



## The role of facultatively nectarivorous birds as pollinators of *Anarthrophyllum desideratum* in the Patagonian steppe: a geographical approach

VALERIA PAIARO<sup>1</sup>✉; ANDREA A. COCUCCI<sup>1</sup>; GABRIEL E. OLIVA<sup>2</sup> & ALICIA N. SÉRSIC<sup>1</sup>

<sup>1</sup>Laboratorio de Ecología Evolutiva y Biología Floral, Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET-Universidad Nacional de Córdoba, Argentina. <sup>2</sup>INTA - Estación Experimental Santa Cruz, Universidad Nacional de la Patagonia Austral, Unidad Académica Río Gallegos, Argentina.

**ABSTRACT.** The endemic shrub *Anarthrophyllum desideratum* appears to be the only ornithophilous plant offering nectar as reward in the extensive Patagonian steppe. The identity of its floral visitor assemblage and to what extent this species depends on bird pollination for sexual reproduction is yet unknown. *A. desideratum*'s vast distribution includes climatic gradients, which may promote geographical shifts in its floral visitors. The aims of this study were to determine the species dependence on pollinators to set fruits, to identify its floral visitor assemblages, to document possible geographical and environmental variation of these assemblages across the species range and to assess their temporal constancy. We conducted different pollination treatments in several populations to assess the species breeding system. Floral visitors were identified in 18 populations distributed along the entire species range and their visitation frequencies were quantified over three flowering periods. We found that the species is self-compatible and that floral visitors do not contribute to fruit set in two marginal populations. Fruit set of open pollinated plants significantly differed among populations, which suggests that the species dependence on pollinators varies among sites. Eight species of non-exclusively nectarivorous birds, mostly passerine, were observed visiting flowers arranged in different assemblages across the species range. Population differences in the proportion of visits by the two most abundant bird species remained constant over time. Geographical differences of bird assemblages were not spatially structured and were weakly associated with climate of the flowering period, suggesting that climatic gradients would not be important as drivers of the geographical variation in *A. desideratum*' floral visitors. Our results indicate that non-exclusively nectarivorous birds visit flowers of *A. desideratum*, revealing for first time the facultative nectarivory behaviour of these bird species in the Patagonian steppe. The potential role of such bird visitors as pollinators of *A. desideratum* appears to vary geographically.

[Keywords: bird assemblage composition, breeding system, climatic factors, floral visitors, geographical structure, passerines, *Phrygilus gayi*, *Zonotrichia capensis*]

**RESUMEN.** El papel de aves no nectarívoras como polinizadoras de *Anarthrophyllum desideratum* en la estepa patagónica: una aproximación geográfica. El arbusto endémico *Anarthrophyllum desideratum* parece ser la única planta ornitófila que ofrece néctar como recompensa en la extensa estepa patagónica. Aún se desconoce la identidad de su ensamble de visitantes florales y en qué medida esta especie depende de la polinización por aves para la reproducción sexual. La distribución amplia de *A. desideratum* incluye gradientes climáticos que pueden promover cambios geográficos en sus visitantes florales. Los objetivos de este estudio fueron determinar la dependencia de esta especie de los polinizadores para la producción de frutos, identificar sus ensambles de visitantes florales, documentar posibles variaciones geográficas y ambientales de estos ensambles a lo largo del rango de distribución de la especie, y evaluar su constancia temporal. Realizamos diferentes tratamientos de polinización en varias poblaciones para evaluar el sistema reproductivo de la especie. Para 18 poblaciones a lo largo de todo el rango de distribución de la especie identificamos los visitantes florales y cuantificamos sus frecuencias de visitas durante tres períodos de floración. Encontramos que la especie es auto-compatible y que los visitantes florales no contribuyen a la formación de frutos en dos poblaciones marginales. La producción de frutos de las plantas bajo polinización abierta difirió significativamente entre poblaciones, lo cual sugiere que la dependencia de la especie de polinizadores varía entre sitios. Ocho especies de aves no exclusivamente nectarívoras, en su mayoría paseriformes, se observaron visitando las flores dispuestas en diferentes ensambles a lo largo del rango de distribución de la especie. Las diferencias entre poblaciones en la proporción de visitas de las dos especies de aves más abundantes se mantuvieron constantes en el tiempo. Las diferencias geográficas en los ensambles de aves no estuvieron espacialmente estructuradas y se asociaron de forma débil con el clima del período de floración, lo que sugiere que los gradientes climáticos no serían importantes como factores determinantes de la variación geográfica en los visitantes florales de *A. desideratum*. Nuestros resultados indican que aves no exclusivamente nectarívoras visitan las flores de *A. desideratum*. Esto revela por primera vez el comportamiento nectarívoro facultativo de estas especies de aves en la estepa patagónica. El papel potencial de estas aves visitantes como polinizadores de *A. desideratum* parece variar geográficamente.

[Palabras clave: composición del ensamble de aves; factores climáticos; estructura geográfica; paseriformes; *Phrygilus gayi*; sistema reproductivo; visitantes florales; *Zonotrichia capensis*]

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✉ vpaiaro@gmail.com

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## INTRODUCTION

Birds adapted to flowers are mainly nectarivorous and include five major groups, each one restricted to a geographical area. Those groups comprise the highly specialized hummingbirds (Trochilidae), which are endemic to the New World, as well as Passerine birds from several families from around the world (e.g., sunbirds (Nectariniidae) in Africa, the Middle East and eastern Asia; sugarbirds (Promeropidae) in Southern Africa; Hawaiian honeycreepers (Drepanididae), endemic to Hawaii, and honeyeaters (Meliphagidae) in Australia and New Zealand (Stiles 1981; Proctor et al. 1996; Willmer 2011 and references therein). Furthermore, other flower-visiting birds adapted in different degrees to nectarivory are also floral visitors and can act as pollen vectors (Faegri and Pijl 1979; Stiles 1981).

In addition to hummingbirds, a wide range of perching birds, not necessarily adapted for nectar-feeding, also visits flowers in search of nectar and can in some cases play an important role in pollination in the Neotropics (Rocca and Sazima 2010, and references therein). Approximately 166 species from 20 families of perching-birds, both Passeriformes and non-Passeriformes, make use of floral resources in the Neotropical region (Rocca and Sazima 2010). Some of these flower-visiting bird species were often regarded as “parasites” on hummingbird-flower systems (Stiles 1981). Compared with hummingbirds, Neotropical flower-visiting passerines exhibit a low to moderate degree of specialization for nectarivory, although nectar may be an important component of the diet of some groups (Sick 1997). On the other hand, while these birds do not rely only on floral resources (Sick 1997), there are plant species in the Neotropical region that do depend exclusively on them for pollination (Rocca and Sazima 2010). In fact, studies in this region have shown that perching birds act as principal pollinators or as co-pollinators in species from many different families, including eight species of Fabaceae (Rocca and Sazima 2010 and references therein). Although perching birds may be more important for pollination than normally considered (Westerkamp 1990), their possible role as pollinators seems to be neglected in the Neotropic studies (Rocca and Sazima 2008).

