FLORAL MORPHOLOGY AND DEVELOPMENT IN *ARAGOA* (PLANTAGINACEAE) AND RELATED MEMBERS OF THE ORDER LAMIALES

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Inflorescence and floral morphology and development were investigated in *Aragoa* (Plantaginaceae) and related genera. Each inflorescence of *Aragoa* is a reduced, axillary raceme, on which the actinomorphic floral apices generally arise successively. The inflorescences of *Aragoa* and *Plantago* are polytelic and lateral. The five sepals emerge from the abaxial to the adaxial side of the floral apex, but at maturity, the calyx is actinomorphic. The four stamens arise simultaneously and before emergence of the petals. The four petals emerge unidirectionally united, but the corolla becomes actinomorphic. Aestivation is cochlear ascendent. The two united carpels initiate simultaneously. The abaxial-adaxial inception of the calyx and corolla during early floral development in genera such as *Aragoa*, *Digitalis*, *Plantago*, and *Veronica* may indicate that the zygomorphic condition is ancestral in those genera. The tetramerous corolla, which is actinomorphic during middle and late development, and the presence of four stamens are possible synapomophies of the clade (*Aragoa* + *Plantago*). Pentamery of the calyx and corolla appears to be plesiomorphic in the broader *Aragoa-Angelonia* clade. Characters related to development and morphology of inflorescences and flowers of *Aragoa* are essentially similar to those found in *Plantago*, which is consistent with the molecular-based sister group relationship between these genera.

Keywords: *Antirrhinum*, *Aragoa*, floral development, floral morphology, *Heliohebe*, Lamiales, *Plantago*, Plantaginaceae, "Scrophulariaceae," *Veronica*.

Introduction

Aragoa Kunth, with 19 species, three subspecies, and four probable hybrids (Fernández-Alonso 1995), is endemic to the páramos of Colombia and Venezuela, between 2500 and 4000 m altitude (Pennell 1937; Fernández-Alonso 1991, 1993, 1995; Nilsson and Hong 1993). The genus was originally described as "Bignoniaceis affinia" by von Humboldt et al. (1819), but its affinities have been in dispute since then (for review, see Fernández Alonso 1995; Bello et al. 2002).

Before recent molecular analyses, most authors included *Aragoa* within Scrophulariaceae *sensu lato* (e.g., Endlicher 1836–1840; Wettstein 1895; Fernández-Alonso 1995), closely related to genera such as *Hebe* Comm. ex Juss. and *Veronica* L. in tribe Veroniceae (Bentham 1846, 1876; Hallier 1903; Pennell 1937; Yamazaki 1957; Thieret 1967; Nilsson and Hong 1993). However, *Aragoa* is atypical among Scrophulariaceae *sensu lato* because all species are shrubs or small trees and have actinomorphic corollas. These characters states led Pennell (1937) to regard *Aragoa* as primitive Veroniceae. Other characters of *Aragoa*, such as the helicoid arrangement of the leaves, the presence of four equal stamens, and the morphology of pollen and seed (Thieret 1967; Nilsson and Hong 1993), are not shared with all other members of the former

tribe Veroniceae. Nilsson and Hong (1993) separated the genus into its own tribe (Aragoeae) based on pollen morphology but did not clarify its closest relationships within Scrophulariaceae. Fernández-Alonso (1995) observed some morphological similarities with *Capraria* L., *Chionohebe* Briggs & Ehrend., *Detzneria* Schltr. ex Diels., and *Hebe* and *Scoparia* L. but did not clearly propose a close relationship with any of them.

Several authors have used floral characters to discuss the systematic relationships of *Aragoa*. For example, Don (1835) segregated the genus into its own family Aragoaceae based on the "convolute" aestivation of the corolla, later described by Bentham (1846) as imbricate. Others (Meisner 1830–1843; Endlicher 1836–1840; Bentham 1846; Rouy 1909) placed the genus within tribe Veroniceae, primarily on the basis of characters such as its racemose inflorescence, five-lobed calyx, the imbricate aestivation of the four-lobed corolla with funnel-rotate shape, and the tetramerous androecium with confluent thecae. Although floral morphology has provided key characters in assessments of the systematic relationships of the genus, a detailed study of its floral development and morphology is lacking, preventing a comparison with related genera that have already been investigated (see, e.g., Payer 1857; Chatin 1873; Saunders 1933; Srinivasan 1940; Yamazaki 1957; Schrock and Palser 1967; Singh 1979; Singh and Jain 1979; Nishino 1983; Awasthi et al. 1984; Leins and Erbar 1988; Endress 1992, 1994, 1998; Wunderlin 1992; Kampny et al. 1993; Kampny and Dengler 1997; Prakash and Singh 1997).

Recent molecular and phytochemical data for *Aragoa* support a previously unsuspected sister group relationship with

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Plantaginaceae, which resulted in the inclusion of *Aragoa* within Plantaginaceae (Bello et al. 2002; Rønsted et al. 2002; Jensen et al. 2003), with *Veronica* as its immediate sister group. The aims of this article are to investigate the floral development and morphology of *Aragoa* and to compare these results with those of genera that are currently included in the clade recognized by Bello et al. (2002), hereafter called the *Aragoa-Angelonia* clade (Veronicaceae *sensu* Olmstead et al. 2001). The hierarchical structure of this clade is as follows: (((((((((*Aragoa* + *Plantago*) *Veronica*) *Hemiphragma*) *Digitalis*) *Globularia*) (*Callitriche* + *Hippuris*) *Antirrhinum*) ((*Chelone* + *Collinsia*) *Tetranema*) and other genera of its sister clade (((*Amphianthus* + *Gratiola*) *Bacopa*) *Angelonia*). *Heliohebe* Garn.-Jones, a member of the *Hebe* complex *sensu* Garnock-Jones (1993), and *Littorella*, a basal genus in the phylogeny of Plantaginaceae s.l. (Rønsted et al. 2002; Hoggard et al. 2003), are here treated as distinct genera in the discussion of floral characters.

The inclusion of *Aragoa* in a clade with *Antirrhinum*, *Plantago*, and *Veronica* makes examination of its floral morphology and ontogeny particularly relevant because recent developmental-genetic work on floral symmetry in *Antirrhinum* has demonstrated that zygomorphy relies on differential expression of two closely related adaxial identity genes *CYCLOIDEA* (*CYC*) and *DICHOTOMA* (*DICH*) in the young floral meristem (Coen and Nugent 1994; Coen 1996; Luo et al. 1996, 1999). This discovery has stimulated a renaissance in studies of floral symmetry patterns (e.g., Neal et al. 1998; Endress 1999; Rudall and Bateman 2003). *Plantago* is often cited as an example of a taxon with actinomorphic flowers that is phylogenetically nested within a clade with taxa that produce predominantly zygomorphic flowers (Coen and Nugent 1994; Donoghue et al. 1998; Endress 2003). Because flowers of *Aragoa* are also regarded as actinomorphic, such different patterns of symmetry in related taxa would merit more rigorous examination in this clade.

Material and Methods

Floral buds of *Aragoa* and *Plantago* were collected in the field at several stages of development, whereas those of *Heliohebe raoulli* were collected from the living collections at the Royal Botanic Gardens, Kew. Various developmental stages covering the complete floral ontogeny of four species (two of them from three different populations) and one putative hybrid of *Aragoa* were examined under the LM and SEM (vouchers cited in table 1). Samples were fixed in FAA and then transferred to 70% ethanol. The samples were then dissected in 90% ethanol under a Wild Heerburg M8 dissecting microscope. The material was then submitted to a dehydration series of 90%, 95%, and 100% ethanol, 1 h in each concentration. The samples used for SEM were critical point dried in a Balzers 030 drier, coated with gold in an Emitech Sputter coater K550, and examined and photographed on a Cambridge Instruments Stereoscan 240 at 15 kV. For light microscopy, the samples were transferred from 100% ethanol through an ethanol-Histoclear series (90 : 10, 70 : 30, 50 : 50, 30 : 70, 10 : 90, 0 : 100) for 18 h. Floral buds were embedded in paraplast for serial sectioning using a rotary microtome (Reichert-Jung 2040). Sections were stained with safranin and Alcian Blue and mounted in DPX resin.

Results

Organography of Aragoa

The flowers of *Aragoa* are bisexual (fig. 1). The five sepals are ovate, oblong, or lanceolate; equally sized (ranging from 2.8 to 8 mm long), (sub)coriaceous, frequently with long hairs on the margin and short hairs along the dorsal side. By preanthesis, the calyx shows a cochlear-ascendent aestivation (figs. 2, 3N). The corolla is tetramerous, actinomorphic, rotate (e.g., in *Aragoa cundinamarcensis* Fernández-Alonso) to subrotate (e.g., in *Aragoa abietina* H.B.K., *Aragoa cleefii* Fernández-Alonso, and *Aragoa cupressina* H.B.K.) or tubular-campanulate (e.g., *Aragoa perez-arbelaeziana* Romero). The corolla is generally white, except in the yellow-flowered *A. perez-arbelaeziana*). The tube is 1.5–17 mm long, and the corolla lobes are obovate or spathulate, 3 to 9 × 2.5 to 7 mm (fig. 1). The marginal indumentum of the petals is different in the species studied. In *A. cleefii* and *A. cupressina* H.B.K., it is formed by dense filiform hairs (fig. 4C); in *Aragoa* x *funzana* Fernandez-Alonso,

Table 1

Species and Material Examined

Species	Voucher	Locality
Aragoa abietina Kunth	COL: *Bello 118, 119, 120*	Colombia: Cundinamarca, Parque Nacional Natural Chingaza
Aragoa cleefii Fernández-Alonso	COL: *Bello 108*	Colombia: Cundinamarca, Villapinzón, Páramo de Gachaneque
Aragoa cundinamarcensis Fernández-Alonso	COL: *Bello 107*	Colombia: Cundinamarca, Villapinzón, Páramo de Gachaneque
Aragoa cupressina Kunth	COL: *Bello 128, 129, 130*	Colombia: Cundinamarca, Páramo de Monserrate
Aragoa x *funzana* Fernández-Alonso	COL: *Bello 113, 115*	Colombia: Cundinamarca, Villapinzón, Páramo de Gachaneque
Heliohebe raoulli (Hook.f.) Garn.-Jones	HK: 1996-23; CHAD 574	Royal Botanic Gardens, Kew
Plantago major L.	COL: *Bello 247*	Colombia: Bogota, campus of the National University

Note.   COL = specimen supported by a voucher in the Colombian National Herbarium, Bogota. HK = material collected from the Living Collections, Royal Botanic Gardens, Kew.

