only in the floral visitors of *E. curvispina* var.  
177 *mutabilis* (Table 2). Regarding bee species in particular, these corresponded to 15 different taxa,  
178 from the five families found in Chile (Andrenidae, Apidae, Colletidae, Halictidae and  
179 Megachilidae). With the exception of a 0.9% of visit on *E. chilensis* var. *albidiflora* due to  
180 introduced *Apis mellifera* all the remaining bee taxa were native.

181       The composition of pollinator assemblages was unique to each *Eriosyce* taxon (Table 2),  
182 being more dissimilar in *E. subgibbosa* in both years (range 2016: 0.000-0.089; 2017: 0.000-  
183 0.031) since the giant hummingbird *Patagona gigas* (Apodiformes: Trochilidae) exclusively  
184 visited its flowers. The contribution of *P. gigas* to the total visits of the pollinator assemblage in  
185 *E. subgibbosa* was 29% (2016) and 86% (2017), and the rest of visits were carried out by

186 Halictidae bees (Appendix 1). The highest similarity (0.844) among the studied taxa was  
187 between *E. chilensis* and *E. chilensis* var. *albidiflora* in 2016 (Table 2), while in 2017, the  
188 similarity between the two taxa decreased to 0.691 (Table 2).

189

190 With regard to fertilization mode and genetic compatibility in *E. chilensis* and *E.*  
191 *chilensis* var. *albidiflora*, we detected an important dependence on pollinator presence (Fig. 2A  
192 and 2B) since bagged flowers produced fewer seeds compared to the control treatment (*E.*  
193 *chilensis* var. *albidiflora*:  $\chi^2 = 16.83$ ;  $P < 0.001$ ; *E. chilensis* var. *chilensis*:  $\chi^2 = 9.91$ ;  $P < 0.001$ ).  
194 However, we detected some degree of genetic self-compatibility and autonomous selfing in *E.*  
195 *chilensis* var. *albidiflora*, since limited seed production was observed in the automatic self-  
196 pollination treatment (Fig. 2A). In contrast, no seeds were collected in the automatic self-  
197 pollination treatments for *E. chilensis*. For *E. curvispina* var. *mutabilis*, we observed a mixed  
198 reproductive system, with no differences in seed production among the compared treatments ( $\chi^2$   
199 = 0.637;  $P = 0.727$ ; Fig. 2C). For *E. subgibbosa*, our results showed significant pollen limitation  
200 (Fig. 2D, Kruskal-Wallis  $\chi^2 = 6.987$ ;  $P < 0.031$ ) since manual cross-pollination produced a more  
201 significant number of seeds than the other treatments, including the control group (Fig. 2D).  
202 Finally, in *E. subgibbosa*, we detected some levels of genetic self-compatibility and autonomous  
203 selfing as seed production was observed only in one individual under the automatic self-  
204 pollination treatment, although this may be the product of pollen contamination (Fig. 2A).

205

## 206 Discussion

### 207 Pollinator assemblages

208 We found contrasting pollinator assemblages among *Eriosyce* species which exhibited floral  
209 morphology associated from ornithophilous (*E. subgibbosa*) to melitophilous syndrome  
210 (remaining taxa). *Eriosyce subgibbosa* was the only species that interact with the Giant  
211 hummingbird *P. gigas* and also was visited by relatively small-sized native bees of Halictidae  
212 sub-family. This ample taxonomic diversity of visitor assemblage in *E. subgibbosa* generated  
213 large inter-annual differences in composition. The pollination syndrome in the *Neoporteria*  
214 section within *Eriosyce sensu lato* has long been hypothesized to be ornithophilic (Walter, 2008),  
215 although this has never been formally tested and remains anecdotal. As in other studies in cacti,  
216 plants with ornithophilic syndromes do not necessarily restrict other pollinators from using their  
217 offered resources (Gorostiague & Ortega-Baes, 2016). Inter-annual variation of *P. gigas* in the  
218 study site may be due to their migration capacity whose residence times in the study site are  
219 unknown. This could impact pollinator availability for specialized species such as *E. subgibbosa*.  
220 Because *E. subgibbosa* have tubular-shape flowers, we expected a null contribution of  
221 hymenopterans in the pollinator assemblage of *E. subgibbosa*. However, native bee determined  
222 as *Dialictus* sp1 may be critical under the detected pollen limitation scenario (i.e., 2017) in *E.*  
223 *subgibbosa* because they may contribute to partially compensating for lower activity of *P. gigas*.  
224 In addition, the presence of plants with massive nectar production may reduce visits in focal  
225 species (Fleming et al., 2001; Chittka & Schürkens, 2001). For instance, genus *Puya*  
226 (Bromeliaceae) can produce massive amounts of nectar that attracts *P. gigas* hummingbird, as  
227 well as other native birds (Reid et al., 2002; Hornung-Leoni, González-Gómez & Troncoso,  
228 2013). Two *Puya* species co-occur in the studied site: *Puya chilensis* Mol. and *Puya venusta*  
229 Phil. ex Baker, this suggest a possible pollination interference against *E. subgibbosa*, which  
230 produce less nectar per flower (mean  $\pm$  estandar error:  $4.93 \pm 0.87$   $\mu$ l; obtained for 14 individuals