Some attributes of birds, such as the capacity to fly long distances and their

high visual acuity, make them excellent pollinators, especially during inclement weather conditions when other pollinators, such as bees, are inactive (Cronk and Ojeda 2008). Birds may, therefore, be important supplemental pollinators in environments where insects have low population densities, such as high-altitude ecosystems (Van der Pijl and Dodson 1966), isolated islands where insect colonization has been poor (Micheneau et al. 2006), and dry environments (Stiles 1978). Nevertheless, compared with tropical and subtropical regions, where bird pollination is particularly common (Willmer 2011), cold, hyper-arid, and nutrient-poor environments have relatively few bird-pollinated plants (Cronk and Ojeda 2008). In fact, species with ornithophilous flowers may represent up to 22% of Angiosperms in tropical communities of the Neotropical region, but only 1.8% in other ecological regions, such as arid ecosystems (Rocca and Sazima 2010 and references therein).

Ornithophily is an expensive syndrome of pollination. Plants must invest in flower shapes big enough to fit pollinators during visits, and also produce a great volume of rich resources (nectar or any other), as birds require more energy than small insects (Cronk and Ojeda 2008). In relation to cost, perching bird flowers may be more expensive than hovering bird flowers, investing more energy into strong inflorescence axis and robust flowers capable of protecting reproductive floral parts against these strong pollinators (Rocca and Sazima 2010). The provision of adequate perches for perching birds also requires more investment. This may in part explain the low proportion of perching bird flowers in dry environments.

Regarding birds, South American arid and semiarid ecosystems have an important number of honeycreepers and icterids, though hummingbirds are usually restricted to humid wooded areas (Willmer 2011). In the southernmost portion of South America, non-exclusively nectarivorous perching birds, which are distributed throughout the Patagonia region, have been occasionally recorded feeding on nectar of few plant species of the temperate forest, severely damaging flowers during the process (Traveset et al. 1998; Smith-Ramírez and Armesto 2003; Devoto et al. 2006). Moreover, the remarkable case of *Calceolaria uniflora*, which offers a corolla appendage as reward that is taken by the frugivorous *Thinocorus rumicivorus* Eschscholtz

(Order Charadriiformes), has been reported for the Patagonian steppe and the forest-steppe ecotone (Sérsic and Cocucci 1996). Nevertheless, *Anarthrophyllum desideratum* (DC.) Benth. (Fabaceae, Papilionoideae), a shrub endemic to the Patagonian steppe, is the only ornithophilous plant offering nectar as reward in this extensive region (Galetto and Bernardello 2003; Paiaro et al. 2012a,b; A. Sérsic and A. Cocucci, personal observation). Its floral traits associated with bird pollination (Paiaro et al. 2012a,b) include a hexoses-dominant nectar (Paiaro et al. 2012b) typical of passerine flowers (sensu Baker and Baker 1983) and also of flowers adapted to generalized bird visitors (sensu Johnson and Nicolson 2008). The species floral visitor assemblage or the extent to which it depends on bird pollination for sexual reproduction is unknown.

*Anarthrophyllum desideratum* is distributed across a broad geographic area and occupies wide environmental gradients across the steppe (González and Rial 2004). In this ecosystem, the species forms dense populations that stand out in the landscape because of their bright red-orange flowers that explain the local name of 'Fire tongue' (Mascó et al. 1998). Some of its floral traits vary considerably among wild populations, showing geographical patterns associated with climatic and edaphic variations throughout the region (Paiaro et al. 2012a,b).

Climatic gradients along which plant populations occur may also determine geographical changes in the abundance and composition of pollinators, especially under adverse weather conditions (Moeller 2005; Devoto et al. 2006; Cosacov et al. 2008; Chalcoff et al. 2012). In arid ecosystems such as the Patagonian steppe, where birds are exposed to harsh environmental conditions, pronounced daily and seasonal thermal fluctuations and largely unpredictable food and water availability, bird assemblage composition can be highly variable in both space and time (Schodde 1982; Dean 1997; Blendinger 2005). Plant pollination studies rarely consider these geographical and temporal contexts (reviewed in Herrera et al. 2006). In addition, the influence of abiotic factors on flower visitors has often been neglected in this type of studies (but see Moeller 2005; Sánchez-Lafuente et al. 2005).

In order to determine the dependence of *A. desideratum* on pollinators to sexual reproduction, we first studied the breeding

system of this species through a set of pollination experiments. Second, we identified *A. desideratum* floral visitors, documented their possible geographical shifts across the species range and assessed their temporal constancy. Finally, we tested the spatial structure of variation in the assemblage composition and used a correlative approach to assess how the climatic gradients present along the species range affect spatial distribution of their floral visitors.

## MATERIALS AND METHODS

### *Study species*

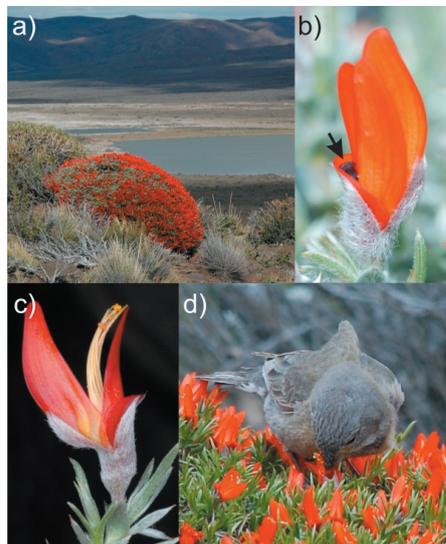
*Anarthrophyllum desideratum* is a perennial shrub endemic to the southernmost regions of Argentina and Chile (Sorarú 1974). It is distributed from 43°10' S down to the Strait of Magellan, covering a latitudinal distance of about 1000 km. It is found in isolated, dense populations mostly in the arid and semiarid steppe across the Patagonian vegetation province, with some populations occurring in the sub-Andean grasslands across the Sub-Antarctic province, which is a narrow strip extending northwards along the Andean Cordillera (Roig 1998). The climate of the region is dry and cold with strong winds, snowfalls in winter, and frosts almost year-round (Cabrera 1971). *A. desideratum* populations are spread along a broad altitudinal range from sea level to 900 m and are exposed to a high variety of environmental conditions spanning broad gradients of temperature and precipitations (Table 1).