Fig. 1 Flowering shoots of *Aragoa*

the hairs are scarce, whereas petal margins of *A. abietina* are glabrous. The corolla aestivation is cochlear ascendent (fig. 4*A*, 4*B*; fig. 5*H*).

The four epipetalous stamens alternate with the corolla lobes (fig. 4*G*) and have filiform, glabrous, or distally pubescent filaments (fig. 5*E*, 5*G*) and dithecal, dorsifixed, short (0.7–1.8 mm long) anthers that are introrse and open by longitudinal slits (fig. 4*G*). The gynoecium is bottle shaped, bilocular, and basally surrounded by a hypogynous, ringlike nectariferous disc (fig. 4*F*, 4*G*; fig. 5*C*, 5*J*). Placentation is axile, and there are four to eight ovules per locule (figs. 4*F*, 5*J*). The style is cylindrical, up to 20 mm long, reaching the same level of the anthers by anthesis (figs. 1*B*, 4*G*). The stigma is slightly bilobed, capitate, dry, and papillate (fig. 4*I*).

Floral Development of Aragoa

Inflorescences. The inflorescences of *Aragoa* are reduced axillary racemes, which are often congested in adjacent nodes of the distal or middle zones of the branches. The racemes and internodes are both extremely short, resulting in a floral arrangement that appears whorled. Several floral pseudowhorls can develop along a single branch, but they are separated by a long vegetative zone. The youngest racemes are closest to the branch apex, which suggests an acropetal sequence of development (fig. 1*A*). Each raceme is subtended by a leaf and consists of two or three flowers (figs. 1*A*, 2). If the raceme has three flowers (e.g., in *A. cundinamarcensis*, *A. cupressina*, and *A. x funzana*), two of them are lateral, and one occupies the median position (fig. 2). Each flower is subtended by a single leaflike bract. This bract is perpendicularly oriented with respect to the subtending leaf in the lateral flowers and opposite to it in the median flower. Within each inflorescence, floral apices generally arise successively, but two can occasionally initiate almost simultaneously (fig. 3*A*, 3*B*). Simultaneous emergence of three floral apices within the same inflorescence was not observed. A dense indumentum of white hairs surrounds the buds, especially during early stages of development (fig. 3*A*).

Calyx. The floral apex is initially radially symmetrical (fig. 3*A*). Calyx development commences with the simulta-

neous initiation of the two abaxial-lateral sepals, followed by initiation of the two adaxial-lateral ones. The gap between the latter two is soon filled by the fifth (adaxial) sepal primordium (fig. 3*B*–3*D*). Thus, the emergence of the sepals is unidirectional, from the abaxial to the adaxial side of the floral apex, indicating a zygomorphic initiation of the calyx. The abaxial-lateral sepals are slightly larger than the adaxial-lateral ones, whereas the adaxial sepal is the smallest (fig. 3*E*, 3*N*); this difference remains evident until preanthesis. The formation of the calyx tube is slightly asymmetric because intercalary growth between the abaxial-lateral sepals occurs earlier than that between the lateral and the adaxial sepals (fig. 3*F*). By preanthesis, the calyx becomes actinomorphic and has cochlear-ascendent aestivation. Sepals have a dense covering of hairs along the margins.

Androecium and corolla. The four stamens primordia arise simultaneously opposite to the abaxial-lateral and the lateral sepals, before the emergence of the alternating petal primordia (fig. 3*G*). There is no evidence of a fifth stamen opposite the adaxial sepal. Initiation of the four petal primordia is unidirectional because the abaxial and the lateral petal primordia emerge simultaneously alternating with the sepals, and the adaxial initiates later opposite the adaxial sepal (fig. 3*H*). Then, the emergence of the petals results in initial bilateral symmetry for the corolla. Just after organogenesis, the petal primordia are separated from each other by the developing stamens (fig. 3*I*–3*K*). Late sympetaly occurs by growth subjacent to the petals and their sinuses. The stamens in these sinuses thus fuse to the corolla (fig. 3*I*, 3*K*, 3*L*). At this time, differentiation of filament and anther becomes apparent, and the corolla is slightly zygomorphic (fig. 3*L*, 3*M*, 3*O*).

Before anthesis, corolla aestivation becomes cochlear ascendent as the lateral and abaxial lobes enlarge over the adaxial one (fig. 4*A*–4*C*). Corolla aestivation in the lateral flowers within an inflorescence forms mirror images (cf. fig. 4*A*, 4*B*). By anthesis, the corolla becomes actinomorphic after the
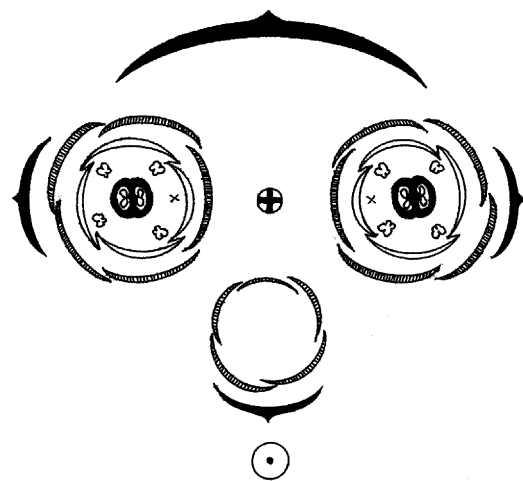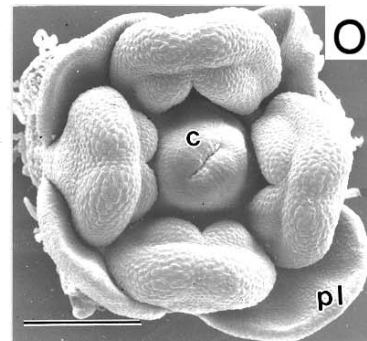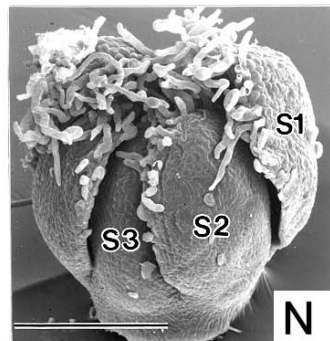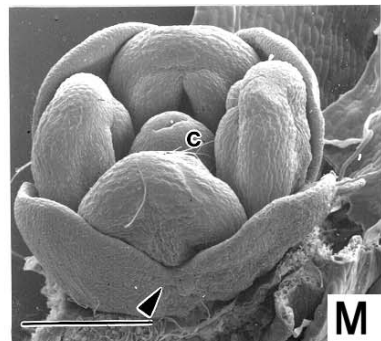

Fig. 2 Inflorescence diagram of *Aragoa* with the two lateral flowers already developed and a third floral apex in median position. Subtending leaf and bracts are in black; sepals are hatched; petals are in white; circle with dot represents shoot axis; circle with cross represents inflorescence axis.
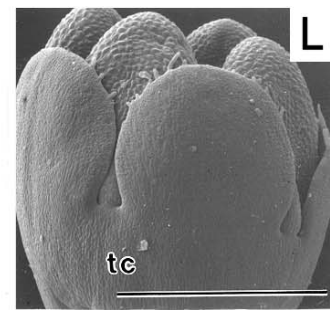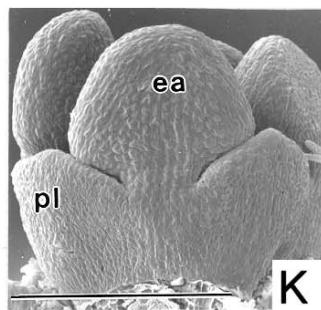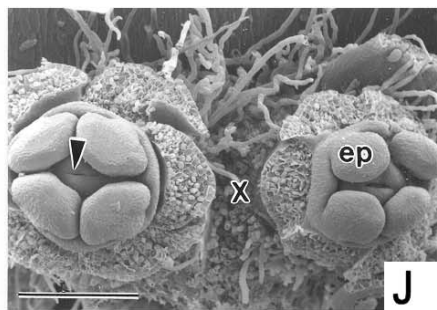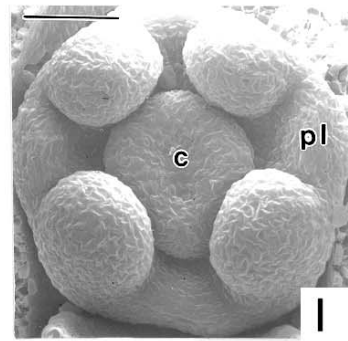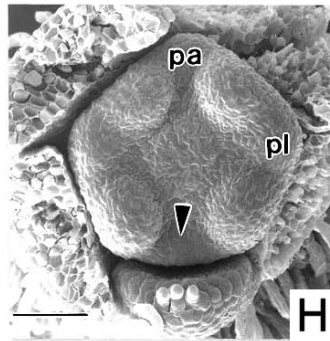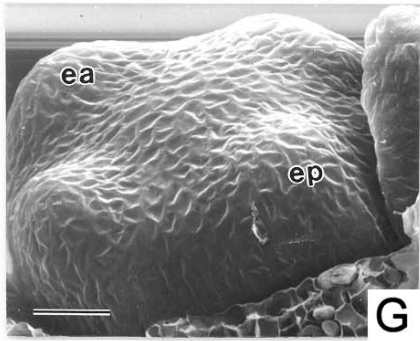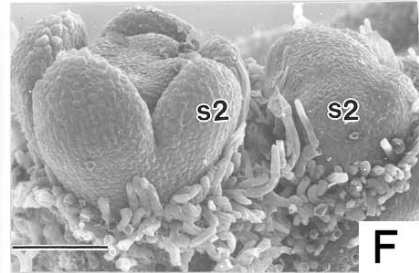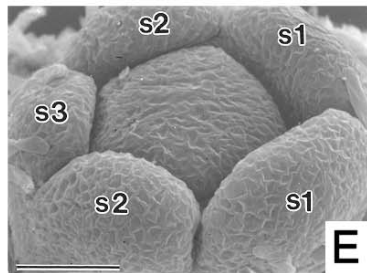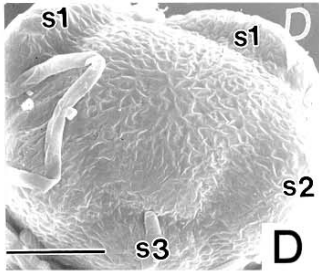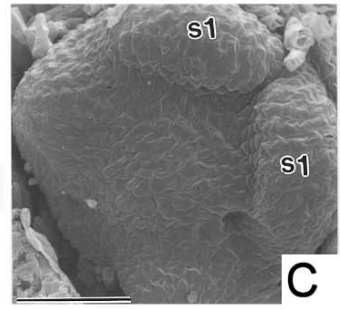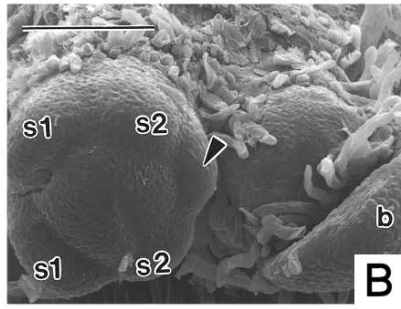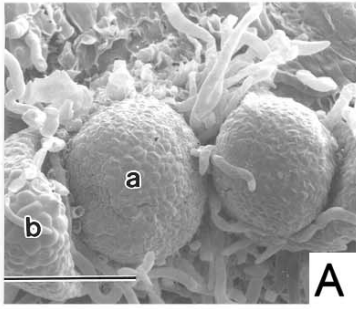
enlargement of petals and the tube, and the four stamens are fully developed and alternate with the corolla lobes (fig. 1).