231 using microcapillary tubes of 75  $\mu$ l) in comparison to *P. chilensis* (358  $\mu$ l) and *P. venusta* Phil.  
232 ex Baker (24  $\mu$ l; *Hornung-Leoni, González-Gómez & Troncoso 2013*). During 2017, a massive  
233 bloom of *P. venusta* and *P. chilensis* occurred in the study area (Authors pers. obs.), which may  
234 have modified *P. gigas* foraging behavior. Peak flowering in *E. subgibbosa* in Pichidanguí  
235 occurs in August, coinciding with the arrival of *P. gigas*, which migrates from the north portion  
236 of Chile, Bolivia, and Peru (*Walter, 2008*). Usually, in winter floral resources for hummingbirds  
237 are scarce, and when no massive flowering of *Puya* spp. occurs, flowers of *E. subgibbosa*  
238 together with some scarce individuals of the hemi-parasitic mistletoe *Tristerix aphyllus*  
239 (*Loranthaceae*) and *Fuchsia lycioides* (*Onagraceae*), are almost the only available floral resource  
240 in the area (Authors pers. obs.). Interestingly, another hummingbird species present in the  
241 studied site at the same time, the Green-backed Firecrown *Sephanoides sephaniodes*, did not  
242 visit *E. subgibbosa* flowers. This result could be related to a dominant territorial exclusion  
243 exerted by the giant hummingbird, which is several times larger than *S. sephaniodes*, the latter  
244 possibly being subordinated and displaced to other floral resources more fitted to its small size  
245 (i.e., the tiny flowers of *Fuchsia lycioides*), which it frequently visits (Authors pers. obs.).

246         Remain of the *Eriosyce* Phil. taxa were strictly bee pollinated, to the best of our  
247 knowledge this is the first time the floral association of these bee species with this cacti genus is  
248 reported. This relationship is consistent with their flower morphology (funnel-form) and  
249 flowering peaks that occur in October and November when pollinator activity increases coupled  
250 with the increase in day temperature (*Walter, 2008*). The high diversity of native bees in the  
251 xeric areas of Chile has long been recognized in the literature (*Michener, 1979*) which are  
252 important pollinators in central Chile (*Montalva & Ruz, 2010; Medel, González-Browne &*  
253 *Fontúrbel, 2018*), some such as *Alloscirtetica lanosa* Urban, 1971 (*Apidae*), *Diadasia chilensis*