The species is a woody, spiny, cushion-like shrub that produces large numbers of solitary apical flowers with the typical zygomorphic papilionate architecture of the Papilionoid Fabaceae (Sorarú 1974) (Figure 1a,b). The flowering season extends from mid-October to mid-November. Flowers are vertically erect, conspicuous, unscented and red to orange in colour. They secrete abundant and dilute hexose-dominated nectar (Paiaro et al. 2012a,b), which is exposed as a large drop spreading between flag and keel (Figure 1b). Corolla size, shape, colour, and nectar traits vary considerably among wild populations, showing geographical patterns associated with climatic and edaphic variations across the species range (Paiaro et al. 2012a,b). The presence of partial protandry suggests that the species depends on pollinators for pollen transfer (V. Paiaro, personal observation). The stems in this species are procumbent

**Table 1.** Geographical and climatic information of the 19 studied populations of *Anarthrophyllum desideratum* in southern Patagonia. Code=population code name; Alt.=altitude (in m above sea level);  $T_{min}$ =mean minimum temperature of the flowering period (i.e., October and November);  $T_{max}$ =mean maximum temperature of the flowering period; Prec.=monthly precipitation of the flowering period. Climatic data obtained from the WorldClim Global Climate GIS database (www.worldclim.org).

**Tabla 1.** Información geográfica y climática de las 19 poblaciones estudiadas de *Anarthrophyllum desideratum* en la Patagonia austral. Code=código del nombre de la población; Alt.=altitud (en m sobre el nivel del mar);  $T_{min}$ =media de la temperatura mínima del período de floración (i.e., octubre y noviembre);  $T_{max}$ =media de la temperatura máxima del período de floración; Prec.=precipitación mensual del período de floración. Datos climáticos obtenidos de la base de datos WorldClim Global Climate GIS (www.worldclim.org).

Code	Population	Latitude (S)	Longitude (W)	Alt. (m)	$T_{min}$ (°C)	$T_{max}$ (°C)	Prec. (mm)	Country	Floral visitors' sampling year
CO	Caleta Olivia	46°41'28"	67°23'0.7"	190	7.2	18.7	11.5	Argentina	2006, 2008
DM	Dos Manantiales	48°15'7.9"	69°46'59.4"	726	3.8	16.7	12.0	Argentina	2006
ET	El Trébol	45°49'55"	67°56'0.3"	557	4.6	17.1	10.0	Argentina	2005
ES	Escarchados	50°27'11"	71°28'0"	700	1.1	13.3	10.5	Argentina	2006
GA	Güer Aike	51°37'45"	69°37'26"	46	2.6	15.3	16.0	Argentina	2008
HE	Helsingfors	49°39'42"	72°51'49"	303	4.9	14.3	50.0	Argentina	2008
LJ	La Julia	49°38'33"	69°23'23"	90	4.9	17.7	11.0	Argentina	2006
MU	Lago Musters	45°28'25"	69°30'31"	671	4.3	17.1	9.0	Argentina	2006
MG	Matagrande	43°42'52"	70°3'4.7"	819	3.0	16.8	9.5	Argentina	2006
ML	Monte León	50°12'13"	68°56'56"	247	3.6	16.4	11.5	Argentina	2006, 2008
PV	Pampa Verdún	46°36'51"	69°36'16"	359	7.7	20.0	15.0	Argentina	2005, 2006
PM	PN Perito Moreno	48°4'39"	71°40'28"	870	2.5	13.6	16.0	Argentina	2006
PA	Potrok Aike	51°56'1.5"	70°23'59.4"	200	2.5	13.7	15.5	Argentina	-
RC	Río Chico	45°30'25"	67°37'24"	524	5.8	18.1	10.5	Argentina	2005, 2006, 2008
SJ	San Julián	49°18'54"	67°46'11"	7	5.4	17.8	15.5	Argentina	2006
SU	Subida Sumich	46°58'41"	70°41'20"	760	3.8	15.6	9.5	Argentina	2005, 2006
TK	Tecka	43°34'11"	70°33'55"	899	2.2	15.7	12.5	Argentina	2005, 2006, 2008
TP	Torres del Paine	50°59'31.6"	72°48'26.4"	316	3.6	13.8	45.5	Chile	2008
TC	Tres Cerros	48°3'41.9"	67°36'49"	172	5.9	18.3	14.0	Argentina	2006, 2008



