Original article

Morphology, nectar characteristics and avian pollinators in five Andean *Puya* species (Bromeliaceae)

C.T. Hornung-Leoni [a], P.L. González-Gómez [b,c,*], A.J. Troncoso [c,d]

[a] *Universidad Autónoma del Estado de Hidalgo, Instituto de Ciencias Básicas e Ingeniería Centro de Investigaciones Biológicas, Herbario HGOM, Mexico*
[b] *Department of Neurobiology, Physiology, and Behavior, University of California, 1 Shields Avenue, Davis, CA 95616, USA*
[c] *Instituto de Filosofía y Ciencias de la Complejidad (IFICC), Los Alerces 3024, Santiago, Chile*
[d] *Departamento de Biología, Universidad de la Serena, Coquimbo, Chile*

A R T I C L E   I N F O

A B S T R A C T

Five Andean *Puya* species (*Puya alpestris*, *Puya chilensis*, *Puya coerulea*, *Puya raimondii* and *Puya venusta*) were studied to determine the relationship between their avian visitors, and plant morphology and nectar characteristics. Our results showed a significant relationship between nectar concentration, presence of sterile apex and avian pollinators's species. In contrast, nectar composition was not related to the frequency of avian visits. We found that *Puya* species were mainly visited by specialist nectarivorous birds such as hummingbirds (i.e., *P. coerulea* and *P. venusta*), lacked a sterile apex and produced high nectar concentration in low volumes. In contrast, species mainly visited by generalist passerines (i.e., *P. chilensis* and *P. alpestris)* were characterized by the presence of a sterile apex and production of highly diluted nectar in large volumes. In a mono-specific group we found that *P. raimondii* produces highly concentrated nectar in large volumes, and its flowers were visited by hummingbirds and passerine birds. We found no effect of nectar composition on bird's visits. Our study highlights the interplay between morphological traits, nectar characteristics and the ecological framework to explain specialized and generalized birds pollination systems.

Sugars, the dominant solutes in nectars, are the primary reward for birds and other pollinators (Percival, 1961; Baker, 1975; Baker and Baker, 1983; Baker et al., 1998). Traditionally the ratio of sucrose, fructose and glucose in nectar (i.e., sugar composition) was used as the key variable to predict animal visitors (Baker and Baker, 1983). For instance, hummingbirds identified as primary pollinators for plant species that produce nectar predominantly composed of sucrose (i.e., sucrose rich; Freeman et al., 1984; Galetto and Bernardello, 2003) whereas passerine birds pollinated plants species that produce nectar with large quantities of glucose and fructose (i.e., hexose-rich; Nicolson, 2002). This pattern was explained as result of the digestive constraints to process different kinds of nectar (Nicolson and Fleming, 2003) considering that in order to process sucrose rich nectars, the presence of sucrase is necessary, an enzyme largely present in hummingbirds (Schondube and Martinez del Rio, 2003). Recently, Johnson and Nicolson (2008) demonstrated that flowers pollinated by specialist nectarivorous passerines are strongly convergent with flowers pollinated by hummingbirds. Rather than the 'hummingbird versus passerine' pollinator dichotomy where hummingbirds pollinate plants with sucrose rich nectars and passerines pollinate plants with hexose rich nectars, it appears that there are differences in nectar traits such as sugar type, concentration and volume between plants pollinated by specialist nectarivores versus plants pollinated by generalists (Johnson and Nicolson, 2008; Brown et al., 2009, 2011). There are also more recent studies pointing out that the lack of sucrase appears to be restricted just to the Fumariidae and Sturnidae-Muscicapoidea lineages (Fleming et al., 2008; Brown et al., 2012). Most generalist and specialist passerine nectarivores have relatively high levels of sucrase (see Lotz and Schondube, 2006; Fleming et al., 2008; Bizaare et al., 2012). Studies of nectar from bromeliad species showed that pollination syndromes (i.e., particular types of pollinators) differed in relationship to nectar sugar composition and concentration (Krömer et al., 2008) and a recent study by Brown et al. (2011) showing the ecotype variation in flower morphology and nectar characteristics within a single species.

Another key factor that may modulate flower visitors is plant morphological characteristics. For example, Johow (1898) suggested that the sterile apex of *Puya* inflorescences, an elongated

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* Corresponding author. Department of Neurobiology, Physiology, and Behavior, University of California, 1 Shields Avenue, Davis, CA 95616, USA.
E-mail addresses: plgonzalezgomez@ucdavis.edu, plgonzalezgomez@gmail.com (P.L. González-Gómez).

erected stem which is an exclusive trait of the *Puya* subgenera, may have adaptively evolved since these sterile apex are used as feeding stations for their perching passerine and hummingbird pollinators (Johow, 1898; Baker and Baker, 1990; Scogin and Freeman, 1984; Foster, 1950). This phenomena has been reported in other species such as *Babiana ringens* (Iridaceae) where the sterile inflorescence axis provides a perch for foraging birds, improving the plant's mating success by causing the malachite sunbird (*Nectarinia famosa*), its main pollinator, to adopt a position ideal for the cross-pollination of its unusual ground-level flowers (Anderson et al., 2005).

