



Parasitism by *Cuscuta chilensis* and gender affect how the nurse cushion *Laretia acaulis* increases diversity in Andean alpine communities

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Nomenclature

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Abstract

Question: Although there is a large amount of information about the anatomy, physiology and auto ecology of parasitic plants, much less is known about how they affect the interaction among other species and if they present gender preferences when the host is dioecious. The little knowledge available is focused on the effects of parasitic plants on negative plant interactions, but their indirect effects on facilitative interactions remains unexplored. We examined whether the parasitic plant *Cuscuta chilensis* showed gender preference on its host, the dioecious nurse cushion species *Laretia acaulis*, and how the presence of the *Cuscuta* affects relationships between *Laretia* and its community of beneficiary species.

Location: Central Chilean Andes.

Methods: We randomly sampled 50 *L. acaulis* cushions infected with *Cuscuta*, 50 *Laretia* cushions free of *Cuscuta* infection, and 50 open sites outside of cushions. We measured the area of each cushion selected, identified cushions as male or female, and identified, counted and recorded the numbers of each other species within cushions and in the open sites. We compared species richness among uninfected *Laretia* individuals, infected *Laretia* individuals and open sites, and between infected and uninfected patches within *Laretia* cushions. Further, we assessed the differences in species assemblage composition between each micro-habitat by means of detrended correspondence analysis.

Results: Fifteen out of 23 species were more common in *Laretia* cushions than in the open, non-cushion habitat. *Laretia* without *Cuscuta* harboured 3.9 ± 0.2 species per cushion in comparison to 2.5 ± 0.2 species in open habitats. Infection of *Laretia* cushions by *Cuscuta* was female-biased and this infection was associated with an increase in the nurse plant effects of *Laretia*, with seven of 23 species more common in *Cuscuta*-infected cushions than in cushions without *Cuscuta*, and a mean of 5.9 ± 0.3 species per *Cuscuta*-infected cushion.

Conclusions: *Cuscuta* infection showed gender preferences, and this infection increased the facilitative effect of *Laretia* nurse cushions. This might be related to a decrease in the competitive resistance of the cushion species to colonization by other species (indirect effect) or direct positive effect due to the presence of the parasitic plant (e.g. increases in available soil N). To our knowledge, this is the first documentation of how the indirect effects of a parasitic plant might increase the facilitative effects of a nurse species.

Introduction

Parasitic plants are present in all terrestrial ecosystems and comprise a large number of species (ca. 4500 species), ranging from small herbaceous plants to large trees

(Heide-Jorgensen 2008; Watson 2009). Although there is a large amount of information about the anatomy, physiology and autecology of parasitic plants (e.g. Press & Graves 1995; Pennings & Callaway 2002; Heide-Jorgensen 2008) much less is known about how parasitic plants may affect

plant community attributes such as diversity and interactions among species (Press & Phoenix 2005; Watson 2009).

Parasitic plants in general can strongly affect plant communities because of disproportional effects among potential host species, contributions to nutrient cycling and interactions with other consumers (Watson 2001, 2002, 2004; Press & Phoenix 2005; Spasojevic & Suding 2011). As consumers, parasitic plants might alter net interactions among plants through preferentially feeding on one of the interacting species (Huntly 1991; Callaway & Pennings 1998). For example, Callaway & Pennings (1998) reported that in a Californian salt marsh the parasite *Cuscuta salina* preferentially infects the dominant competitor *Salicornia virginica*, decreasing its abundance, allowing an increase in abundance of the subordinate competitor *Arthrocnemum subterminale*. Thus, through indirect interactions (i.e. when the strength or direction of the interaction among a pair of species is modified by a third one (Levine 1999; Pagès & Michalet 2003), parasitic plants may have important effects on species co-existence and diversity (Press & Phoenix 2005).

However, virtually all studies to date have focused on how parasitic plants affect competitive interactions, we know far less about how parasitic plants might affect facilitative interactions. On the one hand, parasitic plants may preferentially attack an important nurse or benefactor species and eliminate its facilitative effects. On the other hand, since most benefactor species have both facilitative and competitive effects on their beneficiaries (e.g. Schöb et al. 2014), parasites may reduce competitive effects and thereby enhance facilitative effects. Enhancing facilitative effects may have profound implications for community diversity because positive interactions between plants increase community diversity, particularly in physically stressful environments (Michalet et al. 2006; Cavieres et al. 2014). Recently, Watson (2009) reported that parasitic plants could be considered as facilitators because they indirectly enhance the abundance of other species by modifying nutrient inputs. However, whether parasitic plants directly affect the facilitative effects of a nurse species, thus indirectly affecting the beneficiaries of that nurse, remains unknown.

