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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

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studied populations are the only known for these taxa.

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#### 2 Introduction

Pollinator assemblages are among the main evolutionary driver of mating systems in plants 3 (Kalisz, Vogler & Hanley, 2004; Goodwillie, Kalisz & Eckert, 2005). Animal pollination is a 4 dynamic process that covaries both in spatial and temporal axis with abiotic and biotic factors 5 6 (Eckhart, 1992). Indeed, several studies document differential pollinator composition, pollen flow, and pollination rates along geographical gradients (Herlihv & Eckert, 2005). These 7 gradients generally covary with climatic factors, which in turn may impact the pollination 8 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 Margues et al., 2007; Ferreira et al., 2018). In general, noticeable floral divergence (e.g., phenology and morphology among others) may contribute to differences in 13 14 pollinator guild among evolutionarily closely related taxa that co-occur, and ultimately mediates a reduction in pollination niche overlap (Cuautle & Thompson, 2010; Grossenbacher & Whittall, 15 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 Niet, Johnson & Linder, 2006; Schlüter et al., 2009). Under this scenario, when it is not possible 19 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 drivers may disrupt pollination interactions and contribute to plant pollen limitation and 24 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 change towards self-pollination and genetic self-compatibility can be found in plant taxa exposed 27 to these stressors (Vickery 2008; Roels & Kelly, 2011; Barrett & Harder, 2017). 28 29 Among angiosperms, the species of the Cactaceae family present one of the most impressive evolutionarily labile reproductive systems (Schlumpberger & Renner, 2012; 30 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 pollinators to achieve reproductive success (Valiente-Banuet et al., 1997; McIntosh, 2002; 35 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 & López, 2011; Eggli & Giorgetta, 2015), meanwhile the pollination processes of sympatric 41 42 cacti have received less attention (but see Fleming, Tuttle & Horner 1996; Eggli & Giorgetta, 2017; Ferreira et al. 2018). Increasing knowledge of pollination in sympatric cacti species 43 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 taxa, especially in those with high extinction risk. 46 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. chilensis var. albidiflora), which have reduced their distributional range through the effects of 58 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, we assessed the composition and similarity of flower visitors during two consecutive years (2016 63 and 2017). Then, we characterized the breeding system to determine dependence on pollinators 64 65 and genetic compatibility after fertilization. Specifically, our study aims to contribute to understanding the fate of threatened species within an endangered ecosystem. 66

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 flowering species endemic to central Chile (Lund & Teillier, 2012). Today, 17 plant taxa are 74 recognized as having threatened status within this area (Lund & Teillier, 2012) due to 75 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 composed of globular and sub-columnar species (Fig. 1). Eriosyce chilensis is a narrow endemic 78 species distributed exclusively in this studied site occurring on rocky outcrops near the coast. 79 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 northernmost distributional range of the species and has flowers with white tepals. E. chilensis is 84 classified as Critically Endangered (Faundez et al., 2013). Eriosyce subgibbosa is a globular-85 elongated cactus distributed on the Pacific coastline along ca. 600 km between 31° S to 37° S, 86 presenting pink to pale pink tepals which form a tubular flower, associated with ornithophilous 87 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 with the funnelform flowers of E. chilensis and E. chilensis var. albidiflora. These latter species 92 93 differ in the pigmentation of their flowers, as *E. chilensis* can be seen as pink by the human