254 Spinola, 1851 (Apidae), *Xenochilicola diminuta* Toro and Moldenke, 1979 (Colletidae) and  
255 *Caenohalictus cyanopygus* Rojas and Toro, 2000 (Halictidae). The observed diversity of native  
256 bee species (include all five families found in Chile) may allow a heterogeneous spatial  
257 distribution of pollinators with local specialization of each *Eriosyce* taxa in a group of floral  
258 visitors. This condition is essential considering that the observed bee species are variable in body  
259 size, ranging from medium (i.e., Megachilidae) to mostly small (i.e., Halictidae and Colletidae),  
260 and their foraging spectra may range from poly to oligolectic (*Montalva & Ruz, 2010; Michener,*  
261 *2007*). Even though the studied *Eriosyce* taxa co-occur in a narrow coastal strip (Fig. 1), we  
262 detected changes in bee composition (mostly native species) that may be associated with  
263 different microhabitat occupation. This situation could be more critical regarding *E. chilensis* and  
264 *E. curvispina* var. *mutabilis*, whose floral phenology overlaps during November but which  
265 inhabit different micro-sites with different soil compositions: rocky outcrops (*E. chilensis*) and  
266 coastal terraces (*E. curvispina* var. *mutabilis*). In other systems, micro-habitat conditions mold  
267 pollination richness of butterflies (*Aguirre-Gutiérrez et al., 2015*) and visit rates of  
268 hummingbirds (*Fontúrbel, Jordano & Medel 2015*). In contrast with distribution information  
269 available for hummingbird, distribution of Chilean bees is ambiguous, covering some latitudinal  
270 strips as the potential distributional range (*Montalva & Ruz, 2010*). Furthermore, several frequent  
271 Apoidea pollinators observed in this study seem to correspond to new native bee species (i.e.,  
272 *Anthrenoides* sp. 1, *Liphanthus* sp. 1, M.L. Ruz, per. comm.) whose distributional ranges or  
273 ecological associations have not been described yet. In this sense, knowledge of distribution,  
274 local abundances, and foraging preferences of observed bees is an issue that needs more attention  
275 to understand their dynamic in the studied ecosystem.



276 Despite our study duration was limited to two years, we found a wide variation of  
277 pollinator assemblages among sympatric taxa at a local scale, including significant inter-annual  
278 variation. This variation will contribute to further understanding the network of endemic cacti-  
279 animal pollinator association and help us to comprehend how close-related taxa are capable of  
280 co-existing in sympatry filling the gap on the scarce number of studies in this field.

281

### 282 **Fertilization mode and self-compatibility**

283 Pollen limitation is a widely represented phenomenon in angiosperms (*Bennett et al., 2018*), and  
284 it acts as a significant evolutionary driver since it is a selection agent for floral traits (*Totland,*  
285 *2001*). We detected significant pollen limitation in *E. subgibbosa* inferred by the greater number  
286 of seeds obtained from manual cross-pollination treatment compared to the other the control  
287 group. Probably pollinator richness, abundance, and efficiency during the year 2017 were factors  
288 that explained this pollen limitation for *E. subgibbosa*. On the other hand, automatic self-  
289 pollination treatment in *E. subgibbosa* did not generate seeds except for only one individual,  
290 which may be a problem of the manipulative procedure (e.g., movement of the silk bag)  
291 regarding that individual. We considered that the uniqueness of this result is an exception and  
292 that *E. subgibbosa* should be considered as an auto-incompatible species that largely depends on  
293 pollinator visits to produce seeds. Future studies based on genetic markers could elucidate  
294 paternity of seeds in *Eriosyce* and contributes to determining properly reproductive system of *E.*  
295 *subgibbosa*. Elucidating reproductive output under different pollen limitation regimes in  
296 *Eriosyce* could contribute to understanding potential micro-evolutionary changes in floral traits  
297 in this evolutionarily labile group.

298           Concerning the fertilization mode and genetic compatibility of *E. chilensis* var.  
299 *albidiflora* and *E. chilensis*, we demonstrated their dependence on pollinator visits for  
300 reproductive outcomes (Fig. 2A and 2B) since bagged flowers produced fewer seeds than the  
301 control treatment. However, *E. chilensis* var. *albidiflora* showed some degree of genetic self-  
302 compatibility and autonomous selfing since seed production was observed in automatic self-  
303 pollination treatment. Self-pollination is an expected situation since a previous study has  
304 highlighted that *Eriosyce* subsection *Neoporteria* species have some degree of self-compatibility  
305 (Walter, 2008). Indeed, intermediate mixed mating is frequent among angiosperms, with high  
306 interpopulation variation (Whitehead et al., 2018). The reproductive characteristics of *E.*  
307 *chilensis* var. *albidiflora* could favor reproductive assurance in years when pollinators are scarce,  
308 which is possible in a narrow endemic species with a specialized pollinator assemblage such as  
309 this taxon.

