

Style morphological diversity of some Asteraceae species from Argentina: systematic and functional implications

Carolina Torres · Leonardo Galetto

Received: 8 March 2006 / Accepted: 29 August 2006 / Published online: 28 March 2007
© The Botanical Society of Japan and Springer 2007

Abstract Styler micromorphological diversity of 42 Asteraceae species from Argentina was analysed considering species phylogenetic membership and some floral reproductive functions (pollen presentation and pollen reception). In particular, the morphology and organisation of pollen presenter (sweeping hairs) and pollen receptive structures (stigmatic papillae) were described. Results showed that style morphology of the studied species is far more diverse than the categories previously established for Asteraceae, and that it is problematic to relate the sweeping-hair arrangement of species to the only three modes of pollen presentation described for the family, indicating that the hypothesised relationship could be more complex than was formerly thought. For all species with di- or trimorphic florets, the styles of female florets were more slender and without or with more reduced sweeping hairs than the styles of hermaphrodite florets, and divergences of sweeping hair arrangements and morphology were higher among phylogenetically related species. These results suggest that functional aspects of floral morphology seem to be more important than phylogenetic constraints as selective forces determining styler pollen presentation structures. In contrast, stigmatic-area organisation as well

as the morphology of stigmatic papillae remain identical between female and hermaphrodite florets and among phylogenetically related species. Thus, stigmatic papilla morphology seems to be a phylogenetically constrained character in the studied species.

Keywords Asteraceae · SEM micrographs · Stigmatic-area organisation · Stigmatic papillae · Sweeping hairs

Introduction

In general, the style in Asteraceae is long and at the apex gives off two style branches. This apical portion may be externally smooth and glabrous or clothed with sterile hairs (hereafter called sweeping hairs) which often participate in the pollen presentation process (Small 1915; Ladd 1994). Secondary pollen presentation is the developmental relocation of pollen from the anthers onto another floral organ which then functions as the pollen-presenting organ for pollination (Howell et al. 1993). In this family, the pollen presenter is the sterile tissue (i.e. sweeping hairs) covering the stigmas or, for basal lineages that lack sweeping hairs, it must simply be the style branches (Small 1915; Ladd 1994). Pollen is presented on the terminal section of the modified style where it is actively loaded as it elongates through a connate ring of anthers (Erbar and Leins 1995).

There are three main types of secondary pollen presentation that have been described for this family: pump, brushing, and a combination of these two mechanisms (Yeo 1993; Erbar and Leins 1995). The different pollen presentation mechanisms have been correlated with the arrangement of the sweeping hairs (Leins and Erbar 1990; Yeo 1993; Erbar and Leins 1995, 2000). For species with hairs only at the very tip of the style, the pump mechanism

Electronic supplementary material The online version of this article (doi:10.1007/s10265-007-0081-4) contains supplementary material, which is available to authorized users.

C. Torres (✉) · L. Galetto
Instituto Multidisciplinario de Biología Vegetal (Universidad Nacional de Córdoba-Consejo Nacional de Investigaciones Científicas y Técnicas), Casilla de Correo 495, 5000 Córdoba, Argentina
e-mail: ctores@imbiv.unc.edu.ar

L. Galetto
e-mail: leo@imbiv.unc.edu.ar

was inferred; for those with hairs reaching below the branching of the style, the brushing mechanism was inferred, and for those species with hairs covering only part of the backs of the style-branches an “intermediate” mechanism (part of pollen is pushed out, the other part brushed out) was assigned.

The stigmatic-area organisation (i.e. the papillae arrangement on stigmatic surface) has been separated in two types: it can cover the inside of the style branches or can be separated into two ventro-marginal bands along each style branch (Bremer 1994). The second type is confined to the largest subfamily Asteroideae, but this character is subjected to several reversals (entire surface in parts of Senecioneae and Heliantheae).

Several major clades of Asteraceae are now well established, mainly as a result of cladistic analyses of an increasing body of morphological and molecular data (Bremer 1994, 1996; Baldwin et al. 2002; Panero and Funk 2002; Funk et al. 2005, and citations therein). Among other vegetative and reproductive traits, style and pollen morphology have been important morphological characters in major clade delimitation of the Asteraceae (Bremer 1996).