Gynoecium. After petal initiation, a gynoecium primordium in the center of the young flower is apparent (fig. 3H). Shortly after that, the congenitally fused carpels appear as a ringlike primordium, without protruding median parts (i.e., symplicate carpels); the septum develops later (fig. 3H, 3I). The carpels remain united up to the stigmatic region, where there is a narrow furrow radially oriented with respect to the axis of the inflorescence (fig. 3J, 3O). A narrow style with a central transmitting tract (fig. 5E, 5F, 5H, 5J) is formed after elongation of the upper part of the pistil.

Floral Vasculature and Anatomy of Aragoa

Serial cross sections of *Aragoa* flowers (fig. 5) show that the vascular traces along the pedicel form a continuous stele. At the level of calyx insertion, the median vascular trace of each sepal departs directly from the central stele and remains unbranched (fig. 5A–5E). Additionally, five commissural bundles depart from the stele, and each divides into two traces at the base of the sepals, forming the two lateral traces of adjacent sepals (fig. 5A). As a result, each sepal contains three main vascular bundles.

At the level of insertion of the corolla, eight vascular traces depart from the central stele, four of which supply the corolla and the alternating four supply the stamens (fig. 5B–5E). At this level, the adaxial petal trace divides into three traces; the median of these three traces enters the center of the adaxial corolla lobe, and the two lateral traces irrigate its flanks. The two median carpellary veins are also evident at the level of corolla insertion (fig. 5B, 5C). A short distance above this level, each of the two remaining petal traces also splits into three traces. These traces essentially have the same branching pattern as the adaxial trace. The central traces of each petal further divides into five to eight traces in the distal half of each corolla lobe. In contrast, stamen bundles remain unbranched (fig. 5D, 5E).

The remaining central vascular traces supply the gynoecium. Each of the two carpels is irrigated by a median and two lateral bundles. The ovules are directly supplied by the marginal traces. There are two traces along the style (fig. 5F).

Glandular trichomes consisting of a stalk of two to three cells and a head formed by six cells are scattered on both the outer and the inner corolla surfaces (fig. 4D, 4E, 4H); they are restricted to the distal part of the tube in some species

(e.g., *A. cupressina*). In contrast to the thick cuticle present in mature sepals, petals lack cuticle, at least in some species (fig. 5). The inner epidermis of the petal consists of a single layer of domed (papillate) cells; a dense covering of filiform hairs is also present at the level of stamen insertion (fig. 4G). Stomata were observed on petal surfaces in all species examined. The petal mesophyll consists of a homogeneous parenchyma without intercellular spaces (fig. 5E, 5G, 5H).

The epidermis of the anthers is formed by a thin layer, and the endothecium consists of regularly shaped cells with thickened walls near the dehiscence area. The tapetum is parietal.

Floral Development of Heliohebe

Inflorescence. Inflorescences of *Heliohebe raoulii* are terminal racemes, in which flowers develop spirally and acropetally (fig. 6A). A single bract covers the corresponding floral bud by the time of emergence of the abaxial-lateral sepals.

Calyx. The floral apex is zygomorphic (fig. 6A). The two abaxial-lateral sepal primordia initiate first. They emerge simultaneously. The two adaxial-lateral sepals arise much later (fig. 6B, 6C); they also emerge simultaneously and remain smaller than the abaxial-lateral sepals throughout development (fig. 6C–6F). The whole calyx is therefore zygomorphic. There is no evidence of a fifth sepal primordium. The fusion between the abaxial-lateral sepals is greater than that between the adaxial-lateral ones (fig. 6D–6F). The flanks of the abaxial-lateral sepals partially overlap the adaxial-lateral sepals (fig. 6C–6F). Marginal trichomes become evident on the abaxial-lateral sepals during late development.

Androecium and corolla. During development, these floral whorls are highly zygomorphic in *H. raoulii*. The two stamen primordia initiate simultaneously after the emergence of adaxial-lateral sepals (fig. 6C), opposite them. There is no evidence of additional stamen primordia. Later, three petal primordia simultaneously emerge on the abaxial side of the floral bud; the abaxial petal primordium alternates with the two abaxial-lateral sepals and the two lateral petal primordia opposite them (fig. 6D). Petal growth is delayed because lobes are still included in the young flower even during late development (fig. 6E, 6F). By preanthesis, the corolla aestivation is imbricate because the two lateral petals are external to the medial petals (not shown).

Gynoecium. The gynoecium primordium in the center of the young flower appears to initiate simultaneously with petal initiation. Shortly after that, the congenitally fused