310           Concerning *E. curvispina* var. *mutabilis*, we observed a mixed reproductive system, with  
311 no differences in seed production among treatments. *Eriosyce curvispina* var. *mutabilis*, whose  
312 blooming phenology is close to that of *E. chilensis*, showed a unique pollinator assemblage  
313 compared to the rest of the studied *Eriosyce* (Table 2). Different habitats of this taxon (coastal  
314 terraces) compared with the other *Eriosyce* in the site (rocky outcrops) may explain inter- and  
315 intraspecific pollinator turnover and a potential pollen limitation in *E. curvispina* var. *mutabilis*.  
316 Whether erratic pollination is a common scenario for *E. curvispina* var. *mutabilis*, a mixed  
317 reproductive system with some degree of autonomous selfing and genetic self-compatibility is an  
318 expected situation to allow their reproduction. Some studies in Cactaceae have shown extant  
319 mixed reproductive systems, such as in *Opuntia monacantha*, a partially compatible cactus with  
320 animal-mediated fertilization (Lenzi & Orth, 2012).

321

322 **Conclusions**

323 We found a stretch relationship between *Eriosyce* taxa and several small-sized native bee species  
324 as potential pollinator in the Conservation Priority zone of Los Molles-Pichidangui, besides one  
325 of these species (*E. subgibbosa*) was highlighted as the only one with ornithophilous pollination,  
326 as it flowers were visited by *P. gigas*, the largest farthest-traveling hummingbird, native to  
327 central and South America. This dissimilar floral association may be linked to the varied ecology  
328 of the different *Eriosyce* species studied, and open the door for future inquiries on the  
329 evolutionary steps leading from insect to bird pollination in this lineage. Our study contributes to  
330 the understanding of sympatric variation in the reproductive system of related taxa, which  
331 potentially unveils more general evolutionary mechanisms in plants. This is an important issue,  
332 especially in cacti which are severely threatened group at a global scale (Goettsch *et al.*, 2015),  
333 since understanding plant-pollinator interaction and reproductive consequences contribute to the  
334 conservation and propagation of this clade. Specifically, the reproductive system of the four  
335 endemic cacti studied here showed a spatial-temporal variation of pollinator assemblages and the  
336 existence of different reproductive strategies in a close phylogenetically related group, spanning  
337 from self-incompatibility in *E. subgibbosa* to self-compatibility in *E. curvispina* var. *mutabilis*.  
338 Our results highlight the relevance of considering pollinator diversity in conservation planning of  
339 the studied species, since the reduction of pollinator abundance may have negative impacts in  
340 plant reproduction success (Phillips *et al.*, 2015). This is alarming given the limited distribution  
341 of some species (<17.5 km<sup>2</sup> of occupation area in *E. chilensis* and *E. chilensis* var. *albidiflora*)  
342 and the severe human-originated disturbances of their habitat.

