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## Pollen–pistil relationships and pollen size-number trade-off in species of the tribe Lycieae (Solanaceae)

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**Abstract** Pollen volume may be involved in different associations with other floral traits. Particularly, the literature indicates that pollen volume can be implicated in a functional relationship with pistil length, and that it may be affected by a trade-off with pollen number because of the subdivision of limited resources. To assess these associations, pollen volume was subjected to correlation analyses with pollen number and pistil length in 20 Argentinean and Chilean taxa of the monophyletic tribe Lycieae. Depending on the mechanisms operating on pollen size and number, the variability of these traits may be different. Therefore, their coefficients of variation were compared. Pistil length and pollen volume showed a strong positive correlation. In contrast, pollen grain size and number were significantly correlated at neither inter- nor intraspecific levels. Results suggest that pollen size and pistil length may co-evolve. The central role of this interaction is discussed. The lack of a correlated variation in pollen size and number may be related to the similar constraints experienced by the species and/or because constraints are operating at the plant level and not at the species level. Lower variance in pollen size compared with pollen number denotes that pollen size may be the main trait subjected to natural selection.

**Key words** Lycieae · Pistil length · Pollen size · Pollen number · Solanaceae · Trade-off

### Introduction

The enormous variety of angiosperm floral forms are often adaptations to insure pollen transfer, the subsequent growth of pollen tubes in the pistil, and ovule fertilization.

For the plant, the pistil and pollen grains are particularly important and sensitive target structures for natural selection as changes in one structure would imply obligate changes in the other in order to maintain the functional integrity of the system (Plitmann and Levin 1983). In other words, a pistil length increase will necessarily result in an increase of those provisions contained in the grains (which are translated into larger pollen size), otherwise pollen tubes would simply fail to reach the ovules. Thus, pistil length and pollen size variations need to be heritable for substantial change to take place (Plitmann and Levin 1983).

A positive correlation between pollen size and pistil length has been detected in many plant groups (e.g., Baker and Baker 1982; Plitmann and Levin 1983; Ramamoorthy et al. 1992; Kirk 1993; Harder 1998; Roulston et al. 2000; Torres 2000; Sarkissian and Harder 2001), but not in others (e.g., Cruden and Miller-Ward 1981; Cruden and Lyon 1985). This correlation was interpreted as a functional relationship between the energy storage capacity of pollen grains and the stigma–ovule distance (Baker and Baker 1982). Because many enzymes operate during pollen tube germination and growth, Roulston et al. (2000) speculate that there may be a functional relationship between the amount of enzymes present and the distance or rate of pollen tube growth, considering that pollen grain protein content accounts for more than 60% of its mass.

On the other hand, because resources available for the male function are limited, any increase in pollen size may lead to a trade-off with pollen number. Such a trade-off appears to be primarily determined on a per-flower basis and would counteract the possible directional selection on pollen size by diminishing mating opportunities (Vonhof and Harder 1995). Thus, the characteristic pollen size of a given species may counterbalance the competitive advantages of large pollen versus the numerical advantages of small pollen, given its specific reproductive environment (Sarkissian and Harder 2001). Inverse relationships between size and number of pollen grains have been documented at interspecific (e.g., Mione and Anderson 1992; Vonhof and Harder 1995) and intraspecific level (Vonhof and Harder 1995; Sarkissian and Harder 2001).

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If resources are a limiting factor influencing pollen size-number trade-off, it is possible that some other mechanisms (e.g., pollination mode, pollen tube growth, etc.) affect pollen traits differentially. For example, if selection optimizes pollen size as a function of pistil length and pollen number as a function of pollinator type and efficiency, then the variability of these pollen traits can be different because of the diverse mechanisms involved. Previous studies have found a higher variance in pollen number per flower compared with pollen volume (e.g., Vonhof and Harder 1995). This pattern rises from the expectation that natural selection optimizes resource investment per reproductive unit and not the number of units (Smith and Fretwell 1974; Brockelman 1975; Lloyd 1987).

Against this background, and assuming that successful growth of pollen tubes to fertilize the ovules is predetermined by provisions contained in the pollen grain and that they are a function of pistil length, we expect to find a positive correlation between pollen size and pistil length. On the other hand, if a pollen-number trade-off is operating at the flower level, we expect to observe a negative correlation between the size and number of pollen grains per flower. Finally, we also evaluate and compare the variability of pollen size and number to test the prediction that the size of a reproductive unit is less variable than the number of pollen grains per flower.