Facilitation appears to have particularly important effects in many alpine systems (Callaway et al. 2002; Butterfield et al. 2013; Cavieres et al. 2014). Facilitation by many cushion-forming species has been studied extensively in the Andes of Chile, and the importance of facilitative effects has been shown to vary along altitudinal gradients (Cavieres et al. 2002; 2006; Badano & Cavieres 2006a,b), precipitation gradients (Badano & Cavieres 2006a,b), among benefactor cushion species (Cavieres et al. 2008) and among beneficiary species (Cavieres et al. 2007). In the high Andes of central Chile, the parasitic plant *Cuscuta chilensis* Ker Gawl. (Convolvulaceae), a

native generalist holoparasitic vine, forms dense infections on *Laretia acaulis* (Cav.) Gilles & Hook. (Apiaceae), a dioecious species with strong facilitative effects (Cavieres et al. 2006, 2007). *Cuscuta chilensis* appears to attack few other species within the cushions and never infects species outside of cushions. Other species of *Cuscuta* can strongly suppress host species (Jeschke et al. 1994; Koskela et al. 2002; Grewell 2008), and *C. salina* has been shown to alter the balance of competition in salt marshes (Callaway & Pennings 1998) and enhance species diversity by suppressing community dominants (Grewell 2008).

It is well known that parasite plants show host preferences (Press & Phoenix 2005; Heide-Jorgensen 2008). In the last decades, an increasing number of studies have suggested that males and females of dioecious plants may be differentially affected by parasitism (Ågren et al. 1999; Press & Phoenix 2005). However, field evidence of host gender preferences of a parasitic plant is scarce (e.g. Gehring & Whitham 1992). Thus, taking advantage of *Laretia acaulis* dioecy, it seems relevant to ask if there are gender preferences in *C. chilensis* infection, and if this might have consequences for the facilitative effect of *L. acaulis*.

To explore the effects of a parasitic plant on facilitative interactions, we investigated the effects of *C. chilensis* on species diversity and community composition occurring within *L. acaulis* cushions (hereafter we will refer to studied species by their genus) not infected with *Cuscuta*, *Laretia* cushions infected with *Cuscuta*, between sexes of *Laretia* and in open sites without cushions. We compared species diversity and community composition within and outside of *Cuscuta* patches in infected *Laretia* cushions.

Methods

Study site and species

Fieldwork was conducted at La Parva (33°26'41" S, 70°01'20" W) located in the Chilean Andes 50 km east of Santiago, Chile, at 2800 m a.s.l. The climate is alpine but with a mediterranean-type climate seasonality, thus with low summer rainfall. There is a long snow-free summer period of 5–8 mo, with a mean air temperature of 10.9 °C (Cavieres et al. 2007). Mean total annual precipitation is ca. 445 mm, falling predominately as snow between May and September (Santibáñez & Uribe 1990).

Laretia acaulis is a very flat and tightly knit dioecious cushion species, occurring widely throughout the alpine zone of central Chile. Vegetative growth commences as soon as the ground thaws, and flowering occurs in mid-January, with its leaves dying at the end of the growing season (Hoffmann & Alliende 1984). At the elevation studied, *Laretia* is near the lower limit of its altitudinal range. Alliende & Hoffmann (1985) reported that *Laretia* cushions harbour several herbs species, and Cavieres et al. (2006,

2007) demonstrated that the higher species richness and abundance observed is related to mitigation of extreme temperatures and increased soil nutrients and moisture provided by *Laretia* cushions, which facilitate survival and reproduction of the harboured species. *Laretia acaulis* is frequently infected with *C. chilensis* at this elevation, and *Cuscuta* forms patches of bright yellow-orange, thin leafless stems that cover host *Laretia* leaves in infected portions of cushions. *Cuscuta* do not contain chlorophyll, and their thin stems grow between *Laretia* leaves without forming a closed mat or a distinct 'canopy' from the uninfected portion of *Laretia* cushions. Thus, it seems unlikely that on a single cushion, microclimate conditions in uninfected patches differ from those of infected patches.

Effects of *Cuscuta*

We randomly sampled 50 *L. acaulis* cushions infected with *Cuscuta*, 50 *Laretia* cushions free of *Cuscuta* infection and 50 open sites outside of cushions. All samples were located along eight 100-m long, 5-m wide belt transects, and sampling followed established methods to measure the facilitative effects of the cushions on other plants (Cavieres et al. 2006). Field observations suggested that there is no spatial aggregation between infected and uninfected cushions along the transect (i.e. they were randomly spaced), thus the sampled open areas are representative of alternative microhabitats for both cushion types. We measured the area (length \times width) of each cushion selected, identified cushions as male or female, and identified, counted and recorded the numbers of all other species within cushions and in the open sites. Open areas outside of cushions were randomly located 50 cm east of each of the sampled cushions without *Cuscuta*, and were identical in size and shape to their corresponding cushion. For the 50 cushions infected with *Cuscuta*, we sampled cushions of similar size to the uninfected cushions. The total areas sampled were: uninfected cushions and open area – 21.5 m², infected cushions – 22.2 m².

We measured the sizes of all *Cuscuta* patches within 45 of the *Laretia* individuals that were infected, and sampled the density and area covered by each individual of each species inside of *Cuscuta* patches and outside of *Cuscuta* patches. The total areas sampled were: infected patches within cushions – 4.8 m², and uninfected patches within cushions – 15.1 m². To estimate the preference of *Cuscuta* for *Laretia* and all species found within *Laretia*, we examined all individual plants and all *Laretia* stems within *Cuscuta* patches for infection, based on the presence of at least one *Cuscuta* coil (after Callaway & Pennings 1998). *Cuscuta chilensis* is much smaller than most other *Cuscuta* species and haustoria were not visible without magnification, so we did not count haustoria.