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Fig. 3 Early and midfloral development of *Aragoa*. *A, Aragoa cundinamarcensis*. Two floral apices. *B, Aragoa* x *funzana*. Upper view of inflorescence apex (arrowhead points to primordium of adaxial sepal). *C, Aragoa cleefii*. Floral bud with the abaxial-lateral sepals initiating first. *D, Aragoa cundinamarcensis*. Unidirectional development of sepals. *E, Aragoa cleefii*. Calyx before stamen emergence. *F, Aragoa cundinamarcensis*. Lateral view of inflorescence. *G, Aragoa cupressina*. Stamen emergence. *H, Aragoa cundinamarcensis*. Upper view of floral bud (sepals removed except the adaxial one); arrowhead indicates the site where adaxial petal will develop. *I, Aragoa cundinamarcensis*. Gynoecium initiation. *J, Aragoa* x *funzana*. Upper view of apex inflorescence (sepals removed); the arrowhead shows the intercarpellary furrow. *K, Aragoa cundinamarcensis*. Lateral view of floral bud (sepals removed). *L, Aragoa abietina*. Lateral view of floral bud (sepals removed; adaxial petal on the right). *M, Aragoa cleefii*. Floral bud with margins of petals fusing (arrowhead; sepals removed; adaxial petal on the lower left). *N, Aragoa* x *funzana*. Cochlear aestivation of the calyx. *O, Aragoa cundinamarcensis*. Actinomorphic floral bud (sepals removed). *a* = floral apex; *b* = bract; *c* = gynoecium; *ea* = abaxial stamen; *ep* = adaxial stamen; *pa* = abaxial petal; *pl* = lateral petal; *s1* = abaxial-lateral sepal; *s2* = adaxial-lateral sepal; *s3* = adaxial sepal; *tc* = corolla tube; *x* = inflorescence axis. Scale bars = 100 μm in *A, B, D, E, H, I*; 50 μm in *C, G*; 200 μm in *F, J, K, M–O*; 500 μm in *L*.
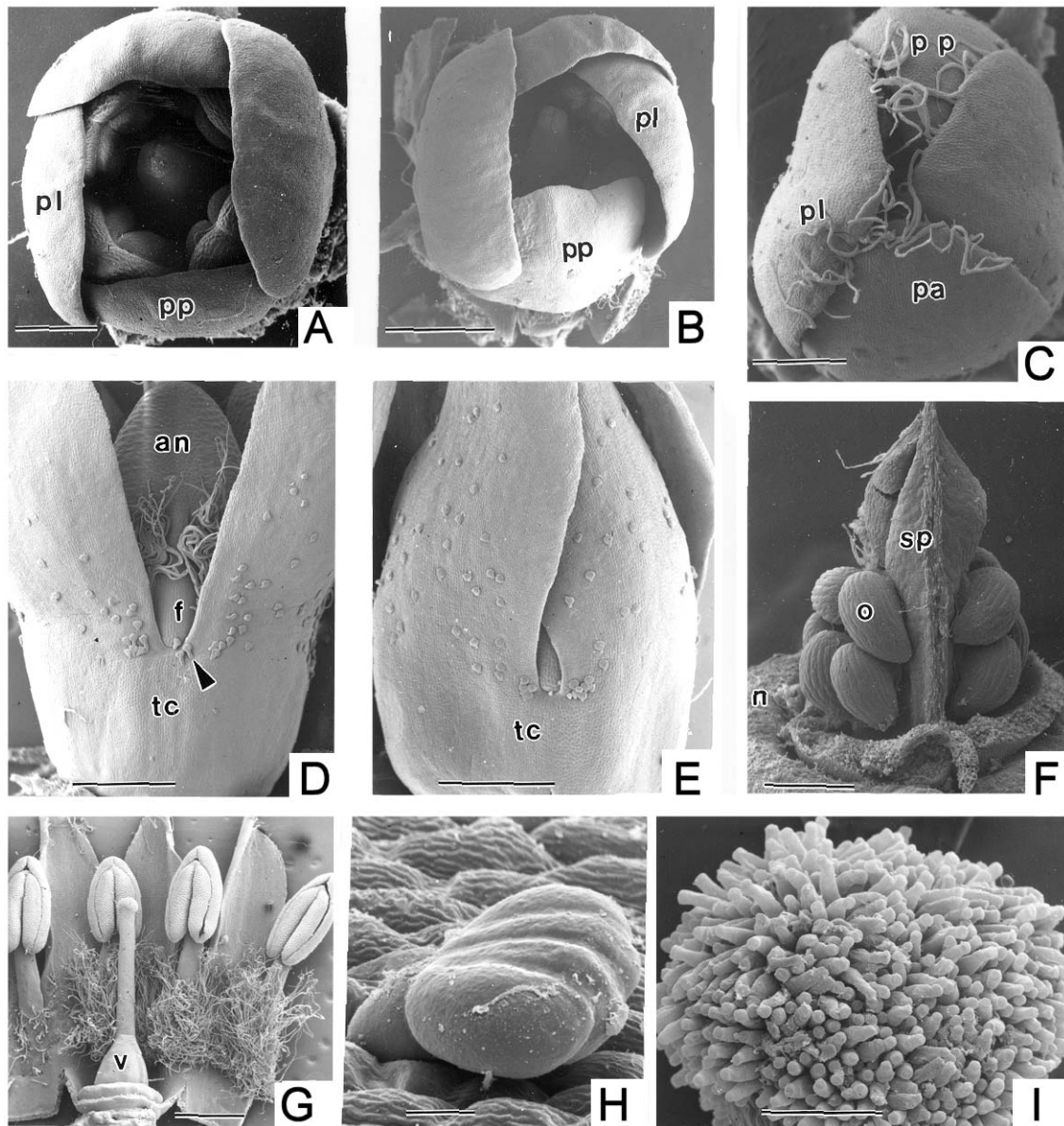
Fig. 4   Late floral development of *Aragoa*. *A, B, Aragoa abietina*. Upper view of mirror image of floral buds (sepals removed). *C, Aragoa cleefii*. Upper view of cochlear-ascendent corolla. *D, E, Aragoa cleefii*. Lateral view of the corolla; capitate hairs arrowed. *F, Aragoa* x *funzana*. Placental septum basally surrounded by a ringlike disk (ovary walls removed). *G, Aragoa cleefii*. Inner view of a mature flower. *H, Aragoa* x *funzana*. Capitate hair. *I, Aragoa cleefii*. Papillary stigma surface. *an* = anther; *f* = filament; *n* = nectarial disk; *o* = ovule; *pa* = abaxial petal; *pl* = lateral petal; *pp* = adaxial petal; *sp* = placental septum; *tc* = corolla tube; *v* = ovary. Scale bars = 500 μm in *A, C, E, F*; 1 mm in *B, D*; 2 mm in *G*; 20 μm in *H*; 100 μm in *I*.

carpels appear as a ringlike primordium (i.e., symplicate carpels; fig. 6*D*). Later development of the pistil was not followed in this species. Mature flowers show a bottle-shaped gynoecium basally surrounded by a ringlike nectariferous disc with glandular-ciliate border (not shown). The stigma is four lobed, narrowly capitate (not shown).

Floral Development of *Plantago*

Inflorescence.   The developing spikes in *Plantago* show congested floral buds that develop acropetally (fig. 7*A*). Each

floral bud is subtended by a bract that covers it even before organogenesis.

Calyx.   Floral apices of *Plantago* are initially radially symmetrical until sepal emergence, which occurs unidirectionally; the initiation of the two abaxial-lateral sepal primordia is followed by the two adaxial-lateral ones (fig. 7*A*, 7*B*). There is no evidence of the emergence of a fifth adaxial primordium. Each calyx lobe soon becomes concave (fig. 7*B*); then, the calyx tube begins to be formed. The calyx tube remains short (fig. 7*E*). The abaxial-lateral calyx lobes are slighly larger than the adaxial-lateral ones (fig. 7*C*–7*E*),
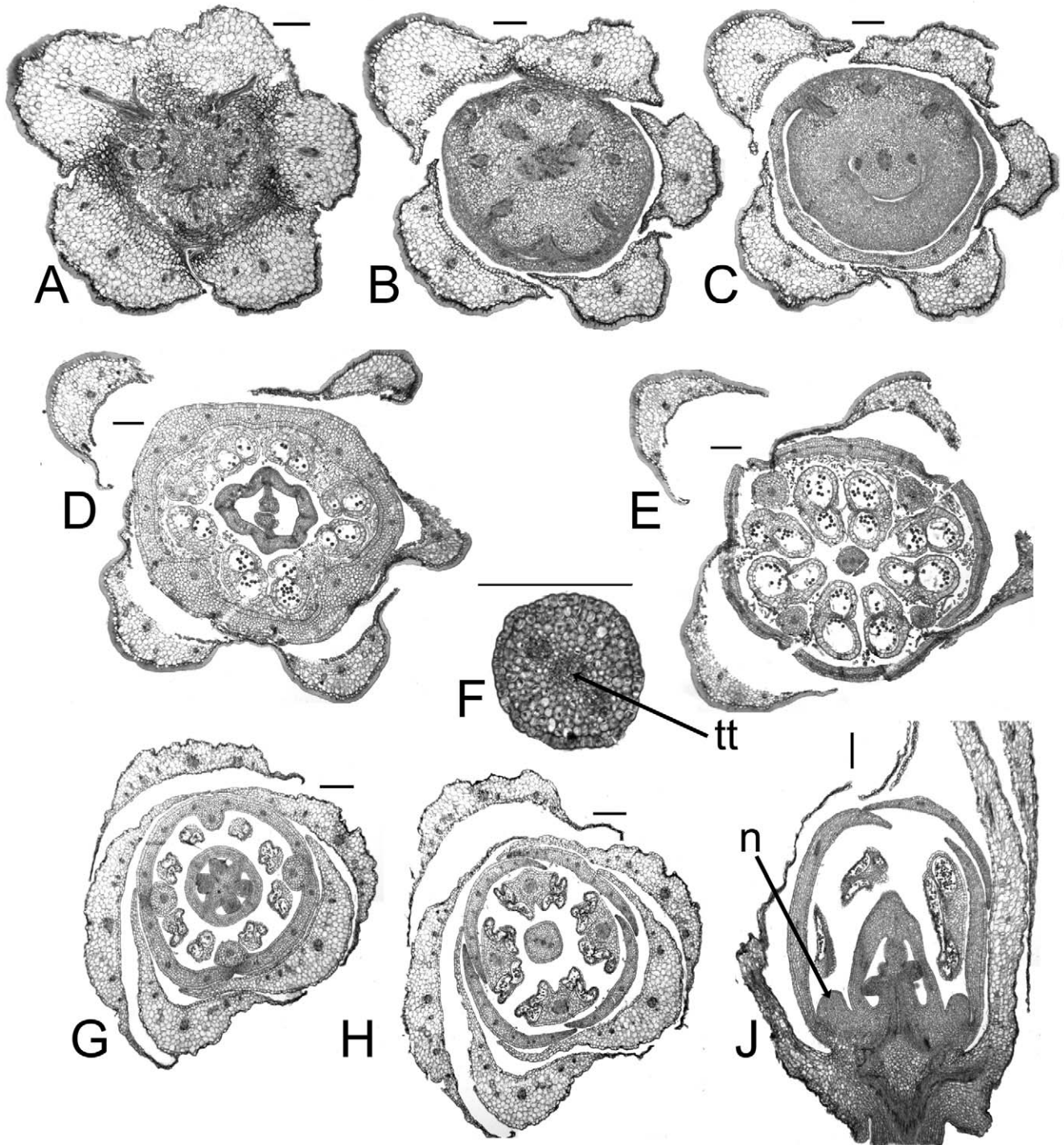
Fig. 5 LMs of flower sections. *A–E, Aragoa cupressina*, series of transverse sections (TS) through a single flower. *F, Aragoa cupressina*, TS style. *G, H, Aragoa abietina*, TS flower bud at preanthesis. *J, Aragoa abietina*, longitudinal sectional of flower bud at preanthesis. *n* = nectariferous disc; *tt* = stylar transmitting tissue. Scale bar = 100 μm.

showing a zygomorphic pattern. Pluricellular trichomes begin to develop on the outer surface (fig. 7D).