The *Puya* genus is an excellent model to examine the role of plant morphology and nectar concentration and composition on the diversity of pollinator visitors (González-Gómez and Valdivia, 2005; Salinas et al., 2007), because it exhibits high interspecific variation in nectar characteristics and morphological traits (Benzing, 2000; Hornung-Leoni and Sosa, 2008). In addition, petal length is correlated with pollinator type (Hornung-Leoni and Sosa, 2005). The genus *Puya,* which includes approximately 219 species (Luther, 2010), was taxonomically divided in two subgenera; *Puya* with 8 species and *Puyopsis* with the remaining species (Smith and Downs, 1974; Hornung-Leoni and Sosa, 2008; but see Jabaily and Sytsma, 2010). In this context, the aims of our study were to: 1) describe the avian visitors of five species of the genus *Puya*, 2) assess the relationship between flower morphological characters and species of avian visitors, and 3) determine if nectar characteristics (volume, concentration, and composition) are related to both flower morphology and avian visitors.

1. Material and methods

1.1. Species and study sites

We analyzed three species traditionally included in the subgenus *Puya*: *P. alpestris*, an endemic and polycarpic species from Chile, *Puya chilensis*, another endemic Chilean species, and *Puya raimondii,* the highest bromeliad in the world, endemic to Peru and Bolivia (Smith and Downs, 1974; Hornung-Leoni and Sosa, 2004). In addition, we studied two species traditionally included in the subgenus *Puyopsis*: *Puya venusta* and *Puya coerulea*, both of which are endemic to Chile.

The study of *P. raimondii* was carried out in two sites in Peru: Huascarán Park, Department of Ancash and in Canchayllo, Department of Canchayllo. Huascarán Park is located in the "Cordillera Blanca" (08°50′—10°40′ S, 77°07′—77°49′ W) with an elevation of approximately 6000 m (Kaser and Georges, 1999) and Canchayllo is located in the "Cordillera Negra" (11°46′ S, 75°42′ W), with elevations of around 3600—4100 m.a.s.l. The populations of *P. raimondii* examined were between 4000 and 4500 m.a.s.l., and data were collected in October 2003.

Puya alpestris was studied in the central Chile in the metropolitan area between Santiago to Farellones (33°17′58″ S, 70°15′06″ W, 1200 m.a.s.l.) and in the Quillota to Limache area (32°56′72.9″ S, 71°20′34.2″ W, 180 m.a.s.l.). For *P. venusta* and *P. chilensis* the observations were carried out on the coast of central Chile, in Zapallar (32°33′07.48″ S, 71°28′09.22″ W), at sea level. Data were collected in October 2007. For information on *P. chilensis* and *P. coerulea* we used previously published data (González-Gómez and Valdivia, 2005). For nectar composition analysis from these species samples were taken in October 2007.

1.2. Floral morphology

To study the morphological characteristics of *Puya*, specimens of each species were examined and described using fresh samples collected in the field and dried specimens from five herbaria US, F, USM, SGO and HDCV. The morphological features were observed and measured from vouchers and the data we collected were supplemented with data from literature. Plant species authors were standardized following the International Plant Name Index (IPNI). The Chilean species we collected were deposited in the HDCV at the Pontificia Universidad Católica de Chile and some duplicates were deposited in SGO (Herbario del Museo Nacional de Historia Natural, Santiago). The Peruvian species, *P. raimondii,* were deposited in USM.

The plant characteristics [plant height (m), petal length (cm), sepal length (cm)] were measured from each voucher and elevation data for each voucher was included. Several vouchers (10—15) were considered for each species. Correlations between floral characteristics and plant size were performed using Pearson's correlations using JMP 10.0.0, SAS Institute Inc. 2012.

1.3. Nectar sampling

To measure nectar volume and concentration, flowers were bagged for 14 h (1800—0800 h) with tulle bags to prevent visitor access and to reduce evaporation. Because the life span of mature flowers is approximately 4 days and flowers open sequentially on inflorescences, we randomly sampled flowers and inflorescences on each day. Total nectar volume produced was assessed nondestructively by repeatedly inserting a 70-μl microcapillary tube into the nectary of each flower until no further nectar could be extracted (Kearns and Inouye, 1993). Flowers were sampled only once. We sampled 10 flowers per plant and 10 plants per species ($N = 100$). To determine variations in volume, we included several flowers in different positions on the inflorescence sampled from base to apex. To estimate total sugar concentration in the nectar a temperature-compensated hand refractometer was used and concentration was expressed as grams of total sugar in 100 g of solution (mass percentage or conventional %w/w). We followed Kearns and Inouye's (1993) methods for determining the "standing crop of nectar". In this manner the quantity and distribution of nectar is determined by randomly sampling patches of flowers, providing a measure of the resources available at a single point in time as mean volume of nectar per flower.