We compared species richness among uninfected *Laretia* individuals, infected *Laretia* individuals and open sites, and between infected and uninfected patches within *Laretia* cushions in two ways. First, we compared the total number of species found in each habitat using ANOVA and post-ANOVA Tukey comparisons to test the three treatments within a factor, and only ANOVA to test the two treatments. Second, we calculated species accumulation curves, first for uninfected *Laretia*, infected *Laretia* and open sites, and second between infected and uninfected patches within *Laretia* cushions. Since our protocol included samples that varied in area, these curves were constructed using sample-based rarefaction techniques to avoid biases due to the sequence in which samples were added to the curves. For each microhabitat (i.e. first for infected *Laretia*, uninfected *Laretia* and open areas, and then for infected and uninfected patches within *Laretia* cushions) we generated a species \times samples matrix, where each cell (i, j) indicated the presence (1) or absence (0) of the *i*th species in the *j*th sample. From these matrices, 5000 re-samples with replacement were randomly drawn for each sample size (from one sample to the maximum number of samples), and the Mao-Tau observed richness estimation and its 95% confidence interval calculated. The average values of species richness were then plotted against the respective sample size to construct a species accumulation curve for each microhabitat. Significant differences were assumed if confidence intervals did not overlap at the asymptote of the species accumulation curves (Gotelli & Colwell 2001). The rarefaction analyses were performed with the software EstimateS v. 8.2 (<http://viceroy.eeb.uconn.edu/estimates/EstimateSPages/AboutEstimateS.htm>). We assessed the differences in species assemblage composition between each type of cushion (i.e. infected or not with *Cuscuta*) by means of a detrended correspondence analysis (DCA), which considered both the difference in the species present in each microhabitat and the difference in abundance for the common species between microhabitats. Ordination analysis was performed with PC-ORD 4.24 (MjM Software Design, Gleneden Beach, OR, US).

Effects of cushion gender

We determined the proportions of male and female *Laretia* cushions infected with *Cuscuta* ($n = 46$) and the proportions of male and female cushions without *Cuscuta* ($n = 46$) in our samples. We compared species richness and density of individuals among male and female *Laretia* using a one-way ANCOVA with cushion area as covariate. To determine whether differences between genders were inherent or likely caused by preferential infection with *Cuscuta*, we compared species richness and density between uninfected male and female *Laretia* with a one-way

ANCOVA with cushion area as covariate. We also performed two-way ANCOVA for total density and richness for all 92 cushions, with *Cuscuta* presence and gender as factors and cushion size and total area occupied by *Cuscuta* as covariates. Even though total density is an area-based variable, we used cushion area as a covariate because area involves more than just space for beneficiary species to occupy. Size is likely to be correlated with cushion age, which provides more time for beneficiary species to colonize cushions, and size may be correlated with increased habitat heterogeneity as cushions mature and within-cushion patchiness develops. Statistical analyses were performed with STATISTICA 6.0 (Statsoft Inc., Tulsa, OK, US).

Results

Cuscuta and the facilitative effect of *Laretia*

There were 23 species, other than *Laretia* and *Cuscuta*, in our samples. Of these, 13 occurred in at least one sample in

the open, whereas all species occurred at least once in the cushions (Table 1). *Cuscuta* was found only within *Laretia* cushions, and did not occur in the open even when patches of other species it sometimes parasitized within cushions, such as *Haplopappus scrobiculatus*, were present in the open. Species diversity was higher in non-*Cuscuta*-infected *Laretia* than in the open, but species diversity was even higher in *Laretia* parasitized by *Cuscuta* than in *Laretia* cushions that did not have *Cuscuta* (Fig. 1a).

Fifteen of the 23 species present in the community (65.2%) were significantly more common in cushions in general than in the open; either more common in uninfected cushions (one species) or equally common in infected and uninfected cushions (Table 1). Eight species were significantly more common in cushions with *Cuscuta* than in cushions without *Cuscuta* or in the open (Table 1), where two species (*Polygala saliciana* and *Lepidium philippianum*) were observed only in *Cuscuta*-infected cushions (Table 1). Only one species, the non-native *Cerastium*

Table 1. Plant species abundance in open areas: in *Laretia acaulis* cushions without *Cuscuta chilensis* and in *Laretia* cushions infected with *Cuscuta*. Results show means and ± 1 SE. Column on the right shows the number of *Cuscuta* coils observed on each species when growing in open areas and *Laretia* cushions on which *Cuscuta* formed coils.