Androecium and corolla. The four stamens emerge simultaneously shortly before petal initiation (fig. 7A). Stamen primordia are opposite the sepals. The emergence of the petal primordia is unidirectional, from the abaxial to the adaxial side (fig. 7B). However, the adaxial petal becomes slightly larger than the others by mid-development (fig. 7C). There is no evidence that the adaxial petal is formed by the fusion of two petal primordia. The corolla lobes emerge independently
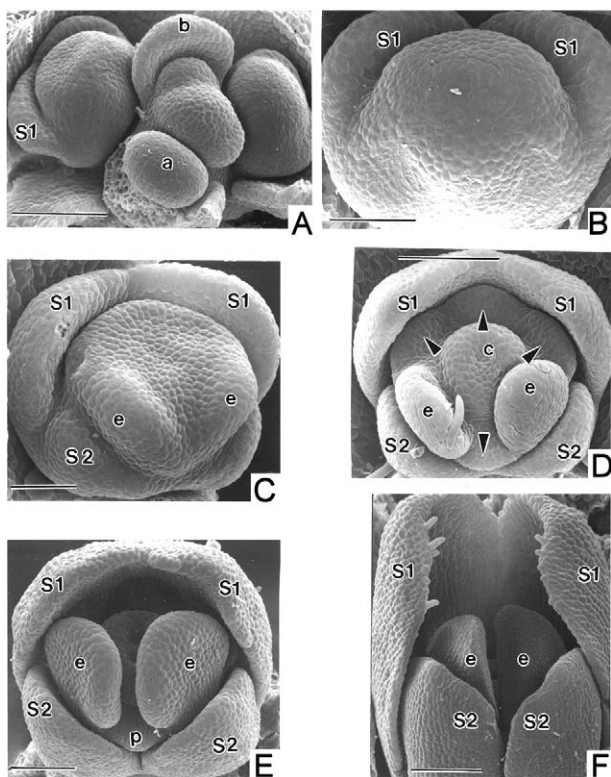
Fig. 6 Floral development of *Heliohebe raoulii*. *A*, Upper view of inflorescence apex with four floral buds. *B*, Frontal view of floral bud with two abaxial-lateral sepals already initiated. *C*, Floral bud with the two abaxial-lateral and two adaxial-lateral sepals; the two adaxial-lateral stamens start initiation. *D*, Floral bud with the four petals (arrowheads) initiated; gynoecium becomes evident. *E*, *F*, Midfloral bud with strongly zygomorphic calyx. *a* = floral apex; *b* = bract; *c* = fused carpels; *e* = stamen; *p* = petal; *s1* = abaxial-lateral sepal; *s2* = adaxial-lateral sepal. Scale bars = 100 μm in *A*, *D–F*; 50 μm in *B*, *C*.

(fig. 7*B*) and become interconnected by interprimordial growth with the alterning stamens, showing late sympetaly. By mid-development (fig. 7*C–7F*), the corolla is actinomorphic, and the stamens are already differentiated in anther and filament (fig. 7*F*). Petal aestivation is imbricate because the abaxial petal is partially covered by the lateral and the adaxial ones (fig. 7*G*).

Gynoecium. Gynoecium development and gross shape of the mature pistil in *Plantago major* is essentially the same to that described in *Aragoa* (fig. 7*A–7E*).

Discussion

Inflorescence

Our results confirm Fernández-Alonso's (1995) report that the inflorescences of *Aragoa* are lateral, extremely condensed racemes (fig. 1). *Plantago* often has lateral spikes (see Weberling and Troll 1998 for a detailed description of inflorescences in Plantaginaceae). Therefore, inflorescences of *Aragoa* and *Plantago* are similar in that they are both lateral

and polytelic. By contrast, *Veronica* and *Hebe* have predominantly polytelic inflorescences (cf. fig. 9 in Weberling and Troll 1998), but they are often terminal.

Flowers have been described as solitary in *Angelonia* Bonpl., *Antirrhinum* L., *Aragoa*, and *Collinsia* Nutt. (Meisner 1830–1843; Bentham 1846, 1876; Lemée 1929). However, they are primarily arranged in polytelic inflorescences, either as lateral (in *Aragoa*) or terminal racemes (in *Angelonia*, *Antirrhinum*, and *Collinsia*; see Wettstein 1895, their fig. 24).

Optimization of polytelic versus monotelic inflorescences in the *Aragoa-Angelonia* clade (not shown in fig. 8) indicates that polytelic inflorescences could be plesiomorphic. However, inflorescence architecture is unknown in genera such as *Amphianthus* and *Hemiphragma*, which makes interpretation of this character difficult in this clade. Inflorescences were described as monotelic in *Tetranema* Benth. ex Lindl. by Weber (1972), which would be autapomorphic for it. Lateral, polytelic inflorescences seem to be plesiomorphic in two subclades: (((((*Aragoa* + *Plantago*) *Veronica*) *Hemiphragma*) *Digitalis*) *Globularia*) and ((*Chelone* + *Collinsia*) *Tetranema*). However, in these subclades, terminal polytelic inflorescences appear in *Collinsia*, *Digitalis*, and some species of *Veronica*. *Littorella* and *Heliohebe* (not sampled in Bello et al. 2002) have lateral and terminal polytelic inflorescences, respectively. In the remaining members of the *Aragoa-Angelonia* clade, solitary flowers appear independently at least twice in the clade *Callitriche* + *Hippuris*, in *Gratiola*, and in *Bacopa*. Furthermore, terminal polytelic inflorescences also appear independently in *Angelonia* and *Antirrhinum*. Inflorescence architecture is unknown in *Amphianthus* and *Hemiphragma*.

Calyx

In some groups of plants, the symmetry of the floral apex determines symmetry during early stages of floral organogenesis (Tucker 1984). This seems to occur in *Digitalis* and *Heliohebe*, which have zygomorphic floral apices before and after organogenesis (Singh 1979; Wunderlin 1992; fig. 6), and in *Collinsia*, which has radial floral apices and a simultaneous initiation of the sepals (Schrock and Palser 1967). However, the floral apices of *Aragoa* and *Plantago* are initially actinomorphic and become zygomorphic because of the abaxial-adaxial emergence of the sepals (fig. 3*A–3E*; table 3). A similar switch in symmetry of the floral apex has been observed in *Angelonia*, *Antirrhinum*, and *Veronica* (Srinivasan 1940; Singh and Jain 1979; Kampny et al. 1993) (table 2), although in *Antirrhinum*, zygomorphy is mainly expressed in the corolla and androecium (Endress 1999). During early and mid-development in *Aragoa*, *Digitalis*, *Heliohebe*, *Littorella*, *Plantago*, and *Veronica*, the abaxial-lateral sepals are larger than either the adaxial sepal (if present) or the adaxial-lateral sepals (Payer 1857; Singh 1979; Kampny et al. 1993; Prakash and Singh 1997; fig. 6*B–6D*; table 3). Similar development is observed in the perianth (treated as calyx in Cronquist 1981; Takhtajan 1997) of *Hippuris vulgaris* (Leins and Erbar 1988).

A switch in symmetry during floral ontogeny was considered unusual by Tucker (1984) and Canne-Hilliker (1987) but has since been reported in a number of plants (Armstrong
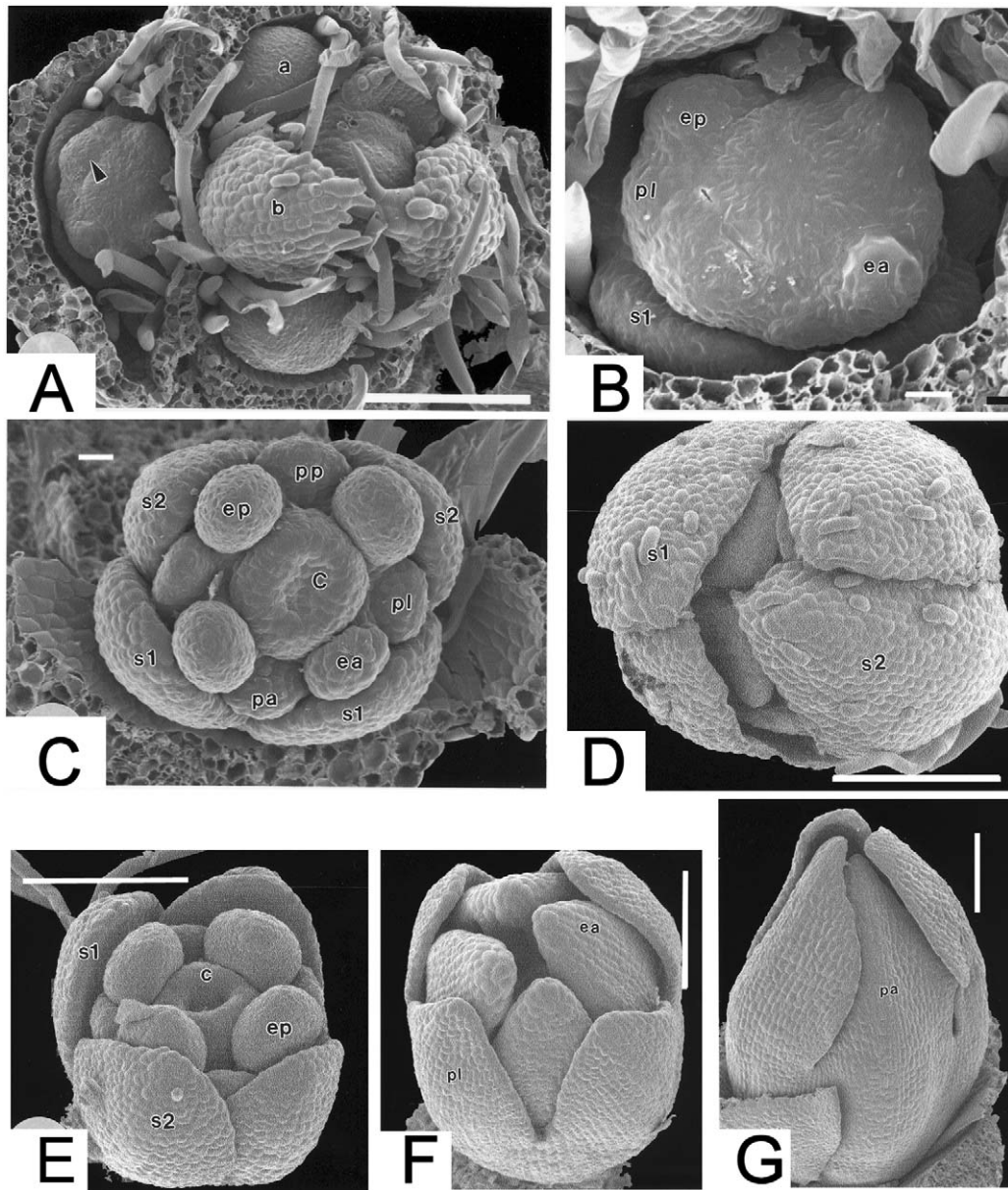
Fig. 7 Floral development of *Plantago major*. *A*, Upper view of an inflorescence; arrowhead on the left floral bud shows an abaxial-lateral stamen primordium. *B*, Floral bud with stamen and petal primordia; the adaxial petal primordia are not yet initiated. *C*, Midfloral development. *D*, *E*, Floral buds showing the zygomorphic calyx. *F*, *G*, Buds at preanthesis (sepals partially removed). a = floral apex; b = bract; c = carpel; ea = abaxial-lateral stamen; ep = adaxial-lateral stamen; pa = abaxial petal; pl = lateral petal; pp = adaxial petal; $s1$ = abaxial-lateral sepal; $s2$ = adaxial-lateral sepal. Scale bars = 100 μm in *A*, *D*–*G*; 10 μm in *B*, *C*.