343

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345

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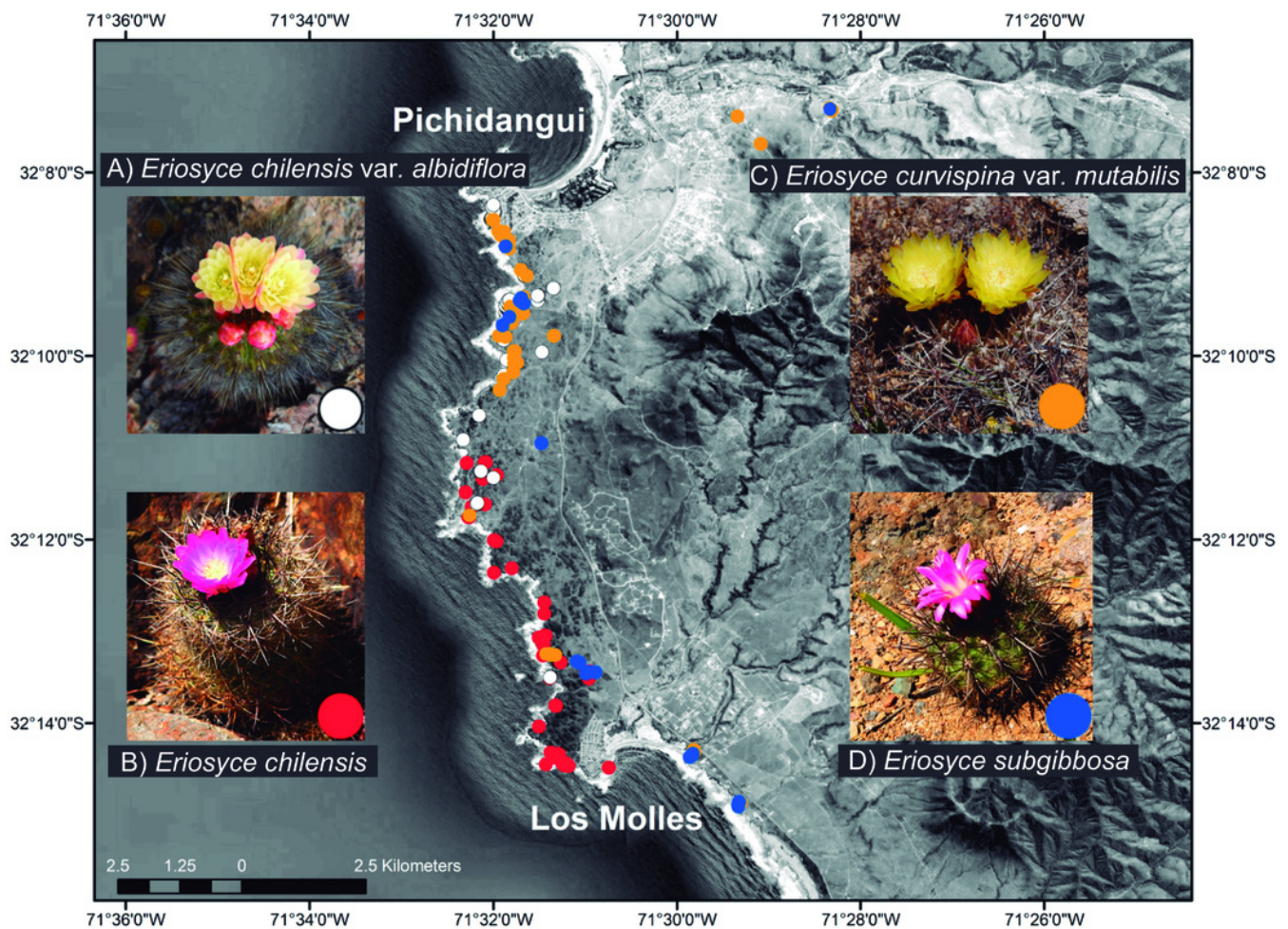
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# Figure 1

Local distribution of *Eriosyce* species between Pichidangui Bay and Los Molles Peninsula, Chile.

The color of dots in the map and within insets refer to different taxa: A) *E. chilensis* var. *albidiflora* (white), B) *E. chilensis* (red), C) *E. curvispina* var. *mutabilis* (orange) and, D) *E. subgibbosa* (blue).