Species Area Sampled	Open Areas (21.5 m ²)	<i>Laretia acaulis</i>		Coils
		Without <i>Cuscuta</i> (21.5 m ²)	With <i>Cuscuta</i> (22.2 m ²)	
Preference for cushions				
<i>Calceolaria biflora</i>	–	0.03 \pm 0.03 ^a	0.33 \pm 0.19 ^b	3/30
<i>Hypochaeris clarionoides</i>	–	0.16 \pm 0.16 ^a	0.13 \pm 0.09 ^a	–
<i>Rytidosperma pictu</i>	–	0.15 \pm 0.11 ^a	0.37 \pm 0.21 ^a	–
<i>Erigeron andicola</i>	–	0.57 \pm 0.33 ^a	0.86 \pm 0.42 ^a	–
<i>Euphorbia collina</i>	–	0.13 \pm 0.13	–	–
<i>Haplopappus scrobiculatus</i>	3.17 \pm 1.13 ^a	7.95 \pm 2.30 ^b	9.40 \pm 2.04 ^b	23/54
<i>Poa</i> sp.	5.54 \pm 0.91 ^a	40.12 \pm 4.48 ^b	48.89 \pm 5.50 ^b	0/4
<i>Cerastium arvense</i> *	1.53 \pm 0.89 ^a	20.61 \pm 5.26 ^b	10.24 \pm 3.90 ^b	0/2
Preference for cushions with <i>Cuscuta</i>				
<i>Polygala saliciana</i>	–	–	0.14 \pm 0.10	–
<i>Lepidium philippianum</i>	–	–	0.11 \pm 0.07	–
<i>Festuca</i> sp.	–	0.62 \pm 1.53 ^a	6.67 \pm 2.57 ^b	0/18
<i>Hordeum comosum</i>	–	1.84 \pm 0.91 ^a	6.58 \pm 1.89 ^b	–
<i>Olsynium philippii</i>	–	0.63 \pm 0.37 ^a	1.92 \pm 0.46 ^a	–
<i>Taraxacum officinale</i> *	0.50 \pm 0.20 ^a	2.28 \pm 0.49 ^a	6.00 \pm 0.83 ^b	–
<i>Acaena pinnatifida</i>	0.20 \pm 0.09 ^a	0.23 \pm 0.14 ^a	2.11 \pm 0.65 ^b	0/5
No preference				
<i>Alstroemeria exerens</i>	0.59 \pm 0.33 ^a	0.48 \pm 0.22 ^a	0.55 \pm 0.28 ^a	0/1
<i>Berberis empetrifolia</i>	2.93 \pm 1.11 ^a	3.22 \pm 1.53 ^a	1.74 \pm 0.91 ^a	0/5
<i>Luzula</i> sp.	0.38 \pm 0.21 ^a	0.09 \pm 0.06 ^a	0.30 \pm 0.16 ^a	0/4
<i>Nassauvia pyramidalis</i>	0.89 \pm 0.53 ^a	1.88 \pm 1.50 ^a	2.34 \pm 1.24 ^a	0/4
<i>Quinchamalium chilense</i>	0.67 \pm 0.28 ^a	1.29 \pm 0.42 ^a	2.55 \pm 0.91 ^a	0/5
<i>Senecio polygaloides</i>	0.03 \pm 0.03 ^a	0.29 \pm 0.25 ^a	0.83 \pm 0.42 ^a	–
<i>Senecio bustillosianus</i>	1.41 \pm 0.59 ^a	2.23 \pm 0.72 ^a	1.92 \pm 1.00 ^a	–
Preference for open				
<i>Chaethanthera euphrasioides</i>	25.69 \pm 8.10 ^a	1.76 \pm 0.81 ^a	0.80 \pm 0.42 ^a	0/4

For each species, shared letters represent no significant differences between means.

*Non-native species.

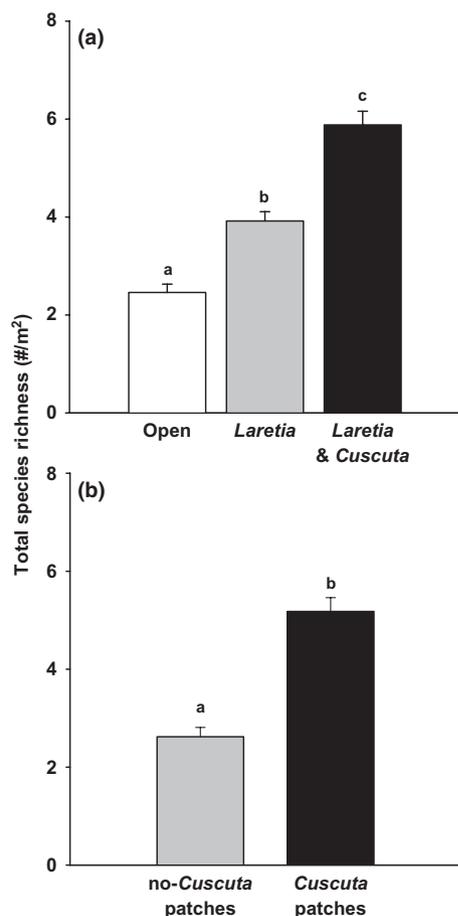


Fig. 1. Total species richness in (a) open areas, in *Laretia* without *Cuscuta* and in *Laretia* infected with *Cuscuta*, and (b) within patches of *Laretia* with no *Cuscuta* infection and within patches of *Laretia* infected with *Cuscuta*. Different letters designate significant differences and bars represent ± 1 SE.

arvense, showed a higher abundance within non-infected cushions than in *Cuscuta*-infected cushions. Further, only the annual herb *Chaethanthera euphrasioides* was significantly more common in the open than in either infected or uninfected *Laretia* cushions.