A comparative analysis of stylar micromorphological diversity is provided in this study of 42 Asteraceae species from Argentina. By considering species phylogenetic membership and some floral reproductive functions (pollen presentation and pollen reception), stylar morphology was analysed. In particular, the morphology and organisation of pollen presenter and pollen receptive structures (i.e. sweeping hairs and stigmatic papillae, respectively) are described.

Materials and methods

Pistil characters were measured on florets (flowers of a capitulum) of 42 species of Asteraceae. Samples were collected from living plants in natural populations from Argentina, in Neuquén, Río Negro, and Córdoba Provinces. Voucher specimens have been deposited in the herbarium of the National University of Córdoba (CORD).

For the study of style morphology, pistils of flowers were preserved in 70% ethanol, dehydrated, critical-point dried, mounted on metal stubs, sputter-coated with gold and observed in a JEOL JSM-5800 LV scanning electron microscope (SEM) at 15 kV. The lengths of five stigmatic papillae and five sweeping hairs from each of three to four florets from different capitula were measured for each species from SEM micrographs. In species with different morphological floret types and in dioecious species, pistil measurements were only performed on hermaphrodite and female florets, respectively.

Species were classified into four categories according to the arrangement of the sweeping hairs on styles, following Bremer’s (1987) descriptions (see sweeping hair arrangement categories in S1 of Electronic Supplementary Material).

A secondary pollen presentation mechanism was assigned to each species considering the relationship proposed by Erbar and Leins’s (1995) between sweeping hair arrangement on styles and the secondary pollen presentation mechanism of flowers. According to these authors, a “pump mechanism” can be assigned to species with hairs only at the very tip of the style, a “brushing mechanism” to those with hairs reaching below the branching of the style and an “intermediate mechanism” when the sweeping hairs cover only part of the backs of the style-branches. Because many of the studied species have shown sweeping hair arrangements very different to these three proposed types (e.g. species with no sweeping hairs or species with a great variation of sweeping hair arrangements that cover only part of the style branches), we decided to leave as “indeterminate” the pollen presentation mechanism of these species.

Means of original data and standard deviations are given in both the text and S1. The phylogenetic diagram proposed by Funk et al. (2005) was used to arrange species in an evolutionary sense. For comparisons, we have considered the species pertaining to Carduoideae and Cichorioideae subfamilies, Barnadesieae tribe and Mutisia clade as early branching taxa, and the species pertaining to the Asteroideae subfamily as late branching taxa (Fig. 5 of Funk et al. 2005).

Results

The four style types in terms of the arrangement of sweeping hairs described for the family (Bremer 1987) can be recognised in the sample of studied species (S1). Papillose and shortly bilobed styles without or with scanty sweeping hairs (type 1) were found in Barnadesieae and most but not all species of Mutisieae clade (Figs. 1a, 3b). Styles with a ring of sweeping hairs on the shaft below the style bifurcation (type 2) were found in the species of the tribe Cardueae (Fig. 1b). Slender styles, with sweeping hairs covering the whole of the back of the style branches and sometimes also part of the stylar shaft (type 3), were found in all Lactuceae and Vernonieae species (Fig. 1c, d) and in *Chaptalia nutans* (Mutisia clade, Fig. 4a). In all species of the Asteroideae subfamily and in *Trixis divaricata* (Mutisia clade), sweeping hairs were concentrated on the upper parts of the style branches or on their tips (type 4) forming more or less developed appendages on the style