and Douglas 1989; Endress 1999). Symmetry during early ontogeny is expressed by several traits as the order of initiation and the phyllotaxis of the floral organs, whereas in later stages, it is expressed by differential growth of the organs (Endress 1999). Similarly, it has been proposed that floral symmetry is affected during early development by the position of the floral buds in the inflorescence; for example, in some plants with actinomorphic flowers, initial development is strongly zygomorphic, especially in spikes or racemes (Endress 1999). Armstrong and Douglas (1989) suggested that zygomorphy produced only by early ontogeny would represent primitive zygomorphy in Scrophulariaceae. This could also be applied to the unidirectional emergence of the sepals of *Aragoa* and related genera such as *Plantago*, which would represent ancestral zygomorphy of the calyx (fig. 9). The unidirectional development of the calyx is present independently in other groups of Scrophulariaceae s.l. such as *Calceolaria* L. (Ritterbusch 1976) and *Lophospermum* D. Don (=*Asarina* Miller; Payer 1857).

Synsepaly and pentamery are often correlated with zygomorphy of the calyx (Endress 1987). However, in *Aragoa*, in spite of early zygomorphy shown by the calyx, it becomes
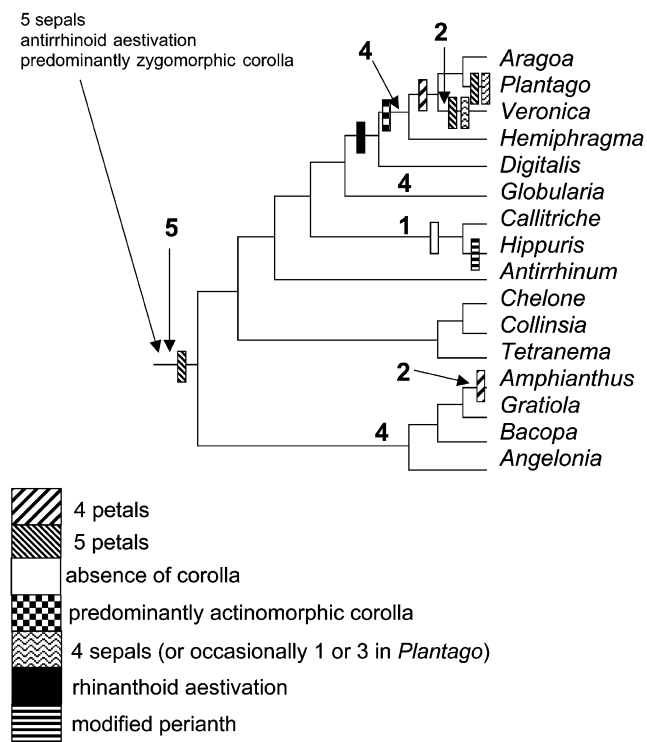
Fig. 8 Distribution of floral characters in the *Aragoa-Angelonia* clade, based on the topology achieved in the molecular analysis of Bello et al. (2002). Numbers above lines indicate number of stamens primordia that initiate in bud. Despite the presence of five petals in some species of *Plantago* and *Veronica*, presence of four petals is optimized as a synapomorphy of (((*Aragoa* + *Plantago*) *Veronica*) *Hemiphragma*), based on species-level phylogenies of *Veronica* and allies (Albach and Chase 2001) and Plantaginaceae (Rahn 1996; Rønsted et al. 2002), in which the basalmost taxa sampled have tetramerous corollas. This suggests that the pentamerous condition could be derived independently in *Plantago* and *Veronica*.

actinomorphic before anthesis; the calyx lobes become similar in size and shape, possibly because there is not a mechanical constriction on their edges as they have cochlear aestivation. This also occurs in *Angelonia*, *Digitalis*, and some species of *Agalinis* and *Antirrhinum* (Wettstein 1895, p. 89; Kampny and Canne-Hilliker 1987; Thompson 1988, pp. 62–66; table 2). However, in *Heliohebe*, *Plantago*, and *Veronica*, genera that primarily have four sepals, the calyx can remain zygomorphic in mature flowers (fig. 6F; table 2). Possibly a tetramerous calyx, with two abaxial-lateral and two adaxial-lateral sepals, allows a permanent zygomorphic symmetry of the calyx during floral development, although *Littorella* and some species of *Plantago* and *Veronica*, with four sepals, exhibit a radial symmetrical calyx (fig. 147H in Harms and Reiche 1895). Moreover, Endress (2003) suggested that in genera such as *Plantago* and *Callitriche*, merosity reduction of the perianth is related to a change from monosymmetry to polysymmetry.

In *Aragoa* and some species of *Veronica*, the adaxial sepal is smaller than the other sepals, whereas in *Heliohebe*, *Littorella*, *Plantago*, and other species of *Veronica*, it is completely suppressed (fig. 6; fig. 7A–7E; tables 2, 3). Taking into ac-

count that the pentamerous condition of the calyx could be plesiomorphic in the *Aragoa-Angelonia* clade (fig. 8; see also Reeves and Olmstead 1998), the presence of four sepals in the latter four genera indicates a suppression of the adaxial sepal (table 3). This type of suppression in an ontogenetic sequence is considered as phase specific; i.e., it is restricted to one whorl only and does not alter subsequent ontogenetic stages (Hufford 1996). Therefore, calyx variation in members of the *Aragoa-Angelonia* clade includes pentamery (i.e., as in *Antirrhinum*, *Aragoa*, *Collinsia*, *Digitalis*, *Hemiphragma*, *Globularia*), tetramery (as in some species of *Plantago* and *Veronica*), trimery (as in some species of *Plantago*), and complete suppression or modification (as in *Callitriche* and *Hippuris*, respectively). Kampny and Dengler (1997) suggested that suppression of the adaxial sepal could be a synapomorphy of *Veronica* and allies, *Pseudolysimachion* (Koch) Opiz and *Hebe* group, with reversals to pentamery in several species of *Veronica*, in *Chionohebe* B. Briggs & Ehrend., *Paedorota* L. and *Paedorotella* (Wulff) Kem.-Nat. However, in the *Aragoa-Angelonia* clade, the presence of four sepals appears independently in *Plantago* and *Veronica* and could be homoplastic in these genera (fig. 8).

Androecium

In most angiosperms, including Euasteridae, the sequence of formation of the floral whorls (calyx, corolla, androecium, and gynoecium) is centripetal (Endress 1994). However, in *Aragoa*, the corolla-androecium sequence of initiation is clearly inverted (fig. 3G; table 3). This change appears independently at least three times in the *Aragoa-Angelonia* clade: (1) in (*Veronica* (*Plantago* + *Aragoa*)) (Kampny et al. 1993; Kampny and Dengler 1997; Prakash and Singh 1997); (2) in *Collinsia* (cf. Schrock and Palser 1967); and (3) in *Angelonia* and *Bacopa* (cf. Srinivasan 1940; Safeeulla and Govindu 1950). The absence of comparative studies on *Amphianthus*, *Chelone*, *Globularia*, *Gratiola*, *Hemiphragma*, and *Tetranema* makes an interpretation of this character in the *Aragoa-Angelonia* clade difficult. Possibly this condition has evolved several times in various groups of Lamiales, although it could be considered as a secondary synapomorphy of the clade ((*Aragoa* + *Plantago*) *Veronica*). The emergence of the stamens before the petals has also been observed in other genera such as *Besseya* (Hufford 1995; Kampny and Dengler 1997), *Calceolaria* (Ritterbusch 1976), *Phlox* (Nishino 1983), *Scrophularia marylandica* (Schertz 1919), *Synthyris* (Hufford 1995; Kampny and Dengler 1997), *Vadellia* (Krishna Iyengar 1940), Plumbaginaceae (Prakash and Singh 1997), and Primulaceae (Duchartre 1844; Schertz 1919; Sattler 1962). Prakash and Singh (1997) suggested that in *Plantago*, this character could indicate a close relationship with Plumbaginaceae rather than with Scrophulariaceae s.l. However, the results of this study and other analyses of floral development in Scrophulariaceae s.l. allow interpretation of independent evolution of this character in different groups of Asteridae. Judd et al. (1999) suggested that the development of the androecium prior to the corolla could be a synapomorphy of Plantaginaceae; however, they included genera such as *Digitalis* in this family. In *Digitalis*, the emergence of the stamen primordia alternates with that of the petal primordia (Singh

Table 2

Main Floral Features of *Aragoa* and Genera Included in the *Aragoa-Angelonia* Clade