To evaluate these assumptions we chose the tribe Lycieae (subfam. Solanoideae), a monophyletic group (Bernardello and Chiang-Cabrera 1998). Its members are three woody genera with hermaphroditic flowers, typical of arid and semiarid environments (Bernardello 1986a; Bernardello and Hunziker 1987; Hunziker 1997): *Lycium* (cosmopolitan with ca. 75 species), *Grabowskia* (American with four species), and *Phrodus* (monotypic and endemic of northern Chile). It is interesting to point out that although the group has a wide variation in flower size, form, type, and color, among other features (Bernardello 1986b, 1987), its species are chiefly visited by three insect orders: Hymenoptera, Diptera, and Lepidoptera (Galetto et al. 1998). Such homogeneity in the pollinator guild overcomes the problem of possible variation of pollen size because of the influence of different pollinators. Taxa studied here come from their main area of diversification and cover the range of habitats where they grow in Argentina and Chile: Patagonia, Chaco, Puna, Pampas, and Paraná rain forests. We examined some varieties of two polymorphic *Lycium* species (*L. chilense* and *L. tenuispinosum*) because these taxa show an interesting variation in flower traits and habitat preference. Thus, these varieties complement the whole range of flower morphology variation.

## Materials and methods

Data were obtained from flowers collected in the field and preserved in formaldehyde, ethyl alcohol, and acetic acid, with the exception of *Phrodus microphyllus* whose measurements were taken from herbarium specimens that

were previously hydrated. Vouchers are deposited at the Botanical Museum of Córdoba (CORD). The species examined and their data are given in the Appendix, and they are all from Argentina unless specified. Each collection number represents one individual analyzed. Data were taken from four to six individuals per species.

The length of the equatorial and polar axes were measured in 50 pollen grains obtained from one dehiscent anther per individual with an ocular micrometer at 400× in a Zeiss Axiolab light microscope. Prior to obtaining the measurements, grains were stained with basic fuchsin. Pollen grain volume was calculated as  $\pi PE^2/6$  (cf. Harder 1998), where  $P$  is polar axis diameter and  $E$  is equatorial axis diameter. Pollen production was calculated from undehiscent anthers. Homogenized total pollen content of each anther was placed on a hemocytometer with a drop of aniline blue and counted under the same microscope. In *Phrodus microphyllus* and *Lycium chandar*, it was not possible to measure pollen number because there were no mature buds available. Pistil length was considered as an approximation to the average distance that a pollen tube must grow to fertilize an ovule. This distance corresponds to the length between the uppermost extremity of the stigma and the center of the ovary. Mean pistil length for each species was calculated with a digital caliper (resolution = 0.01 mm) from five flowers in mature female phase per individual and the assistance of a Zeiss Stemi SV 6 magnifying glass.

## Statistical analyses

All data were log-transformed prior to analyses in order to meet normality. Data were subjected to correlation (Pearson coefficient) and linear regression analyses. Coefficients of variation (CV) were calculated for pollen size and number. *t*-tests were used for CV comparisons between *Lycium* and *Grabowskia* genera for these variables. A paired *t*-test was used to compare the CV between the variables (i.e., pollen size and pollen number). The statistical program SPSS (1992) was used. Means of original data  $\pm$  SD are given in the text and tables.

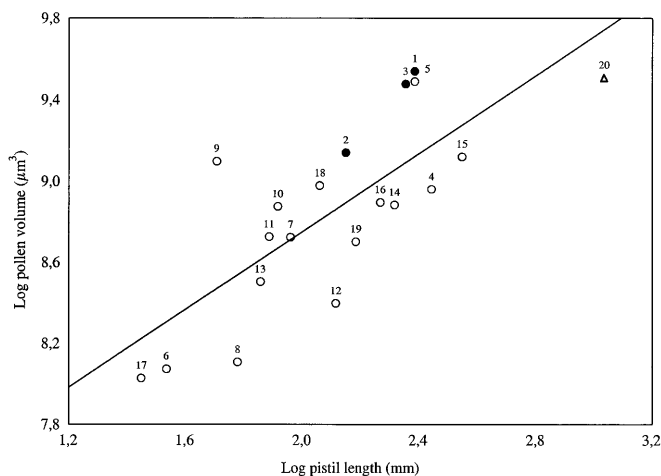
## Results

Pollen size, pollen number, and pistil length varied in a three- to fourfold range (Table 1) in all the studied taxa of tribe Lycieae. The highest values of pollen volume and number of pollen grains per flower were observed in two *Grabowskia* species, whereas the lowest values were detected in species of *Lycium* (*L. chilense* var. *filifolium* and *L. tenuispinosum* var. *friesii* for pollen number; *L. morongii* and *L. chandar* for pollen volume; Table 1). *Phrodus microphyllus* showed the longest pistils whereas *L. morongii* had the shortest.