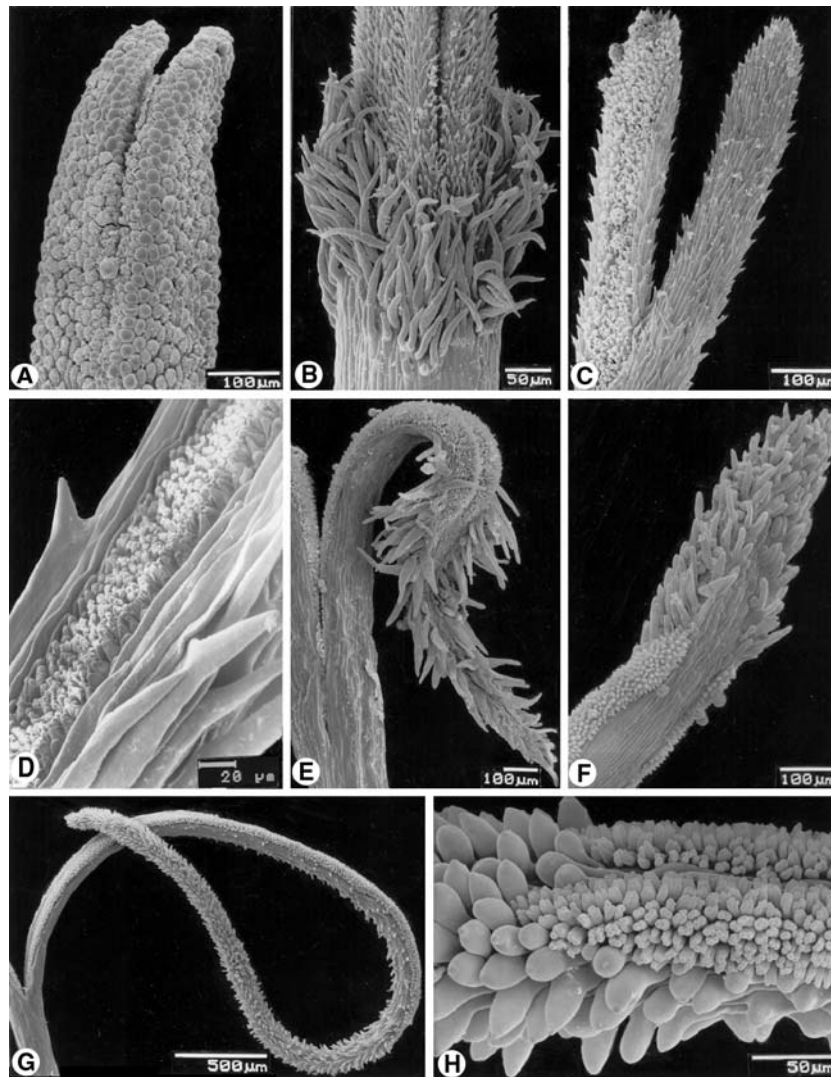


Fig. 1 Style morphological diversity according to the arrangement of sweeping hairs and stigmatic-area organisation in different genera of Asteraceae. SEM micrographs. **a** *Chuquiraga erinacea* (Barnadesieae), style branches with stigmatic surfaces hidden between branches; **b** *Centaurea solstitialis* (Cardueae), bases of the style branches subtended with a ring of sweeping hairs; **c** *Taraxacum officinale* (Lactuceae), style branches with one facing up showing the stigmatic papillae covering the whole inner surface and the other facing down showing the outer surface covered with sweeping hairs; **d** *Vernonia nudiflora* (Vernonieae), detail of a style branch showing the sweeping hairs on the back and the stigmatic papillae covering the

inside of the style branch; **e** *Cosmos sulphureus* (Coreopsidae, hermaphrodite floret), style branch showing stigmatic papillae and sweeping hairs forming a sterile appendage above the stigmatic area; **f** *Grindelia discoidea* (Astereae), style branch end showing stigmatic papillae separated into two ventro-marginal bands and a sterile appendage on the tip; **g** *Stevia satereiifolia* (Eupatorieae), style branch showing sweeping hairs and a long sterile appendage; **h** *Eupatorium clematideum* (Eupatorieae), detail of the style branch showing the stigmatic surface and the beginning of the sterile appendage covered by sweeping hairs

(i.e. sterile structures that are extended above the stigmatic areas; Figs. 1e–g, 2a–e, 3d, f, h, j).

The morphology of stylar appendages was very variable among the species, being triangular-subulate in some Coreopsidae and Astereae species (Figs. 1e, f, 3d) and extremely long in the tribe Eupatorieae (Fig. 1g). In other species with type-4 styles, sweeping hairs formed a truncate brush at the end of the style branches (Figs. 2e, 3f, h, j).