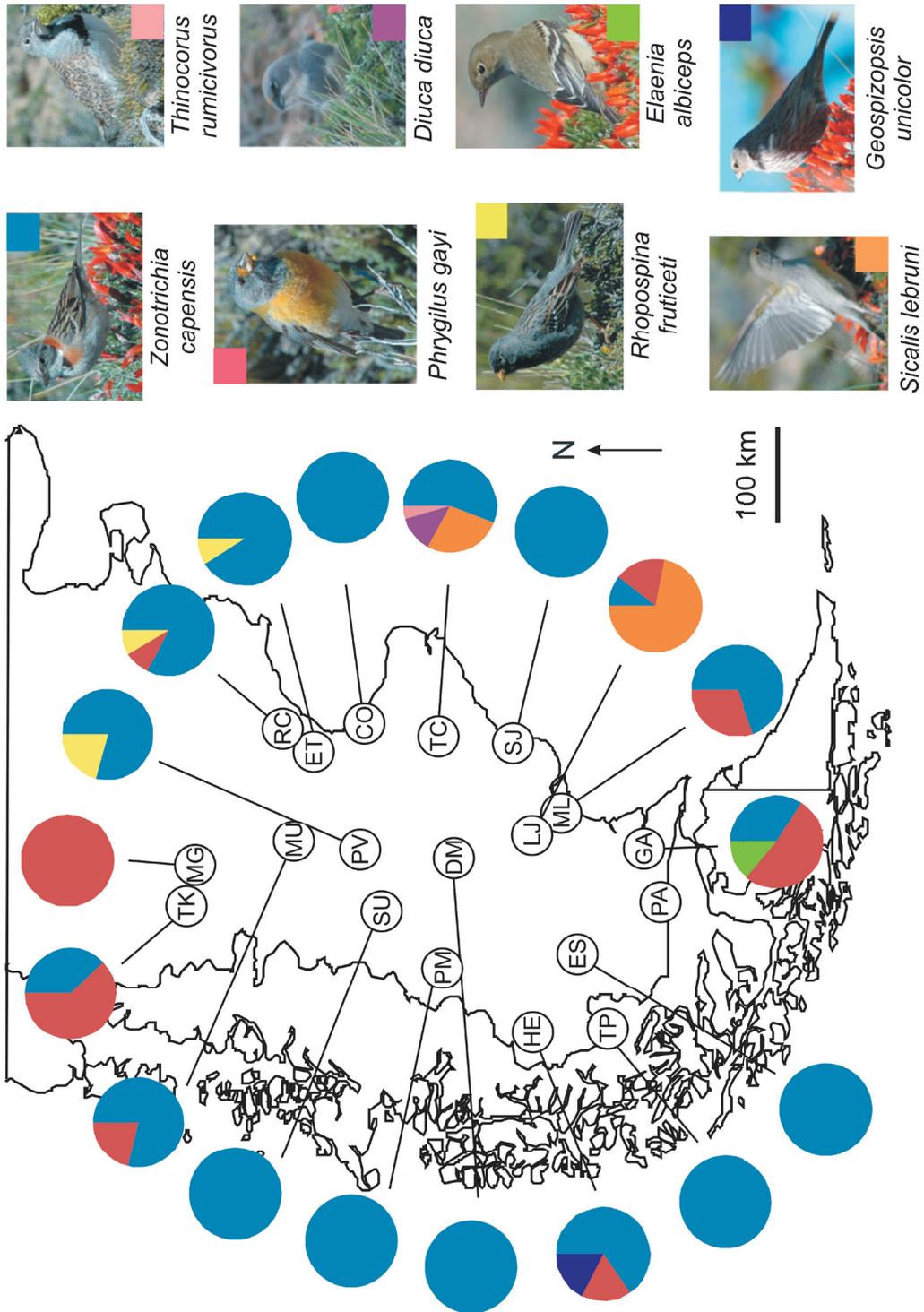
**Figure 1.** a) View of an *Anarthrophyllum desideratum* plant. b) Lateral view of a flower with a nectar drop (denoted with an arrow) between the flag and the keel. c) Open flower with the keel displaced and fertile parts exposed after a bird visit. d) Contact of the bird body with flower fertile parts during a visit.

**Figura 1.** a) Vista de una planta de *Anarthrophyllum desideratum*. b) Vista lateral de una flor con una gota de néctar (indicada con una flecha) entre el estandarte y la quilla. c) Flor abierta con la quilla desplazada y las piezas fértiles expuestas después de la visita de un ave. d) Contacto del cuerpo del ave con las piezas florales fértiles durante una visita.

to erect, branched and sericeous, and are covered by upwardly oriented spiny leaves, providing suitable perches for birds. Seeds have ballistic dispersion due to the explosive dehiscence of pods that, upon drying, throw the seeds within a range of a few meters from the mother plant.

*Breeding system and pollinator dependence*

Pollination experiments were performed following standard protocols provided by Dafni (1992). During the flowering season of 2005, four treatments were conducted on non-visited flowers of 12 plants in one *A. desideratum* population (Escarchados, see Table 1 and Figure 2). Treatments were: 1) open pollination (control) (flowers were exposed to the natural agents of pollination); 2) autonomous self-pollination (buds were bagged throughout the flowering period); 3) manual self-pollination (bagged buds were pollinated by hand with pollen from different flowers of the same individual), and 4) manual cross-pollination (bagged flowers were emasculated before anthesis and pollinated with pollen of recently opened flowers of other individuals located more than 30 m away from the receptor individual). Treatments 1 and 2 were also conducted on flowers of 10 plants



**Figure 2.** Geographical variation in the relative importance of the eight bird visitor species of *Anarthrophyllum desideratum*, estimated as the contributed proportion of total visits to flowering plants. Populations are identified by codes; additional information (population name, geographical coordinates, elevation, climatic conditions, and sampling year of floral visitors) is presented in Table 1.

**Figura 2.** Variación geográfica en la importancia relativa de las ocho especies de aves visitantes de *Anarthrophyllum desideratum*, estimada como la proporción aportada del total de visitas a plantas con flores. Las poblaciones se identifican por códigos. En la Tabla 1 se presenta información adicional (nombre de la población, coordenadas geográficas, elevación, condiciones climáticas y año de muestreo de los visitantes florales).

in other population (Potrok Aike, see Table 1 and Figure 2). Mean number of flowers per plant and total number of flowers and plants used in each treatment at each population are described in Table 2.

In order to assess the capacity of self-pollination in other populations distributed along the species range, 25 buds (one per plant) from seven populations (El Trébol, Monte León, Pampa Verdún, PN Perito Moreno, Río Chico, Subida Sumich, Tecka) growing in an experimental garden (Experimental Station of INTA, Río Gallegos, Argentina) were bagged to test for autonomous self-pollination. For each treatment, the number of fruits produced at each plant was recorded at the end of the flowering season.

In order to evaluate whether fruit set under natural pollination differ among *A. desideratum* populations, the open pollination treatment was tested in four populations (Escarchados, Monte León, Río Chico and Tecka) during the flowering season 2006. At each population, 100-105 plants were randomly chosen, marked and photographed with a Nikon Coolpix 5400 camera. Then, using the UTHSCSA ImageTool ver.3 software, total flower number was estimated as the sum of the open flowers and unopened buds on each plant. After bearing fruit, marked plants were photographed again for fruit counting.

In all cases, fruit set was estimated as the proportion of treated flowers setting fruits. A self-incompatibility index (ISI) was calculated in Escarchados as the ratio between fruit set obtained by manual self-pollination and that obtained by manual cross-pollination (Kearns and Inouye 1993). Species with  $ISI < 0.2$  are

arbitrarily categorized as self-incompatible, whereas higher values indicate that the plant is self-compatible (Dafni 1992).