1.4. Nectar analysis

All nectar samples of the *Puya* species reported in the present study were analyzed through High Performance Liquid Chromatography (HPLC), except for *P. raimondii,* whose nectar sugar content data was published in Hornung-Leoni et al. (2007). Nectar was diluted with HPLC-grade water, sonicated for 15 min, and then filtered using Corning® 0.20 μm- RC membrane syringe filters. Filtered samples were sonicated again for 10 min and analysed by HPLC (Shimadzu LC-9A) coupled to a Refractive Index Detector (Shimadzu RID-10A) using a Pinnacle II Amino column (Restek, 150 × 4.6 mm, 5 μm i.d.). The mobile phase used was a mixture (15:85) of HPLC-grade water and acetonitrile flowing at 1 ml/min. The presence and quantification of nectar sugars was determined by comparison with chromatograms and calibration curves of co-injected sugar standards mixtures (Standards used were: Arabinose, Fructose, Galactose, Glucose, Mannose, Rhamnose, Sucrose and Xylose; all standards were obtained from Aldrich Chem Co.). Nectar was considered "hexose-rich" when the sum of composition percentages of glucose and fructose were more than 50%. We considered nectar as "sucrose-rich" when the percentage of sucrose in it was higher than 50%.

1.5. Floral visitors, morphological and nectar characteristics

Field observations were conducted during the flowering period. Observations of avian visitors were performed by one observer

from 08:00 h to 18:00 h in 10 min periods. Every plant was observed for 6 periods, randomized across the day. We assessed the link among floral visitors, morphology (i.e., plant size and sterile apex length) and nectar characteristics (i.e., nectar volume and nectar concentration) using a Canonical Correspondence Analysis (hereafter CCA). To make the variable values comparable, they were standardized by subtracting the average value for the trait and then dividing it by the standard deviation. Within the CCA, stepwise manual forward selection was used to determine the variables that were significant within the model. Variance in the species data was explained by each variable individually (marginal effects, λ_1) and by variables which explained additional variance (conditional effect, Dodkins et al., 2005). The Monte Carlo permutation test ($N = 9999$ permutations) was used to check the statistical validity of these associations. CCA was performed with CANOCO software (Micro-computer Power, Inc., USA).

2. Results

2.1. Morphological description of species

Floral characteristics varied in form and in size (Table 1, Fig. 1). Taller species (i.e., P. raimondii and P. chilensis) exhibited longer sepals and petals (Table 1), while Puya venusta and, P. coerulea which are shorter, had the smallest flowers (Table 1). This pattern was not related to elevation patterns, since P. raimondii grows at elevations higher than 3000 m, and P. chilensis grows at sea level. Petal and sepal length were positively correlated ($r^2 = 0.835$, $P < 0.05$) as was petal length with plant size ($r^2 = 0.739$, $P < 0.05$).

2.2. Nectar

The volumes of nectar in species of subgenus Puya were three times higher than species of subgenus Puyopsis (Nested GLM, $F_{1,235} = 17.83$, $p < 0.01$, Table 2). Volumes of nectar produced by P. chilensis ($N = 66$ flowers) were significantly higher than the volumes produced by P. raimondii ($N = 18$) and P. alpestris ($N = 60$, Unequal N HSD Post-Hoc, $p < 0.05$). Volumes produced by P. raimondii and P. alpestris were not significantly different (Unequal N HSD, $p = 0.99$). Volumes produced by P. venusta and P. coerulea were not significantly different (Unequal N HSD Post-Hoc $p = 0.89$). However, species of subgenus Puyopsis produced 1.45 times more concentrated nectar than Puya (Nested GLM, $F_{1,185} = 283.97$, $p < 0.001$, Table 2). We did not find significant differences in nectar concentration between P. venusta and P. coerulea (subgenus Puyopsis, unequal N HSD Post-Hoc $p = 0.77$). In contrast, in subgenus Puya, P. raimondii produced significant more concentrated

nectar than P. chilensis and P. alpestris ($p < 0.01$). We did not find significantly differences between P. chilensis and P. alpestris ($p = 0.95$). Four of the five Puya species in this study showed hexose-dominant nectars; P. alpestris was the only relatively species which presented sucrose (Table 2). We found other sugars than fructose, glucose and sucrose exclusively in P. raimondii (Hornung-Leoni et al., 2007, Table 2).

2.3. Flower visitors

The five Puya species were all visited by avian pollinators, but varied in the species visiting (Fig. 2). P. raimondii was visited by hummingbirds and nectar robber birds (Table 3). Nectar robbers were defined as species that can obtain nectar through perforations in the corolla tube of flowers or by destroying the entire flower damaging the reproductive structures not carrying a significant amount of pollen (Inouye, 1980; González-Gómez and Valdivia, 2005). In this study, only hummingbirds were nectar specialists. Potential pollinators of P. raimondii in both areas were hummingbirds and the main floral visitor was Giant hummingbirds (Patagona gigas). Its visits were 25 times higher than Sparking violet-ear (Colibri coruscans) (in both locations) and Andean Hillstar (Oreotrochilus estella) (only in Huascarán). Peruvian Sierra-finch (Phrygilus punensis) was observed robbing nectar only in Huascarán and its abundance was 14 times lower than hummingbirds. The visits of passerine birds to Puya alpestris were 32 times higher than hummingbird visits (Table 3). The most frequent specie was Chilean Mockingbird (Minus thenca), which was observed carrying pollen in the head (González-Gómez et al., 2004). Puya chilensis was visited exclusively by passerine birds whose were observed using the apex of branches as perches (Table 3).