Within *Laretia* cushions infected with *Cuscuta*, species richness was far higher in *Cuscuta* patches than in patches of cushion free of *Cuscuta* (Fig. 1b). There were 22 species in the samples for within-cushion infected vs non-infected patches, and within infected cushions 17 species were more common in *Cuscuta* patches and no species was more common in uninfected patches (Table 2).

Differences in total species richness were also evident in species accumulation curves. Species accumulated significantly faster and to higher levels in uninfected *Laretia* cushions than in the open, but these processes were even higher in *Laretia* infected with *Cuscuta* (Fig. 2). Within infected cushions, species accumulation rates and levels

were far higher in *Cuscuta* patches, despite the much smaller total area occupied by *Cuscuta*, than in *Laretia* patches without *Cuscuta* (Fig. 3).

Laretia had strong effects on community composition, as estimated through ordination, and *Cuscuta* infection caused comparatively minor, but significant, changes in this measure of communities (Fig. 4). *Laretia* cushions either with (106 ± 10 individuals·m⁻²) or without (86 ± 8 individuals·m⁻²) *Cuscuta* infections had higher densities of individual species than open ground (44 ± 8 individuals·m⁻²), but there was no significant difference in beneficiary density between infected and uninfected cushions (ANOVA, $F = 13.58$, $df = 2, 147$, $P < 0.0001$, post-ANOVA Tukey for infected and uninfected cushions vs open, $P < 0.0001$ and $P = 0.002$, respectively, for *Cuscuta* vs no *Cuscuta*, $P = 0.252$).

Laretia gender and nurse plant effects

Of the 46 *Laretia* cushions infected with *Cuscuta*, 12 were male and 34 (74%) were female. Of the 46 *Laretia* cushions not infected with *Cuscuta*, nine (20%) were female and 37 were male. A proportion test ($P < 0.01$) indicated a strong preference for female *Laretia* with *Cuscuta*.

Considering uninfected and infected *Laretia* together, female plants had significantly higher densities of beneficiaries (114 ± 10 vs 84 ± 8 individuals·m⁻²), higher beneficiary richness (4.8 ± 0.3 vs 3.1 ± 0.2 species per sample), and higher richness per area of cushion (12.3 ± 1.3 vs 8.6 ± 0.9 species·m⁻²; Fig. 5) than male plants. Inherent gender differences appeared to determine the gender effect for density, whereas *Cuscuta* preference for female *Laretia* appeared to determine gender differences for richness. First, in a replication-limited ($n = 9$ for females) analysis of uninfected *Laretia* the effect of gender was significant for density (one-way ANCOVA, $F = 5.59$, $df = 1, 43$, $P = 0.023$), but not for richness ($F = 0.70$, $df = 1, 43$, $P = 0.409$) or for richness per area of cushion ($F = 2.84$, $df = 1, 43$, $P = 0.099$). Second, in two-way ANCOVAs the effect of gender was significant for total density but not for infection, while for richness the effect of infection was significant but the effect of gender was not (Table 3).

Discussion

Enhanced community diversity resulting from the presence of nurse, or foundation, species that ameliorate harsh environmental conditions and support a diverse assemblage of other organisms has been widely documented (Callaway 2007 and references therein). Alpine plants with cushion morphologies, such as *Laretia acaulis*, facilitate beneficiary plant species that would otherwise not be present or would be much less common in alpine plant

Table 2. Plant species abundance within *Laretia acaulis* cushions infected with *Cuscuta chilensis*, in *Cuscuta* patches and in patches of cushion free of *Cuscuta*. Results show means \pm 1 SE. Column on the right shows the number of *Cuscuta* coils for each species in patches inside *Laretia* with no *Cuscuta* and patches on which *Cuscuta* had formed coils.