Nearly half of the studied species showed sweeping hair arrangements that could indicate, according to Erbar and Leins (1995), a pump pollen presentation mechanism (Figs. 2e, 3f, h, j, S1) or a brushing mechanism (Fig. 1c, g, S1). For the other half, it was difficult to assign a potential pollen presentation mechanism because: (1) we found a great diversity of sweeping hair arrangements (Figs. 1b, e, f; 2a–c, 3d) that exceed the three types considered in the correlation established by Erbar and Leins (1995) and (2)

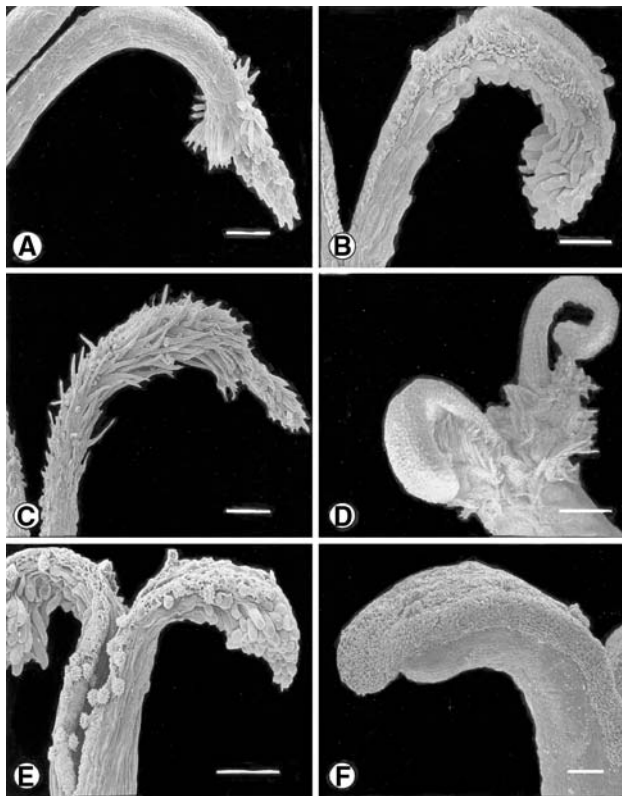


Fig. 2 Style morphological diversity according to the arrangement of sweeping hairs in different genera of Asteraceae. **a** *Gaillardia megapota mica* (Helenieae), style branch apex of hermaphrodite floret; **b** *Tagetes minuta* (Tageteae), style branch apex of hermaphrodite floret; **c** *Wedelia bupthalmiflora* (Heliantheae), style branch apex of hermaphrodite floret; **d** *Zinnia peruviana* (Heliantheae), hermaphrodite floret showing the hairy corolla lobes and a detail of the style branch with sweeping hairs at the tip, respectively; **e** *Schkuhria pinnata* (Bahieae) style branch apex of hermaphrodite floret; **f** *Hyalis argentea* (Mutisia clade), style branch apex of hermaphrodite floret. Scale bars 200 μ m for **a**, **c**, **d** and 100 μ m for **b**, **e**, **f**

some species did not have sweeping hairs on their styles (Figs. 1a, 2f).

Sweeping hair length showed a high variability among the species studied (S1). It was significantly shorter in the tribes Eupatorieae and Lactuceae, and in the Mutisia clade ($t = -5.51$, $P = 0.0001$, S1) than in the other species. Sweeping hairs were absent in *Chuquiraga erinaceae* (tribe Barnadesieae, Fig. 1a) and *Hyalis argentea* (Mutisia clade, Fig. 2f).

All species have papillate stigmatic surfaces. There was little variation in papillae length among species (S1). Papillae were significantly shorter in Barnadesieae, Cardueae, and Senecioneae tribes ($t = -5.6$; $P = 0.003$) than in the other species. In contrast, styles varied among species in their stigmatic-area organisation. The stigmatic papillae covered the inside of the style branches in species pertaining to the Carduoideae and Cichorioideae subfamilies (Fig. 1c, d), Barnadesieae and Senecioneae tribes, Mutisia

clade (except for *Chaptalia nutans*, Fig. 4d, f), one species of the tribe Coreopsideae, and in two species of the tribe Heliantheae (S1). However, stigmatic papillae were shown to be separated into two ventro-marginal bands along each style branch in the Asteroideae subfamily (Figs. 1f, h, 3d), except for the species mentioned above (two Heliantheae, one Coreopsideae, and one Senecioneae species). Figure 4 shows style branch sections of the three different floret types of *Chaptalia nutans* because stigmatic papillae arranged in two bands is not a common characteristic in species of the Mutisia clade.