#### Floral visitors

We recorded floral visitors in a total of 18 georeferenced populations at least 40 km apart from one another throughout the distribution range of *A. desideratum* during peak flowering season of 2005, 2006 and 2008 (Table 1 and Figure 2). Seven populations were surveyed repeatedly (Table 1). For each population, direct observations of flower visitors were conducted intensively in groups of five to 20 plants during periods of 20 min, from 9:00 to 19:00 h of a single day. Observations totaled 370 periods (7400 minutes) accumulated in the flowering seasons of the three study years. During the observation periods, the identity of each flower visitor and the number of plants visited were recorded. Relative frequencies of flower visitors at each population were calculated as the number of plants visited by each species divided by the total of plants visited in the population. The visitation rate for each visitor species was calculated as the number of plants visited by the species divided by the number of observed plants per observation period (i.e., 20 min), and values were averaged for each population. Foraging behaviour, type of visits (legitimate or not) and damage to flowers (if any) were all recorded.

We aimed to assess if bird visitors damaged fertile parts of the flowers when foraging for nectar, as recorded for other plant species of Patagonia (Traveset et al. 1998; Smith-Ramírez and Armesto 2003; Devoto et al. 2006). Given that sometimes after a bird visit flowers of *A. desideratum* remained open with the keel

**Table 2.** Mean number of treated flowers per plant, total number of treated flowers, total number of fruits produced and mean fruiting percentage (%) under different pollination treatments in two populations of *Anarthrophyllum desideratum* (Escarchados and Potrok Aike). Different letters indicate significant differences ( $P < 0.05$ ) among treatments within each population.

**Tabla 2.** Número promedio de flores tratadas por planta, número total de flores tratadas, número total de frutos producidos y porcentaje de fructificación promedio (%) bajo diferentes tratamientos de polinización en dos poblaciones de *Anarthrophyllum desideratum* (Escarchados y Potrok Aike). Letras diferentes indican diferencias significativas ( $P < 0.05$ ) entre los tratamientos dentro de cada población.

Treatment	Mean number of treated flowers per plant $\pm$ SE	Total number of treated flowers	Total number of fruits	Mean fruiting percentage (%) $\pm$ SE
Escarchados				
Open pollination	21.50 $\pm$ 1.71	258	8	3.70 $\pm$ 1.84 a
Autonomous self-pollination	9.92 $\pm$ 0.45	119	3	2.40 $\pm$ 1.27 a
Manual self-pollination	6.83 $\pm$ 0.52	82	12	11.63 $\pm$ 4.67 b
Manual cross-pollination	6.33 $\pm$ 0.70	76	8	10.08 $\pm$ 5.05 b
Potrok Aike				
Open pollination	8.40 $\pm$ 1.36	84	0	0.00 $\pm$ 0.00 a
Autonomous self-pollination	16.90 $\pm$ 2.18	169	2	1.41 $\pm$ 0.99 a

displaced and fertile parts exposed (Figure 1c), we determined whether bird visitation had caused any damage to the pistils by harvesting 90 of those flowers (six from each of 15 individuals) from one population (Matagrande, see Table 1 and Figure 2) and examining their ovaries, styles and stigmas.

In 2008, we placed one mist-net in each of three plant populations (Helsingfors, Monte León and Tecka) to capture birds and check if they carried pollen from *A. desideratum* flowers. Mist-nets were checked regularly and once a bird had been captured, a transparent adhesive strip of ca. 3 x 1 cm was applied to the body parts suspected to transport pollen (throat, forehead), and examined under a microscope.

#### *Geographical and climatic variables*

Geographical coordinates (i.e., latitude, longitude and altitude) from each population were recorded 'in situ' using a GPS (Table 1). Precipitation, minimum and maximum temperature of the months corresponding to the *A. desideratum* flowering period (i.e., October and November) were obtained for each site using the WorldClim Global Climate GIS database ([www.worldclim.org](http://www.worldclim.org)) (Hijmans et al. 2005). These climatic variables correspond to average values obtained from interpolations of data recorded during the period between 1950 and 2000 at a spatial resolution of 1 km<sup>2</sup>. Average values for the flowering season are presented in Table 1.

#### *Statistical analysis*

**Breeding system and pollinator dependence.** We applied generalized linear models (GLM) with binomial error structure in the R 3.3.2 statistical package (R Development Core Team 2016) to test differences in the frequency of flowers producing fruits between pollination treatments at each studied population (Escarchados and Potrok Aike), among populations tested for the open pollination treatment (Escarchados, Monte León, Río Chico and Tecka), and between sampled years (2005 and 2006) for the open pollination treatment in Escarchados.

**Geographical structure of floral visitor assemblages.** To test for spatial structure of floral visitor assemblages we correlated a matrix containing the between-population floral visitor distances determined using the Bray-Curtis dissimilarity index with a matrix containing the between-population

geographical distance (in km) by means of a Mantel test. Linear geographical distances between each pair of populations were calculated based on latitude and longitude coordinates using GPS Track Maker software (Ferreira Júnior 2004). Bray-Curtis dissimilarity index, which is a quantitative modification of Sørensen's index, was calculated using mean values of visitation rate per population. Mantel statistics and significance were determined by performing 999 permutations of the original matrix using the package *vegan* of R 3.3.2 software (R Development Core Team 2016).

**Temporal constancy of spatial differences in floral visitors.** To determine if spatial variation in the proportion of visits of main floral visitors was similar over different flowering seasons, we performed separate Spearman correlations between sampling years for relative visitation frequencies of the most important floral visitor species, including those populations sampled during at least two flowering seasons.

**Environmental correlates of variation in floral visitor assemblages.** We tested for the association between floral visitor assemblage composition and environmental variation using a canonical correspondence analysis (CCA) performed with CANOCO (terBraak and Šmilauer 2002). For this analysis, a dependent matrix of population x visitation rate for different floral visitor species was analysed in relation to a corresponding matrix of explanatory climatic variables. To avoid using different scales and for comparative purposes, climatic variables were standardized to zero mean and unity standard deviation. The significance of the variability explained by each environmental factor was analysed by automatic selection of variables using a Monte Carlo test with 999 permutations. In this procedure, the variable that best fits the data is selected first and then the next best fitting variable is added to the model (terBraak and Šmilauer 2002).