Species	<i>Laretia acaulis</i>		Coils
	No <i>Cuscuta</i> patches (15.1 m ²)	<i>Cuscuta</i> patches (4.8 m ²)	
<i>Laretia acaulis</i>			9796/9796
Preference for <i>Cuscuta</i>			
<i>Festuca</i> sp.	–	30.00 \pm 8.14	18/0
<i>Lepidium philippianum</i>	–	7.24 \pm 2.18	2/0
<i>Calceolaria biflora</i>	–	3.00 \pm 1.78	2/0
<i>Chaethanthera euphrasioides</i>	–	2.58 \pm 1.45	4/0
<i>Hypochaeris clarionoides</i>	–	1.96 \pm 1.49	3/0
<i>Rytidosperma pictu</i>	–	1.22 \pm 0.72	2/0
<i>Polygala salasiana</i>	–	0.89 \pm 0.66	1/0
<i>Acaena pinnatifida</i>	0.04 \pm 0.03 ^a	12.87 \pm 3.90 ^b	6/0
<i>Hordeum comosum</i>	0.18 \pm 0.10 ^a	40.29 \pm 17.32 ^b	22/0
<i>Olsynium philippii</i>	0.16 \pm 0.12 ^a	9.09 \pm 2.33 ^b	11/0
<i>Erigeron andicola</i>	0.13 \pm 0.10 ^a	4.60 \pm 2.77 ^b	–
<i>Quinchamalium chilense</i>	0.16 \pm 0.12 ^a	9.09 \pm 2.33 ^b	3/1
<i>Poa</i> sp.	32.04 \pm 6.45 ^a	116.69 \pm 20.47 ^b	114/0
<i>Taraxacum officinale</i> *	2.60 \pm 0.51 ^a	20.49 \pm 4.11 ^b	23/2
<i>Haplopappus scrobiculatus</i>	3.73 \pm 1.12 ^a	32.18 \pm 10.74 ^b	53/23
<i>Cerastium arvense</i> *	5.07 \pm 2.17 ^a	20.11 \pm 6.69 ^b	29/2
<i>Senecio polygaloides</i>	0.36 \pm 0.16 ^a	12.78 \pm 4.21 ^b	8/1
No preference			
<i>Senecio bustillosianus</i>	1.29 \pm 0.73 ^a	2.98 \pm 1.85 ^a	6/4
<i>Nassauvia pyramidalis</i>	0.73 \pm 0.42 ^a	2.82 \pm 1.98 ^a	14/2
<i>Alstroemeria exerens</i>	0.40 \pm 0.20 ^a	2.24 \pm 1.91 ^a	1/0
<i>Berberis empetrifolia</i>	1.80 \pm 1.57 ^a	3.69 \pm 2.20 ^a	5/0

For each species, shared letters represent no significant differences between means.

*Non-native species.

communities (Cavieres & Badano 2009; Cavieres et al. 2014). The net positive effects of alpine cushion plants on their beneficiaries are primarily due to amelioration of abiotic stress in the alpine environment (e.g. Cavieres et al. 2007), but even this net amelioration does not preclude concomitant strong competitive effects (see Cavieres et al. 2006; De Bello et al. 2011; Dvorsky et al. 2013). Here we found that overall species richness was higher within *Laretia* cushions than in the open, but richness was higher still in *Laretia* parasitized with *Cuscuta* than in non-parasitized *Laretia* cushions. Indeed, within infected cushions, patches with *Cuscuta* contained more species than patches without *Cuscuta*. Similar effects were observed on the abundance of those species found in the three microhabitats, where a majority of the species showed higher abundance on *Cuscuta*-infected cushions. Parasitic plants such as *Cuscuta* spp. can have strong negative effects on their hosts (Pennings & Callaway 2002). Thus, our results suggest that *C. chilensis*, by strongly preferentially infecting *Laretia*, may suppress the competitive effects of *Laretia*, which in turn indirectly enhances the facilitative effects of this cushion plant. Our results add to a growing body of work showing that parasitic plants such as *Cuscuta* can structure plant communities

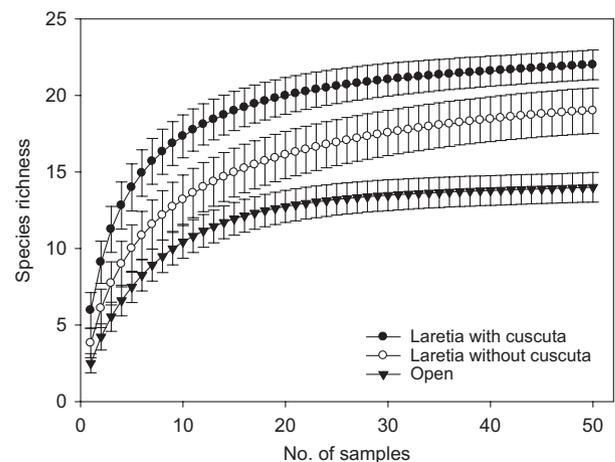


Fig. 2. Species accumulation curves for plots in the open, plots in *Laretia* without *Cuscuta* and plots in *Laretia* with *Cuscuta*. Bars represent 95% confidence intervals.

(Pennings & Callaway 1996; Callaway & Pennings 1998; Marvier 1998; Grewell 2008) and in some cases function as 'ecological keystone species' (Watson 2001, 2004, 2009).

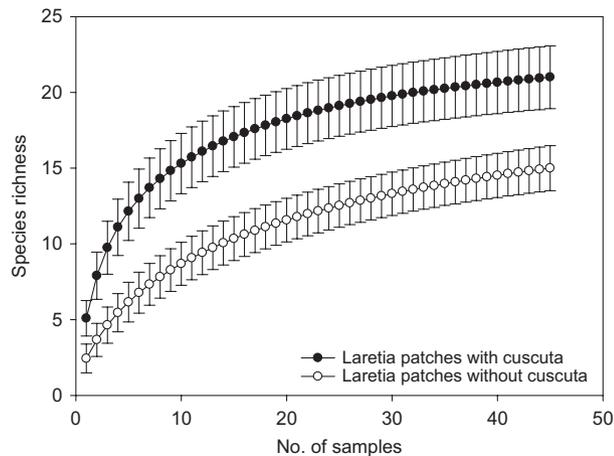


Fig. 3. Species accumulation curves for plots within patches of *Laretia* with *Cuscuta* and in plots within *Laretia* without presence of *Cuscuta*. Bars represent 95% confidence intervals.