For all species with dimorphic florets, we found that the style of female marginal florets was quite different from that of hermaphrodite florets of the same capitulum. The styles of female florets were, in general, more slender and without or with more reduced sweeping hairs than the styles of hermaphrodite florets (Fig. 3a–j). These differences were also detected in species with trimorphic florets in the same capitulum (Fig. 4). However, the stigmatic-area organisation and the morphology of stigmatic papillae remained identical between female and hermaphrodite florets (Fig. 3k, l).

Discussion

This work emphasises that morphological diversity of styles in Asteraceae is far more diverse than the categories previously established for this family (Bremer 1987, 1994; Erbar and Leins 1995 and references therein) and that it is problematic to assign only three modes of pollen secondary presentation to this morphological diversity. In fact, it was not possible to relate the sweeping-hair arrangement of many of the studied species with the three different secondary pollen presentation mechanisms described for the family (Yeo 1993; Erbar and Leins 1995), indicating that the hypothesised relationship could be more complex than was formerly thought.

For some of the studied species, stylar morphology remained similar within some tribes (e.g. Eupatorieae) or some genera (e.g. *Mutisia*, *Vernonia*, S1). In particular for the genus *Zinnia*, the unique species here studied showed a similar corolla and stylar morphology to the congeneric species studied by Yeo (1993). Thus, it is possible that *Zinnia peruviana* has the same exclusive pollen presentation mechanism described by Yeo (1993) for this genus: the truncate style branches with hairs on their tips pushes the pollen forward in the anther-cylinder, so that it is deposited and presented on the hairy inner surfaces of the corolla lobes (Fig. 2d).

However, for many other genera (such as *Tagetes*, *Gaillardia*, *Cosmos*, and *Bidens*), the general description of pollen presentation previously made by Yeo (1993) did not

Fig. 3 Style morphological diversity according to floret type in different genera of Asteraceae. For all species, pairs of SEM micrographs show the style branch of female and hermaphrodite florets. Note the absence or a lesser development of sweeping hairs on female-ray florets when compared with hermaphrodite. However, stigmatic-area organisation as well as the morphology of stigmatic papillae remain identical between female and hermaphrodite florets (**k, l**). **a, b** *Mutisia spinosa* (Mutisia clade), **c, d** *Grindelia anethifolia* (Astereae), **e, f** *Senecio pampeanus* (Senecioneae), **g, h** *Acmella decumbens* var. *affinis* (Heliantheae), **i–l** *Helenium argentinum* (Helenieae)