The visits of hummingbirds to P. coerulea were 27 times higher than Passerine visits. While P. gigas was a pollinator, Curaeus curaeus was a robber, with a double negative effect; it destroyed flowers and also decreased the inflorescence ability to attract hummingbirds, mainly because robbed flowers stop producing nectar (González-Gómez and Valdivia, 2005, Table 3). Puya venusta was frequently visited by Gray hooded sierra-finch (Phrygilus gagi), however, these birds like Rufous-collared sparrow (Zonotrichia capensis) in P. alpestris and P. punensis in P. raimondii, were observed eating anthers and sepals. On the contrary, hummingbirds were pollinators (Table 3).

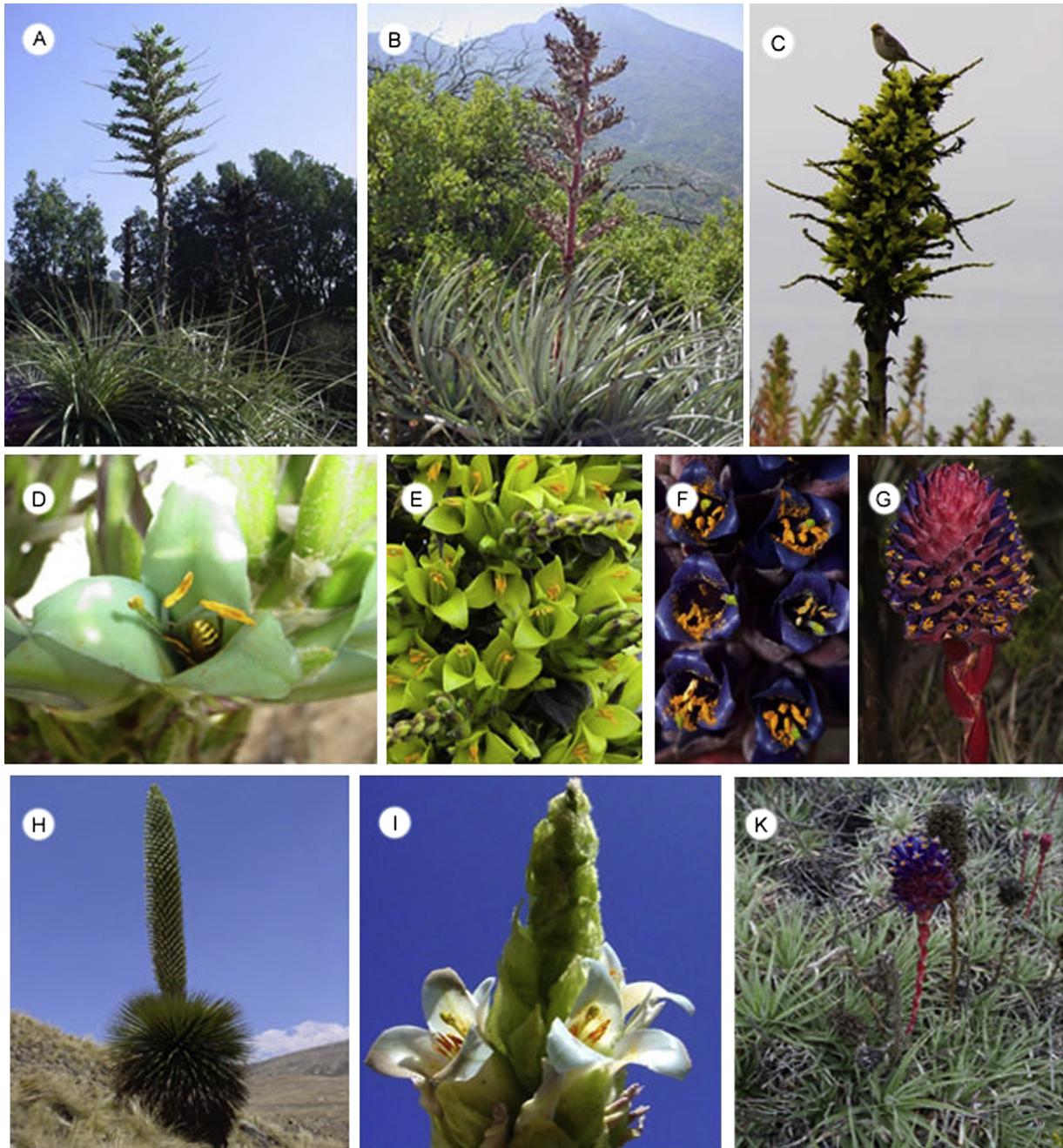### 2.4. Relationship among floral visitors and morphological and nectar characteristics

There is a significant association between Puya species and avian visitors ($\chi^2_{56} = 604.307$, $p < 0.001$). The first two axes in the

Table 1
Morphological characteristics of five Puya species. [a] Taxa included traditionally in Puyopsis subgenus,[b] taxa included in Puya subgenus (sensu Smith and Downs, 1974). Data presented as means ± SEM.