## RESULTS

### *Breeding system and pollinator dependence*

In general, the autonomous self-pollination and open pollination treatments yielded fewer fruits than the manual pollination treatments (Table 2). Only a very low proportion of flowers set fruit after autonomous self-pollination in Escarchados and Potrok Aike (Table 2). On the other hand, none of the flowers under this treatment set fruit

in the seven populations (ET, ML, PV, PM, RC, SU, TK) that grew in the experimental garden (data not shown). In Escarchados, the autonomous self-pollination treatment produced significantly lower amounts of fruits per flower than manual pollination treatments (manual self-pollination:  $z=2.85$ ,  $P<0.01$ ; manual cross-pollination:  $z=2.18$ ,  $P<0.05$ ). Both types of manual pollination did not differ significantly from each other ( $z=-0.77$ ,  $P>0.05$ ) (Table 2). The addition of pollen to flowers significantly increased the reproductive success with respect to open pollination (manual self-pollination:  $z=3.53$ ,  $P<0.01$ ; manual cross-pollination:  $z=2.51$ ,  $P<0.05$ ) in Escarchados (Table 2). The self-incompatibility index (ISI=1.15) indicated that this population was self-compatible. There were no significant differences in frequency of flowers setting fruits between autonomous self-pollination and open pollination in Escarchados ( $z=0.31$ ,  $P>0.05$ ) nor in Potrok Aike ( $z=-0.003$ ,  $P>0.05$ ) (Table 2).

The four populations that were tested for open pollination significantly differed in the proportion of flowers setting fruit ( $P>0.0001$ ) (Table 3). Río Chico and Escarchados showed the lowest fruit sets, while Monte León and Tecka had the highest fruit sets (Table 3). There were no significant differences in fruit set under the open pollination treatment among sampling years (2005 and 2006) in Escarchados ( $z=1.44$ ,  $P>0.05$ ).

*Floral visitors*

A total of 845 plant visits of eight bird species were recorded in the 18 studied populations of *A. desideratum* during the three study years (Figure 2). Among these bird species, seven were passerines (Order Passeriformes), belonging to the families Emberizidae (*Zonotrichia capensis*), Thraupidae (*Phrygilus gayi*, *Rhopospina fruticeti*, *Geospizopsis unicolor*, *Sicalis lebruni*, *Diuca diuca*) and Tyrannidae (*Elaenia albiceps*). The remaining species

(*Thinocorus rumicivorus*) was a member of the family Thinocoridae, in the Order Charadriiformes. The most frequent flower visitor was *Z. capensis* (75.4% of all visits), followed by *P. gayi* (16.6% of visits). The eight visiting bird species perched on the cushion or, less frequently, moved on the ground reaching flowers positioned on lower branches of the shrub, usually foraging for nectar from many flowers per plant (more than 12 flowers on average).

The oil-collecting bee *Centris cineraria* occasionally visited *A. desideratum* flowers, collecting nectar without making contact with the fertile parts of flowers, so they act as nectar thieves. Conversely, birds inserted their beak between the flag and the keel when visiting flowers, causing a backward movement of the keel leaving the stamens and stigma exposed. By doing so, they invariably contacted reproductive organs of flowers with some part of their body, mainly the throat or the forehead (Figure 1d). No flower damage was recorded during visits, and fertile parts were undamaged in 95.5% of the examined flowers that remained open after bird visits in Matagrande site (Figure 1c). Most bird species were directly observed and photographed in the field carrying large amounts of pollen around their beaks. Two individuals of *Z. capensis* were captured in Monte León during 27 hours of mist-netting in three populations. The presence of pollen from *A. desideratum* on their bodies was confirmed, whereas no pollen from other plant species was detected.

*Geographical structure and environmental correlates of variation in floral visitor assemblages*

Populations of *A. desideratum* were visited by bird assemblages that differed in composition and relative contributions of bird species (Figure 2). In most plant populations, only one bird species accounted for >75% of the total recorded visits. *Z. capensis* was the only floral

**Table 3.** Mean fruiting percentage (%) under the open pollination treatment, mean number of flowers per plant, total visitation rate, visitation rate of *Phrygilus gayi* in four populations of *Anarthrophyllum desideratum*. Different letters indicate significant differences ( $P<0.05$ ) among populations.

**Tabla 3.** Porcentaje de fructificación promedio (%) bajo el tratamiento de polinización abierta, número promedio de flores por planta, tasa de visitas totales y tasa de visitas de *Phrygilus gayi* en cuatro poblaciones de *Anarthrophyllum desideratum*. Letras diferentes indican diferencias significativas ( $P<0.05$ ) entre poblaciones.

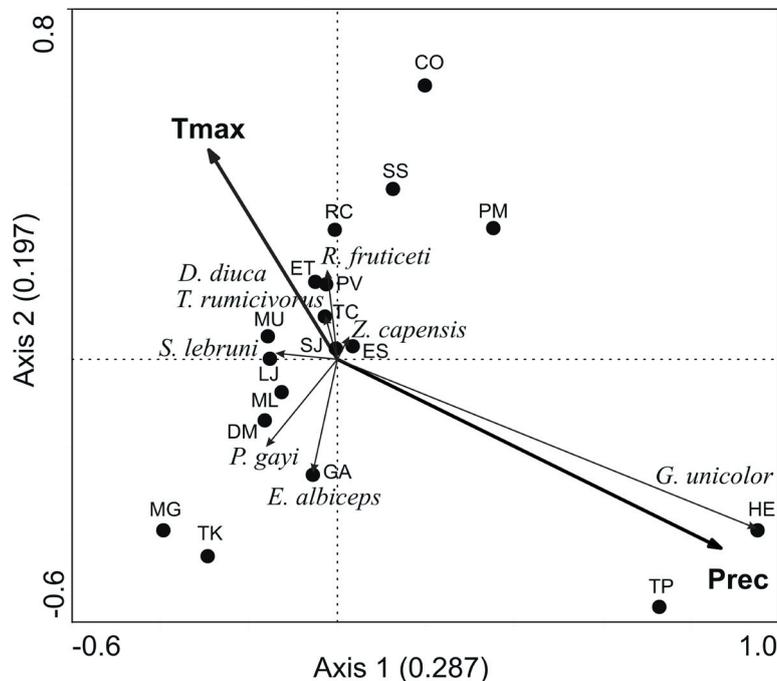
Population	Mean fruiting percentage (%) ± SE	Mean number of flowers per plant ± SE	Total visitation rate	Visitation rate of <i>Phrygilus gayi</i>
Escarchados	5.28 ± 0.45 b	1828.09 ± 103.51	0.43	0.00
Monte León	46.81 ± 2.00 d	532.02 ± 48.03	0.32	0.08
Río Chico	4.11 ± 0.41 a	314.37 ± 23.98	0.30	0.02
Tecka	27.55 ± 2.00 c	271.64 ± 22.54	0.25	0.09

visitor or the dominant one in 14 populations, *P. gayi* was the most frequent species in three populations, and *S. lebruni* was the dominant visitor in the remaining population (Figure 2).