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94 vision while E. chilensis var. albidiflora white and yellowish. Details of diagnostic characters for determination of Chilean Eriosyce are available in Kattermann (1994). 95 96 Finally, *Eriosyce curvispina* var. *mutabilis* is a globular cactus distributed on the coast and in valleys with oceanic influence, and approximately 32° S latitude that has a hemicryptic 97 habit, living semi-buried. In the studied site, E. curvispina var. mutabilis inhabits coastal 98 99 terraces. This species has showy flowers with yellow to orange outward tepals, suggesting bee pollination (Fig. 1). This species differs from others because they have wide bodies compare to 100 the height, bearing less and thicker spines. 101 102 Flower visitors of *Eriosyce*. We performed field observations to determine the main pollinators of each *Eriosyce* taxon during their flower anthesis peak: August (E. subgibbosa), October (E. 103 104 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 centered observations on individuals of each Eriosyce species which have clumped distribution 106 107 forming groups. In the year 2016, we counted the number of individual per group and their opened flower number. Each group contains between 2 - 17 adult individuals (12.7 individuals  $\pm$ 108 109 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 (Fig. 1). In 2017 we centered in the study the same groups. In spite that some species overlap 111 112 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 opened, still in this state. Previous observations after 20:00 hours led us to rule out the presence 118 of nocturnal visitors. Specifically, our observations were performed in periods of 30 min for each 119 group of individuals alternating among groups, covering 540 min of observation each day per 120 121 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 observation. When animals approached flowers, we recorded the time they entered the flowers 123 and collected the animals when feasible. Since we do not evaluate the effectiveness of visitor 124 125 animals as pollinators, we considered all the animals that entered the flowers and contacted reproductive parts (stigma and anthers) to be pollinators. The collected specimens pin-mounted 126 127 and taken to the laboratory for later identification by Apoidea expert Dr. Luisa Ruz Pontificia 128 Universidad Católica de Valparaiso (PUCV), Valparaiso, Chile. Brochure specimens will be deposited at Concepción University Entomology Collection (MZUC-UCCC), Chile. 129 130 Based on flower visits, we built a plant x visitor matrix for each year. We estimated pollinator richness using randomization of matrices (N = 9999 randomizations) based on swap 131 models that find 2 x 2 submatrices that can be swapped holding totals of columns (pollinators) 132 133 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 species) and 1 (two sites do not share any species). Since the Bray-Curtis index (BC) is a 137 138 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 each cacti species during their bloom peak: *E. subgibbosa* in August 2017 (N = 70 individuals); 141 E. chilensis var. albidiflora (N = 32 individuals) and E. chilensis var. chilensis (N = 55 142 143 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 randomly assigned one of the following treatments to each individual: (i) manual cross-146 pollination, where flowers were pollinated with the pollen of individuals located at least 5 m 147 148 away from focal individuals; (ii) automatic self-pollination, where flowers were kept bagged; and (iii) the control, a group of plants whose bags were removed on anthesis day and whose 149 150 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, because the use of more than one pollinated flower may generate resource limitation to make 152 153 offspring (*Charnov*, 1979), which may alter seed production among individuals with a different number of treated flowers. Before performing this experiment, we assessed the stigma receptivity 154 by using the bubble production method, immersing the stigmas in a 3% hydrogen peroxide 155 156 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 (Armbruster 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 *Eriosyce* included in this study showed stigma receptivity even early to anthesis (we assessed the 160 161 receptivity of some stigmas in floral buds). Therefore, *Eriosyce* species may are considered as 162 protogynous. At the time of fruit maturation (ca. 4 weeks after the initiation of treatments), we

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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),

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 <i>E. chilensis</i> and <i>E.</i>
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	<i>chilensis</i> var. <i>albidiflora</i> : $\chi^2 = 16.83$ ; P < 0.001; E. <i>chilensis</i> var. <i>chilensis</i> : $\chi^2 = 9.91$ ; P < 0.001).
194	However, we detected some degree of genetic self-compatibility and autonomous selfing in <i>E</i> .
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 <i>E. subgibbosa</i> , 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 melitophylous 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 Puva 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, 2013). Two Puya species co-occur in the studied site: Puya chilensis Mol. and Puya venusta 228 229 Phil. ex Baker, this suggest a possible pollination interference against *E. subgibbosa*, which 230 produce less nectar per flower (mean + estandar error:  $4.93 + 0.87 \mu$ ); obtained for 14 individuals