Characteristics	Puya species				
	P. venusta[a]	P. coerulea[a]	P. alpestris[b]	P. raimondii[b]	P. chilensis[b]
High plant (m)	0.98 ± 0.09	1.8 ± 0.29	1.97 ± 0.34	9.22 ± 0.34	4.83 ± 0.31
Flowers exposition[a]	Insert or equaling	Exert	Exert	Exert	Exert
Size sepals (cm)	1.62 ± 0.28	2.11 ± 0.14	2.5 ± 0.23	3.82 ± 0.50	3.45 ± 0.66
Petals forms	Obovate	Obovate	Oblong-elliptic	Oblong-elliptic	Oblong-elliptic
Size petals	3.21 ± 0.33	4.21 ± 0.70	4.66 ± 0.45	6.11 ± 1.05	5.85 ± 0.67
Petal color	Deep violet	Blue-deep violet	Blue greenish	Creamy	Yellow
Anthesis	Diurnal	Diurnal	Diurnal	Diurnal	Diurnal
Visible nectar	Yes	No	Yes	Yes	Yes
Floral disposition	Stand out	Stand out	Stand out	Stand out	Stand out
Perch apex[b]	No	No	Yes	Yes	Yes
Elevation range (m.a.s.l)	20–50	500–1495	290–1100	3000–4150	25–70

[a] Related to the floral bract.
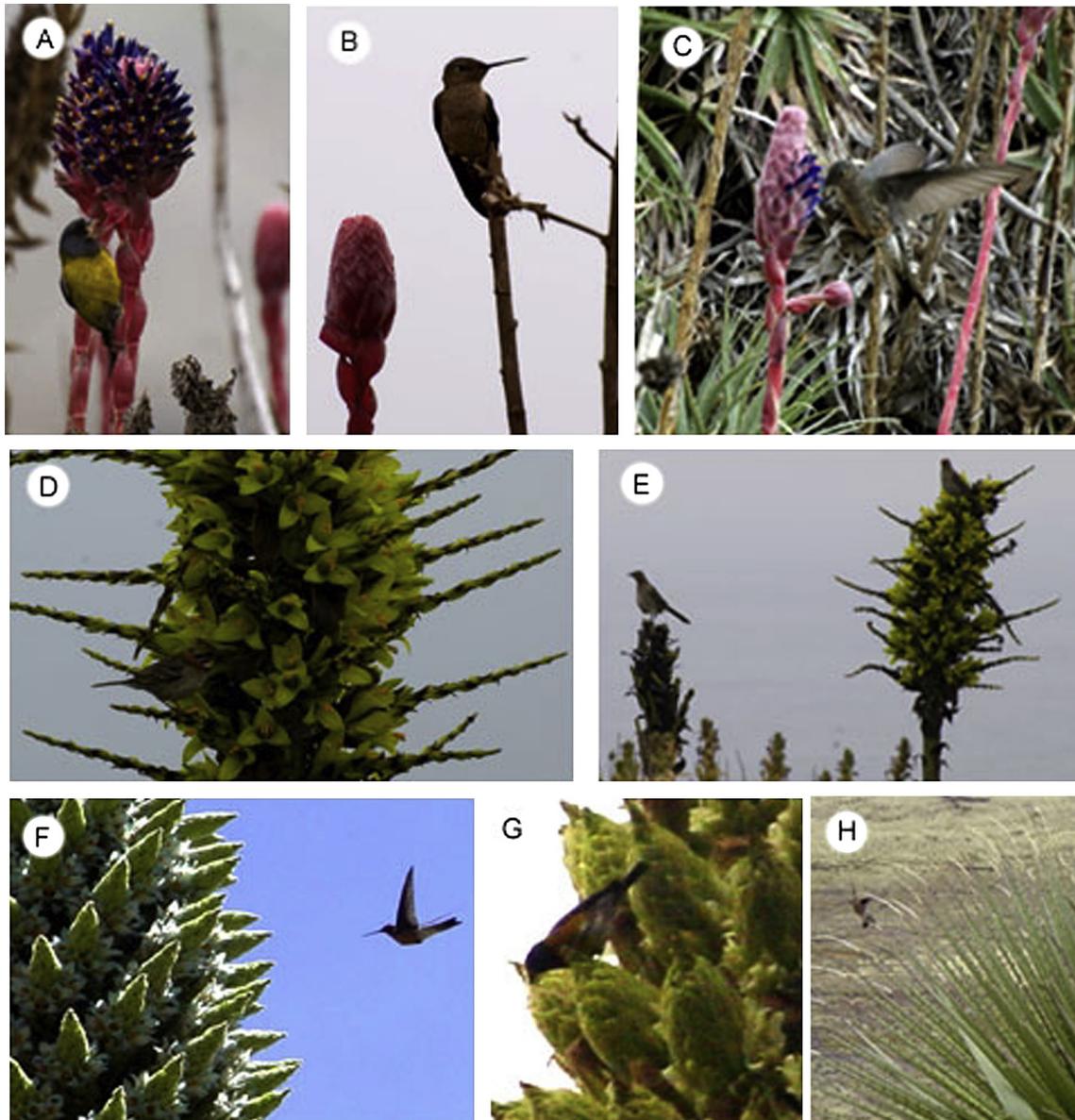[b] Sterile apex of branches.