A strong interspecific linear positive correlation was found between mean pollen grain volume and mean pistil length ( $r = 0.76$ ,  $P = 0.0001$ , Fig. 1). Pistil length ( $l$ ) explained

**Table 1.** Reproductive characters of 20 taxa from the three genera of the tribe Lycieae. *n/d* No data

Reference no.	Species	Pistil length (mm)	Pollen volume ( $\mu\text{m}^3$ )	Pollen number ( $\times 10^3$ )
1	<i>Grabowskia boerhaviaefolia</i>	10.9 $\pm$ 0.3	13,912 $\pm$ 2,053	83 $\pm$ 10
2	<i>G. duplicata</i>	8.6 $\pm$ 1.2	9,336 $\pm$ 729	64 $\pm$ 9
3	<i>G. obtusa</i>	10.5 $\pm$ 1.0	13,807 $\pm$ 1,050	84 $\pm$ 10
4	<i>Lycium americanum</i>	11.5 $\pm$ 0.8	7,795 $\pm$ 979	67 $\pm$ 15
5	<i>L. cestroides</i>	10.8 $\pm$ 0.2	13,233 $\pm$ 1,058	51 $\pm$ 3
6	<i>L. chanar</i>	4.6 $\pm$ 3.5	3,215 $\pm$ 117	n/d
7	<i>L. chilense</i> var. <i>chilense</i>	7.1 $\pm$ 0.9	6,156 $\pm$ 896	52 $\pm$ 6
8	<i>L. chilense</i> var. <i>confertifolium</i>	5.9 $\pm$ 0.3	3,323 $\pm$ 99	69 $\pm$ 6
9	<i>L. chilense</i> var. <i>descolei</i>	5.5 $\pm$ 0.2	8,951 $\pm$ 209	35 $\pm$ 2
10	<i>L. chilense</i> var. <i>filifolium</i>	6.8 $\pm$ 0.4	7,165 $\pm$ 254	30 $\pm$ 4
11	<i>L. chilense</i> var. <i>minutifolium</i>	6.6 $\pm$ 0.4	6,172 $\pm$ 120	50 $\pm$ 2
12	<i>L. ciliatum</i>	8.3 $\pm$ 0.1	4,440 $\pm$ 675	67 $\pm$ 7
13	<i>L. cuneatum</i>	6.4 $\pm$ 0.2	4,939 $\pm$ 478	38 $\pm$ 10
14	<i>L. elongatum</i>	10.1 $\pm$ 0.3	7,215 $\pm$ 305	56 $\pm$ 12
15	<i>L. gilliesianum</i>	12.8 $\pm$ 0.8	9,150 $\pm$ 1,778	50 $\pm$ 8
16	<i>L. infaustum</i>	9.6 $\pm$ 0.5	7,313 $\pm$ 186	77 $\pm$ 11
17	<i>L. morongii</i>	4.3 $\pm$ 0.4	3,073 $\pm$ 170	43 $\pm$ 6
18	<i>L. tenuispinosum</i> var. <i>friesii</i>	7.8 $\pm$ 0.3	7,940 $\pm$ 234	32 $\pm$ 3
19	<i>L. tenuispinosum</i> var. <i>tenuispinosum</i>	8.9 $\pm$ 0.3	6,022 $\pm$ 1,075	50 $\pm$ 9
20	<i>Phrodus microphyllus</i>	20.8 $\pm$ 1.0	13,487 $\pm$ 1,539	n/d

**Fig. 1.** Correlation analysis between pistil length and pollen grain volume of 20 taxa of the tribe Lycieae: empty circle *Lycium* spp., filled circle *Grabowskia* spp., empty triangle *Phrodus microphyllus*. The numbers in the figure correspond with the species listed in Table 1

significant proportions of pollen volume variation ( $v$ ):  $\log v = 2.968 + 0.764 \log l$ ;  $r^2 = 0.562$ ,  $P < 0.0005$ .