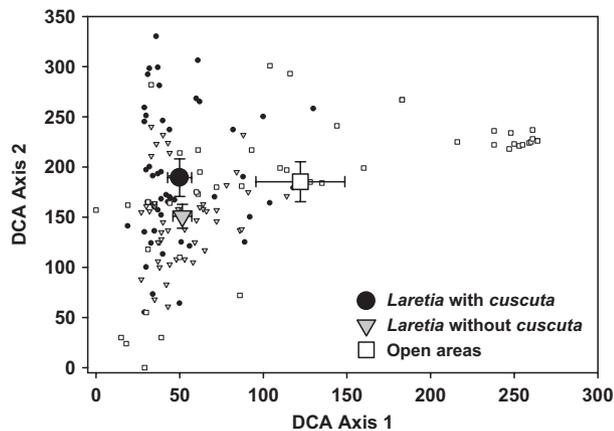


Fig. 4. Detrended correspondence analysis ordination of plots in open areas, in *Laretia* cushions *Cuscuta* infection, and in *Laretia* cushions without *Cuscuta* infection. Total inertia = 3.96, Eigenvalues = 0.82 and 0.42 for axes 1 and 2, respectively. Large symbols represent centroids and bars represent 95% confidence intervals.

Even though most parasitic plants, including *Cuscuta* spp., are generalists, it is well established that *Cuscuta* spp. commonly exhibit host preferences (Pennings & Callaway 1996; Callaway & Pennings 1998; Koskela et al. 2002; Grewell 2008). For instance, Koch et al. (2004) found that for *Cuscuta campestris*, the quality of resources acquired from a host is host-dependent, and that *C. campestris* selectively parasitized hosts having the largest rewards. Runyon et al. (2006) discovered that seedlings of *Cuscuta pentagona* use volatile chemical cues emitted by neighbouring plants to direct growth towards suitable hosts. We found that *C. chilensis* only occurs within *Laretia* cushions and did not occur in the open, even when patches of other species it some-

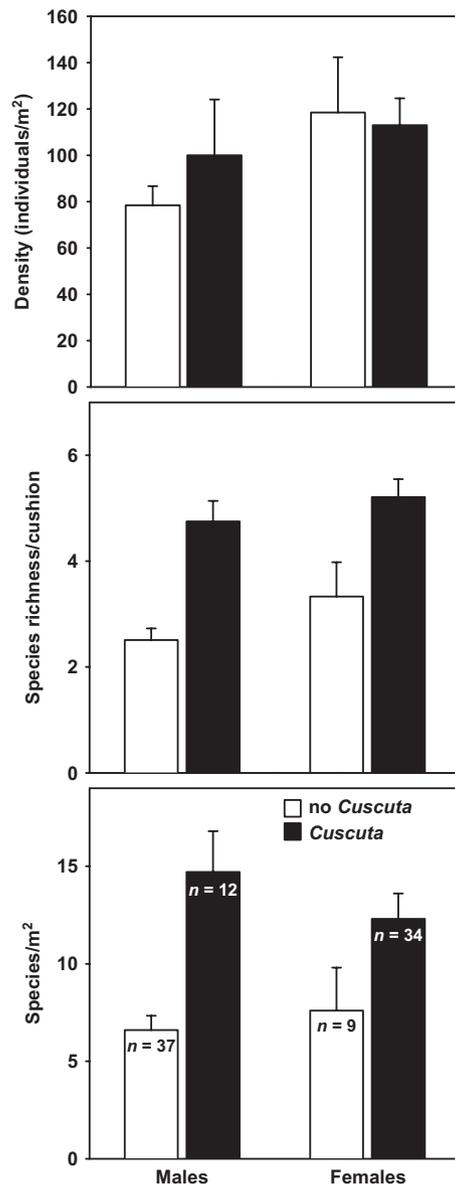


Fig. 5. Density of all species, richness per *Laretia* cushion, and species per square metre in male and female *Laretia* cushions either infected with *Cuscuta* or not infected with *Cuscuta*. Bars represent ± 1 SE; statistics are presented in Table 3.

times parasitized within cushions, e.g. *Haplopappus scrobiculatus*, were present in the open. Thus *C. chilensis*, by selectively parasitizing *Laretia*, increases its net facilitative effects, affecting the overall richness and structure of this alpine plant community.

The promotion of other species by *C. chilensis* may be due to more than competitive suppression of *Laretia*. The growth of plants in arctic and alpine ecosystems is commonly constrained by nutrient availability, particularly nitrogen (N) (Bowman et al. 1993), and parasitic plants often speed the recycling of N because they typically have

Table 3. ANCOVA tables for total density and species richness of cushion using the gender of *Laretia acaulis* and *Cuscuta chilensis* infection status as factors (see Fig. 3).