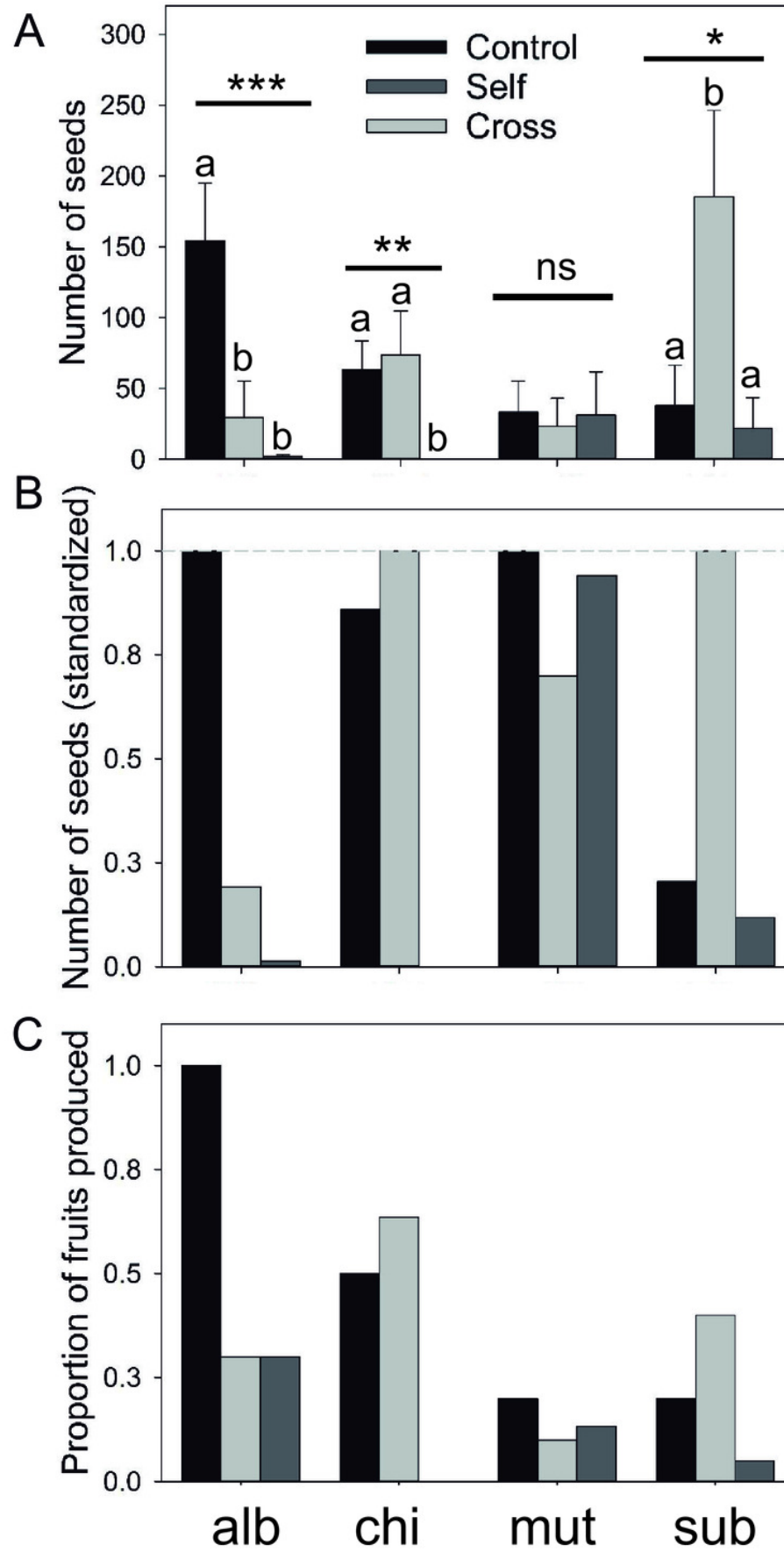




## Figure 2

Reproductive output of *Eriosyce* species. A) Production of seeds (mean  $\pm$  standard error), B) standardized seed number and C) proportion of fruits produced.

For each panel, bars indicate the result of pollination treatments: unmanipulated plants (Control), manual cross-pollination (Cross) and automatic self-pollination (Self). Taxa names were summarized as follow: *E. chilensis* var. *albidiflora* (alb), *E. chilensis* (chi), *E. curvispina* var. *mutabilis* (mut) and *E. subgibbosa* (sub). For seed production, we reported statistical significance of Kruskal-Wallis test that compare differences among treatment for each species (\*\* $P < 0.01$ ; \*  $P < 0.05$ ; ns: non-statistical differences). Letters above seed number bars are result of Nemenyi a posteriori test; different letters indicate statistical differences among compared treatments.



**Table 1** (on next page)

Pollinator richness and number of visits for four *Eriosyce* taxa in two consecutive years, central Chile.

Values in parenthesis in species richness are 95% confidence intervals estimated after matrix randomization (N = 9999).

1 **Table 1:**

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Taxon	Year 2016		Year 2017	
	Species richness	Number of visits	Species richness	Number of visits
<i>E. chilensis</i>	4 (3 - 8)	32	8 (4 - 10)	74
<i>E. chilensis</i> var. <i>albidiflora</i>	7 (3 - 9)	32	4 (4 - 10)	97
<i>E. curvispina</i> var. <i>mutabilis</i>	7 (4 - 11)	85	6 (4 - 10)	118
<i>E. subgibbosa</i>	4 (3 - 9)	50	2 (2 - 7)	14
Total	11 (4 - 10)*	199	12 (5 - 11)*	303

3 \* P &lt; 0.05

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**Table 2** (on next page)

Similarity of pollinator assemblage (based on Bray-Curtis index) among *Eriosyce* during 2016 (below diagonal) and 2017 (above diagonal).