The mean number of pollen grains per flower and mean pollen volume were not significantly correlated ( $r = -0.07$ ,  $P = 0.78$ ). Number and size of pollen grains correlation remained non-significant when analyzing *Lycium* and *Grabowskia* separately ( $r = -0.18$ ,  $P = 0.52$ ;  $r = 0.97$ ,  $P = 0.16$ , respectively). We also checked for intraspecific pollen size-number correlations. Although intraspecific sample sizes preclude from making conclusive statements, there are trends that may be interesting to point out. Mean pollen volume did not seem to correlate with pollen grain number per flower for most taxa (Table 2). *Grabowskia duplicata*

was the only species that showed a significant inverse relationship between pollen number and size (Table 2).

A considerable pollen number and pollen volume variability was found within species (Table 2). In general, pollen grain volume within species showed less variability than pollen number, except for five species where pollen volume variations were larger than those of pollen number (Table 2). *Lycium cuneatum* and *L. gilliesianum* had the largest coefficients of variation (CV) for pollen number and pollen volume respectively, whereas *L. chilense* var. *minutifolium* showed the smallest for both variables (Table 2). Average CV of pollen volume was lower than pollen number per flower ( $8.5 \pm 5.8$  and  $13.3 \pm 5.9$  respectively), a statistically significant difference (paired  $t$ -test:  $t = 2.57$ ,  $P = 0.05$ ). The average CVs for pollen number were comparable between *Grabowskia* and *Lycium*, but average CV of pollen volume was lower in *Grabowskia*. Nevertheless, CV comparisons of these variables between genera did not show significant differences (Table 3).

## Discussion

The literature supports the positive correlation between pollen size and pistil length for a large sample of unrelated plant taxa (e.g., Baker and Baker 1982; Plitmann and Levin 1983; Williams and Rouse 1990; Ramamoorthy et al. 1992; Kirk 1993; Harder 1998; Bigazzi and Selvi 2000; Roulston et al. 2000; Torres 2000; this work). The interpretation of this correlation is based on a functional relationship between the energy storage capacity of pollen grains, which is directly related to their size, and the stigma-ovule distance (Baker and Baker 1982). Contrary to this interpretation, Cruden and Lyon (1985) argued that pollen tubes obtain

**Table 2.** Coefficients of variation (CV) for pollen number and pollen volume for 18 *Lycieae* taxa. Values are ranked from the smallest to the largest. *n* Number of individuals studied, *r* correlation coefficient

Species ( <i>n</i> )	Pollen number		Pollen volume		Intraspecific size-number trade-off
	CV (%)	Rank	CV (%)	Rank	
<i>Grabowskia boerhaviaefolia</i> (5)	12	8	14.8	15	$r = -0.23$ ; $P = 0.71$
<i>G. duplicata</i> (5)	14	12	7.8	9	$r = -0.87$ ; $P = 0.05$
<i>G. obtusa</i> (5)	12	9	8	11	$r = -0.45$ ; $P = 0.44$
<i>Lycium americanum</i> (6)	22.4	17	12.6	13	$r = -0.04$ ; $P = 0.95$
<i>L. cestroides</i> (6)	5.9	3	8	10	$r = -0.28$ ; $P = 0.64$
<i>L. chilense</i> var. <i>chilense</i> (6)	11.5	7	14.5	14	$r = -0.01$ ; $P = 0.98$
<i>L. chilense</i> var. <i>confertifolium</i> (5)	8.7	4	3	4	$r = -0.58$ ; $P = 0.30$
<i>L. chilense</i> var. <i>descolei</i> (5)	5.7	2	2.3	2	$r = -0.89$ ; $P = 0.10$
<i>L. chilense</i> var. <i>filifolium</i> (5)	13.3	10	3.5	6	$r = 0.19$ ; $P = 0.76$
<i>L. chilense</i> var. <i>minutifolium</i> (5)	4	1	2	1	$r = 0.22$ ; $P = 0.72$
<i>L. ciliatum</i> (4)	10.4	6	15.2	16	$r = 0.5$ ; $P = 0.39$
<i>L. cuneatum</i> (4)	26.3	18	9.7	12	$r = 0.23$ ; $P = 0.71$
<i>L. elongatum</i> (5)	21.4	16	4.2	7	$r = 0.39$ ; $P = 0.20$
<i>L. gilliesanum</i> (6)	16	14	19.4	18	$r = -0.48$ ; $P = 0.41$
<i>L. infaustum</i> (6)	14.3	13	2.5	3	$r = 0.04$ ; $P = 0.95$
<i>L. morongii</i> (6)	14	11	5.5	8	$r = 0.36$ ; $P = 0.55$
<i>L. tenuispinosum</i> var. <i>friesii</i> (5)	9.4	5	3	5	$r = -0.17$ ; $P = 0.78$
<i>L. tenuispinosum</i> var. <i>tenuispinosum</i> (6)	18	15	18	17	$r = -0.3$ ; $P = 0.62$