Differences among populations in the proportion of plants visited by the two major floral visitors remained temporally constant in the localities sampled during two seasons, as revealed by significant correlations between years for the relative visitation frequency of *Z. capensis* ( $r=0.94$ ,  $n=6$ ,  $P=0.04$ ) and *P. gayi* ( $r=0.91$ ,  $n=7$ ,  $P=0.03$ ).

The Mantel test showed no significant association between similarity in floral visitor assemblages and geographical

distances among populations ( $r=-0.02$ ,  $P>0.05$ ,  $n=18$ ), indicating that differences in bird composition were not spatially structured across the *A. desideratum* range. The CCA exploring the association between floral visitor assemblages and climatic variables revealed that precipitation ( $F=2.82$ ,  $P=0.086$ ) and maximum temperature of the flowering period ( $F=2.30$ ,  $P=0.057$ ) made a marginally significant contribution to the model (Figure 3). The arrow representing the direction of maximum change for each explanatory variable showed that populations from humid and cold sites were visited by *G. unicolor*, whereas populations from warm and dry localities were visited by *R. fruticeti*, *S. lebruni*, *D. diuca* and *T. rumicivorus* (Figure 3).



**Figure 3.** Biplot of the first two axes of the CCA ordination of 18 populations of *Anarthrophyllum desideratum* (dots) based on visitation rates of eight bird species (thin arrows), including the explanatory environmental factors (thick arrows) that were marginally significant ( $P<0.1$ ) determinants of variation in floral visitor composition. Eigenvalues associated with each axis are provided in parentheses. Prec.=monthly precipitation during the flowering period;  $T_{max}$ =mean maximum temperature of the flowering period. Population codes and names are given in Table 1.

**Figura 3.** Gráfico de los dos primeros ejes de ordenación del CCA de 18 poblaciones de *Anarthrophyllum desideratum* (puntos) a partir de las tasas de visita de ocho especies de aves (flechas delgadas), incluyendo los factores ambientales explicativos (flechas gruesas) que fueron determinantes marginalmente significativos ( $P<0.1$ ) de la variación en la composición de visitantes florales. Los eigenvalores asociados a cada eje se proporcionan entre paréntesis. Prec.=precipitación mensual durante el período de floración;  $T_{max}$ =temperatura media máxima del período de floración. Los códigos y nombres de las poblaciones figuran en la Tabla 1.

## DISCUSSION

### *Breeding system and pollinator dependence*

The pollination treatments evaluated in one population (Escarchados) showed that *A. desideratum* has a self-compatible breeding system, setting fruits similarly through manual self- and manual cross-pollination. The

occurrence of geitonogamy may be affected in general by floral display and pollinator behaviour (Harder and Barrett 1996), and it may occur in plants that display numerous flowers simultaneously, and whose pollinators move between flowers of the same plant, as is the case for birds visiting *A. desideratum*. Despite its self-compatibility, the species

showed a limited capacity for autonomous self-pollination, as fruit sets under this treatment were low in Escarchados and Potrok Aike sites and null in the seven populations at the experimental garden, including those that were also sampled for open pollination (i.e., Monte León, Río Chico and Tecka).

Moreover, our findings suggest that floral visitors do not contribute to sexual reproduction at neither of the two studied populations (i.e., Escarchados and Potrok Aike), where fruit set did not differ significantly between autonomous self-pollination and open pollination. In addition, reproductive success appears to be strongly pollen-limited under natural conditions in Escarchados, as indicated by the significantly increased fruit set when pollen was added to flowers in comparison to open pollination flowers. This pollen-limitation appears to be temporally constant in this population, where the low fruit set under the open pollination treatment did not differ among consecutive years. However, it is important to notice that plants at this population display a really large number of flowers (Table 3), which might limit the chances of each flower of being pollinated by birds in the open pollination treatment, even with the highest visitation rate (Table 3). On the other hand, our results of pollinator dependence are based on two populations located near the southern limit of the species distribution (Paiaro et al. 2012a), where pollen limitation may increase and reproduction eventually fail (Chalcoff et al. 2012). In line with this idea, we found lower reproductive success under open pollination in populations located in the south-western (i.e., Escarchados) and north-eastern (i.e., Río Chico) edges of the species distribution (Paiaro et al. 2012a) in relation to the two central populations that were studied (i.e., Monte León and Tecka). In addition, these populations had different visitor assemblages (Figure 2) and visitation frequencies (Table 3), supporting the idea that dependence on pollinators for setting fruits varies among sites differing in abundance and composition of floral visitors (Moeller 2006; Cosacov et al. 2008; Chalcoff et al. 2012). In this sense, it is interesting to notice that reproductive success under open pollination was higher in those populations where *Phrygilus gayi* was present in a high proportion (>30%) and had a higher visitation rate (i.e., Monte León and Tecka), suggesting the potential role of this bird species as pollinator of *A. desideratum* in

these populations. In addition, considering the *A. desideratum* pollen found on the individuals of *Z. capensis* captured with mist-nets in Monte León, this bird species could also be acting as an effective pollinator in this population, where reproductive success under open pollination was the highest. It is possible that the effectiveness of each bird species as pollinator differs among sites, taking into account that the same bird species appears to handle flowers in different manner at distinct populations (A. Cocucci, personal observation).

#### *Floral visitors*

Eight bird species outside the main nectarivorous groups were observed visiting and feeding on the nectar of *A. desideratum* flowers. As expected based on *A. desideratum* floral characters, flower visitors were mostly passerine. None of the eight visiting bird species recorded in this study is exclusively nectarivorous. Most of them have a mainly granivorous diet that also includes insects (*Zonotrichia capensis*, *Phrygilus gayi*, *Sicalis sp.*, and *Diuca diuca*), whereas *Thinocorus rumicivorus* is a frugivorous and seed-eating species, and *Elaenia albiceps* is mainly frugivorous in the temperate forest (Aizen et al. 2002; Amico and Aizen 2005; García et al. 2010) and mainly insectivorous in the Monte desert (Blendinger 2005). Thus, bird species reported here do not depend exclusively on flower nectar and appear to be only opportunistic flower visitors of *A. desideratum*. Possibly these birds were led to exploit nectar from flowers of this species while searching for other food resources (Willmer 2011). *A. desideratum* produces abundant nectar (440 flowers per plant and 8  $\mu$ L of nectar per flower average, Paiaro et al. 2012b), that is available early in the spring and could represent a seasonally important food and water source relative to other food sources that are scarce during this season. In fact, seasonal changes in diet according to food availability have been reported for *Z. capensis* (Novoa et al. 1996; Sabat et al. 1998), the most common flower visitor of *A. desideratum*. On the other hand, the onset of flowering of this ornithophilous species overlaps with the beginning of the reproductive activity of most of its flower visitors, which start egg laying between mid-October and mid-November (Blendinger 2005). Therefore, birds might depend on this species nectar during their reproductive season when energy demand is highest.