Fig. 1. Species of *Puya* studied: A) *Puya alpestris* plant B) *P. coerulea* C) *P. chilensis* inflorescence (with passerine birds) D) *P. alpestris* flowers with bees inside; E) *P. chilensis* flowers; F) *P. venusta* flowers, G) *P. venusta* inflorescence. H) the giant *P. raimondii*, I) *P. raimondii* branch with basal flowers, J) *P. venusta* plant, K) *P. venusta* inflorescence. Photos A, B, H, I by C. Hornung-Leoni. Photos C, F, G, K by Pablo Necochea.

Table 2

Nectar characteristics of five *Puya* species.* In *P. raimondii* were found (Hornung-Leoni et al., 2007) and additional 4.68% of xylose and xylose-derived compounds (4.68%), and mannose (0.21%). We did not find other sugars in the remaining *Puya* species.

Puya species	Volume (μl) (min–max)	Volume (μl, mean ± se)	Sugar concentration range (°Bx min–max)	Sugar concentration average (°Bx)	Nectar composition (%)			$S/(G + F)$ ratio
					Fructose	Glucose	Sucrose	
P. venusta	0–178	23.65 ± 2.69	19.2–34	22.93 ± 2.93	47.23	53.27	0	0
P. coerulea	0–315.5	62.78 ± 8.35	16.8–28.8	22.78 ± 0.35	49.40	50.60	0	0
P. alpestris	0–813.8	120.88 ± 21.65	4.7–23.3	12.16 ± 0.51	9.66	56.1	34.27	0.521
P. raimondii								
Huascarán	30.5–80.0	49.5 ± 17.82	12.6–25.0	20.1 ± 0.60	9.58	85.94	0.04*	0.0004
Canchayllo	15.0–30.0	21.7 ± 4.40	21.0–21.4	21.2 ± 0.60				
P. chilensis	0–1200	358.27 ± 293.66	9–15.2	12.56 ± 1.63	70.14	29.86	0	0

Fig. 2. Floral visitors: A) *P. gayi* in *P. venusta*, B–C) *P. gigas* in *P. venusta*; D) *Z. capensis* in *P. chilensis* E) *M. thenca* in the top of *P. chilensis* inflorescence; F) *P. gigas* in *P. raimondii*, G) *P. punensis* in P. raimondii; H) *C. coruscans* in *P. raimondii*. Photos A–E by Pablo Necochea; F–H by C.T. Hornung-Leoni.

CCA ordination were significant ($P < 0.05$) with eigenvalues of 0.925 and 0.888. The third and fourth axes had eigenvalues of 0.796 and 0.689, respectively. CANOCO analyses yielded two variables that were significant to explain the variance in avian floral visitors, nectar concentration (F-ratio = 1.53, $p = 0.026$, $\lambda_1 = 0.90$) and the sterile apex (F-ratio = 1.42, $p = 0.074$, $\lambda_1 = 0.85$, Fig. 3). Those traits explained 66% of variance in avian visitors. In contrast, neither nectar volume (F-ratio = 0.78 $p = 0.5$, $\lambda_1 = 0.56$) nor plant size (F-ratio = 0.72, $p = = 0.57$, $\lambda_1 = 0.58$) were significant to explain variance in avian visitors. *P. alpestris* and *P. chilensis* which were primarily visited by passerine birds (Table 3), exhibited long sterile apexes, and large volumes of diluted nectar (Table 2). In contrast, species mainly visited by hummingbirds showed no sterile apex (as *P. coerulea* and *P. venusta*), and higher nectar concentrations (Tables 2 and 3). The giant *P. raimondii* represents a complex species with the highest nectar concentration in the five *Puya* species examined, has a small sterile apex and is visited primarily by hummingbirds (Tables 2 and 3).

3. Discussion

It has been traditionally argued that hummingbirds and passerine birds select for different nectar properties exerting selective pressures over nectar traits and in turn, bird-pollinated plants co-evolve producing nectar and morphologies that facilitate their pollination for different bird's groups (Baker and Baker, 1982). Our data strongly support the ideas proposed by Johnson and Nicolson (2008) distinguishing between specialized and generalized bird pollination systems instead of the traditional dichotomy hummingbird-passerine pollination. It has been shown that flowers adapted for nectar specialists produce reduced volumes (~ 20 uL) and higher concentration (~ 25% w/w) and sucrose content (~ 40–60% of total sugar) than flowers adapted to generalized bird pollinators, characterized by large volumes (~ 100 uL) of extremely dilute (~ 10%) nectar with minimal sucrose (approx. 0–5%). In our study we found a correlation among plant morphology, nectar concentration and avian visitors in *Puya* species. In this

Table 3

Frequency of passerine and hummingbird floral visitors to inflorescences of five *Puya* species. (P = pollinator, PR = pollen rober, NR = nectar rober, PB = perching bird). Sucrase activity (S), absence of sucrase activity (A) or unknown sucrase activity (U) in the gut are shown under each species (Ramirez-Otarola and Sabat, 2011; Ramirez-Otarola et al., 2011). Data presented as means ± SEM.