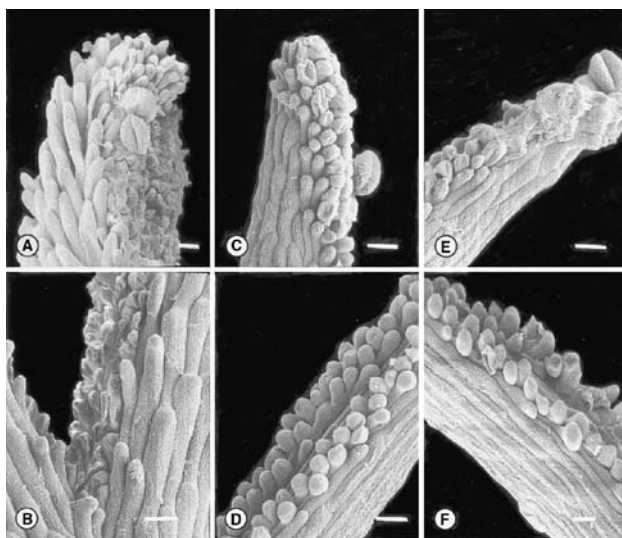
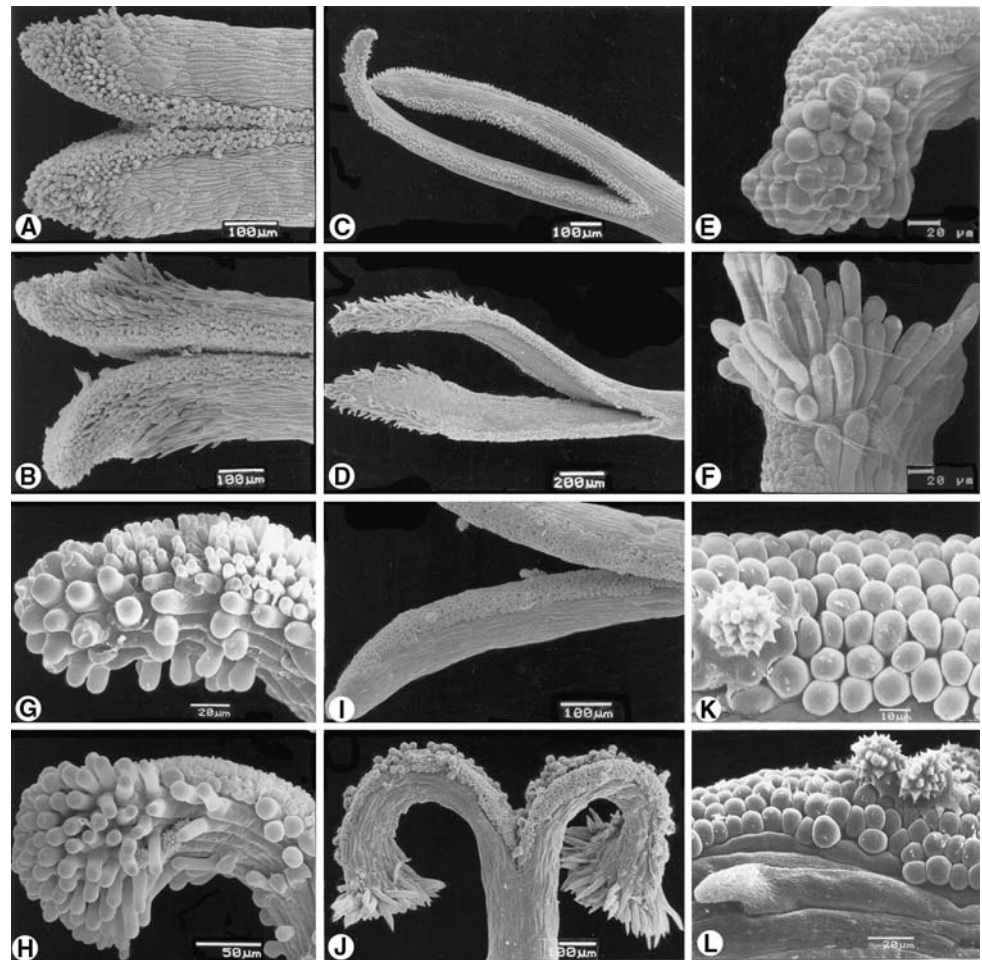


Fig. 4 Style morphological diversity according to floret type in *Chaptalia nutans* (Mutisia clade). Style branch sections of hermaphrodite (**a, b**), female filiform (**c, d**), and female ligulate (**e, f**) florets. Scale bars 20 μ m

coincide with the stylar morphology of congeneric species studied here. For this reason, it is possible that interspecific variation of sweeping hair arrangements occurs within some genera. In addition, results showed that the different pollen presentation mechanisms were not restricted to a particular subfamily. For example, brushing, pump and intermediate mechanisms have been recognised within Asteroideae (S1).

In addition, discrepancies between Yeo's (1993) descriptions of pollen presentation and the data presented here become more evident when congeneric species with "intermediate morphologies" are considered. In this way, it is possible that these discrepancies were due not only to the congeneric variation stated above but also to the subjectivity in the interpretation of the diverse "intermediate morphologies".