Genera	Inflorescences	Floral apex symmetry	Sepal emergence	Sepal primordia	Final symmetry of calyx	Stamen emergence	Vestigial stamens	Fertile stamens	Petal emergence	Petal primordia	Sympetaly	Final symmetry of corolla	Corolla aestivation	References
Amphianthus	Polytelic?	?	?	5	?	?	None	2	?	4	?	Bilateral	Antirrhinoid	1, 3, 4, 6, 15
Angelonia	Polytelic terminal	Radial	Abaxial-adaxial	5	Radial	Abaxial-adaxial	None	4	?	5	?	Bilateral	Antirrhinoid	1, 3, 4, 15, 35
Antirrhinum	Polytelic terminal	Radial	Abaxial-adaxial	5	Bilateral radial	Abaxial-adaxial	Adaxial	4	Simultaneous?	5	Late	Bilateral	Antirrhinoid	1, 2, 3, 4, 7, 8, 13, 15, 24, 25, 29, 31, 32, 35
Aragoa	Polytelic lateral	Radial	Abaxial-adaxial	5	Radial	Simultaneous	None	4	Abaxial-adaxial	4	Late	Radial	Rhinanthoid	1, 3, 6, 12, 15, 38
Bacopa	Solitary flowers	?	?	5	?	?	None	4	?	5	?	Bilateral	Antirrhinoid	1, 3, 4, 15, 34
Callitriche	Solitary flowers	?	?	Absent	...	?	None	1–2	...	...	...	...	...	1, 3, 4, 15, 17, 32
Chelone	Polytelic lateral	?	?	5	?	?	Adaxial	4	?	5	?	Bilateral	Antirrhinoid	1, 3, 4, 15, 18, 32
Collinsia	Polytelic terminal	Radial	Simultaneous	5	Radial	Simultaneous	Adaxial	4	?	5	?	Bilateral	Antirrhinoid	1, 3, 4, 15, 27, 32
Digitalis	Polytelic terminal	Radial or bilateral	Abaxial-adaxial	5	Radial	Abaxial-adaxial	Adaxial	4	Abaxial-adaxial	5	Late	Bilateral	Rhinanthoid	1, 3, 4, 15, 28, 32, 35, 36
Globularia	Polytelic lateral	?	?	5	?	Abaxial-adaxial	None	4	?	5	?	Bilateral	?	3, 4, 15, 30
Gratiola	Solitary flowers	?	?	5	Radial	?	Two abaxial	2	?	5	?	Bilateral	Antirrhinoid	1, 3, 4, 15, 35
Heliohebe	Polytelic terminal	Bilateral	Abaxial-adaxial	4	Bilateral	Simultaneous	None	2	Abaxial-adaxial	4	Late	Radial	Rhinanthoid	38
Hemiphragma	Solitary flowers?	?	?	5	?	?	None	4	?	5	?	Radial	Rhinanthoid	1, 3, 4, 6, 15
Hippuris	Solitary flowers	?	Abaxial-adaxial	?	Radial	...	None	1	...	...	...	...	...	5, 30
Littorella	Polytelic lateral	?	Abaxial-adaxial	4	Radial	?	None	4	?	4	?	Radial	?	6, 10, 11, 12, 15, 23, 26
Plantago	Polytelic lateral	Radial	Abaxial-adaxial	4	Radial, bilateral	Simultaneous	None	(1–3) 4	Abaxial-adaxial	4	Late	Radial, bilateral	Antirrhinoid	1, 6, 10, 11, 16, 19, 20, 21, 22, 23, 30, 32, 38
Tetranema	Monotelic lateral	?	?	5	?	?	Adaxial	4	?	5	?	Bilateral	Antirrhinoid	1, 3, 4, 15, 33, 35
Veronica	Polytelic lateral and terminal	Radial	Abaxial-adaxial	5–4	Radial, bilateral	Simultaneous	None	2	Abaxial-adaxial	4	Late	Radial, bilateral	Rhinanthoid	1, 3, 6, 12, 14, 15, 16, 26, 32, 34, 37

Note. References for data in this table are as follows: 1 = Armstrong and Douglas 1989; 2 = Awasthi et al. 1984; 3 = Bentham 1846; 4 = Bentham 1876; 5 = Cronquist 1981; 6 = Endlicher 1836–1840; 7 = Endress 1992; 8 = Endress 1999; 9 = Fernández-Alonso 1995; 10 = Harms and Reiche 1895; 11 = Hoggard et al. 2003; 12 = Hong 1984; 13 = Kampny and Canne-Hilliker 1988; 14 = Kampny et al. 1993; 15 = Lemeé 1929; 16 = Payer 1857; 17 = Philbrick and Anderson 1992; 18 = Pilger 1913; 19 = Prakash and Singh 1997; 20 = Rahn 1981; 21 = Rahn 1984; 22 = Rahn 1985; 23 = Rahn 1996; 24 = Ronse-Decraene 1992; 25 = Ronse-Decraene and Smets 1994; 26 = Saunders 1939; 27 = Schrock and Palser 1967; 28 = Singh 1979; 29 = Singh and Jain 1979; 30 = Takhtajan 1997; 31 = Thompson 1988; 32 = Walker-Larsen and Harder 2000; 33 = Weber 1972; 34 = Weberling and Troll 1998; 35 = Wettstein 1895; 36 = Wunderlin 1992; 37 = Yamazaki 1957; 38 = this study.

Table 3

Summary of Floral Development in *Aragoa* and Some Related Genera

Taxa	Sequence of floral organ development										References
Aragoa	$Se_{al(2)}$	$Se_{pl(2)}$	$Se_{p(1)}$	$St_{(4)}$	$Pe_{a(1)}$	$Pe_{al(2)}$	$Pe_{ad(1)}$		*	Ca	This study
Digitalis	$Se_{al(2)}$	$Se_{pl(2)}$	$Se_{p(1)}$	$Pe_{a(1)}$	$St_{al(2)}$	$Pe_{al(2)}$	$St_{pl(2)}$	$Pe_{pl(2)}$	$Stm_{ad(1)}$	Ca	Chatin 1873; Singh 1979; Wunderlin 1992
Heliohebe	$Se_{al(2)}$	$Se_{pl(2)}$	*	$St_{(2)}$	$Pe_{a(1)}$	$Pe_{al(2)}$	$Pe_{ad(1)}$		*	Ca	This study
Plantago	$Se_{al(2)}$	$Se_{pl(2)}$	*	$St_{(4)}$	$Pe_{a(1)}$	$Pe_{al(2)}$	$Pe_{ad(1)}$		*	Ca	Payer 1857; Prakash and Singh 1997; this study
Veronica	$Se_{al(2)}$	$Se_{pl(2)}$	*	$St_{(2)}$	$Pe_{a(1)}$	$Pe_{al(2)}$	$Pe_{ad(1)}$		*	Ca	Payer 1857; Chatin 1873; Erbar 1991; Kampny et al. 1993

Note. Sequence of events is from left to right. a = abaxial; al = abaxial-lateral; Ca = carpels; p = adaxial; Pe = petals; pl = adaxial-lateral; Se = sepals; St = stamens; Stm = staminode. Numbers in parentheses indicate number of whorl parts. An asterisk indicates missing organs.

1979; Wunderlin 1992; table 3), which indicates the high residual lability of this character in closely related genera.

The emergence of the stamens is simultaneous in (*Veronica* (*Plantago* + *Aragoa*)), *Collinsia* (Schrock and Palser 1967) and *Heliohebe* (fig. 6C) and unidirectional (from abaxial to the adaxial side) in *Angelonia* (Srinivasan 1940), *Antirrhinum* (Endress 1999), *Digitalis* (Singh 1979; Wunderlin 1992), and *Globularia* (Chatin 1873). In the *Aragoa-Angelonia* clade the simultaneous emergence of the stamens appears independently at least twice and could be derived from a unidirectional pattern (table 2).

In *Antirrhinum*, *Digitalis*, and the ((*Chelone* + *Collinsia*) *Tetranema*) clade, five stamen primordia emerge, but the adaxial one remains rudimentary (Schrock and Palser 1967; Singh 1979; Endress 1999; Walker-Larsen and Harder 2000; table 2). In (*Aragoa* + *Plantago*), *Angelonia*, *Bacopa*, *Globularia*, *Gratiola*, and *Hemiphragma* only four stamens emerge, although in *Gratiola* the two abaxial are rudimentary (Chatin 1873; Wettstein 1895). This implies that the tetramerous androecial condition has evolved at least three times in the *Aragoa-Angelonia* clade (fig. 8). In *Amphianthus*, *Heliohebe*, and *Veronica* there are only two adaxial-lateral stamen primordia (fig. 6; table 2), a condition proposed as synapomorphic in Veroniceae (Kampny and Dengler 1997), which apparently evolved twice in the *Aragoa-Angelonia* clade. However, a plesiomorphic condition for tetramery cannot be ruled out because of the absence of information about androecial development in genera such as *Bacopa*, *Gratiola*, and *Hemiphragma* (see also Reeves and Olmstead 1998). The dimerous and/or tetramerous androecial condition has been proposed as derived from a pentamerous state in Asteridae, including Lamiales (Ronse Decraene 1992). However, in Lamiales, the tetramerous androecium is independently derived in different groups (Ronse Decraene 1992; Endress 1994, 1996; Walker-Larsen and Harder 2000), probably related to different constraints, including specific pollination syndromes, reduction in floral apex size, zygomorphy, and unilateral mechanical constraints during ontogeny (Delpino 1886; Ronse Decraene 1992; Kampny and Dengler 1997; Endress 1998).

Corolla

Petal primordia emerge in an abaxial-adaxial direction in all members of the clade ((*Aragoa* + *Plantago*) *Veronica*) and

in *Digitalis* and *Heliohebe* (Kampny et al. 1993) (figs. 3H, 6C, 7B; table 3). This character strongly suggests early zygomorphy in the corolla of *Aragoa* and *Plantago*, despite its predominantly actinomorphic symmetry during later development of the floral buds. The corollas in these genera become actinomorphic during enlargement as the tube grows uniformly and the lobes reach the same size and shape (fig. 3L, 3M; fig. 7F; fig. 9). The actinomorphic and tetramerous corolla of *Aragoa* is consistent with Endress's (2003) hypothesis that merosity reduction of the perianth is related to a change from monosymmetry to polysymmetry.