231 using microcapillary tubes of 75 µl) in comparison to *P. chilensis* (358 µl) and *P. venusta* Phil. 232 ex Baker (24 µl; 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 have modified P. gigas foraging behavior. Peak flowering in E. subgibbosa in Pichidangui 234 235 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 are scarce, and when no massive flowering of Puya spp. occurs, flowers of E. subgibbosa 237 together with some scarce individuals of the hemi-parasitic mistletoe Tristerix aphyllus 238 239 (Loranthaceae) and Fuchsia lycioides (Onagraceae), are almost the only available floral resource in the area (Authors pers. obs.). Interestingly, another hummingbird species present in the 240 241 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 exerted by the giant hummingbird, which is several times larger than S. sephaniodes, the latter 243 244 possibly being subordinated and displaced to other floral resources more fitted to its small size (i.e., the tiny flowers of *Fuchsia lycioides*), which it frequently visits (Authors pers obs.). 245 Remain of the *Eriosyce* Phil. taxa were strictly bee pollinated, to the best of our 246 247 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 (funnelform) 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 xeric areas of Chile has long been recognized in the literature (*Michener*, 1979) which are 251 252 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 Caenohalictus cyanopygus Rojas and Toro, 2000 (Halictidae). The observed diversity of native 255 bee species (include all five families found in Chile) may allow a heterogeneous spatial 256 distribution of pollinators with local specialization of each Eriosyce taxa in a group of floral 257 258 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), and their foraging spectra may range from poly to oligolectic (Montalva & Ruz, 2010; Michener, 260 261 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 different microhabitat occupation. This situation could be more critical regarding E. chilensis and 263 264 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 pollination richness of butterflies (Aguirre-Gutiérrez et al., 2015) and visit rates of 267 hummingbirds (Fontúrbel, Jordano & Medel 2015). In contrast with distribution information 268 269 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 Apoidea pollinators observed in this study seem to correspond to new native bee species (i.e., 271 272 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, local abundances, and foraging preferences of observed bees is an issue that needs more attention 274 275 to understand their dynamic in the studied ecosystem.

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

281

#### 282 Fertilization mode and self-compatibility

Pollen limitation is a widely represented phenomenon in angiosperms (Bennett et al., 2018), and 283 284 it acts as a significant evolutionary driver since it is a selection agent for floral traits (Totland, 2001). We detected significant pollen limitation in *E. subgibbosa* inferred by the greater number 285 286 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 that explained this pollen limitation for E. subgibbosa. On the other hand, automatic self-288 289 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 *Eriosyce* could contribute to understanding potential micro-evolutionary changes in floral traits 296 297 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 reproductive outcomes (Fig. 2A and 2B) since bagged flowers produced fewer seeds than the 300 control treatment. However, E. chilensis var. albidiflora showed some degree of genetic self-301 302 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. *chilensis* var. *albidiflora* could favor reproductive assurance in years when pollinators are scarce, 307 308 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 compared to the rest of the studied *Eriosyce* (Table 2). Different habitats of this taxon (coastal 313 314 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 as potential pollinator in the Conservation Priority zone of Los Molles-Pichidangui, besides one 324 325 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 endemic cacti studied here showed a spatial-temporal variation of pollinator assemblages and the 335 336 existence of different reproductive strategies in a close phylogenetically related group, spanning from self-incompatibility in *E. subgibbosa* to self-compatibility in *E. curvispina* var. *mutabilis*. 337 Our results highlight the relevance of considering pollinator diversity in conservation planning of 338 the studied species, since the reduction of pollinator abundance may have negative impacts in 339 340 plant reproduction success (Phillips et al., 2015). This is alarming given the limited distribution of some species (<17.5 km<sup>2</sup> of occupation area in *E. chilensis* and *E. chilensis* var. albidiflora) 341 342 and the severe human-originated disturbances of their habitat.

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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 ± 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 < 0.001; \*\* 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.

### NOT PEER-REVIEWED



### 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:**

2

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

3

\* P < 0.05

4

### 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:**

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

2

### 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

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

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Calladitadaa	Xenochilicola						1 (0 10/)		
Coneditadae	Moldenke, 1979						1 (0.1%)		
Halictidae	<i>Caenohalictus</i> <i>cyanopygus</i> Rojas and Toro, 2000	1 (0.2%)							
Halictidae	Caenohalictus rostraticeps (Friese, 1917)	3 (0.6%)					2 (0.2%)	1 (0.3%)	
Halictidae	Caenohalictus sp. 1				7 (0.5%)				
Halictidae	Corynura herbsti (Alfken, 1913)						1 (0.1%)		
Halictidae	Dialictus sp. 1	6 (12%)	2 (14%)	27 (31%)	13 (11%)			1 (0.3%)	1 (0.1%)
Halictidae	Dialictus sp. 2						1 (0.1%)		
Megachilidae	<i>Trichothurgus</i> <i>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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