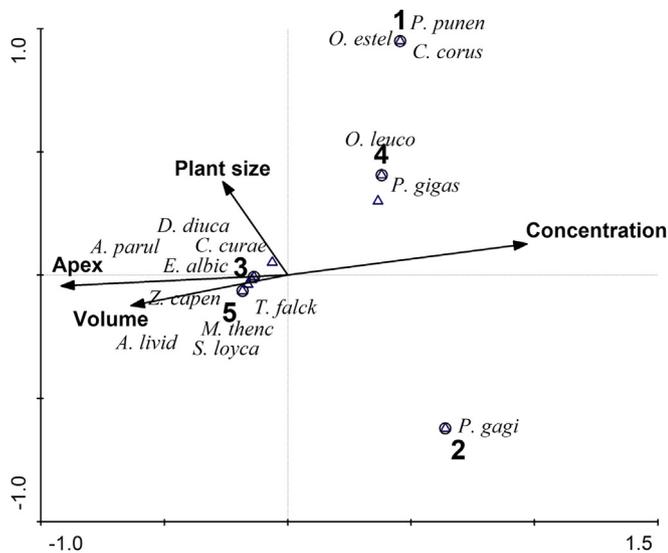| Floral visitors	P. venusta	P. coerulea	P. alpestris	P. raimondii	P. chilensis
Order Apodiformes					
Colibri coruscans				0.25 ± 0.42	
(S)				(P)	
Oreotrochilus estella				0.15 ± 0.34	
(S)				(P)	
Oreotrochilus leucopleurus		0.01 ± 0.00			
(S)		(P)			
Patagona gigas	0.034 ± 0.18	0.26 ± 0.04	0.03 ± 0.01	1 ± 0.00	
(S)	(P)	(P)	(P)	(P)	
Order Passeriformes					
Anairetes parulus			0.01 ± 0.01		
(S)			(PB)		
Agriornis livida					0.08 ± 0.28
(U)					(PB)
Curaeus curaeus		0.01 ± 0.01 (NR)	0.06 ± 0.02		
(S)			(P)		
Diuca diuca			0.12 ± 0.03		
(S)			(PB)		
Elaenia albiceps			0.03 ± 0.01		
(S)			(P)		
Mimus thenca			0.61 ± 0.05		0.15 ± 0.38
(A)			(P)		(P)
Phrygilus punensis				1 ± 0.00	
(S)				(NR)	
Phrygilus gagi	0.15 ± 0.44				
(S)	(PR)				
Sturnella loyca					0.15 ± 0.38
(S)					(PB)
Turdus falcklandii					0.08 ± 0.28
(A)					(PB)
Zonotrichia capensis			0.13 ± 0.02		0.15 ± 0.38
(S)			(PB)		(P)

context it is possible to distinguish three groups of species: a) one group includes *P. venusta* and *P. coerulea* with high nectar concentration, low nectar volumes and no sterile apex, mainly visited by hummingbirds, b) the second group includes *P. alpestris* and *P. chilensis,* with low nectar concentration, large nectar volumes and sterile apex, mainly − but not exclusively-visited by generalist passerine birds; and c) the third group is represented by *P. raimondii* that is characterized by large nectar volume and concentration visited by both hummingbirds (mainly) and passerine birds. In this context, our study strongly suggests that both nectar concentration and presence of a sterile apex robustly explain the abundance and diversity of avian visitors in *Puya* species.

In terms of avian nectar consumption, most birds prefer hexoses at low concentrations and switch to a preference to sucrose or no preference at high concentrations (Jackson et al., 1998b; Schoundube and Martinez del Rio, 2003; Brown et al., 2010a; b; c, Odendaal et al., 2010). When nectar concentrations are high, sucrotic birds like hummingbirds tend to reduce their osmotic stress by feeding on sucrose rather than hexose (Lotz and Schondube, 2006; Brown et al., 2008, 2010b), but this effect is less important at lower nectar concentrations, such as those reported in our study. At lower concentrations, hummingbirds in general, show preference for hexose solutions (Schondube and Martinez del Rio, 2003; but see Fleming et al., 2004). *P. coerulea*, *P. venusta* and *P. raimondii* produce hexose-dominant nectar and were pollinated primarily by hummingbirds, suggesting that in an environment with few available sources of sucrose, hummingbirds use hexose-dominated resources even at concentrations under which they normally would prefer sucrose-dominated nectars. In contrast, *P. alpestris*, the only species that presented a significant amount of sucrose and *P. chilensis* (hexose-dominant) both produced nectar in significantly lower concentrations and were mainly visited by generalist passerine birds. This finding support the idea that generalist prefer lower concentrations when given a choice, suggesting selective pressure by them on plants to produce lower nectar concentration. It could act as a filter to exclude specialists (Johnson et al., 2006), who cannot often maintain energy budgets on low concentration nectar (Brown et al., 2010b). We found a large number of generalist passerine birds visiting *Puya* sp. and especially *P. alpestris*. Consistently, all visitors of this species -saving *Mimus thenca* − appear to have the enzyme sucrase necessary to digest sucrose (Ramirez-Otarola et al., 2011; Ramirez-Otarola and Sabat, 2011). The lack of sucrase activity in members of the superfamily Muscicapoidea, like the Austral thrush (*Turdus falcklandii*) and the Chilean mockingbird (*M. thenca*) in this study have been pointed out as a selective pressure that these birds can exert on the plants whose seeds they disperse and whose flowers they visit (Gatica et al., 2006). Consistently food preference by thrushes is significantly biased toward glucose and fructose, showing scant to nil consumption of sucrose (Gatica et al., 2006). Our findings of *M. thenca* feeding from nectar containing sucrose need further examination, however this result could be coincident with findings showing species of the Sturnidae-Muscicapoidea lineage like Red-winged starlings (*Onychognathus morio*) which lack of sucrase can tolerate solutions containing until 11−15% sucrose content (Brown et al., 2012).