It is interesting to analyse the observed differences of sweeping hair arrangements and morphology between hermaphrodite and female florets within the same capitula of some species. These differences (already mentioned by Bremer 1994) suggest a close relationship between style

morphology and the pollen presentation function of flowers. In addition, divergences of sweeping hair arrangements and morphology are higher among phylogenetically related species. Thus, data suggest that functional aspects of floral morphology seem to be more important than phylogenetic constraints as selective forces determining stylar pollen presentation structures.

The morphological divergences among pollen presentation structures contrast with the homogeneity of stigmatic surfaces between hermaphrodite and female florets in the species with di- or trimorphic florets—as previously noted by Bremer 1994; Ladd 1994—but also with the homogeneity of stigmatic surfaces among phylogenetically related species. Thus, stigmatic papilla morphology seems to be a phylogenetically constrained character in the studied species.

Results presented here suggest that style morphological characteristics of Asteraceae represent a highly complex system in which not only female but also male reproductive functions of the flower are closely integrated. The large variability of stylar morphologies probably reflects compromises between phylogenetic constraints and the different functional aspects of plant reproduction.

Acknowledgments We thank Peter Leins, Claudia Erbar and Ramiro Aguilar for useful comments on early versions of this manuscript, and Gerardo Robledo, Ana María González and Ricardo Munch for technical support in SEM micrographs. The study was supported by “Consejo Nacional de Investigaciones Científicas y Técnicas” (CONICET), “Agencia Nacional de Promoción Científica y Tecnológica”, “Secretaría de Ciencia y Tecnología de la Universidad Nacional de Córdoba”, and “Agencia Córdoba Ciencia”. Thanks are due to “Consejo de Investigaciones Científicas y Técnicas de la Provincia de Córdoba” and the “Academia Nacional de Ciencias Exactas, Físicas y Naturales” for the fellowships to the first author. C.T. and L.G. are members of CONICET.

References

- Baldwin BG, Wessa BL, Panero JL (2002) Nuclear rDNA evidence for major lineages of helenioid Heliantheae (Compositae). *Syst Bot* 27:161–198
- Bremer K (1987) Tribal interrelationships of the Asteraceae. *Cladistics* 3:210–253
- Bremer K (1994) *Asteraceae: cladistics and classification*. Timber Press, Oregon
- Bremer K (1996) Major clades and grades of the Asteraceae. In: Hind DJN, Beentje HJ (eds) *Compositae: systematics*. Proceedings of the international compositae conference, Kew, 1994, vol 1, Royal Botanical Gardens, Kew, pp 1–7
- Erbar C, Leins P (1995) Portioned pollen release and the syndromes of secondary pollen presentation in the Campanulales–Asterales-complex. *Flora* 190:323–338
- Erbar C, Leins P (2000) Some interesting features in the capitulum and flower of *Arnaldoa macbrideana* Ferreyra (Asteraceae, Barnadesioideae). *Bot Jahrb Syst* 122:517–537
- Funk VA, Bayer RJ, Keeley S, Chan R, Watson L, Gemeinholzer B, Schilling E, Panero JL, Baldwin BG, Garcia-Jacas N, Susanna A, Jansen RK (2005) Everywhere but Antarctica: using a supertree to understand the diversity and distribution of the Compositae. *Biol Skr* 55:343–374
- Howell GJ, Slater AT, Knox RB (1993) Secondary pollen presentation in Angiosperms and its biological significance. *Aust J Bot* 41:417–438
- Ladd PG (1994) Pollen presenters in the flowering plants—form and function. *Bot J Linn Soc* 115:165–195
- Leins P, Erbar C (1990) On the mechanisms of secondary pollen presentation in the Campanulales–Asterales-complex. *Botanica Acta* 103:87–92
- Panero JL, Funk VA (2002) Toward a phylogenetic subfamilial classification for the Compositae (Asteraceae). *Proc Biol Soc Wash* 115:909–922
- Small J (1915) The pollen-presentation mechanism in the Compositae. *Ann Bot* 29:457–470
- Yeo PF (1993) Secondary pollen presentation. Form, function and evolution. *Pl Syst Evol Supp* 6, Wien, New York