Diagonal depict intra-specific similarity comparing years 2016 and 2017. Similarity index were contrasted with null models (see text for details); indices that deviated from null expectation were marked with asterisks (\*  $P < 0.05$ ).

1 **Table 2:**

	<i>E. chilensis</i> var. <i>albidiflora</i>	<i>E. chilensis</i>	<i>E. curvispina</i> var. <i>mutabilis</i>	<i>E. subgibbosa</i>
<i>E. chilensis</i> var. <i>albidiflora</i>	0.403 (0.050 – 0.851)	0.690 (0.070 – 0.837)	0.242 (0.027 – 0.857)	0.018 (0 – 0.851)
<i>E. chilensis</i>	0.844 (0.051 – 0.847)	0.453 (0.073 – 0.833)	0.104* (0.154 – 0.860)	0 (0 – 0.718)
<i>E. curvispina</i> var. <i>mutabilis</i>	0.291 (0.165 – 0.868)	0.342 (0.049 – 0.843)	0.187 (0.136 – 0.872)	0.030 (0 – 0.776)
<i>E. subgibbosa</i>	0.032* (0.064 – 0.833)	0 (0 – 0.862)	0.089 (0.050 – 0.852)	0.438 (0 – 0.811)

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**Table 3** (on next page)

Hummingbird and bees species visiting frequency on flowers of four *Eriosyce* during two consecutive years in coastal central Chile.

## 1 Appendix 01

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Subfamily	Species	Relative frequency							
		<i>Eriosyce subgibbosa</i>		<i>Eriosyce curvispina</i> var. <i>mutabilis</i>		<i>Eriosyce chilensis</i>		<i>Eriosyce chilensis</i> var. <i>albidiflora</i>	
		2016	2017	2016	2017	2016	2017	2016	2017
Trochilinae	<i>Patagonas gigas</i> Giant hummingbird	40 (80%)	12 (85%)						
Andrenidae	<i>Anthrenoides</i> sp. 1			2 (0.2%)	91 (81%)	14 (41%)	6 (0.8%)	13 (40%)	25 (26%)
Andrenidae	<i>Liphanthus</i> sp. 1			30 (35%)		13 (40%)	52 (70%)	12 (37%)	70 (72%)
Apidae	<i>Alloscirtetica lanosa</i> Urban, 1971				3 (0.2%)				
Apidae	<i>Apis mellifera</i> Linnaeus 1758							3 (0.9%)	
Apidae	<i>Diadasia chilensis</i> (Spinola 1851)			1 (0.1%)					
Colleditidae	<i>Chilicola mantagua</i> Toro and Moldenke 1979			8 (0.9%)					
Colleditidae	<i>Chilicola</i> sp. 1			11 (12%)	2 (0.1%)	3 (0.9%)	9 (12%)	1 (0.3%)	



Colleditidae	<i>Xenochilicola diminuta</i> Toro and Moldenke, 1979							1 (0.1%)	
Halictidae	<i>Caenohalictus cyanopygus</i> Rojas and Toro, 2000	1 (0.2%)							
Halictidae	<i>Caenohalictus rostriceps</i> (Friese, 1917)	3 (0.6%)						2 (0.2%)	1 (0.3%)
Halictidae	<i>Caenohalictus</i> sp. 1				7 (0.5%)				
Halictidae	<i>Corynura herbsti</i> (Alfken, 1913)							1 (0.1%)	
Halictidae	<i>Dialictus</i> sp. 1	6 (12%)	2 (14%)	27 (31%)	13 (11%)				1 (0.3%) 1 (0.1%)
Halictidae	<i>Dialictus</i> sp. 2							1 (0.1%)	
Megachilidae	<i>Trichothurgus dubius</i> (Sichel, 1867)			6 (0.7%)	2 (0.1%)	2 (0.6%)	2 (0.2%)		1 (0.3%)
	Total	50	14	85	118	32	74	32	96

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