Source	df	Type III SS	Mean Square	F-value	P > F
Density (not transformed)					
Cushion area	1	19559.0	19559.0	18.44	<0.0001
<i>Cuscuta</i> area	1	2756.0	2756.0	2.60	0.111
Infection status	1	21.3	21.3	0.20	0.888
Gender	1	4392.8	4392.8	4.14	0.045
Infection × gender	1	1506.8	1506.8	1.42	0.237
Error: MS (Error)	86	91206.8	1060.5		
Richness (not transformed)					
Cushion area	1	36.38	36.38	16.57	<0.0001
<i>Cuscuta</i> area	1	3.08	3.08	1.60	0.240
Infection status	1	44.02	44.02	20.05	<0.0001
Gender	1	0.66	0.66	0.30	0.585
Infection × gender	1	0.45	0.45	0.21	0.651
Error: MS (Error)	86	188.79	2.20		

much more N-rich leaves than non-parasitic plants in the same community (Quested et al. 2003; Watson 2009). For example, Quested et al. (2003) found evidence that the hemiparasite *Bartsia alpina* produced nutrient-rich, rapidly decomposing litter in sub-arctic communities, which were estimated to increase the total annual N input from litter to the soil by 53%. Similarly, in a temperate grassland system, Bardgett et al. (2006) found that parasitism of dominant, fast-growing grasses by the parasitic plant *Rhinanthus minor* resulted in changes to the soil microbial community that increased N mineralization and increased the availability of mineral N in the soil solution, the N source preferred by plants in temperate systems (see also Spasojevic & Suding 2011). The presence of the parasitic plant also increased plant community diversity, most likely due to the increase in available soil N along with the suppressive effect of *R. minor* on the dominant grass competitors. Yu et al. (2009) demonstrated that selective parasitism by *Cuscuta campestris* of the invasive *Mikania micrantha* increased soil resource availability and facilitated native plants. In our system, *Cuscuta* may also indirectly increase mineral nutrient availability to species growing with *Laretia*. As an annual, *Cuscuta* is likely to provide a pulse of N-rich litter towards the end of each growing season when it senesces, and this nutrient-rich litter may result in increased rates of nutrient cycling in *Cuscuta* patches within *Laretia*. However, further studies are needed to disentangle the exact mechanisms behind the direct and indirect relationships between *Cuscuta*, *Laretia* and the beneficiary species.

We found that infection of dioecious *Laretia* cushions with *Cuscuta* was female-biased, and there are several direct and indirect mechanisms that might determine this bias. As fruits, seeds and parts of plants of *Cuscuta* spp. are sometimes dispersed by birds and other mammals (Parker

& Riches 1993), it is possible that generalist frugivorous birds that forage on *Cuscuta* fruits and the fruits of female *Laretia* may asymmetrically distribute *Cuscuta* seeds by disproportionately visiting female plants, as observed in other studies (e.g. Tewksbury & Nabhan 2001; Carlo & Aukema 2005; Montesinos et al. 2007). Also, trade-offs often exist among sexes between allocation to growth, defence and reproduction due to limited resources (Herms & Mattson 1992). Male plants of dioecious perennial species are often larger and faster growing than female plants, because female plants commonly allocate more resources to reproduction than males (Jing & Coley 1990; Sarkissian et al. 2001). Even within a monocious species, interspecific competition may cause a shift in the sex ratio towards a larger proportion of male inflorescences due to the higher cost of female reproduction (Holzapfel & Mahall 1999). It is possible that a higher rate of *Cuscuta* infection of female *Laretia* results from the added costs of fruiting, because allocation of resources to reproduction may reduce allocation to defence. Gehring & Whitham (1992) found that female *Juniperus monosperma* growing under conditions of high abiotic stress averaged three times higher levels of infection by the parasitic mistletoe *Phoradendron juniperinum* than male trees, but observed no differences in mistletoe infection between male and female trees growing in less stressful conditions. Similarly, Koskela et al. (2002) found that *Cuscuta europaea* infecting female *Urtica dioica* host plants had higher biomass compared to parasites grown with male hosts.

In theory, due to the higher cost of female reproduction, female plants would occupy more favourable micro-sites while males might be restricted to less favourable micro-sites (Dawson & Ehleringer 1993). For instance, Eppley (2006) found that seedlings of the dioecious grass *Distichlis spicata* were twice as large when grown with male vs female conspecific competitors, and that female-dominated micro-environments are more nutrient-rich than male-dominated micro-environments. Thus, it is also possible that female *Laretia* cushions occupy more favourable micro-sites within our study site. However, *Laretia* sexes were thoroughly intermixed spatially, and recent analyses indicated there is no spatial structure in the distribution of sexes in *Laretia* (MC García and L.A. Cavieres, unpubl. results).

We found that facilitative patterns associated with the dioecious nurse plant *Laretia acaulis* increase substantially when the benefactor is parasitized by the holoparasite *Cuscuta chilensis*. Interestingly, female *Laretia* cushions were more infected with *Cuscuta*, and hence showed the strongest change in their positive effect on the beneficiary plant species assemblage. The role that parasitic plants play in promoting community diversity in the context of the continuum of facilitative to competitive interactions has

not previously been addressed. Our results suggest that parasitic plants can have positive effects on whole-community diversity even when selectively parasitizing an important nurse plant. The results also suggest that indirect interactions can play important roles in determining plant community structure, as highlighted in some recent studies (Pagès & Michalet 2003; Pagès et al. 2003; Xiao & Michalet 2013).

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