In addition, avian visits could be related with water availability; diluted nectar in open flowers can act as a good source of water in xeric environments such as our study sites (Symes et al., 2007). Large nectar volumes at low concentrations such as in *P. alpestris*,

Fig. 3. Triplot of the canonical correspondence analysis (CCA) ordination using morphological (sterile apex, petals and sepals length, inflorescence size, plant high) and nectar characteristics (volume and concentration). Circles represent Puya species (1. *P. raimondii*, 2. *P. venusta*, 3. *P. chilensis*, 4. *P. coerulea*, 5. *P. alpestris)* positions in the ordination space. Triangles represent the positions of avian visitors: Peruvian sierra-finch (*P. punensis*), Gray hooded sierra-finch (*P. gayi*), Sparkling violet-ear hummingbird (*C. coruscans*), Andean hillstar hummingbird (*O. estella*), White-sided hillstar hummingbird (*O. leucopleurus*), Giant hummingbird (*P. gigas*), Rufous-collared sparrow (*Z. capensis*), Great shrike-tyrant (*A. livida*), Long-tailed meadowlark (*S. loyca*), Austral thrush (*T. falkandii*), Chilean mockingbird (*M. thenca*), Tufted Tit-tyrant (*A. parulus*), Common diuca finch (*D. diuca*), White-crested elaenia (*E. albiceps*), Austral blackbird (*C. curaeus*). The vector lines reflect the relationship of significant environmental variables to the ordination axes, and their length is proportional to their relative significance. The eigenvalues are 0.921 and 0.276 for the first and second axis.

P. chilensis, and *P. raimondii*, could also be beneficial, by limiting nectar evaporation or promoting the movement of water into nectar (Johnson and Nicolson, 2008). On the other hand, *P. coerulea* and *P. venusta* inhabit the same areas and have significantly smaller volumes of nectar along with tubular flowers a trait which may prevent evaporation too (Baker, 1978).

The composition of species visiting the flowers was also related to flower morphology. The presence or absence of the sterile apex of branches played an important role in the avian visitors. The presence of this trait was proposed by as an adaptation that allowed perching birds to pollinate *Puya* species (Johow, 1898; Anderson et al., 2005). Our study supports this argument; species with sterile apex had a significantly higher rate of visits by passerine birds than species with no sterile apexes, suggesting that this structure provides a landing surface for passerine birds that are unable to hover. The evolutionary history of the sterile apex trait is not clear yet and at least two hypotheses have been proposed (see Hornung-Leoni and Sosa, 2008; Jabaily and Sytsma, 2010). This trait could have evolved in a convergent fashion in *Puya* in response to pressure from similar passerine pollinators (Johow, 1898) or evolved once and subsequently lost multiple times in the remainder of *Puya* (Jabaily and Sytsma, 2010). Comparison in the inflorescence development between *P. alpestris* (with sterile inflorescence tips) and *P. coerulea* and *P. venusta* (with nonsterile tips) show that abortion of the terminal flowers took place at early developmental stages in *P. alpestris*. In contrast, the entire axis of the inflorescences of both *P. coerulea* and *P. venusta* were fertile throughout (Jabaily and Sytsma, 2010). This observation supports the idea that this trait evolved once and it has been lost in species that are mainly visited by hummingbirds. However, further studies are necessary to understand and test both ideas. In addition to the sterile apex, other morphological characteristics, as floral morphology was correlated with avian visitors, as traditionally argued (Stiles, 1981). *Puya coerulea* and *P. venusta*, which both have tubular flowers, were visited mainly by hummingbirds whereas *P. chilensis* and *P. alpestris*, both with more opened flowers, were visited by passerine birds. However, *P. raimondii* with long opened flowers was mainly visited by hummingbirds.

In summary, our study strongly suggests that pollination syndromes are complex phenomena that should be studied integrating morphological traits, nectar characteristics and the ecological framework where plants and pollinators co-evolved. An integrative view could help us to understand what kind, and how strong, are the selective pressures acting on *Puya* species and the evolutionary trajectory of traits as sterile apex and nectar composition.

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