**Table 3.** Coefficients of variation (CV) for pollen number and pollen volume for *Grabowskia* and *Lycium* taxa

Variables	<i>Grabowskia</i> ( <i>n</i> = 3)	<i>Lycium</i> ( <i>n</i> = 15)	Statistical analysis
CV (%) of pollen grain number per flower	13.6 ± 6.8	13.0 ± 1.8	$t = 0.28$ , $P = 0.78$
CV (%) of pollen volume	8.3 ± 6.3	16.0 ± 8.7	$t = -1.46$ , $P = 0.26$

resources from the style, thus pollen size is functionally related to stigma depth (which reflects the distance a pollen tube has to grow to reach the transmission tissue in the style) and not to the style length. The positive relationship we found between pollen size and style length, as previous findings support, does not contradict the fact that pollen tubes uptake nutrients and reserves from the female tissue as they grow along the style (e.g., Herrero and Hormaza 1996; Cheung 1995, 2000). Pollen volume itself does not explain how far a pollen tube can develop, but it can reflect the storage capacity of particular nutrients that do affect pollen tube growth (Roulston et al. 2000). In a model of pollen competition, Mulcahy (1983) showed that increased style length intensifies male to male competition and selects for faster pollen tube growth rate. Moreover, there is evidence suggesting that larger pollen grains may give rise to faster growing pollen tubes (Ottaviano et al. 1983; Lord and Eckard 1984). Recently, studies of selection on pollen size in *Brassica rapa* have elicited correlated responses in several flower traits, suggesting that pollen size cannot evolve independently (Sarkissian and Harder 2001). Positive correlations between pollen size and style length, in particular, may be the result of a widespread gametic-phase disequilibrium that arises from nonrandom fertilization success of large pollen in pistils with long styles (Sarkissian and Harder 2001).

In contrast, we did not find a correlation between pollen size and pollen number per flower among *Lycieae* taxa. Sim-

ilar results were found in several interspecific studies (e.g., Cruden and Miller-Ward 1981; Plitmann and Levin 1983; Knudsen and Olesen 1993; but see Mione and Anderson 1992; Vonnhof and Harder 1995). Considering that *Lycieae* taxa belong to a small monophyletic group (Bernardello and Chiang-Cabrera 1998) and that they are visited by similar pollinators (Galletto et al. 1998), the absence of a pollen size-number trade-off among the species may be a result of the similar constraints experienced by the species and/or because constraints are operating at the plant level and not at the species level.

The lack of intraspecific pollen size-number correlation in *Lycieae* taxa may be taken cautiously because of the limited sample size used. Although the few studies currently available preclude to asseverate any generalizations, there are previous intraspecific studies – using larger sample sizes than our study – that verified a negative correlation between pollen size and number (e.g., Vonnhof and Harder 1995; Sarkissian and Harder 2001; but see Stanton and Preston 1986). Such intraspecific pollen size-number trade-off has been explained as a consequence of the subdivision of limited resources in the plant (Vonnhof and Harder 1995). Moreover, a negative correlated response of pollen number, found after artificial intraspecific selection on pollen size, may reflect the genetically determined and physically constrained pattern of resource allocation in the plant (Sarkissian and Harder 2001). Conversely, negative genetic variations between the size and number of competing enti-

ties, such as pollen grains (e.g., Stanton and Young 1994), may be compensated by the effects of genes that control the acquisition of resources (Houle 1991), which can eliminate or reverse genetic correlations between competing entities (e.g., Young et al. 1994; Fenster and Carr 1997).