In Lamiales secondary actinomorphy occurs in a number of taxa (Endress 1999). The clade (((*Aragoa* + *Plantago*) *Veronica*) *Hemiphragma*), in which the corolla is actinomorphic at maturity, is nested within a group of genera with strongly zygomorphic corollas. Thus, within the *Aragoa-Angelonia* clade, the actinomorphic corolla in mature flowers could be interpreted as a derived condition. The zygomorphic corollas of some species of *Plantago* and *Veronica* could represent a symplesiomorphy. It has been found that mutation of the

Whorl	Calyx			Corolla		
Stage	O	E	A	O	E	A
Aragoa	gray ellipse	gray ellipse	open circle	gray ellipse	open circle	open circle
Plantago	gray ellipse	gray ellipse	gray ellipse	gray ellipse	open circle	open circle
Veronica	gray ellipse	gray ellipse	open circle	gray ellipse	gray ellipse	open circle
Digitalis	gray ellipse	gray ellipse	open circle	gray ellipse	gray ellipse	gray ellipse
Antirrhinum	gray ellipse	gray ellipse	open circle / gray ellipse	gray ellipse	gray ellipse	gray ellipse

Fig. 9 Differences in symmetry of calyx and corolla during different stages of floral development in *Aragoa* and related genera. O = organogenesis; E = enlargement; A = anthesis; open circle = actinomorphic; gray ellipse = zygomorphic.

genes *CYCLOIDEA* and *DICHOTOMA* in *Antirrhinum* produces polysymmetric flowers (Coen and Nugent 1994; Coen 1996; Luo et al. 1996, 1999), so it is possible that mutation of orthologous genes in related genera could be responsible for the secondary actinomorphy within the *Aragoa-Angelonia* clade.

The prevalent actinomorphic tetramerous corolla in *Aragoa* and *Plantago* during late stages of development and the tetramerous androecium are apparently synapomorphies of these two genera (fig. 3*M*, 3*O*; fig. 4*A*, 4*B*; fig. 7*B*–7*G*). Although *Hemiphragma* possesses four stamens and an actinomorphic corolla at anthesis, its corolla is pentamerous. In *Heliohebe* and *Veronica*, the corolla is strongly zygomorphic during development, which results from the presence of three abaxial and one adaxial petal primordia and only two stamens (fig. 6*C*, 6*D*). If ontogeny frequently recapitulates phylogeny, differences in timing of a character state during development, such as changes of symmetry of calyx and corolla in *Antirrhinum*, *Aragoa*, *Digitalis*, *Plantago*, and *Veronica* (fig. 9), would indicate that zygomorphy of both whorls is the plesiomorphic condition for these genera. However, heterochronic shifts may well reflect complex developmental constraints and can undermine apparently straightforward ontogenetic sequence transformations (Hufford 1996).

A reduction from pentamerous to tetramerous corollas in Lamiales can occur by fusion of the adaxial-lateral petals. For example, in *Digitalis* (Wunderlin 1992), *Orobanche*, and *Pedicularis* (Meier-Weniger 1977), the adaxial corolla lobe is produced by two basally united primordia; the resulting corolla lobe is frequently broader than the others (Endress 1998). Assuming that a pentamerous corolla is ancestral in Lamiales (Endress 1994; Reeves and Olmstead 1998), reduction in petal number has occurred at least three times in the *Aragoa-Angelonia* clade: (1) in *Callitriche* and *Hippuris*, in which the corolla is totally absent (Leins and Erbar 1988); (2) in *Amphianthus*, with a tetramerous corolla; and (3) in ((*Aragoa* + *Plantago*) *Veronica*), also with tetramerous corollas. During floral development of *Aragoa*, *Plantago*, and *Veronica*, there is no direct evidence of fusion of two primordia to form the adaxial petal, although the adaxial petal primordium is slightly broader than the others in early development of *Aragoa* (fig. 3*H*), *Plantago* (fig. 7*C*; see also Eichler 1878; Saunders 1939; Endress 1998), and *Veronica* (Hufford 1992; Kampny et al. 1993). The tetramerous corolla could be synapomorphic for the clade ((*Aragoa* + *Plantago*) *Veronica*) (fig. 8). The presence of five petals in some species of *Plantago* (Rahn 1996) and *Veronica* (Saunders 1939; Yamazaki 1957) could be homoplastic because species-level phylogenies of these genera show that their basalmost members have tetramerous corollas (Rahn 1996; Albach and Chase 2001; Rønsted et al. 2002).

Within the *Aragoa-Angelonia* clade, late sympetaly occurs in *Antirrhinum*, *Aragoa*, *Collinsia*, *Globularia*, *Digitalis*, *Plantago*, and *Veronica* (Schrock and Palser 1967; Singh 1979; Erbar 1991; this study). In these genera, the petal primordia emerge independently and become interconnected by interprimordial growth of the alternate stamens (fig. 3*K*–3*M*). However, there are some differences between the late sympetaly of *Digitalis* and *Veronica*; Erbar (1991) showed that in *Digitalis* the two upper petals arise separately and fuse later,

whereas in *Veronica* sympetaly occurs during formation of the upper and lower lips. Erbar (1991) suggested that in these variations, the original condition may become blurred by events such as dorsiventrality and abortion in the androecium. In order to analyze sympetaly in a phylogenetic context, this character needs to be carefully studied in other genera of *Aragoa-Angelonia* clade such as *Amphianthus*, *Angelonia*, *Bacopa*, *Chelone*, *Collinsia*, *Gratiola*, and *Tetranema*.

Corolla aestivation has been considered an important diagnostic character in Scrophulariaceae s.l. (Pennell 1935) because in most Antirrhinoideae, the adaxial lobes are external, whereas in Rhinanthoideae, the abaxial lobes are external in bud. Armstrong and Douglas (1989) showed that this character is evident even during early ontogeny; stronger growth occurs in the adaxial lobes in Antirrhinoideae and in the abaxial lobes in Rhinanthoideae. As *Aragoa* exhibits rhinanthoid aestivation (fig. 4*A*–4*C*), it has traditionally been included in the latter subfamily (cf. Takhtajan 1997). The distribution of this character in the *Aragoa-Angelonia* clade shows that antirrhinoid aestivation occurs in most of the basal genera (*Amphianthus*, *Angelonia*, *Antirrhinum*, *Bacopa*, *Chelone*, *Collinsia*, *Globularia*, and *Gratiola*) (fig. 8; table 2). Thus, rhinanthoid aestivation could be a synapomorphy of the clade (*Digitalis* (*Hemiphragma* (*Veronica* (*Plantago* + *Aragoa*)))), although this character needs detailed ontogenetic study, particularly in *Plantago*.

Gynoecium

The general pattern of gynoecial structure and development seems to be relatively uniform in Lamiales (Kampny and Canne-Hilliker 1988; Kampny et al. 1993; Endress 1994). The unilocular gynoecium (in *Gratiola*, *Hippuris*, and some species of *Plantago*) and tetralocular gynoecium (present in *Callitriche* by formation of a false septum; see Lemée 1929; Leins and Erbar 1988) are apomorphic in the *Aragoa-Angelonia* clade, whereas the bilocular gynoecium, which occurs in *Aragoa*, is plesiomorphic. Seeds of *Aragoa* and *Plantago* are remarkably similar because they are flattened, winged, and reticulate (M. A. Bello, personal observation; cf. also Fernández-Alonso 1995 and Rahn 1996), thus providing a set of possible synapomorphies between the two genera. As for the ringlike nectariferous disk with glandular-ciliate border found in *Heliohebe raoulii*, it is remarkably similar to that found in *Heliohebe hulkeana* (F. Muell.) Garn.-Jones (cf. fig. 1*A* in Garnock-Jones 1993).

Interspecific Differences

Fernández-Alonso (1995) found that gross morphological characters of the mature flowers of *Aragoa* are useful to propose five species groups. The species examined here represent three of these groups: *Aragoa abietina* belongs to the Abietina group; *Aragoa cleefii* and *Aragoa cupressina* belong to the Cuppressina group; and *Aragoa cundinamarcensis* belongs to the Lycopodioides group. However, we did not observe differences in early development that would suggest deep ontogenetic differentiation between these species groups. The slight differences that we have detected are evident only during late stages, when the corolla reaches its definite shape and indumentum; corollas of *A. cupressina*, *A. cleefii*, and *A.*

abietina are subrotaceous and have a dense indumentum on the inner side of the tube, whereas in *A. cundinamarcensis*, it is rotaceous with a glabrous tube. This observation is consistent with Tucker's (1984, 1997) theory that floral characters that are variable at a suprageneric level often arise during early stages of development, whereas those that are variable at generic or infrageneric levels emerge during medium and late developmental stages. In other genera of Lamiales such as *Agalinis* Raf., the appearance of characters that are variable at a specific level also occurs during late development (Kampny and Canne-Hilliker 1987; see also Armstrong and Douglas 1989 for further examples). However, this theory requires more rigorous testing; such hierarchical levels are artificial and may not be equivalent in different groups.

Floral Vasculature

Floral vascularization provides contradictory evidence in members of the *Aragoa-Angelonia* clade. In some species of *Veronica* and *Veronicastrum*, the upper lip of the tetramerous corolla is irrigated by two traces, which could be taken as evidence that this lip is produced from two fused petal primordia (Saunders 1933; Yamazaki 1957; Nishino 1983;

Armstrong 1985). By contrast, in *Digitalis*, the staminode is not vascularized (Henslow 1890), which indicates that, in this case, the vasculature disappeared before the loss of the external organ. Floral vascularization of *Aragoa* does not show vestigial traces of lost floral parts (fig. 5). It seems that the reduction of vascular traces versus external organs is distinct in different taxa (Ronse-Decraene 1992). This requires that such reductions be evaluated with care (Sattler 1973), especially in Lamiales, in which floral synorganization of sepals, petals, and stamens is expressed in diverse combinations of reduction and fusion.

Acknowledgments

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