Some of the bird species observed in the present study (*Z. capensis*, *P. gayi*, *E. albiceps* and *T. rumicivorus*) were also recorded visiting flowers of plant species from other South American regions (Bernardello et al. 1994; Vieira and de Carvalho-Okano 1996; Roitman et al. 1997; Willis 2002), including the Andean-Patagonian forest and the sub-Andean grasslands (Sérsic and Cocucci 1996; Traveset et al. 1998; Smith-Ramírez and Armesto 2003; Devoto et al. 2006). *Phrygilus* species (*P. gayi* and/or *P. patagonicus*) have been observed visiting flowers of *Embothrium coccineum* and *Fuchsia magellanica* in the Patagonian temperate forest, but causing severe damage to flowers while foraging for nectar (Traveset et al. 1998; Devoto et al. 2006). *Elaenia albiceps* have been registered visiting flowers of *Embothrium coccineum* in the Chilean side of the temperate Andean forest (Smith-Ramírez and Armesto 2003; Devoto et al. 2006). Finally, *Thinocorus rumicivorus* has been reported foraging on the corolla appendage (i.e., a food body) of *Calceolaria uniflora* in the Patagonian steppe and the forest-steppe ecotone (Sérsic and Cocucci 1996). However, these bird species do not seem to find other nectar-secreting ornithophilous species that co-occur with *A. desideratum* in the Patagonian steppe (Galletto and Bernardello 2003; A. Sérsic and A. Cocucci, personal observation), as the pollen found on the two individuals of *Z. capensis* captured with mist-nets was only from this species. Even when our pollination experiments casted doubts on the effectiveness of the birds as pollinators in some populations, our photographs and direct observations show that all the bird species appeared to behave as pollinators by touching anthers and stigma while collecting nectar and by carrying pollen on their bodies. In addition, our results confirmed that *P. gayi* does not damage fertile organs of flowers while visiting them in Matagrande, where it was the only visitor species observed. This observation contrasts with studies on other plant species in the Patagonian temperate forest whose flowers appeared damaged after visits of *P. gayi* and/or the sister species *P. patagonicus* (Traveset et al. 1998; Devoto et al. 2006).

*Geographical structure and environmental correlates of variation in floral visitor assemblages*

Composition of bird assemblages varied spatially across *A. desideratum*'s range, as both identity and relative contributions of bird species shifted amongst plant populations.

Few species appeared to be the most important floral visitors across the plant species range, at least in terms of abundance. In addition to the Emberizidae *Z. capensis* (Rufous-Collared Sparrow), which was widespread and the most frequent floral visitor throughout the entire plant species range, two other birds, *S. lebruni* and *P. gayi* (Thraupidae), seem to be locally important floral visitors of *A. desideratum* in one and three populations, respectively. The remaining flower visitors were restricted to few populations, and in most cases occurred in a low frequency. Our results agree with those found in the Monte desert of Argentina, where species composition of bird assemblages was highly variable at the regional scale (Blendinger 2005). Differences among populations in the proportion of visits by the two major floral visitors of *A. desideratum* (*Z. capensis* and *P. gayi*) were constant over time, suggesting that local abundances of these bird visitors are spatially variable but temporally constant within localities, probably responding to similar food supply during the flowering season year after year.

Geographical differences in assemblage composition can be partially attributed to the incomplete overlap with the plant species distribution range. For instance, the exclusive presence of *G. unicolor* in a southwestern population (HE) reflects its narrow distribution in high Andean steppes and sub-Andean grasslands (de la Peña and Rumboll 2001). In contrast, the passerine *Elaenia albiceps*, which is a very frequent species in the temperate Andean forest (Aizen et al. 2002; Amico and Aizen 2005; García et al. 2010), was observed visiting *A. desideratum* flowers only in one dry steppe population at the southern most extreme of the bird species distribution (GA). Moreover, taking into account that the ranges of the other bird species recorded include and exceed the distribution of *A. desideratum* (de la Peña and Rumboll 2001), the geographical variation in floral visitor assemblages cannot be explained solely by the geographic ranges of the bird species. It is possible that idiosyncratic responses of these species explain the spatial changes in their abundances along the geographic range of *A. desideratum*, as it was suggested for granivorous birds of the Monte desert (Blendinger and Ojeda 2001). Alternatively, shifts in pollinator abundance and composition may respond to ecological factors varying over large spatial scales, such as climate (e.g., Moeller 2005; Devoto et al. 2006; Chalcoff et al. 2012). However, shifts in bird assemblages did not show a geographical

structure across the *A. desideratum* range and spatial variations in floral visitor composition were only weakly associated with precipitation and maximum temperature of the flowering period. Such weak association could be explained by the exclusive presence of *G. unicolor* in a population located in the most humid and one of the coldest sites of the studied gradient, which is surely related to the restricted distribution of this species (de la Peña and Rumboll 2001). Therefore, changing climatic conditions across the *A. desideratum* range would not be important as drivers of the geographical variation in floral visitor assemblage composition. Considering that plant phenotypic traits may be as important, or even more, than environmental factors as determinants of plant–pollinator interactions (Sánchez-Lafuente et al. 2005), an ongoing study explores whether geographical variations in plant and flower phenotype of *A. desideratum* (Paíaro et al. 2012a,b) affect spatial changes in its floral visitors.

In summary, our study provides new information on the reproductive biology of *A. desideratum*, explores the spatial and temporal variation of floral visitor assemblages as well as one of their possible causes. Our results indicate that non-exclusively nectarivorous birds visit flowers of *A. desideratum*, revealing the facultative nectarivory behaviour of these bird species in the absence of other food supplies. In addition, we show the first records of these bird species as nectar-feeding floral

visitors in the Patagonian steppe. According to our data, climatic gradients along which populations of *A. desideratum* occur would not determine geographical changes in the composition of their floral visitors. On the other hand, dependence on pollinators of this plant species appears to vary geographically, suggesting the importance of considering the spatial context in plant pollination studies. Our findings indicate that despite the opportunistic behavior of bird visitors, their potential role as pollinators should not be rejected. In fact, flower traits of *A. desideratum* seem to have evolved in response to these opportunistic flower-visiting birds in the Patagonian steppe.

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