Pollen grain size within most *Lycieae* taxa varied considerably less than the number of pollen grains produced per flower. A similar pattern was observed by Vohnhof and Harder (1995) when studying species of *Fabaceae*. This trend would indicate that there is a stronger resilience to variations of reproductive resources of pollen size compared with pollen number (Vohnhof and Harder 1995, and citations therein). This corroborates the fact that pollen size is phenotypically less variable than most other floral traits (Creswell 1998), and that pollen size may be the primary target of natural selection (Sarkissian and Harder 2001).

In summary, our data in a small monophyletic group with a restricted guild of pollinators support the positive correlation between pistil length and pollen size, but not the trade-off predicted for pollen number and size. Although floral traits involved in plant reproduction may interact and eventually co-evolve, there are selective pressures determining compromises and constraining character evolution differentially by limiting the viable options, such as pollen–pollen and/or pollen–pistil trait combinations.

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## Appendix

### Voucher specimens

*Grabowskia boerhaviaefolia* Schltdl. *Hunziker* 4003, 25523, Catamarca; *Hunziker* 25617, Salta; *Hunziker* 13711, Tucumán; *Galetto* 113, Salta. *G. duplicata* Arn. *Hunziker* 9284, 9288, San Luis; *Hunziker* 12875, 12982, San Juan; *Hunziker* 12475, Entre Ríos. *G. obtusa* Arn. *Hunziker* 9998, Catamarca; *Hunziker* 12977, San Juan; *Hunziker* 13078, San Luis; *Bernardello* 161, Córdoba; *Bernardello* 792, Santa Fe. *Lycium americanum* Jacq. *Bernardello* 179, Córdoba; *Bernardello* 263, 264, 265, Córdoba; *Hunziker* 23147, 23149, Santiago del Estero. *L. cestroides* Schltdl. *Bernardello* 150, 268, 824, 878, 882, 883, Córdoba. *L. chanar* Phil. *Galetto* 268, 271, 272, Mendoza; *Hunziker* 23273, San Juan. *L. chilense* Miers ex Bertero var. *chilense* *Bernardello* 100, 102, 125, 757, Córdoba; *Bernardello* 845, 865, Chile. *L. chilense* var. *confertifolium* (Miers) Barkley *Bernardello* 139, 780, La Pampa; *Bernardello* 837, San Juan; *Bernardello* 860, Chile; *Galetto* 270, Mendoza. *L. chilense* var. *descolei* Barkley *Bernardello* 231, Santa Cruz; *Bernardello* 194, 785, 786, 787, Chubut. *L. chilense* var. *filifolium* (Miers) *Bernardello* *Bernardello* 111, 126, La Pampa; *Bernardello* 253, 756, 758,

Córdoba. *L. chilense* var. *minutifolium* (Miers) Barkley *Bernardello* 781, La Pampa; *Bernardello* 850, 851, 854, 862, Chile. *L. ciliatum* Schltdl. *Bernardello* 876, *Galetto* 98, 101, 596, Córdoba. *L. cuneatum* Dammer *Galetto* 182, 185, Salta; *Bernardello* 793, 812, Santa Fe. *L. elongatum* Miers *Bernardello* 879, 880, 881, Córdoba; *Galetto* 111, Córdoba; *Hunziker* 23385, San Juan. *L. gilliesianum* Miers *Bernardello* 112, 115, 119, 120, San Luis; *Bernardello* 256, 258, La Pampa. *L. infaustum* Miers *Bernardello* 104, 105, 106, La Pampa; *Bernardello* 181, 192, Córdoba; *Galetto* 235, Córdoba. *L. morongii* Britton *Bernardello* 186, 188, Córdoba; *Bernardello* 486, 487, 489, 493, Formosa. *L. tenuispinosum* var. *friesii* (Dammer) C. L. Hitchc. *Bernardello* 98, 109, 114, Córdoba; *Galetto* 262, 263, San Juan. *L. tenuispinosum* Miers var. *tenuispinosum* *Bernardello* 759, Córdoba; *Galetto* 250, La Pampa; *Galetto* 255, 256, 257, 261, San Juan. *Phrodus microphyllus* (Miers) Miers *Krapovickas* 5761, Chile; *Bernardello* 847, 852, 853, Chile; *Di Fulvio* 713, 720, Chile.

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